Growth of Yeast Colonies on Solid Media

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Colonies on nutrient agar of the aerobic yeast *Candida utilis* show linear increases in diameter and height with time throughout most of the growth cycle. The concentration of glucose in the agar has a negligible effect on radial growth rate although an increase in the glucose concentration prolongs the linear radial growth phase. The rate of increase in height of the colony is proportional to the square root of the initial glucose concentration. A new model that considers both glucose diffusion and oxygen diffusion in the colony is consistent with the observed colony profiles.

INTRODUCTION

There have been numerous studies of colonies of bacteria and filamentous fungi but little attention has been given to yeast colonies. Pirt (1967) formulated the physical and chemical factors affecting the growth rate of colonies (mainly bacterial) on solid media; since then there have been several experimental studies (Cooper *et al.*, 1968; Johnson *et al.*, 1970; Palumbo *et al.*, 1971; Rieck *et al.*, 1973; Burchard, 1974; Pirt, 1975; Reyolle & Letellier, 1979; Wimpenny, 1979; Wimpenny & Parr, 1979; Lewis & Wimpenny, 1981). Wimpenny & Lewis (1977) used actual oxygen consumption rates to predict the penetration depth of oxygen into the colony. Oxygen profiles in colonies and in the solid medium have been measured using microelectrodes (Wimpenny & Coombs, 1983; Bungay *et al.*, 1983; Peters *et al.*, 1987). Fraleigh & Bungay (1986) modelled oxygen and substrate gradients in the colony.

Wimpenny (1979) found that near the periphery or leading edge of a growing colony, the height rises relatively steeply to a ridge. Beyond the point of inflexion marking this ridge, the height rises much less steeply to a flat or domed centre. He proposed that growth is exponential and unrestricted by diffusion in young colonies and in the leading edge region of older colonies. At the change in the slope of the profile near the ridge between the two areas, nutrient becomes less available possibly because of depletion of a narrow layer adjacent to the colony base. The growth rate in this area depends on the rate of diffusion of one or more growth limiting nutrients through the agar. Lewis & Wimpenny (1981) also reported variation in colony size and growth rates due to oxygen availability and different nutritional conditions.

According to Pirt (1967), when nutrients have a long diffusion path to the top of a colony, growth is restricted to the outer peripheral zone. However, Palumbo *et al.* (1971) found an increase in colony height throughout the entire growth cycle. There are several similar observations (Wimpenny, 1979; Wimpenny & Parr, 1979; Lewis & Wimpenny, 1981). Reyrolle & Letellier (1979) found two zones of growth – a peripheral zone responsible for the increase in diameter and a central zone that accounts for the increase in height. In the peripheral zone, the main factor for growth was bacterial mobility.

An approximately linear increase in colony height, which later slows down, has been reported to occur during the earlier period of growth (Palumbo *et al.*, 1971; Wimpenny, 1979; Lewis & Wimpenny, 1981). Pirt (1967) postulated that at steady state, the width of the growing zone would remain constant and hence the radial growth rate would become constant. This is called the 'linear' or 'radial' growth law and has been observed by many other workers. This phase is

found to continue until the colony reaches about 80% of its final size. The linear growth phase eventually switches to a slower phase in which the colony area increases linearly with time (Cooper *et al.*, 1968). It was postulated that the onset of strict diffusional resistances caused by nutrient depletion is responsible for this phase and may explain the morphological changes observed in older colonies.

At very low glucose concentrations (0.01–0.1%), Rieck *et al.* (1973) found that colonies exhibited transition points or times at which the initial linear phase changed to a second and slower linear phase. As the concentration of glucose was lowered, colonies had earlier transition points and slower radial growth rates. Growth of the colonies after the transition point was shown to be limited by the rate of diffusion of glucose and by its availability to the colony.

Gray & Kirwan (1974) studied the effect of the boundary walls on the growth of colonies of *Saccharomyces cerevisiae* by varying the size of the culture dish. Transition from radial to area growth rate laws did indeed relate to nutrient depletion. In addition, growth rate was independent of the size and shape of the inoculum and it was concluded that the radial growth law holds for longer periods of time in larger dishes.

Jones & Gray (1978*a, b*, 1979) exposed yeast colonies on a gel to a continuously flowing nutrient solution from below, resulting in a constant nutrient concentration at the colony–gel interface. Rather than confirming the property of indefinite linear–radial growth, maturing colonies in this apparatus showed an exponential increase in diameter with time. An eventual departure from exponential growth was observed and attributed to such factors as buildup of toxic materials (ethanol) and depletion of oxygen and glucose.

For both bacterial and yeast colonies there is a minimum substrate concentration below which no growth occurs, and a linear relationship between the growth rate, k_r , and the square-root of the substrate concentration (Pirt, 1967). The threshold concentration seems to be peculiar to bacterial colony growth and is not found with filamentous fungi (Pirt, 1975). Colonies of filamentous fungi or moulds are inherently different from those of bacteria and yeast because of hyphal extensions. Bull & Trinci (1977) and Prosser (1982) offer excellent reviews.

Pirt (1967) derived equations for the linear growth law and the area law

$$r = \mu wt + r_0 \quad (1)$$

$$r^2 = 2\mu bt + r_0^2 \quad (2)$$

where r = the radius of the colony, r_0 = the initial radius, μ = the specific growth rate coefficient, w = the width of peripheral growth zone, b = a constant and t = time. The main assumptions are constant colony height, growth only in the periphery, and that the cross-section of the growing zone is rectangular.

The above model is not strictly valid, but the rate of increase in colony height is much smaller than the radial growth rate so that the model fits actual data well. Palumbo *et al.* (1971) modelled the colony as a spherical segment. This choice is reasonably accurate for small colonies. The radius R of the sphere of which a colony of radius r and height h is a segment can be calculated using the formula

$$R = \frac{(r^2 + h^2)}{2h} \quad (3)$$

and the colony volume is given by

$$V = \frac{1}{6}\pi h (3r^2 + h^2) \quad (4)$$

Using the same assumption, Wimpenny & Coombs (1983) calculated the volume of the aerobic surface layer, i.e. the growing zone of a colony (V_0) as

$$V_0 = \frac{\pi}{6} \{3(h+r)^2d - 6(h+r)d^2 + 4d^3\} \quad (5)$$

where d is the depth of oxygen penetration into the colony.

The gradual changes in a colony with time permit the assumption that at any time a pseudo-steady-state exists with regard to the gradients of nutrients. Mass continuity equations governing the gradients of nutrients within the microbial colony can be described by

$$\nabla \cdot (D_i \nabla c_i) = Q_i \quad (6)$$

where c , Q and D are the concentration, rate of consumption and mass diffusivity of species i respectively, provided the appropriate boundary conditions are specified.

Gray & Kirwan (1974) modelled colony growth with a sink term for glucose (the limiting nutrient) of the form

$$Q = \mu n(s - s_i) \quad (7)$$

where n is the concentration of the organisms, s is the concentration of the substrate and s_i is the threshold concentration. They considered the one-dimensional propagation of a colony, in the x direction, the plane front of the colony moving with a velocity K_r to develop the equation

$$K_r = k \sqrt{cD} \sqrt{s_0 - s_i} \sqrt{\mu} \quad (8)$$

where k is a constant. This model has the following drawbacks: (a) its one-dimensional nature; (b) the assumption of only one growth-limiting nutrient; and (c) the assumed relation for the consumption rate of the substrate is overly simplistic to be truly applicable to a biological system.

Colonies of aerobic species receive glucose (carbon and energy source) from the medium below while oxygen (terminal electron acceptor) diffuses from the gaseous phase. The counter-diffusion and uptake of these two species means that there is a possibility that in certain regions, metabolism is limited by oxygen availability while in others, glucose is lacking. Fraleigh (1985) sought to overcome the limitations of the model of Gray and Kirwan by simulating oxygen and glucose gradients within a microbial colony using a more realistic two-dimensional approach and also considering double-substrate (glucose and oxygen) limited growth kinetics. He too used the spherical segment approximation of the colony shape.

In this paper we describe the growth on nutrient agar of colonies of the aerobic yeast *Candida utilis*.

METHODS

Candida utilis (ATCC 42416) was grown at 25 °C in 500 ml shake flasks with 150 ml of medium comprising D-glucose (10 g l⁻¹), peptone (5 g l⁻¹) and yeast extract (5 g l⁻¹), sterilized together for 20 min at 121 °C. For growth on Petri dishes (10 cm diameter, 1.5 cm high), medium containing the same concentrations of peptone and yeast extract was solidified with 15 g agar l⁻¹; the glucose concentration used was 1, 5, 8, 10, 15 or 20 g l⁻¹. This range was chosen because, in another study of yeast colonies (Gray & Kirwan, 1974), the threshold concentration was found to be 2.4 g l⁻¹, and beyond about 12 g l⁻¹ there was a departure from the linear relationship between K_r and $\sqrt{s_0}$. The Petri dishes were inoculated to give 20–25 colonies per plate. Growth was at 25 °C.

The colonies were measured with a probe (electrode) and micromanipulator. A model 610C solid state electrometer (Keithley Instruments) and a voltage source were connected to a reference electrode of silver coated with AgCl and an ordinary sewing needle that served as the probe. The Petri dish was held in position on the micromanipulator stand by a small amount of putty at the edges. The reference electrode was embedded into the solid agar medium, and the probe (needle) was centred over a selected colony. A reference point was established above the agar surface, and the probe was lowered to contact the agar along the line of measurement near the ends of the colony to determine the inclination of the agar. Touching either the colony or the agar completed the electrical circuit and caused a current to pass through the electrometer. Colony diameter was measured in two perpendicular directions and averaged.

RESULTS AND DISCUSSION

Typical colony profiles at various times after inoculation on different glucose concentrations are shown graphically in Fig. 1. None of the colonies was completely flat even after long growth periods and most had reasonably circular profiles. A common feature was a point of inflexion close to the edge of the colony, as observed by Wimpenny (1979). The profiles at different times were reasonably parallel, at least during the period when the colony height was increasing.

Fig. 1. Typical measured colony profiles at various times on nutrient agar with glucose.

Fig. 2. Measured colony dimensions versus time at selected glucose concentrations ($\square$, 1 g l⁻¹; $\blacktriangle$, 5 g l⁻¹; $\bullet$, 8 g l⁻¹; $\blacksquare$, 10 g l⁻¹; ∇ , 15 g l⁻¹; $\circ$, 20 g l⁻¹).

Colony radius increased linearly with time at all glucose concentrations (upper portion of Fig. 2), but the findings of Pirt (1967) and Gray & Kirwan (1974) of a radial growth rate proportional to the square of the rate of substrate concentration were not confirmed. We postulate that glucose concentration at the periphery of our colonies was above the value of the half-saturation constant until a colony was large enough to deplete a sizeable region of the agar.

There was no systematic trend in the variation of radial growth rate, K_r , with s_0 . In fact, the K_r values were much the same. The linear phase extended over a longer time period as the glucose concentration was increased. This is to be expected since the eventual departure from linearity is caused by glucose depletion. For the case of 8 g glucose l⁻¹, two linear phases were observed as was reported earlier by Rieck *et al.* (1973), but only at very low glucose concentrations. The constancy of K_r with respect to s_0 is not entirely surprising. In the linear phase, growth is assumed to be restricted to the peripheral region (Pirt, 1967), where the concentration of glucose is nearly equal to s_0 . Hence, unless s_0 itself is very small, glucose will not be a limiting factor.

Colony height increased linearly with time over most of the growth period at all glucose concentrations studied (lower portion of Fig. 2). Although the data were erratic, there is a definite increasing trend of rate of height increase, K_h , with glucose concentration.

Development of a mathematical model

The shape of the colony is assumed to be a spherical segment (Palumbo *et al.*, 1971; Wimpenny & Coombs, 1983). This implies that the profiles at various times will be parallel as was observed in this study. The radius of the sphere bounding the segment is given by equation 3. The following relationship holds

$$dh = dr \cos \theta \quad (9)$$

where

$$\cos \theta = \frac{r}{R} = \frac{2rh}{r^2 + h^2} \quad (10)$$

and

$$\frac{dr}{dh} = \frac{r^2 + h^2}{2rh} \quad (11)$$

Substituting $r = vh$ leads to

$$\frac{dh}{h} = \frac{2vdv}{1 - v^2} \quad (12)$$

On integrating the above expression and simplifying, the following solution is obtained

$$h(v^2 - 1) = \text{constant} = k \quad (13)$$

Hence, the final equation for the colony profile is:

$$\frac{r^2 - h^2}{h} = k = \frac{r_0^2 - h_0^2}{h_0} \quad (14)$$

which is the equation of a hyperbola. This can be rewritten in the following forms

$$r = \sqrt{h(h + k)} \quad (15)$$

$$h = \frac{k}{2} \{ \sqrt{1 + (2r/k)^2} - 1 \} \quad (16)$$

Thus, by specifying the value of the parameter k , the colony profile is determined. The value of k depends on the species of micro-organism and many other factors (Jones *et al.*, 1978*a, b*, 1979).

Case of constant glucose concentration at the agar surface. The assumptions are as follows. (a) The thickness to which a diffusing nutrient can penetrate into the colony is given by the following equation derived by Pirt (1967)

$$h = \sqrt{2Ds_0/q} \quad (17)$$

where h is the colony height, D is the diffusion coefficient, s_0 is the concentration of nutrient at the surface, and q is the metabolic coefficient (g nutrient consumed per cm³ of colony per second). (b) Since s_0 is constant for both the nutrients glucose and oxygen, the depth, d , of penetration of oxygen into the colony as well as the height, h_g , to which glucose from the solid medium can diffuse are assumed to be constant. (c) It is further known that d is less than h_g (Pirt, 1967).

Fig. 3 portrays growing zones in a colony as shaded areas. Four phases can be distinguished in the growth of the colony, depending on the height of the colony.

Phase 1 ($h \leq d$). When the colony height is less than the oxygen penetration depth, d , the entire colony grows exponentially (Fig. 3A), since both glucose and oxygen are available throughout the colony. This is the initial exponential phase predicted by Pirt (1967).

Phase 2 ($d < h \leq h_g$). When the colony height increases beyond d and until it reaches h_g , glucose is available throughout the colony, but oxygen is available only on the colony surface resulting in an annular region of constant thickness d , which is the growth zone in this phase (Fig. 3B).

Fig. 3. Growth phases in a colony. A, Growth throughout colony, no limitation. B, No growth in regions remote from air. C, Growth in top portion limited by glucose, lower portion limited by oxygen. D, Growth only at periphery; overlap of limitations. E, More severe glucose depletion when not supplied continuously. F, Glucose not supplied continuously; overlap of limitations.

Phase 3 ($h_g < h < h_g + d$). When the colony height increases beyond h_g , there are regions in the colony which are deficient in glucose (at the top of the colony) as well as regions where oxygen cannot diffuse. This leads to a growth zone in the form of a truncated annular spherical segment (Fig. 3C). This phase continues until the colony height reaches a value of $(h_g + d)$, at which point the lower growth front touches the glucose line (i.e. the hypothetical line which defines the maximum height to which glucose can diffuse). At this height, there is no point on the colony centre line where both glucose and oxygen are adequate. Of course, reduced glucose or oxygen does not necessarily halt growth completely, and slow growth in the limited zones will distort the colony from the shape predicted by this simplified model. Hence, it is proposed that the final height of the microbial colony is equal to $(h_g + d)$.

Phase 4 ($h = h_g + d$). In this phase, the colony height remains constant at the value $(h_g + d)$. Here, only the outer peripheral region of constant thickness d and constant height h_g is the growth zone (Fig. 3D). Thus, we can see that this model predicts the experimentally observed point of inflexion at height h_g , since the portion above the glucose line remains as a spherical segment while the lower portion expands outwards.

The advantage of the spherical segment assumption is that the mathematical formulation becomes relatively simplified although numerical solutions are still required. If the density of biomass is assumed to be constant, the equation for colony growth (equation 17) can be rewritten as

$$\frac{dV}{dt} = \mu V_g \quad (18)$$

where V is the total colony volume and V_g is the volume of the growth zone.

Up to the end of phase 3, the colony shape is that of a spherical segment. Hence, for phases 1–3, the total colony volume V is given by equation 9. In phase 1, V_{g1} is equal to V . In phase 2, the volume of the growth zone V_{g2} is given by

$$V_{g2} = \frac{\pi}{6} [h(3r^2 + h^2) - (h - d)\{(h - d)^2 + 3(h - d)(h - d + k)\}] \quad (19)$$

The volume of the growth zone in phase 3 can be derived as

$$V_{g3} = V_{g2} - \frac{\pi}{6} (h - h_g)\{(h - h_g)^2 + 3(h - h_g)(h + h_g + k)\} \quad (20)$$

The volume of the growth zone in phase 4 is the volume of the segment of constant height h_g and constant thickness d . Finally, the total colony volume in phase 4 is equal to the constant volume of the spherical segment of height $(h_g + d)$, which is the volume at the end of phase 3, plus the volume of the truncated annular segment of constant height h_g and thickness $(r - r_3)$, where r_3 is the radius at the end of phase 3 obtained by using $h = (h_g + d)$ in the relation between h and r (equation 16). Thus, substituting these expressions for V and V_g for the various phases into the growth equation (equation 20), the following equations are obtained, which can be solved in conjunction with the relationship between r and h (equations 14, 18 and 19).

Phase 1.

$$3(r^2 + h^2) \frac{dh}{dt} = \mu(3r^2 + h^2) \quad (21)$$

An analytical solution leads to the following relation between h and t

$$\ln[h^2(4h + 3k)/\{h_0^2(4h_0 + 3k)\}] = \mu t \quad (22)$$

The colony radius r is related to t using equation 18.

Phase 2.

$$12rh \frac{dr}{dt} = \mu V_{g2} \quad (23)$$

where V_{g2} is given by equation 21.

Phase 3.

$$12rh \frac{dr}{dt} = \mu V_{g3} \quad (24)$$

where V_{g3} is given by equation 22.

Phase 4. Substituting for V and V_g into equation 18 and simplifying produces the following equation

$$\begin{aligned} 6 \left[rf + \frac{df}{dr} \{r^2/2 + 5f^2/2 + (k - 2h_g)f - kh_g\} \right] \frac{dr}{dt} = \mu \left[f[3r^2 + f^2 + (f - h_g) \right. \\ \left. \{ (f - h_g)^2 + 3(f - h_g)(f + h_g + k) \} - \{ (f - d)((f - d)^2 + 3(f - d)(f - d + k)) \} \right. \\ \left. + (f - d - h_g)\{ (f - d - h_g)^2 + 3(f - d - h_g)(f - d + h_g + k) \} \right] \end{aligned} \quad (25)$$

The equations for phases 2 to 4 have been solved numerically (Kamath, 1987), and some simulation results are shown in Fig. 4. From Fig. 4A and 4B, we see that an increase in k leads to a significant increase in the radial growth rate K_r , but has essentially no effect either on the rate of height increase K_h or on the final height attained. In this model, the final colony height is a function only of h_g and of d . Fig. 4B does not show the plateau for maximum colony height at extended times. From Fig. 4C and 4D, we observe that an increase in μ results in an increase in both K_r and K_h , which is to be expected. A surprising prediction of the model is that there is no effect of h_g and consequently s_0 on either K_r or K_h , although the final colony height increases with increase in h_g (Fig. 4E). This is partly corroborated by the experimental finding of this study that K_r is independent of s_0 . Fig. 4F shows the obvious effect of height reached by glucose on the final height of the colony. Also of interest, we found that increasing h_0 has no effect on either K_r or K_h . In summary, this model predicts that the radial growth rate K_r is a function (approximately linear) of the parameter k , the depth of oxygen penetration d and the specific growth rate μ , and is insensitive to changes in the initial substrate concentration s_0 and the initial colony height h_0 . The rate of height increase K_h is a function (approximately linear) of only d and μ . We expected K_h to be independent of h_0 but did not anticipate that k and s_0 would have such small effects.

Fig. 4. Simulation results for growth versus time. A, Effect of parameter k on radial growth; numbers refer to k in mm. B, Effect of parameter k on colony height; same k values as for (A). The plateau at 1.78 mm at extended time is not shown. C, Effect of specific growth rate coefficient μ on radial growth; numbers refer to μ in reciprocal h . D, Effect of specific growth rate coefficient μ on colony height; same μ as (C). E, Effect of oxygen penetration depth d , on radial growth; numbers refer to d in mm. F, Effect of height for glucose diffusion h_g on colony height; numbers refer to h_g in mm. The plateau for 1.644 mm is not shown. Values when held constant: $k = 20$; $\mu = 0.5$; $d = 0.04$; $h_g = 1.644$; initial colony height assumed = $4 \mu\text{m}$.

Case of varying glucose concentration at agar surface. Here again, it is assumed that the thickness to which a diffusing nutrient can penetrate into the colony is given by equation 17 and that the depth of oxygen penetration d is constant, since for oxygen s_0 is constant. But the height h_g to which glucose can diffuse varies with time since the glucose concentration at the agar surface directly in contact with the base of the colony s_b is always less than the initial concentration s_0 . This variation in h_g with time makes the problem much more complex than the earlier one, necessitating the quantification of the change in s_b with colony growth. It is assumed that s_b is a function only of the radial distance along the agar surface since Robson *et al.* (1987) reported that there is no significant variation in the glucose concentration in the agar with depth for fungal colonies. Assuming an expression for the uptake of glucose by the biomass and substituting into the equation for simultaneous diffusion and reaction (equation 6), the change in s_b with changing colony radius can be obtained. In the simplest case of a zero-order uptake, the variation in s_b with radius r is found to be parabolic. A further assumption is that the glucose in the agar that is directly under the colony alone is depleted. This is a reasonable assumption although at very low glucose concentrations Rieck *et al.* (1973) found it invalid. In this case also, growth can be divided into three or four phases, depending on the conditions of growth.

Cell physiology and metabolism may shift because of ageing and because of inhibition by cell products. The exact effects on supply and demand for glucose and oxygen are beyond the scope of this simple model. Since h_g decreases with time, it is not necessary that d is less than h_g at all times. At this point, it is convenient to define the critical height at which the hypothetical glucose line (actually a curve in this case) touches the tip of the colony as h_c . We now have subcase 1, where $h = d < h_c$ at some region, and subcase 2, with $h = h_c < d$ at some region, which is likely to occur only at very low glucose concentration. In subcase 1, exponential growth occurs throughout the colony until the colony height h becomes equal to d . This is phase 1, the exponential phase (Fig. 3A). When h is between d and h_c , in phase 2, the growth zone is an annular segment of constant thickness d (Fig. 3B). When h increases beyond h_c and until it reaches the final value of $\{h_g(t) + d\}$, the growth zone is as shown in Fig. 3E. This is phase 3. In the final phase (phase 4), the growth zone is as shown in Fig. 3F. Depending on the uptake rate of the substrate, the tip of the colony may either be flattish or the colony may assume a bimodal shape with two peaks and a valley at the central point of the colony. Thus, this model can accommodate the experimentally observed bimodal nature of some type of colonies.

In subcase 2, the exponential phase (phase 1) will occur until $h = h_c$. Beyond h_c and until $h = h_g(t) + d$, the growth zone will be as shown in Fig. 3E. In this case, there is a possibility that the s_b may go to zero before the height $h_g(t) + d$ is attained. This is phase 2. Finally, in phase 3, the growth zone will be similar to that in Fig. 3F. Thus, there are only three phases of growth in subcase 2. An appropriate expression for the substrate uptake is needed for solving the above equations using numerical techniques.

Our model for colony growth used the spherical segment approximation for colony shape. Both the simple case of constant glucose concentration at the bottom surface of the colony and the case of nutrient gradients were considered. These models support the experimental findings and help explain the shapes commonly observed with microbial colonies.

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