Experimental verification of a mathematical model for pelleted growth of *Streptomyces coelicolor* A3(2) in submerged batch culture

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A published mathematical model for growth of pellets of filamentous micro-organisms has been tested by comparison of model predictions with experimental data on growth of *Streptomyces coelicolor* in liquid batch culture. The original model considered the classification of pellets into a range of size classes. Growth resulted in movement of pellets to classes of increasing size, while shear forces produced mycelial fragments which entered the smallest size class, from which they grew to form further pellets. This model did not correctly describe changes in pellet size distributions during growth and was therefore modified in two ways. In the first, new pellets were assumed to be formed by the break-up, by shear forces, of existing pellets into two pellets of equal size, rather than removal of small hyphal fragments from the pellet surface. The second modification assumed that the outer shell of active mycelial biomass had a density less than 1 g cm^{-3} and that hyphal density within this shell decreased with distance from the pellet centre. The modified model generated predictions which agreed closely with experimental data on biomass concentration, pellet size distribution, pellet number and pellet radius during batch growth, thereby supporting the assumptions on which the model was based. The model did not accurately describe final biomass concentration, through lack of consideration of autolysis of mycelia at the centre of larger pellets in which growth was limited by diffusion of nutrients. Attempts to incorporate autolysis into the model improved prediction of biomass concentration but were not based on sound biological assumptions and increased the complexity of the model. Further experimental work is required for accurate description of the effects of autolysis on pellet growth.

Keywords: streptomycetes, mycelial pellets, filamentous growth, mathematical model

INTRODUCTION

The growth form of filamentous micro-organisms in submerged culture ranges from discrete compact pellets to homogeneous dispersed mycelia. The relative quantities of each type, together with pellet surface interactions, influence the viscosity, mass transfer and rheological characteristics of the broth. The viscosity of pelleted cultures is low and consequently mixing and mass transfer within the broth are maintained during growth. Harvest-

ing is also made simpler by virtue of the improved filtration characteristics of the broth. Substrate and product concentration gradients within pellets do, however, introduce structural and physiological heterogeneity within the biomass, complicating quantitative analysis of their growth kinetics. This contrasts with dispersed mycelial growth for which such gradients do not exist. This growth form does, however, present disadvantages in that mycelia may wrap around impellers and probes and may cause blockages, leading to contamination of sampling and overflow lines.

The ability to control culture morphology is of particular importance in the prediction of rheological properties,

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downstream processing requirements and product yields. Such control is potentially possible since pellet formation is influenced by both biological and physicochemical factors (Metz *et al.*, 1979; Prosser & Tough, 1991). Factors considered of particular importance include agitation, composition of the growth medium and inoculum concentration.

Although the kinetics of mycelial growth on solid media have been well characterized, growth in liquid culture has been little studied (for review, see Prosser & Tough, 1991). Growth of dispersed mycelia is considered to be equivalent to that of unicells and homogeneous distribution of biomass, substrates and products lead to exponential growth at a constant specific rate in batch culture, where substrates are in excess. In batch culture, biomass (M) increases as a cubic function of time (t) (Marshall & Alexander, 1960; Pirt, 1966)

$$M^{\frac{1}{3}} = kt + M_0^{\frac{1}{3}}$$

where M_0 represents initial biomass and k is a constant. This is due to restricted diffusion of material from the liquid phase to the pellet centre, while unrestricted growth is limited to hyphae in the outer shell of the pellet. Pirt (1966) demonstrated that, for a culture with n spherical pellets, of equal radius r and density ρ , and with an active outer growing shell of thickness w , growing at a specific rate μ , the constant k is given by

$$k = \left(\frac{4}{3} \pi \rho n \right)^{\frac{1}{3}} \mu w$$

Cube-root kinetics will therefore be accompanied by a linear increase in pellet radius through growth within the outer shell of active mycelium enclosing a central non-growing core. More complex mathematical models of pellet growth incorporate oxygen and nutrient gradients within the biomass to predict the likely effects on metabolic activity. Such models have not been applied to the growth of streptomycetes.

Tough *et al.* (1995) presented a finite element model for pellet growth in which the pellet population was distributed between a number of theoretical compartments containing pellets with a particular range of radii. Pellets are assumed to be spherical and growth leads to a linear increase in pellet radius, and individual growing pellets therefore move to compartments representing pellets of increasing size. Fragmentation, through shear forces, also leads to the transfer of pellets to compartments of smaller size. The model considers the development of a population of pellets from an inoculum of spores or mycelial fragments and generates experimentally testable predictions of increases in biomass concentration during growth and changes in pellet size distributions. One prediction of the model is the generation of a bimodal frequency distribution for pellet size. This arises from fragmentation of pellets to form small particles, which subsequently develop to form larger pellets. The culture therefore consists of populations of large, well-developed pellets and small fragments. In this study, the predictions of this

model are tested for pelleted cultures of the filamentous actinomycete *Streptomyces coelicolor* A3(2).

METHODS

Organism and growth media. *Streptomyces coelicolor* A3(2), supplied by Professor D. A. Hopwood, John Innes Institute, Norwich, UK, was routinely cultured and maintained as described elsewhere (Allan & Prosser, 1983). Growth experiments were carried out in a glucose mineral salts broth, adapted from Noack (1986) and containing [g (l distilled water)⁻¹] KH₂PO₄, 2.72; Na₂PO₄·2H₂O, 7.16; NaCl, 5.097; Na₂SO₄, 1.065; MgCl₂·6H₂O, 0.04; FeCl₂, 0.005; NH₄Cl, 0.4; Casamino acids (Difco), 5; D-glucose, 0.5. All components, except glucose, were sterilized by autoclaving for 15 min at 121 °C (15 p.s.i.; 103.5 kPa). Glucose was added to autoclaved medium after filter-sterilization (Millipore, pore size 0.22 µm). MnCl₂ was omitted from the original formulation because of the possibility of oxidation under alkaline conditions affecting pellet formation. Casamino acids were incorporated to increase both specific growth rate and final biomass concentration.

Preparation of inocula. Spore suspensions were prepared by a method modified from Hopwood *et al.* (1985) in which a glass wool filter was used in place of non-absorbent cotton wool, eliminating the need for filtration under vacuum. Similarly, the holed test-tube originally described was replaced by a glass filter funnel to ease transfer of the spore suspension. The filtrate was washed twice in sterile distilled water and resuspended in sterile distilled water for use as an inoculum. Spore concentration was estimated using a haemocytometer counting chamber.

Growth studies. Growth experiments were carried out in shake flask culture and in stirred tank reactors. For the former, 50 ml medium in a 250 ml Erlenmeyer flask was inoculated to give a final spore concentration of 10⁶ spores ml⁻¹. Flasks were incubated at 28 °C on a reciprocal shaker at 250 r.p.m. with a 25 mm stroke. All experiments were performed in triplicate. The pre-germination procedure described by Hopwood *et al.* (1985) was not used as it was found to increase the lag phase of growth in defined medium.

Stirred tank reactor experiments were performed in a 1.6 l stainless steel/glass fermenter vessel (Bioflo II, New Brunswick Scientific) of 1 l working volume maintained at 28.0 ± 0.1 °C and pH 7.00 ± 0.02. Air was supplied at a rate of 2 vols (vol. culture)⁻¹ min⁻¹ by three Hyflo model C pumps (Medcalf Bros) and was pre-filtered through glass wool before sterilizing through a 0.2 µm hydrophobic polytetrafluoroethylene filter (Sartorius). The vessel was fitted with a water-cooled condenser incorporating a similar filter. Baffles were removed to minimize secondary flow and to reduce the surface area available for hyphal entanglement, pellet impaction and possible biofilm formation. Oxygen was monitored by an NBS Series 900 galvanic oxygen electrode and pH by a steam-sterilizable glass membrane pH electrode (Ingold). Dissolved oxygen concentration was maintained within 70–95% of saturation, the lower concentration occurring temporarily on the addition of anti-foam.

Temperature was maintained by an outer water jacket and measured by a platinum resistance electrode within a glycerol-filled stainless steel jacket.

Set point control was used for all parameters, pH being maintained by addition of either 2 M NaOH or 2 M HCl. Although dissolved oxygen tension could be maintained by automatically varying agitator speed, this method was not used because of its possible effect on pellet formation. Consequently

it was ensured that the air-flow rate employed was well in excess of culture requirements. Foam, occurring during the later stages of growth, was controlled by the addition of Dow Corning Antifoam RD Emulsion at a rate of 0.1 ml h⁻¹ using an LKB 2132 Microperpex pump.

The system was autoclaved for 30 min at 121 °C, except for acid and base solutions and growth medium, which were autoclaved for 15 min at 121 °C. Medium was inoculated to give a final spore concentration of 10⁶ ml⁻¹. Samples were removed for analysis of biomass, glucose and ammonium concentrations and pellet size.

Analysis of samples. Biomass concentration was measured by filtering samples through dry, pre-weighed cellulose nitrate filters (Whatman, 0.22 µm pore size). Mycelia were washed in distilled water and dried at 55 °C to constant weight. Glucose concentration in the medium was determined enzymically using Sigma kit 510-A. Ammonium nitrogen was determined colorimetrically using a Technicon Autoanalyser II system. The biomass density of pellets was determined by sizing 25 pellets, calculating their volume prior to drying and determining biomass after drying at 55 °C to constant weight.

To determine pellet size distributions, video images of approximately 200 pellets, suspended in excess broth (to prevent shrinkage due to drying), were traced onto acetate sheets and scaled against a stage micrometer. Outlines of pellets were then digitized using a Hewlett Packard HP9111A digitizing tablet, passing co-ordinates to a controlling HP-85 Hewlett Packard microcomputer. Pellets were assumed to be spherical, enabling calculation of pellet radius and representation of pellet size frequency distributions for each sample with associated statistical analysis.

Electron microscopy. Low temperature scanning electron microscopy was used to examine hydrated pellets and hyphae for shear damage. Washed specimens were blotted dry and frozen under nitrogen slush in a Hexland cryo unit for 10–15 s, preventing damage that would otherwise be caused by boiling nitrogen. Surface ice was removed from the specimens by controlled sublimation, eliminating the need to defrost the main body of the specimen. Specimens were sputter coated with gold at -185 °C in an atmosphere of argon for 5 min at 4 mA before being observed under a Jeol JSM 840 microscope at voltages of 11–25 kV.

RESULTS AND DISCUSSION

Growth characteristics and kinetics

In shake flask cultures, on glucose mineral salts medium, growth took the form of pellets. The increase in biomass concentration could be described equally well by cube-root and exponential kinetics, the former giving a rate constant of 0.03 mm⁻³ h⁻¹ and a correlation coefficient of 0.986 between 8 and 24 h. Over the same period the exponential rate constant, equal to the maximum specific growth rate, was 0.14 h⁻¹, with a correlation coefficient of 0.996. The experimental data were not sufficiently precise to distinguish between cube-root and exponential growth kinetics.

Pellets were also formed during growth in stirred tank reactors, at a specific growth rate (assuming exponential growth kinetics) of 0.22–0.25 h⁻¹ (see below). This higher specific growth rate may be due to more efficient oxygen supply in stirred reactors. Alternatively, differences in

Fig. 1. Changes in biomass (●), glucose (▲) and ammonia (■) concentrations during growth of *S. coelicolor* A3(2) in batch culture in a stirred tank reactor.

shear forces in the two types of vessel may have produced pellets with different morphologies. Differences in specific growth rates between flask cultures and stirred tank reactors are a further indication that application of exponential growth kinetics to pellet growth may not be valid. Values obtained from dispersed mycelia, where biomass will be less heterogeneous, are likely to provide more reliable estimates of specific growth rate.

Typical batch growth in stirred tank reactors is illustrated in Fig. 1 and was accompanied by a decrease in glucose concentration and an increase in ammonia, through deamination of Casamino acids. In the absence of pH control, glycolysis led to a reduction in the pH of the medium. This has also been reported for other streptomycetes, e.g. *Streptomyces alboniger* (Surowitz & Pfister, 1985), *Streptomyces antibioticus* (Lishnevskaya *et al.*, 1986), *Streptomyces hygroscopicus* (Grafe *et al.*, 1975) and *Streptomyces thermoviolaceus* (James & Edwards, 1988) and is associated with over-production of pyruvate and/or α -keto acids. Although ammonia was produced throughout growth, glycolysis prevented alkalization of the medium. When glucose was fully utilized, and in the absence of pH control, the pH of the medium rose by approximately 0.3 pH units and foaming occurred. Catabolite repression by glucose prevented pigment production (Hopwood *et al.*, 1986) during early growth but following exhaustion of glucose, and at acid and neutral pH, pellet cores developed a pink colouration due to production of the isochromanequinone antibiotic actinorhodin (Cole *et al.*, 1987). Under alkaline conditions this acid-base indicator appeared blue, providing an indicator of culture ageing in the absence of pH control.

Effect of agitation rate on pellet size distribution and specific growth rate

Pellet size distributions obtained on the cessation of batch growth at agitation rates ranging from 250 to 999 r.p.m. are illustrated in Fig. 2(a), and all followed unimodal

Fig. 2. Experimental (a) and predicted (b, c) pellet size distributions during batch growth of *S. coelicolor* A3(2) at agitation rates of 250 (▨), 500 (▤), 750 (□) and 999 (■) r.p.m. Assumptions on which Model 1 and Model 2 are based are described in the text.

normal distributions. The model of Tough *et al.* (1995) predicted a bimodal distribution consisting of a population of large pellets, due to pellet growth, and smaller pellets arising from fragmentation through shear forces. A bimodal distribution was not observed experimentally due to the relatively short period of growth in batch culture, which did not provide sufficient time for significant production of hyphal and mycelial fragments before complete utilization of the medium. Modal pellet radius decreased linearly with agitation rate (Fig. 3). Higher agitation rates are thought to reduce spore aggregation and thereby increase the number of particles capable of

developing into pellets. Since only a finite amount of nutrient was available, the mean pellet radius was inversely related to total pellet number and, therefore, to agitation rate. It is not clear why modal pellet radius and agitation rate should be linearly related, nor why pellet number and agitation rate should be exponentially related (Fig. 3), but both relationships were statistically significant with correlation coefficients of 0.997 and 0.981, respectively.

Growth at an agitation rate of 250 r.p.m. did not provide sufficient biomass for analysis of specific growth rates. In

Fig. 3. Effect of agitation rate on modal pellet radius (■), final biomass concentration (▲) and pellet concentration (●) following batch growth of *S. coelicolor* A3(2).

Fig. 4. Low temperature scanning electron micrograph of hyphae on the surface of a pellet of *S. coelicolor* A3(2) showing no signs of shear damage. Bar, 1 μm .

the range 500–999 r.p.m., specific growth rates, calculated assuming exponential growth kinetics, were unaffected by agitation rate (Table 1) and correlation coefficients were significant. Correlation coefficients for cube-root kinetics were also statistically significant for agitation rates up to 750 r.p.m., but cube-root growth constants decreased as agitation rate increased.

Final biomass concentration increased with increasing agitation rate (Fig. 3), as found by Wang & Fewkes (1977) for *Streptomyces niveus* NRLL 2466. This effect has previously been attributed to improved aeration at higher agitation rates (Maxon, 1959), but in our study oxygen was in excess at all agitation rates. Mycelial damage may also reduce yields (Steel, 1959) at low agitation rates but low temperature scanning electron microscopy did not provide any evidence of such damage (Fig. 4). An alternative explanation based on consideration of pellet size and activity is presented below.

Modification of the mathematical model

Discrepancies between predicted and observed pellet size distributions led to modification of the original model of Tough *et al.* (1995) before further testing. The model assumed fragmentation of pellets to form particles of the smallest size class, which subsequently developed to form larger pellets, and therefore predicted a bimodal distribution, which was not observed experimentally. This represents the situation in which hyphal fragments are effectively chipped away from the pellet surface. In simulations this led to the production of a large number of fragments which rapidly utilized substrate, preventing their maturation into true pellets. Experimentally, hyphal fragments were shown to be absent from batch cultures. Indeed it was only in continuous culture studies (data not presented) that mycelial, rather than hyphal, fragments were observed.

Table 1. Experimental and predicted values of specific growth rate and cube-root growth constant during batch growth of *S. coelicolor* A3(2) at agitation rates of 250, 500, 750 and 999 r.p.m.

Experimental values at 250 r.p.m. could not be determined due to production of insufficient biomass. ND, Not determined; r^2 , correlation coefficient. Assumptions on which Model 1 is based are described in the text.

Agitation rate (r.p.m.)	Specific growth rate (h^{-1})				Cube-root growth constant ($\text{mm}^{-3} \text{h}^{-1}$)			
	Experimental data		Model 1		Experimental data		Model 1	
	Mean	r^2	Mean	r^2	Mean	r^2	Mean	r^2
250	ND	ND	0.21	0.96	ND	ND	0.067	0.99
500	0.22	0.93	0.27	0.97	0.059	0.99	0.091	0.99
750	0.22	0.98	0.45	0.99	0.049	0.97	0.109	0.99
999	0.25	0.98	1.34	0.98	0.030	0.84	0.389	0.99

Table 2. Thickness of the non-pigmented outer growing shell of pellets of *S. coelicolor* A3(2) following growth in batch culture at a range of agitation rates

Agitation rate (r.p.m.)	Thickness of active biomass shell (mm)	
	Mean	Standard error
250	0.039	0.007
500	0.033	0.002
750	0.030	0.001
999	0.030	0.001

The model was therefore modified such that pellets were assumed to be broken into two spherical particles of equal mass (M), with radii (r) given by

$$r = \left(\frac{3M}{4\rho\pi} \right)^{\frac{1}{3}}$$

where ρ represents the biomass density within the pellet, initially assumed to be 1 g cm^{-3} . While it may be unlikely that breakage of pellets will result in two particles of identical size, this assumption provides a means of testing alternative mechanisms of breakage, removal of many small fragments from the pellet surface and splitting into a small number of larger particles.

A second modification considered the effect of substrate concentration on the specific growth rate and radial growth rate of pellets. The concentration of limiting substrate (s) available after a time interval dt is the difference between the initial concentration (s_0) and that utilized for growth

$$s = s_0 - \frac{\mu x dt}{Y_{x/s}}$$

where $Y_{x/s}$ is the biomass yield on limiting substrate s ,

and x is the concentration of actively growing biomass. Pellets were assumed to consist of a dense central core of inactive biomass surrounded by a shell of active biomass, of thickness w , capable of utilizing substrate. The concentration of active biomass could therefore be calculated as the mass of small pellets, in which growth was not substrate limited, plus the mass contributed by the outer growing shells of larger pellets experiencing substrate limitation.

This revised model (Model 1) was simulated by iterative solution of equations for growth, loss and fragmentation of pellets in each compartment, as described by Tough *et al.* (1995), and mycelial fragments produced by fragmentation were transferred to the appropriate size class at each time interval. At each iteration the specific growth rate, μ , was calculated according to the Monod equation

$$\mu = \mu_m s / (K_s + s)$$

where μ_m is the maximum specific growth rate. The limiting substrate was assumed to be Casamino acids and, in the absence of published data, a value of 10^{-5} M was used for the half-saturation constant K_s . The thickness of the outer growing shell was assumed to be equivalent to the region of the pellet able to utilize glucose and consequently lacking pigment. This thickness was determined microscopically and there was some evidence for a reduction in thickness with increasing agitation rate (Table 2). This reduction was not large, model predictions were not sensitive to values in the observed range and a thickness of 0.032 mm was used for simulation of the model at all agitation rates.

Predictions of the modified mathematical model (Model 1)

In the absence of any experimental evidence for hyphal or mycelial fragmentation, pellet size distributions at 250, 500, 750 and 999 r.p.m. were obtained by simulation of Model 1 with inocula of 109, 206, 1258 and 7750 equally sized initial fragments, respectively. These values are the

Table 3. Experimental and predicted values of final pellet radius during batch growth of *S. coelicolor* A3(2) at agitation rates of 250, 500, 750 and 999 r.p.m.

Experimental values at 250 r.p.m. could not be determined due to production of insufficient biomass. ND, Not determined; SD, standard deviation. Assumptions on which Model 2 is based are described in the text.

Agitation rate (r.p.m.)	Pellet radius (mm)								
	Experimental data			Model 1			Model 2		
	Mean	Mode	SD	Mean	Mode	SD	Mean	Mode	SD
250	ND	ND	ND	0.24	0.21	0.11	0.16	0.15	0.034
500	0.12	0.13	0.02	0.19	0.17	0.10	0.14	0.13	0.031
750	0.09	0.09	0.018	0.11	0.09	ND	0.10	0.09	0.038
999	0.06	0.05	0.01	0.06	0.03	ND	0.07	0.05	0.032

Fig. 5. Predicted changes in pellet radius (●), pellet concentration (◆), biomass concentration (■) and substrate concentration (▲) during batch growth of *S. coelicolor* A3(2) at agitation rates of (a) 250, (b) 500, (c) 750 and (d) 999 r.p.m.

final pellet numbers observed experimentally in batch culture and demonstrate the effect of agitation rate on spore agglomeration and/or hyphal aggregation prior to the development of true pellets. The experimentally determined value for specific growth rate, 0.22 h^{-1} , was used for simulations. The model predicts changes in the

distribution of pellet radii within the population and changes in biomass concentration, pellet number, pellet radius and substrate concentration, which were compared with experimental data.

As previously described, simulations were carried out using a density value of 1 g cm^{-3} for actively growing biomass at the periphery of pellets. This implies a solid homogeneous mass of mycelium and simulations predicted rapid substrate consumption and negligible radial pellet growth. In reality, hyphae are not tightly packed and the model was modified on the assumption that the outer growing shell had a biomass density less than 1 g cm^{-3} . In the absence of experimental data on biomass density in this region, the value was varied by trial and error by finite intervals until optimal fit was obtained with a value of 0.004 g cm^{-3} . Pellet size distributions obtained using this value are illustrated in Fig. 2(b) and distribution statistics are summarized in Table 3. The model predicts the experimentally observed decrease in pellet radius with increasing agitation rate and the distribution is unimodal, rather than bimodal as predicted by the original model. Model 1 therefore provides a better qualitative description of the experimental data, but there remain quantitative differences. In particular, pellet radius is over-estimated and the predicted pellet size distributions show much greater variability than those observed experimentally.

Organization of biomass within the outer growing shell (Model 2)

A further modification was made to assumptions about properties of the outer growing shell to account for discrepancies between experimental and predicted results. Smaller, immature pellets were assumed to be composed largely of loosely packed hyphae all of which are capable of utilizing substrate. This contrasts with larger more mature pellets with a central impermeable core and only a proportion of the biomass capable of growth and substrate utilization. The thickness of the outer growing shell was assumed to be inversely related to the pellet radius. Initially, in the absence of experimental data, this inverse relationship was assumed to be linear, and a constant of proportionality of 0.0035, obtained by trial and error, gave optimal fit. In addition, the amount and arrangement of hyphae within the outer growing shell will affect growth and substrate uptake. To simulate this factor the density of biomass both within the outer growing shell and in any mycelial fragments was defined. For simplicity, density was assumed to be constant and represented the mean hyphal density within the growth zone capable of utilizing substrate. A mean density of 0.0119 g cm^{-3} was found appropriate when simulating population development at 500, 750 and 999 r.p.m. At 250 r.p.m., a better fit was obtained with a mean density of 0.0063 g cm^{-3} reflecting a further decrease in hyphal density. Although hyphal frequency increases exponentially with distance from the margin of surface colonies of streptomycetes (Allan & Prosser, 1986), application of this relationship to pellet growth failed to improve predicted size distributions. This is not surprising as the factors affecting hyphal

Fig. 6. Low temperature scanning electron micrograph of *S. coelicolor* A3(2) pellet structure indicating reduced hyphal density within the pellet. Bar, 50 μm .

density and physiology are different in the two growth forms. On solid medium, the peripheral growth zone is related to properties of individual hyphae and mechanisms of transport of material to hyphal tips (Trinci, 1971). In pellets, the thickness of the outer growing shell depends on the rates of diffusion and utilization of substrates and oxygen through biomass.

The predicted effects of agitation rate on biomass and substrate concentrations of this revised model, Model 2, are illustrated in Fig. 2(c) and Fig. 5. Final biomass concentration, final pellet number and the rate of substrate uptake increased with increasing agitation rate, as observed experimentally. The predicted standard deviation remained higher than that determined experimentally and failed to decrease with increasing agitation rate. This may reflect the lack of consideration of the effects of pellet density and structure on pellet strength and susceptibility to shear damage. Predicted modal pellet radii were consistent with experimental values but predicted mean radii were slightly overestimated. Correlation coefficients were high for both cube-root and exponential kinetic relationships but the highest correlation was obtained for a linear relationship between biomass concentration and time. In all cases, however, contrary to experimental observations, growth rate is predicted to increase with increasing agitation rates. Whilst growth rates predicted at agitation rates of 250 and 500 r.p.m. were consistent with experimental values, those predicted for 750 and 999 r.p.m. were much greater than observed values. Predicted final biomass concentrations were also greater than experimental values. These discrepancies were thought to arise out of a lack of consideration of the effects on pellet density of hyphal arrangement and autolysis occurring at the centre of pellets. Mature pellets may, for example, consist of a hollow central core surrounded by a solid outer shell (Fig. 6).

Fig. 7. Relationship between observed biomass density and radius for pellets of *S. coelicolor* A3(2) after growth in batch culture (●). The predicted densities of pellets with shell thicknesses of 1.6 ($\square$), 3.0 ($\blacklozenge$) and 19.4 μm ($\circ$) are shown.

Cell autolysis within pellets

To determine whether variations in pellet density were significant in determining final predicted biomass concentrations, the densities of pellets of different radii were calculated (Fig. 7). A lack of information concerning both the kinetics of autolysis and the arrangement of hyphae within pellets allowed the model to be used to test possible explanations for the inverse relationship encountered between pellet density and pellet radius. Initially pellets were considered to be comprised of a central hollow core enveloped in a solid shell of constant thickness ($\rho = 1 \text{ g cm}^{-3}$). Pellet densities were calculated as the ratio of wall mass to pellet volume while the amount of biomass capable of utilizing substrate was defined as above. No attempt was made to simulate the arrangement of hyphae within the pellet since the model is incapable of distinguishing between a thin outer growing shell of densely packed hyphae and a thick shell of sparsely packed hyphae, both of which may have the same biomass density. For experimental and theoretical values to agree, pellets with radii of, for example, 0.3 mm and 0.8 mm would have to contain amounts of biomass equivalent to shell thicknesses of approximately 1.6 μm and 19 μm , respectively (Fig. 7). It should be noted that the use of a biomass density of 1 g cm^{-3} produces a result which may also be derived from a more meaningful value of r and a greater shell thickness. A similar relationship with density exists in defining the thickness of the outer growing shell and the transition radius (below).

It is evident from Fig. 7 that as pellet radius increases, there is less variation in pellet density associated with shell thickness. For radii greater than 0.5 mm, and for the purposes of the model, a shell of constant thickness might be considered to exist. The high density of small pellets may result from initial unlimited hyphal branching in the presence of excess substrate. Larger pellets may experience a reduction in hyphal density at the pellet core, possibly as a consequence of lytic products escaping from these densely packed, substrate-limited, hyphae. Such an ex-

Fig. 8. Relationship between biomass density and pellet radius used to simulate the effects of increasing pellet density as a result of hyphal growth in small, young pellets and the reduction in hyphal density associated with larger, older pellets. Experimental values (●) are also shown.

Fig. 9. Predicted (▨) final biomass concentrations simulated by incorporating the effects of increasing pellet density as a result of hyphal growth in small, young pellets and the reduction in hyphal density associated with larger, older pellets. Experimentally determined final biomass concentrations are also shown (■).

planation has been proposed by van Suijdam (1980) to account for variations in the pellet densities of *Aspergillus niger* and *Penicillium chrysogenum*.

In order to simulate this hypothesis, pellets of radii greater than 0.18 mm (the transition radius) were considered to have the structure described above, with a shell thickness of 0.0175 mm. These values were chosen to optimize fit with experimentally determined biomass concentrations. For those pellets with radii less than 0.18 mm the following empirical equation was used to describe an exponential increase in pellet density

$$\rho = \exp\left(\frac{1}{r} \times 0.18 \times 1.35\right)$$

where ρ is the pellet density and r the pellet radius. Calculated values of ρ incorporated into Model 2 are illustrated in Fig. 8.

The general trend of final biomass concentrations predicted by this modified model agreed with experimental data but predicted values were low at agitation rates below 750 r.p.m. and higher than observed at 999 r.p.m. Whilst predicted final biomass concentrations were greatly improved at the level of the population (Fig. 9), providing some support for this model, large differences between actual and predicted densities of individual pellets (Fig. 8) reflect our ignorance of pellet structure.

Evaluation of the model

The aim of this study was to test the model of Tough *et al.* (1995) for pellet growth of filamentous organisms using experimental data for *S. coelicolor* in batch culture. The original model did not describe the experimental data well, indicating deficiencies in the assumptions on which the model was based. Microscopic observations of pellets indicated that assumptions regarding the structure of the outer shell of active mycelium were too simplistic and invalid. Two successive modifications of these assumptions greatly increased the quality of fit between predicted and experimental values of modal pellet size, pellet

number and pellet size distribution. The major discrepancies are in prediction of final biomass concentration and its rate of increase. This is believed to be due to lack of consideration of autolysis of biomass at the centre of larger pellets, where growth has ceased due to restrictions to substrate and oxygen diffusion. Modifications to the model to describe autolysis were partially successful but increased the complexity of the model and necessitated inclusion of mathematical terms which were empirical rather than based on biological mechanisms. A more rigorous approach to the modelling of autolysis requires additional experimental work, to provide a sounder base on which to build assumptions. This, however, will lead to greater model complexity and could be counter-productive, making critical testing of the model more difficult and increasing the likelihood of introducing significant rounding errors during simulations as a consequence of the iterative method of solution.

The model contains a number of simplifying assumptions which might also be considered in further development. For example, all pellets are considered to be spherical and to increase in diameter at a constant rate. Such assumptions are necessary but the quality of fit obtained between experimental and predicted results indicates that they are valid and that any deviations from these assumptions are not critical for prediction of pellet development. Similarly, errors which might be introduced by the iterative method of simulation may be controlled, for example by varying the time and distance intervals employed, and did not significantly affect model predictions.

In conclusion, a mathematical model is presented, based on a relatively small number of simplifying biological assumptions, which describes growth of pellets of *S. coelicolor* in liquid batch culture. In particular, it predicts the effects of agitation rate on pellet radius, number and biomass concentration. The model extends our understanding of the nature of the outer growing mycelia of

pellets, and of the means by which new pellets are generated, and highlights deficiencies in quantitative descriptions of autolysis within pellets.

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