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MORPHOLOGY-BASED MODELLING OF PENICILLIUM CHRYSOGENUM FED-BATCH CULTIVATIONS

J. MEYERHOFF and K.-H. BELLGARDT

Institut für Technische Chemie
Universität Hannover
Callinstraße 3, 30167 Hannover, Germany
Fax: (0)511/762-3004;
E-Mail: nhchpelt@ rrzn-user.uni-hannover.de

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Abstract

The presented models combine microscopic and macroscopic descriptions of pellet development. There are still some assumptions in the model which are difficult to verify experimentally like the inactivation of the biomass as a consequence of mechanical damage. However, we presented a novel approach which allows to study influences on the overall process such as substrate limitations within the pellets, hyphal morphology or mechanical forces.

Keywords: penicillium chrysogenum fed-batch cultivations

1. Introduction

The cultivation of pellet-forming microorganisms is advantageous compared to filamentous morphologies because of a lower cultivation broth viscosity the facilitation of the biomass separation. On the other hand, nutrients and oxygen can enter the inner parts of a pellet only diffusively. Thus, large pellets (> 400 μm diameter) often become hollow in the centre due to oxygen or nutrient starvation (WITTLER et al., 1986).

The first comprehensive theoretical study on modelling pellet-containing bioprocesses was published by METZ (1976). His models, however, had some serious restrictions, for example, the assumption of a constant biomass density within the pellet. A model which primarily considers the mycelial growth was presented by YANG et al. (1992a, b). This model is capable of describing early pellet development in detail but does not consider internal substrate limitations. BUSCHULTE (1991) expressed the pellet development by a set of partial differential equations. None of these models was applied for process simulations. It was the aim of our work to build a statistical model which not only describes the morphology of single pellets but also an ensemble of pellets to represent the biomass in a process.

2. Mathematical Models

The model is subdivided into three parts: a morphologically detailed model considering each hyphal subunit of the pellet, a simplified model for pellet development, and finally a pellet ensemble representing the pellet population in a fed-batch fermentor.[1]

2.1. Single Hyphae Model

This part of the model is largely adopted from YANG et al. (1992a, b). It describes growth, septation and branching of single hyphae. The growth of the hyphal network is simulated in three dimensions. We added expressions for oxygen and glucose diffusion into the pellet. As soon as one of the substrates in the pellet centre drops below a critical concentration, the biomass is inactivated and lyses. Furthermore, the model describes the chipping of hyphae from the pellet surface.

2.2. Simplified Layer Model

Since the detailed model makes great demands on the computer capacity, the detailed model for single pellets had to be simplified. The spherical space in which the pellet grows is subdivided into layers of equal thickness. Biomass density, the hyphal growth unit, concentrations of substrates and tips are averaged within a layer. The model avoids partial differential equations; all calculations are discretisized in time and space. Some of the expressions were empirically modified in order to make the correspondence to the single hyphae model as close as possible. Except for the detailed description of the mycelial structure, the features of layer model are equivalent to the single hyphae model.

2.3. Model for Pellet-Containing Bioprocesses

The simulations of the layer model are extended to an ensemble of 100 or more pellets, each of which represents a subpopulation of the biomass. Balances were set up for a *Penicillium chrysogenum* fed-batch process and compared to experimental data. Spore germination at different times (PAUL et al., 1993) and pellet breakup by mechanical forces were included

[1] In order to keep the text concise, all mathematical formulae were omitted. Further information is available upon request.

so as to explain pellet size distributions. Pellet disruption was assumed to cause cell damages which diminish the growth activity. Penicillin production was coupled to the morphology: In the model, all active hyphae except the first 20 μm adjacent to a tip produce penicillin with first order rate.

3. Results

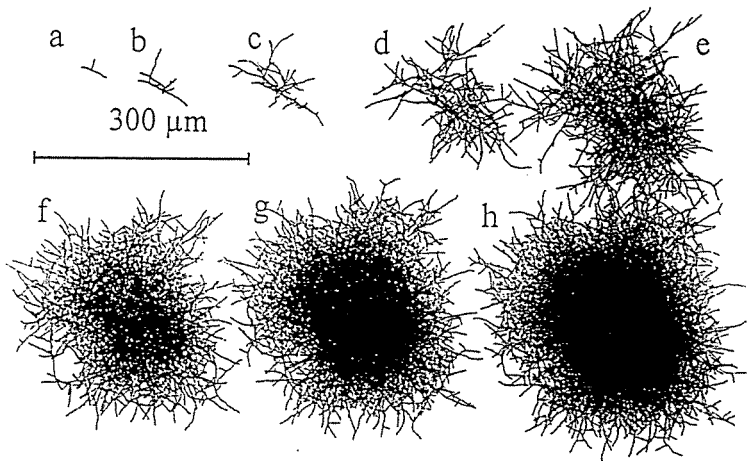

Fig. 1. Two-dimensional projection of the mycelial structure during pellet development without influence of shear force. a) 2 compartments/13 h, b) 10/32 h; c) 50/51 h; d) 250/69 h; e) 1250/84 h; f) 4000/96 h; g) 10000/103 h; h) 17000/110 h.

Fig. 1 shows a two-dimensional projection of the developing hyphal structure of a pellet. Abrasing of hyphae from the surface is not included in this simulation. Studies with the single hyphae model showed that the maximal hyphal growth rate is crucial in determining the maximum volume density and the radius of the pellet. Glucose is not limiting unless the medium concentration drops below about 100 mg l^{-1}. The functional relation of the oxygen diffusion coefficients to the mycelial volume density is not as decisive for lysis as the oxygen concentration in the medium.

Fig. 2 shows the simulated development of a single pellet represented by profiles of the local cell volume density. It also shows that both the single hyphae and the layer model yield similar results. The sharp edge in the inner parts of the older pellets is due to lysis which follows an all-or-nothing mechanism.

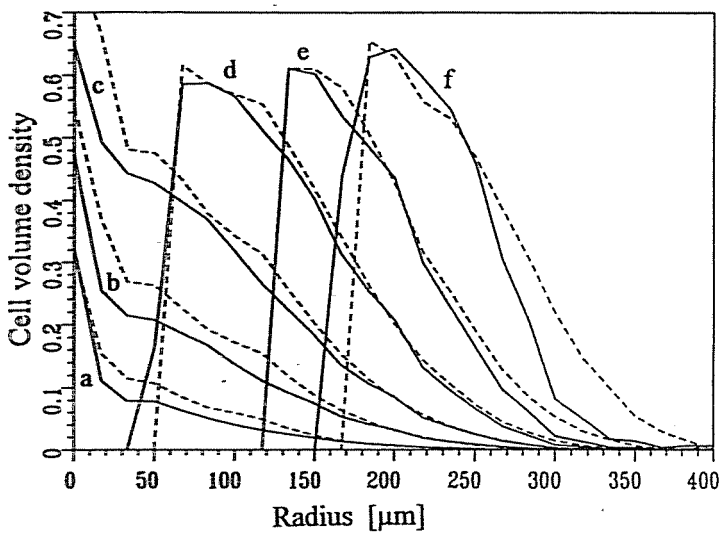

Fig. 2. **Comparison between results of simulations with the single hyphae model (dashed line) and the layer model (solid line). Cell volume densities after a) 90 h; b) 105 h; c) 115 h; d) 125 h; e) 135 h; f) 149 h.**

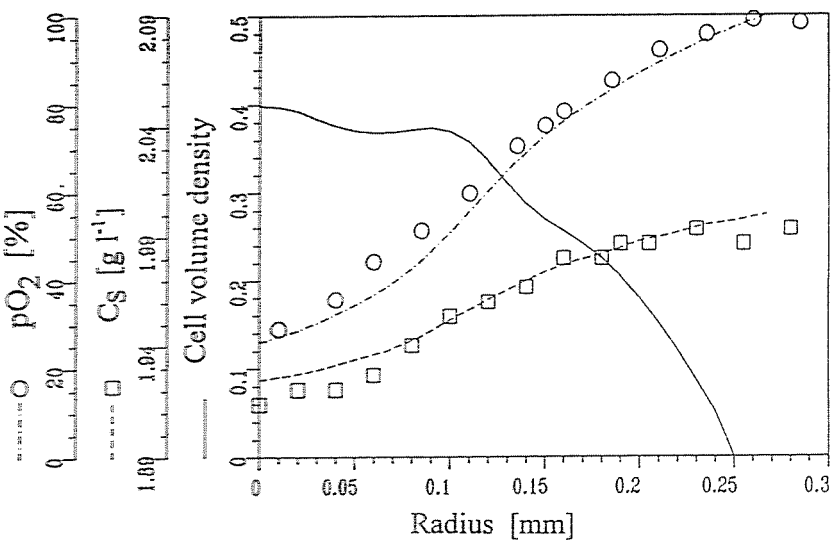

Fig. 3. **Comparison between oxygen and glucose profiles as obtained from microprobe measurements (symbols) and simulations (dashed lines) with the layer model. The cell volume density profile is obtained from digital image processing. C_s: concentration of glucose.**

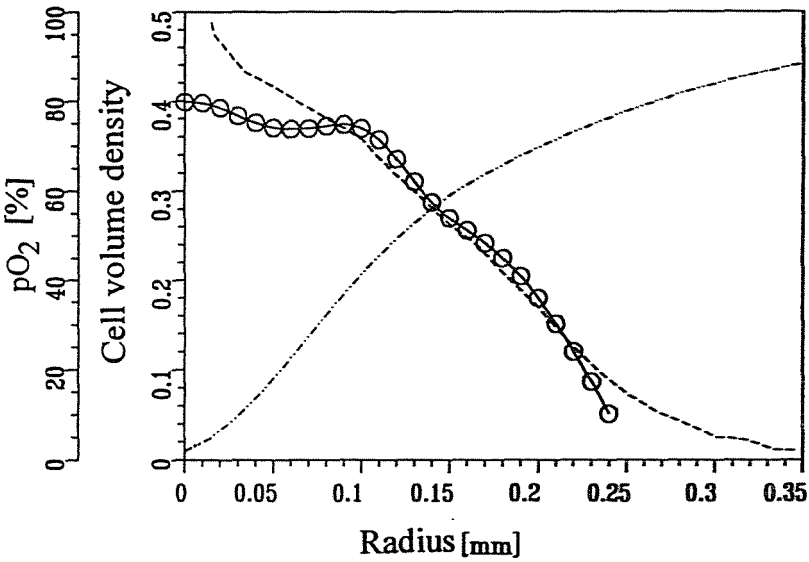

Fig. 4. De novo simulation of a pellet. (– – – – –) **Simulation results of cell volume** density; (○) image processing density **measurements connected by cubic spline** interpolation (——————); (– · – · – · –) **simulated oxygen profile.**

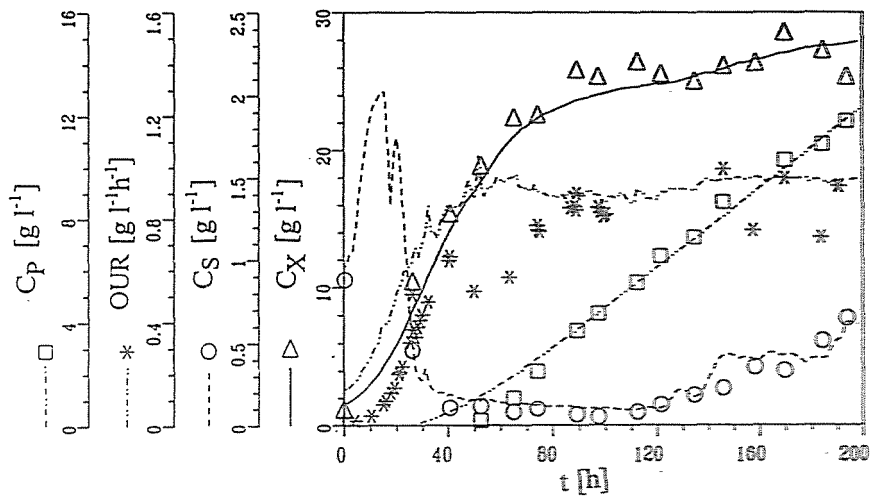

Fig. 5. Time course of biomass, glucose and product concentrations of a *Penicillium chrysogenum* fed-batch cultivation. Symbols refer to experimental data. C_P, C_S, C_X: concentrations of penicillin, glucose and dry biomass, respectively; OUR: oxygen uptake rate.

Experimental data from microprobe measurements and digital image processing were used to verify the layer model (CRONENBERG, 1994; CRONENBERG et al., 1994, POTTEL and BELLGARDT, 1992). The image processing method yielded cell volume density profiles after the pellets had been cut into thin slices and stained. *Fig. 3* shows the comparison of simulated and measured oxygen and glucose profiles using the experimentally measured cell density profiles. *Fig. 4* shows that the cell volume density profiles can also be obtained by *de novo* simulation. The simulated time corresponds to the time of sampling the examined pellet.

Experimental fed-batch cultivations of *Penicillium chrysogenum* were described by an ensemble of 80 pellets (*Fig. 5*). The same data were used in a conventional segregated model by TILLER et al. (1994). The pellet model presented here achieves similar results. In addition, the pellet model explains the experimentally observed pellet size distribution. The inhibition of growth caused by pellet breakage turned out to be most important for the development of the

C_x-plateau after $t = 80$ h. This corresponds to the assumption of an inactive fraction of the biomass in the model by TILLER et al. (1994).

A sensitivity analysis showed that the penicillin productivity is strongly dependent on the morphology since the number of tips per mycelial length determines the size of the fraction of producing biomass.

4. Conclusion

The presented models combine microscopic and macroscopic descriptions of pellet development. There are still some assumptions in the model which are difficult to verify experimentally like the inactivation of the biomass as a consequence of mechanical damage. However, we presented a novel approach which allows to study influences on the overall process such as substrate limitations within the pellets, hyphal morphology or mechanical forces.

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