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Influence of age and structure of Pencillium chrysogenum pellets on the internal concentration profiles

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Abstract Pellets of Penicillium chrysogenum which were spontaneously formed after a certain stage of a batch fermentation, displayed a considerable structural change in course of their lifetime. Microelectrode studies showed the internal mass transport properties of these pellets (diameter 1–3 mm) to be highly effected by their morphological structure. Relatively young pellets, in an early stage of the batch fermentation, possessed a homogeneous and dense structure. These pellets were only partly penetrated by oxygen (ca. 70 μm) at air saturated bulk conditions. Older pellets, in a final stage of the batch fermentation, were stratified and fluffy. They were completely penetrated by oxygen due to a decreased activity and a higher diffusivity. Investigations with glucose microelectrodes revealed that glucose consumption inside pellets of all lifetimes exclusively occurred in the periphery, indicating that growth was restricted to these regions only.

List of symbols

C	mol/m^3	Concentration
De	m^2/s	Effective diffusion coefficient in pellet
Dgel	m^2/s	Effective diffusion coefficient in gel
D	m^2/s	Effective diffusion coefficient obtained from transient state experiment
k	mol/(m^3 s)	Zero order rate constant
Q	mol/(m^3/s)	Oxygen uptake rate
Q'	mol/(m^3 s)	Effective oxygen uptake rate
R	m	Radius of the pellet
r	m	Distance from the centre of the pellet
v	m/s	Average fluid velocity
y		Dimensionless oxygen concentration
ϕ		Thiele modulus: $R(k/C_{i,o}De_o)^{1/2}$
λ	m	Oxygen penetration depth
ρ		Dimensionless distance

Subscripts

b	Bulk liquid
c	Centre of the pellet
g	Glucose
i	Liquid-pellet interface
o	oxygen

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1 Introduction

Filamentous mycelia, like Penicillium chrysogenum can grow either in suspension, or in the form of submerged pellets. The formation of mycelial pellets results in a heterogeneous physiology of the culture as a consequence of poor substrate and oxygen penetration in the pellet. In the growth phase, the mycelia in the peripheral shell of the pellet grow exponentially, while, due to diffusional limitations of the oxygen transfer, growth of the inward lying biomass is prevented, which may eventually end in autolysis and void formation in the centre of the pellet [2]. Due to growth and lysis processes the physical and biochemical properties of the pellets alter during the course of the fermentation.

Numerous papers and reviews have described kinetics and mass transfer properties of mycelial pellets [9, 11, 18]. Wittler et al. [17] investigated oxygen concentration profiles in pellets with microelectrodes in the presence and absence of turbulence and convective flow of the bulk medium. In their paper, a convective contribution to mass transfer in the peripheral shell of the pellet was demonstrated. From the influence of the external mixing intensity on the internal mass transport rates it might be explained why the effective diffusion coefficients for oxygen De_o, reported in literature, exhibit such a large variation: Values range from 0.29×10^{-9} to 19.2×10^{-9} m^2/s [8, 12]. Furthermore, it seems plausible that differences in pellet structure are an important reason for the reported variations. Due to aging the structure of a pellet will change during the fermentation, thereby effecting the internal mass transfer rates.

Our aim was to investigate changes in mass transport properties and kinetics of Penicillium chrysogenum pellets as a function of the fermentation time, using a recently developed glucose microsensor [4]. For this purpose a batch cultivation was carried out in a laboratory scale fermenter, inoculated with spores of Penicillium chrysogenum. Samples of the reactor contents were taken at various cultivation times for microelectrode measurements. Oxygen and glucose concentration profiles and penetration depths were determined in some 40 different pellets with sizes ranging from 0.5 to 3 mm.

2 Materials and methods

2.1 General

An industrial strain of Penicillium chrysogenum *S2*, donated by Hoechst Co. (FRG), was grown at conditions which produce mycelial pellets. A 2 l fermenter was directly inoculated with lyophilized spores without a separate preculture to realise a well

defined start. The pH and the dissolved oxygen concentration were controlled continuously. The first pellets developed after 100 hours, and onwards 25 ml samples were taken periodically for microelectrode measurements and microscopic observations. Conditions and medium composition for the fermentation and the measurements are given in Table 1.

After circa 200 hours the apparent viscosity of the culture had increased considerably, due to a steady increase of the mycelial mass, while the glucose was nearly consumed. It indicated the transition from the growth to the production phase, and 18 kg/m^3 lactose, 6.6 kg/m^3 phenoxyacetic acid, and 2.0 kg/m^3 ammoniumsulfate was added to the culture medium. After 300 hours 10 kg/m^3 of penicillin was produced.

To study the morphology of pellets, histological slices with a thickness of 1–10 μm were prepared by imbedding pellets in a synthetic resin, and cutting with a microtome. The slices were coloured with toluidine blue and photographed through a microscope.

2.2
Microelectrodes

The preparation and operation of oxygen microelectrodes with a selective silicon membrane and an internal reference electrode has been described previously [15]. The resolution of the electrode signal was approximately 2×10^{-3} mol/m^3 oxygen. The simple and shielded type of microelectrodes used for the glucose profile measurements had a 5 to 10 μm tip and has been described recently [4, 5, 6].

2.3
Steady-state concentration profiles measurements

The measurements of concentration profiles were conducted in a rectangular 100 ml cell containing a pellet mounted on entomological specimen needles. The micro-electrode was introduced in the biofilm with a motordriven micromanipulator (Owis, Freiburg) with an accuracy of 1 μm. Since the filamentous structure at the outside of pellets complicated visual determination of the pellet surface, the method of Huang and Bungay [8] was used to define its exact position. The micro-electrode was slowly driven to the pellet surface after draining the fluid from the cell. According to this method, the point of liquid junction with the pellet, as apparent from the change in the electrode signal, was taken as zero. Then medium was supplied to the cell again. Filaments that first stuck to the surface stretched and pointed in radial direction (Fig. 1f). The average length of these hairy structures as well as the diameter of the whole pellet was determined with a microscope with an eyepiece micrometer.

Two mixing rates were employed in the cell, using a stirrer and an aerator. Average fluid velocities (v) around the pellet were measured by flow line visualization [13], and estimated as 0.02 m/s and 0.1 m/s respectively. After each glucose measurement the pellet was turned around for the measurement of an oxygen profile. In this way, the possible damage caused by the glucose electrode did not disturb the oxygen measurement. If an anoxic core in the pellet was present, the penetration depth λ was defined as the distance from the pellet surface to the position where the oxygen concentration amounted to 3×10^{-3} mol/m^3.

Table 1. Conditions and media for fermentation and measurements

	Unit	Fermentation	Measurements
Conditions:			
temperature	°C	25	25
pH	–	6.0	6.5
O_2	mol/m^3	0.08–0.25	0.25
fluid velocity	m/s	0–0.75	0.02 and 0.1
Medium:			
glucose	mol/m^3	150	2.5 and 5.0
lactose	mol/m^3	30	0
KH_2PO_4	mol/m^3	3.0	3.0
$(NH_4)_2SO_4$	mol/m^3	15.1	15.1
$CaCO_3$	mol/m^3	50	0
pharmamedium	kg/m^3	10	0
cornsteep	kg/m^3	30	0
yeast extract	kg/m^3	10	1.0

2.4
Determination of oxygen uptake rates

After completion of a microelectrode measurement, the total oxygen consumption rate of the intact pellet was investigated in a Biological Oxygen Monitor (BOM). The BOM consisted of a 1 ml vessel mounted with a commercial oxygen electrode (YSI 5331) and a 3 mm magnetic stirring bean. Care was taken to establish comparable flow conditions in the BOM and in the microelectrode flowcell ($v = 0.1$ m/s).

The pellet was incubated in the BOM for half an hour, in continuously aerated measuring medium containing 5 mol/m^3 glucose. Then the oxygen supply was stopped, and the decreasing dissolved oxygen concentration was registered in time.

2.5
Diffusivity measurements

Average diffusivity rates of glucose and oxygen in the pellet were obtained by stimulus response experiments [5]. For this purpose the pellet was deactivated in a 0.1% (w/v) $HgCl_2$ solution for 2 hours. After rinsing and equilibration in the measuring cell in 0.1 M phosphate buffer at pH 6.8, the micro-electrode was positioned at a certain depth in the pellet. The liquid in the cell was mixed intensively ($v = 0.1$ m/s).

Glucose and oxygen concentration steps of respectively 0 to 1.0 mol/m^3, and 0.02 to 0.25 mol/m^3 were employed. The resulting change of the electrode response in time was recorded. The average diffusivity rate of glucose and oxygen at a certain position in the biological matrix was calculated by comparison of the measured and theoretical response [3, 16]. Eventually, the average diffusivity rate in the pellet was compared with the experimental rate found in spherical gels ($R = 1.60$ mm) which values are comparable to those in water. The gel beads were prepared from a dilute agar-agar solution (10 kg/m^3 w/v) as described previously [5].

3
Results and discussion

3.1
Microscopic observations

The first pellets, formed after approximately 100 hours of cultivation, showed a dense structure and appeared relatively

robust and difficult to disrupt. Entrapped solid particles could be observed under the microscope (Fig. 1a); they probably consisted of $CaCO_3$ crystals present in the medium. In some cases these particles caused breaking of the microelectrodes during penetration of the pellet.

The structure of the pellets gradually changed during cultivation. Although these changes appeared uniformly, it might be that pellets were continuously formed and disrupted. After 200 hours the pellets were fluffy and easy to deform (see Fig. 1d and 1f). The outer 200–500 μm zone of these older pellets had changed significantly: The photographs clearly show a very loose structure. Obviously, the biomass concentration in the pellets had decreased significantly in time. Some pellets appeared to be hollow, while some other microtome slices showed a dark coloured zone in the centre of the pellets, probably resulting from mineralization of dead biomass.

3.2
Steady state microprofiles

The glucose microprofiles due to consumption were characterized by a gradient in a small layer near the interface, and the absence of a gradient deeper inside the pellet (Figs. 2 and 3). Although glucose penetrated the whole pellet almost at bulk level, no glucose consumption was observed in the core. It was concluded that the glucose consumption was located only in the outer shell of the pellet, with a thickness of only 100–200 μm.

No significant change was displayed in the general shape of the glucose profiles in the course of the cultivation. However, the glucose concentration difference ΔC_g between the interface and the centre of the pellet did decrease; for 1 mm pellets from approximately 0.07 mM at 150 hours to 0.04 mM at 250 hours. Furthermore, the pellet size had no significant influence on the general shape of the glucose profile. In Fig. 4, a profile measured in the relatively small pellet from Fig. 1c is given. Like in larger pellets, the glucose consumption mainly took place in the outer zone. Since the glucose consumption for all pellets investigated remained restricted to the outer, most hairy zones, it can be concluded that here the most active mycelia were present, and that growth only occurred in the periphery.

Contrary to the glucose profiles, the oxygen profiles changed significantly during the batch culture. As shown in Fig. 2, the pellets that developed firstly exhibited steep profiles and an oxygen penetration depth less than 200 μm. Consequently, anoxic conditions prevailed in the largest part of these pellets. Since respiration of Penicillium chrysogenum under these circumstances is irreversibly inhibited [10], the biomass in the centre lost its activity, as confirmed by the glucose microelectrode measurements.

In a later stage of the culture, the oxygen penetration increased. In Fig. 3 it is shown that after 240 hours oxygen

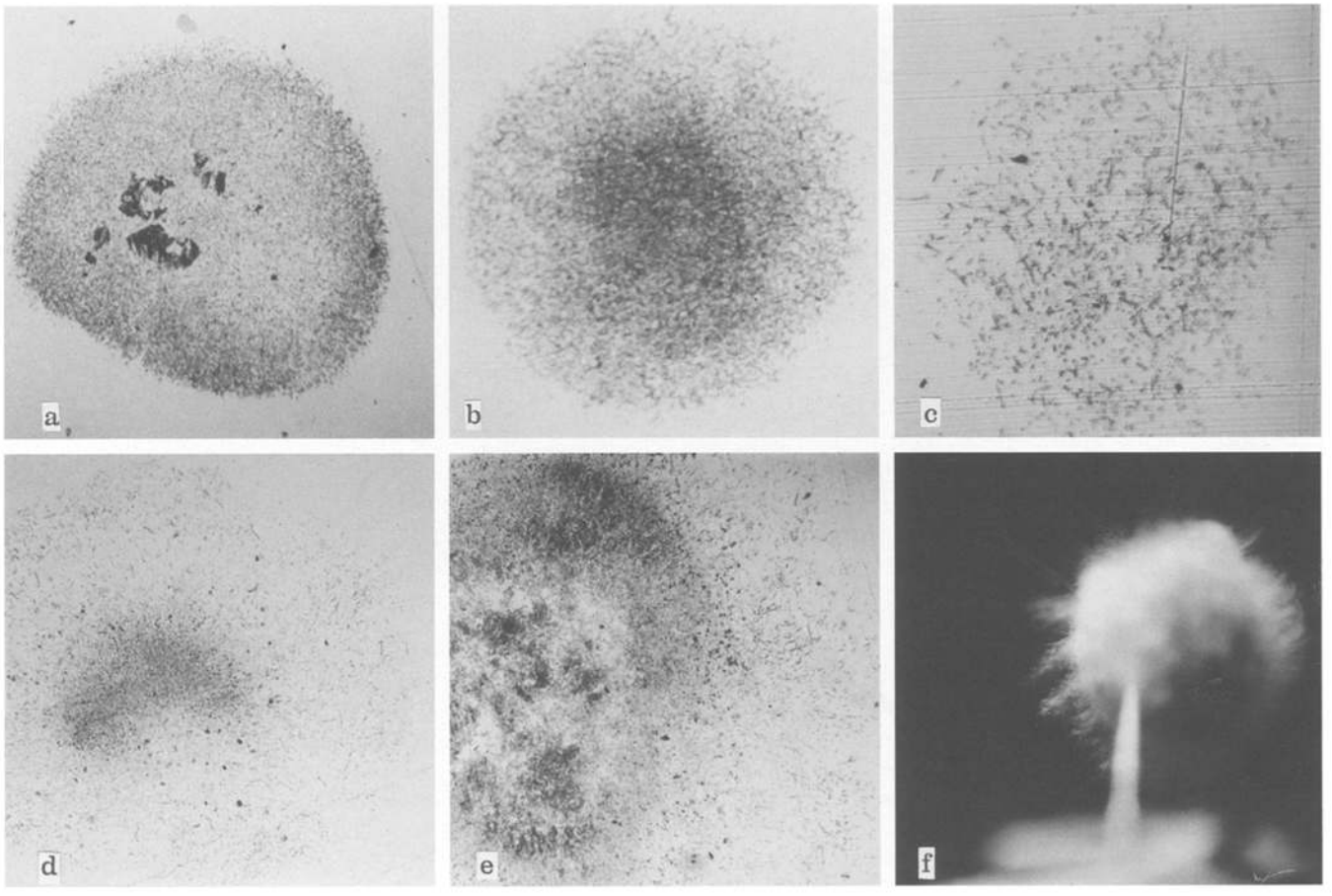

Fig. 1a–f. Photographs of microtome-slices with a thickness of a few microns of different pellets at a different fermentation time, and a photograph of a whole pellet in the measuring cell. **a** $R = 830$ μm, 144 h; **b** $R = 800$ μm, 164 h; **c** $R = 240$ μm, 216 h; **d** $R = 1440$ μm, 240 h; **e** $R = 1560$ μm, 211 h; **f** $R = 900$ μm, 240 h

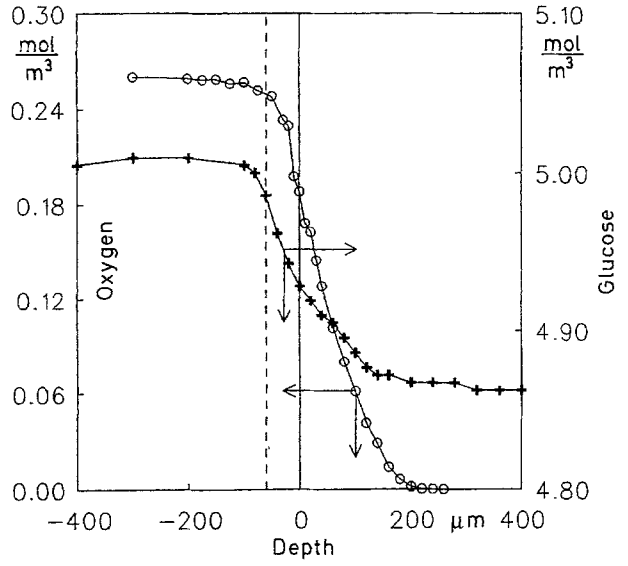

Fig. 2. Micro profiles in a pellet ($R = 800$ µm) after 144 h (Fig. 1b): (+) glucose, (○) oxygen. The solid line indicates the pellet surface in air; the dashed line indicates the surface of the outermost filaments with a length of 60 µm. Mixing rate: $v = 0.02$ m/s

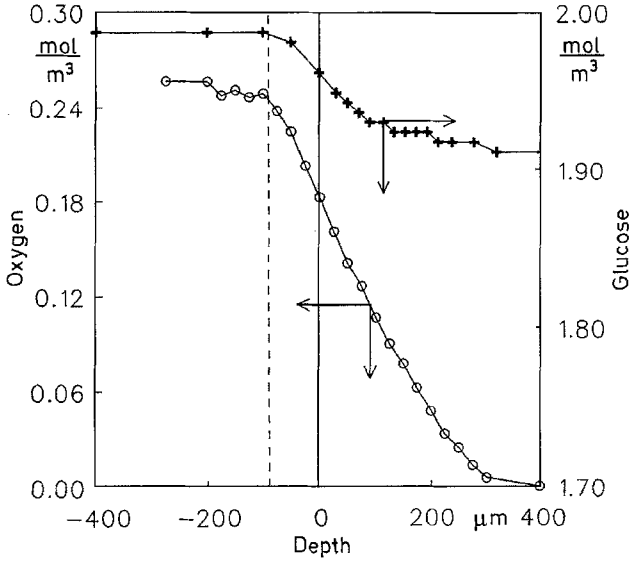

Fig. 3. Micro profiles in a large pellet ($R = 1560$ µm) after 240 h (Fig. 1d): (+) glucose, (○) oxygen. The solid line indicates the pellet surface in air; the dashed line the surface of the outermost filaments with a length 90 µm. Mixing rate: $v = 0.02$ m/s

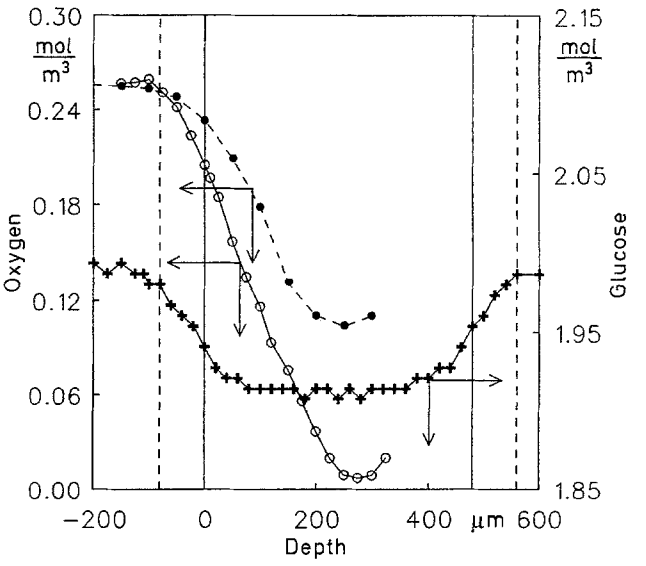

Fig. 4. Micro profiles in a small pellet ($R = 240$ µm) after 216 h (Fig. 1c): (+) glucose, and (○) oxygen at a mixing rate $v = 0.02$ m/s. The dashed curve indicates the oxygen profile at $v = 0.1$ m/s. The solid lines indicate the pellet surface in air; the dashed lines indicate the surface of the outermost filaments with an average length of 80 µm

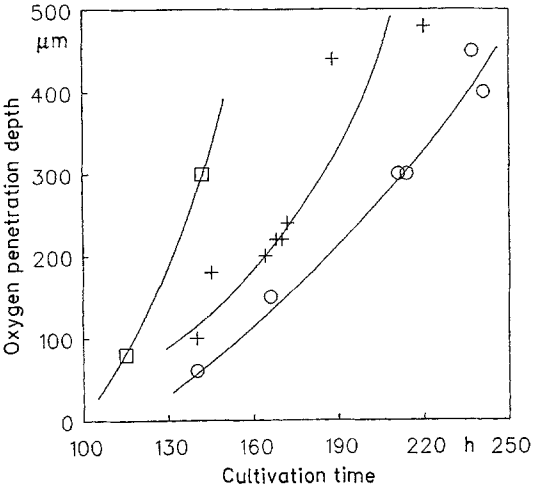

Fig. 5. Increase of the oxygen penetration depth as a function of the fermentation time for different size classes: (□): 480 µm $< R <$ 500 µm; (+): 800 µm $< R <$ 1000 µm; (○): 1200 µm $< R <$ 1400 µm; $v = 0.02$ m/s

penetrated in a large pellet more than 350 µm. These larger pellets became even fully oxygenated after some 260 hours of cultivation. In Fig. 5, the values of the oxygen penetration depth λ are plotted versus the cultivation time for three pellet radius classes: 480–500, 800–1000, and 1200–1400 µm. Clearly, an increase in penetration depth is observed during the batch culture, indicating a decrease in specific oxygen uptake rate and/or a significant facilitation of oxygen transport in the pellet. In Fig. 6 classification of fully and partly penetrated pellets according to their cultivation time and radius shows a distinct separation, indicating a consistent change of properties of all pellets during the culture. Because glucose is consumed only in the periphery, it is assumed that the oxygen uptake deeper inside the pellet was not caused by primary metabolic processes, but must be ascribed to other processes like the oxidation of storage materials or lysis products. The glucose and oxygen concentration differences between the pellet interface and the centre of the pellet (ΔC_g and ΔC_o) may be compared quantitatively. A very simple approach is chosen, assuming diffusive mass transfer only (effective diffusion coefficients De_g and De_o), and a stoichiometry of 6 moles oxygen per mole glucose. Then, the following steady-state mass balance should

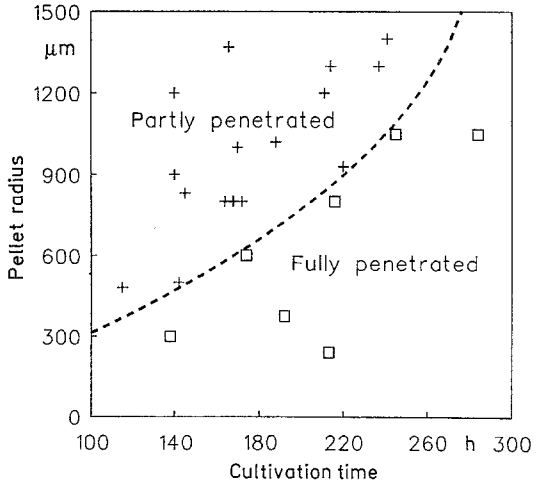

Fig. 6. Maximal pellet size for a full oxygen penetration as a function of the fermentation time. (□) fully penetrated with oxygen. (+) oxygen limited pellet with anaerobic core: $v = 0.02$ m/s

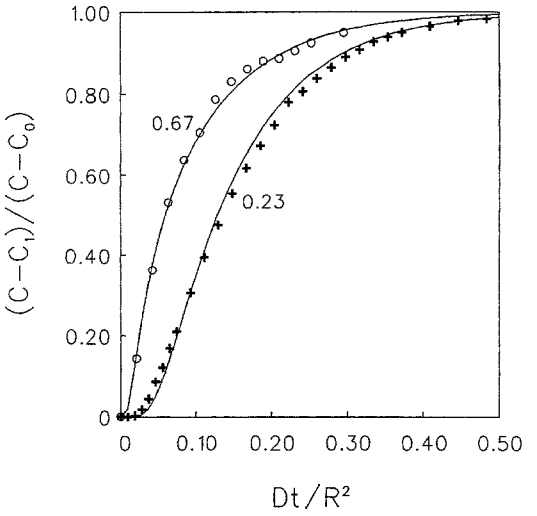

Fig. 7. Glucose diffusivity experiment in a pellet with $R = 1100$ µm at 164 h.The solid curves represent the theoretical solutions for $r/R = 0.67$ and $r/R = 0.23$. To fit the data points with the theoretical curves, D_g values of respectively 5.1×10^{-10} m²/s (○), and 4.5×10^{-10} m²/s (+) were used

hold:

$$\Delta C_o / \Delta C_g = 6(De_g / De_o). \quad (1)$$

If De_g/De_o is set equal to the ratio of the corresponding molecular diffusion coefficients of 0.3 [14], $\Delta C_o/\Delta C_g$ should equal 1.8. Obviously, the experimentally determined value was considerably larger; from Figs. 2 and 3, a value $\Delta C_o/\Delta C_g$ of 3 is obtained, while a value of 5 is found for the pellet in Fig. 4. There are two possible explanations for these deviations: (1) More oxygen is consumed than balanced by the glucose oxidation due to oxidation of *e.g.* lysis products, (2) De_g/De_o is larger than 0.3 due to convective flows, since these relatively increase the mass transfer rate of the slowest diffusing component.

3.3 Diffusivity measurements

To compare the effective diffusion coefficients in pellets at different stages of the fermentation in more detail, transient-state measurements were carried out in pellets at a fermentation time of 164, 188, 211, 237 and circa 300 hours. For this purpose the response to a step function of the tracers glucose and oxygen were measured at different positions r/R in deactivated pellets. Characteristic results for glucose and oxygen are given in Figs. 7 and 8, respectively. The solid curves in the figures represent the theoretical path if a single value for the effective diffusion coefficient D is assumed throughout the whole pellet. The data points were fitted with the theoretical curves by varying the value of D. As can be seen in Figs. 7 and 8, good fits were obtained between the transient state measurements and the theoretical relation. The D values obtained in pellets were compared with those determined in the agar gels, and their ratio is given in Table 2.

In young pellets (164 h), D_o and D_g amounted to ca. 80% of the diffusion coefficients in agar gels, being 2.2×10^{-9} m²/s (±7%) for oxygen and 0.65×10^{-9} m²/s (±5%) for glucose. Furthermore, the value of D_g is quite independent of the position in the pellet. These observations indicate that mass transfer at this stage of the fermentation is due to molecular diffusion.

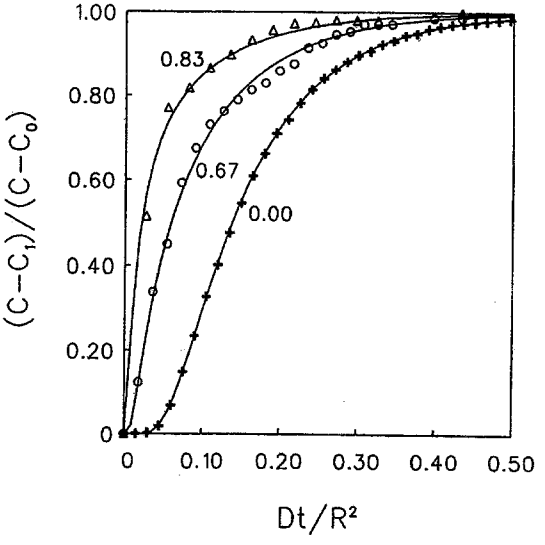

Fig. 8. Oxygen diffusivity experiment in a pellet with $R = 1560$ µm at 211 h. The solid curves represent the theoretical solutions for $r/R = 0.83$, $r/R = 0.67$, and $r/R = 0.00$. To fit the data points with the theoretical curves, D_0 values of respectively 33×10^{-10} m²/s (Δ), 22×10^{-10} m²/s (○), and 18×10^{-10} m²/s (+) were used

For older pellets (211 and 237 h) the tracer experiments revealed the diffusivity to be position dependent. In Table 2 an increase of D_g and D_o is seen as a function of r/R, upto values larger than the corresponding diffusivity in gel beads. For glucose, the solute with the lowest molecular diffusion coefficient, D/Dgel was larger than for oxygen. This probably indicates, that a certain degree of convection played an additional role in the mechanism of internal mass transport, as the relative transport rate of the slowest diffusing compound will be accelerated by convection mostly. In the pellet at 300 h, having a very loose structure, convection played a more significant role, as the oxygen transport rate was twice as high as in a gel bead.

3.4
Oxygen uptake rates

The oxygen uptake experiments performed in the BOM revealed an activity decrease in the course of the fermentation. Pellets at the end of the fermentation exhibited an oxygen uptake rate Q of only half the original rate. The penetration depth in the pellets measured with the microelectrodes were used to calculate the uptake rate Q', based solely on the pellet volume supplied with oxygen. From Table 3 it can be concluded that during the fermentation the decrease of this specific uptake rate Q' is far more pronounced than the decrease of Q, since the more active, younger pellets had only small penetration depths compared to older pellets.

The changes in the kinetic behaviour of the pellets might be related to changes in fermentation conditions. The viscosity of the culture had increased significantly during the fermentation. Therefore it is assumed that oxygen transfer to the pellets was worse than in the measuring cell, although the oxygen bulk concentration in the fermenter was nearly air saturated (0.2 mol/m^3) at $t = 200$ h. The less ideal mixing of the fermenter liquid and probably the occurrence of anoxic zones [10] might have caused that only the periphery in the older pellets was continuously supplied with oxygen. As a consequence, the biomass in the core could have been subject to oxygen starvation.

Table 2. Diffusivities of glucose and oxygen at different positions in the pellets determined with a transient-state method. The ratio of the experimental effective diffusion coefficient and the corresponding coefficient measured in agar gel ($Dgel_g$ and $Dgel_o$) is given for comparison. $Dgel_g = 0.65 \times 10^{-9}$ m^2/s; $Dgel_o = 2.2 \times 10^{-9}$ m^2/s

Time [h]	R [μm]	Position r/R	$D_g/Dgel_g$	$D_o/Dgel_o$
164	1100	0.67	0.8	0.8
		0.23	0.7	–
164	800	0.41	0.7	–
164	880	0.49	0.7	–
188	1160	0.40	1	–
188	1400	0.04	1	–
211	1560	0.83	–	1.5
		0.67	–	1
		0.00	–	0.8
211	1380	0.23	1.1	0.9
237	1600	0.53	1.6	0.8
		0.00	0.7	0.6
300	1100	0.00	–	2

During the microelectrode measurements, chemical oxidation of this dead core material, was observed in addition to respiration by the active mycelium. Likely, chemical oxidation in the centre was more pronounced under the well aerated conditions of the measuring cell than under the fermentation conditions.

The oxygen uptake experiments performed with disrupted pellets confirmed the potential of the core material for chemical oxidation, and resulted in an overestimation of the pellet activity. For this reason only intact pellets were used to measure the initial oxygen uptake rate Q.

3.5
Estimation of steady-state mass transfer rates

In the literature several models have been proposed for the description of concentration profiles within mycelial pellets. Kobayashi et al. [9] used an inhomogeneous activity distribution to describe pellet kinetics, while Wittler et al. [17] introduced a model where the mass transfer rate properties are a function of the position within the pellet. Although it is clear, that both aspects are important, we used a very simple model without these refinements simply and solely to assess changes in the kinetics and transport properties of the pellets in course of their lifetimes.

For this purpose, a spherical pellet is assumed, and both the respiration rates and the transport properties are considered constant throughout the pellet. The mass transfer rates are described by an effective diffusion coefficient De_o, while the local conversion rate is described by a constant k (zero order kinetics), whose value is equal to Q' in the periphery of the pellet and zero in the inactive core. Then, the concentration profile is described by the following differential equation:

$$\frac{\delta^2 y}{\delta \rho^2} + \frac{2}{\rho}\frac{\delta y}{\delta \rho} = \phi^2 \qquad (2)$$

where $y = C_o/C_{i,o}$, $\rho = r/R$ and $\phi = R(k/De_o C_{i,o})^{1/2}$, being the zero order Thiele number.

Depending on the boundary conditions Eq. (2) yields two different solutions [18] for partly and fully penetrated pellets. The value of ϕ was calculated from the measured interface concentration and penetration depth. From ϕ the value of De_o was estimated

As can be concluded from Table 3, in young pellets De_0 was close to the value of the diffusivity as determined in the agar gel ($Dgel_o = 2.2 \times 10^{-9}$ m^2/s). In old and fluffy pellets, a much larger value was found, up to more than 5 times the value for $Dgel_o$. Based upon these observations it can be concluded that, in

Table 3. Results of oxygen uptake experiments in different pellets. The interface and centre concentrations $C_{o,i}$ and $C_{o,c}$ and the penetration depth λ were determined with microelectrodes. The corresponding oxygen consumption rate Q of the pellet is determined with the BOM. From Q and λ the consumption rate Q' based upon the active pellet volume is obtained, De_o is calculated assuming a zero-order reaction rate [18]

Time [h]	R [μm]	$C_{o,i}$ [mol/m^3]	$C_{o,c}$ [mol/m^3]	λ [μm]	Q [10^{-3} mol/m^3 s]	Q' [10^{-3} mol/m^3 s]	ϕ [1]	De_o [10^{-9} m^2/s]
166	1370	0.180	0	190	8.0	22	11	2
172	1290	0.221	0	185	13	35	10	2.5
190	1530	0.220	0	240	8.3	21	9	2.4
284	1050	0.231	0.072	1050	5.4	5.4	2	6
310	1250	0.227	0.116	1250	5.0	5.0	2	12

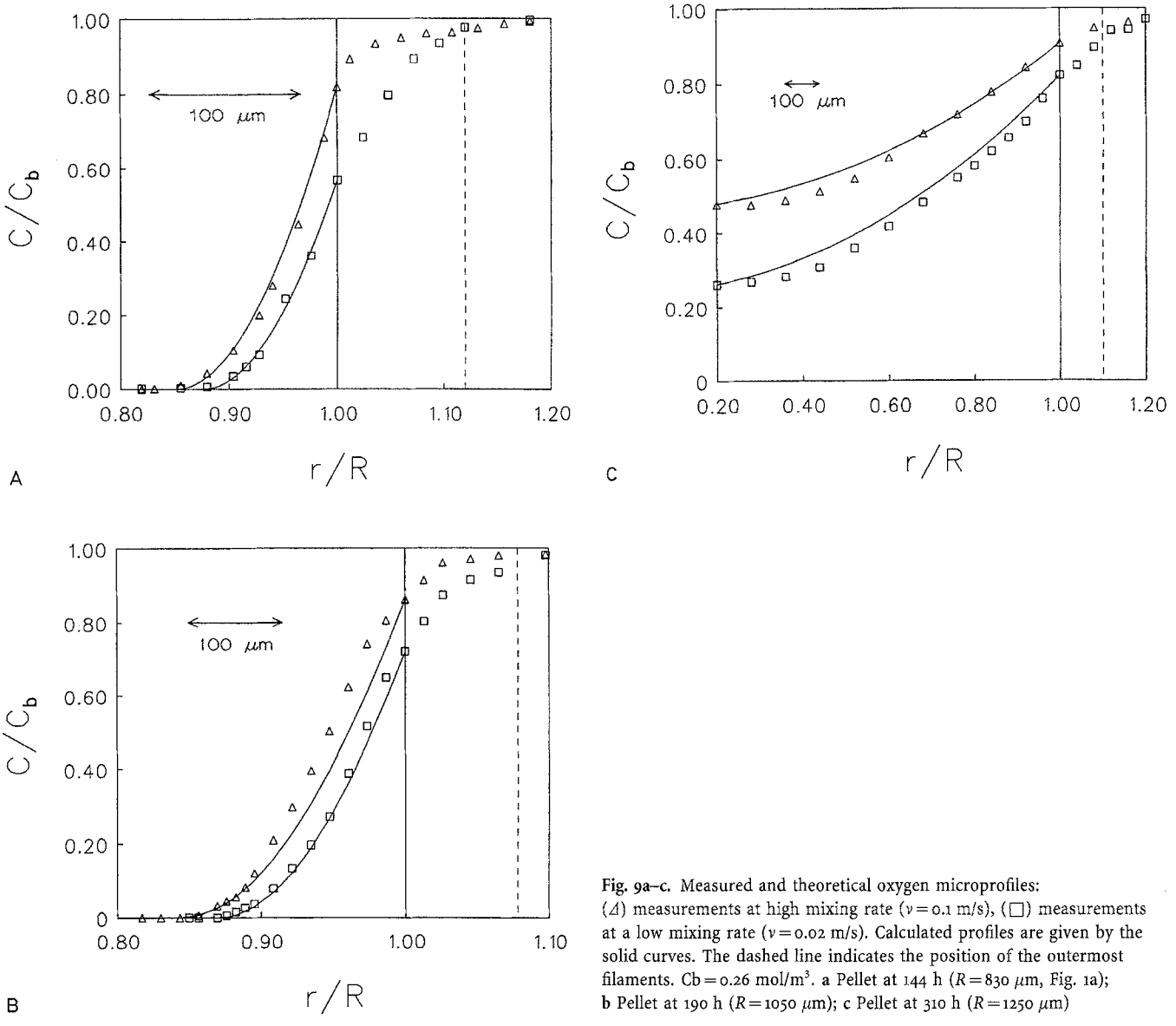

Fig. 9a–c. Measured and theoretical oxygen microprofiles: (△) measurements at high mixing rate ($v = 0.1$ m/s), (□) measurements at a low mixing rate ($v = 0.02$ m/s). Calculated profiles are given by the solid curves. The dashed line indicates the position of the outermost filaments. Cb = 0.26 mol/m^3. **a** Pellet at 144 h ($R = 830$ μm, Fig. 1a); **b** Pellet at 190 h ($R = 1050$ μm); **c** Pellet at 310 h ($R = 1250$ μm)

agreement with the tracer experiments, the internal resistance for mass transfer in the latter pellets is considerably decreased, probably by convective transport inside the pellet.

Theoretical oxygen profiles for a low and high mixing intensity were calculated according to the solution of Eq. (2); typical examples are represented in Fig. 9 by the solid curves. The markers indicate the measurements.

In dense and young pellets, present after some 140 h, the measured and theoretical profiles showed a good correspondence, indicating that a zero order approach down to a very low detected oxygen concentration of circa 0.003 mol/m^3 is very reasonable. In literature considerably higher critical oxygen concentrations of 0.022 mol/m^3 are reported [1].

In these pellets the concentration profiles changed as expected after increasing the mixing intensity of the bulk liquid (Fig. 9a). The interface concentration $C_{i,o}$ increased significantly as the external mass transfer rate was increased, and consequently an increase of the penetration depth is observed.

The mixing rate strongly influenced the general shape of the oxygen profile in old and more fluffy pellets (>190 h), since a considerable decrease of the interfacial concentration gradient was observed (Figs. 9b & 9c). This effect can only be ascribed to increased internal mass transport due to convection.

It should be emphasized that the fact that the theoretical profiles and the measured data were in reasonable agreement (Fig. 9c) is rather misleading, and wrongly suggests the pellets were homogeneous. Concentration profiles are the ultimate result of the combined interaction between local reaction rates and mass transport. As was already concluded in this work, the specific activity k, as well as the mass transfer properties (De_o) were different in the centre of the pellet and in the periphery.

4 Conclusions

By following the gradual changeover from compact to fluffy pellets of *P. chrysogenum* during the course of a fermentation process, distinct relations between the internal structure, the mass transfer properties, and the activity were observed. On the basis of these observations it might be explained why diffusion

coefficients in mycelial pellets, reported in literature exhibit such a large variation [7].

Our work showed, that the compact and homogeneous pellets, present in an early stage of the fermentation, were characterized by high Thiele numbers; for oxygen, these young pellets exhibited a high specific activity, in combination with relatively slow mass transport rates which are based on molecular diffusion. Except for the periphery, most of the mycelial mass was subject to oxygen starvation. The glucose gradients measured in these pellets were in reasonable accordance with the oxygen gradients.

Due to changing culture conditions, the pellets present in later stages of the fermentation became fluffy, and exhibited a stratified structure. In the outer regions the mass transport resistances were decreased, because the loose and open structure enables convective transport in this region. Since the peripheral oxygen uptake rates decreased as well, oxygen penetrated the pellets much deeper. Nevertheless, the glucose consumption occurred in the periphery only indicating no growth took place in the deeper regions. Therefore the oxygen consumption in deeper zones was not caused by respiration of active cells, but might be ascribed to the chemical oxidation of autolysis products. The exact nature of this process, nor the importance of it for other fermentations is clear yet.

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