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# Penicillin Fermentation in a 200-Liter Tower Fermentor Using Cells Confined to Microbeads

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## Abstract

The scale-up of the penicillin fermentation through cell confinement in a 200-L tower fermentor is described. *P. chrysogenum* spores were adsorbed into Celite microbeads having diameters greater than 180 μm. Fed-batch fermentations were performed using both free and confined cells. Cell growth and penicillin concentrations were measured during the fermentation. In addition, the oxygen transfer rate, the aeration rate, and the level of dissolved oxygen were also measured. Significant improvement in the mass transfer coefficient was found when the cells were anchored onto the microbeads. This improved oxygen transfer rate was accompanied by higher production of penicillin at a lower aeration rate. Besides the improved oxygen transfer rate into the mycelial broth, a reduction of the energy input for the oxygen transfer was observed. The confinement of the cells to this microcarrier furthermore allowed the intermittent harvesting of fermentation broth without reducing the cell mass in the fermentor.

**Index Entries:** Penicillin fermentation, in tower fermentor; *Penicillium chrysogenum*, immobilized; air tower fermentor; confinement of mycelia, to microbeads; microbeads, for immobilization; Celite, as microcarrier; oxygen transfer, improved; power input per oxygen transfer; harvesting, intermittent; fermentor, penicillin fermentation in a tower; mycelia, confinement on microbeads; fermentation, of penicillin on microbeads.

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## Introduction

One of the major limitations that governs productivity in mycelial processes in submerged culture is the gas-to-liquid-oxygen transfer. This is caused by the filamentous structure of the mycelial organisms that lead to highly viscous non-Newtonian suspensions. When the oxygen transfer into the fermentation broth becomes diminished during the fermentation, the dissolved oxygen levels drop, growth of the culture ceases, and the specific productivity of secondary metabolites is severely reduced.

To overcome this limitation, most mycelial fermentations are carried out in stirred-tank reactors using a very high specific power input. As an alternative, the concept of confining mycelial growth to beads was proposed (*1,2*). This technique is envisaged to restructure the filamentous morphology of the mycelia and to reduce the viscosity in the non-Newtonian fermentation broth in order to overcome the limitation of the gas-to-liquid-oxygen transfer. As a model system, the growth of *Penicillium chrysogenum* and the production of penicillin was chosen.

In previous work with *P. chrysogenum* confined to celite or diatomaceus earth beads (*1,2*), it could be shown that in shake flasks and in a small 3-L air bubble column a higher oxygen transfer coefficient $k_{La}$ and increased cell concentrations were indeed obtained. The air bubble fermentor was chosen because of the shear sensitivity of the highly porous celite beads (70% porosity). In a recent publication (*3*), the use of an air tower fermentor with the formation of pellets has been proposed as a fermentation system possibly having a lower specific power input and lower cooling costs compared with the conventional stirred-tank fermentation.

The objectives of the present research were to scale-up the system with the confined mycelial cells (*1,2*) into a 200-L tower fermentor and to investigate first whether a higher oxygen transfer per energy input can be obtained; second, whether a higher productivity in cell mass or specific productivity can be observed, and third, whether it is possible to harvest fermentation broth without reducing the confined cell mass in the fermentor.

## Methods

A 5-m high, 30-cm diameter stainless steel tube was used as a tower fermentor (Fig. 1). Two spargers, one facing upwards, one facing downwards into the corners, with a total of 32 air holes were mounted at the bottom of the fermentor. The temperature and the pH were controlled automatically. Since no pH-electrode could be attached directly to the tank, a small continuously pumped loop was built for pH-control. Dissolved oxygen (DO) was measured on the bottom, in the middle, and on top. From a 200-L autoclavable tank the medium, and later the feeding solution, could be pumped into the tower fermentor. The spore solution and the initial glucose solution that was autoclaved separately from the medium were stored in 5-gal glass bottles. Their content could be added with peristaltic pumps directly into the fermentor.

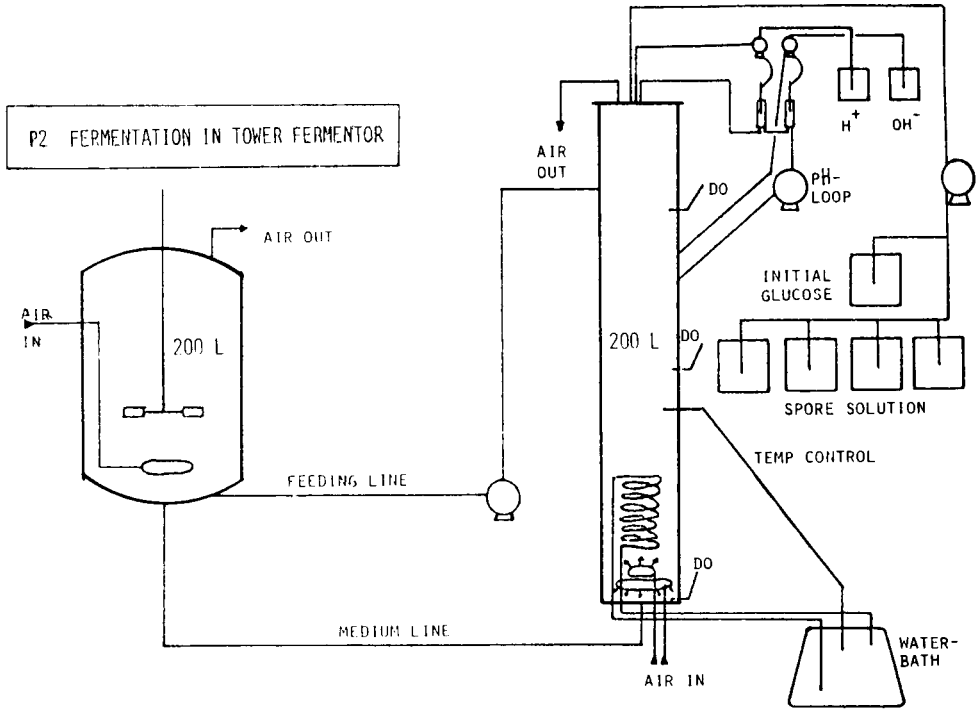


Fig. 1. A 200-L tower fermentor (height 5 m, diameter 30 cm) with 200-L stirred-tank fermentor for sterilizing medium and feeding solution. The tower fermentor has a pH and temperature control. Initial glucose and spore solution are added from 5-gal glass bottles with peristaltic pumps.

The strain *Penicillium chrysogenum* P2 (Panlabs Inc., Fayetteville, NY) was used. Celite beads (Celite grade 560, John Manville Corp.) were sieved and the size range over 180 μm diameter served as microcarrier. The characteristics of this bead material with respect to mycelial binding is described elsewhere (*1,2*). No pretreatment or washing of beads after adding the spore solution was found necessary. The quantity of spores used as inoculum is characterized in Table 1 and the medium composition is shown in Table 2. Fermentations were run in the tower fermentor with and without celite beads as microcarrier. In the fermentation with celite beads, 20 kg celite (100 g/L) were first autoclaved and then air dried in the tower fermentor. Then 60 L of penicillin spore solution (Table 1) were pumped on the beads. After a 3-h incubation time, the sterile medium (Table 2) (concentrated in 130 L) and the initial glucose solution (concentrated in 10 L) were transferred in the tower fermentor. During the first 40 h of the fermentation, the medium tank was cleaned, and the glucose feeding solution (200 g/L glucose) containing the precursor (8 g/L phenylacetic acid) was prepared and autoclaved. From 40 to 320 h, this feeding solution was added continuously with a calculated constant flow rate of about 0.5 L/h. In the fermentation without beads, the same procedures was used without adding the 20 kg celite beads in the tower fermentor.

The cell masses were determined as dry cell weight (DCW) by the difference of the weights of the total sample on a filter paper minus the filter paper and the cal-

TABLE 1
Characteristics of Beads and Spore Solution

| | |
|---|---|
| Celite beads | 100 g/L |
| Diameter beads | 180–500 μm |
| Spore loading | About 125 spores/bead, or $5 \times 10^8$ spores/L fermentation volume |
| Total spores | 13 Slants/200 L fermentation volume |
| Inoculum | Spores of 13 slants in 60 L $H_2O$ |

cined microbeads. Total penicillin was measured by the hydroxylamine methode (*4*). Galvanic probes (*5*) were used for dissolved oxygen measurements. The oxygen uptake rates (OUR) in the fermentor were determined (*6*) by measuring the inlet and exit $O_2$ and $N_2$ with a Perkin Elmer mass spectrometer. In addition the so-called oxygen uptake rates in vitro were determined during the course of the fermentations. Samples of fermentation broth with known DCW were transferred into a magnetically stirred flask equipped with a dissolved oxygen probe. This flask was then filled up to the rim with a medium containing 5 g/L glucose and 1 g/L magnesium sulfate. After airtight closure of the flask the decrease of the DO-level in the flask per time was measured and the OUR was determined (*6*). This in vitro OUR expresses the oxygen demand of the culture at that specific time.

## Results

In order to have an idea about the oxygen transfer capability of the tower fermentor shown in Fig. 1, oxygen transfer rates were measured with the sodium sulfite oxidation technique (*6*). The data using the lower or upper sparger separately are shown in Fig. 2. There was little difference when using the upper, the lower, or both spargers. As expected, linear relationships were obtained between the logarithms of air flow rate, oxygen uptake rate (OUR), and oxygen transfer coefficient, $k_{La}$. Using both spargers, the compressor allowed air flow rates up to 550 L/min or

TABLE 2
Composition of Medium
(per 200 L)

| | |
|---|---|
| 35 g/L | Corn steep liquor |
| 7 g/L | Initial glucose |
| 4 g/L | Lard oil |
| 1 g/L | P2000 antifoam |
| 4.5 g/L | $(NH_4)_2SO_4$ |
| 3 g/L | $KH_2PO_4$ |
| 4 g/L | $MgSO_4 \cdot 7\ H_2O$ |
| 1.8 g/L | $CaSO_4 \cdot 2\ H_2O$ |
| 0.3 g/L | $FeSO_4 \cdot 7\ H_2O$ |
| 0.015 g/L | $ZnSO_4 \cdot 7\ H_2O$ |
| Adjusted to pH 5.5 with NaOH | |

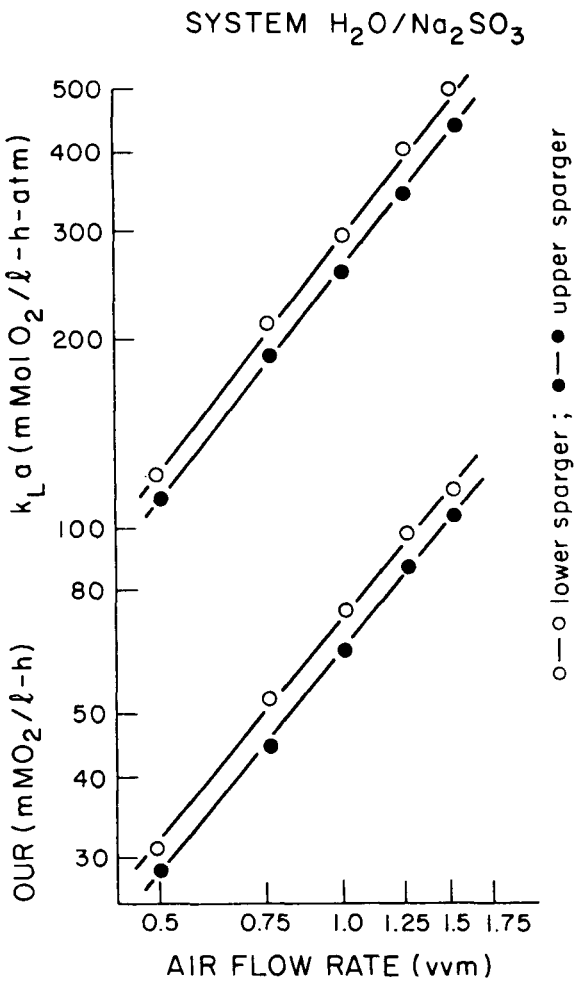


Fig. 2. Oxygen transfer capabilities of the 200-L tower fermentor using upper (●) or lower (○) air sparger ring. The solution containing sodium sulfite (*6*) was used to measure the oxygen uptake rate (OUR) and the oxygen transfer rates ($k_{La}$) as a function of the aeration (vvm = volume gas per volume solution per minute).

2.7 vvm, which corresponds to a superficial gas velocity $V_s$ of 13 cm/s or a power input of 1.1 kW/m$^3$. This power input, maximally used in the following fermentations, is well below the 5 kW/m$^3$ reported for penicillin fermentations with the conventional stirred-tank fermentor (*3*).

Figure 3 compares the two fermentations with and without binding of the cell mass to celite beads. Since the same medium and feeding were employed, the cell growth is very similar in both fermentations, reaching about 34 g/L. The penicillin production, however, is different because, in the case without beads, the specific productivity drops after 120 h to zero and no more penicillin was produced because of severe oxygen limitation. In the fermentation with celite, the fermentation broth remained less viscous, the gas-to-liquid-oxygen transfer occurred more easily, and the penicillin concentration increased up to 180 h. The proof that oxygen limitation was the cause of the lower penicillin productivity (3.6 g/L vs 5.2 g/L) in the fer-

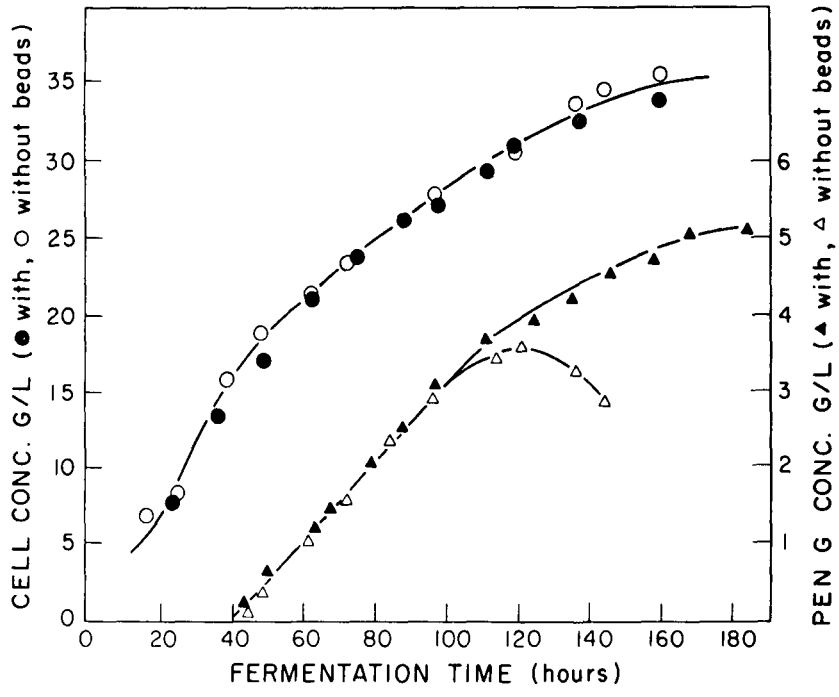


Fig. 3. Comparison of penicillin fermentations in 200-L tower fermentor with free cell mass (open symbols) and with cell mass confined to microbeads (closed symbols). The dry cell weights (round symbols) and the penicillin concentrations in the broth (triangles) are plotted as a function of the fermentation time.

mentation without beads is given in Figs. 4 and 5. In the fermentation without beads—even with a strong aeration of 450 L/min—the dissolved oxygen (DO) fell and remained below 5% after 120 h fermentation time (Fig. 4) and penicillin productivity ceased (Fig. 3). In the fermentation with beads, a higher DO of about 20% could be maintained between 120 and 140 h using less aeration (only about 320 L/min) (Fig. 4) and penicillin was still produced in this case (Fig. 3). In Fig. 5 the Oxygen Uptake Rates (OUR) measured in the fermentor are compared to the oxygen demand of the culture measured in vitro in a separate vessel and medium. In the fermentation with beads (top of Fig. 5) higher OUR were observed at the peak of the growth phase; afterwards oxygen demand and supply were more or less in balance during the course of the fermentation. In the fermentation without beads, however, the maximal OUR was smaller at the peak because of the viscosity of the broth, and the oxygen supply was significantly lower than the demand after 100 h of fermentation time. As a result of this discrepancy in supply and demand of oxygen, the DO-level fell (Fig. 4) and the penicillin productivity decreased (Fig. 3). In comparing the very similar in vitro OUR of free and confined cells, it can be demonstrated that there was probably no limitation of oxygen transfer from the liquid phase to the mycelia on the surface or to the inside of the celite beads. Therefore, in removing some of the gas-to-liquid-oxygen transfer limitation with the beads, no new liquid-to-solid-oxygen transfer limitation was introduced.

Figure 6 shows the dissolved oxygen levels and the oxygen uptake rates (OUR) as a function of the air flow rates. These measurements were made at different

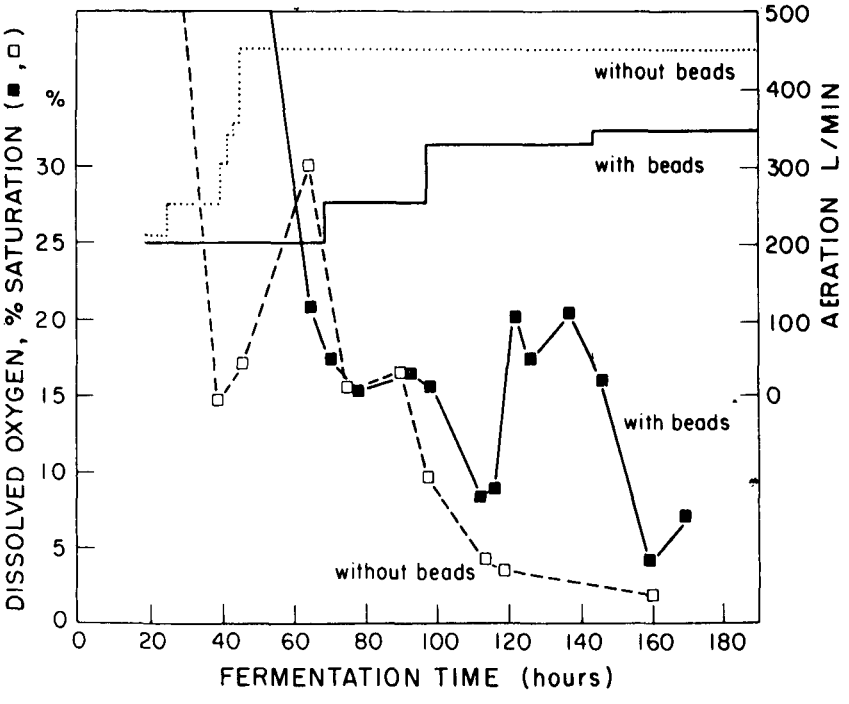


Fig. 4. Dissolved oxygen levels (% of saturation, square symbols) and the aeration (L/min) used in the two fermentations shown in Fig. 3. The closed symbols apply again for the fermentation with confined cell mass and the open symbols to the one without beads.

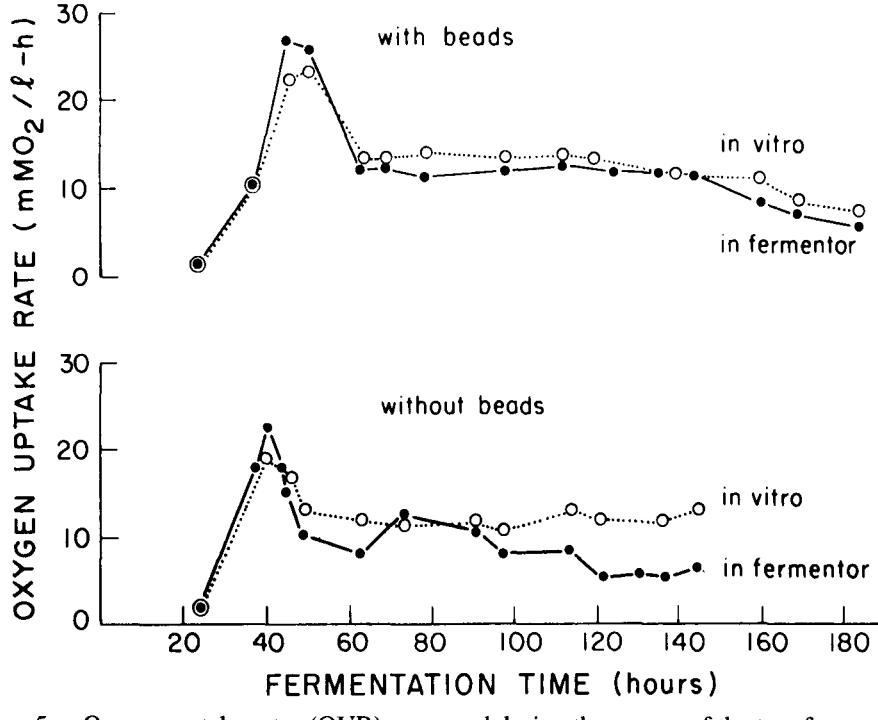


Fig. 5. Oxygen uptake rates (OUR) measured during the course of the two fermentations with confinement of the cell mass to beads (top) and without beads (bottom). In each fermentation, the OUR were measured in the fermentor as oxygen supply to the culture and in vitro (see methods) as oxygen demand of the culture.

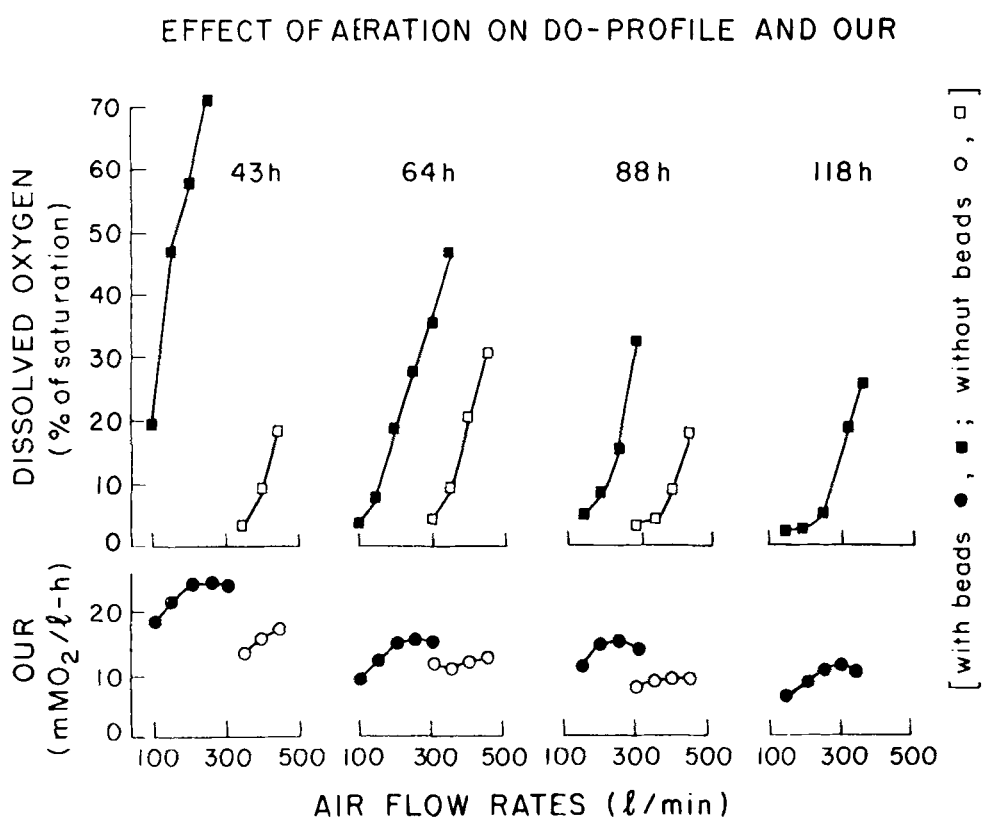


Fig. 6. Effect of the aeration on the dissolved oxygen level (DO) and on the oxygen uptake rate (OUR) measured at 43, 64, 88, and 118 h of the fermentation. At these four fermentation times the aeration was varied from 100 to 500 L/min (0.5–2.5 vvm) and the corresponding OUR and DO were recorded. The solid symbols again belong to the fermentation with beads and the open symbols refer to the fermentation without beads.

times during the fermentation (at 43, 64, 88, and 118 h) in both fermentations with and without beads. The cell masses in the two fermentations are very similar at these times, as can be seen in Fig. 3. With increasing air flow rates, which were varied between 100 and 500 L/min, the OUR increases until a maximal value is reached. This value corresponds to the OUR found in vitro, which expresses the oxygen demand of the culture at that time point. As soon as no more oxygen is used by the culture, the DO-level rises sharply in the fermentation broth when the aeration is further increased. Again it can be seen that the OUR decreases in the course of the fermentation. This is straightforward and expected. Interestingly, however, the "critical dissolved oxygen level" (the level where the maximal OUR is reached) decreases during the fermentation. In the later stage of the fermentation, therefore, a lower DO-level (below 10 or even 5%) seems sufficient to provide the necessary maximal oxygen uptake rate for the mycelial cells. Furthermore, Fig. 6 shows again clearly that in the fermentation with beads (solid symbols) the oxygen uptake and the DO-level are higher with less aeration or power input compared to the fermentation without beads (open symbols).

In order to test the third possibility, whether the harvesting of fermentation broth is possible without reducing the mycelial cell mass in the fermentor, the experiment shown in Fig. 7 was made. The fermentation time was extended to 330 h, and three times during this period (at 115, 190, and 285 h) a quarter of the fermentor broth (50 L) was harvested and replaced with fresh medium (Table 2). A 20-cm diameter porcelain funnel was covered with a 500- μm stainless steel screen and was mounted upside down at half-height in the tower fermentor. Using some overpressure (10 psi) in the fermentor and/or some vacuum on the exit line of the funnel, clear penicillin broth could be harvested without changing the aeration in the fermentor. As shown in Fig. 7, this intermittent harvesting could be done without reducing the cell mass in the fermentor due to the confinement of the cell mass on the 500 μm screen. Dry cell weights (DCW) of over 40 g/L were obtained. Unfortunately, the penicillin productivity ceased after about 130 h at 3 g/L (Fig. 7, lower curve) because of oxygen limitation. This is proven in Fig. 8, where the dissolved oxygen levels (DO) and the corresponding aeration are shown on top and the oxygen uptake rates (OUR) at the bottom. The DO-level dropped below 5% at 120 h and never recovered even though high aeration rates up to 550 L/min were used. Oxygen limitation, therefore, is the reason for the loss in penicillin produc-

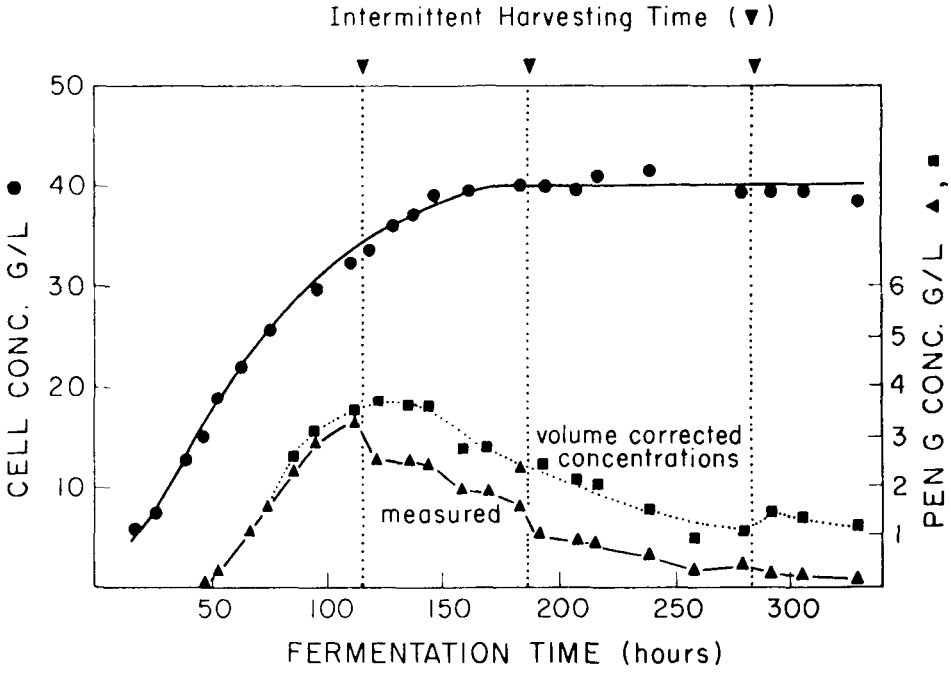


Fig. 7. Penicillin fermentation with cell mass confined to microbeads. After 115, 190, and 285 h a quarter of the fermentor volume (50 L of fermentation broth) was harvested and replaced with fresh medium. The experimental dry cell weights (●) are shown on top, the measured penicillin concentrations (▲) are given below. Since changes of volumes were made frequently by harvesting or taking samples, volume-corrected penicillin concentrations (■) give a better picture of the actual penicillin productivity. The decrease of concentrations after the production of penicillin stopped around 125 h is the result of slow penicillin degradation (*7*).

tivity. This can be further demonstrated in comparing the actual OUR in the fermentor to the oxygen demand of the culture (OUR in vitro): the crossing point is at 125 h; after that moment the oxygen demand was never satisfied anymore in this fermentation (Fig. 8, bottom). Three factors contributed to this oxygen limitation even though beads were used: (a) during the harvesting periods of about 3 h, the actual cell concentration in the fermentor rose by one-third. At 120 h for instance, an actual cell concentration of 43 g/L (32 g/L of 200 L in a reduced volume of 150 L) is suddenly present, which is too much for the oxygen transfer capabilities of the fermentor; (b) after adding 50 L of new medium at 120 h, a new growth phase that demanded extra oxygen started (see peak of in vitro OUR at 150 h); (c) after 200 h, a cell mass of over 40 g/L was reached, which apparently is too much for this fermentor. A proportional reduction of substrate by about 20% should overcome the oxygen problems encountered in this experiment. In order to demonstrate the different power input necessary for a sufficient oxygen transfer in the two systems

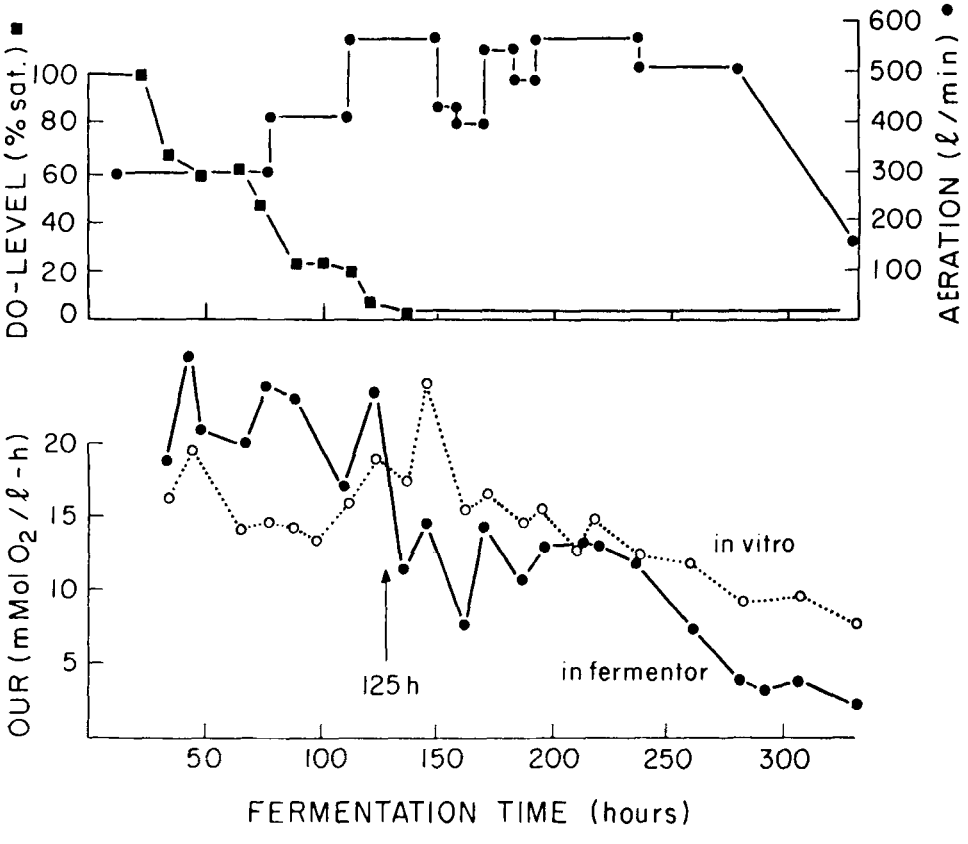


Fig. 8. Dissolved oxygen levels (DO), air flow rates, and oxygen uptake rates (OUR) of the fermentation shown in Fig. 7. On top of the figure the DO-levels (■) and the air flow rates (●) are given. On the bottom of the figure the OUR measured during this fermentation are plotted as oxygen demand (○) in vitro and as oxygen supply (●) in the fermentor (see methods). After 125 h fermentation time, the oxygen demand was no longer met and the DO remained correspondingly low.

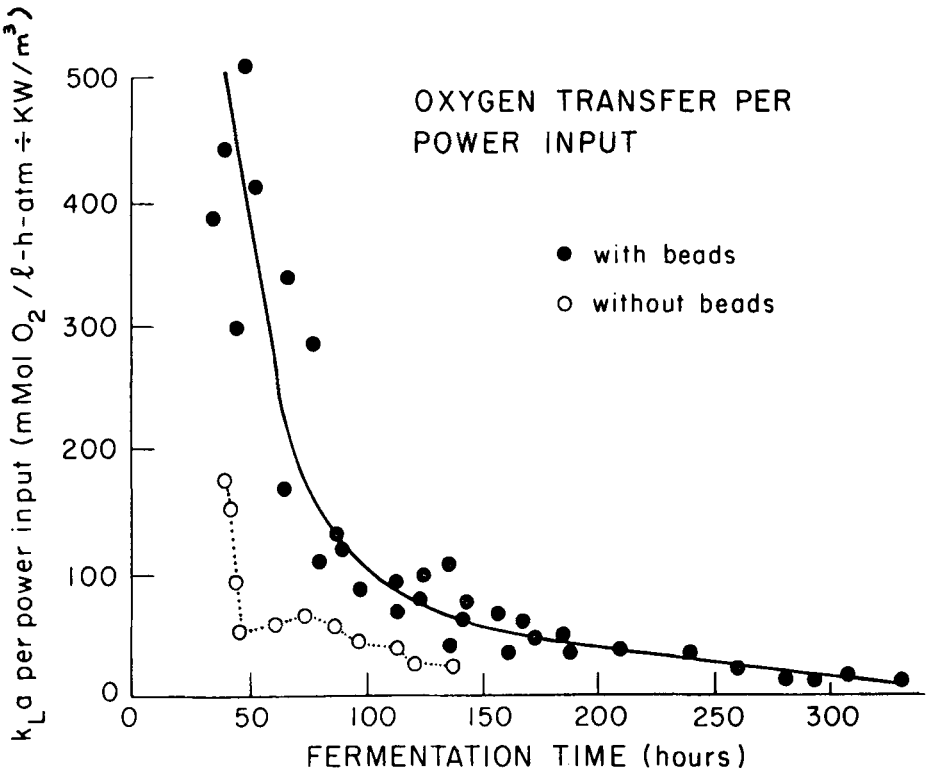


Fig. 9. Oxygen transfer per power input. The oxygen transfer rates $k_{La}$ (mMol $O_2$/L-h-atm) measured in the three fermentations of Fig. 3 and 7 were divided by the corresponding power input used (kW/m$^3$). The oxygen transfer per power input is at least a factor 2 higher in the system with beads (●) compared to the system without beads (○).

with and without beads, the $k_{La}$ measured in the different experiments were compared in Fig. 9. Since different aeration were used in the three experiments (Figs. 4 and 8), the oxygen transfer per power input ($k_{La}$ per kW/m$^3$) is plotted as a function of the fermentation time. In both cases (with and without confinement of the cell mass to beads), $k_{La}$ decreases during the course of the fermentation because of the increasing cell mass and viscosity of the broth. However, the oxygen transfer per power input is at all times and cell concentrations higher by at least a factor two when the cell mass is bound to celite (Fig. 9, upper curve).

## Conclusions

The present investigation showed that the penicillin fermentation with cell mass confined to microbeads (*1,2*) could successfully be scaled up in a 200-L tower fermentor. Comparing the fermentation with confined cell mass to the fermentation with free mycelia, it could be shown that an increased oxygen transfer per power input was obtained (Fig. 9), that a higher penicillin productivity was observed as a result of less limitation of gas-to-liquid oxygen transfer (Fig. 3 and 4)

and, finally, that intermittent harvesting of fermentation broth is possible without reducing the cell mass in the fermentor (Fig. 7).

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