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Application of an Airlift Bioreactor to the Nystatin Biosynthesis

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Summary

Pilot plant studies were performed using a concentric-tube airlift bioreactor of 2.5 m^3 fermentation volume. The results have proven the relative merits of such a system in the biosynthesis of nystatin, produced by *Streptomyces noursei*, in submerged aerobic cultivation and batch operation mode. The results were compared to those obtained in a pilot-scale stirred tank bioreactor of 3.5 m^3 fermentation volume.
The fermentation processes in the two fermentation devices were similar with respect to substrate utilization, biomass production and nystatin biosynthesis.
In the riser section, the dissolved oxygen concentration was higher than that in the downcomer.
The volumetric oxygen mass transfer coefficient was dependent on the rheological behaviour of the biosynthesis liquids, which was not constant during the fermentation process.
The total energy consumption for nystatin production in the airlift bioreactor was 56% of that in the stirred tank, while the operating costs represented 78% of those in the stirred tank bioreactor.

Introduction

The airlift bioreactor is a device which has potential in a wide range of biochemical processes from waste-water treatment to the production of biological metabolites, owing to the lack of mechanical rotating parts and reduced damage of contamination. It is also well suited because of its mass and heat transfer characteristics.
Many papers have appeared on the behaviour, design, application and simulation of the airlift reactor [1–4].
The application of the airlift principle has been reviewed by SITTING and FAUST [1], ONKEN and WEILAND [2], MARGARITIS and WALLACE [3] and SIEGEL *et al.* [4].
Use of the airlift bioreactor has increased significantly along with overall developments in the fields of bio- and genetic engineering. The bioreactor provides the necessary growth environment for the new strains of different microorganisms suitable for the efficient production of secondary metabolites of pharmaceutical value.
The application of the airlift reactors to fermentation processes with non-Newtonian media has been reported by several researchers [5–8].
In this work, the concentric tube airlift bioreactor is investigated on a pilot-plant scale. This was in order to assess the potential of such a device in the fermentation, i.e. in the

biosynthesis of antibiotics using a non-Newtonian high viscous broth, and to exploit the numerous attractive features associated with its operation.
The main objective of this study is to investigate whether nystatin can be obtained successfully in airlift fermenters and whether this type of bioreactor shows advantages for this process over stirred fermenters. It also points out the energy saving obtained by using the airlift bioreactor in the nystatin biosynthesis.

Materials and Methods

Fermentation Equipment

The pilot-plant internal-loop airlift bioreactor (ALR) is made of stainless steel and consists of a cylindrical part containing the draft tube and the top portion with the degassing zone. The ratio between the cross-sectional areas of the riser and downcomer is 1.25, and the ratio between the draft tube and reactor diameter, i. e. D_T/D_C is 0.67.
The bioreactor contains several ports for measuring the dissolved oxygen concentration, the pH value and temperature, as well as ports for the removal of the exhaust gas and for the addition of antifoam agents and substrates. As a heat exchanger a coil was used, which was inserted in the draft tube column. In this way, the heat transfer of fermentation heat could be realized easily, and this would also be a way to warm the fermenter content in case, heating should be required. The flow of the fermentation broth was also not affected by additional devices for heat transfer. The bioreactor content was aerated by compressed air, presterilized, measured with a rotameter and sparged by a multiple-pipe gas distributor into the draft tube.
Steam lines were connected to the fermenter to allow live steam to pass through the vessel for sterilization.
A schematic diagram of the airlift bioreactor and its ancillary equipment are presented in Fig. 1. For comparison, a stirred tank bioreactor (STR) was used.
The energy consumption for agitation was measured using a wattmeter. Some geometrical elements of the ALB and STR are given in Tabs. 1 and 2, respectively.

Tab. 1. Dimensions of the airlift bioreactor

Geometrical parameters	Symbols	Units	Values
Reactor:			
Liquid volume	V_L	m^3	2.500
Total height	H	m	10.516
Cylindrical part:			
Height	H_c	m	10.078
Inner diameter	D_c	m	0.600
Draft-tube diameter	D_T	m	0.400
Bottom part with sparger:			
Height	H_B	m	0.216
Sparger diameter	d_S	m	0.380
Serpentine:			
Height	H_s	m	1.297
Diameter	D_s	m	0.400
Exchange area	A_s	m^2	7.000
Length	L_s	m	85.000

Tab. 2. Geometrical parameters of the stirred tank bioreactor

Geometrical parameters	Symbols	Units	Values
Tank diameter	D	m	1.600
Impeller diameter	d	m	0.530
Liquid height	H_L	m	2.000
Baffle width	W	m	0.160
Baffle number	n_W	–	4.000
Impeller number	n_I	–	2.000
Number of blades/impellers	n_B	–	6.000
Blade length	l_B	m	0.190
Blade width	h_B	m	0.190
Distance between impellers	L	m	0.900
Liquid volume	V_L	m^3	3.500

The strain *Streptomyces noursei* ICCF-S80, obtained from the Research Centre for Antibiotics, Jassy, and the corresponding fermentation procedure were the same as for a conventional process, developed in a stirred tank bioreactor. The main culture medium contains corn steep, starch, carbohydrates and minerals. Industrially similar fermentation conditions have been used for simple, reproducible and comparable fermentations in airlift and stirred tank bioreactors, and not for the highest possible nystatin productivity. All fermentations were carried out in a batch operating mode at 26–28 °C and at 1.6–1.8 at for 150–170 h and at aeration rates of between 0.9–1.0 vvm.

The fermentation medium was prepared and sterilized in an additional stirred vessel. After cooling to approximately 30 °C, the medium was transported into the airlift and stirred tank bioreactors, respectively, previously sterilized *in situ* with steam (2.3 at/121 °C). Then, the aeration was started at a preset value, (usually 1.00 vvm) and the seed culture was added through the inoculating line. During the fermentation, the dissolved oxygen concentration, pH and temperature levels were monitored. Samples of fermentation broths were taken regularly at 8-hour intervals. The sugar and nitrogen levels and the cell mass concentration were measured by specific methods [9, 10]. The concentration of nystatin was determined by a spectrophotometric assay. Rheological parameters of the broths were determined using a rotational cylinder viscosimeter (Rheotest 2.1, MLW, PRÜFGERÄTE MEDINGEN) at the fermentation temperature. The rheological data were adjusted to the power-law rheologic model [11]:

$$\tau = K\gamma^n \tag{1}$$

In the airlift bioreactor, the apparent viscosity, η_{app}, was given by:

$$\eta_{app} = \tau/\gamma = K\gamma^{n-1} \tag{2}$$

The average shear rates were calculated from the superficial gas velocity of the riser, based on the relation proposed by NISHIKAWA *et al.* [12] for bubble columns:

$$\gamma = 5{,}000\, v_{SGR} \tag{3}$$

For the stirred vessel, the apparent viscosity under mixing conditions was calculated using the following Equation [13]:

$$\eta_{am} = \frac{K}{(11\,N)^1}\left[\frac{(3n+1)}{4n}\right]^n \tag{4}$$

All the calculations were done using the upward part of the rheograms. The nystatin biosynthesis liquids displayed non-Newtonian behaviour and showed thixotropy.

The mass transfer coefficients were first evaluated in tap water with the dynamic gassing-in technique [14], using polarographic oxygen electrodes (INGOLD type), connected to a recorder. The probes were attached at two points in the riser, one of them at 0.9 m above the level of the air sparger and at 4 m

below the draft tube upper edge and the other one in the downcomer at 4 m above the lower edge of the draft tube. The oxygen concentration at different positions varied by no more than 3–5% at any time. This was within the experimental measuring error, indicating excellent mixing and, therefore, the validity of a point-calculated k_La. The oxygen mass transfer coefficient values were obtained from the slope of the plot $\Delta C_L/\Delta$ t vs. $(C_s - C_L)$ determined from C_L variations vs. time. It was also assumed that C_s was constant throughout the fermenter and during the fermentation and equal to 2.252×10^{-4} mole/m^3 [15–17]. It was also assumed that all the oxygen transfers occurred in the riser section of the ALB [18]. The absence of a significant delay in the apparent dynamic response time of the dissolved oxygen electrode was taken into account as well. The k_La measurements were performed in the airlift bioreactor at 27 °C and 1.7 at. In the stirred tank bioreactor, the k_La was evaluated following a procedure developed by the authors [19, 20].

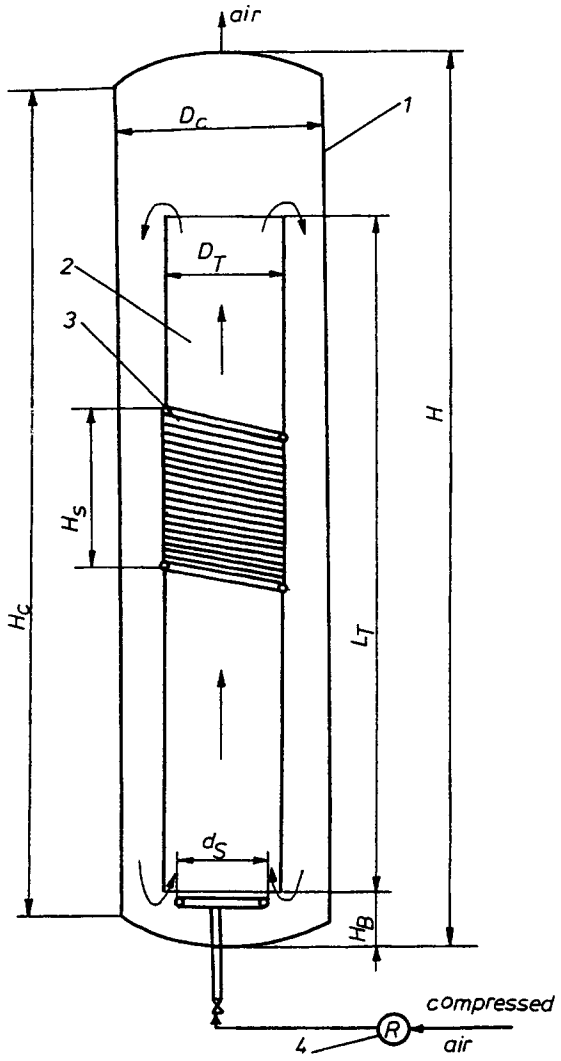

Fig. 1. Schematic diagram of the pilot-scale concentric-tube airlift bioreactor
1 – bioreactor body; 2 – draft-tube; 3 – serpentine; 4 – rotameter.

The mass transfer coefficient during the biosynthesis process was determined by a direct method based upon oxygen balance in inlet and off-gas streams around the fermenter [13].

Results and Discussion

Effect of Operating Parameters on k_La Values

Before the biosynthesis started, some preliminary experiments to determine the mass transfer possibilities of the ALB and STR bioreactors were performed.
In the airlift device, the superficial velocity of the air was varied between 0.02 to 0.04 m/s, corresponding to aeration rates between 0.0628–1.256 vvm. The measurements of k_La were taken with an oxygen probe at a height of 4 m in the riser section, using water and the initial unseed cultivation medium with viscous non-Newtonian behaviour. The apparent viscosity of the unseed medium is presented in Fig. 2 as a function of the superficial velocity of the gas and as an average of five experiments. The viscosity of this liquid is not a constant, being a function of the superficial velocity of the gas in the airlift bioreactor (Eq. 3, Fig. 2). At a constant aeration rate, it was a function of the agitator speed in the stirred tank bioreactor, as shown in Fig. 3. The effect of v_{SGR} on k_La in the ALB is also presented in Fig. 2. In Fig. 3, the effect of the impeller speed on k_La in the STR at 1.00 vvm is clearly visible.

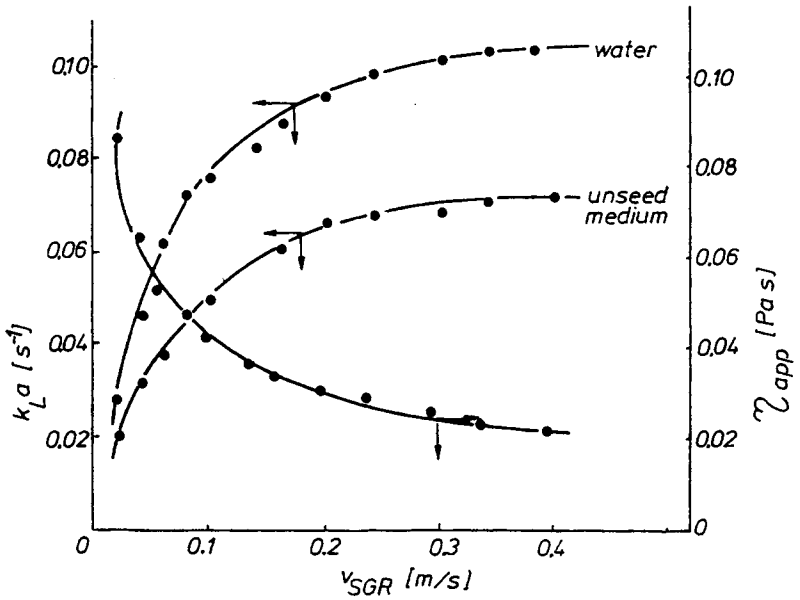

Fig. 2. Variation in the k_La, depending on the v_{SGR} in water and the initial unseed culture medium, together with that of the apparent viscosity, η_{app}, of the unseed medium in the airlift bioreactor

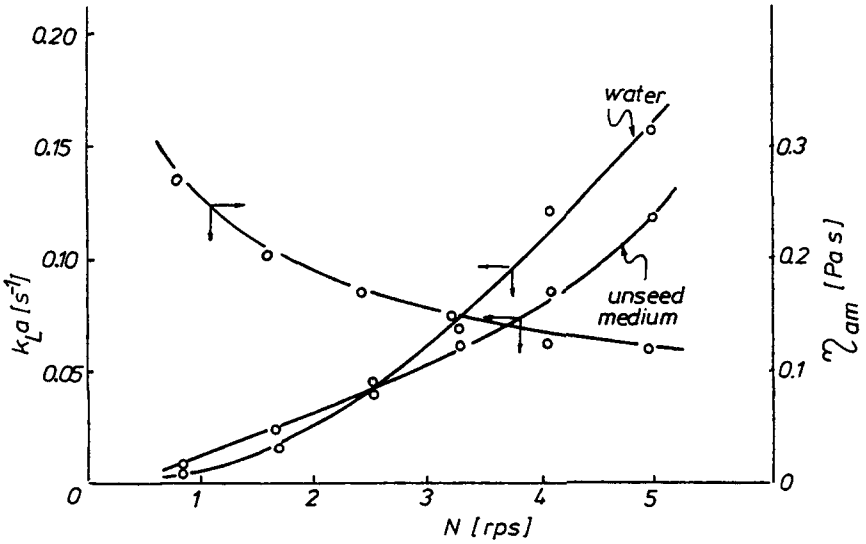

Fig. 3. Dependence of the k_La on the agitator speed in water and the unseed culture medium, in parallel with that of the apparent viscosity under mixing conditions in unseed medium, determined in the stirred tank bioreactor

The same value of k_La in water is realized at 0.34 m/s in an ALB and at 230 rpm and 1.00 vvm in an STR, as shown in Figs. 2 and 3. This k_La value of 400 h^{-1} was obtained at a pressure of 1.7 at. From Fig. 2 it is evident that k_La is reduced by approximately 50% of that value in water in the initial medium, owing to the effect of the rheological behaviour of the unseed medium. In an STR and at 1.00 vvm, the reduction is visible after 150 rpm, which corresponds to the minimum impeller speed required to overcome flooding.

To conclude, the operating parameters of the ALB correspond to the developed churn-turbulent flow, while in an STR they correspond to the turbulence range outside the impeller flooding regime.

Fermentation Results

The average results of the three fermentations performed in the airlift bioreactor are plotted in Fig. 4, which indicates close similarities between the two-fermentation modes with respect to substrate utilization (a), cell mass production (c), pH change (b) and antibiotic concentration (d). The curves in Fig. 4 e, showing the dissolved oxygen concentration as a function of the fermentation time, reveal some differences. Airlift fermentations provide better aeration to the portion of liquid in the riser zone than STR fermentations provide to the overall liquid volume. This is probably due to the fact that in the airlift bioreactor the distribution of the energy dissipation rate is fairly uniform in contrast to the stirred tank bioreactor. Here the energy dissipation rate varies from the impeller edge to the reactor wall by a factor of about a hundred [6, 8]. In this case, the oxygen transfer from the gas phase to the aqueous phase is increased in the airlift bioreactor, and the oxygen levels remain higher in the riser than in the stirred tank. In

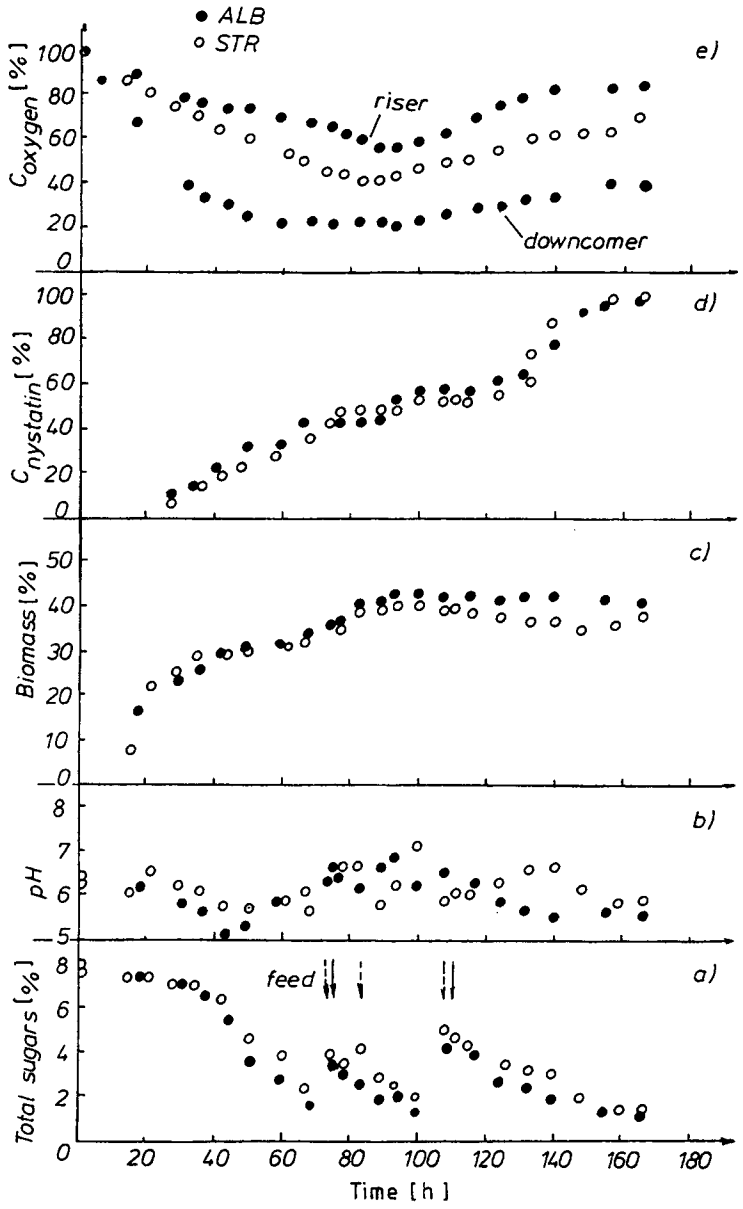

Fig. 4. Comparison of the ALB and STR with respect to
a – substrate utilization; b – pH values; c – biomass growth; d – nystatin concentration and e – dissolved oxygen level in the course of nystatin biosynthesis

the downcomer, the dissolved oxygen levels fall to approximately 20% of that of saturation. This value is higher than that obtained in the downcomer part of a pilot-plant airlift reactor with an external recirculation loop, in which the oxygen concentration

dropped significantly below 10% of that of saturation during bacitracin biosynthesis [8]. This is probably due to the fact that in the gas separator of the concentric-tube air-lift bioreactor the gas bubbles from the liquid phase are not completely disengaged during circulation, and an important number of bubbles are recirculated through the downcomer. At 1.00 vvm, corresponding to 0.34 m/s of the superficial velocity of the gas, the depth of bubbling penetration represents more than $^2/_3$ of the downcomer height [21].

Once the growth rate declines in the latter stages of the runs, dissolved oxygen concentrations in both ALB and STR bioreactors rise to near the saturation value.

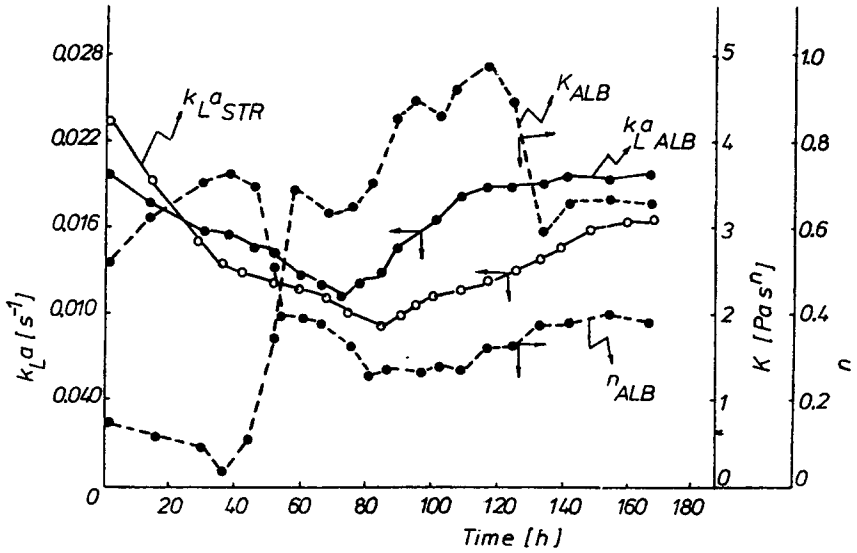

Fig. 5. Variation in the consistency index *(K)* and flow behaviour index *(n)* during nystatin biosynthesis in the ALB in parallel with a k_La variation in the ALB and STR

Change of k_La during the Fermentation

Data on the changes of the oxygen mass transfer coefficient, k_La, during nystatin biosynthesis, obtained by the balance method, indicate that k_La values generally decrease from the initial values. With increasing biomass concentration, the medium gradually becomes viscous because of the formation of filamentous mycelia. This reduces the volumetric mass transfer coefficient with respect to its initial value, due to the formation of large bubbles and a reduction of the specific interfacial area. In Fig. 5, the k_La variation with the biosynthesis time is plotted; k_La decreases quickly in the ALB and the STR, particularly in the first period of fermentation. Variation of the consistency index *(K)* and the flow behaviour index *(n)* during the process, as shown in Fig. 5 for the ALB, induces the k_La variation as well [11, 20].

The apparent viscosity during biosynthesis is not a constant either (Fig. 6). It is evident that a maximum appears after the culture age of 40 h, which coincides with the maximum growth rate of the biomass. This maximum corresponds to a more pronounced

decrease in k_La. It must be stressed that the morphology of the mycelia in the airlift bioreactor and the stirred tank fermenter observed at the microscope were not different.

Energy Expenses in the Airlift Bioreactor Compared to the Stirred Tank Reactor

From the data presented above, it is evident that the pilot-scale airlift bioreactor offers several advantages over the more traditional stirred tank bioreactor in the nystatin biosynthesis. The airlift column has proved equally effective as the STR in the conversion of substrates, biomass growth, etc., in antibiotic production. It has also been shown to be capable of handling the viscous broth, commonly observed in the fermentation of the filamentous mould *S. noursei* progresses.

Throughout the airlift fermentations, the oxygen levels in the fermentation broth remained higher than in the stirred tank runs due to the increased mass transfer characteristics inherent in such a system.

Based on the information gathered, it is possible to draw a comparison of energy costs with operating costs in the two fermenters. In Tab. 3, the energy consumption for 164 h of fermentation is presented. Considering the total power involved in the fermentation in the STR as 100%, the energy saving for the nystatin biosynthesis in the airlift bioreactor would be 54%.

Considering the operating costs per kg nystatin required in the stirred vessel of 100%, the operating costs per kg nystatin required in the ALB would represent 78% of those in the STR.

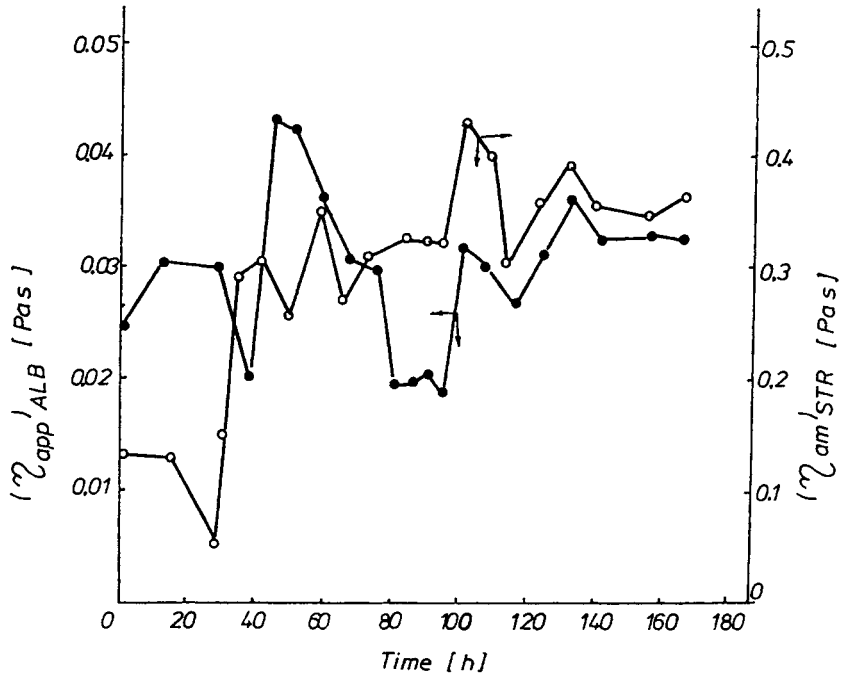

Fig. 6. Variation in the apparent viscosity of a liquid culture of nystatin during the biosynthesis in the ALB and STR

Tab. 3. Energy consumption for the nystatin biosynthesis in airlift and stirred tank bioreactors for 164 h of fermentation

Bioreactor	Power consumption [kWh]		
	Aeration	Agitation	Overall
ALB	2,240	—	2,240
STR	2,240	1,772	4,012

It is apparent that in the light of the current emphasis on energy conservation, airlift fermentation devices should enjoy a favourable position in the future growth of biotechnology and particularly of fermentation technology.

Conclusions

On the basis of our comparative studies it is evident that the airlift bioreactors are not confined to the more conservative cultivation of yeasts or bacteria, being able to perform fermentations which include filamentous moulds.
Following the considerations of the pre-requisite for the reactor comparison and the fundamental differences between stirred tank and airlift bioreactors, their performances are compared in the production of the secondary nystatin metabolite from *Streptomyces noursei* in submerged aerobic cultivation. Under the conditions of aeration and agitation which gave comparable k_La values in non-biological media, the results of the present study show that a considerable reduction in net energy consumption can be gained by the use of the airlift fermenter, even for the cultivation of filamentous microorganisms. Moreover, the lack of rotating mechanical devices in the airlift system provides safety and a more gentle environment for the cultivation of microorganisms.

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Symbols

ALB – airlift bioreactor
K – consistency index [Pas^n]
k_La – volumetric oxygen mass transfer coefficient [h^{-1}, s^{-1}]
N – agitator speed [rpm, s^{-1}]
n – flow behaviour index [–]
t – time [h, s]
v_{SGR} – gas superficial velocity in the riser zone of ALB [m/s]

Greek symbols

η_{app} – apparent viscosity in ALB [Pas]
η_{am} – apparent viscosity under mixing conditions [Pas]

γ – shear rate [s^{-1}]
τ – shear stress [Pa]

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