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Effect of air flow rate on scleroglucan synthesis by *Sclerotium glucanicum* in an airlift bioreactor with an internal loop

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Abstract The effect of air flow rate upon scleroglucan production in batch cultures of *S. glucanicum* NRRL 3006 cultivated in airlift reactor with internal loop was studied using air flow rates from 0.25 m^3/h to 1.00 m^3/h. Biomass formation was favoured at high air flow rates (with maximum biomass at 0.75 m^3/h), whilst scleroglucan synthesis was highest at relatively low air flow rates (maximal at 0.40 m^3/h). *S. glucanicum* had a maximum specific growth rate of 0.018 h^{-1} at 0.75 m^3/h.

The values of the key process optimisation parameters (scleroglucan yield, productivity and specific productivity) were all maximal at 0.4 m^3/h. Since achievement of an adequate cell mass is essential for subsequent biopolymer synthesis, a bi-staged process was developed. In the first phase biomass formation was maximised by a high air flow rate of 0.75 m^3/h, while in the second phase a lower air flow rate of 0.40 m^3/h was used to stimulate biopolymer synthesis and minimise operational costs.

1 Introduction

Scleroglucan is an extracellular polysaccharide secreted by certain fungi, including species from the genera *Sclerotium, Corticium* and *Sclerotinia*. It is a linear chain of β-(1,3) linked D-glucopyranosyl groups, with single D-glucopyranosyl groups linked β-(1,6) to every third residue of the main chain etc [1, 2]. Due to its rheological characteristics, scleroglucan has potential as a suspending, stabilizing, coating or thickening agent in the food industry [3, 4]. It could also be used as an emulsifier, lubricant, thickening agent or mobility-control agent in enhanced oil recovery [5–7] and has potential applications in the agricultural, cosmetic and ceramic industries [8–10].

Recently, great interest has centred on the role of highly purified scleroglucan as an antitumour and an antiviral agent [3, 11]. Scleroglucan is reportedly active against a number of tumour types in relatively low doses [12], and thus is a promising biological response modifier (BRM). Despite prolonged interest in scleroglucan, it has failed in some areas of application to supplant traditional plant-derived polysaccharides and synthetic polymers due to its high production costs. Thus, in order to reduce production costs and to improve the competitive position of scleroglucan, the conventional batch fermentation must be improved.

In the optimisation of biopolymer production by fermentation the key parameters are productivity, specific productivity, yield and, finally, product broth concentration. In a number of studies in STRs, various authors have indicated that cell growth and scleroglucan formation are alternative fates for the C source, thus there is a need to control these activities. This has been attempted in STR's by nutrient limitation, aeration [13] control of pH [14] and process temperature [15].

The stirred tank bioreactor (STR) is the workhorse of the fermentation industry and is extensively used in bioreaction processes at both research and production scale [16]. It is normally thought to have a number of advantages relative to other bioreactor types, which include flexibility of use, its relatively well characterised nature, industrial familiarity and the potential for high oxygen transfer rates [17]. Conversely, it also has a number of drawbacks including mechanical complexity and high power consumption [16, 17]. In addition, problems with mass, momentum and heat transfer may occur [17]. Such difficulties increase in intensity as the scale of the bioreaction process increases and are especially pronounced in viscous fermentation fluids which display non-Newtonian behaviour, such as those containing filamentous fungi and/or exopolysaccharide producing microorganisms [18, 19]. Additionally, since it has been estimated that agitation and aeration costs may account for up to 20% of the overall production costs in conventional reactors, there is a clear incentive to develop simpler, less energy intensive alternatives [20].

In order to successfully carry out a bioreaction involving such microorganisms at lower energy costs, a wide variety of alternative bioreactor designs have been developed [21]. A number of these were based on the air-lift principle and have shown some promise [22]. Such air-lift reactors (ALRs) are mechanically simpler than STRs, and because of the absence of mechanical agitation, are less costly to run. Against this, oxygen transfer rates are generally lower than in STRs and such systems are commonly

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characterised as low shear, and poorly mixed [23], however, mixing in these reactors can be improved by the introduction of internal or external loops.

Very few studies have been carried out examining scleroglucan production in non-mechanically agitated vessels. However, since it has been shown that scleroglucan synthesis is stimulated by low O_2 levels, the major drawback of ALRs (relatively low O_2 transfer rate) is not a difficulty in this process. To date, scleroglucan production has not been studied in an ALR with an internal loop (ALR-IL), despite the ALR-IL being superior in some aspects to both the STR and the ALR-EL, e.g. in the region above the central draught tube, shear stress (and thus mixing) can be significantly higher than in an equivalent ALR-EL [24, 25].

The objective of this work was to study the fermentation process in ALR-ILs and evaluate the effect of air flow rate on cell growth and scleroglucan formation in the cultures of *Sclerotium glucanicum*, particularly in terms of the critical process parameters, yield, productivity and specific productivity.

2 Materials and methods

2.1 Microorganism

The organism used in this study was *Sclerotium glucanicum* NRRL 3006 (US Department of Agriculture, Peoria, Illinois). This was supplied as a freeze-dried culture and was resuscitated on Potato Dextrose Agar plates (Oxoid Ltd, Basingstoke, England). These were incubated at 28 °C, for 7 days. A 10 ml cell suspension prepared from such plates were used to inoculate 200 ml of sterile liquid medium in a 500 ml shake flask, which was subsequently incubated at 28 °C, 175 rpm, for 2 d on a rotary shaker. One flask was used to inoculate the airlift reactor. The composition of the fermentation medium was as follows: (g/l): sucrose, 30.0; $(NH_4)_2SO_4$, 1.0; $KH_2PO_4{\cdot}7H_2O$, 1.0; $MgSO_4{\cdot}7H_2O$, 0.5; yeast extract (Oxoid Ltd), 1.0; $FeSO_4{\cdot}7H_2O$, 0.01. This medium, with the addition of 0.5 g/l KCl, was also that used for the inoculum.

2.2 Bioreactor

The 8 l volume air-lift reactor with an internal loop used in this work is shown in Fig. 1. It was made up of 2 borosilicate glass sections sealed with teflon gaskets and held together by mild steel flanges. The height of the reactor was 0.9 m. The nominal internal diameter of the central draught tube was 0.036 m and the external diameter of the main body of the reactor was 0.1 m. Temperature was monitored by a Pt resistance thermometer fixed on the top of the reactor, linked to a controller, heating by means of an electrical heating element at the reactor base, or cooling via a cooling finger. Culture pH was controlled precisely by automatic addition of either 1 M NaOH or 10% (v/v) H_2SO_4. Air flow rate was regulated by means of a pressure regulator and variable area flowmeter giving flow rates in the range of 0.25 m^3/h to 1.00 m^3/h (approximately 0.75 to 3.33 vvm).

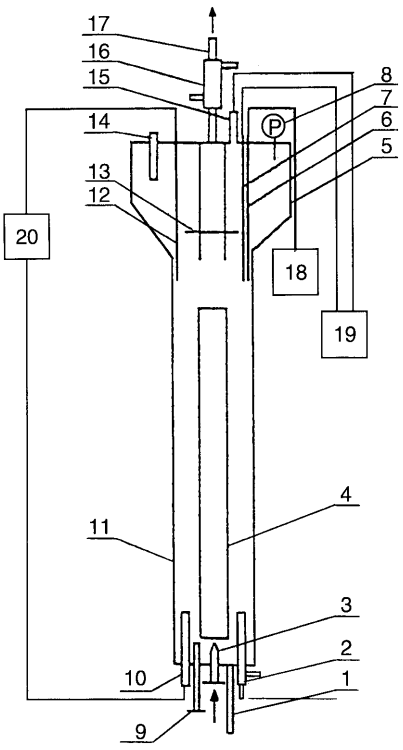

Fig. 1. Line diagram of the airlift reactor with internal loop; 1. liquid exit; 2. cooling finger; 3. gas nozzle; 4. central tube; 5. expanding section; 6. D.O. probe; 7. pH probe; 8. pressure gauge; 9. sampling port; 10. heating element; 11. reactor body; 12. thermometer; 13. baffle; 14. liquid inlet; 15. alkali and acid input; 16. condenser; 17. gas exit; 18. D.O. meter; 19. pH controller and recorder; 20. temperature controller

2.3 Analytical methods

The biomass concentration was measured by means of dry weight estimation involving filtration of broth samples through pre-dried and weighed GF-C filter discs (Whatman Ltd, Maidstone, UK). The biomass was then washed, dried (20 min at defrost in a 650 W microwave oven), cooled in a desiccator and weighed.

Exopolysaccharide (EPS) concentration was measured by precipitation of scleroglucan from cell free broth samples by addition of two volumes of absolute ethanol. The precipitated scleroglucan was then filtered, washed, dried and weighed. Sucrose concentration in the EPS free filtrate was then measured using the method of Dubois et al. [26]. The determination of NH_4^+ was described by Weatherburn [27].

3 Results and discussion

3.1 Fermentation kinetics

The time course of a typical batch scleroglucan process in terms of cell mass, scleroglucan, residual sucrose and residual NH_4^+ concentrations at an air flow rate of 0.40 m^3/h is shown in Fig. 2. As shown in Fig. 3, dissolved oxygen tension (DOT) decreased from 100%, at the start of the

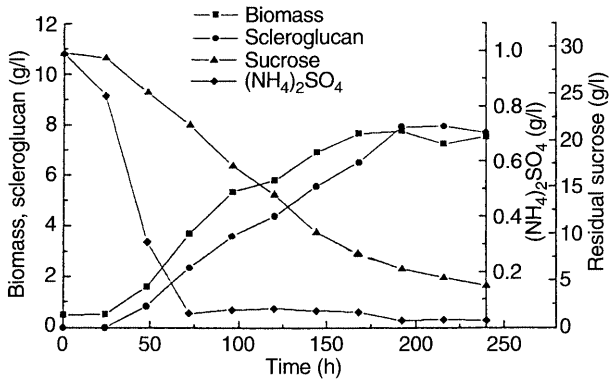

Fig. 2. Biomass, scleroglucan, residual sucrose and residual $(NH_4)_2SO_4$ vs time at air flow rate of 0.4 m^3/h

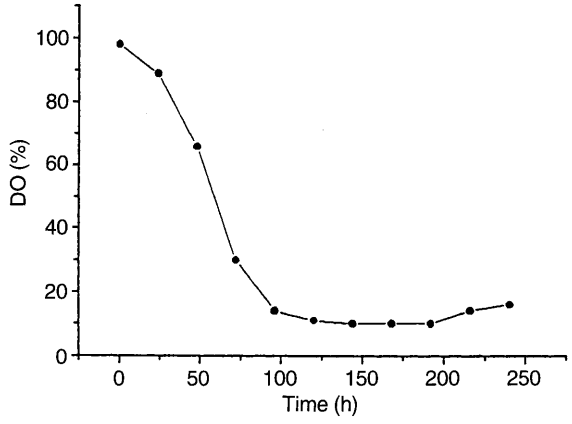

Fig. 3. Dynamic change of dissolved oxygen tension of the fermentation at air flow rate of 0.4 m^3/h

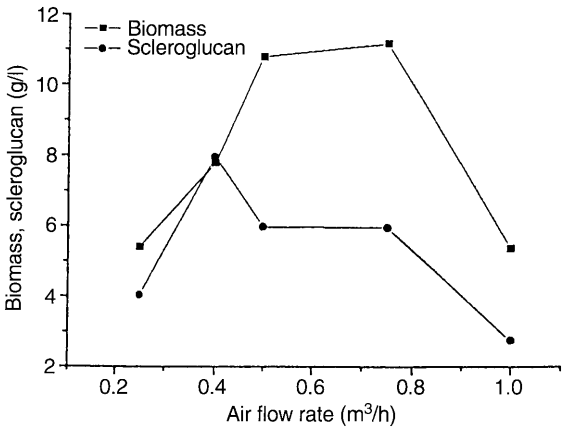

Fig. 4. Effect of air flow rate on maximum biomass and scleroglucan

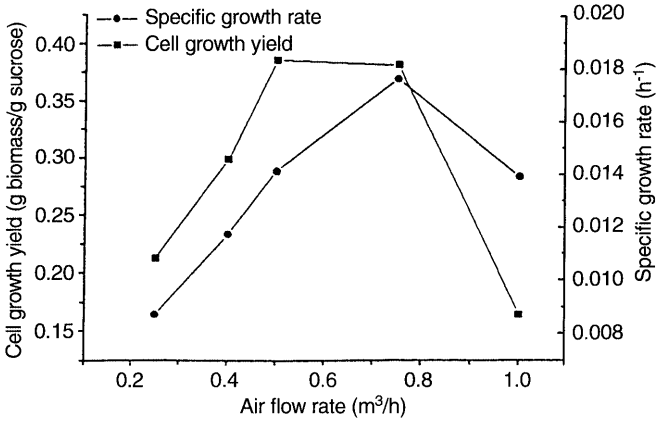

Fig. 5. Effect of air flow rate on cell growth rate and specific growth rate

process, to a minimum of 10% in the stationary phase. This decrease was due to fast O_2 consumption for cell growth and increased broth viscosity which substantially reduced O_2 transfer rate. A range of batch fermentations was carried out at air flow rates between 0.25 m^3/h and 1.00 m^3/h and the results are discussed below.

3.2
Effect of air flow rate on cell growth

3.2.1
Maximum cell density

Figure 4 shows the effect of air flow rate on maximal biomass and scleroglucan concentrations. It can be seen that the final cell density increased with increased air flow rate in the range of 0.25 m^3/h to 0.5 m^3/h. The maximum biomass concentrations (10.78 g/l to 10.95 g/l) were obtained when the air flow rates vary in the range of 0.5 m^3/h to 0.75 m^3/h. In the gas bubble zone between the top of the central draught tube and the baffle, high air flow rates can lead to high gas hold-ups, enhanced bulk mixing and improved dO_2 and mass transfer which promote growth. However, when the air flow rates exceed 0.75 m^3/h, the maximal cell concentrations decreased. This is because very high air flow rates will produce a zone of high shear stress, especially in the gas bubble zone [24, 25], which can potentially lead to cellular damage.

3.2.2
Cell growth yield and specific growth rate

In this instance, the cell growth yield at each point is determined by plotting cell density vs. sucrose concentration, the slope of the resulting line equals the cell growth yield. As can be seen in Fig. 5, cell growth yield increased with increasing air flow rate, and reached a maximum value about 0.38 g biomass/g sucrose when air flow rates are between 0.5 m^3/h and 0.75 m^3/h. Cell growth yield decreased when air flow rates were higher than 0.75 m^3/h and decreased to 0.16 g biomass/g sucrose when the air flow rate reached 1.00 m^3/h.

The specific growth rate (μ) was determined from the slope of semi-logarithmic plot of the cell density vs. fermentation time. Figure 5 shows that the specific growth rate increased as air flow rates increased from 0.25 m^3/h to 0.75 m^3/h and then decreased dramatically with further increase in the air flow rate. The maximum specific growth rate was 0.018 h^{-1} at an air flow rate of 0.75 m^3/h. Again, emphasising that high air flow rates lead to restricted cell growth.

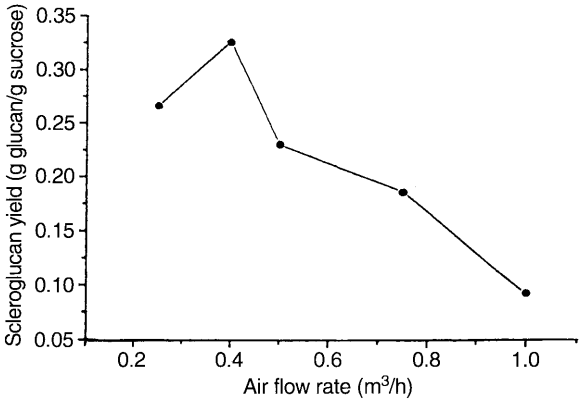

Fig. 6. Effect of air flow rate on scleroglucan yield

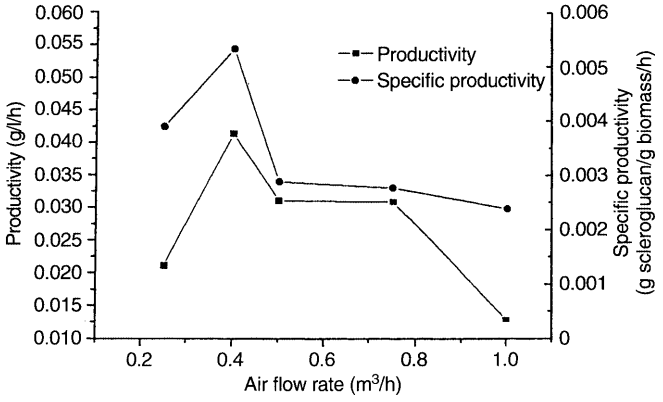

Fig. 7. The effect of air flow rate on productivity and specific productivity

3.3
Effect of air flow rate on scleroglucan production

3.3.1
Maximal scleroglucan concentration and scleroglucan yield

The maximal scleroglucan concentration increased with air flow rates ranging from 0.25 m^3/h to 0.40 m^3/h and then decreased with further increases in air flow rates. The maximum scleroglucan concentration was 7.93 g/l, at an air flow rate of 0.4 m^3/h, but only 2.75 g/l scleroglucan was obtained when the air flow rate was increased to 1.00 m^3/h. One reason for this could be that the higher air flow rates led to improved oxygen supply, and the subsequent production of scleroglucan was inhibited [26]. Rau et al. [28] concluded that oxygen limitation is a stimulatory factor for scleroglucan production. On the other hand, at high air flow rates, shear stress will be high, resulting in damage to the cells, thereby reducing their ability to produce scleroglucan.

Figure 6 shows the relationship of scleroglucan yield and air flow rate. Scleroglucan yield was determined from the linear plot of the scleroglucan concentration vs. sucrose concentration; the yield can be obtained from the slope of the resulting line. It can be seen from Fig. 6 that scleroglucan yield increased from 26.6%, at an air flow rate of 0.25 m^3/h, to 32.6%, at an air flow rate of 0.40 m^3/h. Thereafter, scleroglucan yield decreased with increasing air flow rate. When air flow rate was 1.00, scleroglucan yield decreased to 9.3%. Apart from the influence of the oxygen inhibition and cell damage mentioned above, another reason for this very low yield could be that higher air flow rates resulted in higher respiration rates [29], potentially leading to a significant decrease in C source availability for any other purpose, including scleroglucan synthesis.

3.3.2
Scleroglucan productivity and specific productivity

The effect of air flow rate on scleroglucan productivity and specific productivity are shown in Fig. 7. Scleroglucan productivity was simply defined as the maximum scleroglucan concentration divided by the correspondent fermentation time. Specific scleroglucan productivity was obtained by dividing scleroglucan productivity by the maximum cell density. It can be seen that scleroglucan productivities increased with air flow rates between 0.25 m^3/h to 0.40 m^3/h, then declined above 0.40 m^3/h. The specific scleroglucan productivity trend was identical. The maximum values of scleroglucan productivity and specific scleroglucan productivity are 41.3×10^{-3} g scleroglucan/l/h and 5.32×10^{-3} g scleroglucan/g biomass/l/h respectively at the same air flow rate of 0.40 m^3/h. High productivity and specific productivity indicate decreased running costs for fermenter operation (shorter process times) and a potential decrease in the costs of separation of biomass from biopolymer. Since recovery costs of biopolymers can be a significant cost factor, this can have a profound economic impact on the process.

3.4
Optimal air flow rates

From the above result it is clear that there are two distinct air flow rate optima, one which optimises growth, between 0.5 and 0.75 $m^3\ h^{-1}$, whilst the optimum for scleroglucan synthesis is lower, at only 0.4 $m^3\ h^{-1}$. The maxima reported for scleroglucan concentration (a key factor in recovery costs, since high concentrations minimise solvent costs, yield, productivity and specific productivity are equal to those reported in batch STR processes [30, 31], indicating that production of this biopolymer in ALR-IL reactors is practical.

This would have the additional process benefit of minimising the costs of compressed air provision, a major contributor to the costs of running large scale airlifts [23].

3.5
Bi-staged air flow rate process

It can be seen from Figs. 2–8 that the formation of adequate cell mass (and thus glucan synthesising enzymes) is clearly critical to the success of this process, thus, a process initially optimised for biomass formation by high air flow rate of 0.75 m^3/h for 96 h, followed by low air flow rate of 0.40 m^3/h following nitrogen source exhaustion, may offer some promise, and could well be a cheaper alternative than constant operation at high air flow rate. The results of such an operational pattern upon the process are

Table 1. Comparative results between constant air flow rate processes and bi-staged air flow rate process*

Air flow rate (m^3/h)	X_m (g/l)	Y_c (g X/g S)	P_m (g/l)	Y_p (g P/g S)	$P_r \times 10^3$ (g Pl/h)	$P_s \times 10^3$ (g P/g X/h)
0.25	5.23	0.212	4.05	0.266	21.09	3.89
0.4	7.77	0.299	7.93	0.326	41.30	5.32
0.5	10.78	0.386	5.97	0.231	31.09	2.88
0.75	11.15	0.382	5.93	0.186	30.89	2.77
1.00	5.35	0.164	2.75	0.093	12.73	2.38
0.75 → 0.4	9.41	0.396	10.2	0.422	46.58	4.95

* Symbols: X_m, Maximum cell concentration; Yc, cell growth yield; P_m, maximum scleroglucan concentration; Y_p, scleroglucan yield; P_r, scleroglucan productivity; P_s, specific scleroglucan productivity; X, biomass, S, sucrose and P, scleroglucan

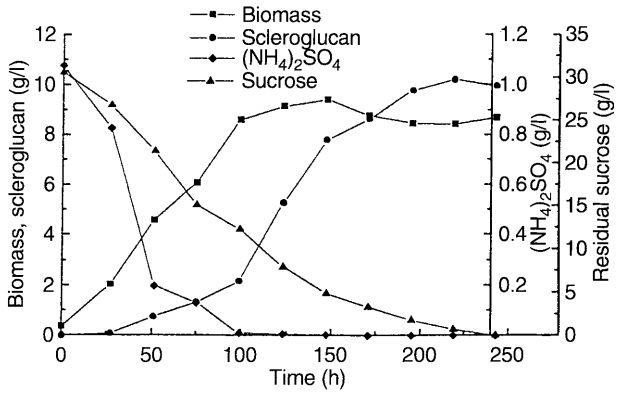

Fig. 8. Biomass, scleroglucan, residual sucrose, residual $(NH_4)_2SO_4$ vs time in bi-staged air flow rate fermentation (0.75 m^3/h before 96 h, 0.40 m^3/h thereafter)

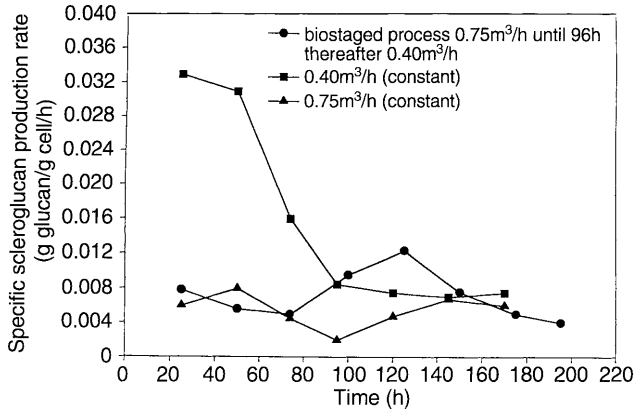

Fig. 9. Specific scleroglucan formation rate vs time at various aeration rates

shown in Fig. 8, which indicates that significantly better results can be obtained.

The final scleroglucan concentration of this process was 10.2 g/l, much higher than those obtained at constant air flow rates of either 0.4 m^3/h or 0.75 m^3/h. The change in specific scleroglucan formation rate versus time under varying aeration conditions can be seen in Figs. 9. The pattern of scleroglucan synthesis per unit biomass in the bi-staged process is clearly distinct from both of the processes at constant aeration, and a marked increase in specific scleroglucan synthesis rate following the decrease in the aeration rate is clear. This clearly indicates that, in the presence of sufficient C source, reduced provision of oxygen does lead to an increase in the synthesis of scleroglucan (as proposed by Rau et al. [28] and Wang [30]). More significantly, by focusing on the impact of reduced aeration on the specific rates of scleroglucan synthesis in these processes, it is clear that the increase in maximal scleroglucan concentration in the bi-staged process is not due simply to the diversion of C source from biomass formation to scleroglucan synthesis (as proposed by Taurhesia and McNeil [31]) but actually involves a direct increase in glucan formation per unit biomass, that is, a direct stimulatory mechanism.

Table 1 summarises some of the important data from this air flow rate shift experiment and compares them with the results from batch fermentation at constant air flow rate. Both the scleroglucan yield and productivity were increased significantly by a shift-down in the air flow rate. The increases were mainly due to the achievement of a suitable cell concentration in the growth phase and the stimulation of scleroglucan production by oxygen limitation in the synthetic phase. It can also be seen that the specific scleroglucan productivity of the bi-staged air flow rate process is only little lower than at the constant air flow rate of 0.4 m^3/h. Nevertheless, air flow rate shift from 0.75 m^3/h to 0.4 m^3/h at an appropriate fermentation time gave the best overall performance (high scleroglucan concentration, yield and productivity) among all the batch processes examined.

4 Conclusions

Air flow rates between 0.5 m^3/h and 0.75 m^3/h were favourable to cell growth while an air flow rate of 0.4 m^3/h was best for scleroglucan production. A bi-staged air flow rate fermentation process with air flow rate shift from 0.75 m^3/h to 0.4 m^3/h when nitrogen source was consumed was superior to the conventional batch fermentation at constant air flow rate. The scleroglucan productivity obtained in ALR-IL was comparable to those in STRs [28, 29] or in ALR-EL [13], but equipment investment and operational costs were much lower than in STR. Thus, the use of ALR-IL in the production of scleroglucan is feasible economically and technically.

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