Journal of Industrial Microbiology, 9 (1992) 19–26
Elsevier

SIM 00389

Enhanced glucan formation of filamentous fungi by effective mixing, oxygen limitation and fed-batch processing

U. Rau, E. Gura, E. Olszewski and F. Wagner

Institute of Biochemistry and Biotechnology, Section A, Technical University of Braunschweig, Braunschweig, Germany

(Received 2 October 1990; accepted 22 October 1991)

Key words: Schizophyllum commune; *Sclerotium glucanicum*; Branched β-1,3-glucan; Fan-impeller; Oxygen limitation; Process design

SUMMARY

Glucan formation of *Schizophyllum commune* and *Sclerotium glucanicum* were investigated. Process data obtained during batch cultivation are presented. Glucan release can be improved by oxygen limitation. Thus, growth and glucan release are influenced by oxygen in opposite ways. Possible pathways of this oxygen-dependent regulation are discussed. A draft-tube/propeller system, rushtonturbine-, fan- and helicon-ribbon-impeller as well as a fundaspin and intermig agitator were tested. The 4-bladed fan impeller with $d^* = 0.64$ yielded the best results, since effective bulk mixing is much more important than bubble break up (micromixing) with regard to this system. Fed-batch cultivation always resulted in higher rates of glucan formation than the batch process.

INTRODUCTION

Schizophyllum commune and *Sclerotium glucanicum* secrete a neutral homoglucan that consists of a backbone chain of 1,3-β-D-glucopyranose units linked with single 1,6-bounded β-D-glucopyranoses at about every third glucose molecule in the basic chain. These gums are called "Schizophyllan" and "Scleroglucan" by their common names, respectively. Due to their nearly identical rheological properties [2,12,17,36] the uniform term "glucan" will be applied here.

Most information on this glucan is related to molecular and rheological characteristics. It dissolves in water as a triple helix [12,17,36] with protruding pendent β-1,6-linked D-glucose residues originating from the outside of the triplex [2]. In DMSO and at a pH > 12 the triple helix melts to single randomly coiled strains, equivalent to reduction of the average molecular weight by one-third [17]. Aqueous solutions show thixotropic, pseudoplastic [22] and viscoelastic behaviour. These characteristics are not markedly influenced by the genus of the producing fungus but extremely affected by the conditions of cultivation and down-stream processing [10,23].

Many fungi are able to secrete this branched glucan. First claims with respect to this glucan and methods of its production using species of filamentously growing fungi of the two different genera *Sclerotium* and *Corticum* were patented in 1967 by Halleck [11]. However, these fungi secrete glucans with relatively low molecular weight (up to 250 000 g/mol equivalent to a degree of polymerization of 1570). Yet there is little information in recent literature concerning cultivation conditions and parameters for production of this glucan. Only Griffith and Compere [5,9] report about the production of a highly viscous polysaccharide by *Sclerotium rolfsii*. Stephan [30] investigated the same fungus in greater detail by using a broad substrate spectrum and discussing viscometric data. Steiner et al. [29] described cellulase and xylanase production of *S. commune* and noted relationship of growth to polysaccharide formation. In this study we therefore report on the influence of cultivation conditions (mixing, oxygen) and process design (batch, fed-batch) on glucan formation.

MATERIALS AND METHODS

Microorganisms and conditions of cultivations

The wood rotting basidiomycete *Schizophyllum commune* ATCC 38548 and the fungus imperfectus *Sclerotium glucanicum* CBS 52071 were employed to produce extracellular glucans. The fungi were grown at room temperature on agar slants supplemented with 39 g/l potato dextrose agar and 5 g/l yeast extract. After one week the covered slants were stored at 4 °C. New slants were inoculated at intervals of 4 weeks.

Correspondence: U. Rau, Institute of Biochemistry and Biotechnology, Section A, Technical University of Braunschweig, Konstantin-Uhde-Straße 5, 3300 Braunschweig, F.R.G.

The medium used for shake flask and bioreactor cultivations consisted of (per l deionized water): *S. commune*: glucose 30 g; yeast extract 3 g; KH_2PO_4 1 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; pH = 5.3 at 27 °C. *S. glucanicum*: glucose 30 g; $NaNO_3$ 3 g; yeast extract 1 g; KH_2PO_4 1; KCl 0.5; $MgSO_4 \cdot 7H_2O$ 0.5; $FeSO_4 \cdot 7H_2O$ 0.05 g; pH = 3.5 at 22 °C. The sulphates were added separately after sterile filtration. Cultivations were performed without pH control.

For the first subculture a piece of covered agar was used to inoculate 100 ml of medium in 500-ml shake flasks. After 4 days on a rotary shaker the second seed with 250 ml in 1-l shake flasks was inoculated with 10 ml of homogenized (Ultra Turrax, Ika, Staufen im Breisgau, F.R.G.) culture broth and was cultivated for 2 days as described above.

Bioreactor cultivations were performed in vessels with a working volume 10, 30, and 50 l (B. Braun-Diessel-Biotech, Melsungen, F.R.G.). The vessels possessed a uniform width/height ratio of two. Using intermig, rushton turbine and fan impellers four baffles were mounted inside the bioreactor and three stirrers of each type were installed on the shaft at a distance equivalent to 2/3 of the stirrer diameter. The medium was inoculated with 5% (v/v) culture suspension. All data presented refer to at least two independent experiments.

Analysis

Cell dry weight was determined gravimetrically after homogenization, centrifugation and drying. Polysaccharide was precipitated from the supernatant by i-propanol and subsequently dried to constant weight. The procedure is described in detail elsewhere [23].

Exhaust analysis of carbon dioxide and oxygen was performed by Unor 6N and Oxygor 6N (Maihak, Hamburg, F.R.G.), respectively.

Determination of amino groups was carried out by the colour reaction with ninhydrin [25]. L-glutamine was used as standard.

Viscometry

Shear viscosities of aqueous glucan solutions were measured by a rotational viscometer (Rotovisco RV 100, Haake, Karlsruhe, F.R.G.). All measurements were carried out at 25 °C.

RESULTS AND DISCUSSION

Batch cultivation and oxygen limitation

A characteristic course of a batch cultivation of *S. commune* is shown in Fig. 1.

30 g/l of glucose were consumed after approximately three days of glucan production associated with growth.

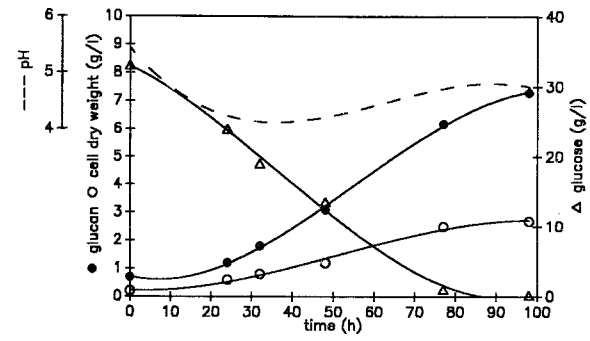

Fig. 1. Batch cultivation of *S. commune* in a 30-l bioreactor equiped with three fan impellers at 100 rpm, 27 °C, an initial pH of 5.3 and anaeration rate of 0.083 v/v.

Compared to xanthan [23], a substantially lower weight ratio of biomass : glucan (up to 1 : 4) was achieved, facilitating subsequent cell separation for down-streaming of glucan. The pH was not adjusted as pH-static cultivations resulted in poor productivities [15]. At the time when the partial pressure of oxygen in the liquid phase decreased to almost 0% of maximum (30 h), the pH went through a minimum of 4.2 and increased subsequently to 4.8 at the end of cultivation. After onset of oxygen limitation the maximum productivity of glucan (2.3 g/dl) was reached at 65 h. With a cell mass of 1.7 g/l at this time, specific productivity of 1.35 g glucan per g biomass per day was observed. Cultivation was terminated after substrate consumption. Prolonged cultivation under carbon limited conditions led to the release of glucan degrading enzymes. These enzymes cause a slight increase in glucose concentration accompanied by a decrease in concentration of the glucan and a sharp drop in the viscosity of glucan. Small fragments of the degenerated glucan serve as carbon source for the fungus.

The molecular weight runs through a broad maximum [23] and covers a range of $6–15 \cdot 10^6$ g/mol (degree of polymerization 37000–92500 glucopyranose units). Previous investigations have shown a strong dependence of intrinsic viscosity $[\eta]$ and molecular mass M_r as expressed by the Mark-Houwink-equation (Müller, R.J., private communication):

$$[\eta] = 4.45\ 10^{7} \cdot M_r^{1.49} \text{ (glucan-water, 25 °C)}$$

Therefore, we concluded that intrinsic viscosity is mainly affected by a change in molecular weight and does not ensue from an altered glucan conformation. The high exponent of 1.49 indicates an increased stiffness of the polymer (0.5 = random coil, 2 = rodlike behaviour). For comparison, xanthan as a highly branched polysaccharide shows rather random coil characteristics at an exponent

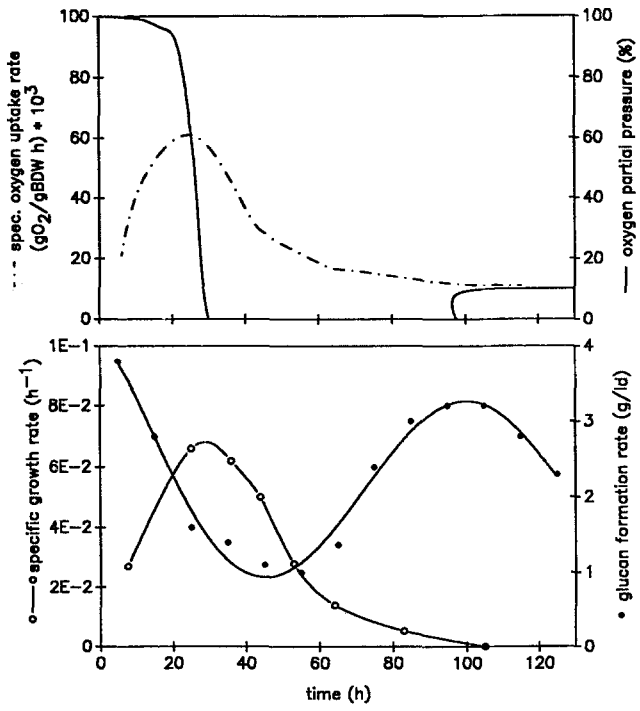

Fig. 2. 50-l batch cultivation of *S. commune* with glucose as carbon source and a draft-tube/propeller system (internal loop) at 500 rpm. For further cultivation conditions see Fig. 1.

of 0.9. The influence of oxygen on glucan and biomass formation is shown in Fig. 2.

In order to start cultivation the medium was inoculated with an oxygen-starved preculture incubated in shake flasks. Therefore, high rates of glucan formation were observed at the beginning. At first, oxygen supply of the cells is sufficient, resulting in a rise in the specific growth rate and a decrease in the rate of exocellular glucan formation. After 30 h oxygen partial pressure of the liquid phase was reduced to almost zero. The fungus responds to this oxygen limitation by declined growth and increased glucan formation. Glucose was consumed after 95 h in coherence with an increase in oxygen partial pressure.

The maximum specific rate of oxygen uptake of 60 mgO_2/gBDW · h was achieved when oxygen partial pressure was almost zero. The volume related transfer coefficient $k_l a$ drops sharply from 400 h^{-1} to 12 h^{-1} during the first 30 h and subsequently decreased only slightly to approximately 5 h^{-1} at the end of cultivation. This type of decreased influence of polymers and filamentous cells on $k_l a$ at increasing concentration was also described by Schügerl [26] and Tuffile [33].

Improved glucan formation under oxygen limited conditions was also observed in the case of *S. glucanicum*.

TABLE 1

10-l batch cultivation with *S. glucanicum* at different air flow rates using a draft-tube propeller system (600 rpm)

Air flow rate (m^3/h)	Sec. growth rate[1] (h^{-1})	Glucan formation rate[1] (kg/m^3 d)
0.03	0.036	3.1
0.3	0.063	1.4

[1] Maximum data.

The comparison of two batch cultivations in a 10-l bioreactor equipped with a draft-tube/propeller system with different air flow rates showed a change in growth and glucan formation (Table 1).

In order to discuss the oxygen-dependent regulation of hyphal growth on the one hand and glucan release on the other, the structure and growth of a fungal cell wall have to be considered. Viewing a cross section of the cell wall of *Schizophyllum commune* [36] and beginning from the outside a mucilage is loosely associated with the cell wall or is released into the medium, consisting of a branched (1-3)-β-D/(1-6)-β-D-linked glucan. This glucan is soluble in water or aqueous alkalic solutions and is called "S-glucan" by its trivial name [14]. A more rigid sphere of S-glucan incorporating small amounts of a α-linked glucan adheres further inside. The next layer consists of a fibrillar network of alkali resistent "R-glucan" and chitin. R-glucan comprises (1-3)-β-D/(1-6)-β-D-linkages as well but offers a different structure because of higher branching than S-glucan [28]. Diverse structures of R-glucan are still under discussion and are not mentioned here.

The composition of the cell wall determines the shape of the cell. A plastic wall results in a rounded cell and is found at the hyphal tip. This plasticity is thought to be due to the reduced SH-groups [16] that result from the anaerobic metabolic state found in the apex of filamentous fungi [34]. In contrast the sub-apical wall is rigid, perhaps due to the oxidized -S-S-bonds as a consequence of the oxidative state [6] and/or through changes in the dimensions of microfibrills and the insertion of β-D-glucan and the glycoprotein reticulum, respectively. Thus, β-D-glucan is not primarily deposited in the extension zone at the advancing hyphal tip, a site wherein the microfibrillar chitin is deposited, but rather in the thickening areas adjacent to it [4].

The 1–2 μm apical zone lacks all organelles except numerous cytoplasmic vesicles, which partly fuse with the plasma membrane. These vesicles are thought to contain cell wall and plasma membrane precursors, lytic enzymes (cellulase, β-1,3-glucanase, protease) and β-glucan syn-

thase. Chitin synthase has been localized in spherical particles called chitosomes [7]. One model of the mechanism of tip growth proposed by Bartnicki-Garcia [1] describes the movement of vesicles to the hyphal tip, their fusion with the plasma membrane and the release of their contents into the wall matrix. The degrading enzymes weaken the microfibrils while the cell's turgor pressure causes the wall to expand. In addition, cell wall synthesizing enzymes react with the precursors to rigidify the expanded wall. Tip growth can thus be considered as a dynamic balance between wall lysis, wall synthesis and turgor pressure. Yet it is unknown how oxygen influences these complex interactions resulting in enhanced glucan release. Two possible pathways are discussed.

i) The glucan synthase system might not be influenced by oxygen.

When oxygen supply is sufficient, cell wall synthesis is unlimited and the required glucan is incorporated. Under oxygen limitation essential constituents or precursors are formed more slowly and exceeding glucan which cannot be incorporated into the cell wall is released.

ii) The glucan synthase system of new cells might be influenced by oxygen.

The cultivation starts with an oxygen limited preculture. Thus, appropriately high glucan formation rates are observed. Old cells produce glucan at a continously high rate, but new cells at first show a declining activity of glucan synthase up to the point of oxygen limitation of the culture.

Yeast extract was used as the sole N-source. Preliminary investigations [15] had shown that at varying yeast extract concentrations of 3 to 10 g/l the yielded cell dry weight and glucan concentration were approximately constant. Only yeast extract concentrations lower than 3 g/l led to reduced yields. Complex nitrogen sources as peptone, casamino acids, molasses or corn steep liqueur do not contribute to higher yields [15]. Amino-N determinations and elementary analysis resulted in 0.033 g N and 0.1 g entire N per g yeast extract, respectively. Related to the input glucose concentration of 30 g/l a high C/N ratio of 40 : 1 is obtained corresponding to the very low N-percentage of 2.2% (w/w) of the cell dry weight. Furthermore, only 60% of the amino-N was consumed after 100 h. On account of these results we excluded further limitation by nitrogen.

Generally most polysaccharide producing bacteria and fungi [18,31] need at least one form of limitation for enhanced productivity (e.g. phosphorus, potassium, sulphur and magnesium) as well as a high carbon : nitrogen ratio. However, contrary to our results, oxygen has to be sufficiently provided especially for *Xanthomonas campestris* (Xanthan) [19], *Alcaligenes faecalis* (Curdlan) [14] and *Aureobasidium pullulans* (pullulan) [24]. Information on polysaccharide promoting effects by oxygen limitation is rarely found in the literature. One such example is *Rhizobium trifolii* [32] which shows increased exopolymer production and decreased biomass production under oxygen limited conditions. Yet strictly anaerobic conditions or resting cells (without nitrogen) of *S. commune* prevent growth and glucan formation.

Types of impellers

Impellers applied for shear thinning fluids without microorganisms were studied intensively [3,13,20,27]. However, when a filamentously growing fungus is used to produce highly viscous pseudoplastic polysaccharide suspensions, not only short mixing times and high mass transfer but also shear stress, which depends on the type of impeller used and stirrer speed influencing physiology of the fungus, have to be considered. In other words, in proportion to the agitator used the impeller has to present a compromise between micro- and macromixing and glucan release from the cell wall on the one hand and low shear stress for the fungus and glucan on the other. Furthermore, the impeller should enable easy construction for a subsequent scale up. Batch cultivations with various types of impellers (Fig. 3) were therefore carried out in 50- and 30-l bioreactors.

During cultivation impeller speed was not varied. The chosen speed for each impeller resulted from measurements of mixing times at a concentration of the glucan of 1 g/l. These individual speeds yielded mixing times of 8 to 15 s at a mixing homogeneity of 95%. It should be emphasized that the only constant parameter was the mixing time when comparing different types of impellers because macromixing is the decisive factor during cultivation.

As can be seen from Table 2 the fan impeller $d^* = 0.64$ yielded the highest rate of glucan formation at each volume. The cultivation at 50 l with draft-tube/propeller system and intermig resulted in only slightly lower productivities. However, the intermig possessed a high local dissipation energy and therefore it degraded the molecular structure of the glucan, resulting in decreased specific shear viscosity. Furthermore, increased damage of the hyphal cells was observed (microscope), hindering subsequent cell separation. The draft-tube/propeller system with internal loop shows only low shear stress, since the propeller acts as a pump. Yet this system is not suitable for scale up. The latter could also be said for the fundaspin. The low specific viscosity observed when using the helicon ribbon impeller during 30-l cultivation is inexplicable. The impeller commonly possesses low shear stress. Previous investigations have shown that glucan is not released from the cell wall without shear stress. Possibly, the shear stress of the helicon ribbon impeller is too

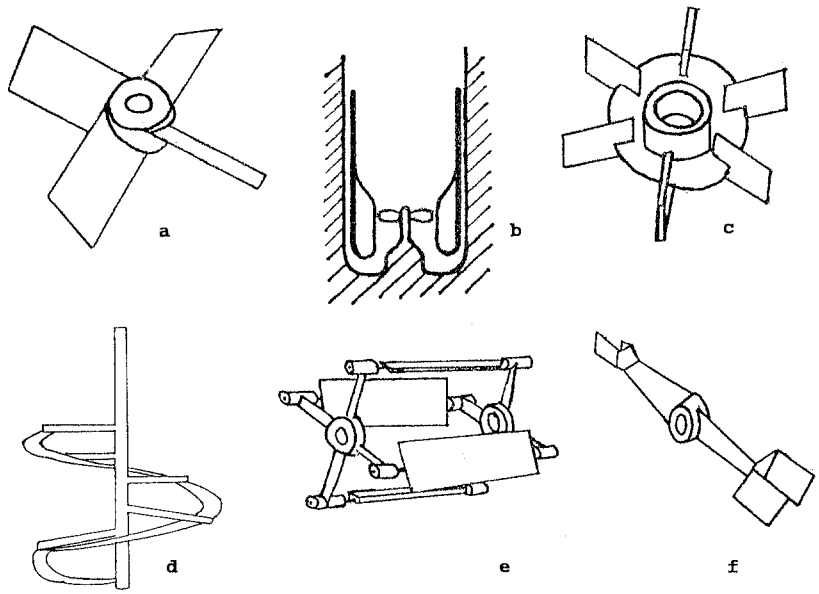

Fig. 3. Agitators applied for batch cultivation: (a) fan; (b) draft-tube/propeller; (c) rushton-turbine; (d) helicon-ribbon; (e) fundaspin (Chemap, Volketswil, Switzerland); (f) intermig (Ekato, Schopfheim, F.R.G.).

low, so only low molecular glucans were released while high molecular ones adhere to the cell wall. By using a gear pump (type 2032, Verder, Düsseldorf, F.R.G.) in an external loop, productivity is markedly increased. Once a day the three-fold medium volume was recirculated in 1 h under sterile conditions. The smooth shear stress of the gears is likely to provide the compromise mentioned above between glucan release from the cell wall and cell damage. Thus, this procedure results in a homogeneous culture suspension without pellets and with appropriately increased specific interfacial area.

Due to these results the 4-bladed fan impeller d* = 0.64 with a blade pitch of 45° is very suitable. Its easy and inexpensive construction favours its application

TABLE 2

Process data of batch cultivations with *Schizophyllum commune* depending on the type of impeller (t = 27 °C, aeration rate 0.083 v/v, initial pH 5.3)

Vol. (l)	Impeller	d* (–)	rpm (min^{-1})	Cell dry weight[1] (g/l)	Glucan[1] (g/l)	Glucan formation rate (g/ld)	Spec. visc.[2] (mPas)
50	Draft-t./prop.	–	500	1.8	9	3.2	160
50	Intermig	0.66	250	5.9	12.6	3.3	74
50	Fan	0.64	100	5.4	8.4	3.6	150
50	Rushton-t.	0.4	250	3.8	6.7	1.4	110
30	Rushton-t.	0.4	250	3.8	8.3	1.8	120
30	Fundaspin	0.64	100	3.1	7.1	2.6	153
30	Hel.-rib.	0.8	150	2.4	3.4	1.1	50
30	Hel + gearp.	0.8	150	1.9	8.0	2.1	178
30	Fan	0.73	100	3.4	7.8	2.2	153
30	Fan	0.64	100	2.8	7.8	2.8	196
30	Fan + gearp.	0.64	100	2.1	8.2	3.1	152
30	Fan	0.4	250	2.5	7.4	2.6	140

[1] At the moment of glucose consumption.

[2] Specific shear viscosity of cell-free aqueous glucan solutions (0.3 g/l, shear rate 0.3 s^{-1}).

in scale up. Other authors presented similar results for pseudoplastic and viscoelastic culture suspensions when using a hydrofoil impeller of slightly different construction [8]. They also stated that bubble break-up (micromixing) is much less important for viscous cultivations and it is just as important to improve bulk mixing. Furthermore, fan impellers do not generate high local shear stress. This will provide an advantage for shear-sensitive microorganisms. Whether a gear pump is additionally connected or not depends on the shear sensitivity of the strain used. Both fungi investigated in this study showed positive effects by additional shearing.

Fed-batch cultivation

Fed-batch processing was carried out as follows. When sufficient glucan and biomass concentration were reached, a fraction α of the culture suspension V_f, that contains cells and glucan was removed at time intervals, t_b, in approximately zero time. This volume was replaced by an equal fraction (V_r) containing substrate. The fed batch reactor can be compared with the chemostat using the apparent dilution rate D', which in this case is:

$$D' = \alpha/t_b \text{ with } \alpha = V_r/V_f$$

Considering this, a fed-batch cultivation was conducted using fan impellers (Table 3).

The fed batch process was terminated after 12 days. The substrate composition in the fed volumes was equal to batch cultivation. Only glucose was added in such a way that 30 g/l were present at the beginning of each cycle. Different apparent dilution rates resulted from empirically varying the time intervals of each batch cycle for achieving stable conditions. Thus, after achieving a stable process, i.e. constant data of biomass and glucan after each renewal, highest rates of glucan formation are yielded at an apparent dilution rate of 0.014 to 0.018 h^{-1}. All GFR calculated were on an average higher than in the case of the batch process [23]. A possible explanation for this behaviour could be the prolonged oxygen limitation the fungus was subjected to, so oxygen dependent glucan release was intensified.

TABLE 3

Fed batch cultivation with *S. commune* and glucose as carbon source in 50-l bioreactor with three fan impellers (d* = 0.64, 100 rpm) at an aeration rate of 0.083 v/v.

α	t_b (h)	D' (h^{-1})	GFR (kg/m^3 d)	$C_{g,e}$ (kg/m^3)
0.5	22.8	0.022	5.2	14.3
0.5	26.7	0.018	8.2	19.3
0.5	45.2	0.011	3.2	13.2
0.5	48.4	0.010	6.1	20.3
0.33	21.3	0.015	7.8	15.9
0.33	22.7	0.014	8.7	19.8
0.33	25.3	0.013	6.6	17.9

α, renewal volume/volume of culture broth; t_b, time of a batch cycle; GFR, glucan formation rate; $C_{g,e}$, glucan concentration at the end of a batch cycle.

REFERENCES

1 Bartnicki-Garcia, S. 1973. Fundamental aspects of hyphal morphogenesis. Symp. Soc. Gen. Microbiol. 23: 245–267.
2 Bluhm, T.L., Y. Deslandes and R.H. Marchessault. 1982. Solid-state and solution conformation of scleroglucan. Carbohydr. Res. 100: 117–130.
3 Brauer, H. 1971. Grundlagen der Ein- und Mehrphasenströmung. Sauerländer Verlag, Frankfurt a.M., F.R.G.
4 Catley, B.J. 1983. Regulation of yeast and fungal polysaccharides excluding chitin and cellulose. In: Progress in industrial microbiology, (Bushel, M.E., ed.) 18: 129–200, Elsevier, Amsterdam.
5 Compere, A.L. and L. Griffith. 1981. Scleroglucan biopolymer production, properties and economics. In: Advances in Biotechnology (Moo-Young, M., ed.) 3: 541–546, Pergamon Press, New York.
6 Evans, B. 1980. Relationships between morphology and metabolism in the dimorphic fungus Mycothypha. Ph. D. Thesis, Technical University of Braunschweig, F.R.G.
7 Garraway, M.O. and R.C. Evans (eds.). 1984. In: Fungal nutrition and physiology, John Wiley and Sons, New York.
8 Gbwonyo, K., D. Dimasi and B.C. Buckland. 1986. The use of hydrofoil impellers to improve oxygen transfer efficiency in viscous mycelial fermentations. In: Int. Conf. on Bioreactor Fluid Dynamics, pp. 281–299, Cambridge, England.
9 Griffith, W.L. and A.L. Compere. 1978. Production of a high viscosity glucan by *Sclerotium rolfsii* ATCC 15206. Dev. Ind. Microbiol. 19: 609–617.
10 Haarstrick, A., U. Rau and F. Wagner. 1990. Cross flow filtration as a method of separating fungal cells and purifying the polysaccharide produced. Bioproc. Eng., submitted.
11 Halleck, F.E. 1967. Polysaccharides and methods for production thereof. US Patent 3.301.848.
12 Kashiwagi, Y., T. Norisuye and H. Fuyita. 1981. Triple helix of *Schizophyllum commune* polysaccharide in dilute solution. Macromol., 14: 1220–1225.
13 Kipke, K.D. and E. Todtenhaupt. 1982. Rühren von nicht-Newtonschen Flüssigkeiten. Verfahrenstechnik 16: 497–503.
14 Lawford, H. and J. Rousseau. 1989. Effect of oxygen on the rate of β-1,3-glucan microbial exopolysaccharide production. Biotechnol. Lett. 11: 125–130.
15 Münzer, S. 1989. Produktion und Charakterisierung eines von *Schizophyllum commune* ATCC 38548 gebildeten extrazellulären β-1,3-glucans. Ph. D. Thesis, Technical University of Braunschweig, F.R.G.
16 Nickerson, W. and F. Falcone. 1956. Identification of protein

disulfide reductase as a cellular division enzyme in yeasts. Science, 124: 722–723.

17 Norisuye, T.,T. Yanaki and H. Fujita. 1980. Triple helix of a *Schizophyllum commune* polysaccharide in aqueous solution. J. Poly. Sci. 18: 547–558.

18 Pace, G.W. and R.C. Righelato. 1980. Production of extracellular microbial polysaccharides. In: Adv. Biochem. Eng. (Fiechter, A., ed.), 15: 41–70, Springer Verlag, Hamburg, F.R.G.

19 Peters, H.U., H. Herbst, E. Widjayanti, A. Schumpe and W.-D. Deckwer. 1990. The influence of agitation rate on xanthan production rate by *Xanthomonas campestris*. Biotechnol. Bioeng., submitted.

20 Pörtner, R. and U. Werner. 1989. Betrachtungen zur Effektivität von Rührern bei Homogenisierprozessen und viskoelastischen Flüssigkeiten. Chem.-Ing.-Tech. 61: 250–251.

21 Rau, U. and F. Wagner. 1987. Non-Newtonian flow behaviour of colloid dispers glucan solutions. Biotechnol. Lett. 9: 95–100.

22 Rau, U., E. Gura, A. Schliephaake and F. Wagner. 1989. Influence of processing on the formation of exopolysaccharides by filamentously growing fungi. In: Dechema Biotechnology Conferences (Behrens, D. and A.J. Driesel, eds.), 3 part B: pp. 571–574.

23 Rau, U., R.-J. Müller, K. Cordes and J. Klein. 1990. Process and molecular data of branched 1,3-β-D-glucans in comparison with *Xanthan*. Bioproc. Eng. 5: 89–93.

24 Rho, D., A. Mulchandini, J.H.T. Luong and A. Le Duy. 1988. Oxygen requirement in pullulan fermentation. Appl. Microbiol. Biotechnol. 28: 361–366.

25 Rosenthal, G.A. 1985. Colorimetric and fluorimetric detection of amino acids. In: Chemistry and Biochemistry of Amino Acids (Barret, G.C., ed.), pp. 573–590, Chapman and Hall Inc., London.

26 Schügerl, K. 1981. Oxygen transfer into highly viscous media. In: Advances in Biochemical Engineering (Fiechter, A., ed.) 19: 71–174.

27 Shamlou, P.A. and M.F. Edwards. 1985. Power consumptions of helical ribbon mixers in viscous Newtonian and non-Newtonian fluids. Chem. Eng. Sc. 40: 1773–1781.

28 Sietsma, J.H. and J.G.H. Wessels. 1977. Chemical analysis of the hyphal wall of *Schizophyllum commune*. Biochim. Biophys. Acta. 496: 225–239.

29 Steiner, W., R.M. Lafferty, I. Gomes and H. Esterbauer. 1987. Studies on a wild strain of *Schizophyllum commune*. Biotechnol. Bioeng., 30: 169–178.

30 Stephan, D. 1986. Bildung von β-1,3-Glucanen aus Mono-, Di- und Polysacchariden mit *Sclerotium rolfsii* und deren physikochemische Charakterisierung. Ph. D. Thesis, Technical University of Braunschweig, F.R.G.

31 Sutherland, I.W. 1983. Extracellular polysaccharides. In: Biotechnology (Rehm, H.J. and G. Reed, eds.) 3: 531–574.

32 Thompson B.G. and W.T. Leps. 1986. Effect of dissolved oxygen on growth and production of exopolysaccharide by *Rhizobium trifolii*. J. Ferment. Technol. 64: 335–338.

33 Tuffile, C.M. and F. Pinho. 1970. Determination of oxygen-transfer coefficients in viscous streptomycete fermentations. Biotechnol. Bioeng. 12: 849–871.

34 Turian, G. 1976. Reducing power of hyphal tips and vegetative apical dominance in fungi. Experentia, 32: 989–991.

35 Wessels, J.G.H. and J.H. Sietsma. 1979. In: Fungal walls and hyphal growth (Burnett, J.H. and A.P.J. Trinci, eds.), pp. 27–48, Cambridge University Press, Cambridge.

36 Yanaki, T., T. Kojima and T. Norisuye. 1981. Triple helix of scleroglucan in dilute aqueous sodium hydroxide. Pol. J., 13: 1135–1143.