Biotechnology Letters Vol 11 No 2 125-130 (1989)
Received as revised February 2

EFFECT OF OXYGEN ON THE RATE OF ß-1,3-GLUCAN MICROBIAL EXOPOLYSACCHARIDE PRODUCTION

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SUMMARY

Examination of the relationship between the rate of oxygen transfer and the rate of polymer production revealed an unexpectedly high requirement for oxygen. At a cell density of about 3 g (dry wt)/L, the threshhold value for OTR for optimal synthesis of polymer is about 50 mmoles O_2/L.hr. Whereas Rushton turbines are efficient at transfering oxygen to solution, their use reduces the quality of the recovered polymer. Although better quality polymer can be produced in a reactor employing an agitator which causes less shear stress, the productivity can be compromised due to the inefficiency in OTR. The present study describes operating conditions for the provision of sufficient OTR in a system compatible with the production of high-quality polymer whereby turbine impellers were replaced with a marine-type propeller and mass transfer was assisted by means of a gas dispersion device.

INTRODUCTION

Water-insoluble ß-1,3-glucan EPS with unique rheological and thermal gelling properties are produced by certain species of two different genera of bacteria, *Alcaligenes* and *Agrobacterium*. The uncharged polymer can be recovered as an insoluble gel upon neutralization of the alkalinized cell-free fermentation broth. "*Curdlan*" is a specific ß-1,3-glucan EPS produced by a selected mutant (strain K) of *Alcaligenes faecalis* var *myxogenes* 10C3 (Harada *et al.*, 1966, 1968). Claims with respect to the superior nature of thermal gels formed by other *curdlan-like* polymers exist primarily in the patent literature (Kimura *et al.*, 1973). In contrast to the extensive literature relating to the chemistry and physical properties of these ß-glucan exopolymers (for reviews see Harada, 1972; Harada, 1977; Harada, 1979), there is little information concerning the physiology and biochemistry of the process organisms. In addressing this deficiency we have described various bioengineering strategies for increasing productivity in ß-1,3-glucan EPS fermentations (Phillips, 1982; Phillips *et al.*, 1983, Phillips & Lawford, 1983b; Lawford *et al.*, 1983; Lawford, 1982). Earlier investigations into the influence of bioreactor design on fermentation kinetics and product yield (Lawford *et al.*, 1986a & 1986b; Orts, 1987; Orts *et al.*, 1987) focused attention on the simultaneous effect of agitation and mixing on the *quality* of the recovered polymer and it was observed that designs which enhanced productivity were often associated with the production of an inferior quality polymer (Lawford, 1988; Lawford *et al.*, 1988).

In batch fermentations, the production of ß-1,3-glucan EPS occurs following growth in a nitrogen-deficient medium with excess carbon source (Phillips *et al.*, 1983). From theoretical considerations with respect to oxygen demand associated with growth, maintenance metabolism and polymer biosynthesis (Phillips & Lawford, 1983a), it was assumed that even systems with relatively low capacity for oxygen transfer would be adequate in terms of meeting the significantly reduced oxygen requirement of a stationary-phase culture that was producing exopolymer. However, experiments using shake flasks suggested EPS production was oxygen limited and suggested a correlation between the availability of oxygen and the rate of EPS production (A. Kligerman, personal communication).

Our objective was to examine the requirement of this EPS fermentation for oxygen and to quantify the relationship between the rate of oxygen transfer (OTR) and the specific rate of recoverable exopolymer biosynthesis (q_p) in fermentors designed to yield a high-quality product.

MATERIALS and METHODS

A. faecalis ATCC 31749 was cultured aerobically in a defined mineral salts medium (Phillips *et al.*, 1983) with glucose (50g/L) as the sole carbon source. The bacterial cell density was determined gravimetrically by ultrafiltration of a 10 ml aliquot of culture at the onset of stationary-phase and was proportional to the amount of assimilable nitrogen (as NH_4Cl) in the medium. The standard amount of ammonium chloride was 28mM giving a biomass concentration of about 3 g dry weight/L. Batch fermentations were conducted in Erlenmeyer shake flasks (500ml) and variously configured bench-top, stirred tank bioreactors (STR) equipped with sensing/control devices for agitation (RPM), air sparging (V/V/M), pH and temperature. Operating conditions for batch fermentations are described in Table 1 and different apparatus are illustrated in Figure 2. Procedures for exopolymer recovery were as described previously (Phillips *et al.*,1983). OTR was determined by the standard chemical method using sulphite oxidation with cupric ions as catalyst (Cooper *et al.*, 1944). The helical propeller was from BioLafitte (USA)

RESULTS and DISCUSSION

The different experimental systems, designed to examine the effect of aeration and agitation on the kinetics of ß-1,3-glucan EPS (specifically *PS-31749*) batch fermentations, are listed in Table 1 and illustrated graphically in Figure 2. Experiment 2 typifies our previous standard operating conditions and Figure 1 shows a typical batch fermentation using a standard commercial, bench-top, stirred tank reactor fitted with baffles and flat-blade Rushton turbine impellers. Figure 3 summarizes the observations with respect to the specific rate of glucose utilization (q_s), the specific rate of recovered polymer production (q_p), the product yield ($Y_{p/s}$) and the oxygen transfer rate (OTR) for each of the 7 different experimental systems listed in Table 1. Using experiment 2 as the common reference, these results point to a correlation between q_p and OTR suggesting that, perhaps with the exception of experiments 6 and 7, all others were oxygen-limited (Figs. 3b & 3d). For the most part, the effect of oxygen limitation is equally reflected in

qs (Fig. 3a) and q_p (Fig. 3b), with the possible exception being experiment 3, where the value for $Y_{p/s}$ (the ratio of q_p:q_s) is significantly lower than in all the other systems tested (Fig. 3c). The relationship between q_p and OTR is shown in Fig. 4.

Previously it had been shown that productivity could be increased relative to the standard operating procedure represented in Fig. 1 (expt. 2 in Table 1 & Fig. 2) by replacing the upper two turbine impellers with a single marine-style propeller (Lawford *et al.*, 1986). However, this improvement had been accomplished by increasing the rotational speed of the agitator from 850 to 1000 RPM and it was subsequently discovered that this had a serious deleterious effect on the 'quality' of the isolated polymer product as judged by intrinsic viscosity (in 3N alkali), MW and gel strength (Lawford *et al.*, 1988). When the same configuration (expt. 3) was operated at a lower rotational speed (at constant ventilation rate), the q_p was compromised due to oxygen limitation (Fig. 3). Experiments 6 and 7 represent designs which are known to be compatible with the production of high-quality polymer (Lawford, 1988; Lawford *et al.*, 1988). Experiments 4, 5 and 6 were conducted in the same fermentor (single propeller with a sintered glass disk air sparger) at different air sparging rates (0.08, 0.15 and 0.33 V/V/M respectively) to assist in describing the relationship between OTR and q_p (Fig. 4), with the conditions in expt. 5 appearing to mimic those in the shake flask (expt.1) with respect to both OTR and q_p (Fig. 3).

These observations were unexpected in the sense that it would appear that the stationary-phase culture, which was actively synthesizing ß-1,3-glucan polymer, had a much higher oxygen demand than would be predicted from our theoretical estimation of energetic requirements associated both with maintenance metabolism and EPS biosynthesis (Phillips & Lawford, 1983a). Unlike xanthan and pullulan, ß-glucan ('curdlan') is insoluble and should be less constraining to O_2 transfer from gas to liquid (Lawford *et al.*, 1988). However, the possiblility that this cell-associated exopolymer presents a barrier to mass transfer from liquid phase to the cell, thereby requiring unusually high levels of dissloved oxygen to provide the requisite driving force for diffusive entry into the cell, is the subject of our continuing investigation.

Acknowledgements

This project was supported by grants from the Ontario Ministry of Colleges & Universities (URIF) and the Natural Sciences & Engineering Council of Canada (Strategic Grants Prgm. G1730) and through a R&D Contract from **George Weston Limited** (Toronto). We are grateful for the technical assistance provided by Hans Preusting, Wendy Chow and Ruth Wallace. We also wish to thank Anne Kligerman (Weston Research Ctr.) for preliminary data relating to shake flask experiments, Steve Lawford for preparing the computer-generated drawings and Kim Phillips (Connaught Research Institute) for providing the helical propeller.

Figure 1 Typical Batch Fermentation for the Production of B-1,3-Glucan PS 31749 by A.faecalis

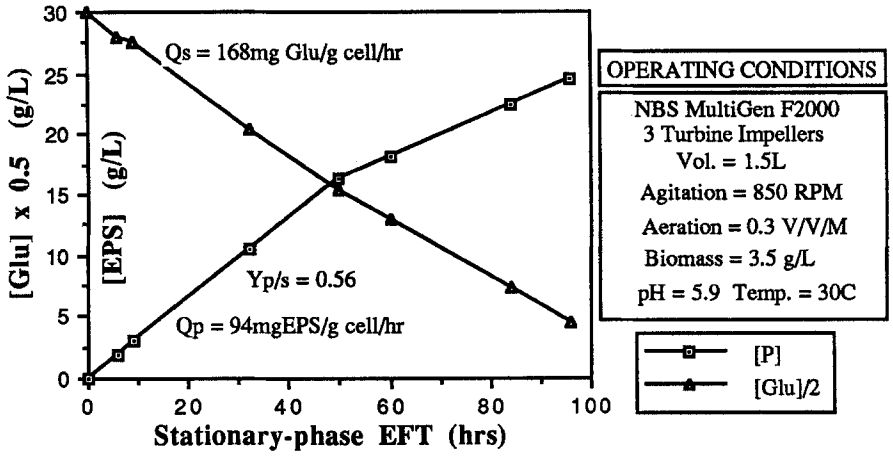


Figure 3a

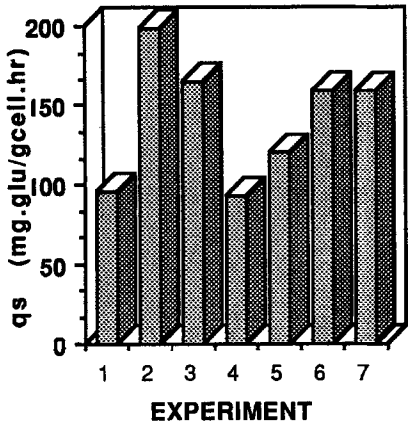


Figure 3b

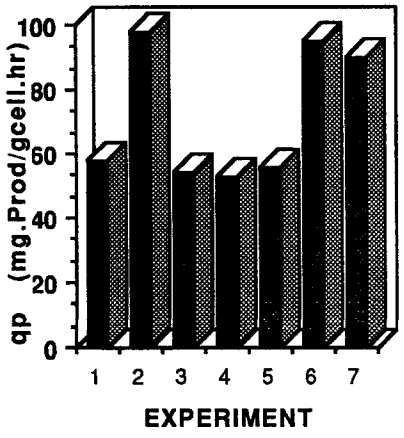


Figure 3c

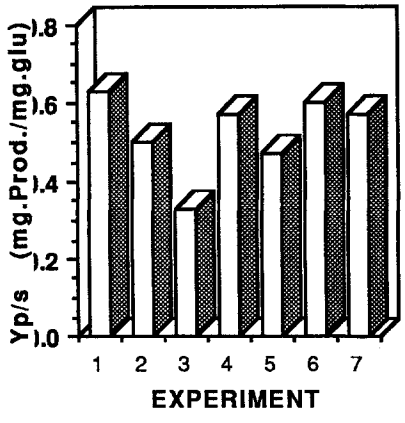


Figure 3d

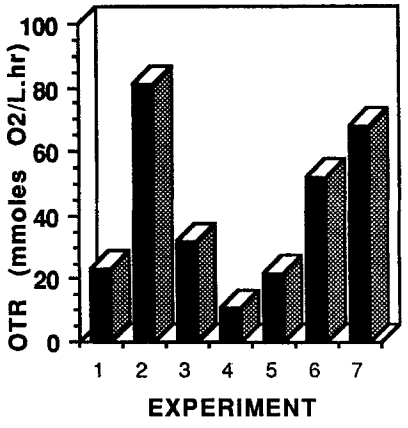
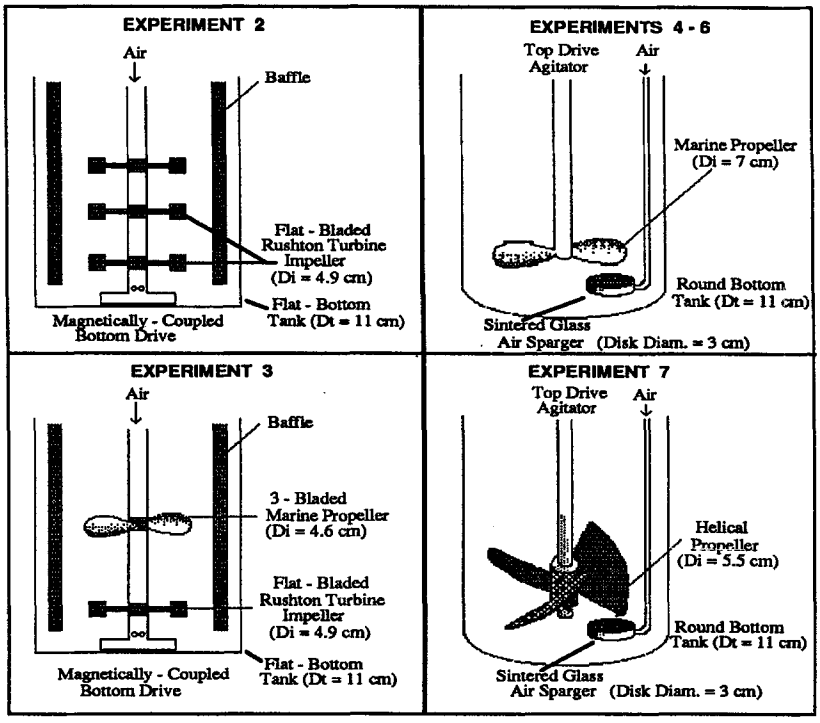


FIGURE 3
Influence of bioreactor design with respect to agitation and aeration on ß-1,3-glucan production by A.faecalis ATCC 31749 (qs, qp, Yp/s, OTR)

FIGURE 2 EXPERIMENTAL DESIGN and FERMENTOR CONFIGURATION with respect to AERATION and AGITATION



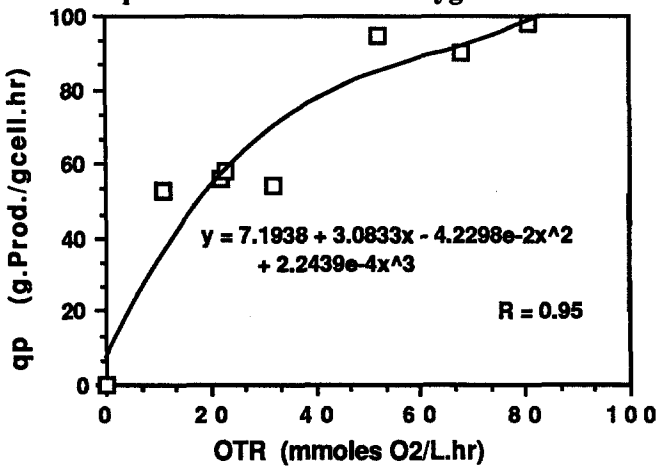
NOTE: OPERATIONAL PARAMETERS FOR EACH EXPERIMENT ARE DESCRIBED IN TABLE 1

TABLE 1 DETAILS OF EXPERIMENTAL DESIGN and FERMENTOR CONFIGURATION with respect to AERATION and AGITATION

FERMENTOR DESIGN and CONFIGURATION	OPERATIONAL PARAMETERS					
	Liquid Vol. (ml)	Di/Dt	Air Flow Rate (V/V/M)	Rotational Speed (RPM) Growth	Stat.-phase	Tip Velocity (cm/sec)
1. Erlenmeyer Flask (500ml size)	50		NA	(250)	(250)	NA
Air sparged through holes at bottom of central shaft						
MultiGen F2000 3 turbine impellers Diam. = 4.9 cm	1500	0.45	0.33	600	850	218
3. MultiGen F2000 1 prop (4.6 cm) + 1 imp (4.9 cm)	1500	0.45	0.33	750	750	181
Air sparged through sintered glass disk (3 cm) under propeller						
4. Round bottom tank Single prop (7 cm)	1500	0.64	0.08	600	500	183
5. Round bottom tank Single prop (7 cm)	1500	0.64	0.15	600	500	183
6. Round bottom tank Single prop (7 cm)	1500	0.64	0.33	600	500	183
Air sparged through sintered glass disk (3 cm) under propeller						
7. Round bottom tank Helical prop (5.5 cm)	1500	0.50	0.33	600	500	144

The 500ml Erlenmeyer flask was incubated in a shaking (reciprocating type) water bath at 250 strokes/min ; D_i = diam. of agitation device, either Rushton turbine impeller, helical or marine-type propeller; D_t = diam. of fermentation tank. The NBS MultiGen F2000 glass fermentor is a magnetically-coupled bottom drive unit.The modified F2000 (round bottom) was fitted with a variable speed, top-drive motor (different configurations are illustrated in Fig. 2).

Figure 4 Relationship between specific rate of polymer production and rate of oxygen transfer



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