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EFFECT OF OXYGEN AND AIR FLOW ON XYLANOLYTIC AND CELLULOLYTIC ACTIVITY FROM BACTERIAL CULTURES GROWN ON BAGASSE PITH.

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SUMMARY

The xylanolytic and cellulolytic activity from *Cellulomonas* were reduced by high 0_2 concentrations in continous culture as well as by an air flow passed trough the samples, suggesting an inhibition or inactivation of enzymes in such conditions.

INTRODUCTION

Experimental observations in our laboratories have shown that after a short period without stirring, *Cellulomonas* cultures on bagasse pith undergo a faster growth and higher enzymatic activity when the stirring conditions are recovered, this effect lasting for a few hours run on. The inactivation of fungal cellulases by shaking has been observed by different authors (Reese, 1980, Wase et al. 1985, Mukata et al., 1988). However, for cellulolytic bacteria this effect has not been reported.
In this paper the effect of different dissolved oxygen concentrations as well as of an air flow on the xylanase and cellulase enzyme systems from *Cellulomonas* is reported.

MATERIALS AND METHODS

STRAINS.- A *Cellulomonas Xanthomonas* mixed culture, (with *Cellulomonas* being responsible for xylanases and cellulases production De la Torre and Casas, 1984), and the strain of *Cellulomonas* sp. IIbc (Enríquez, 1981) were employed in this study.

MEDIA AND CULTIVATION CONDITIONS.- For shaker runs saline MO medium (Rodríguez et al., 1983) was used with 1% alkali treated bagasse pith as carbon and energy source. For continous cultivation the medium described by De la Torre and Casas (1984) was employed with 0.5% alkali treated bagasse pith, previously milled to particle size of approximately 60 mesh. Shaker cultures were carried out at 37°C and 100 rpm. Continous cultivation was at the following conditions: effective volume, 2 l: temperature 37°C, dilution rate 0.1 h^{-1} and stirring 400 rpm. The air flow was controlled such that 0_2 level at the desired concentration could be maintained.

ANALYSIS.- Growth was followed by measuring absorbance at 600 nm and by dry weigh determination (Rodríguez et al., 1983). CMC-ase, FP- and β-glucosidase activities were assayed as described previously (Rodríguez and Volfová, 1984). The xylanases were assayed according to Richard and Laugling (1980).

EFFECT OF AIR FLOW.- From an exponential phase culture of *Cellulomonas* sp. IIbc, supernatants as well as cell extracts were prepared (Rodríguez and Volfová, 1984). The samples were submitted to a 1 l/h air flow through a No. 1 porosity sintered glass, in 500 ml Erlenmeyer flasks containing 30 ml of sample. At different times samples were withdrawn for enzymatic activity determinations.

RESULTS AND DISCUSSION.

EFFECT OF OXYGEN CONCENTRATION.- Table 1 shows the biomass production together with enzymatic activity of the *Cellulomonas Xanthomonas* culture at different 0_2 concentrations in continous cultivation. At the lower O2 concentrations growth was markedly affected but the enzymatic activity shows a different behaviour. Xylanase activity was markedly increased when the 0_2 concentration was reduced to 52%, both in supernatants and total culture samples. These values kept constant after a new drop in 0_2 to 40%. The FP- activity increased with the decrease in 0_2 level to 52% in the total activity but not in the supernatant samples, which suggests an increase in the cell and/or substrate bound enzymes. The enzymatic level remained constant again when lowering the O2 to 40 % . On the other hand, the CMC-ase activity remained unaffected by the variations in 0_2 level.

TABLE I. ENZYMATIC ACTIVITY OF *Cellulomonas Xanthomonas* MIXED CULTURE AT DIFFERENT O_2 CONCENTRATION DURING CONTINOUS CULTIVATION.

O_2 (%)	Biomass (g/l)	Activity (IU/mg cell)					
		CMCase		FP		Xylanase	
		SN	TC	SN	TC	SN	TC
76	1.16	0.35	0.52	0.012	0.013	0.100	0.099
70	1.16	0.33	0.47	0.012	0.014	0.096	0.097
52	0.58	0.32	0.49	0.010	0.020	0.185	0.199
40	0.59	0.35	0.55	0.015	0.020	0.170	0.178

SN: Supernatant

TC: Total culture

TABLE II. EFFECT OF AIR FLOW ON ENZYMATIC ACTIVITY FROM *Cellulomonas* sp IIbc.

Sample	Exposition Time (h)	Activity (IU/ml)			
		CMCase	FP	β-glucosidas	Xylanase
Supernatant	0	0.0190	0.0101	0.0048	0.3610
	1	0.0186	0.0099	0.0039	0.3522
	2	0.0192	0.0099	0.0040	0.2844
Cell extract	0	0.0256	0.0016	--	0.0603
	1	0.0241	0.0010	--	0.0497
	2	0.0259	0.0012	--	0.0476

Robinson (1984) observed an oppossite effect of 0_2 levels in the range 50-10% saturation on xylanase and CMC-ase activity from *Trichoderma reesei*. In our case, it seems that a high 0_2 concentration reduces the xylanolytic and some components of the cellulolytic complex of the *Cellulomonas* strain. This effect could be due to the action of 0_2 itself or to the physical effect of the agitation forces due to the different air flows employed. Negative effects of shearand agitation on fungal cellulases has been reported (Reese, 1980, Wase et al., 1985, Mukataka, et al., 1988). However, in our case, the changes in the overall agitation caused by the changes in the air flow are relatively small in comparison with the agitation caused by the mechanical stirring, so, the possibility of a direct effect of 0_2 should be taken into account.

To clarify whether the effect is due to changes in the enzyme activity or in their synthesis,an experiment in which the air flow was passed through cell free samples was carried out. The results are shown in Table II. The xylanase activity decreased with increased exposure to air in both supernatants and cell extracts. The FP activity decreased in the cell extracts but not in the supernatant (as the previous experiment, where only the bound activity varied with the 0_2 changes). The β-glucosidases also dropped only in the cell extracts. Also coinciding with the continous culture results, the CMC-ase activity was not affected.

The results suggest that the decrease in xylanase and FP-activity observed in the continous culture with the increase in 0_2 level (Table I) should be due to an effect of inhibition or inactivation more than on the enzymes production.

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