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SOLVENT PRODUCTION BY CLOSTRIDIUM PASTEURIANUM IN MEDIA OF HIGH SUGAR CONTENT

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SUMMARY

The fermentation end products of Clostridium pasteurianum ATCC 6013 are normally acetic and butyric acids. When grown in media of high sugar content however, significant quantities of solvents (acetone, butanol and ethanol) were produced. Solvent production was not stimulated by added acetic and butyric acids, nor was the effect due to a low water activity of the medium *per se*.

INTRODUCTION

As part of a general study of the biotechnological utility of a number of clostridia (Morris 1982), we appreciated that their special advantages might best be realised in reactors of high biomass density. To this end we have studied the growth of Clostridium pasteurianum in alginate gels (Lovitt *et al.* 1985) and, in a parallel study, we also investigated the growth of this organism in a bioreactor with cell recycle. An unexpected finding was that, under certain conditions, acetone, butanol and ethanol were produced in significant amounts, and it was therefore of interest more fully to characterise this behaviour and its mechanistic basis. The present article describes these studies.

METHODS

Clostridium pasteurianum ATCC 6013 and its oligosporogenous, non-granulose-forming mutant MR505 (Robson *et al.* 1974) were grown anaerobically in a mineral salts medium described previously (Kell *et al.* 1981). The concentration of the carbon source was varied as described in the legends to the figures. Porton-type fermentors incorporating pH-control (3M KOH) were used and the temperature was 37°C. When cell recycle was employed, the rate of medium addition corresponded to a "dilution rate" of 0.9 h^{-1} and cells were recycled (retentate:filtrate ratio = 4) by means of a Pellicon filter cassette system (Millipore) incorporating a HVLP000C5 filter. The working volume was in this case 800 ml, otherwise 500 ml. Metabolic end-products were determined by gas-liquid chromatography and glucose by a glucostat procedure, as described (Gottschal & Morris 1981a). Biomass was estimated turbidimetrically, 1 O.D. unit at 680nm corresponding to 0.40 g. dry wt/l.

RESULTS AND DISCUSSION

Fig. 1 shows the production of biomass, acetate, butyrate, butanol and ethanol, and the concentration of glucose, in a continuous flow culture of Clostridium pasteurianum MR505 incorporating cell recycle, when glucose was present in the medium reservoir at a concentration of 4% (w/v) and the pH was maintained at 6. This concentration of glucose was chosen to ensure that it would not be limiting to glycolysis even at the highest biomass densities encountered. It is evident, especially during the terminal phases of the experiment, that ethanol and butanol were produced in significant quantities. Since the ability to produce such concentrations of solvents is not normally associated with this organism (Thauer et al. 1977, Clarke et al. 1982, but see Nakas et al. 1983), and foaming and mixing problems made it difficult to maintain well-defined and homogeneous environmental conditions in the cell recycle fermentor, we decided to investigate this phenomenon in pH-controlled batch cultures. Fig. 2 shows the changes in biomass, acetate, butyrate, acetone, butanol, ethanol and glucose concentrations during a batch fermentation of Clostridium pasteurianum ATCC 6013 in a minimal medium initially containing 5% (w/v) glucose at pH 5.5; in this case, more than 80mM total solvents were produced after 24 hr.

Solvent productivity was dependent upon the concentration of glucose provided in the medium, such that solventogenesis was negligible under the more usual culture conditions (glucose concentration $\leq$ 2% w/v) but marked in media in which the initial glucose concentration exceeded some 6% w/v. Hahn-Hägerdal et al. (1982) have pointed out that a low water activity of the cellular micro-environment might be responsible for stimulating solvent (ethanol) production in immobilised yeast cells (and see Hahn-Hägerdal 1986). That the present effect was not due simply to the low water activity of the media containing high sugar concentrations, however, is shown by the fact that it could not be mimicked by the non-metabolisable sugar alcohol xylitol, nor by KCl (R.Mulder, unpublished), nor by membrane-permeant solutes such as acetamide (R.P.Walter, unpublished). The concentrations of acetate and butyrate are important in determining the solventogenic activity of Clostridium acetobutylicum (e.g. Gottschal & Morris, 1981b), but supplementation of the medium with these acids did not enhance solvent production by C. pasteurianum (Table 1).

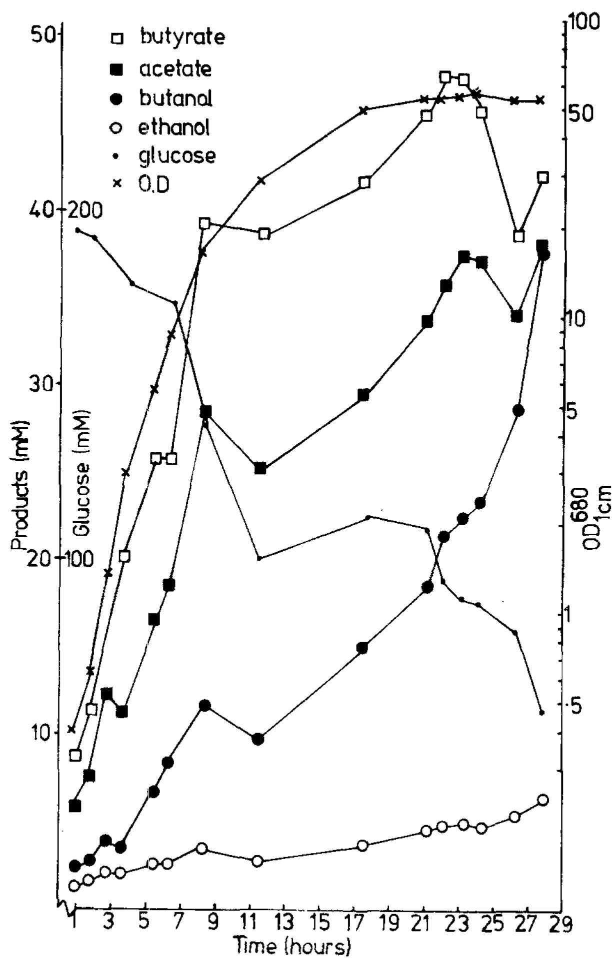

Fig.1. Fermentation of 4% glucose by C. pasteurianum MR505 grown with cell recycle

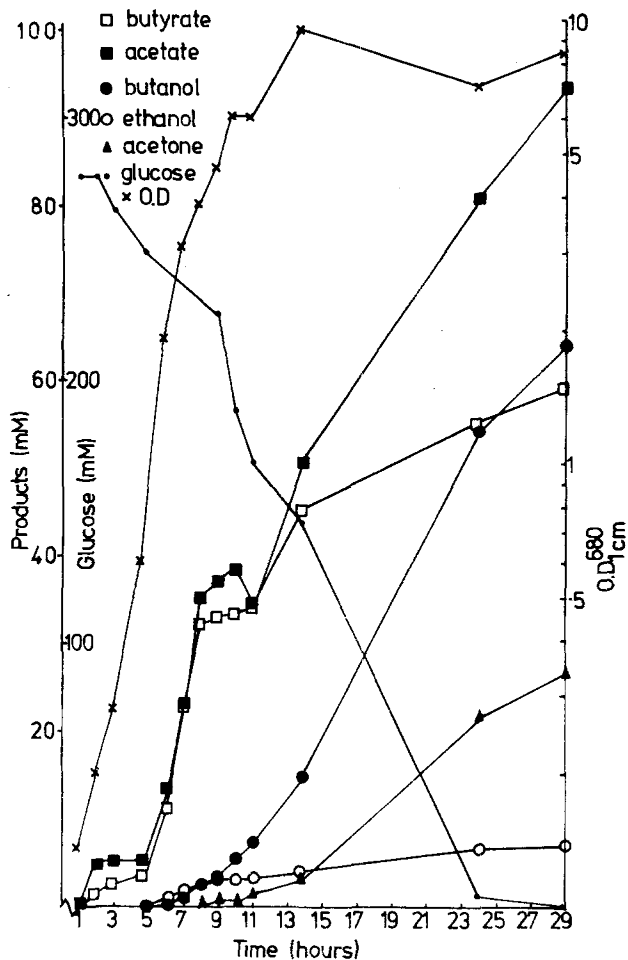

Fig.2. Batch fermentation of 5% glucose by C. pasteurianum 6013 at pH 5.5

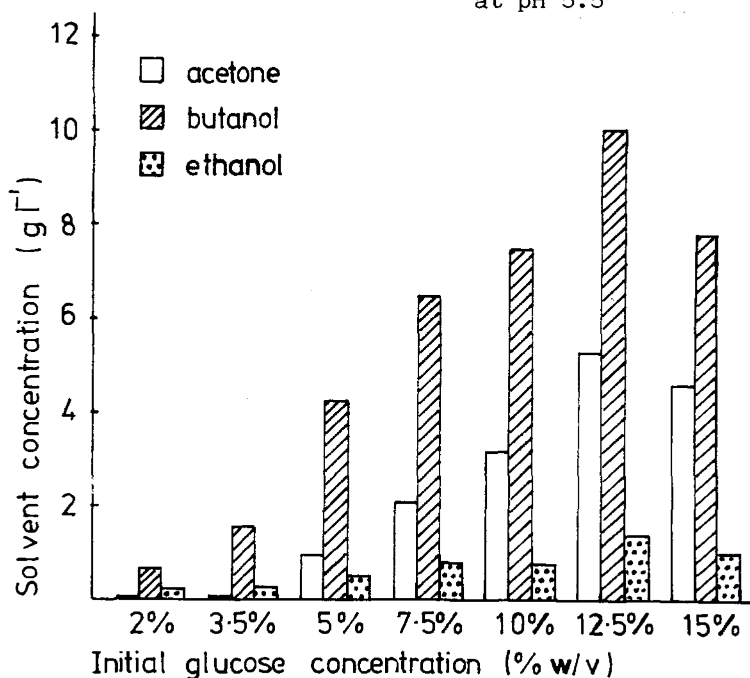

Fig.3. Effect of glucose concentration on total solvent production by C. pasteurianum 6013 grown at pH 5.5 in batch culture

ADDITIONS	PRODUCTS				
	Acetate	Butyrate	Acetone	Butanol	Ethanol
None	128.7	76.2	15.9	56.9	10.3
70mM Acetate	161.6	77.0	18.3	51.6	16.9
70mM Butyrate	114.1	141.0	2.0	32.1	10.7
35mM Acetate and 35mM Butyrate	115.5	100.1	7.5	32.4	11.3

TABLE 1. Lack of effect of acetate and butyrate on solventogenesis by *Clostridium pasteurianum* ATCC 6013. Cultures were grown at pH 5.5 and their end products analysed as described in Methods. End product concentrations are given in mM.

Future studies will be directed towards a study of the role of glucose concentration in solventogenesis by washed and immobilised cell suspensions derived from cells grown in media of differing glucose concentrations.

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