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THE RELATION BETWEEN REDOX POTENTIAL AND D-XYLOSE FERMENTATION BY CANDIDA SHEHATAE AND PICHIA STIPITIS

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SUMMARY

Fed-batch xylose fermentations with the yeasts *Candida shehatae* and *Pichia stipitis* were conducted, using stirrer speed variation with the redox potential as control index to maintain oxygen-limited conditions. The best results were obtained with *C. shehatae* at 300 (±10) mV (relative to the standard hydrogen electrode), and these fermentation parameters compared favourably with those obtained previously with the dissolved oxygen tension as control variable. Redox control of *P. stipitis* fermentations proved especially difficult. Cell growth during the fermentation was probably a major factor affecting redox potential.

INTRODUCTION

Limited aeration is required to enhance the rate and efficiency of ethanol production from D-xylose by the yeasts *Candida shehatae* and *Pichia stipitis* (Du Preez and Van der Walt, 1983; Du Preez *et al.*, 1984; Slininger *et al.*, 1985; Delgenes *et al.*, 1986; Sreenath *et al.*, 1986; Ligthelm *et al.*, 1988a). Oxygen probably acts as an electron acceptor to prevent an overproduction of NADH which apparently occurs to a degree (Du Preez *et al.* 1988a), despite a closed cycling between NADH and NAD^+ in these yeasts (Bruinenberg *et al.*, 1984; Van Dijken and Scheffers,, 1986; Ligthelm *et al.*, 1988b). The critical dissolved oxygen tension (DOT) for an optimal xylose fermentation by these yeasts apparently lies at the extreme sensitivity limit of the commercial DOT electrode (Du Preez *et al.*, 1988a). Similarly, ethanol production by *Pachysolen tannophilus* commenced when the DOT approached zero (Schvester *et al.*, 1983; Chung and Lee, 1986).

Although the reduction-oxidation (redox) potential of a culture is a complex parameter resulting from the combined effects of free oxygen and hydrogen, pH, temperature and the presence of compounds acting as reduction-oxidation couples (Jacob, 1974; Kjaergaard, 1977), the redox probe can provide a sensitive indication of very low DOT levels because of the logarithmic relationship of the redox potential to the DOT; as such, the redox potential has been used as a control index of oxygen supply in oxygen-deficient aerobic fermentations (Ishizaki *et al.*, 1974; Shibai *et al.*, 1974; Radjai *et al.*, 1984; Chung and Lee, 1986; Memmert and Wandrey, 1987). Dahod (1982) regards the redox

potential as a particularly useful parameter in that the redox probe measures the overall feedback effect of oxygen availability on the culture.

In this article we report on the use of the redox potential as the controlled variable for conducting oxygen-limited D-xylose fermentations with *C. shehatae* and *P. stipitis*. The redox potential served as control signal for the oxygen supply rate, which was regulated by continuous adjustment of the fermentor stirrer speed.

MATERIALS AND METHODS

Yeasts and media. The source and maintenance of *Candida shehatae* CSIR-Y981 (formerly designated CSIR-117A/1) and *Pichia stipitis* CSIR-Y633 (CBS 7126) were mentioned elsewhere (Du Preez *et al.*, 1988b). The fermentation medium contained D-xylose (50 $g.l^{-1}$), casamino acids, NH_4Cl, mineral salts and vitamins as described elsewhere (Du Preez *et al.*, 1988b). Actively growing shake flask cultures were used as inocula (Du Preez *et al.*, 1988b).

Culture conditions. Fed-batch fermentations were conducted in a 2-litre bench-top fermentor controlled at pH 4.5, 30°C, with a feed of sterile 50% (w/v) D-xylose solution, as described elsewhere (Du Preez *et al.*, 1988b). The DOT was monitored with a polarographic electrode (Du Preez *et al.*, 1988b). The electronic unit used previously for DOT control by continuous regulation of the stirrer speed (Du Preez *et al.*, 1988b) was slightly modified so as to use the culture redox potential as control signal instead of the DOT reading. In the initial experiments, where stated, the output from the oxygen electrode was used for DOT control. The redox potential of the culture was constantly monitored with an Ingold platinum ring combination redox eletrode (Argenthal reference system) connected to a pH/mV meter (model 1003, Digital Data Systems, Johannesburg) with a 1 mV resolution. This electrode was calibrated in Ingold redox buffer (no. 9881), and at 30°C had a potential of 203 mV with respect to the standard hydrogen electrode. All redox values are expressed relative to the standard hydrogen electrode by adding 203 mV to the measured voltage.

Analytical procedures. The analyses of xylose, xylitol, ethanol and dry cell mass were as described elsewhere (Du Preez and Van der Walt, 1983; Du Preez *et al.*, 1984).

RESULTS

Initial fed-batch fermentations at low DOT levels (using the oxygen probe output as control index) showed that the maximum volumetric ethanol productivity with *P. stipitis* was attained at redox potential values of 277 to 327 mV, while the maximum productivity of *C. shehatae* was in a similar but narrower range of 325 to 331 mV (Fig.1). These redox values served as guideline for the control settings used in the subsequent fermentations run at controlled redox potential values.

Fermentations were conducted with *C. shehatae* using redox control settings of 300 and 310 mV, respectively. The best fermentation and degree of control was obtained at the 300 mV setting, where the desired redox potential was maintained within ±10 mV (Fig. 2). Despite this control, the DOT initially increased up to 16% air saturation before dropping to a zero reading. During the second half of the fermentation the DOT increased to measurable levels (Fig. 2), briefly increasing to a value of 3% towards the end of the fermentation. The fermentation parameters are summarised in Table 1. In the fermentation controlled at 310 mV excessive cell growth occurred with a concomitant lower ethanol yield because of the more aerobic conditions (Table 1). During the first 10 h of this fermentation the DOT reading was zero, but oscillated up to a value of 36% in the subsequent 12 h period, returning to *ca.* 1 to 2% during the final 15 h of the fermentation.

Table 1. Fermentation parameters for fed-batch xylose fermentation by *Candida shehatae* and *Pichia stipitis* at various redox potential (RP) or dissolved oxygen tension (DOT) setpoints. Nomenclature: μ_{max}, maximum specific growth rate; Q_p and q_p, maximum volumetric and specific rates of ethanol production, respectively; Y_e, Y_{xyl} and Y_x, respective yield coefficients for ethanol, xylitol and biomass

Yeast	Fermentation parameter								
	μ_{max} h^{-1}	Q_p $g.(l.h)^{-1}$	q_p h^{-1}	Y_e $g.g^{-1}$	Y_{xyl} $g.g^{-1}$	Y_x $g.g^{-1}$	Ethanol $g.l^{-1}$	Xylitol $g.l^{-1}$	Biomass $g.l^{-1}$
C. shehatae									
RP 300 mV	0.13	0.73	0.33	0.38	0.09	0.06	35.0	6.4	5.8
RP 310 mV	0.25	1.52	0.18	0.2	0	0.3	19.3	0	28.4
DOT 0.7%[a]	0.08	0.65	0.35	0.37	0.13	0.03	44.3	13.1	4.0
P. stipitis									
RP 283 mV[b]	0.07	0.31	0.23	0.47	0	0.09	32.8	0	6.6

[a]Data from Du Preez *et al.* (1988b)

[b]No redox control possible during most of the fermentation; see text

In similar experiments with *P. stipitis*, control of the redox potential proved ineffective. At a 300 mV setting redox control was only possible during the initial 19 h of the fermentation, after which the value decreased to about 260 mV despite high rates of aeration (1.5 $l.min^{-1}$) and agitation (800 $r.min^{-1}$), resulting in a DOT increase to 47%, which rapidly decreased to about 1% for the remainder of the fermentation as a consequence of the high oxygen uptake rate by the growing cells. These aerobic conditions favoured growth above ethanol production (26.5 $g.l^{-1}$ cells *vs* 14.6 $g.l^{-1}$ ethanol upon termination of the experiment). This effect was more pronounced and redox control more ineffectual in an experiment using a 320 mV setting. By contrast, at a setting of 283 mV the measured values (290 to 340 mV) tended to remain above this setpoint (with a zero DOT reading) throughout the greater part of the fermentation; consequently the control system was not activated and the fermentor operated at a constant minimal stirrer speed (220 $r.min^{-1}$) and aeration rate (0.06 $l.min^{-1}$). Only towards the end of the fermentation did the redox potential decrease with the agitation speed increasing in response to maintain the setpoint of 283 mV. This resulted in the DOT increasing to 40%, however, with concomitant ethanol assimilation. The results of this fermentation are presented in Table 1.

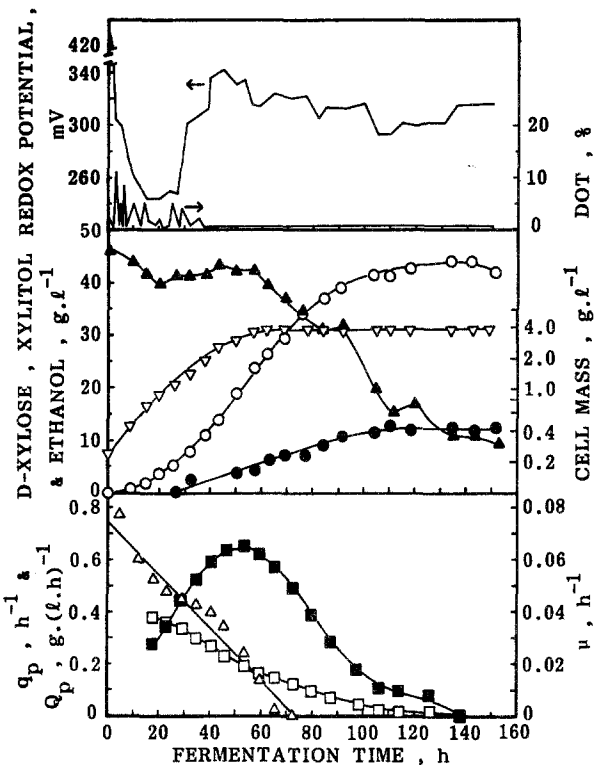

Fig. 1 Fermentation profile of *C. shehatae* CSIR-Y981 in a fed-batch xylose fermentation at a DOT setpoint of 0.7% air saturation. Symbols: ○ , ethanol; ● , xylitol; ▲ , D-xylose; ▽ , dry cell mass; □ , specific rate (q_p) and ■ , volumetric rate (Q_p) of ethanol production; △ , specific growth rate (μ).

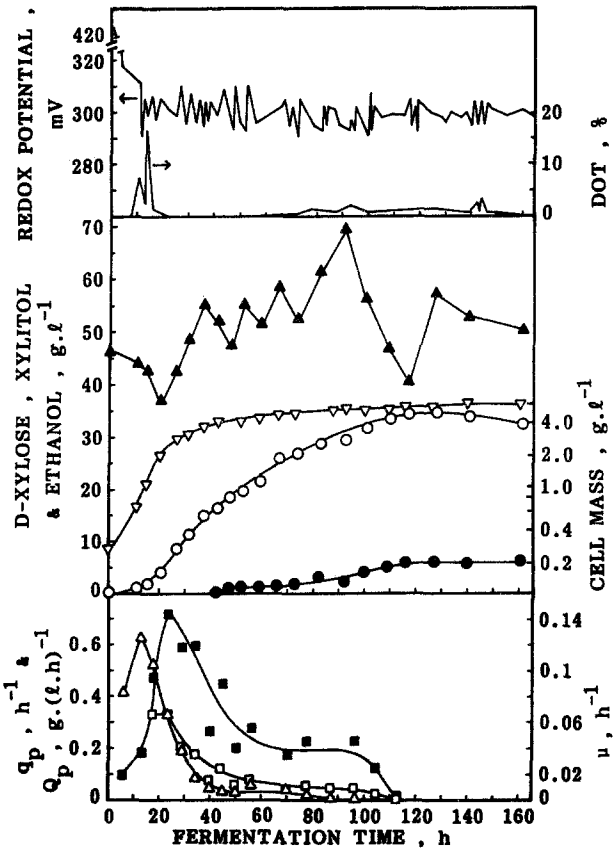

Fig. 2 Fermentation profile of *C. shehatae* CSIR-Y981 in a fed-batch xylose fermentation at a controlled redox potential of 300 mV. Symbols as in Fig. 1.

Chung and Lee (1986) showed that oxygen was the main factor influencing the redox potential of a non-growing *Pa. tannophilus* culture. They found a distinct correlation between fermentation parameters, such as ethanol, cell and xylitol yields, and the redox potential at which the culture had been controlled. Furthermore, their data indicated that product formation was directly related to the specific oxygen uptake rate rather than the redox potential. They nevertheless had success in conducting fermentations using the redox potential as control parameter.

In our redox-controlled fermentations only limited success was obtained. This was probably because we worked with growing cultures, the rate and yield of cell mass produced depending largely on the degree of aeration, whereas Chung and Lee (1986) had used non-growing cultures. The metabolic activity of cells can influence the redox potential (Kjaergaard, 1977).

Our results indicated that a difference as small as 10 mV in the redox potential setting had a substantial effect on the fermentation profile of these yeasts. This is probably related to the above observation: a small increase in redox setpoint causes an increase in the degree of aeration, enhancing cell growth which in turn depresses the measured redox potential, as shown by Chung and Lee (1986). This causes the redox controller to respond by increasing the agitation speed, further promoting the oxygen transfer rate and growth rate, thus amplifying the effects of a small change in redox setting on the controller. In this instance the cellular metabolic activity apparently obscured the correlation between DOT and redox potential.

The difficulty in culturing *P. stipitis* at a controlled redox potential is in accordance with earlier findings that this yeast strain was more sensitive to the DOT level and required a lower DOT than *C. shehatae* for an optimal xylose fermentation (Du Preez *et al.*, 1988a).

The fermentation parameters of *C. shehatae* attained during cultivation at a controlled redox potential of 300 mV compared quite favourably with those recorded previously using DOT control (Table 1). Although the ethanol yields were similar, in this work the final ethanol concentration was considerably lower than that obtained previously (Du Preez *et al.*, 1988b). In this case the residual xylose concentration was about 50 $g.l^{-1}$, as opposed to *ca.* 10 $g.l^{-1}$ in the previous fed-batch experiments. This higher sugar concentration could possibly have depressed the final ethanol concentration; a high substrate concentration can enhance ethanol toxicity in *Saccharomyces cerevisiae*, probably by increasing the osmotic pressure (Mota *et al.*, 1984; Letourneau and Villa, 1987).

In conclusion, it appears that optimal control of xylose fermentations using redox potential as control variable is a delicate and complicated matter. It may be possible to use this parameter if the relation between redox potential and fermentation behaviour of a given process is determined empirically. It may also be required to change the redox setpoint as the fermentation proceeds, using a computer programme in which both redox potential and DOT are employed as control parameters.

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