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NOTES

pH-Dependent Polyol Production in *Moniliella tomentosa*

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Production of polyols by the yeastlike fungus *Moniliella tomentosa* could be increased by growing the organism at constant low pH. Up to 54% increase in yield was obtained. Growth at low pH also results in the production of D-arabitol which is not found in normal media.

Osmophilic yeasts were shown to produce the polyhydric alcohols glycerol, erythritol, arabitol, and mannitol in yields varying with the yeast strain and the conditions of growth (5, 11). Factors known to influence polyol production are aeration, temperature, and medium composition (4, 6-8, 12). The present study was undertaken to investigate the effect of the pH on the polyhydric alcohol production by *Moniliella tomentosa* var. *pollinis* (CBS 1040), normally producing ethanol, glycerol, and erythritol (1). The pH-dependent production of D-arabitol is reported for the first time.

Fermentations were conducted at 30 C in a 10-liter fermentor equipped with a pH-stat and containing 3 liters of medium. The pH of the media was continuously adjusted to pH 6.4 or 3.4 with 5 N NaOH or N HCl. Aeration rates were chosen to obtain the highest polyol yield. Cell growth, residual glucose, and polyol production were determined at different time intervals, and the fermentations were stopped when most of the glucose was consumed. Total polyols were determined by the periodate oxidation method (3) and were calculated as erythritol. Individual polyols were separated on Whatmann 3MM paper with ethyl acetate-acetic acid-water (60:30:20, v/v/v) as developing solvent (9), eluted with water, and determined by the periodate oxidation method. Known amounts of polyols chromatographed simultaneously were used to calculate the polyol concentration in the samples. Glucose was determined by the method of Somogyi-Nelson (3). Polyol yield is always expressed as percentage of glucose consumed.

Cell growth and fermentation time in 10% glucose, 1% yeast extract, 0.1% urea media are roughly the same at either pH 6.4 or 3.4 (Fig. 1). In both experiments polyol yield increases as cell mass increases and remains constant when cell growth has stopped, but the final yields of polyols are quite different: 24% at pH 6.4 and 37% at pH 3.4.

The same effect was observed in 30% glucose, 1% yeast extract, 0.1% urea media (Table 1). However, the polyol yield was now more than twofold higher at pH 3.4 than at pH 6.4. The patterns of cell growth and polyol yield were similar to those given in Fig. 1, but the fermentation time was three times longer. The latter could be reduced from 240 to 120 hr by

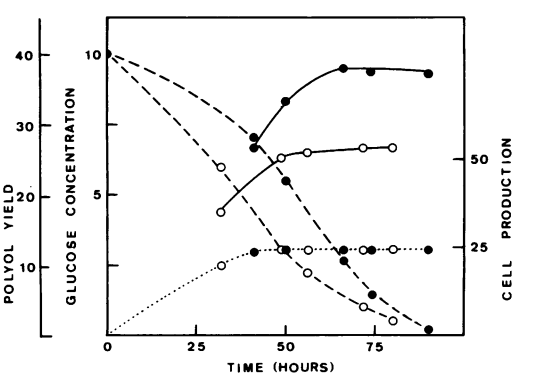

FIG. 1. *Polyol production by M. tomentosa at pH 6.4 (○) and at pH 3.4 (●) in 10% glucose, 1% yeast extract, 0.1% urea media. Solid lines represent polyol yield in relation to glucose used (percent), dashed lines residual glucose concentration (grams/100 ml of medium), and dotted lines cell production (milligrams of dry weight/100 ml of medium).*

raising the yeast extract concentration to 2%, causing a doubling of the cell production but without an effect on the polyol yield (Table 1).

Still higher yields of polyols were obtained in 50% glucose, 2% yeast extract, 0.1% urea media: 32% at *p*H 6.4 and 57% at *p*H 3.4. When the *p*H of the medium at *p*H 6.4 was changed after 180 hr to *p*H 3.4, a pronounced increase in polyol yield was observed (Fig. 2), showing that the effect of the *p*H on polyol yield is not due to influences on cell growth. This was confirmed by experiments with resting cells in shaken cultures, with calcium carbonate as *p*H regulator. Polyol yield decreased from 62% in the absence of calcium carbonate to 42% in its presence.

Analysis of the media with respect to individual polyols revealed that different types and amounts were produced as a function of the *p*H and the glucose concentration (Table 1). At *p*H 6.4 and 10% glucose, only erythritol was detected. Glycerol appears when the *p*H is lowered or the glucose concentration raised. At each glucose concentration, lowering of the *p*H causes an increase of both glycerol and erythritol production. At constant *p*H, only the

TABLE 1. *Fermentations of Moniliella tomentosa under different cultural conditions*[a]

*p*H[b]	Medium composition (%)			Aeration rate[c]	Cell growth[d]	Polyol yield[e]				Fermentation time (hr)
	Glucose	Yeast extract	Urea			Total	Glycerol	Erythritol	Arabitol	
6.4	10	1	0.1	1.6	2.9	24	0	24	0	80
6.4	30	1	0.1	1.6	2.8	23	2	20	0	240
6.4	30	2	0.1	4.1	5.9	27	5	22	0	120
6.4	50	2	0.1	4.1	5.6	32	11	21	0	275
3.4	10	1	0.1	1.6	2.4	37	4	33	1	80
3.4	30	1	0.1	1.6	2.4	50	16	32	3	240
3.4	30	2	0.1	4.1	5.6	49	16	32	1	120
3.4	50	2	0.1	4.1	5.6	57	22	29	6	275

[a] The analyses were made at the end of the fermentations when most of the glucose was consumed.
[b] The *p*H of the media was kept constant during the entire course of the fermentations.
[c] Liters air per minute.
[d] Milligrams (dry weight) per 100 ml of medium.
[e] Percent of glucose used.

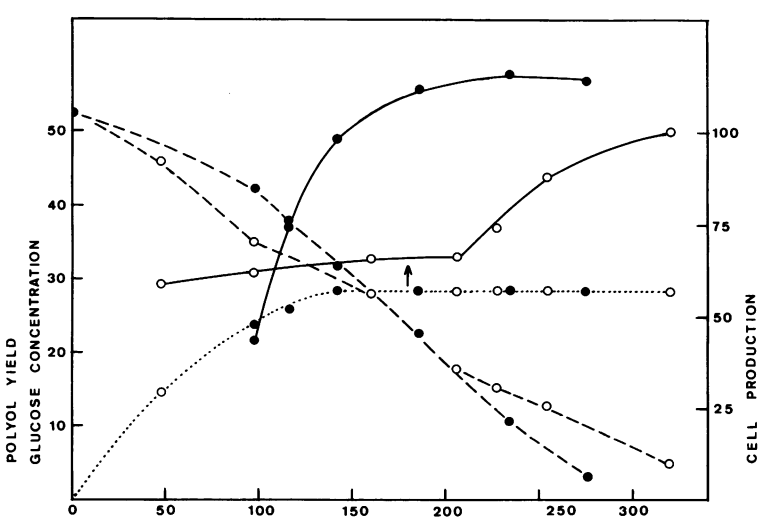

FIG. 2. *Polyol production by M. tomentosa in 50% glucose, 2% yeast extract, 0.1% urea media. Symbols as in Fig. 1. Arrow indicates a change in pH from constant 6.4 to constant 3.4.*

glycerol yield is increased with increasing glucose concentration. In the media at *p*H 3.4, a third polyol was detected in amounts up to 10% of the total polyols. The infrared spectrum, paper chromatography in different solvent systems, and the determination of melting point and optical rotation (2) indicated that this compound was D-arabitol.

These results indicate that the *p*H of the culture media greatly affects polyol production by *Moniliella tomentosa*. The unexpected production of different types of polyols as a function of the *p*H and especially the production of arabitol at *p*H 3.4 might be interesting for further studies on the problem of biosynthesis of specific polyols in different strains of osmophilic yeasts. As pointed out by Spencer (10) a controlling mechanism for the formation of polyhydric alcohols in these yeasts must exist. The *p*H could be such a controlling factor.

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