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EFFECT OF TEMPERATURE ON ETHANOL PRODUCTION FROM SUCROSE BY BACTERIA *Zymomonas mobilis*

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ABSTRACT

It seems that *Z. mobilis* is capable of tolerating high concentrations of sucrose than of glucose on molar basis. The ethanol yields by *Z. mobilis* on molasses are low as compared to pure substrates like glucose, fructose or sucrose. Efforts have been made to improve its fermentation efficiency. These observations are based on the study of effect of temperature on fermentation of sucrose by *Zymomonas mobilis*. The optimum pH range and temperature for fermentation is between 5 to 7 and 30^{o}C, respectively. This necessitates sterilization of fermentation medium.

Key words: Sucrose, Ethanol, Bacteria, *Zymomonas mobilis*.

INTRODUCTION

Z. mobilis is one of the few facultatively anaerobic bacteria metabolizing glucose by Enter Duodroff (E-D) pathway which is usually found in aerobic micro-organisms. *Z. mobilis* is capable of tolerating high concentrations of sucrose than of glucose. The sucrose hydrolyzing activity seems to be stimulated by sucrose and fructose[1].The hydrolysis rate of sucrose and rate of transfructosylations are shown to be higher than the sugar uptake rate of *Z. mobilis*[2,3].

Temperature is likely to play an important role in the optimal control of ethanol production, particularly where cell recycle, vacuum operation or simultaneous saccharification and fermentation are contemplated. Therefore, if the optimum temperature for *Zymomonas* could be increased from 30^{o}C, the process would be more economical overall. Viikari et al[4] found that their strains did not grow above 37^{o}C. ZM4 proved to be the most temperature tolerant but it produced only 4.6 % w/v ethanol[5]. Thermotolerant mutants have also been described by Berthalin et al.[6] The yields of ethanol are reduced, when sucrose or sucrose based raw materials are fermented by *Zymomonas mobilis* due to formation of by

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products[7-9]. Serzedelo et al.[10] have recently reported improved ethanol yield from sugarcane juice.

Kositonont et al.[11] reported fructokinase negative mutant derived from *Z. mobilis* UQM 2716[12] for production of ethanol plus fructose sweetner. Doelle and Greenfield[13] have described fermentation pattern of *Z. mobilis* at high sucrose concentrations. The studies on continuous culture with sucrose as substrate[14] and with equimolar mixture of glucose and fructose[15] have demonstrated that important parameter governing is sorbitol formation.

EXPERIMENTAL

Materials and methods

(i) *Zymomonas mobilis* ZM4 was obtained from CNRS, Marselli (France)

(ii) *Zymomonas mobilis* NRRLB-4286, NRRLB 806 from Northern regional research centre, Peoria, Illinois, 61604 U.S.A.

(iii) Medium preparation – Sucrose; Yeast extract; KH_2PO_4; $(NH_4)_2SO_4$; $MgSO_4$

(iv) Sugar estimation - DNS method[16]

(v) Glucose estimation - o-Toluidine method[17]

(vi) Ethanol estimation - Chromic acid method[18]

The effect of temperature on the kinetics of fermentation was studied using temperature 30, 35, and 38^0C. The data are shown in Fig. 1, 2 and 3, respectively. The kinetic parameters were calculated from these data (Table 1).

RESULTS AND DISCUSSION

It is seen that the kinetic parameters μ, q_s, q_p and $Y_{x/s}$ are relatively constant at a temperature of 30 and 35^0C but these parameters are reduced at 38^0C. However, parameters related to growth are more significantly affected than those for ethanol production. This shows the role of temperature in uncoupling metabolism from growth as reported by Forest et al.[19] Ethanol production is reduced by increasing the temperature from 30 to 38^0C from 47 to 40 g/L. The decrease in ethanol yield at higher temperature indicates an increased production of aldehydes and lactic acid. Lee et al.[20] reported loss of viability of cells at higher temperature due to ethanol accumulation leading to reduced yields during fermentation of glucose. Similar observations of the effect of temperature on ethanol inhibition have been reported by various authors with strains of *S. cerevisae*[21,22].

Table 1: Effect of temperature on fermentation of sucrose medium by *Zymomonas mobilis* ZM4

Kinetic and overall parameters	Temperature (^{0}C)		
	30	35	38
Specific growth rate μ (h^{-1})	0.14	0.13	0.07
Specific ethanol productivity q_p ($gg^{-1}h^{-1}$)	4.40	4.53	2.82
Specific substrate uptake rate q_s ($gg^{-1}h^{-1}$)	8.58	10.29	5.71
Cell yield $Y_{x/s}$ (gg^{-1})	.016	.015	.010
Ethanol yield $Y_{p/s}$ (gg^{-1})	0.50	0.49	0.47
Final Biomass (g/L)	1.53	1.26	0.90
Final ethanol (g/L)	47.0	43.0	40.0
Substrate utilized (%)	94.8	89.1	87.6
Fermentation efficiency (% of theoretical)	97.8	93.9	90.0
Fermentation time (h)	24	24	24

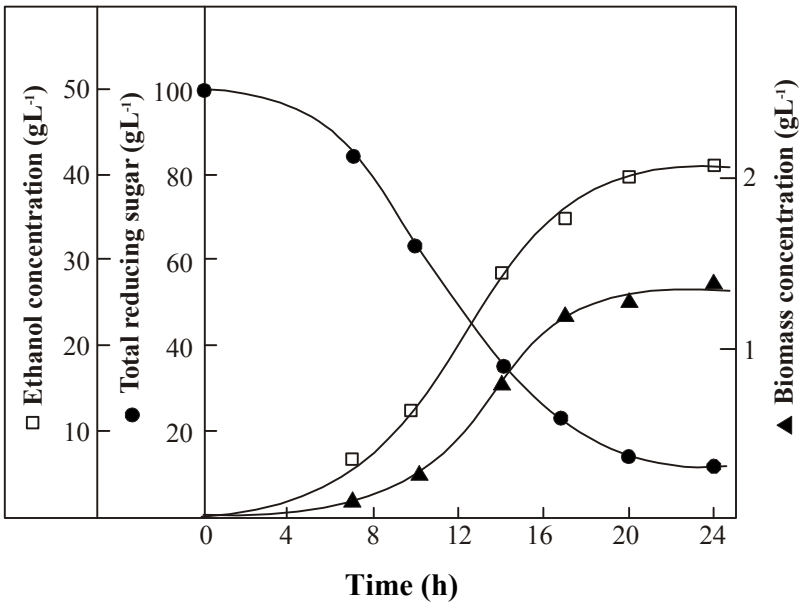

Fig. 1: Fermentation of sucrose medium I (Roger's) by *Zymomonas mobilis* ZM4 (temp. 30°C, pH 5) (●) Total reducing sugar, (□) Ethanol, (▲) Biomass

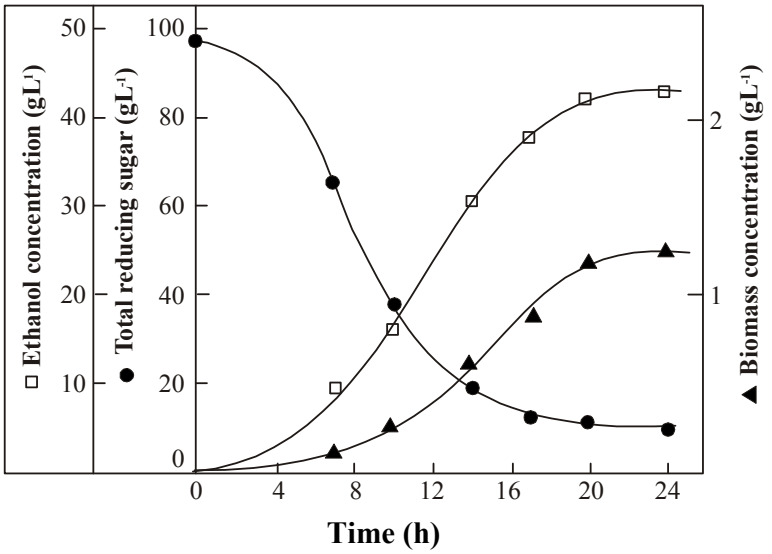

Fig. 2: Fermentation of 100 gL^{-1} sucrose by *Zymomonas mobilis* ZM4 (temp. 35^{o}C, pH 5) (●) Total reducing sugar, (□) Ethanol, (▲) Biomass

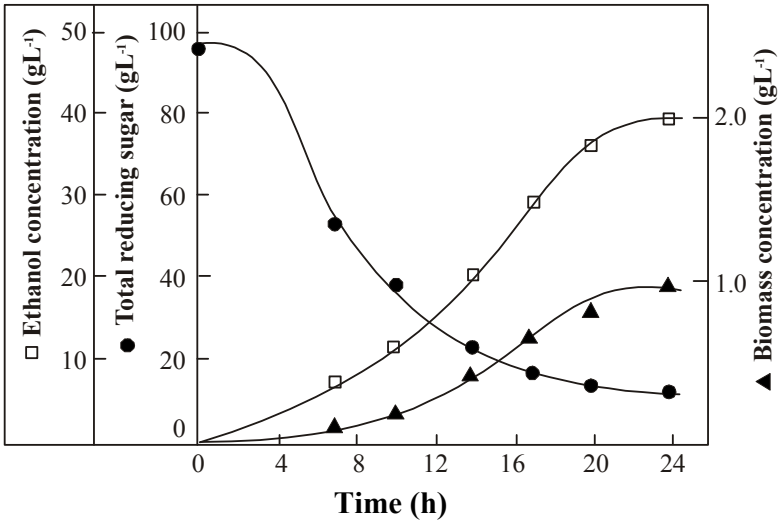

Fig. 4: Fermentation of 100 gL^{-1} sucrose by *Zymomonas mobilis* ZM4 (temp. 38^{o}C, pH 5) (●) Total reducing sugar, (□) Ethanol, (▲) Biomass

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