J. Biosci., Vol. 10, Number 2, June 1986, pp. 181-186. © Printed in India.

Fermentation pattern of *Zymomonas mobilis* strains on different substrates—a comparative study

P. GUNASEKARAN, T. KARUNAKARAN and M. KASTHURIBAI

Department of Microbiology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

MS received 10 September 1985; revised 15 March 1986

Abstract. The optimum conditions (pH and initial sugar concentration) of fermentation for the production of ethanol by 4 strains of *Zymomonas mobilis* (ATCC 10988, ATCC 12526, NRRL B 4286 and IFO 13756) were studied. An initial sugar concentration of 15 % (w/v) at pH 7·0 was found to be optimal for the first two strains and 20 % (w/v) initial sugar at pH 7·0 was found to be optimal for the last two strains. The fermentation pattern of these strains on synthetic medium, cane juice and molasses were compared. Strain NRRL B 4286 showed maximum ethanol production on synthetic medium while on cane juice ATCC 10988 and ATCC 12526 performed well. However, all the strains fermented molasses poorly.

Keywords. *Zymomonas mobilis*; optimization of condition; fermentation; synthetic medium; cane juice; molasses.

Introduction

Zymomonas mobilis is a rod shaped, gram negative, non-spore forming, motile bacteria. As an alternative organism for the production of ethanol, *Z mobilis* has several advantages over yeast. These are: higher rates of glucose uptake and ethanol production, higher ethanol yields and ethanol tolerance (Lee *et al*., 1979; Rogers *et al*., 1979). This bacterium uses a modified Entner-Doudroff pathway to rapidly produce upto 1·9 mol of ethanol per mol of glucose used. Thus *Z. mobilis* is a promising candidate for large scale production of ethanol (Gibbs *et al.*, 1951, 1954; Swings and DeLey, 1977). In this paper we report optimization experiments (pH and initial sugar concentration) for the production of ethanol by strains of *Z. mobilis* and compared the fermentation pattern of these strains on various substrates.

Materials and methods

Strains of *Z. mobilis* were obtained from Oak Ridge National Lab, Tennesse, USA (ATCC 10988 and ATCC 12526), Northern Regional Lab, Illinois, USA (NRRL B 4286) and Institute for Fermentation, Osaka, Japan (IFO 13756). These cultures were maintained as stab cultures in YPS agar medium (yeast extract 1 %; peptone 1 %; sucrose 2% and agar 2%) at 4°C.

The synthetic fermentation medium contained sucrose 20 % (w/v), yeast extract 0·5% (w/v), ammonium sulphate 0·1 % (w/v), potassium dihydrogen ortho phosphate 0·1%

(w/v) and magnesium sulphate 0·05 % (w/v). Cane juice was used as such. Generally, the reducing sugar concentration in the cane juice was 18 % (w/v). The molasses medium was prepared by diluting the molasses as required. The pH of all these media was adjusted as required and then fermentation media were sterilized at 10 pounds for 30 min. For batch fermentation, the primary inoculum was prepared in YPS broth and incubated at room temperature for 18 h. Then the fermentation broth was inoculated with 10% (v/v) of seed culture and fermentation process carried out at 30°C.

Total sugar was estimated colorimetrically as described by Dubois *et al*. (1956). Ethanol was estimated by gas chromatography (Model 5830A, Hewlett Packard, USA) according to Robinson *et al*. (1980) with the following modifications. Six feet 10% carbowax 20 m column was maintained at 80°C for 2 min and increased to 140°C at the rate of 25°C per min and held for 5 min. Injector temperature was 150°C and detector temperature was 160°C. Nitrogen gas was used as carrier at 25 ml/min and ethanol as standard.

Results and discussion

Effect of initial pH on fermentation

In order to find out the optimum pH for the fermentation with *Z. mobilis* the initial pH of the fermentation medium was adjusted to different levels and fermentation carried out for 72 h. At the end of this period samples were collected and ethanol and residual sugar concentration were estimated. Table 1 shows the effect of initial pH on the fermentation parameters of various strains of *Z. mobilis*. In all, the fermentation efficiency was maximum at pH 7·0 but low at pH 4·0. With the increase of initial pH, the substrate utilization and fermentation efficiency increased. Hence, the optimum initial pH for fermentation by *Z. mobilis* is 7·0. This result is consistant with the result of Ohta *et al*. (1980).

Effect of initial sugar concentration on ethanol production

To find out optimum sugar level for fermentation, batch fermentation was carried out with varying levels of sugar. Table 2 shows ethanol produced by 4 strains of *Z. mobilis* in varying initial sugar concentration. The maximum efficiency of fermentation was observed at 15% (w/v) initial sugar concentration for strain ATCC 10988 and ATCC 12526. Increasing the sugar concentration from 15–20% decreased the fermentation efficiency of these strains. However IFO 13756 and NRRL B 4286 showed a marked increase in the fermentation efficiency at 20 % initial sugar, but no significant increase in the utilization of substrate was observed. At 25 % (w/v) initial sugar concentration, both the substrate utilization and fermentation efficiency decreased in all the strains.

Fermentation pattern of Z. mobilis on various substrates

Synthetic medium: Figure 1 and table 3 show the fermentation pattern of 4 strains of *Z. mobilis* at optimum conditions in synthetic medium. Eventhough NRRL B 4286 and IFO 13756 showed low fermentation efficiency till 12 h, the fermentation efficiency was higher compared to others (ATCC 10988 and ATCC 12526). But IFO 13756 rapidly

Table 1. Effect of pH on ethanol production by various strains of *Z. mobilis*.

pH	Fermentation parameters	Strains ATCC 10988	ATCC 12526	NRRL B 4286	IFO 13756
	P	2·7	2·2	4·0	3·5
	Su	74·4	68·1	85·6	75·0
4.0	g/gs	0·18	0·16	0·23	0·23
	E	26·37	21·48	39·06	34·18
	P	2·9	3·2	4·2	4·4
	Su	ND	ND	87·5	81·3
5.0	g/gs	ND	ND	0·24	0·27
	E	28·32	31·25	41·02	42·97
	P	3·9	4·0	4·3	4·7
	Su	80·6	81·2	87·5	88·1
6.0	g/gs	0·24	0·25	0·25	0·27
	E	38·09	39·06	41·99	45·9
	P	6·6	6·3	5·2	6·4
	Su	82·77	79·0	88·1	92·5
7.0	g/gs	0·40	0·40	0·30	0·35
	E	64·45	64·51	50·29	62·5

P, Productivity of alcohol % (v/v); Su, substrate utilized % (w/v); g/gs, gram alcohol produced/g of substrate utilized; E, percentage of theoretical yield; ND, not determined.

Table 2. Effect of initial sugar concentration on ethanol production by various strains of *Z. mobilis*.

Initial sugar (% w/v)	Fermentation parameters	Strains ATCC 10988	ATCC 12526	NRRL B 4286	IFO 13756
15	P	6·6	6·3	5·1	6·1
	Su	82·77	79·0	88·0	93·3
	g/gs	0·43	0·44	0·31	0·33
	E	68·75	65·63	53·13	63·54
	P	5·9	6·1	10·15	8·85
20	Su	73·0	75·0	89·0	91·0
	g/gs	0·32	0·33	0·46	0·39
	E	46·09	47·65	79·29	69·14
	P	5·2	5·5	7·9	7·2
25	Su	76·0	78·0	84·8	75·6
	g/gs	0·22	0·23	0·30	0·30
	E	32·05	34·38	49·38	45·0

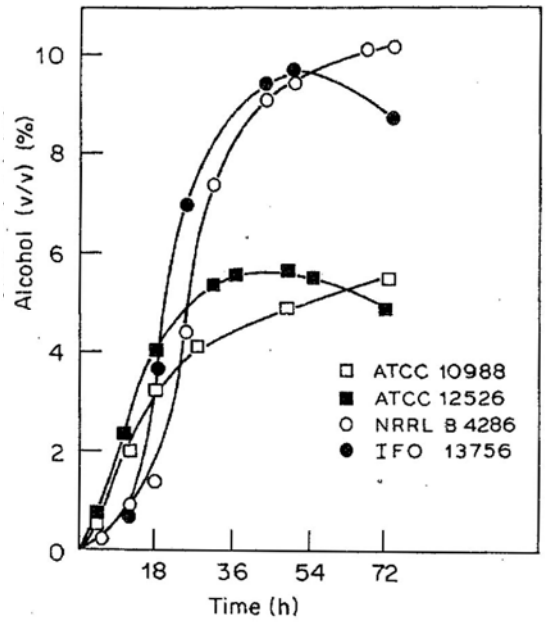

Figure 1. Ethanol production by strains of *Z. mobilis* on synthetic medium.

Table 3. Ethanol production by strains of *Z. mobilis* on various substrates.

Substrate	Fermentation parameters	Strains ATCC 10988	ATCC 12526	NRRL B 4286	IFO 13756
Synthetic medium	P	5·9	6·1	10·15	8·85
	Initial sugar	20·0	20·0	20·0	20·0
	Su	73·0	75·0	89·0	91·0
	g/gs	0·32	0·33	0·46	0·39
	E	46·09	47·65	79·29	69·14
Cane juice	P	10·3	10·3	9·2	10·3
	Initial sugar	18·0	18·0	18·0	18·0
	Su	95·0	95·0	97·2	97·2
	g/gs	0·48	0·48	0·42	0·47
	E	89·4	89·4	79·86	89·4
Molasses	P	3·05	2·9	3·29	1·97
	Initial sugar	10·0	10·0	10·0	10·0
	Su	ND	ND	ND	ND
	g/gs	ND	ND	ND	ND
	E	47·66	45·31	51·41	30·78

fermented after 12 h and the fermentation ceased at 48 h and thereafter the ethanol content decreased. This could be due to the oxidation of alcohol to acetic acid (Belaich and Senez, 1965; Swings and De Ley, 1977). Among all 4 strains of *Z. mobilis*, NRRL B 4286 showed higher fermentation efficiency (10·15 %, v/v) in synthetic medium. The specific productivity of ethanol was maximum (0·46) in this strain and minimum in ATCC 10988.(0·32).

Cane juice: Figure 2 and table 3 show the fermentation pattern of4 strains of *Z. mobilis* in cane juice. The strain which showed better fermentation in synthetic medium (NRRL B 4286) was a poor fermentor in cane juice compared to others. This strain showed a delay of 12 h while in the others no such delay was observed. The strain ATCC 10988 rapidly fermented and fermentation ceased at 48 h. Lyness and Doelle (1981) reported that ethanol yield of *Z. mobilis Z* 7 in cane juice medium was 60–88 % in 20–29 h. The present study shows that the fermentation of ATCC 10988 lasted for 48 h and resulted in 82–94% ethanol. The fermentation continued upto 66 h in strain ATCC 12526 resulting in 10·3 % (v/v) ethanol.

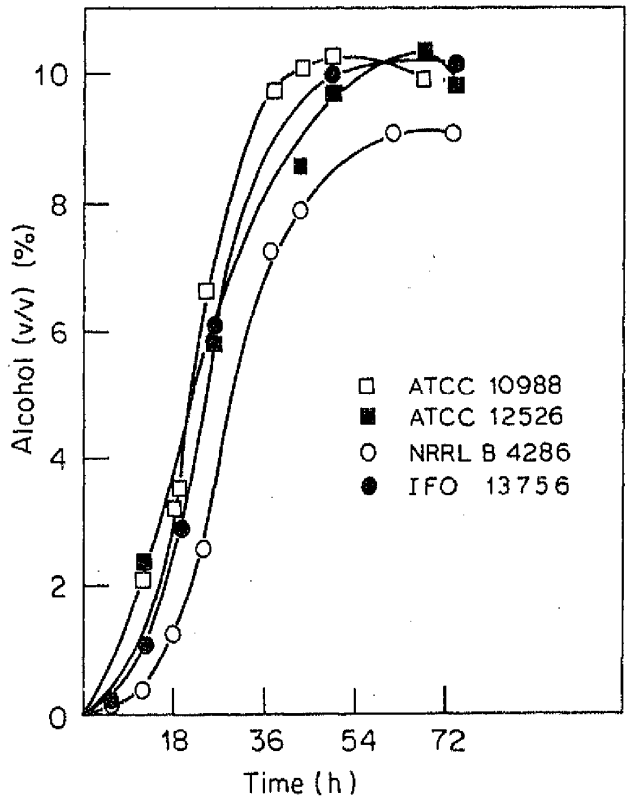

Figure 2. Ethanol production by strains of *Z. mobilis* on cane juice.

Molasses: Table 3 shows the ethanol production by strains of *Z. mobilis* in molasses with 10% (w/v) initial sugar concentration. The strains ATCC 10988 (3·05% v/v) and NRRL B 4286 (3·29 % v/v) showed maximum productivity whereas IFO 13756 showed minimum productivity (1·97 % v/v). *Z. mobilis* strain ATCC 10988 was well studied for its performance at different initial sugar concentrations of molasses (table 4). It showed

Table 4. Ethanol production at different initial sugar concentration of molasses by *Z. mobilis* strain ATCC 10988.

Initial sugar (% w/v)	Ethanol production (% v/v)	Theoretical yield (%)
3	1·25	65·1
5	2·0	62·5
7	2·5	55·8
10	3·05	47·66
15	3·35	34·9

maximum ethanol production (3·35%, v/v) at 15% (w/v) initial sugar concentration where substrate utilization was also found to be maximum (42% w/v). Above 15 % (w/v) initial sugar concentration of molasses, the ethanol production decreased (data not shown). In general, in all 4 strains of *Z. mobilis*, the ability to produce ethanol from molasses was very poor compared to other substrates. This observation is consistent with the result of Van Vuuran *et al.* (1982) and this could be due to the presence of high concentration ofions ($Mg^{2+}$and K^{+}) in molasses (Skotnicki *et al.,* 1982). Furthermore, investigations are in progress to improve the fermentation ability of *Z. mobilis* in molasses.

References

Belaich, J. P. and Senez J. C. (1965) *J. Bacteriol.*, **89**, 1195.
Dubois, M., Gills, K. A., Hamilton, J. K., Robert, F. A. and Smith, F. (1956) *Anal. Chem.,* **28**, 350.
Gibbs, M. and Demoss, R. D. (1951) *Arch. Biochem. Biophys*., **34,** 478.
Gibbs, M. and Demoss, R. D. (1954) *J. Biol. Chem.*, **207,** 689.
Lee, K. J., Tribe, D. E. and Rogers, P. L. (1979) *Biotechnol. Lett.,* **1**, 421.
Lyness, E. and Doelle, H. W. (1981) *Biotechnol. Lett.*, **3**, 257.
Ohta, K., Supanwong, K. and Hayashida, S. (1981) *J. Ferm. Technol*., **59**, 435.
Robinson, C. W., Mooyoung, M. and Lamptey, J. (1980) *Adv. Biotechnol*., **2**, 106.
Rogers, P. L., Lee, K. J. and Tribe, D. E. (1979) *Process Biochem.*, **15,** 7.
Skotnicki, M. L, Lee, K. J., Tribe, D. E. and Rogers, P. L. (1982) *Appl. Environ. Microbiol.*, **42,** 270.
Swings, J. and DeLey, J. (1977) *Bacteriol Rev*., **41,** 1.
Van Vuuran, H. J. and Meyer, L. (1982) *Biotechnol. Lett*., **4**, 253.