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RAISIN: A SUITABLE RAW MATERIAL FOR ETHANOL PRODUCTION USING *Zymomonas mobilis*

A.A. Koutinas[1], M. Kanellaki[1], M.A. Typas[2] and C. Drainas[3]

1. Department of Chemistry, Sector of Analytical, Environmental and Applied Chemistry, University of Patras, Patras, Greece
2. Department of Biochemistry, Molecular & Cellular Biology and Genetics, University of Athens, Athens 15701, Greece
3. Department of Chemistry, Sector of Organic Chemistry and Biochemistry, University of Ioannina, Ioannina, Greece

SUMMARY

A batch fermentation process for the production of ethanol from raisin using *Zymomonas mobilis* is described. This process shows significant advantages in ethanol production compared with yeasts, such as, faster fermentation time and higher ethanol productivity and yield. Moreover, fermentation of the raisin extracts by *Z.mobilis* gave three-fold higher ethanol productivity than of standard synthetic media of the same invert-sugar concentration.

INTRODUCTION

Raisin is widely used in Greece as raw material for ethanol production employing fermentation by the traditional baker's yeast *Saccharomyces cerevisiae*. The development of a more productive fermentation process of this aboundant raw material, which is rich in invert sugar content, is always of great economical importance. *Zymomonas mobilis* is a promising processing microorganism here, as it ferments glucose, fructose or sucrose to ethanol (Swings and DeLey, 1977), and, is more advantageous than baker's yeast (Rogers et.al., 1982). Various *Z.mobilis* strains may grow and produce ethanol as efficiently on simple chemically defined minimal media (Galani et.al., 1985). This justifies *Z.mobilis* as a potential fermenting microorganism of practically any natural raw material containing glucose, fructose or sucrose. A disadvantage here may be the production of non-fermentable sorbitol and levan by *Z.mobilis* when grown in sucrose or invert-sugar containing synthetic media (Viikari, 1984). However, recently it was proposed that production of sorbitol and levan may be minimized

by physical parameter control (Doelle and Greenfield, 1985). In the present report it is demonstrated that *Z.mobilis* may ferment raisin extracts to ethanol more efficiently than baker's yeast. This fermentation process gives higher ethanol productivities than in synthetic media of the same invert-sugar concentration.

MATERIALS AND METHODS

Preparation of raisin extracts. A two years stock of Greek raisin, variety Trechoumena, was used. Raisins and tap H_2O to give the desired invert-sugar content were place in conical flasks and extracted at 72°C for 4 h. The pH of the extracts was 3.5. This was adjusted as desired by NaOH. Extracts were used in fermentations without sterilization or addition of nutrients.

Strains and growth. *Z.mobilis* strain ATCC 10988 and baker's yeast were used. *Z.mobilis* was grown as described before (Galani et.al., 1985). *Z.mobilis* inocula were prepared as follows: In a 500 ml culture at late log phase, 1 l raisin extract of 6°Be, pH 5 were added. The culture was incubated without agitation in 30°C until late log. Cells were separated by centrifugation and transferred to 2 l fresh raisin extract as above. 15-20 g of pressed wet weight of *Z.mobilis* cells, harvested at early stationary phase, were produced by this procedure and used directly for the fermentation process. These inocula could be used over again for several fermentations (up to 7 tested here) without any effect on the kinetic parameters. Baker's yeast was supplied in cakes from bakeries.

Fermentation. Batch fermentation was followed in 1 l flasks without agitation. 500 ml of raisin extract or synthetic complete glucose medium (Galani et.al., 1985) were inoculated with the appropriate inoculum (in g wet weight) of *Z.mobilis* or Baker's yeast, and incubated in 30°C.

Determination of Kinetic parameters. Quantitative determination of ethanol was made in a Variant 1400 Gas Liquid Chromatographer using Porapac S as column material with N gas carrier (40ml/min). Ethanol productivity (EP) is given in g ethanol/l produced in 24 h. The ethanol yield factor (EYF) is the ratio of g ethanol/g of utilized sugar. Biomas productivity (BP) is expressed in g dry weight/l produced in 24 h. The biomas yield factor (BYF) is the ratio of g biomas/g of utilized sugar. Residual sugar was determined by the Lane Eynon analytical method (Egan et. al., 1981). Fermentation time (FT) was given in h and was followed by optical density at 700 nm. All values were the mean of six repeats. The standard deviation for ethanol productivity was $< \pm 10$ and for biomas and residual sugar $< \pm 0.2$.

RESULTS AND DISCUSSION

The fermentation kinetic parameters were studied in batch fermentations at industrial ethanol production standards.

Ethanol productivity was significantly higher and fermentation time shorter when *Z.mobilis* was used instead of baker' yeast (Tables 1,2 and 3). This productivity increases about 2-fold at initial cell concentration above 20 g/l (Table 1).

Table 1. Effect of initial cell concentration on kinetic parameters of raisin extract fermentations

	Z.mobilis					baker's yeast					
ICC	FT	EP	BP	EYF	BYF	RS	FT	EP	BP	EYF	BYF	RS
5	45	35.8	3.8	0.45	0.018	4.2	55	30.0	1.5	0.46	0.023	3.8
10	34	47.4	3.3	0.45	0.031	5.8	27	50.0	4.0	0.37	0.030	4.2
20	14	106.4	4.6	0.40	0.018	4.4	31	52.1	0.9	0.45	0.008	4.2
30	13	104.8	11.1	0.38	0.040	4.4	27	52.1	2.4	0.39	0.018	4.0
40	11	148.4	5.9	0.46	0.018	4.2	16	97.4	0.0	0.43	0.000	3.8

ICC= initial cell concentration g/l, initial pH 6 and initial invert-sugar concentration 150g/l.

Fermentation time and ethanol productivity were not affected by low pH as no differences were observed at pH 6 and 3.5 (Table 2). These results imply that the pH of the raisin extracts do not need to be adjusted before fermentation.

Table 2. Effect of pH on kinetic parameters of raisin extract fermentations.

pH	FT	EP	BP	EYF	BYF	RS	FT	EP	BP	EYF	BYF	RS
6.0	11	148.4	5.9	0.46	0.018	4.2	16	97.4	0.0	0.43	0.00	3.8
5.2	25	55.0	6.6	0.37	0.045	3.9	16	89.2	0.0	0.38	0.00	4.0
4.5	19	77.8	8.8	0.40	0.047	5.5	16	91.7	0.0	0.40	0.00	3.8
3.5	10	147.8	13.6	0.41	0.038	5.6	16	94.1	0.0	0.41	0.00	3.8

Initial cell and sugar concentration 40 g/l and 150 g/l respectively.

However, a decrease of ethanol production is observed at pH 5.2 and 4.5.

Ethanol productivity and yield of *Z.mobilis* fermentations are significantly higher than yeast at high initial sugar concentrations (Table 3). At concentrations over 176 g/l the yields obtained by *Z.mobilis* are very close to the theoretical value. This suggests that *Z.mobilis* fermentation of raisin extract, which is rich in invert-sugar, did not produce non-fermentable by-products as reported by Viikari (1984) for synthetic media. Fermentation of raisin extract gave higher ethanol productivity and yield than fermentation of synthetic media with the same initial sugar concentration (Table 4).

Moreover, addition of a small amount of raisin extract (optimum 6%) to the synthetic medium, resulted to dramatic increase of ethanol productivity (over 3-fold) and decrease of fermentation time. These results, in agreement with earlier report on fermentation of sugar cane mollases (Doelle and Greenfield, 1985) indicate that unidentified physiological factors in the raisin extract may enhance the fermentation process.

Table 3. Effect of initial invert sugar concentration on kinetic parameters of raisin extract fermentation

	Z.mobilis						baker's yeast				
ISC	FT	EP	BP	EYF	BYF	RS	FT	EP	BP	EYF	BYF	RS
118	15	89.8	13.9	0.50	0.047	2.6	16	74.4	2.8	0.43	0.073	2.3
150	10	147.8	13.6	0.41	0.038	5.6	16	94.1	0.0	0.41	0.000	3.8
176	13	166.0	13.9	0.51	0.065	4.0	35	56.6	1.4	0.47	0.012	3.6
227	21	137.0	13.1	0.51	0.049	4.9	35	73.5	5.7	0.47	0.038	4.7
269	29	108.0	7.5	0.49	0.033	6.3	40	69.1	5.8	0.43	0.036	5.3

ISC= Initial sugar concentration, pH 6, initial cell concentration= 40 g/l.

Table 4. Effect of raisin extract on *Z.mobilis* fermentation of synthetic medium

RE(%)	FT	EP	EYF	BP	BYF	RS
0.0	90	18.4	0.46	0.6	0.016	5.0
0.5	62	26.9	0.45	1.2	0.019	4.2
3.0	65	26.6	0.46	1.3	0.023	4.0
6.0	25	67.6	0.45	2.0	0.014	1.0
9.0	36	48.0	0.46	2.8	0.027	3.0
100.0	34	47.4	0.45	3.3	0.030	5.8

Initial cell concentration: 10 g/l, initial sugar concentration: 150 g/l pH 6, RE: percentage of raisin extract added in syntetic glucose complete medium.

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