Comparative Study of Kissiris, γ-Alumina, and Calcium Alginate as Supports of Cells for Batch and Continuous Wine-Making at Low Temperatures

V. Bakoyianis, A. A. Koutinas, K. Agelopoulos, and M. Kanellaki*

Department of Chemistry, Section of Analytical, Environmental and Applied Chemistry, University of Patras, GR-265 00 Patras, Greece

Kissiris, γ-alumina, and calcium alginate were the supports for immobilization of a cryotolerant and alcohol-resistant strain of *Saccharomyces cerevisiae* species. Continuous wine-making with immobilized cells on each above support was performed at ambient and low temperatures. To evaluate the results of continuous wine-making with immobilized cells, batch fermentations were performed using immobilized and free cells separately, in the same range of temperatures. Fourfold higher ethanol productivities at room temperature and 10-fold higher productivities at low temperatures were obtained by continuous wine-making, in comparison to batch fermentations performed with free cells. Specifically, at 7 °C, ethanol productivities achieved by continuous wine making were 16.7, 13.8, and 23.2 g L^{-1} day^{-1} and by batch wine-making were 4.5, 5.1, and 5.6 g L^{-1} day^{-1}, respectively, for kissiris, γ-alumina, and calcium alginate. For free cells ethanol productivity was 1.5 g L^{-1} day^{-1} at 7 °C. The three continuous systems were operated continuously for 80 days without any infection and diminution of the ethanol productivity. Also, the wines were produced with low total and volatile acidities.

Keywords: *Alcoholic fermentation; wine; continuous; batch; immobilized cells; Saccharomyces cerevisiae; psychrotolerant strain*

INTRODUCTION

The use of immobilized cells for alcoholic fermentation is an area that has received great attention, particularly in the past 15 years. The selection of a suitable support for cell adsorption is difficult, because a number of factors (nature of support and microbial cell, environmental conditions) are known to influence the cell−support interactions (Margaritis and Merchant, 1984). In wine-making the technique of immobilized cells was used for the second fermentation for sparkling wine (Fumi et al., 1987; Godia et al., 1991; Crapisi et al., 1992; Malik et al., 1993). Also, immobilized cells were used for batch wine-making of white wines (Nakanishi and Yokotsuka, 1987; Shimobayashi and Tominaga, 1987). An alcohol-resistant and cryotolerant *Saccharomyces cerevisiae* strain was immobilized on delignified cellulosic material (Bardi and Koutinas, 1994) and on gluten pellets (Bardi et al., 1996) for batch wine-making. Although the immobilization of yeast is widely studied for alcohol production (Margaritis and Merchant, 1984), publications concerning continuous wine-making by immobilized cells are not abundant in the literature (Keniichi, 1980; Ogbonna et al., 1989; Laffoirque et al., 1992; Iconomou et al., 1996).

Manufacturers have the experience that the wine produced at low temperatures has desirable odor and taste. However, publications concerning continuous wine-making by immobilized cells at low temperatures, <10 °C, to our knowledge, are unusual in the literature. In our previous studies (Kana et al., 1989a,b) γ-alumina was used as support for the immobilization of *S. cerevisiae* for ethanol production and mineral kissiris supported biocatalyst for low-temperature continuous wine-making (Bakoyianis et al., 1992). Because continuous fermentors may remain in uninterrupted operation for weeks or months, they have many economic advantages (Jackson, 1994). Generally, continuous processes can achieve substantial improvements in the efficiency of the process and product quality and subsequently higher productivities and lower operating costs. If a continuous fermentation is carried out with low-temperature-adapted and immobilized yeast cells, then the method can be industrialized and the products have commercial value. Therefore, a comparative study of cheap and abundant kissiris and γ-alumina against calcium alginate used as supports of immobilization for batch and continuous wine-making at low temperatures is also necessary. This work particularly focuses on the extensive study of continuous wine-making at low temperatures.

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Microorganism. All investigation was carried out with Visanto, an alcohol-resistant *S. cerevisiae* strain, isolated from the Greek agricultural area (Argiriou et al., 1996). The growth medium composition was given previously (Bardi and Koutinas, 1994).

Must. The must used for the fermentations was from grape**s,** variety Roditis (11.2−12.0 °Be) and was sterilized at 130 °C for 15 min.

Analyses. The analytical methods for ethanol, residual sugars, and total and volatile acids contents of wine were as described previously (Bardi and Koutinas, 1994). Ethanol productivity, given in Tables 1−3, was expressed as grams of ethanol per liter produced in 1 day and calculated on the basis of total working volume, by multiplying the dilution rate by ethanol concentration. Dilution rates were calculated by dividing the flow rate of liquid by the total volume of the fermentors. Ethanol productivity, given in Table 4, was calculated on the basis of liquid volume.

Supports for Immobilization. As immobilization supports for the above yeast strain were used separately (i) kissiris, a porous volcanic rock; (ii) porous γ-alumina pellets

S0021-8561(97)00192-1 CCC: $14.00

(length = 5 mm and diameter = 1.5 mm); and (iii) calcium alginate beads. Alginic acid was obtained from Sigma Chemical Co.; kissiris and γ-alumina were repeatedly washed with distilled water, sterilized at 130 °C, and dried. The immobilization of cells of the strain Visanto on kissiris and γ-alumina separately was obtained as it was described in previous papers (Kana et al., 1989a,b).

Pilot Plant. The experimental bioreactor for yeast immobilization and wine-making was described previously (Bakoyianis et al., 1992). For each support such a bioreactor was used, and three such bioreactor systems were operated simultaneously for three supports.

The temperature was gradually decreased, and when steady state was attained, samples were collected at 20, 16, 13, 10, and 7 °C per 24 h. The samples were analyzed for ethanol, residual sugar, and total and volatile acidity. The systems operated for 80 days without any contamination or loss of their activity. After that, the systems were returned to operate again at 27 °C. For kissiris and γ-alumina results are presented in Tables 1 and 2.

Cells of the Visanto yeast strain were entrapped in calcium alginate beads according to the procedure of Kierstan and Bucke (1977). The two bioreactors were fed with must only, because the synthetic culture medium, which contains phosphates, may disrupt the beads (Cheetham, 1979). The wine-making process was the same as in the case of kissiris and γ-alumina.

Repeated Batch Fermentations. Kissiris supported biocatalyst (156 g) and γ-alumina supported biocatalyst (200 g) were introduced separately into 300 mL of must (11.5 °Be) in two 500 mL glass cylinders. This was performed so that the cell concentrations would be equal. The glass cylinders for each fermentation batch were incubated at the temperatures indicated in Table 4. For each supported biocatalyst, before the fermentation was completed, the liquid was decanted, the support was washed three times with must, and must was added for the next fermentation. In a parallel way, fermentations using free cells (6 g of cells/300 mL of must) were performed (Table 4). The quantity of free cells was determined and was the same as that immobilized on these solids supports contained in the same liquid volume. Each fermentation was repeated a minimum of three times.

Two hundred milliliters of calcium alginate beads, containing the same amount of yeast cells as in γ-alumina and kissiris supported biocatalyst, were washed with must (12 °Be) to remove free $CaCl_2$ and were added into 300 mL of must (12 °Be) for the fermentation. The following process was the same as in the above paragraph, and the results are presented in Table 4. All values were the mean of three repeats. The standard deviation for ethanol concentration was $<\pm 0.2$, for ethanol productivity $<\pm 10$, for residual sugar $<\pm 2$, and for total and volatile acidity $<\pm 0.1$.

Electron Microscopy. The γ-alumina supported biocatalyst was washed with water and dried overnight at room temperature. It was then coated with gold in a Balzers SCD 004 sputter coater for 3 min to increase the electron conductivity. The samples were examined in a Cambridge Stereoscan 120 scanning electron microscope.

RESULTS AND DISCUSSION

Continuous Fermentation. The same must (11.2−12.0 °Be) was pumped into the three pairs of the bioreactors at a flow rate of 600 mL/day. The three systems of the bioreactors operated at 27 °C for 12 days, and then the temperature was decreased gradually to 7 °C. The total operation time was 80 days, and the results are presented in Figure 1 and Tables 1−3.

At 27 °C, the °Be densities of the three effluents (wine) were 0 °Be. At 20 °C, the °Be densities of the two effluents (from kissiris and calcium alginate beads) remained constant (0 °Be), but a slight increase of the °Be density (0.5 °Be) of the effluent was reported in the case of γ-alumina supported biocatalyst. Likewise, the same behavior was reported for the three systems of bioreactors at 16 °C. At 13 °C, the °Be densities of the effluents were 0, 1.5, and 3 °Be for calcium alginate, kissiris, and γ-alumina supported biocatalysts, respectively. As the temperature was reduced, the °Be densities of the effluents were increased and at 7 °C the °Be densities were about 2.5, 4, and 6 for calcium alginate, kissiris, and γ-alumina supported biocatalysts, respectively. If we increased the bioreactor volume and the temperature and flow rate remained constant, we could produce dry wine in industrial scale. After the above operation, which lasted ~70 days, the temperature was increased again to 27 °C and the three systems of bioreactors were operated; the densities of the effluents from the three bioreactors were all 0 °Be (Figure 1).

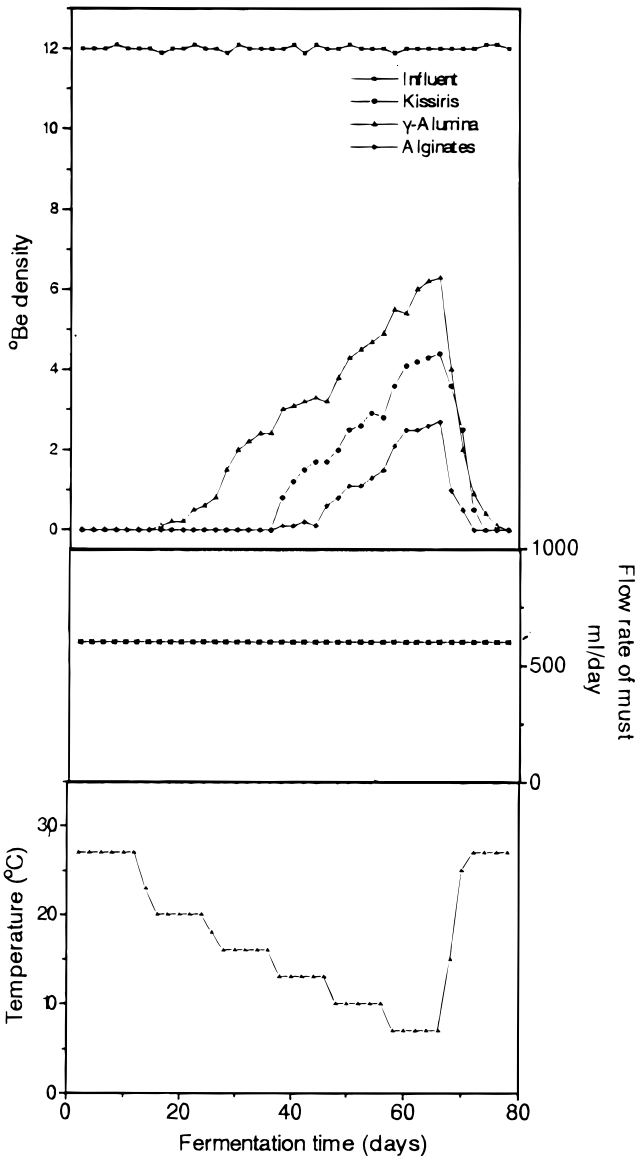

Figure 1. Flow rate of must and °Be density converted as related to temperature in the range 7−27 °C.

The immobilization procedures and supports maintained the cells in a viable state, and the psychrophile character of the solid supported biocatalysts resulted in the operational stability of the systems even at low temperature (7 °C). Tables 1−3 show that the ethanol productivities of the biocatalysts ranging from greatest to least were in the order calcium alginate, kissiris, and γ-alumina at each temperature. The ethanol productivities of the continuous system of kissiris supported

Table 1. Characteristics of the Wine Produced by Continuous Fermentation with Immobilized Yeast Cells on Kissiris

temp (°C)	prodtn rate (mL/day)	EtOH concn (% v/v)	resid sugar (g/L)	EtOH prodtn (g L^{-1} day^{-1})	total acidity of the wine (g of tartaric acid/L)	volatile acidity of the wine (g of acetic acid/L)
20	600	11.6	8.5	18.3	4.3	0.24
20	600	12.0	4.9	19.0	4.9	0.25
20	600	11.4	10.7	18.0	4.8	0.23
20	600	11.6	8.7	18.3	4.3	0.19
16	600	11.6	9.8	18.3	4.0	0.21
16	600	11.6	7.3	18.3	4.4	0.22
16	600	11.4	13.1	18.0	4.3	0.24
16	600	11.2	15.4	17.7	4.9	0.21
13	600	9.5	32.2	15.0	4.3	0.26
13	600	9.2	37.1	14.6	4.6	0.26
13	600	9.1	40.2	14.4	4.1	0.28
13	600	9.3	34.8	14.7	3.9	0.25
10	600	8.4	48.2	13.2	4.0	0.24
10	600	8.3	52.4	13.1	3.9	0.26
10	600	8.0	54.7	12.6	3.6	0.25
10	600	8.1	50.1	12.8	3.9	0.28
7	600	7.8	62.3	12.3	3.9	0.25
7	600	7.4	58.3	11.7	3.5	0.22
7	600	7.0	72.6	11.0	3.6	0.24
7	600	7.1	70.5	11.2	3.5	0.21

Table 2. Characteristics of the Wine Produced by Continuous Fermentation with Immobilized Yeast Cells on γ-Alumina

temp (°C)	prodtn rate (mL/day)	EtOH concn (% v/v)	resid sugar (g/L)	EtOH prodtn (g L^{-1} day^{-1})	total acidity of the wine (g of tartaric acid/L)	volatile acidity of the wine (g of acetic acid/L)
20	600	11.2	8.8	17.7	2.3	0.46
20	600	10.8	18.5	17.0	2.4	0.25
20	600	11.0	14.2	17.4	2.4	0.30
20	600	10.9	19.5	17.2	2.4	0.28
16	600	9.8	33.4	15.5	2.3	0.47
16	600	9.4	36.8	14.8	2.6	0.34
16	600	9.1	35.2	14.4	2.5	0.34
16	600	9.0	40.6	14.2	2.6	0.38
13	600	8.8	49.6	13.9	2.7	0.46
13	600	8.5	52.6	13.4	2.8	0.41
13	600	8.3	58.3	13.1	3.8	0.55
13	600	8.4	57.4	13.3	3.9	0.43
10	600	7.4	67.2	11.7	4.3	0.37
10	600	7.6	65.3	12.0	4.5	0.44
10	600	7.1	70.4	11.2	4.4	0.40
10	600	7.3	69.8	11.5	4.5	0.37
7	600	6.2	81.2	9.8	3.7	0.46
7	600	6.0	83.6	9.5	3.9	0.40
7	600	5.7	89.4	9.0	3.7	0.42
7	600	5.4	90.4	8.5	3.8	0.41

biocatalyst at ~7 °C and that of γ-alumina at 10 °C were about equal to that obtained by free cells at temperature in the range 22−25 °C (Table 4). Comparison of the ethanol productivities for each continuous system (Table 4) at 27 and 7 °C showed decrease of 71%, 77%, and 80% in the case of calcium alginate, kissiris, and γ-alumina, respectively.

The ethanol concentration of the wines was decreased as the temperature was diminished. In wine-making by γ-alumina supported biocatalyst a greater decrease of ethanol concentration was obtained as compared with kissiris (Tables 1 and 2). However, less decrease is reported in wine-making by calcium alginates (Table 3).

Different behaviors of the total acidity are reported for each support. In the case of kissiris supported biocatalyst, the total acidity of the wine is decreased as the temperature is diminished (Table 1). Especially at 7 °C, the total acidity is 54% of that of must. It is estimated that this reduction is higher than that obtained in wines produced by natural fermentation.

Table 3. Characteristics of the Wine Produced by Continuous Fermentation with Immobilized Yeast Cells on Alginates

temp (°C)	prodtn rate (mL/day)	EtOH concn (% v/v)	resid sugar (g/L)	EtOH prodtn (g L^{-1} day^{-1})	total acidity of the wine (g of tartaric acid/L)	volatile acidity of the wine (g of acetic acid/L)
20	600	12.5	6.5	19.7	4.6	0.37
20	600	12.4	5.5	19.6	5.1	0.41
20	600	12.9	4.6	20.4	5.2	0.37
20	600	12.9	4.3	20.4	5.2	0.37
16	600	12.5	5.8	19.7	4.7	0.34
16	600	12.9	3.5	20.4	4.7	0.32
16	600	12.8	4.2	20.2	4.8	0.32
16	600	12.9	4.1	20.4	4.7	0.32
13	600	12.8	3.8	20.2	4.4	0.44
13	600	12.6	4.9	19.9	5.2	0.40
13	600	12.7	5.5	20.0	5.5	0.37
13	600	12.7	5.1	20.0	5.4	0.36
10	600	11.2	9.7	17.7	5.0	0.37
10	600	11.0	14.2	17.4	4.9	0.40
10	600	10.6	18.7	16.7	4.8	0.42
10	600	10.5	20.4	16.6	4.7	0.35
7	600	10.0	28.5	15.8	4.9	0.38
7	600	9.5	35.4	15.0	4.2	0.46
7	600	9.8	38.4	15.5	4.6	0.41
7	600	9.9	36.2	15.6	4.5	0.37

Table 4. Ethanol Productivities in Fermentation of Must by Free and Immobilized Cells on Kissiris, γ-Alumina, and Alginates at 7, 13, and 27 °C

	EtOH prodtn (g L^{-1} day^{-1})						
		immobilized cells					
		batch fermentation			continuous fermentation		
temp (°C)	free cells	kissiris	γ-alumina	alginates	kissiris	γ-alumina	alginates
27	17.3	48.6	48.8	60.3	73.4	68.5	80.6
13	5.8	13.7	13.6	15.8	22.8	19.9	30.0
7	1.5	4.5	5.1	5.6	16.7	13.8	23.2

This is an advantage in the case of must with high total acidity, which must be reduced to obtain better organoleptic character. The reduction of the total acidity can be attributed to the increase of crystallization of monopotassium tartate as the temperature drops. In the case of γ-alumina supported biocatalyst, the total acidity of the wine is increased as the temperature is decreased (Table 2) to 10 °C. By cooling at 7 °C, the total acidity was increased and reached that of kissiris. At 7 °C the total acidity is ~56% of that of must. Probably, the amphoteric character of γ-alumina plays an important role in this effect. In the case of calcium alginate supported biocatalyst, the total acidity remained constant in general as the temperature was decreased (Table 3).

Volatile acidity fluctuates for each support: 0.19−0.28 g of acetic acid/L for kissiris (Table 1), 0.25−0.55 g/L for γ-alumina (Table 2), and 0.32−0.46 g/L for calcium alginate (Table 3).

Electron Microscope Study. We proceeded to an examination of γ-alumina pellets supported biocatalyst by electron microscopy just after the end of the operation of the bioreactors at 7 °C to explain the lower ethanol productivities obtained at low temperatures as compared with kissiris and alginates. This reduced productivity was not observed at temperatures >20 °C. Figure 2 shows electron micrographs taken at two magnifications. One can observe on the surface of the immobilized cells compounds deposited on them. Probably, such compounds (diluted γ-alumina, potassium sodium tartrate, etc.) inhibit the full operation of the immobilized cells and result in lower ethanol productiv-

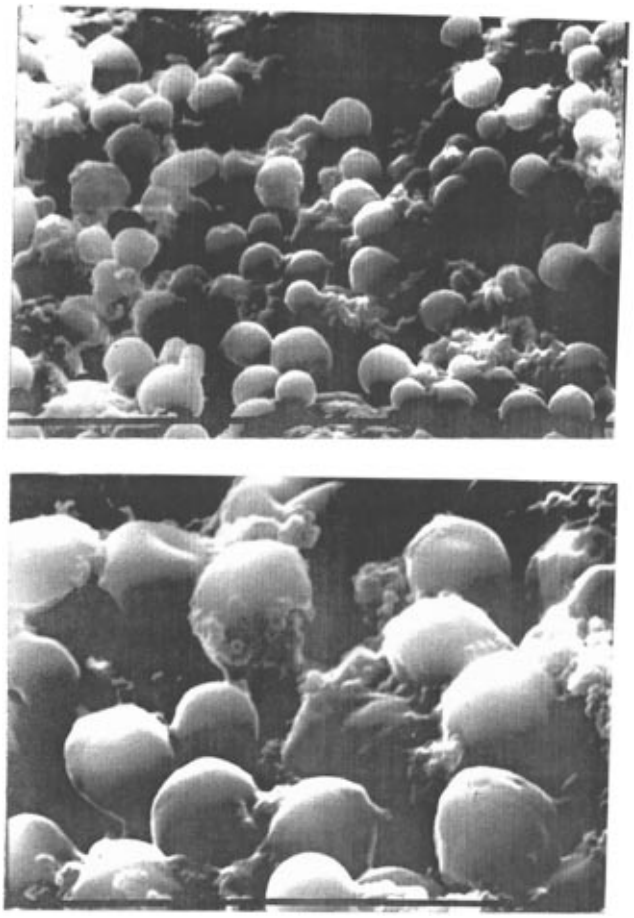

Figure 2. Electron micrograph showing immobilized cells on γ-alumina at 2100× and 5200×. (Figure is reproduced here at 67% of the original.)

ity at low temperatures as compared with kissiris and alginate supported biocatalyst. It is obvious that at high temperatures such compounds are not crystallized and not deposited.

Repeated Batch Fermentations. For a better evaluation of the results of the continuous process, repeated batch fermentations by free cells and by cells immobilized on the three supports separately were carried out at 7, 13, and 27 °C with the strain of yeast and must as in the continuous process. Must (300 mL) was added into a glass cylinder of 500 mL and 6 g of free cells was suspended in it, equal to 6 g of immobilized cells on each support. The fermentations were carried out at 27, 13, and 7 °C, and the results are presented in Table 4 and in Figures 3−5. In all cases the fermentation rate of immobilized cells was 2−3-fold higher than that of free cells. Especially, the fermentation rates were decreased according to the series calcium alginate to kissiris to γ-alumina, and the difference between the fermentation rates was increased as the temperature was decreased (Figures 3−5).

Table 4 presents the ethanol productivities of all cases of fermentations. The ethanol productivities of immobilized cells were higher than those of the free cells system and were especially higher in the continuous process than in the batch process as reported by other researchers (Tyagi and Ghose, 1982). As the temperature was decreased, the ethanol productivity of the continuous system was 2−4-fold higher than that of the batch system.

Comparing the ethanol productivities between free cells and immobilized cells in the continuous process

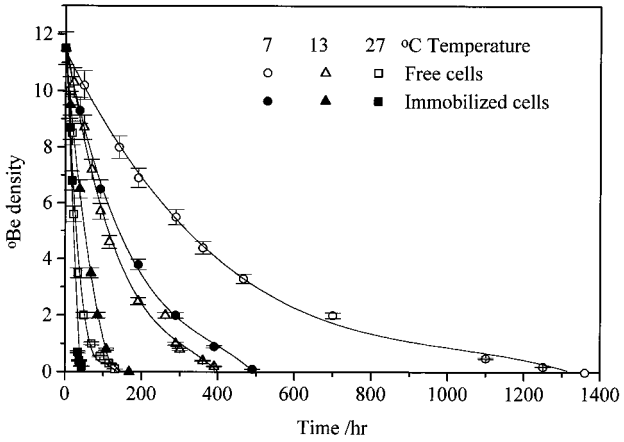

Figure 3. Alcoholic fermentation kinetics of must, observed at temperatures of 7, 13, and 27 °C and carried out in batch reactors separately by free and immobilized cells on mineral kissiris.

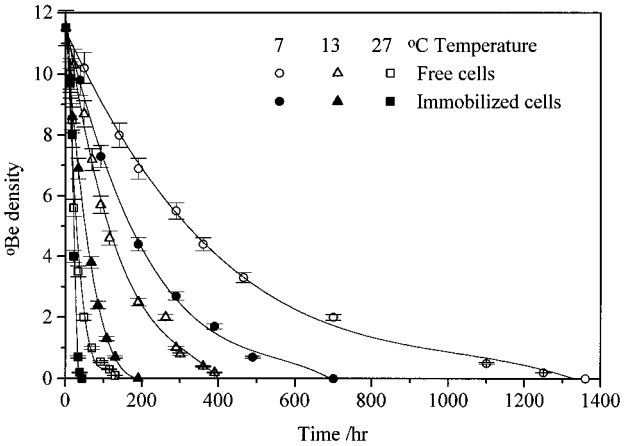

Figure 4. Alcoholic fermentation kinetics of must, observed at temperatures of 7, 13, and 27 °C and carried out in batch reactors separately by free and immobilized cells on γ-alumina.

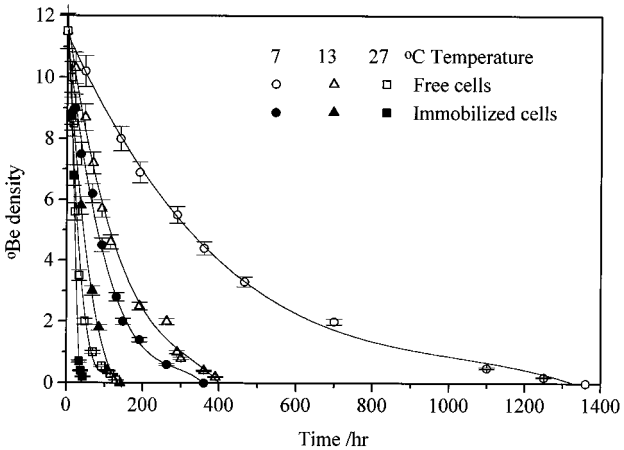

Figure 5. Alcoholic fermentation kinetics of must, observed at temperatures of 7, 13, and 27 °C and carried out in batch reactors separately by free and immobilized cells entrapped in calcium alginate beads.

(Table 4), one can observe that the ethanol productivity in the continuous process for the three supports is 3−4 times higher than that for free cells at 27 °C and 11, 9, and 15 times higher for kissiris, γ-alumina, and calcium alginate, respectively, than that for free cells at 7 °C. The decrease of the temperature affected the ethanol productivity of immobilized cells in continuous processes much less than that of the free cells.

It is obvious (Figures 3−5) that the three supports increase the ethanol production rate as compared with the fermentation without them.

Finally, we can say that the three supports, calcium alginate, kissiris, and γ-alumina, can be used for the immobilization of the cryotolerant yeast strain Visanto and the obtained three supported biocatalysts can be used for batch and continuous alcoholic fermentations separately at ambient and low temperatures. From the three solid supported biocatalysts, calcium alginate presented increased fermentation rate and ethanol productivity at low temperatures with respect to the other two supports. Probably, the entrapped cells in the beads of caclium alginate were in a more steady environment (no change in pH) and there was not the risk of cell wash out and cell loss by the turbulence caused by CO_2 evolution. However, the use of adsorbed yeast cells for the production of ethanol employs a cheap and simple method of retaining high cell densities. Also, the immobilization procedure maintains the cells in a viable state (Margaritis and Merchant, 1984). For the three supports we must evaluate other parameters. There is their market cost as well as the cost for yeast immobilization and the cost to avoid infections should the column become infected. Kissiris is an abundant volcanic raw material, and cheap ($60/ton). γ-Alumina, an industrial product, is more expensive than kissiris, and alginic acid is more expensive than γ-alumina. The technique for yeast immobilization on kissiris and γ-alumina is simple and cheap, but in the case of alginates the process is complicated. In the case of infected supports, the regeneration of a pure culture for kissiris and γ-alumina can be done by washing with hot water or chemical treatment. It is obvious that a simple process such as that cannot be applied for calcium alginate beads, due to their destruction by the hot water and possible diffusion problems. In the case of destruction of calcium alginate beads the process for recovering alginic acid is difficult to impossible and an environmental problem of disposal is created. It is evident that the most environmentally friendly supports are the inorganic kissiris and γ-alumina. Moreover, for the final consideration of a support for cell immobilization must also be estimated the quality of the product (byproducts).

LITERATURE CITED

Argiriou, T.; Kaliafas, A.; Psarianos, K.; Kanellaki, M.; Voliotis, S.; Koutinas, A. A. Psychrotolerant *Saccharomyces cerevisiae* stains after an adaption treatment for low-temperature wine making. *Process Biochem*. **1996**, *31* (7), 639−642.

Bakoyianis, V.; Kanellaki, M.; Kaliafas, A.; Koutinas, A. A. Low- temperature wine making by immobilized cells on mineral kissiris. *J. Argic. Food Chem*. **1992**, *40* (7), 1293−1296.

Bardi, E. P.; Koutinas, A. A. Immobilization of yeast on delignified cellulosic material for room temperature and low-temperature wine making. *J. Agric. Food Chem*. **1994**, *42* (1), 221−226.

Bardi, E. P.; Bakoyianis, V.; Koutinas, A. A.; Kanellaki, M. Room temperature and low-temperature wine making using yeast immobilized on gluten pellets. *Process Biochem*. **1996**, *31* (5), 425−430.

Cheetham, P. S. J. Physical studies on the mechanical stability of columns of calcium alginate gel pellets containing entrapped microbial cells. *Enzyme Microb. Technol*. **1979**, *1* (3), 183−188.

Crapisi, A.; Pasine, G.; Borin, G.; Versini, G.; Spettoli, P. Use of immobilized yeasts for the production of Colli Euganei sparkling wines. *Vignevini* **1992**, *10*, 65−68.

Egan, H.; Kirk, R. S.; Sawyer, R. *Pearson's Chemical Analysis of Foods*, 8th ed.; Churchill Livingstone: Edinburgh, 1981; pp 150−153.

Fumi, M. D.; Trioli, G.; Colagrande, O. Preliminary assessment on the use of immobilized yeast cells in sodium alginate for sparkling wine processes. *Biotechnol. Lett*. **1987**, *9*, 339−342.

Godia, Fr.; Casas, C.; Sola, C. Application of immobilized yeast cells to sparkling wine fermentation. *Biotechnol. Prog*. **1991**, *7*, 468−470.

Iconomou, L.; Kanellaki, M.; Voliotis, S.; Agelopoulos, K.; Koutinas, A. A. Continuous wine making by delignified cellulosic material supported biocatalyst: An attractive process for industrial applications. *Appl. Biochem. Biotechnol*. **1996**, *60*, 303−313.

Jackson, R. S. In *Wine Science: Principles and Applications*; Academic Press: New York, 1994; pp 245, 233.

Kana, K.; Kanellaki, M.; Paradimitriou, A.; Psarianos, C.; Koutinas, A. A. Immobilization of *Saccharomyces cerevisiae* on γ-alumina pellets and its ethanol production in glucose and raisin extract fermentation. *J. Ferment. Bioeng*. **1989a**, *68* (3), 213−215.

Kana, K.; Kanellaki, M.; Psarianos, K.; Koutinas, A. A. Ethanol production by *Saccharomyces cerevisiae* immobilized on mineral kissiris. *J. Ferment. Bioeng*. **1989b**, *68* (2), 144−147.

Keniichi, O. Wine Making. Jpn. Kokai Tokkyo Koho 80,158,-789, 1980.

Kierstan, M.; Bucke, C. The immobilization of microbial cells, subcellular organelles and enzymes in Calcium alginate gels. *Biotechnol. Bioeng*. **1977**, *19*, 387−397.

Laffoirque, D.; Stilhart, C.; Goma, Y. Novel procedure for the manufacture of alcoholic beverages by continuous fermetation in a membrane bioreactor: Pettilant de raisin. *Sci. Aliment*. **1992**, *12*, 393−413.

Malik, F.; Krasny, S.; Nahalka, J.; Minarik, E. Immobilized yeasts for secondary fermentation with wine. *Mitt. Klosterneuburg* **1993**, *43*, 8−11.

Margaritis, A.; Merchant, F. J. A. Advances in ethanol production using immobilized cell systems. *CRC Crit. Rev. Biotechnol*. **1984**, *1* (4), 339−393.

Nakanishi, K.; Yokotsuka, K. Fermentation of white wine from Koshu grape using immobilized yeast. *Nippon Shokuhin Koguo Gakkaishi* **1987**, *34*, 362−369.

Ogbonna, J. C.; Amano, Y.; Nakamura, K.; Yokotsuka, K.; Shimazu, Y.; Watanabe, M.; Hara, S. A multistage bioreactor with replaceable bioplates for continuous wine fermetation. *Am. J. Enol. Vitic*. **1989**, *40*, 292−297.

Shimobayashi, Y.; Tominaga, K. Application of biotechnology in the food industry. I. Brewing of white wine by a bioreactor. *Hokkaidoritsu Kogyo Shikenjo Hokoku* **1987**, *285*, 199−204.

Tyagi, R. D.; Ghose, T. K. Studies on immobilized *Saccharomyces cerevisiae*. I. Analysis of continuous rapid ethanol fermentation in immobilized cell reactor. *Biotechnol. Bioeng*. **1982**, *24* (4), 781−795.

Zoeckle, B.; Fugelsang, K.; Gump, B.; Nury, F. Volatile acidity. In *Production Wine Analysis*; Van Nostrand Reinhold: New York, 1990; pp 105−110.

Received for review March 12, 1997. Revised manuscript received July 17, 1997. Accepted September 25, 1997.[⊗]

JF970192Q

[⊗] Abstract published in *Advance ACS Abstracts*, November 1, 1997.