Effect of Carbohydrate Substrate on Fermentation by Kefir Yeast Supported on Delignified Cellulosic Materials

I. Athanasiadis,† D. Boskou,† M. Kanellaki,‡ and A. A. Koutinas*,‡

Laboratory of Food Chemistry and Technology, Department of Chemistry, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece, and Food Biotechnology Group, Department of Chemistry, University of Patras, Patras, Greece

The suitability of delignified cellulosic (DC) material supported kefir yeast to ferment raw materials that contain various single carbohydrates, for the production of potable alcohol and alcoholic drinks, is examined in this investigation. Results are reported of fermentations carried out with sucrose, fructose, and glucose in synthetic media. Repeated batch fermentations at various initial sugar concentrations of sucrose, fructose, and glucose were performed at 30 °C in the presence of the aforementioned biocatalyst. The results clearly show feasible yields in the range of 0.38−0.41 g/g, alcohol concentrations of 7.6−8.2% v/v, fermentation time of 90−115 h, and conversion of 92−96%. DC material supported kefir fermented 11-fold more rapidly than free cells and 9-fold more rapidly in comparison to kissiris supported kefir. The main volatile byproducts such as amyl alcohols (mixture of 2-methyl-1-butanol and 3-methyl-1-butanol), ethanal, and ethyl acetate were formed in all sugar fermentation products. The formation of 65−110 ppm of ethyl acetate is as high and even higher than that obtained with traditional wine yeasts. The increase of the initial concentration of sugar in the fermentation media resulted in an increase in contents of volatiles. The fine aroma that was obtained in the product of fructose could be attributed to the high percentage of ethyl acetate on total volatiles. The efficiency of DC material supported kefir was the same for the fermentations of individual sugars or a mixture of fructose, sucrose, and glucose. When whey with raisin extracts was fermented, lower yields were obtained but the aroma of the product was even better.

Keywords: *Kefir; fermentation; glucose; sucrose; fructose; immobilization*

INTRODUCTION

Great effort has been made during the past decades to study through *Saccharomyces cerevisiae* all biotechnological processes concerning fuel-grade alcohol, potable alcohol, alcoholic beverages, and SCP production. During the past two decades the bacterium *Zymomonas mobilis* (*1*) has been extensively studied, mainly for fuel-grade alcohol production, due to its higher productivity and yield (*2*) in comparison to those of *Saccharomyces cerevisiae*, which has been traditionally employed in the food industry. Still, the bacterium has not yet been used in industrial processes due to its difficulty in handling. Kefir yeast is a known culture that ferments lactose and seems to have a potential for potable alcohol using milk whey. The fermentation of mixtures of milk whey with other raw materials, such as molasses and raisin extracts, will avoid the condensation of whey and, consequently, the high energy demand due to steam consumption. However, these mixtures need a yeast which hydrolyzes lactose, a property that is not shown by *S. cerevisiae*. Because molasses contains sucrose, raisin extracts contain glucose and fructose, and starch is hydrolyzed to glucose, a study of the fermentation of these oligosaccharides with kefir yeast is necessary. Kefir yeast is a mixed culture of various species of genera *Kluyveromyces*, *Candida*, *Saccharomyces*, and *Pichia* and various lactic acid bacteria of the genus *Lactobacillus* (*3*). The use of milk whey in mixtures with molasses or raisin extracts may significantly decrease potable alcohol production cost and may lead to a novel alcoholic drink. Many researchers have reported alcohol production from lactose using *Klyuveromyces fragilis* (*4*−*6*), strains of *Klyuveromyces marxianus* (*7*, *8*), and *K. fragilis* (*9*, *10*), immobilized on alginates. *K. fragilis* was also immobilized on beech wood and pellets of activated carbon (*11*). Recombinant DNA techniques (*12*, *13*) and co-immobilization using *S. cerevisiae* with β-galactosidase (*14*) have also been examined. Recently, an effort has been undertaken to examine the behavior of kefir in the low-temperature alcoholic fermentation of glucose by delignified cellulosic (DC) material supported kefir yeast (*15*). However, a more systematic study is needed to prove its suitability for the fermentation of mixtures consisting of molasses, raisin extracts, and glucose-containing raw materials.

MATERIALS AND METHODS

Wet DC material was used as support for the immobilization of cells of kefir yeast (*16*). DC material is sawdust from which lignin has been removed after its treatment with 1% w/v aqueous sodium hydroxide solution at the boiling point. Kefir yeast commercial product, which is used in Caucasus for homemade kefir drink, was employed in this study. Cell growth was performed as in a recent study (*15*). Glucose was a Riedel-de Häen product, and sucrose and fructose were commercial products. The porous mineral kissiris was also used as a support of immobilization of kefir yeast, and the immobilization was performed as described in the study of Kana et al. (*17*).

* Author to whom correspondence should be addressed [telephone 0030(0)61 997104; fax 0030(0)61 997105; e-mail A.A.Koutinas@upatras.gr).

† Laboratory of Food Chemistry.

‡ Food Biotechnology Group.

10.1021/jf0006628 CCC: $20.00
Published on Web 12/23/2000

Glucose fermentations by DC material supported kefir, kissiris supported kefir, and free cells of kefir yeast were performed separately in parallel by taking into account the same biomass concentration for immobilized and free cells, calculated according to the results of previous studies (*16*, *18*). Amounts of 120 g wet weight of DC material supported kefir yeast, 160 g of kissiris supported kefir yeast, and 6 g wet weight of free cells were introduced into 250 mL of liquid medium containing glucose and having initial 10 °Be density (pH adjusted to 4.7), each separately in a 1 L glass cylinder. The glass cylinders were incubated at 30 °C, and kinetics of fermentations were performed by measuring the °Be density at various time intervals.

Fermentations. For each fermentation an amount of 120 g wet weight of DC material supported kefir yeast, prepared by immobilization of cells, was introduced into 250 mL of synthetic liquid medium containing separately fructose, glucose, and sucrose and 1 g/L $(NH_4)_2SO_4$, 1 g/L KH_2PO_4, 5 g/L $MgSO_4 \cdot 7H_2O$, and 4 g/L yeast extract. The initial sugar concentrations (ISC) studied for each sugar were 50, 70, 120, and 170 g/L, and for each ISC and sugar, seven repeated batch fermentations were performed. The pH was adjusted to 5.6, and the 1 L cylinders containing the above fermentation broth and DC material supported kefir were incubated at 30 °C. The fermentations were carried out without agitation, and kinetics of fermentations were plotted by measuring the °Be density at various time intervals. Just before the fermentation was completed, the liquid was filtered with a Büchner funnel and the support was washed three times with 400 mL of the liquid medium containing the sugar that was going to be studied. The biocatalyst was pressed and then used for the next fermentation batch. In addition, synthetic media of initial 7.3 °Be density, containing 40 g/L each of fructose, sucrose, and glucose, was fermented as above using DC material supported kefir. Furthermore, mixtures of 45% whey and 55% raisin extracts having initial 7 °Be density were also fermented by the same biocatalyst, without addition of nutrients. Samples of each fermentation were analyzed for ethanol, residual sugar, and volatile byproducts as described below. When the amount of the biocatalyst was decreased by 10% in repeated batch fermentations, the appropriate amount of DC material was added. All values were the mean of three runs. The standard deviation for fermentation time was $<\pm 10$, for ethanol concentration $<\pm 0.6$, for residual sugar $<\pm 2.5$, for yield $<\pm 0.03$, for ethanol productivity $<\pm 0.05$, and for conversion $<\pm 7$.

Ethanol and Byproduct Determination. Residual sugar and ethanol were determined in all samples by high-performance liquid chromatography (HPLC). A Shimadzu HPLC chromatograph, model LC-9A, connected with an integrator, C-R6A Chromatopac, column SCR-101N (packed with a cation-exchange resin-sulfonated polystyrene−divinylbenzene copolymer), CTO-10A column oven, and refractive index detector RID-6A were employed. Triple-distilled and filtered water was used for the elution. The determination was performed using a pressure of 78−82 atm, and the flow rate of the mobile phase was 0.8 mL/min. An oven temperature of 60 °C was used. Samples of 0.5 and 2.5 mL of 1% 1-butanol as internal standard were added in a 50 mL volumetric flask with distilled and filtered water. This solution was filtered using microfilters of 0.45 μm pore size and injected directly into the column. Kinetic parameters such as ethanol productivity, residual sugar, conversion, and yield were calculated as described in previous studies (*18*, *19*).

Determination of Volatiles. Volatile byproducts were analyzed using a Shimadzu gas chromatograph GC-8A, connected with the integrator C-R6A Chromatopac. Amyl alcohols (total amount of 2-methyl-1-butanol and 3-methyl-1-butanol), isobutyl alcohol, 1-propanol, ethanal, and ethyl acetate were determined using a stainless steel column (4 m long, 0.125 in. i.d.), packed with Escarto 5905 [consisting of squalene 5%, Carbowax 300 90%, and bis(2-ethylexyl) sebacate 5% v/v, with N_2 as the carrier gas (20 mL/min)]. The injection port and detector temperatures were 210 °C, and the column temperature was 58 °C. The internal standard was butanol, at a concentration of 0.5% v/v. Samples of 2 μL of the fermented liquid were injected directly in the column.

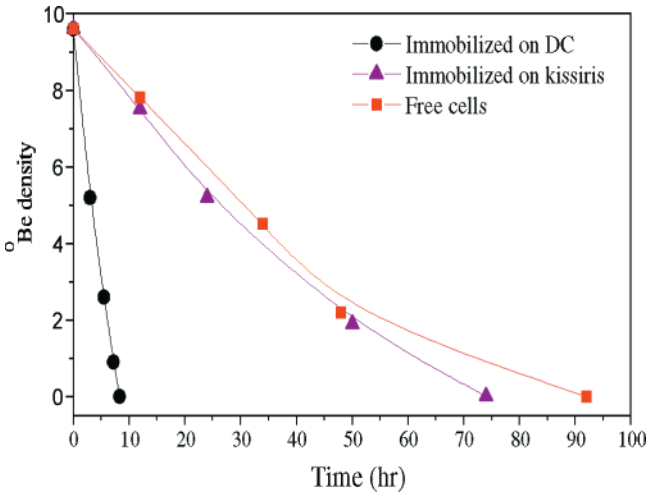

Figure 1. Kinetics of glucose fermentation by the presence of DC material supported kefir yeast compared with kissiris supported kefir and its free cells.

RESULTS AND DISCUSSION

The systematic study of potable alcohol production by DC material supported kefir yeast should include (i) fermentations of sucrose, fructose, and glucose and (ii) study of the formed volatile byproducts. Various sugars were studied because they derive from different raw materials, such as molasses (sucrose) and raisin extracts (glucose and fructose). Furthermore, the operational stability was examined through repeated batch fermentations at various ISC.

Before these experiments, comparative fermentations were performed with DC material supported kefir, kissiris supported kefir, and free cells of kefir yeast. The results are presented in Figure 1 and were determined to evaluate the behavior of kefir yeast immobilized in promising organic and inorganic supports. The reason these supports were chosen was that DC material and the foaming mineral kissiris satisfy most of the prerequisites for a cost-effective immobilization. Figure 1 shows that the fastest fermentation of glucose was obtained by DC material supported kefir. This justifies the detailed study of glucose, fructose, and sucrose fermentation by DC material supported kefir yeast.

The production of alcohol and the effect of ISC are presented in Table 1. This table shows that DC material supported kefir yeast produced maximum ethanol concentrations (v/v) of 8.2% on fructose, 7.6% on sucrose, and 7.0% on glucose. Therefore, the biocatalyst showed effectiveness in repeated batch fermentations, in all sugars and ISC that were studied. This is indicated by the relative operational stability that was obtained from batch to batch in all cases that were studied. This operational stability can be proved by the stability of fermentation time and productivity, as well as by yield, residual sugar, ethanol concentration, and conversion. Yield was increased as the ISC increased. Glucose fermentation was faster than that of fructose and sucrose, whereas fructose fermentation rates were higher than those obtained by using sucrose. This is proved by the kinetic parameters and especially by the kinetics of fermentations presented in Figure 2.

Because potable alcohol is employed as the raw material to produce various kinds of alcoholic drinks

Table 1. Effect of ISC on the Kinetic Parameters of Fermentation of Various Carbohydrates by DC Materials Supported Kefir Yeast

carbohydrate	ISC (g/L)	repeated batch fermentation	fermentation time (h)	EtOH concn (% v/v)	residual sugar (g/L)	yield (g/g)	EtOH productivity (g/L/h)	conversion (%)
glucose	50	1	6.3	0.6	1.2	0.10	0.75	98
		3	4.3	0.5	1.2	0.08	0.92	98
		5	6.0	0.4	0.2	0.06	0.53	100
		7	4.3	0.8	0.2	0.13	1.47	100
	70	1	5.3	1.2	1.6	0.14	1.79	98
		3	4.8	0.7	5.3	0.10	1.16	92
		5	4.0	0.8	2.8	0.09	1.58	96
		7	4.3	1.1	2.6	0.13	2.03	96
	120	1	7.0	2.0	3.7	0.14	2.26	97
		3	7.0	4.2	3.8	0.29	4.75	97
		5	7.0	4.4	6.4	0.31	4.98	95
		7	7.0	3.7	4.0	0.25	4.19	97
	170	1	10	6.6	11.2	0.33	5.23	93
		3	8.5	5.4	7.8	0.26	5.03	95
		5	9.0	6.5	0.3	0.30	5.72	100
		7	10	7.0	2.4	0.33	5.54	99
fructose	50	1	25	2.4	1.3	0.39	0.76	97
		3	28	1.0	1.6	0.16	0.28	97
		5	29	1.1	1.1	0.18	0.30	98
		7	27	1.5	2.9	0.25	0.44	94
	70	1	36	1.5	3.2	0.18	0.33	96
		3	35	1.9	1.5	0.22	0.43	98
		5	33	1.8	2.4	0.21	0.43	97
		7	34	2.0	2.9	0.23	0.47	96
	120	1	78	5.4	4.4	0.37	0.55	96
		3	78	5.5	5.6	0.38	0.56	95
		5	75	4.8	2.8	0.33	0.51	98
		7	72	4.6	4.0	0.32	0.51	97
	170	1	115	7.7	15.7	0.40	0.53	91
		3	113	7.6	12.0	0.38	0.53	93
		5	95	7.8	7.4	0.39	0.65	96
		7	90	8.2	7.1	0.41	0.72	96
sucrose	50	1	96	1.3	2.1	0.21	0.11	96
		3	63	1.1	1.2	0.18	0.14	98
		5	30	1.5	0.8	0.24	0.40	99
		7	29	1.7	0	0.27	0.46	100
	70	1	47	2.6	1.1	0.30	0.44	98
		3	33	2.0	1.1	0.23	0.48	99
		5	34	2.7	1.2	0.31	0.63	98
		7	35	2.7	0.3	0.31	0.61	99
	120	1	56	5.9	1.8	0.40	0.83	99
		3	59	5.3	5.2	0.37	0.71	96
		5	67	4.6	8.9	0.33	0.54	93
		7	60	5.0	1.9	0.34	0.66	98
	170	1	75	7.4	8.9	0.36	0.78	95
		3	94	6.9	12.4	0.34	0.58	93
		5	112	7.6	13.1	0.38	0.54	92
		7	97	7.6	11.7	0.38	0.62	93

such as liqueurs, sweet wines, and distillates, an expansion of the study on the formation of volatile byproducts, aromatic compounds of alcoholic drinks, is also necessary. The effects of ISC on the formation of the main volatile compounds at batch fermentations of sucrose, fructose, and glucose by DC material supported biocatalyst were studied by placing the reactor in an incubator at 30 °C, studying successively the initial concentrations of 50, 70, 120, and 170 g/L. At every ISC, samples were collected after the end of fermentation and analyzed for ethanol, amyl alcohols (total amount of 2-methyl-1-butanol and 3-methyl-1-butanol), ethyl acetate, and ethanal. The results are summarized in Figures 3−5.

The concentration of amyl alcohols (total amount of 2-methyl-1-butanol and 3-methyl-1-butanol) in products obtained by batch fermentations by DC material supported kefir was higher for sucrose compared to fructose and glucose. The concentration of amyl alcohols was increased as the ISC of each sugar increased. The ethyl

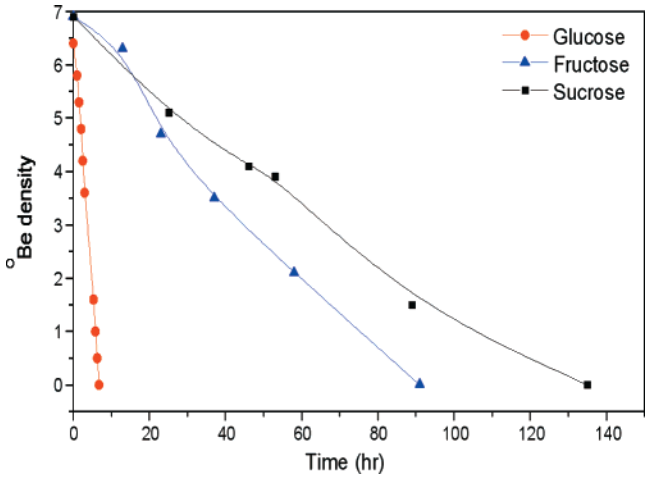

Figure 2. Effect of carbohydrates on kinetics of the alcoholic fermentation by DC material supported kefir yeast.

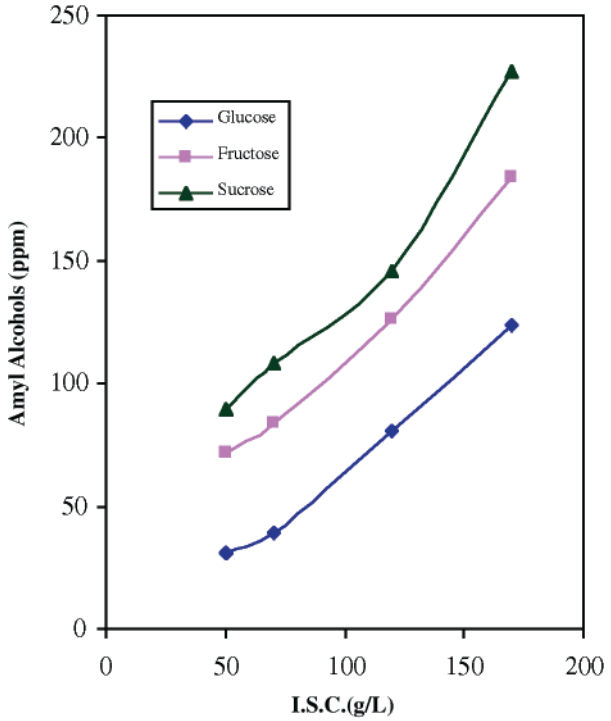

Figure 3. Effect of carbohydrates on the formation of amyl alcohols in the alcoholic fermentation by DC material supported kefir yeast.

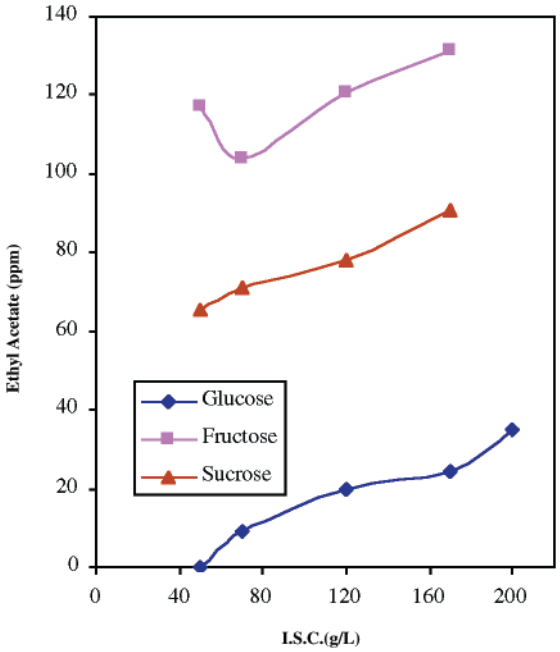

Figure 4. Effect of carbohydrates on the formation of ethyl acetate in the alcoholic fermentation by DC material supported kefir yeast.

acetate content of the products produced by batch fermentation of sucrose, fructose, and glucose by DC material supported kefir increased as the ISC increased. According to Figure 4, glucose gave the lowest concentration of ethyl acetate, fructose gave the highest, and sucrose gave values in between. The aroma of alcoholic drinks depends on the concentration of certain volatile compounds; therefore, the main volatiles formed during the fermentation were determined, and the percentage of each of them on total volatiles was calculated separately. Graphs versus ISC were plotted. The effect

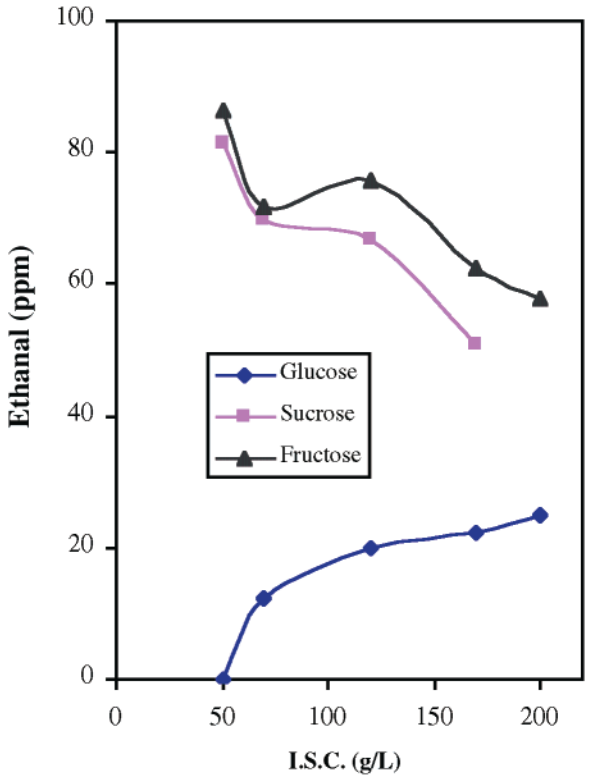

Figure 5. Effect of carbohydrates on the formation of ethanal in the alcoholic fermentation by DC material supported kefir yeast.

of ISC must be known in order to know the changes in aroma compounds when we have changes of ISC in potable alcohol or alcoholic drink production. The results are presented in Figures 6−8. Glucose fermentations resulted in products that have constant percentage contents of ethanal, ethyl acetate, and amyl alcohols on total volatiles in ISC in the range of 50−200 g/L glucose. However, fructose and sucrose fermentations lead to a decrease of ethanal and ethyl acetate and an increase of amyl alcohols as the ISC increases.

From these results, it is obvious that DC material supported kefir produces alcohol from sugars such as fructose, glucose, and sucrose, which raw materials of potable alcohol contain. The alcohol yield obtained at a relatively high ISC is close enough to that used in industrial processes, which is considered to be >0.40 g/g, and this is usually the lower limit for industrial applications. The reduced yield at low ISC is due to the cell growth of kefir yeast. This is related to low osmotic pressure. Cell growth is reduced as the ISC increases. Therefore, osmotic pressure does not favor cell growth. On the other hand, cell growth is favored by the composition of synthetic medium that is used as fermentation broth. The alcohol concentration that is obtained is of industrial level. This is a prerequisite for the industry as low alcohol contents lead to high oil demand in the alcohol distillery and therefore to high cost and environmentally unfriendly technology. Concentrations of sugars in the range of 120−170 g/L, which are considered to be of an industrial scale, resulted in ethanol production yields that sometimes are close to 0.40 g/g. This ethanol yield is relatively high, taking into account that the broth is a synthetic medium and accommodates cell growth. The difference between high ethanol yield and the low concentration of ethanol of commercial kefir drinks can to be attributed to (i) the

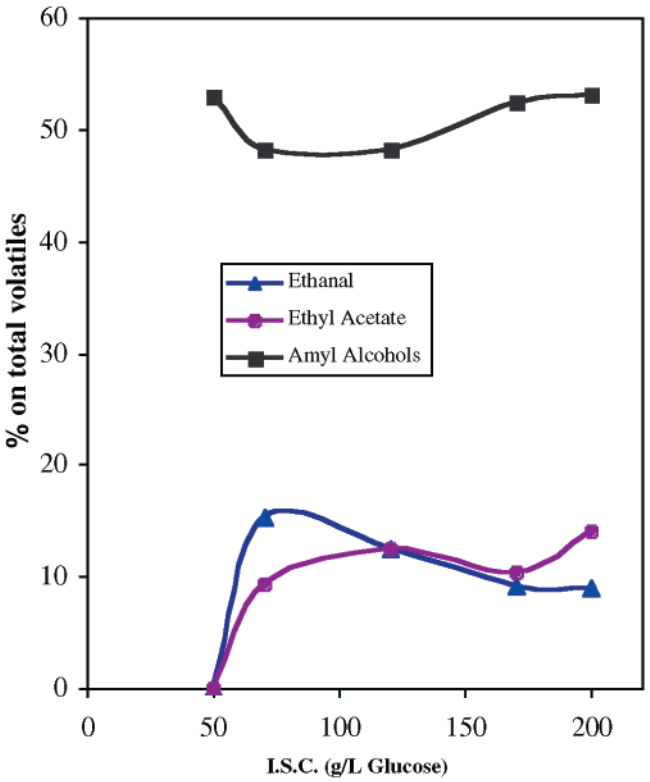

Figure 6. Effect of ISC in glucose fermentation by DC material supported kefir yeast on percent of the most important volatiles on total volatiles determined.

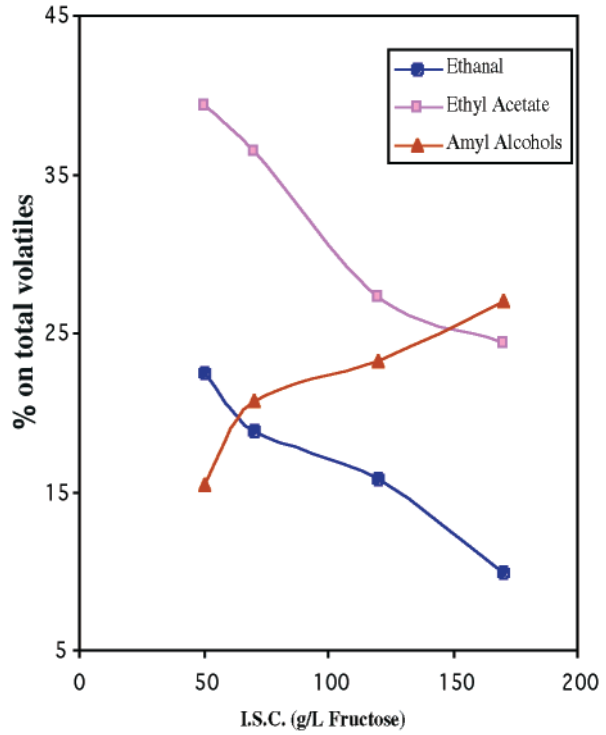

Figure 7. Effect of ISC in fructose fermentation by DC material supported kefir yeast on percent of the most important volatiles on total volatiles determined.

nature of sugar, (ii) the higher sugar concentration in the starting material, (iii) the composition of the medium, and (iv) the cell immobilization. For example, high sugar concentration inhibits cell growth of lactic bacteria, which are present in the microflora of kefir, and this leads to the reduction of lactic acid formed in the

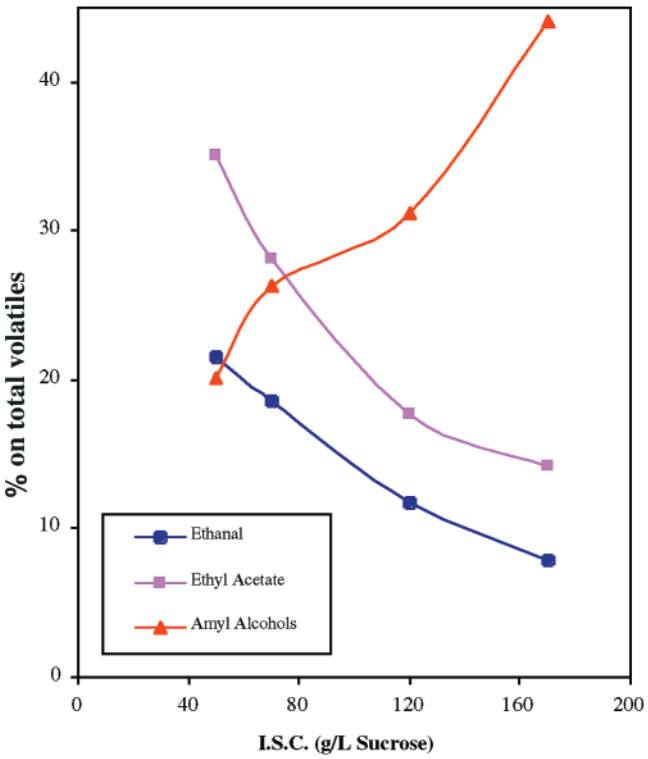

Figure 8. Effect of ISC in sucrose fermentation by DC material supported kefir yeast on percent of the most important volatiles on total volatiles determined.

kefir drink. The increase in ethanol yield can be also attributed to the fact that DC materials promote alcoholic fermentation, as was reported by Iconomou et al. (*20*).

Furthermore, DC material supported kefir, as does DC material supported *S. cerevisiae* (*21*), forms from all industrial sugars (sucrose, fructose, glucose) the main volatile byproducts such as ethanal, ethyl acetate, amyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol), 1-propanol, and isobutanol. This means that the organoleptic character of the drinks that will be produced should be close to that of traditionally used yeasts. The increase of the concentration of volatile byproducts as the ISC increased may be due to physiological factors of yeast cells because of the higher osmotic pressure that is created. However, even though the concentration of volatile byproducts is increased by the increase of ISC, mainly the percentage of each volatile compound on total volatiles remains constant or is decreased. The decrease of the percentage of ethyl acetate and the increase of the percentage of amyl alcohols on total volatiles in the case of fructose does not agree with the fine aroma of the fermented liquid that was observed by the researchers. This may be due to the high percentages of ethyl acetate on total volatiles that were determined, which were in the range of 25−40%, as well as other compounds that are formed. This requires further research by using GC-MS analysis. However, the formation of ethyl acetate is considered to be of the same level in all sugars and clearly higher than the concentrations obtained by traditional wine yeasts, which at 7 °C were found to be <10 mg/L (*21*, *22*).

The DC material used in this investigation seems to be a convenient support for the industrialization of cell immobilization because it satisfies prerequisites of abundance in nature, low cost, food grade purity, inability to be destroyed in the bioreactor (as are

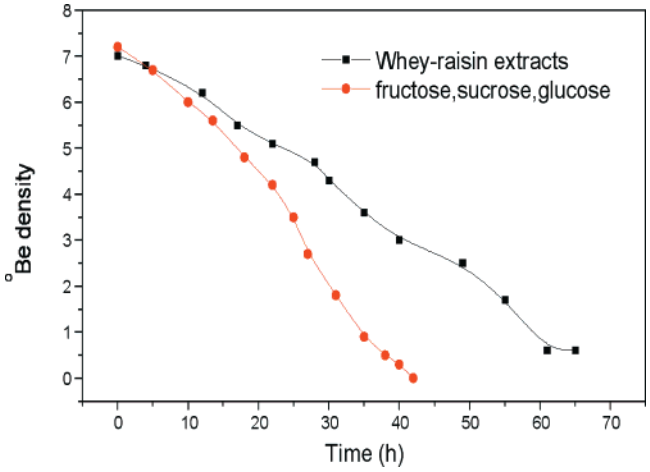

Figure 9. Fermentation kinetics observed using DC material supported kefir yeast in mixtures of fructose, sucrose, and glucose as well as whey with raisin extracts.

alginate beads), and ease of handling on an industrial scale due to the fact that it is light and porous.

The industries may use raw materials that are the source of more than one carbohydrate. Consequently, it was quite interesting to study fermentations of mixtures of fructose, sucrose, and glucose, as well as mixtures of whey with raisin extracts using DC material supported kefir. Figure 9 shows the efficiency of this biocatalyst to ferment these mixtures. The alcohol concentration achieved was 5.9% v/v, whereas the residual sugar was 2.3 g/L. Mixtures of whey and raisin extracts demanded greater fermentation time and gave lower alcohol, 4.4% v/v. In comparison to fermentations of fructose and sucrose, mixtures were fermented more quickly. This is attributed to the significantly lower fermentation time of glucose and shows that the biocatalyst is more suitable for raw materials that contain glucose. Fermented products obtained from mixtures of whey with raisin extracts had a fine aroma. This could be considered for the production of a novel drink product.

Finally, the kinetic parameters obtained for fructose, glucose, sucrose, and their mixtures could be accepted for consideration for scale-up of an industrial fermentation process for potable alcohol production. Contents of the main volatile byproducts that are formed by DC material supported kefir are of the same level of those produced by DC material supported *S. cerevisiae* (*21*). The above results also show that DC material supported kefir yeast could be employed for alcoholic drink production using mixtures of raisin extract with whey.

LITERATURE CITED

(1) Swings, J.; De Ley, J. The Biology of *Zymomonas*. *Bacteriol. Rev*. **1977**, *41*, 1−46.
(2) Rogers, P. L.; Lee, K. J.; Skotnicki, M. L.; Tribe, D. E. Ethanol production by *Zymomonas mobilis*. *Adv. Biochem. Eng.* **1982**, *23*, 37−84.
(3) Luis, A.; Lopez, E.; Lema, C. Microflora present in Kefir grains of the Galician region. *J. Dairy Res.* **1993**, *60*, 263−267.
(4) O'Leary, V. S.; Sutton, C.; Bencivengo, M.; Sullivan, B.; Halsinger, V. H. Influence of lactose hydrolysis and solids concentration on alcohol production by yeast in acid whey ultrafiltrate. *Biotechnol. Bioeng.* **1977**, *19*, 1689−1702.
(5) Gawel, J.; Kosikowski, F. Improving alcohol fermentation in concentrated ultrafiltration permeates of cottage cheese whey. *J. Food Sci.* **1978**, *43*, 1717−1719.
(6) Guillaume, M.; Moulin, G.; Galzy, P. Alcohol production by yeast in whey ultrafiltrate. *Biotechnol. Bioeng.* **1980**, *22*, 1277−1281.
(7) Marwaha, S. S.; Kennedy, J. F.; Seghal, V. K. Simulation of process conditions of continuous ethanol fermentation of whey permeate using alginate entrapped *Kluyveromyces marxianus* NCYC 179 cells in a packed-bed reactor system. *Process Biochem*. **1988**, *24*, 17−22.
(8) Marchant, R.; Nigam, P.; Brandy, D.; McHale, L.; McHale, P. Ethanol production at 45 °C by *Kluyveromyces marxianus* IMB3 immobilized in magnetically responsive matrices. *Biotechnol. Lett.* **1996**, *18*, 1213−1216.
(9) Hahn-Haegerdal Baerbel. Enzymes coimmobilized with microorganisms. The bioconversion of whey permeate to ethanol with *β*-galactosidase and *Saccharomyces cerevisiae*. *Appl. Biochem. Biotechnol.* **1982**, *7*, 43−45.
(10) King, V. A.; Zall, R. R. Ethanol fermentation of whey using calcium alginate entrapped yeasts. *Process Biochem*. **1983**, *18*, 17−22.
(11) Gianneto, A.; Berruti, F.; Glick, B.; Kempton, A. The production of ethanol from lactose in a tubular reactor by immobilized cells of *Kluyveromyces fragilis*. *Appl. Microbiol. Biotechnol.* **1986**, *24*, 277−281.
(12) Sreekrishna, K.; Dickson, R. C. Construction of strains of *Saccharomyces cerevisiae* that grow on lactose. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7909−7913.
(13) Poro, D.; Martegani, E.; Ranzi, B. M.; Alberghina, L. Lactose/whey utilization and ethanol production by transformed *Saccharomyces cerevisiae* cells. *Biotechnol. Bioeng.* **1992**, *39*, 799−805.
(14) Roukas, T.; Lazarides, H. N. Ethanol production from deproteinized whey by *β*-galactosidase coimmobilized cells of *Saccharomyces cerevisiae*. *J. Ind. Microbiol.* **1991**, *7*, 15−18.
(15) Athanasiadis, I.; Boskou, D.; Kanellaki, M.; Koutinas, A. A. Low temperature alcoholic fermentation by delignified cellulosic materials supported kefir yeast. *J. Agric. Food Chem*. **1999**, *47*, 4474−4477.
(16) Bardi, E.; Koutinas, A. A. Immobilization of yeast on delignified cellulosic material for room temperature and low-temperature wine making. *J. Agric. Food Chem*. **1994**, *42*, 221−226.
(17) Kana, K.; Kanellaki, M.; Psarianos, C.; Koutinas, A. A. Ethanol production by *Saccharomyces cerevisiae* immobilized on mineral kissiris. *J. Ferment. Bioeng.* **1989**, *68* (2), 144−147.
(18) Bakoyiannis, V.; Kanellaki, M.; Kaliafas, A.; Koutinas, A. A. Low temperature wine making by immobilized cells on mineral kissiris. *J. Agric. Food Chem*. **1992**, *40*, 1293−1296.
(19) Koutinas, A. A.; Gourdoupis, C.; Psarianos, C.; Kaliafas, A.; Kanellaki, M. Continuous potable alcohol production by immobilized *Saccharomyces cerevisiae* on mineral kissiris. *Appl. Biochem. Biotechnol.* **1991**, *30*, 203−216.
(20) Iconomou, L.; Psarianos, C.; Koutinas, A. A. Ethanol fermentation promoted by delignified cellulosic material. *J. Ferment. Bioeng.* **1995**, *79* (3), 294−297.
(21) Bardi, E.; Koutinas, A. A; Psarianos, C.; Kanellaki, M. Volatile by-products formed in low-temperature wine-making using immobilized yeast cells. *Process Biochem*. **1997**, *32* (7), 579−584.
(22) Bakoyiannis, V.; Kana, K.; Kaliafas, A.; Koutinas, A. A. Low-temperature wine making by kissiris supported biocatalyst: volatile by-products. *J. Agric. Food Chem*. **1993**, *41*, 465−468.

Received for review May 31, 2000. Revised manuscript received October 12, 2000. Accepted October 29, 2000. We acknowledge financial support from the Greek Government (General Secretariatof Research and Technology, Program EPET II) in the frame of EU social funds. A.I. thanks the Institute of Governmental Scholarships (I.K.Y.) for financial support.

JF0006628