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A Qualitative Outline to Industrialize Alcohol Production by Catalytic Multistage Fixed Bed Tower (MFBT) Bioreactor

A. A. KOUTINAS,* V. BAKOYIANIS, T. ARGIRIOU, M. KANELLAKI, AND S. VOLIOTIS

Department of Chemistry, University of Patras, Greece

ABSTRACT

The possibility for industrialization of the catalytic Multistage Fixed Bed Tower (MFBT) bioreactor using the mineral kissiris as a promoting material is examined in a large-scale pilot plant using 7000 and 100,000 L bioreactors separately. The stability of kissiris' structure, the viability of culture, and the formation of volatile compounds were studied from batch to batch. This mineral, being a volcanic and foaming silicious rock with an average cost of $60/t, was examined by X-ray defractometer and found to mainly consist of anorthite. The stability of the catalyst not being infected was studied by an electron microscope and the viability of culture was estimated by the percentage of dead cells and the percentage of bacteria. Therefore, viability of culture was at a good level and the structure of catalyst, as related with low-residual sugar, remained constant from batch to batch, even after a preservation process of at least 6 mo. Likewise, ethyl-acetate was increased and higher alcohols were decreased in the overall period of experiments.

Index Entries: Industrialization; alcohol; catalytic; bioreactor.

INTRODUCTION

Molasses is one of the world's raw main materials for potable alcohol production. Free-cell systems using traditional *Saccharomyces cerevisiae* continue to be the unique methodology in the industry, although considerable attention has been paid to cell immobilization in recent research. However, recent reports have referred to the improvement of alcohol productivity and distillation requirements in molasses fermentation using free cells by

*Author to whom all correspondence and reprint requests should be addressed.

the presence of the mineral kissiris *(1)*, a volcanic and foaming silicious rock with an average cost of 60$/t. Therefore, research was undertaken in Greece for the industrial-scale development of a process for molasses fermentation—using kissiris as a promoting material—that would increase the fermentation rate as well as final ethanol concentration. This research was financed by the European Union and the Greek government. In this research, regeneration *(2)* of this catalyst, and development of a catalytic multistage fixed bed tower (MFBT) bioreactor *(3)* of 100,000 L capacity were studied. Regeneration of catalyst was essential because laboratory results showed the activity to be reduced after a few repeated batch fermentations using the same amount of kissiris from batch to batch. Particle size of this mineral varies in the range of 1.5–2.5 cm and no pressure-drop problems were created when the bioreactor was filled by an up-flow stream.

Our experience with molasses alcoholic fermentation in the presence of kissiris in installed industrial scale pilot plants has shown:

1. excellent operational stability of the reactor for many batches;
2. successful regeneration of catalyst by hot-water treatment;
3. necessary substitution of the amount of kissiris which corresponds to its destruction from batch to batch; and
4. a 30% higher final ethanol concentration as compared with free-cell systems.

Likewise, the formation of volatile by-products in fermentations performed in the laboratory by free and immobilized cells in molasses fermentation were reported *(4,5)*. It has recently been reported that volatiles formed in the continuous wine making by 4-L capacity bioreactor contained immobilized yeast cells on kissiris *(6)*. Although chemical elements contained in the kissiris have been reported in recent publications *(7)*, the chemical and crystal structure of this mineral has not been examined in detail. Because of the aforementioned results, and because volatile by-products of alcoholic fermentation are mainly responsible for the flavor of alcoholic beverages *(8)*, it is apparent that the study of the volatiles formed in MFBT bioreactor is needed. The chemical and crystal structure of kissiris, as well as its effect on residual sugar analyzed in detail in industrial scale, and microbiological study of the process are also necessary. Therefore, the study of the volatile by-product formation, structure of catalyst, its effect on residual sugar, and the microbiological requirements of this industrial process are the aims of the present investigation.

EXPERIMENTAL

Materials and Methods

Fresh baker's yeast (*Saccharomyces cerevisiae*) and Greek sugar beet molasses were used in pilot-plant operations.

Quantitative determinations of by-products were made with a Shimadzu gas chromatograph GC-8A connected with an integrator C-R6A Chromatopac. Specifically, acetaldehyde, ethyl-acetate, propanol-1, isobutyl-alcohol, and amylalcohols (total amount of 2-methyl-butanol-1 and 3-methyl-butanol-1) were determined using a stainless steel column, packed with Escarto 5905 [consisting of Squalene 5%, Carbowax 300 90%, and bis(2-ethyl-hexyl)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 3-pentanol at a concentration of 0.5% v/v. Samples of 2 μL of the fermented liquid after centrifugation were injected directly into the column (e.g., without extraction).

Residual sugar was estimated analytically to its content of glucose, fructose, and sucrose. Determinations were performed by high-performance liquid chromatography (HPLC) using a Shimadzu Liquid Chromatograph with LC-9A high-pressure pump, RID-6A Refractive Index Detector, and CTO-10A Column Oven for temperature control of column SCR-101N (packed with a cation exchange resin—sulfonated polystyrene-divinylbenzene copolymer). The mobil phase was deionized, distilled as well as water filtrated and the diluted air was removed by passing helium. The flow rate was 0.8 mL/min; column temperature was controlled at 60°C. The chromatographs were obtained by a Shimadzu CR6-A Chromatopac integrator and results were obtained by using calibration curves designed for each sugar analyzed. Pentanol-3 was used as an internal standard.

Biomass, obtained by centrifugation of samples and cell mass as pressed yeast, was determined by weight. Biomass productivity (BP) is expressed in grams dry weight per liter, daily produced. Biomass yield factor (BYF) is the ratio of grams dry weight biomass per grams of sugar utilized. Cell concentrations were given in grams of biomass wet weight per liter. Infection was calculated as percent content of bacteria cells on the cells of *Saccharomyces cerevisiae*, both determined in every sample by a microscope. Likewise, dead cells were calculated as percent content of dead yeast cells on total cells, determined by microscopic study.

Pilot Plant Operations

Fermentations were performed by two pilot plants. The first was installed with a 7000 L two-stage, fixed-bed bioreactor and the second with a 100,000 L capacity-catalytic MFBT bioreactor. All installations were made at the factory of the alcohol distillery B. G. Spiliopoulos S.A., Patras, Greece. The design of bioreactors and plants as well as the description of processes were conducted as previously described *(3)*.

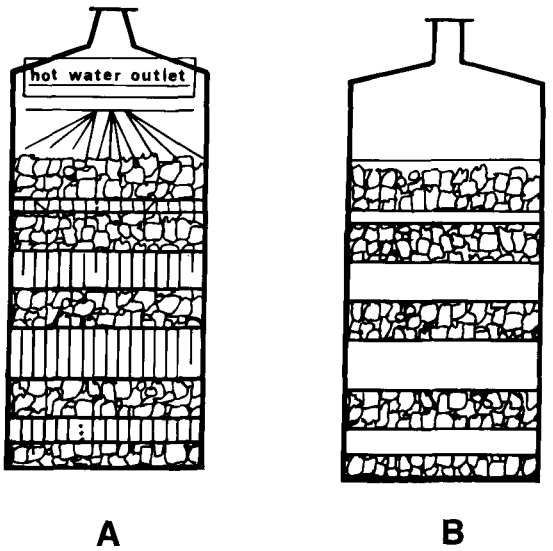

Fig. 1. Layered kissiris in the MFBT bioreactor of 100,000 L. **(A)** The layered kissiris is regenerated or is preserved by hot water treatment. **(B)** Layered kissiris during fermentation.

Preservation of the System

In order to conserve the system of bioreactor containing layered kissiris (Fig. 1) and to protect it from infections during the time when the plant does not operate, the following treatment was performed. The bioreactor of 100,000 L was pumped with 5,000–6,000 L water at 70°C. This water was then circulated for at least 30 min with the bioreactor system creating a shower bath on the surface of the kissiris. This treatment was repeated once every week for a period of 5 mo.

Structure of the Catalyst

The structure of kissiris and its stability during the process was studied by X-ray powder diffraction patterns using a NONIUS PDS 120 X-ray powder diffraction system with a JCPDS database.

Electron Microscope Study

Two samples of kissiris were used. One was obtained after 54 repeated fermentation batches, after which the bioreactor was left untreated throughout the preservation process, a period of at least 6 mo. The other sample was obtained from the 100,000 L bioreactor after 10 repeated batch fermentations, remained and treated by the preservation process for 6 mo also. These samples were studied by an electron micro-

scope. The materials were individually coated with gold in a BAL-Tech SCD 004 sputter coater under argon pressure 10^{-2} mBar so as to obtain an increase of the electron conductivity. The prepared samples were studied in a Jeol JSM-5200 Scanning Electron Microscope.

RESULTS AND DISCUSSION

Structure of Kissiris and Stability

Because kissiris is a promoter *(1,3)* of molasses alcoholic fermentation, a more detailed study of structure and its stability during the process was necessary. Therefore, the starting material and samples of kissiris during the 54 repeated batch fermentations were analyzed by X-ray powder diffraction methods. These patterns indicated that the main component is the mineral anorthite $(Ca,Na)(Al,Si)_2Si_2O_8$, but can hardly identify which of the five different forms, existing in the JCPDS database, it concerns. The first results showed no real modifications from batch to batch concerning the chemical constitution and structure of the crystal phase of the catalyst. Moreover, this point is related and confirmed by the stability of the catalytic effect *(3)*.

Occasionally, alcoholic beverage factories must slow or stop their activities and production. A stop in production will lead to infection of the catalyst, as shown in Fig. 2B and C. A negative point for the industrialization of using the catalytic MFBT bioreactor is the cost and difficulties of charging and emptying. If the bioreactor is emptied and charged with kissiris every year, the ethanol-production cost is increased by 0.3%. It is obvious that when the time is reduced to a month except of the cost increase there are problems handling kissiris. Likewise, it is obvious that since time is increased more than a year, cost is not affected and handling problems are significantly diminished. In the last case, a cost decrease of 10–20% is obtained by the increase of the ethanol productivity and final alcohol concentration. Consequently, a preservation process with very low cost is needed to increase further the time of using the same amount of kissiris. A relative process is presented in the experimental part of the paper and after applying for a long time resulted not to have infected the catalyst, as presented in Fig. 2A, in comparison with a non-treated sample (Fig. 2B,C). This does not mean that this treatment refers to aseptic processing but it preserves the system for as long as needed for the industrialization.

Viability of Culture

The operational stability of the system from batch to batch is related with the possibility of yeast culture infection as well as by the viability of yeast cells. Likewise, yield in ethanol production is dependent on cell growth and residual sugar. To prove that the process is suitable for indus-

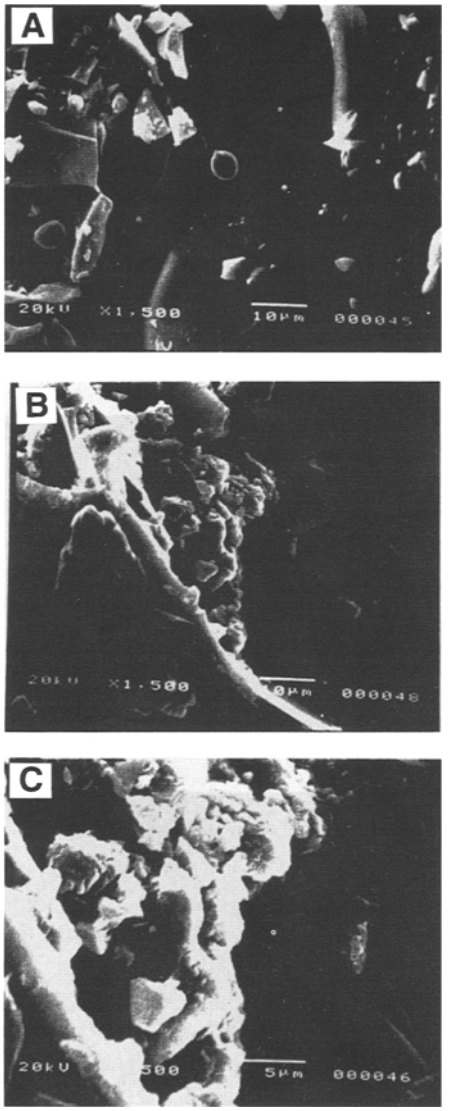

Fig. 2. Electron micrographs: **(A)** at ×1500 showing mineral kissiris after treatment by the preservation process. **(B)** at ×1500 performance of mineral kissiris without treatment and **(C)** indicates (B) at ×3500.

trial application, measurements (from batch to batch) of residual sugars, biomass kinetic parameters, percent bacteria, and percent dead cells were performed. These results are presented in Tables 1 and 2.

Table 1 shows that, for the long period of the experiments, cell growth was lower than 20% and BP, as well as BYF, were also low even though batch fermentations were performed. These results were confirmed in the fermentations of 100,000 L bioreactor. The relatively low cell growth in this

Table 1
Kinetic Parameters of Biomass, Infection, and Viability of Culture

Bioreactor type	Batch fermentations[a]	Initial concentration, g/L	Final cell concentration, g/L	BP, g/L/d	BYF, g/g	Bacterial, %	Dead cells, %
Two-stage	1	20	24	3.4	0.0078	5	11
fixed-bed	5	25	28	2.6	0.0051	6	12
bioreactor	7	25	28	2.0	0.0051	10	10
of	10	24	26	1.6	0.0035	10	12
7,000 L	11	22	25	2.6	0.0055	8	12
	12	23	24	0.9	0.0017	6	14
	17	22	25	1.2	0.0050	7	15
	19	20	23	1.6	0.0052	12	15
	22	23	25	1.1	0.0035	10	11
	24	20	22	1.4	0.0037	9	14
	25	21	24	1.8	0.0053	10	15
	27	21	24	1.9	0.0053	11	14
	28	20	23	2.1	0.0053	10	15
	30	22	25	1.4	0.0050	8	10
	31	20	23	1.0	0.0038	18	8
	35	35	38	1.9	0.0048	11	13
	37	33	38	0.8	0.0095	12	10
	38	26	29	4.2	0.0062	15	10
	40	25	28	2.0	0.0053	22	10
	42	27	30	2.0	0.0053	—	—
	46	20	25	2.6	0.0095	14	10
	47	26	31	2.8	0.0076	8	8
	54	27	29	1.6	0.0045	21	17
Catalytic	1	23	25	1.1	0.0035	8	9
MFBT	2	24	26	1.2	0.0033	7	10
bioreactor	3	23	25	1.1	0.0037	—	—
of	4	21	25	3.2	0.0065	8	7
100,000 L	5	21	24	1.8	0.0053	7	8
	6	18	20	1.2	0.0037	8	9
	7	21	22	1.0	0.0036	7	7

[a] The ethanol concentration was for all fermentation batches in the range 9.2–10.8% v/v.

Table 2
Residual Sugar Analyzed in Detail

Bio-reactor	Batch fermen-tations	Initial density, °Be	Glucose, g/L	Fructose, g/L	Sucrose, g/L	Residual sugar, g/L
Two stage	1	14.3	4.0	0		4.0
fixed-bed	5	16.0	1.6	0	0	1.6
bioreactor	7	15.9	1.7	0	0	1.7
of 7000 L	10	15.8	1.8	0	0	1.8
	11	15.2	3.1	0	0	3.1
	12	15.7	3.9	0	0	3.9
	17	16.6	1.6	4.9	0	6.5
	19	15.8	1.8	0	0	1.8
	22	15.8	3.3	0	0	3.3
	24	15.3	4.5	10.6	0	15.1
	25	15.7	2.9	8.7	0	11.6
	31	16.0	3.7	5.1	0	8.8
	35	16.0	1.1	0	0	1.1
	37	15.3	1.0	0	0.9	1.9
	38	15.6	1.0	0	0.9	1.9
	40	16.0	1.6	2.1	0	3.7
	42	16.1	1.5	3.5	0	5.0
	46	15.2	2.0	3.5	0	5.5
	47	15.7	2.0	2.8	0	4.8
	52	15.5	2.9	1.3	traces	4.2
	53	15.5	0.6	1.2	0	1.8
	54	16.0	0	0	0	0
Catalytic	1	15.7	1.2	0.8	0	2.0
MFBT	2	16.5	1.4	1.2	0	2.6
bioreactor	3	15.4	1.4	0.7	0	2.1
of	4	16.0	0.8	1.0	0	1.8
100,000 L	5	16.0	0.9	1.2	0	2.1
	6	14.6	0.8	1.3	0	2.1
	7	15.5	0.6	1.6	0	2.2

process can be attributed to relatively high initial °Be density and therefore to higher osmotic pressure and higher ethanol concentrations. Specifically, the ethanol concentration for all fermentation batches was found to be within the range of 9.2–10.8%. The last parameters affected low percent bacteria obtained in yeast cultures after the end of fermentations. However, although the final ethanol concentrations *(3)* were 30–40% higher than those usually obtained by free cells, the percent dead cells were at a low level. This is due to higher ethanol fermentations rates *(1,3)* obtained in the presence of kissiris as compared to those of free cells.

Residual sugar was also at a low level (Table 2) for the long period of the experiments and it was also confirmed by the industrial scale experiments (bioreactor of 100,000 L). Although final ethanol concentrations and initial °Be densities were higher than those obtained by free cells in industrial practice, residual sugar was at about the same level. This is of industrial importance, since it has a positive effect on the alcohol production yield. Sucrose was not found in residual sugar. This is in agreement with previous work *(8)*.

Volatile By-Products

The quality of potable alcohol is an important parameter for the market and it is dependent on volatiles formed during fermentation. To prove that the process was suitable for industrial application, analysis of volatile by-products was also necessary. Therefore, acetaldehyde, ethyl-acetate, propanol-1, isobutyl-alcohol, and amyl-alcohols were determined from batch to batch and the results are presented in Table 3. This table shows that the results were confirmed in the case of industrial scale bioreactor. More specifically, amyl-alcohols were low and about half of them obtained by free cells *(4)* and propanol-1 and isobutyl-alcohol were reduced too. In contrast, ethyl-acetate was substantially higher. These results contribute to an improved quality of the distillate, since the reduced higher alcohols and the increase of the main ester ethyl-acetate will result in a better aroma.

The results presented in this work concerned the preservation process, cell growth, infections, and viability of yeast culture as well as volatiles, and confirm the conclusion that this process can find industrial application *(3)*.

CONCLUSIONS

The main structure of kissiris is the same as anorthite. Its chemical constitution and crystal structure are not affected from batch to batch or after hot water treatment for a long period as in system preservation. The overall process and mechanical construction do not lead to any infection of the yeast culture for a long period. Finally, the process gives lower content of higher alcohol and higher concentration of ethyl-acetate. The latter is in relation to the higher final alcohol concentrations and the increase of the ethanol production rate obtained *(3)*.

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Table 3
Volatile By-Products Formed

Bio-reactor	Batch fermen-tations	Acetal-dehyde, mg/L	Ethyl-acetate, mg/L	Propa-nol-1, mg/L	Isobutyl-alcohol, mg/L	Amyl-alcohol, mg/L	Total volatiles deter-mined, mg/L
Two	1	42	55	44	37	77	255
stage fixed-	5	84	28	59	42	87	300
bed bio-	7	55	27	54	47	85	268
reactor	10	—	—	—	—	—	—
of 7000 L	11	44	35	61	34	81	255
	12	—	—	—	—	—	—
	17	126	15	49	35	76	301
	19	65	25	44	27	69	230
	22	36	17	36	29	57	175
	24	traces	18	38	18	65	139
	25	44	17	38	19	60	182
	37	92	100	37	32	52	313
	38	142	104	37	26	50	359
	40	34	92	29	24	40	219
	42	—	—	—	—	—	—
	46	32	96	19	10	26	183
	47	—	0	—	—	—	—
	52	traces	57	31	20	33	84
	53	40	131	26	12	35	170
	54	89	—	22	11	32	285
Catalytic	1	traces	58	12	19	50	139
MFBT	2	traces	56	13	18	48	135
bio-	3	135	127	18	13	53	346
reactor of	4	46	220	18	11	36	331
100,000 L	5	traces	58	22	12	38	130
	6	traces	290	19	14	35	358
	7	—	—	—	—	—	—

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