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Fermentation Efficiency of Cells Immobilized on Delignified Brewers' Spent Grains after Low- and High-Temperature Thin Layer Thermal Drying

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Abstract Low-cost dried yeasts immobilized on delignified brewers' spent grains for use in wine making and brewing were produced by simple thermal drying techniques. To optimize the thermal drying process, vacuum and air stream conditions were examined. Drying of thin layers of the biocatalysts was performed at low (30–38 °C) and high temperatures (40–70 °C). The fermentation efficiency of the thermally dried biocatalysts was acceptable, with immobilized cells showing a significantly higher thermotolerance compared with free cells. Immobilized cells dried at high temperatures presented slightly improved glucose fermentation efficiency compared with the low-temperature dried biocatalysts. Gas chromatography–mass spectrometry analysis of aroma volatiles of the fermented products revealed an increase of esters, lower higher alcohol formation, and significantly lower concentration of carbonylic compounds.

Keywords Thermal drying · Brewers' spent grains · Yeast · Immobilization · Volatiles

Introduction

Drying is a highly energy-intensive process with total cost largely determined by the cost for energy consumption, which can be minimized by the application of maximum-efficiency heating techniques [1]. Extended shelf-life of dried microorganisms can be achieved by reducing the moisture content to levels below those required for metabolic activity. This information is also essential for modeling, designing, and optimizing the drying process, evaluating storage stability and microbiological safety, determining moisture changes during storage, and selecting appropriate packaging materials [2]. Thermal drying of microorganisms is more attractive than freeze-drying due to its lower cost. In this respect, low-temperature thermal drying of yeast was recently reported [3], resulting in accepted fermentation efficiency of the dried cells. Immobilization of yeast on solid carriers (e.g., delignified cellulosic materials, gluten pellets, dried fruit, brewery solid wastes, etc.) exhibited a protective effect on cell viability and

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operational stability, resulting in feasibility of extremely low-temperature fermentations [4–9]. Therefore, the investigation for production of such immobilized biocatalysts, easy to preserve and commercialize, was necessary, and freeze-drying was a successful first approach [5, 6]. However, the cost for freeze-drying both for energy demand and equipment is high, and therefore, the replacement of such expensive processes with lower-cost simple thermal drying methods in the industry of dry culture production is necessary. The stability of cells during thermal drying, according to the current data, may possibly be higher if they are immobilized on a solid support (such as cellulosic material), and this is evaluated in the present study.

Brewer's spent grains (BSG) are a solid agro-industrial waste material produced after the mashing stage of the brewing process. BSG generated as wastes by the member countries of the European Community are millions of tons annually, but their utilization still remains limited. Many researchers have focused on alternative uses of this agro-industrial by-product, including evaluation as raw material for food or feed production, energy production, and other biotechnological applications [10, 11]. Specifically, the use of fresh (nontreated) or delignified BSG (DBSG) as solid supports for yeast immobilization led to the production of biocatalysts highly efficient for alcoholic fermentation processes, even at very low temperatures [8, 9, 12, 13]. Therefore, to combine the need for both utilization of BSG and production of commercial biocatalysts efficient for alcoholic fermentation processes at extreme conditions, an investigation on a simple technique for low-temperature thermal drying of yeast immobilized on DBSG was conducted. Specifically, in the present study, DBSG were used as support for the immobilization of the cryotolerant and alcohol-resistant strain *Saccharomyces cerevisiae* AXAZ-1, and thermal drying of the biocatalyst in a thin layer and in a broad range of temperatures was examined. The fermentation efficiency of the dried biocatalyst as well as of dried free yeast cells was evaluated. The development of such commercial dried biocatalysts is expected to produce added value, provided that their production is cost-effective.

Materials and Methods

Yeast Strain and Fermentation Media

S. cerevisiae AXAZ-1 was an alcohol-resistant and cryotolerant strain isolated from an agricultural wine producing area in Greece [14]. It was grown in medium containing (in percent weight per volume): 0.1 $(NH_4)_2SO_4$, 0.1 KH_2PO_4, 0.5 $MgSO_4 \cdot 7H_2O$, 0.4 yeast extract, and 4.0 glucose monohydrate, and harvested by centrifugation at 5,000 rpm for 10 min (wet-weight biomass of 70% moisture). Media containing the above composition of salts and 18% *w/v* glucose were used for alcoholic fermentation experiments. All media were sterilized at 130 °C and 1–1.5 Atm for 15 min.

Preparation of the Support and Immobilization of Yeast

BSG were obtained from the Athenian Brewery S.A. They contained 79.2% *w/w* moisture, 25.6% *w/w* crude protein (on dry matter), 2.5°P remaining extract (washable), and had pH 6.0. Delignification of BSG was carried out as described by Kopsahelis et al. [8]. In brief, 170 g of BSG were mixed with 1.7 L of 1% *w/v* NaOH solution and were heated for 3 h at 80 °C with continuous stirring. After that, the DBSG were collected, washed well with water, and sterilized by autoclaving at 120 °C for 10 min. Yeast immobilization on DBSG was carried out by suspending 16 g wet *S. cerevisiae* AXAZ-1 cells in 800 mL of glucose synthetic medium (12% *w/v*) and mixing with 170 g DBSG. Soft mixing was achieved by the produced CO_2 during

fermentation. The immobilization process lasted 6–8 h until all sugar was utilized. The liquid was then decanted and the biocatalyst washed twice with 200 mL of fresh glucose medium.

Enumeration of Immobilized Yeast Cells

In the used immobilization process, 16 g of *S. cerevisiae* AXAZ-1 were used per 170 g DBSG. Therefore, 0.94 g of yeast corresponded to 10 g of support. When the immobilization process was completed, 10 g of the biocatalyst was blended with 90 mL of sterile Ringer's solution. Similarly, amounts of 0.55, 0.56, 0.57, 0.58, 0.59, 0.60, 0.61, and 0.62 g of free yeast cells were diluted in 90 mL Ringer's solution and successive dilutions were prepared from each solution. The enumeration of viable cells (in colony-forming units), immobilized or free, was made on malt agar with the following (in percent weight per volume) composition: 4 glucose, 0.4 yeast extract, 0.1 $(NH_4)_2SO_4$, 0.1 KH_2PO_4, and 0.5 $MgSO_4 \cdot 7H_2O$, after incubation at 30 °C for at least 48 h. The number of cells immobilized on DBSG corresponded to 0.59 g of free cells. Consequently, an amount of 0.59 g out of the 0.94 g of cells that were used initially was immobilized per 10 g of DBSG.

Drying of the DBSG–Biocatalyst in Thin Layer

Amounts of 170 g of the DBSG–biocatalyst were placed in thin layers on glass plates and drying was performed using three different drying methods at different temperatures (30, 35, and 38 °C). Specifically, drying was implemented by: (a) simple placement in a drying oven, (b) by air stream, and (c) under vacuum. Furthermore, drying experiments were carried out at relatively high temperatures of up to 70 °C. Drying kinetics were performed by monitoring the weight loss at various time intervals, until constant weight. The average percentage of weight loss was 85%.

Storage

Dried *S. cerevisiae* AXAZ-1 immobilized on DBSG was placed in a receptacle and stored at 4 °C for 4 months. Subsequently, dried samples were used for repeated batch fermentations of glucose solutions to evaluate the effect of storage time on their activity.

Fermentations

Repeated batch fermentations of 400 mL glucose media using the dried biocatalysts were performed at 15 °C in 1-L bioreactors. Experiments were carried out in triplicate. Repeated batch fermentations using the dried biocatalysts after storage were also performed at 15 °C. At the end of each fermentation batch, the liquid was collected and the biocatalyst was washed twice with fresh glucose medium and was used for a subsequent fermentation batch. Fermentation kinetics were monitored by measuring the °Be density at various time intervals. The fermented liquids were analyzed for ethanol, residual sugar, and headspace volatiles by solid-phase microextraction (SPME) gas chromatography–mass spectrometry (GC-MS).

Assays

Ethanol concentration and residual sugar were determined by GC and high-pressure liquid chromatography (HPLC), respectively. Ethanol was determined on a Shimadzu GC-8A Gas Liquid Chromatograph system with a Porapac-S column and N2 as carrier gas (40 mL/min).

The column temperature was 130 °C increased by 10 °C/min. The temperatures of the injector and flame ionization detector were 210 and 220 °C, respectively. Samples of 2 μL were injected directly into the column. All determinations were performed by means of standard curves. Residual sugar was determined by HPLC, on a Shimadzu LC-9A Liquid Chromatograph, consisting of a SCR-101 N stainless steel column, a LC-9A pump, a CTO-10A oven (60 °C), and a RID-6A refractive index detector. Three times distilled and filtered water was used as mobile phase (0.8 mL/min) and butanol-1 was used as internal standard. Amounts of 0.5 mL of sample and 2.5 mL of 1% *v*/*v* solution of butanol-1 were diluted to 50 mL, and 40 μL of the final solution was injected into the column after filtration through 0.2 μm microfilters. Residual sugar (grams per liter) and ethanol (in percent volume per volume) concentrations were calculated using standard curves. Ethanol productivity was calculated as grams of ethanol per liquid volume produced per day (grams per liter per day).

Headspace SPME-GC-MS

The headspace aroma volatiles of the fermented samples were determined by means of GC-MS. A SPME sampling method was used, employing a 2-cm fiber coated with 50/30 mm divinylbenzene/carboxen on poly(dimethylsiloxane) and bonded to a flexible fused silica core (Supelco, Bellefonte, PA, USA). The conditions of headspace SPME sampling were: 10 mL liquid sample, 2.2 g NaCl, and internal standard (4-methyl-2-pentanol) were transferred into a 20-mL headspace vial, which was sealed and shaken for 5 min at 60 °C. The SPME fiber was then exposed to the headspace for 45 min. Desorption of volatiles took place in the GC injector in splitless mode at 240 °C for 3 min. The fiber was exposed to the injection port between assays for 5 min to remove any volatile residues. GC-MS analysis was performed on a Shimadzu GC-17A chromatograph coupled to a Shimadzu MS QP5050 mass spectrometer. Helium was used as carrier gas (1.8 mL/min). Separation of compounds was performed on a Supelcowax-10 capillary column (60 m/0.32 mm i.d./0.25 μm film thickness). The oven temperature was programmed at 35 °C for 5 min and then raised to 60, 200, and 250 °C with a rate of 2, 5, and 25 °C/min, respectively. The injector and interface temperature was 240 °C. The MS scan range was 45–400 *m*/*z*. Identification of compounds was done by comparing (a) the linear retention indices of even *n*-alkanes (C10–C24) with those of standard compounds and the retention indices reported in the literature and (b) the MS data with those of standard compounds and those in Wiley and NIST libraries. Semiquantitative analysis was performed by dividing the peak area of a compound with the peak area of the internal standard and multiplying the result with the concentration of the internal standard (1.62 mg/L).

Scanning Electron Microscopy

Dried samples were coated with gold in a Balzers SCD 004 sputter coater (Oerlikon-Balzers, Balzers, Leichtenstein) for 3 min, to increase electron conductivity, and then studied in a Jeol model JSM-6300 scanning electron microscope (JEOL Ltd., Tokyo).

Results and Discussion

Rationale

The development of low-cost, simple, and mild drying techniques is essential for commercialization of biocatalysts such as the one proposed in the present study (yeast

immobilized on BSG). Drying ensures increased shelf-life, easier handling and transportation, lower weight, and protection from contamination/spoilage, so that the dried biocatalysts can be produced commercially and be available for various applications (from small to industrial scale). Since drying can affect the viability, activity, and shelf-life of the biocatalyst, as well as have an impact on final product quality, it is important to evaluate all these attributes. From the experience gained through extended research efforts on cells immobilized on natural lignocellulosic carriers for use in alcoholic or lactic acid fermentation processes, it has been shown that the delignified form of the carriers is far more efficient than the nontreated ones due to: (a) removal of lignin, which can negatively affect product quality and be toxic to cells, and (b) increase of porosity, which allows more cells to be immobilized. This is why the present work focused on the use of the delignified form of BSG, although previously it was found that, either fresh or delignified, this material is very efficient as yeast immobilization support [8, 9].

Specifically, in this study, a biocatalyst was prepared by immobilization of the cryotolerant strain *S. cerevisiae* AXAZ-1 on DBSG and was used for alcoholic fermentation of synthetic glucose media. To investigate the potential of commercialization of the proposed biocatalyst, a simple thermal drying process at mild conditions was evaluated, as an alternative to expensive methods like freeze-drying, a common drying method in industrial applications. A recent study on low-temperature thermal drying of thin layers of free yeast cells [3] showed that the dried cultures had acceptable fermentation efficiency, retained even after storage for 1 month. Therefore, the study of thermal drying of immobilized cells was encouraged. BSG and DBSG have been previously used successfully as yeast immobilization supports for use in alcoholic fermentation processes (brewing and wine making), leading to increased productivity and good quality of final products [8, 9, 12]. Drying of the immobilized yeast biocatalyst at a broad range of temperatures was carried out to optimize the process in terms of temperature, biocatalyst activity, and cost associations.

Low-Temperature Thermal Drying of Immobilized Yeast

Drying of the cryotolerant strain *S. cerevisiae* AXAZ-1 immobilized on DBSG was done at low temperatures (30, 35, and 38 °C). The biocatalyst was spread on glass plates in the form of thin layers to accommodate drying. Drying kinetics were monitored (Fig. 1), showing that drying by air stream was almost complete in 5 h, while thermal drying under vacuum required more time. On the other hand, higher amounts of moisture were removed under vacuum (87%) compared with the other tested methods (83%), which could be further improved by higher air supply rates. In the case of air drying, the heat was transferred fast on the surface of the biocatalyst, which was rapidly dehydrated forming a crust, resulting in slower moisture loss internally. Obviously, the moisture removal by the examined drying methods was not complete. To examine if the residual moisture affected the biocatalyst, its fermentation efficiency after storage was evaluated and verified (Tables 1 and 2; Figs. 1 and 3). The moisture levels of commercial active dry yeasts are never lower than 5–8%, since irreversible damage to metabolic functions occurs by further removal of chemically bound water [15]. The electron micrographs of the dried biocatalyst (Fig. 2) show undamaged yeast cells attached on the surface of DBSG. Although thermal drying under vacuum is a more stressful method, cells do not appear to be seriously damaged (Fig. 2c), and comparing the three drying methods, the yeast cells seem to have retained better shape and cell wall integrity in the case of drying by air stream (Fig. 2b). The rate of drying in the case of DBSG was higher compared with free cells, probably due to the porous nature and increased surface of the support material.

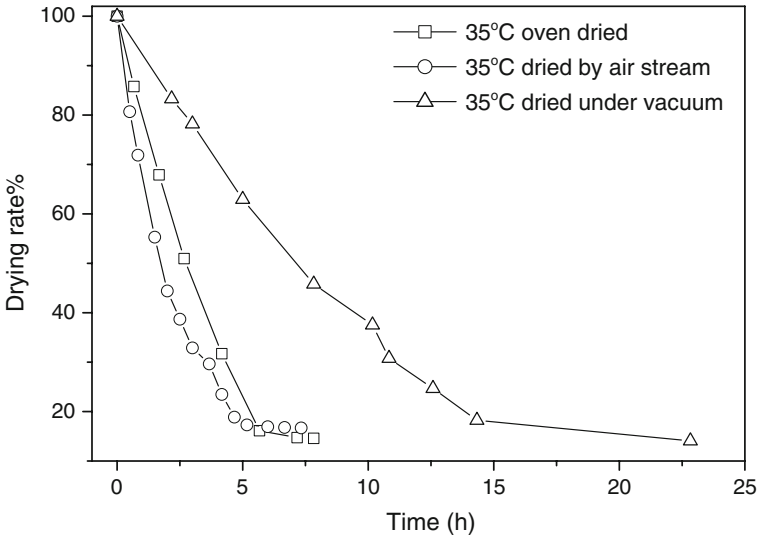

Fig. 1 Kinetics of low-temperature (35 °C) drying of yeast immobilized on DBSG (oven drying, drying by air stream, and drying under vacuum)

Fermentation Efficiency of Low-Temperature Thermally Dried Biocatalysts

Batch fermentations of glucose were carried out at 15 °C, and the fermentation activities of the oven-dried, dried by air stream, and dried under vacuum biocatalysts are shown in Fig. 3. The initiation of the fermentations required a few hours in all cases. The biocatalysts that were dried under vacuum were activated after about 60 h, while the other biocatalysts required only 10–17 h. This is also justified by the electron micrographs. The fermentations were completed in 4–7 days. The kinetic parameters of glucose fermentations are recorded concisely on Table 1. Specifically, fermentations of solutions with high initial °Be density yielded relatively high alcohol concentrations, showing suitability of the dried DBSG–biocatalyst, e.g., for wine making. The conversion of sugar to ethanol was almost complete,

Table 1 Kinetic parameters of glucose fermentations at 15 °C by yeast immobilized on DBSG: (a) oven dried, (b) dried by air stream, and (c) dried under vacuum at 30, 35, and 38 °C.

Biocatalyst	Temperature (°C)	Initial density (°Be)	Fermentation time (h)	Residual sugars (g/L)	Alcohol concentration (% *v*/*v*)	Alcohol yield (g/g)	Alcohol productivity (g/Ld)	Conversion (%)
Oven dried	30	10.0	102	0.19	9.6	0.29	11.50	99.06
	35	10.1	102	0.04	10.0	0.51	20.70	99.92
	38	10.0	116	0.02	10.0	0.51	17.93	99.99
Dried by air stream	30	10.3	139	0.10	10.1	0.53	15.94	99.59
	35	10.1	92	0.08	10.1	0.52	23.06	99.68
	38	10.1	131	0.05	10.1	0.42	13.18	99.90
Dried under vacuum	30	9.0	158	0.27	8.5	0.49	11.26	98.38
	35	9.3	119	0.22	9.0	0.52	16.29	98.81
	38	9.3	142	0.20	8.5	0.42	11.04	98.90

Table 2 Kinetic parameters of repeated batch fermentations of glucose at 15 °C by dried yeast immobilized on DBSG: (a) oven dried, (b) dried by air stream, and (c) dried under vacuum at 30, 35, 38 °C, after storage at 4 °C for 4 months.

Biocatalyst	Temperature (°C)	Batch	Initial density (°Be)	Fermentation time (h)	Residual sugars (g/L)	Alcohol concentration (% *v*/*v*)	Alcohol yield (g/g)	Alcohol productivity (g/Ld)	Conversion (%)
Oven dried	30	1	10.0	89	0.07	9.1	0.42	19.38	99.74
		2	10.0	53	0.02	10.0	0.53	40.99	99.99
		3	10.0	40	0.02	10.0	0.52	53.09	99.98
	35	1	10.0	89	0.03	10.0	0.46	21.20	99.96
		2	10.0	53	0.02	10.0	0.54	41.27	99.99
		3	11.0	45	0.21	9.7	0.33	32.49	98.94
	38	1	9.3	119	0.23	8.8	0.50	15.70	98.73
		2	9.3	47	0.30	7.9	0.40	31.64	98.23
		3	9.3	34	0.35	7.8	0.40	44.19	97.90
Dried by air stream	30	1	11.5	168	0.30	10.5	0.47	13.03	98.62
		2	10.0	65	0.15	9.5	0.40	24.83	99.43
		3	9.5	45	0.04	9.0	0.57	49.33	99.86
	35	1	10.0	116	0.04	9.9	0.48	15.96	99.84
		2	10.0	58	0.08	9.8	0.37	23.80	99.57
		3	10.0	44	0.06	9.9	0.36	25.88	99.72
	38	1	12.0	167	0.09	11.4	0.21	16.11	99.62
		2	10.0	65	0.08	9.7	0.05	33.37	99.56
		3	9.5	45	0.03	9.0	0.25	21.38	99.89
Dried under vacuum	30	1	9.3	112	0.02	9.3	0.51	17.12	99.99
		2	9.3	50	0.13	9.1	0.52	39.81	99.22
		3	9.3	48	0.11	9.2	0.48	37.92	99.35
	35	1	9.3	160	0.09	8.9	0.41	9.59	99.54
		2	9.3	65	0.12	9.2	0.46	26.75	99.27
		3	9.3	67	0.14	9.1	0.48	27.01	99.19
	38	1	9.4	138	0.06	8.5	0.22	6.17	99.73
		2	9.3	59	0.12	8.0	0.35	22.58	99.31
		3	9.3	60	0.14	7.7	0.23	14.70	99.16

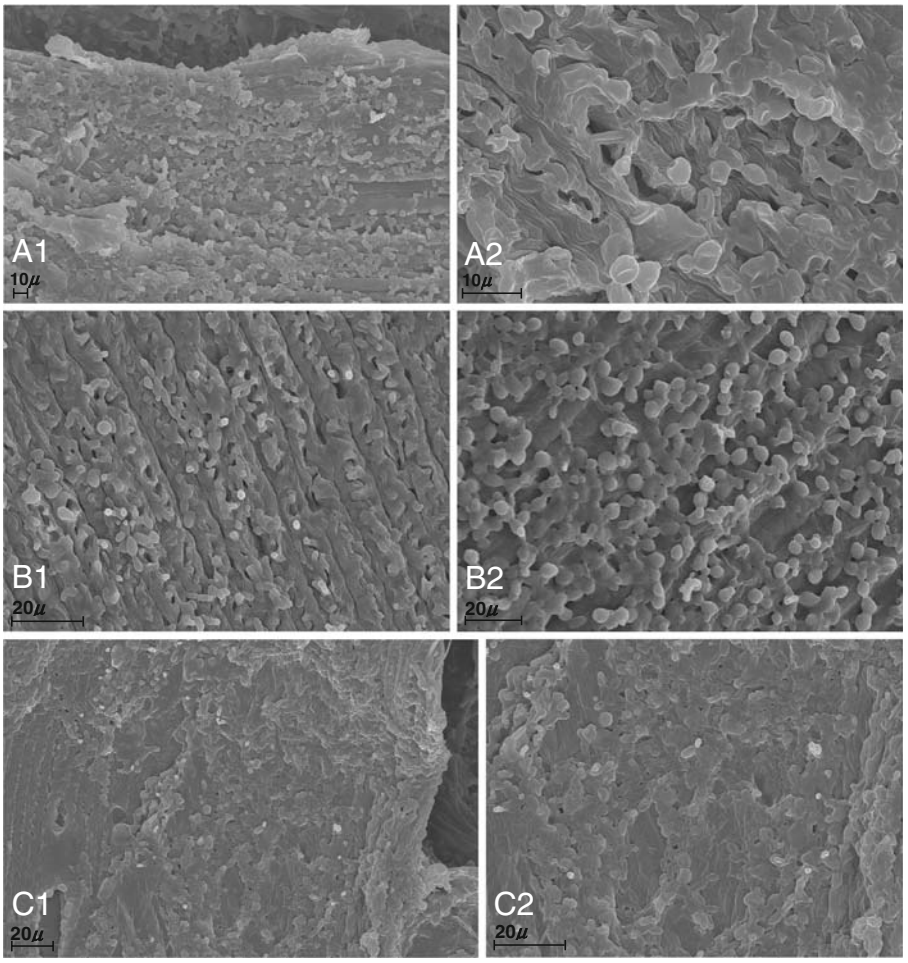

Fig. 2 Scanning electron microscopy of yeast immobilized on DBSG **a** oven dried, **b** dried by air-stream, and **c** dried under vacuum at 35 °C

and hence, residual sugar concentrations are inconsiderable. The biocatalysts dried under vacuum presented lower fermentation activity.

To examine the effect of storage on the fermentation activity of the dried biocatalysts, repeated batch fermentations were performed after storage for 4 months. Comparison of kinetics (Fig. 3) shows that the fermentation time was not affected by storage. Also, an important observation is the twofold decrease of fermentation times as the repeated fermentation batches proceeded, which was about threefold in the case of the biocatalysts dried by air stream (Table 2). This can be attributed to rehydration and new cell growth during the first batch. The fermentation activity was not affected by storage, for the given period of 4 months, as also shown by the stability of sugar conversion. Residual sugar and alcohol concentrations were also at the same levels as in the case of the fresh biocatalysts. Alcohol productivity was significantly increased during the second and third batches. The obtained high yields and productivities show that the thermally dried biocatalysts were efficiently active even after 4 months of storage.

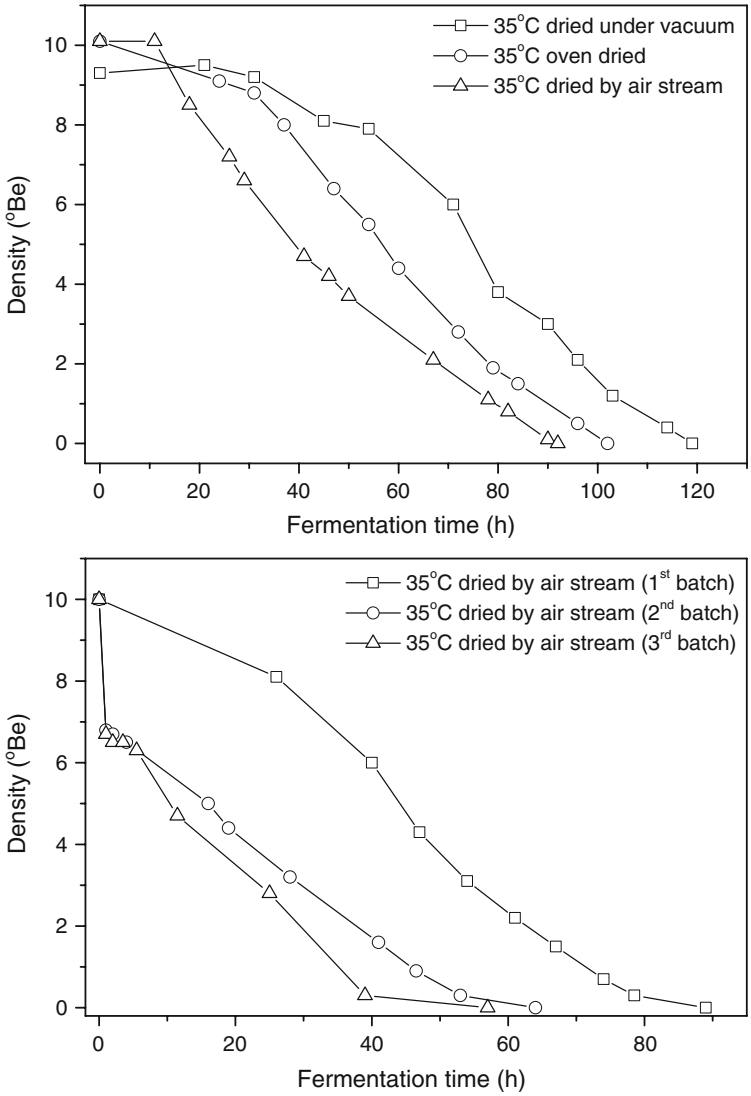

Fig. 3 Kinetics of batch fermentations of glucose at 15 °C by low-temperature dried yeast immobilized on DBSG before storage and after storage for 4 months

Volatile by Products

Analysis of the volatile compounds in the liquids fermented by the dried immobilized biocatalysts was performed by GC-MS (Table 3). A big number of compounds were identified, the majority of which were esters commonly found to most wines and beers and known to contribute to fruity, floral, and caramel-like flavors. Higher alcohols and acids followed esters in abundance. The semiquantitative analysis showed that the total amount of volatiles was similar for the tested biocatalysts, except the one dried by air stream for which the sum of esters was higher. More specifically, the biocatalyst dried by air stream at 30 °C resulted to higher amounts of esters and lower concentrations of higher alcohols. The

Table 3 GC-MS analysis of volatiles (in milligrams per liter) in glucose media fermented by dried yeast immobilized on DBSG: (a) oven dried, (b) dried by air stream, and (c) dried under vacuum at 30, 35, and 38 °C.

R.T. (min)	Compound	Oven dried (ppm)			Dried by air stream (ppm)			Dried under vacuum (ppm)		
		30 °C	35 °C	38 °C	30 °C	35 °C	38 °C	30 °C	35 °C	38 °C
Esters										
4.355	Ethyl acetate	36.71	10.22	17.06	75.76	32.45	12.69	15.01	39.50	28.77
10.608	Ethyl butanoate	–	–	0.54	–	1.31	0.62	–	–	1.01
11.307	3-Methylbutyl acetate	0.04		13.20	62.86	17.47	7.21	9.12	32.47	22.69
20.096	Ethyl hexanoate	1.62	4.26	5.70	18.87	10.73	8.30	9.27	4.32	5.16
27.550	Ethyl-2-hydroxy-propanoate	0.19	–	–	–	2.04	–	1.38	1.76	–
29.846	Ethyl octanoate	0.03	8.67	13.10	21.25	11.47	11.23	6.02	0.16	5.17
33.250	Ethyl nonanoate	–	–	0.08	–	–	–	–	–	–
36.130	Ethyl decanoate	0.13	5.36	13.92	14.56	7.06	6.85	7.87	12.12	7.37
36.674	3-Methylbutyl octanoate	0.07	0.21	0.40	0.58	0.28	0.13	0.86	0.45	0.38
37.523	Ethyl-dec-9-enoate	0.15	0.37	2.16	1.74	0.87	0.82	1.69	3.30	0.03
40.100	Ethyl benzoate	0.04		0.18		0.10			0.36	0.08
40.836	2-Phenylethyl acetate	0.47	4.00	12.00	20.37	9.62	13.19	9.39	23.29	11.17
41.361	Ethyl dodecanoate	0.09	2.09	3.20	6.34	1.96	2.78	3.69	5.03	3.69
41.850	3-Methylbutyl pentadecanoate	–	0.08	0.49	–	–	–	0.91	0.31	0.50
45.917	Ethyl tetradecanoate	0.31	–	–	–	–	–	–	–	–
49.017	Ethyl hexadecanoate	0.23	0.15	0.72	–	–	0.39	0.36	0.40	–
49.400	Ethyl-9-hexadecenoate	–	0.20	1.38	–	–	0.63	0.89	0.68	0.58
	Identified esters	13	11	15	9	12	12	13	14	13
	Total ester content	40.12	35.60	84.10	222.33	95.35	64.85	66.46	124.15	86.58
Alcohols										
8.854	1-Propanol	15.94	0.75	0.52	2.41	0.30	0.40	4.82	–	2.05
15.483	2-Methyl-1-propanol	8.20	–	4.61	–	4.30	0.84	2.54	4.26	5.27
19.508	1-Butanol	–	–	–	–	–	–	–	–	0.11
23.283	1-Pentanol	0.23	–	–	–	–	–	–	–	–
26.292	1-Hexanol	0.12	0.12	0.06	0.15	–	–	–	0.12	–
30.823	1-Heptanol	–	–	0.18	0.33	0.20	–	1.46	–	0.24
31.890	2-Ethyl-1-exanol	–	–	–	0.21	–	–	–	–	–
33.647	2,3-Butanediol	0.22	0.17	1.32	2.74	0.85	1.09	7.90	1.90	0.93
34.725	1,3-Butanediol	–	–	0.38	–	–	0.24	1.64	–	–
34.942	1-Octanol	0.75	–	–	–	–	–	–	–	–
43.100	2-Phenylethanol	0.16	13.21	10.64	11.09	11.49	10.78	15.79	15.30	4.37
	Identified alcohols	7	4	7	6	5	5	6	4	6
	Total alcohol content	25.66	14.24	17.71	16.93	17.24	13.35	34.14	21.57	12.97
Acids										
42.400	Hexanoic acid	2.97	–	2.78	12.74	–	4.27	3.18	7.86	0.26
46.437	Octanoic acid	21.48	14.31	23.15	22.33	25.34	30.59	47.50	34.80	31.45
48.337	Nonanoic acid	0.21	0.04	0.25	2.61	0.79	0.50	6.51	2.48	0.46
49.936	Decanoic acid	0.01	0.35	5.04	2.42	–	6.55	11.75	17.93	6.46
	Identified acids	4	3	4	4	2	4	4	4	4
	Total acid content	24.68	14.70	31.22	40.10	26.13	41.91	68.95	63.07	38.63

Table 3 (continued).

R.T. (min)	Compound	Oven dried (ppm)			Dried by air stream (ppm)			Dried under vacuum (ppm)		
		30 °C	35 °C	38 °C	30 °C	35 °C	38 °C	30 °C	35 °C	38 °C
Carbonyl compounds										
3.316	Acetaldehyde	–	0.47	0.62	2.31	0.43	0.50	3.22	1.12	0.74
33.015	Benzaldehyde	0.10	–	0.13	0.45	–	–	0.67	–	0.10
45.817	Dihydro-5-pentyl-2(3H) furanone	0.10	–	–	0.16	–	–	–	0.84	–
	Identified carbonyls	2	1	2	3	1	1	2	2	2
	Total carbonyl content	0.20	0.47	0.75	2.91	0.43	0.50	3.89	1.96	0.84

relatively high contents of acids observed in the case of this drying temperature cannot downgrade the sensory properties of a potential fermentation product, since, e.g., wines normally contain much higher amounts. An advantageous observation is the low concentrations of carbonyl compounds that are known to adversely affect quality. From the presented data, it can be concluded that the method of drying by air stream gave the best results. Furthermore, the temperature of 30 °C, which is a very low drying temperature, was effective for drying the porous DBSG-supported biocatalyst in a thin layer, in terms of both fermentation activity and formation of volatiles.

High-Temperature Thermal Drying of Immobilized Yeast

For comparison reasons, drying of the immobilized biocatalyst at high temperatures (up to 70 °C) was also evaluated. The idea was based on the observation that, since cells immobilized on delignified materials are more alcohol-resistant [16] and cryotolerant [4] compared with free cells, they would also be tolerant when subjected to high temperatures. The high-temperature dried biocatalysts were used in the same manner as described above to ferment glucose media for the evaluation of their fermentation activity. Likewise, comparisons with free cells were also carried out (Fig. 4). The DBSG–biocatalyst dried at 70 °C led to similar fermentation times with the thin layer thermally free cells dried at low temperatures (35–40 °C). Drying at high temperature in the range 42–60 °C gave only slightly lower fermentation times compared with low-temperature drying at 30–38 °C. This indicated that the viability and activity of the immobilized cells dried at high temperatures was not affected significantly. This can be attributed to the protection rendered to the attached and entrapped cells by the porous matrix of DBSG due to the low thermal conductivity of cellulose.

Conclusions

This investigation is a scientific and technological contribution to the efforts for the introduction of immobilized cells to the food and fermentation industry, based on the following claims: (1) the development of low-temperature, low-cost drying techniques for immobilized cells; (2) the development of high-temperature cell-drying technology using more tolerant immobilized biocatalysts; (3) the increase of the drying rate due to the increased surface of immobilized biocatalysts; (4) the increase of added value through utilization of BSG and production of low-cost immobilized wine and brewing yeasts; and

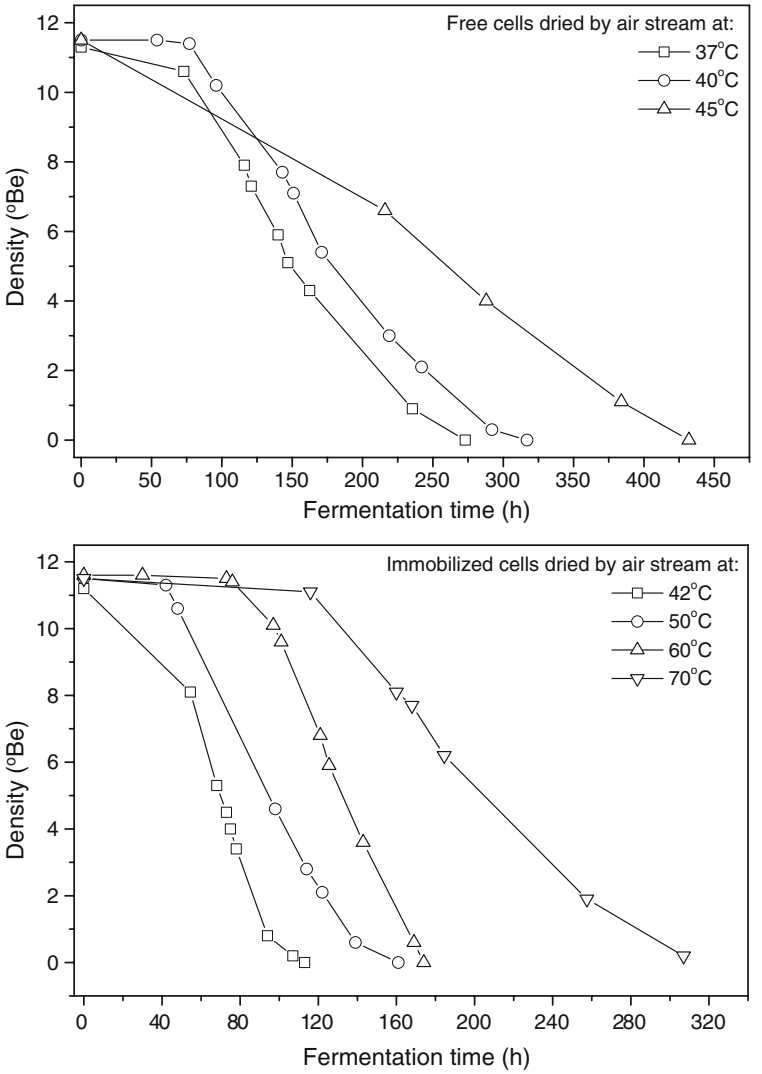

Fig. 4 Kinetics of batch fermentations of glucose at 15 °C by high-temperature air-dried yeast immobilized on DBSG

(5) the production of ready-to-use and easy-to-preserve commercial dry immobilized biocatalysts of proved higher fermentation activity compared to free cells. Specifically, the thermally dried DBSG–biocatalyst is advantageous compared with thermally dried free cells in terms of thermal stability, extremely low-temperature fermentation ability, improvement of product quality, and lower cost due to the fact that, although only 6% of its weight corresponds to immobilized microbial biomass, its productivity is higher compared with free cells [4, 8, 9, 12]. Likewise, the proposed technology is environmentally friendly. The porosity of the DBSG–biocatalyst and its thermal stability improves the potential of thin layer drying increasing the possibility of industrial application.

Low-temperature drying is attractive due to increased cell viabilities and lower energy demands. However, high-temperature drying, which was successful in the case of the

DBSG–biocatalyst, will facilitate the development of thermally dried wine, brewing, or baker's yeast technology taking into account the aforementioned advantages. The potential industrial application of thermally dried yeasts is also determined by their demand in brewing, wine-making, baking, and ethanol production processes. The proposed drying methodology is simple and of lower cost compared with energy-demanding and expensive-equipment techniques like freeze-drying. The fermentation kinetics and analysis of volatiles indicate that the quality of potential wine and beer products made with dried immobilized cells will be similar with that of traditional products. The increase of esters and low higher alcohol concentration, as well as significantly lower amounts of carbonyls obtained in the case of low-temperature drying (30 °C) also indicate the potential for improved wine and beer quality [3].

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