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Yeast Biomass Production in Brewery's Spent Grains Hemicellulosic Hydrolyzate

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Abstract Yeast single-cell protein and yeast extract, in particular, are two products which have many feed, food, pharmaceutical, and biotechnological applications. However, many of these applications are limited by their market price. Specifically, the yeast extract requirements for culture media are one of the major technical hurdles to be overcome for the development of low-cost fermentation routes for several top value chemicals in a biorefinery framework. A potential biotechnical solution is the production of yeast biomass from the hemicellulosic fraction stream. The growth of three pentose-assimilating yeast cell factories, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, and *Pichia stipitis* was compared using non-detoxified brewery's spent grains hemicellulosic hydrolyzate supplemented with mineral nutrients. The yeasts exhibited different specific growth rates, biomass productivities, and yields being *D. hansenii* as the yeast species that presented the best performance, assimilating all sugars and noteworthy consuming most of the hydrolyzate inhibitors. Under optimized conditions, *D. hansenii* displayed a maximum specific growth rate, biomass yield, and productivity of 0.34 h^{-1}, 0.61 g g^{-1}, and 0.56 g l^{-1} h^{-1}, respectively. The nutritional profile of *D. hansenii* was thoroughly evaluated, and it compares favorably to others reported in literature. It contains considerable amounts of some essential amino acids and a high ratio of unsaturated over saturated fatty acids.

Keywords *Debaryomyces hansenii* · Biomass · Single-cell protein · Hemicellulosic hydrolyzate · Brewery's spent grains · Agro-industrial residues upgrading · Yeast extract

Introduction

Yeast single-cell protein (SCP) and yeast extract (YE), in particular, are two products that have many food, feed, pharmaceutical, and biotechnological applications. However, many of these applications, e.g., yeast extract as nutrient source for industrial growth of

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microorganisms, are limited by the market price. Actually, yeast extract is one of the most relevant media supplements, and its replacement is one of the major technical hurdles to solve for developing low-cost fermentation routes to obtain several top value chemicals from biomass in a biorefinery framework, e.g., 3-hydroxypropionic acid, glutamic acid, and itaconic acid [1], polyols [2], and lactic acid production [3]. For the latter, an economic analysis showed that YE was the largest contributor accounting for 38% of the total production cost.

A possible cheap production approach for SCP and YE is the management of yeast biomass derived or produced within the biorefinery framework, namely, from the upgrading of the hemicellulosic fraction stream.

For this biotechnological process, there are several required traits for a yeast-cell factory, namely, the yeast should fully consume the different carbon sources present in the hydrolyzates, especially pentoses, exhibiting high yield and productivity. Other required traits include minimal byproduct formation, high tolerance to inhibitors, and to process hardiness. Simultaneous sugar utilization, minimal nutrient supplementation, and tolerance to both low pH and high temperature are also desirable traits [4]. Due to the potential food and feed applications of these products, the GRAS or QPS [5] status are additional requirements for any yeast strain.

The yeasts currently used and/or studied for SCP production, e.g., *Candida utilis*, are not able to utilize all sugars present, namely, arabinose, and as they belong to the *Candida* genus [6–8], they are not suitable for QPS status. It is thus important to develop new YE/SCP producing processes based on different yeasts.

Among other possibilities, three naturally pentose-assimilating yeasts, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, and *Pichia stipitis* can be considered as strong potential candidates for cell factory. These yeasts have been used as model microorganisms mainly for the production of ethanol (*P. stipitis* and *K. marxianus*) [9, 10], SCP, and heterologous enzymes (*K. marxianus*) [11, 12] and polyols (*D. hansenii*) [2, 13]. Furthermore, they are prone, although at different levels, to genetic manipulation to achieve higher productivities from pentose metabolism [9, 14]. Moreover, *D. hansenii* and *K. marxianus* have already a QPS status [5].

In this work, we comparatively assessed the potential of brewery's spent grains (BSG) hemicellulosic hydrolyzate for YE and SCP production using *D. hansenii*, *K. marxianus*, and *P. stipitis*.

Materials and Methods

Feedstock and Hydrolysis

BSG was obtained from a local brewery (SCC-Sociedade Central de Cervejas e Bebidas, Vialonga, Portugal). The feedstock material was pretreated in an autoclave (Uniclave 88, AJC, Lisbon, Portugal) for residual starch removal, as described before [15]. The pretreated BSG was subjected to a two-step process hydrolysis consisting of an autohydrolysis followed by a sulfuric acid catalyzed posthydrolysis, as optimized before [16]. Autohydrolysis was carried out for 2.5 min at 190 °C in a 2-l stainless steel Parr reactor model 4532 (Moline, Illinois, USA), with a liquid to solid ratio of 8:1. The oligosaccharide-containing liquor was separated from the residual solid by filtration (Whatman no. 1 filter paper). Posthydrolysis were carried out in autoclave at 121 °C for 15 min after H_2SO_4 was added to the liquor to reach a final concentration of 2% (*w*/*w*).

The pH of the acid hydrolyzates was increased to 5.5 (cultivation pH) by the addition of $Ca(OH)_2$. After 1 h at pH 5.5, the precipitate was removed by centrifugation at 7,500×*g* for 25 min (Beckman Coulter, Fullerton, USA).

Microorganisms and Maintenance

The yeast strains used were *D. hansenii* CCMI 941, *P. stipitis* CBS 5773, and *K. marxianus* CBS 6556, obtained from the National Collection of Yeasts Cultures (UK), as NCYC 2597. The strains were maintained on YM agar slants containing 20 g l^{-1} glucose, 3 g l^{-1} yeast extract, 3 g l^{-1} malt extract, 5 g l^{-1} peptone, and 20 g l^{-1} agar.

Medium Preparation

To compare the performance of the three yeast species and to prevent growth limitations due to any nutritional deficiency, the BSG hydrolyzate was supplemented with several mineral nutrients and vitamins to reach the concentrations described in Duarte et al. [17]. *D. hansenii* biomass production for subsequent studies was carried out using a previously optimized medium containing 0.5 g l^{-1} KH_2PO_4 as the only supplement to BSG hydrolyzate [2]. To prevent nutrient thermal decomposition, all media were filter sterilized using a 0.22-μm Gelman membrane filters (Pall Corporation, Ann Arbor, MI, USA).

Cultivation Conditions

A 24-h-grown YM slant was used to seed 100 ml of hydrolyzate medium in a 1,000 ml baffled Erlenmeyer flask capped with cotton wool stopper. After 17 h, 2.5 ml of this culture was used to seed a similar flask and medium. Initial cell dry weight concentration was about 0.4 g l^{-1}. All cultures were carried out aerobically, in an Infors® Unitron (Bottmingen, Switzerland) orbital incubator set at 30 °C and 150 rpm. All cultivation assays were done at least in duplicate, and the mean values are reported.

At preset fermentation times, samples were withdrawn for high performance liquid chromatography (HPLC) analysis, pH, and cell growth measurements. To have enough biomass for macromolecular, amino acids and fatty acid analysis, several shake flask cultivations in media containing only 0.5 g l^{-1} KH_2PO_4 were performed. At the end of fermentation period (24 h), cells were harvested by centrifugation (Sigma, Osterode am Harz, Germany) at 9,000×*g*, 4 °C and 15 min, washed twice with 0.9% (*w*/*v*) NaCl, and freezed until further use.

Analytical Methods

D-glucose, D-xylose, L-arabinose, formic, acetic and levulinic acids, ethanol, HMF, and furfural were analyzed by HPLC using an Aminex HPX-87H column from Bio-Rad (Hercules, CA, USA). The HPLC system was a Waters LC1 module l plus (Millfort, MA, USA) equipped with both a refractive index and an ultraviolet detector set at 280 nm (used to detect HMF and furfural). The mobile phase was 5 mM H_2SO_4, the column temperature 50 °C, and the flow rate 0.4 ml/min. The system was equipped with a Micro-Guard Cation-H Refill Cartridge from Bio-Rad (Hercules, CA, USA) before the HPX-87H column. Due to the partial overlap of arabinose, xylitol and arabitol, samples were also analyzed by HPLC using a Waters Sugar Pak 1 column (Millfort, MA, USA). Also used was a Merck Hitachi HPLC

system (Tokyo, Japan) equipped with a refractive index detector (L-7490). The mobile phase was 50 mg l^{-1} calcium ethylenediaminetetraacetic acid (EDTA), the column temperature 90 °C, and the flow rate 0.5 ml/min. As this method does not allow to distinguish between D- and L-arabitol, the latter was used as arabitol standard. All samples were filtered by 0.45 μm Gelman membrane filters before analysis.

Phenolic compounds were quantified spectrophotometrically by a modification of the Prussian blue method as described by Graham [18]. Tannic acid was used as calibration standard.

Cell mass was followed spectrophotometrically (OD_{600nm}), diluting when necessary. At the beginning and at the end of fermentations, biomass dry weight was determined gravimetrically, by filtration of 5 ml of culture broth through 0.45 μm Gelman membrane filters, washing with a double volume of water and drying overnight at 100 °C to constant weight.

All assays were done at least in duplicate.

Macromolecular Composition

Quantitative acid hydrolysis with 72% (*w*/*w*) H_2SO_4 [19] was used to characterize the BSG feedstock. The monosaccharides and acetic acid were determined by HPLC to estimate (after corrections for stoichiometry and sugar decomposition) the contents of glucan (cellulose) and hemicelluloses (xylan, arabinan, and acetyl groups) in the sample. The acid-insoluble residue after hydrolysis was recovered by filtration and considered as Klason lignin after correction for the acid-insoluble ash. Protein was determined by the Kjeldahl method [20] using the $N \times 6.25$ conversion factor.

Protein content in the yeast biomass was also determined by the Kjeldahl method using the conversion factor of 6.25. Total carbohydrates in yeast biomass were determined by the anthrone method [21]. RNA content was determined by the Schmidt-Thannhauser method as described in Benthin et al. [22]. Fat content was determined in dried cells by a Soxhlet extraction procedure using petroleum ether (60–80 °C) as solvent [23]. Ash content was determined by igniting the samples at 575 °C for 5 h, both for the feedstock and yeast biomass. All results are reported on the dry basis.

Amino Acid Analysis

Amino acid content of the hydrolyzate and cell mass were determined in dried biomass according to Commission Directive [24] using a Biochrom 20 (Pharmacia Biotech) amino acid analyzer equipped with a photometric detector (440 nm for proline and 570 nm for all others). An external standard method was used.

Fatty Acid Analysis

Yeast biomass was freeze–dried and grounded. Fatty acid extraction and preparation of methyl esters were carried out according to Lepage and Roy [25]. Samples (100 mg) were transmethylated with 5 ml of methanol/acetyl chloride (95:5 *v*/*v*). The mixture was sealed in a light-protected Teflon-lined vial under nitrogen atmosphere and heated at 80 °C for 1 h. The vial contents were then cooled, diluted with 1 ml water, and extracted with 2 ml of *n*-heptane. The heptane layer was dried over Na_2SO_4, evaporated to dryness under nitrogen atmosphere and redissolved in heptane, which contained the methyl esters.

The methyl esters were then analyzed by gas–liquid chromatography as described before [26], on a VARIAN (Palo Alto, USA) 3800 gas-liquid chromatograph equipped with a flame ionization detector. Separation was carried out on a 0.32 mm×30 m fused silica capillary column (30 m, 0.32 mm ID, film 0.32 μm) Supelcowax 10 (SUPELCO, Bellafonte PA, USA) with helium as carrier gas at a flow rate of 1.3 ml min^{-1}. The column temperature was programmed at an initial temperature of 200 °C for 10 min, then increased at 4 °C min^{-1} to 240 °C and held there for 16 min. Injector and detector temperatures were 250 and 280 °C, respectively, and split ratio was 1:100. Peak identification and response factor calculation were carried out using known standards (Nu-Chek-Prep, Elysian, USA). For each sample, two independent derivations were prepared and injected twice.

Calculations

The specific growth rate (μ, h^{-1}) was calculated by linear regression of the $\ln(OD/OD_i)$ vs time for the exponential growth phase. The biomass volumetric production rate (productivity), Q_x (g l^{-1} h^{-1}), was calculated, at 24 h, based on cell dry weight produced per liter of culture medium per hour. The cell yield, Y_x (g/g), was calculated at 24 h, as the amount of cell dry weight formed per gram of all consumed sugars. The relative sugar consumption was calculated at 24 h, as the ratio of the amount of monosaccharide consumed to the individual initial monosaccharide amount.

Results and Discussion

Feedstock and Hydrolyzate Composition

Brewery's spent grains macromolecular composition varies much, as it is not a defined product from a single raw material; rather, it is a by-product from a mixture of several raw materials that can be processed in the brewery under quite variable conditions. The average composition of the used BSG is presented in Table 1.

Minerals and vitamins are usually found in BSG [27]. The mineral elements include aluminum, barium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, silicon, sodium, strontium, sulfur, and zinc, typically all in concentrations lower than 0.5%, except for silicon that is the major mineral present. The vitamins include: biotin, choline, folic acid, niacin, pantothenic acid, riboflavin, thiamine, and pyridoxine. Although, many of the vitamins can be destroyed during the hydrolysis

Table 1 Average macromolecular composition of brewery's spent grains on a dry weight basis (%).

Component	BSG
Glucan	21.2
Hemicellulose	30.4
Xylan	19.8
Arabinan	9.8
Acetyl groups	0.8
Klason lignin	22.2
Protein	24.6
Ash	1.1

Table 2 Composition of brewery's spent grains hydrolyzate.

Compound	Concentration (g l^{-1})
Glucose	5.2
Xylose	14.9
Arabinose	6.2
Acetic acid	1.3
Formic acid	0.8
Levulinic acid	0.16
Furfural	0.64
HMF	0.05
Total phenolic compounds	1.3
Protein	1.2

processes, a part may become available for microbial growth, together with some nitrogen compounds.

The used BSG hydrolyzate composition is shown in Table 2. It has approximately 26 g l^{-1} of monosaccharides and a low level of microbial inhibitors, specially aliphatic acids, and furan derivatives compared to similar hemicellulosic hydrolyzates used for SCP production, e.g., eucalyptus wood [28] and sugar cane bagasse [7,8]. Also, it has a low content of phenolic compounds. Crude protein has a concentration of about 1.2 g l^{-1}. Much of this nitrogen (about half) is in ammonia form, the rest as amino acids (data not shown).

The impact of pH correction to 5.5 on the chemical composition of the hydrolyzate has been studied before [29] and leads to a decrease of about 6% in monosaccharide content and a more considerable removal of furan derivatives and phenolic compounds (10–15%). Aliphatic acid contents are not significantly affected.

Yeast Growth in Hemicellulosic BSG Hydrolyzate

All three yeast species, *D. hansenii*, *K. marxianus*, and *P. stipitis* were able to grow in fully supplemented BSG hydrolyzate medium without any detoxification step. Biomass production starts after a short lag phase, and specific growth rates are higher during glucose assimilation. It could be observed that there is a decrease in growth rate after glucose depletion, but markedly diauxic type growth was not observed for any yeast. Stationary phase was completely set in at 24 h.

Overall biomass productivity and yield differ much among the yeasts (Table 3) and reflect the ability to metabolize the different sugars and other compounds present. The high

Table 3 Kinetic and stoichiometric parameters, of *P. stipitis*, *K. marxianus*, and *D. hansenii* growth in supplemented brewery's spent grains hydrolyzate.

Parameter	*D. hansenii*	*K. marxianus*	*P. stipitis*
μ (h^{-1})	0.35	0.30	0.19
Q_x (g l^{-1} h^{-1})	0.47	0.20	0.32
Y_x (g g^{-1})	0.60	0.41	0.46
Consumed Glc (%)	100	100	100
Consumed Xyl (%)	100	70	100
Consumed Ara (%)	100	45	31

μ Specific growth rate, Q_x biomass productivity, Y_x biomass yield

D. hansenii biomass productivity reflects the ability of this yeast to fully make use of all monosaccharides present in the medium.

The yeasts exhibited a similar sugar utilization pattern (data not shown). Glucose and xylose were assimilated simultaneously, the former at a higher rate, enabling higher growth rates. Xylose was completely assimilated by *P. stipitis* and *D. hansenii*, but not by *K. marxianus*, which only consumed 70%. Arabinose was also assimilated by all yeasts simultaneously with late xylose assimilation, being only completely exhausted by *D. hansenii*. The other two yeasts, *Pichia stipitis* and *K. marxianus*, only consumed 31 and 45% of available arabinose, respectively. Furfural, acetic, and formic acid were also consumed (data not shown).

As expected for aerobic conditions, no metabolic products were found in significant amounts in the cultures supernatants, except for ethanol that was slightly produced by *P. stipitis* (maximum, 1 g l^{-1}).

Among these three yeasts, *D. hansenii* presented the best overall performance, and this comparison can be extended for other microorganisms grown in hemicellulosic hydrolyzates, e.g., *Candida tropicalis* [7] and *Paecilomyces variotii* [28]. *Candida langeronii* grown in sugar cane bagasse presented a higher biomass productivity of 0.97 g l^{-1} h^{-1} [8], but under fully controlled oxygen and pH conditions and using a higher sugar content. Nevertheless, the biomass yield for *C. langeronii* was 0.40 g g^{-1}, which is lower than that obtained for *D. hansenii*. Furthermore, *D. hansenii* has been reported to achieve, in chemostat cultures, a higher cell productivity of 2.5 g l^{-1} h^{-1} in chemically defined media using xylose as sole carbon and energy source [30].

D. hansenii has some other advantages over other yeasts, as it also tolerates high concentrations of inhibitors and is able to grow with a high biomass yield and productivity, with minimum supplement requirements. For BSG hemicellulosic two-step hydrolyzate, 0.5 g l^{-1} KH_2PO_4 was identified as the sole required supplement [2]. This has significant advantages at industrial level. Furthermore, besides its use as a polyol producer, it has many other biotechnological applications [31], namely, in food processing, e.g., cheese making, yoghurt, and meat products. Recently, it has also been reported that *D. hansenii* spent yeast was used to successfully replace yeast extract in growth media supplementation [32]. Hence, there is a need for developing efficient and economically viable procedures for its large-scale biomass production. Considering these advantages altogether with *D. hansenii* performance in BSG hydrolyzate makes this yeast species the proper choice for the subsequent analysis.

For optimized BSG supplemented media, *D. hansenii* displayed a maximum specific growth rate, biomass yield, and productivity of 0.34 h^{-1}, 0.61 g g^{-1}, and 0.56 g l^{-1} h^{-1}, respectively (data not shown).

D. hansenii Macromolecular Composition

The macromolecular composition of *D. hansenii* biomass after 24 h growth in optimized BSG medium is presented in Table 4. The total protein content of 31.8% is compared to 31.3 % reported for *C. tropicalis* [7], 37% for *Kluyveromyces fragilis* [23], and 48.1% for *C. langueronii* [8]. The protein yield per sugars consumed obtained, of 0.20 g g^{-1}, was similar to the reported value for shake flask cultures of *C. blankii* grown on xylose (0.22 g g^{-1}) [6]. Carbohydrate content was higher than the values reported for *C. langeronii* [8], and *K. fragilis* [23], but in the range of *C. blankii* [6]. These values are typical of carbon excess growth conditions for this yeast [33], and hence they can be further improved, namely, the protein content.

Table 4 Macromolecular composition of *D. hansenii* biomass grown on brewery's spent grains hydrolyzate.

Component	Relative composition (%, *w*/*w*)
Protein	31.8
Carbohydrates	41.6
Ash	10.8
Fat	2.3
RNA	9.0
Rest	4.5

The values obtained for the RNA content (that only include stable RNA, i.e., rRNA and tRNA) compares well to the values reported for *C. langeronii* [8], *C. kruseii* SO1, and *Saccharomyces* sp. LK3G [34] and are close to the upper end of the range (6–11%) previously reported [23]. Although this can be somewhat disadvantageous for human nutrition due to the negative health effects of a high nucleic acid intake that induces overproduction of uric acid crystals [23, 35], it may be interesting for biotechnological purposes, as in some cases, YE is used as a source of nucleic acids [36, 37]. DNA was not measured, as it usually has a fairly constant and low level regardless of the growth conditions. Typical reported values for DNA levels are 0.57 [23] and 1.4% [8]. Ash content of *D. hansenii* dried cells was found to be 10.8 %, which is within the range usually described in the literature (7–18%) [23, 38–40]. The rest value accounts for the DNA content and for pools of different building blocks and metabolites.

Amino Acid Composition

D. hansenii amino acid profile is presented in Table 5. *D. hansenii* protein contains considerable amounts of the essential amino acids. The profile compares favorably with the

Table 5 Amino acid composition (% of total protein) of *D. hansenii* biomass grown on brewery's spent grains hydrolyzate as compared to the FAO standard[a].

Amino acid	*D. hansenii*	FAO
Alanine	5.70	
Arginine	3.74	
Aspartic acid	8.51	
Cysteine	1.06	2.00
Glutamic acid	11.32	
Glycine	4.70	
Histidine	1.95	
Isoleucine	3.93	4.20
Leucine	6.00	4.80
Lysine	6.48	4.20
Methionine	0.99	2.20
Phenylalanine	3.71	2.80
Proline	3.42	
Serine	4.82	
Threonine	4.55	2.80
Tryptophan	N.d.	1.40
Tyrosine	4.88	2.80
Valine	5.21	4.20

N.d. Not determined

[a] [8, 28, 35, 45]

FAO food protein standard. All essential amino acids are present in amounts above or close to the required levels, with the exception of the sulfur-containing amino acids (methionine and cystein), which was expected, as yeast protein are described to have a low content of these amino acids [8, 23, 38]. Nevertheless, methionine and/or cysteine levels are above that described in literature for other yeast [7, 8, 23, 34, 40]. Among the nonessential amino acids, glutamic and aspartic acid are present in higher amounts as previously found for other yeasts [7, 23, 38]. Considering the overall amino acid composition, there seems to be interesting prospects for *D. hansenii* biomass use as a food/feed supplement.

Fatty Acid Composition

The distribution of fatty acids is presented in Table 6. Vaccenic acid (18:1ω7) was the major fatty acid found, which is consistent to the reported fatty acid for other *D. hansenii* strains where C18:1 was the major type of fatty acid identified [41]. The second most abundant fatty acid was linoleic acid (18:2ω6), which has been reported as the major fatty acid present in *K. fragilis* PC8002 [38]. Altogether, *D. hansenii*, compared to other yeasts, exhibited a much higher content of C18 fatty acids (more than 73%) and low levels of C16 fatty acids, which are usually the second most common fatty acids [23, 38, 41].

Concerning the degree of unsaturation, *D. hansenii* unsaturated fatty acid content is five times higher than the saturated fatty acids. This ratio is higher than the reported fatty acid content for other yeasts [23, 38], which can be advantageous as unsaturated fatty acids, namely, ω-3 and ω-6, are considered essential fatty acids that must be obtained from the diet because humans lack the anabolic processes for their synthesis [42]. Relatively to the polyunsaturated acid linolenic (C18:3ω3), *D. hansenii* has a higher content than *K. fragilis* PC8002 [38] but considerably lower than *K. fragilis* MTCC 188 [23], although the latter yeast has been reported to have a very low content of other unsaturated fatty acids. This high content of unsaturated fatty acids, specifically C18 unsaturated fatty acids, can also be an advantageous trait for a SCP/YE product. Actually, in *Saccharomyces cerevisiae*, unsaturated fatty acid composition is a significant determinant of ethanol tolerance [43]. Growing *S. cerevisiae* cells with a higher content of C18:1 fatty acids, either produced endogenously by the yeast or added as supplement to the growth media exhibit better efficacy in overcoming the toxic effects of ethanol. These results are consistent with the

Table 6 Fatty acid composition of *D. hansenii* biomass grown on brewery's spent grains hydrolyzate.

Fatty acid	Composition (% of total)
C10:0 Capric	0.36
C16:0 Palmitic	6.35
Iso C17:0 5-methyl hexadecanoic	1.66
C17:0 Margaric	2.30
C17:1 Heptadecenoic	7.86
C18:0 Stearic	3.19
C18:1ω7 Vaccenic	46.5
C18:1ω9 Oleic	0.49
C18:2ω6 Linoleic	19.5
C18:3ω3 α-Linolenic	0.70
C18:3ω6 γ-Linolenic	3.61
C20:0 Arachidic	0.35
Unidentified	7.10

current knowledge that yeast ethanol tolerance is resulting from the specific incorporation of C18:1 fatty acids in the lipidic membranes leading to a compensatory decrease in membrane fluidity. A similar trend was found in *Mucor fragilis*, where γ-linolenic acid production has increased when ethanol was added to the medium [44].

Conclusions

In non-detoxified fully supplemented BSG hemicellulosic hydrolyzate, *D. hansenii* assimilates all sugars and consumes most of the inhibitors, presenting both superior kinetic and stoichiometric performance compared to the other tested yeast species. This performance is maintained for minimal (optimized) supplemented BSG medium, and it is expected that this performance can be further improved for more controlled oxygen and pH growth conditions.

The nutritional profile of *D. hansenii* was thoroughly evaluated, and it compares favorably to others reported in literature. It contains considerable amounts of the essential amino acids and a high ratio of unsaturated over saturated fatty acids. These, together with the potential QPS and GRAS status of this yeast species, strongly support the use of *D. hansenii* as a suitable cell factory for using hemicellulosic hydrolyzates toward YE/SCP production in a biorefinery framework leading to valuable co-upgrade solutions from lignocellulosic byproducts.

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