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Efficient chemoselective biohydrogenation of 1,3-diaryl-2-propen-1-ones catalyzed by *Saccharomyces cerevisiae* yeasts in biphasic system

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ABSTRACT

A series of chalcones (**1–9**) was synthesized by base catalyzed aldol condensation with 50–94% yields. These α,β-unsaturated carbonyl compounds were used as substrates in biotransformation reactions mediated by three industrial *Saccharomyces cerevisiae* strains in biphasic systems. Several reaction parameters were evaluated, such as yeast concentration, temperature, pH, substrate concentration, organic solvent, volume of aqueous and organic phases and the influence of substituent groups on chalcones **1–9**. The biotransformation was chemoselective and formed only the corresponding saturated ketones. The highest conversion (>99%) to the dihydrochalcone was obtained at 30–45 °C and pH above 5.5, while the cellular and substrate concentrations also showed a strong influence on the biohydrogenation reaction. Organic solvents with log *P* >3.2 (hexane or heptane) were the most appropriate, and 40–80% of aqueous phase allowed the highest conversions probably by maintaining the yeast enzymes catalytically active. The influence of substituents on rings A and B of chalcones **1–9** was low and no correlation between the donor or withdrawing electron groups was observed.

1. Introduction

Whole cells of various microorganisms have been employed to catalyze the asymmetric reduction of ketones, aldehydes, β-ketoesters, imines and α,β-unsaturated systems with C=C bonds activated by strongly polarizing groups, such as nitro, carbonyl, or hydroxyl groups [1–8]. Whole cells are useful tools in the production of chiral synthons as they are able to catalyze reactions exhibiting high chemo-, regio- and enantioselectivity [9,10]. The application of whole cells (growing or resting cells) in the reduction reactions has clear advantages, as the separation of enzyme and the regeneration of NADH are no longer required [11]. These biocatalysts are also able to work under mild conditions, such as room temperature and atmospheric pressure, minimizing problems of isomerization, racemization, epimerization and rearrangement that may occur during traditional chemical catalysis. Therefore, biocatalytic asymmetric synthesis attracts considerable attention due to its simple, cheap and benign methodologies that combine green chemistry with high efficiency [12–14].

Quite a large number of papers and reviews have been published describing the microbiological reduction of α,β-unsaturated carbonyl compounds [15–18]. The available literature data indicate that the microbiological reduction of these compounds in many cases gives a mixture of saturated ketone or aldehyde, saturated alcohol or allylic alcohol, indicating that several enzymes may catalyze the reduction of C=C and C=O double bonds competitively. For example, the reduction of 2-ethylhexen-2-enal mediated by baker's yeast in aqueous media gave a mixture of the corresponding saturated alcohol and allylic alcohol in a ratio of 46:54 [19]. In the case of the reduction of an α,β-disubstituted enone (3-methyl-4-phenyl-3-buten-2-one) mediated by whole cells of *Rhodotorula rubra* M18D3 yeast in a 2% glucose solution the corresponding (*S*)-saturated chiral ketones were the major products with 32–49% conversion yields; while the corresponding saturated and unsaturated alcohols were produced in small amounts [20]. Rodrigues *et al.* used *Phicia stipitis* CCT 2617 yeast adsorbed on Amberlite XAD-7 for the reduction in aqueous media of an acyclic α-methyleneketone (2-ethyl-1-phenylprop-2-en-1-one), obtaining the corresponding (*S*)-unsaturated alcohol as the single product (ee_p >99%) with a conversion yield of 65% [21].

As shown above, water is the natural solvent of choice for whole-cell biocatalysis. However, performing the reduction of α,β-unsaturated carbonyl compounds with whole cells in water has several drawbacks, including low solubility of organic substrates and products, undesired side reactions such as hydrolysis, and difficult separation of products [22]. One of the technical solutions to this problem is the application of two liquid-phase reaction systems in which the substrate solubilizes in the organic solvent [23,24], and

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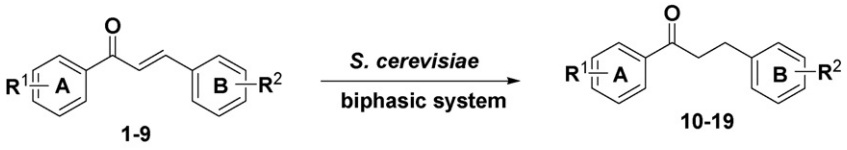


1, 10 - R^1= H, R^2= H
2, 11 - R^1= 4-OCH_3, R^2= H
3, 12 - R^1= 3,4-*di*-OCH_3 R^2= H,
4, 13 - R^1= 3,4-(OCH_2O), R^2= H
5, 14 - R^1= 4-NO_2, R^2= H
6, 15 - R^1= H, R^2= 4-OCH_3
7, 16 - R^1= H, R^2= 3,4-*di*-OCH_3
8, 17 - R^1= H, R^2= 3,4-(OCH_2O)
9, 19 - R^1= H, R^2= 4-NO_2

Scheme 1. The biohydrogenation of chalcones **1–9** catalyzed by *S. cerevisiae*.

thus this organic phase reservoir permits the storage of a high concentration of substrates or products. Furthermore, this approach allows *in situ* removal of the products, thus improving the overall process productivity [25]. The disadvantage of organic–aqueous biphasic systems is the effect of solvent toxicity on the activity and stability of the biocatalyst [26]. In whole-cell systems, the solvent might be incorporated within the membrane lipids, which results in the disruption of membrane functions, inactivation or denaturation of membrane-bound enzymes, collapse of transport mechanisms, or even complete cell lysis [25,27]. Therefore, one of the key factors in the implementation of an effective aqueous–organic bioconversion is the selection of an appropriate solvent, which has to be biocompatible, to provide an adequate substrate pool and product sink, exhibiting the required mass transfer characteristics. An empirical rule, correlating the toxicity of the organic solvent with its log *P* values, is in current use, and the log *P* values are considered a measure of the solvent hydrophobicity. However, this correlation is still not completely understood and does not hold true for all organic solvents [28–31].

In this report whole cells of *Saccharomyces cerevisiae* were used for the biohydrogenation of the activated double bonds of chalcones in a biphasic system to obtain the corresponding dihydrochalcones. These compounds belong to the flavonoid family and display an impressive array of biological activities such as anti-malarial, anti-cancer, anti-tuberculosis, cardiovascular, anti-leishmanial, anti-hyperglycemic, anti-inflammatory and anti-fungal [32–35]. The operational conditions for the biohydrogenation of chalcone **1**, such as amount of biocatalyst, reaction temperature, pH values, substrate concentration, organic solvents, and effect of the volume ratio of the aqueous phase to the organic phase (V_{aq}/V_{org}), were analyzed to improve the conversion into the corresponding dihydrochalcones. The influence of substituent groups on both aromatic rings of chalcones **2–9** was also examined (Scheme 1).

2. Experimental

2.1. Microorganisms

Three commercial *S. cerevisiae* yeast strains were used in their "active-dry-yeast" form: a baker's yeast (BY) from Fleischmann, and two industrial yeast strains (CAT-1 and PE-2) which are currently being used for efficient ethanol production in Brazil [36]. These two industrial fuel ethanol yeasts have been commercially available since the late 1990s, distributed initially by Lallemand Inc. from Canada [37], and more recently by LNF Latino Americana Ltda. from Brazil [38]. The pellets of dehydrated yeast cells were directly used in the biohydrogenation reactions as described below.

2.2. Chemicals

The following chemicals were used as received. Benzaldehyde (>99%), and potassium phosphate buffer were purchased from Vetec; 3′,4′-(methylenedioxy)acetophenone (>98%), 3,4-(methylenedioxy)benzaldehyde (>99%), 3,4-dimethoxybenzaldehyde (>99%), 3,4-dimethoxyacetophenone (>98%), and 4-methoxybenzaldehyde (>98%) from Aldrich-Sigma; acetophenone (>98%) was purchased from Riedel-di-Haen; 4′-methoxyacetophenone (>98%) and sodium borohydride from Merck; 4-nitroacetophenone (97%), 4-nitrobenzaldehyde (>98%) and citric acid from Acros; sodium hydroxide from Grupo Química and deuterium chloroform and acetone-d_6 from Cambridge Isotope Laboratories. All organic solvents were obtained from commercial sources and were analytical grade.

2.3. Preparation of 1,3-diaryl-2-propen-1-ones ***1–9***

The 1,3-diaryl-2-propen-1-ones **1–9** (Scheme 1) were prepared by aldol condensation in a medium with 50% (m/v) KOH using equimolar amounts of substituted benzaldehyde with substituted acetophenones according to the typical procedures described in the literature [34]. The yields varied from 60 to 94%.

(2*E*)-1,3-diphenyl-2-propen-1-one (**1**): 3.5 g, 80% yield, m.p. 53–54 °C (55–56 °C) [39];
(2*E*)-1-(4-methoxyphenyl)-3-phenyl-2-propen-1-one (**2**): 3.5 g, 70% yield, m.p. 100 °C (104–105 °C) [33];
(2*E*)-1-(3,4-dimethoxyphenyl)-3-phenyl-2-propen-1-one (**3**): 4.1 g, 72% yield, m.p. 82–83 °C (85–87 °C) [40];
(2*E*)-1-(1,3-benzodioxol-5-yl)-3-phenyl-2-propen-1-one (**4**): 4.3 g, 78% yield, m.p. 97 °C (97–98 °C) [41];
(2*E*)-1-(4-nitrophenyl)-3-phenyl-2-propen-1-one (**5**): 3.2 g, 60% yield, m.p. 146–147 °C (145–147 °C) [40];
(2*E*)-3-(4-methoxyphenyl)-1-phenyl-2-propen-1-one (**6**): 3.1 g, 62% yield, m.p. 70 °C (75–77 °C) [39];
(2*E*)-3-(3,4-dimethoxyphenyl)-1-phenyl-2-propen-1-one (**7**)**:** 4.7 g, 84% yield, m.p. 83–84 °C (87–89 °C) [40];
(2*E*)-3-(1,3-benzodioxol-5-yl)-1-phenyl-2-propen-1-one (**8**): 4.7 g, 94% yield, m.p. 118 °C (117 °C) [39];
(2*E*)-3-(4-nitrophenyl)-1-phenyl-2-propen-1-one (**9**): 3.1 g, 84% yield, m.p. 160 °C (160–163 °C) [39].

2.4. Preparation of racemic alcohols

The racemic alcohols were prepared from the chalcones **1–9** by reduction with $NaBH_4$ as described on the literature and were used as standards on the ^{1}H NMR and chiral gas chromatography analysis [42,43]. For example, the racemic alcohol (*R*,*S*)-1,3-diphenyl-2-

propen-1-ol was obtained in 84% yield, after purification. m.p. 49 °C (56–57 °C) [43]. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.26–7.40 (10H, m), 6.66 (1H, dd), 6.40 (1H,m), 5.14 (1H,m), 1.61 (1H, s). IR (KBr) cm^{-1} 3345, 3027, 1599, 1493, 1450, 1010, 966, 746, 696. Chiral-GC t_R 18.1 and 18.9 min.

2.5. Biohydrogenation reactions

The biohydrogenation of 1,3-diaryl-2-propen-1-ones **1–9** in a biphasic system was performed in a 125-mL shake flask. In general 2–6 g of active dry yeast were suspended in 0–60 mL of potassium phosphate/citric acid buffer (adjusted to the desired pH), and 0–60 mL of organic solvent containing 0.5–5.0 mmol of substrate was added, being the total volume of 60 mL (and V_{aq}/V_{org} expressed in %). The reaction mixture was incubated at 25–50 °C with constant magnetic stirring. Aliquots were withdrawn at specified time intervals from the reaction mixture, extracted with diethyl ether (2× 10 mL), and analyzed by gas chromatography to evaluate the percentage of conversion. Substrate and product peak areas were compared, and the sum of both areas was considered as 100%.

The dihydrochalcone **10**, was prepared using 6 g of active dry yeast, 1.0 mmol of chalcone **1** in 30 mL of *n*-hexane and 30 mL of potassium phosphate/citric acid buffer (V_{aq}/V_{org} 1:1%), at pH 5.5 and 35 °C. After 3 h reaction the mixture was extracted with diethyl ether (4× 20 mL), and after the solvent evaporation the crude product was crystallized in absolute ethanol forming **10** in 85% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.24–8.00 (10H, m), 3.33 (2H, t), 3.16 (2H,t). IR (KBr) cm^{-1} 2922, 2853, 1450, 1171, 741, 694. Chiral-GC t_R 14.4 min.

2.6. Analytical methods

The reactions were monitored by thin-layer chromatography (TLC) using *n*-hexane:ethyl acetate (9:1, v/v) as the eluent, and a gas chromatograph (GC-14B Shimadzu) equipped with a chiral column (CP-chirasil-Dex CB, packed β-cyclodextrin, 25 m × 0.25 mm × 0.25 mm, CHROMOPACK) and H_2 was used as the carrier gas with a pressure of 75 kPa. The temperatures of the injector and detector were 250 °C and 275 °C, respectively. A column was set to temperature ramps of 100–150 °C (10 °C/min) and 150–200 °C (5 °C/min). The retention times of dihydrochalcone **10**, chalcone **1** and the corresponding racemic alcohol (*R*,*S*)-1,3-diphenyl-2-propen-1-ol were 14.4, 17.3 and 18.1–18.9 min, respectively. The substrate and products were fully characterized by infrared spectra (PerkinElmer FT-IR 1600) and ^{1}H NMR (Varian AC 400F-400 MHz). Melting points were determined on a Microquímica melting point apparatus and were not corrected.

3. Results and discussion

We initially synthesized a series of 1,3-diaryl-2-propen-1-ones by base catalyzed aldol condensation, and applied them in biohydrogenation reactions using *S. cerevisiae* cells in water/organic solvent biphasic systems. Biphasic systems, consisting of an aqueous phase and water immiscible organic phase, are often chosen to avoid the inhibition of the reaction by substrate and products that may occur in aqueous systems [24,44]. Three commercial active dry yeasts were used, including a baker's yeast (BY) and two fuel ethanol strains (CAT-1 and PE-2) selected for their excellent yields and high tolerance towards industrial stresses [36].

The biohydrogenation of (2*E*)-1,3-diphenyl-2-propen-1-one (**1**) in a water/*n*-hexane biphasic system by all three yeasts was completely chemoselective, producing only the corresponding saturated ketone 1,3-diphenyl-1-propanone (**10**). The choice of *n*-hexane to be used as organic solvent was based on our previous results on the reduction of ethyl acetoacetate by yeasts, and some other literature data [4,11,27]. No alcohol was detected by chiral-GC analysis, even after 168 h of reaction, and the dihydrochalcone produced was also confirmed by IR and ^{1}H NMR analysis. In fact, the only known report describing the hydrogenation of chalcones by microorganisms also obtained the corresponding saturated ketone when the reaction was catalyzed by *Corynebacterium equi* IFO3730 in aqueous medium [45,46].

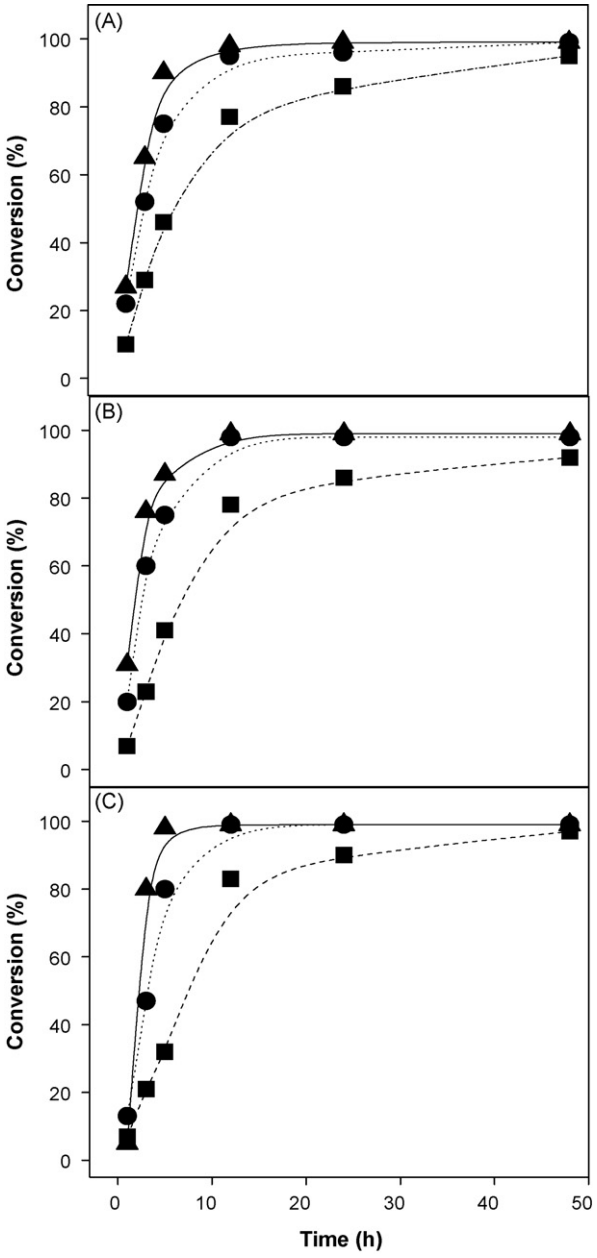


Fig. 1. Time course of (2*E*)-1,3-diphenyl-2-propen-1-one biohydrogenation in a water/*n*-hexane biphasic system using 33.3 g/L (■), 66.7 g/L (●), and 100 g/L (▲) of whole yeast cells of BY (A), CAT-1 (B), and PE-2 (C). Reaction conditions: 208.0 mg (1 mmol) substrate, 30 mL potassium phosphate/citric acid buffer (0.2 M/0.1 M, pH 4.5), 30 mL *n*-hexane (V_{aq}/V_{org} 1:1%), 25 °C.

Fig. 1 shows the conversion rates of **1** into **10** as a function of time and yeast cell concentration. The initial rates increased with the cell concentration, and the highest values were obtained using 100 g/L of whole yeast cells. All three biocatalysts were capable of similar and highly efficient reduction of this chalcone, with initial reaction rates of 6.6, 7.0 and 7.6 × 10^{-5} mmol L^{-1} min^{-1} for strains BY, CAT-1 and PE-2, respectively. Considering these results, the reac-

tion time of 3 h and a cell concentration of 100 g/L of BY, CAT-1 and PE-2 were used for the subsequent experiments aiming at the optimization of other operational conditions for the biohydrogenation of (2*E*)-1,3-diphenyl-2-propen-1-one mediated by whole cells of *S. cerevisiae*. After defining the best reaction conditions, a study of substituent effects on both rings A and B of the chalcones **1–9** was also performed.

3.1. Effect of substrate concentration

Some previous reports have demonstrated that a change in the substrate concentration in the medium may affect the reaction rate, and sometimes the stereoselectivity of a particular process, as substrate inhibition might occur at different substrate concentrations for different enzymes present in the yeasts [47,48]. Thus, different substrate concentrations ranging from 8.3 to 83.3 mM were used in the biohydrogenation of **1** in the *n*-hexane/water biphasic system mediated by *S. cerevisiae* yeasts.

As observed in Fig. 2A, the conversions into **10** remained constant as the substrate concentration increased from 8.3 to 25 mM, forming the product at >99% conversion. At higher substrate concentrations, however, the conversion into **10** decreased, especially in the case of BY yeast cells, but still reached conversions of 75 and 77% at the highest substrate concentration tested using CAT-1 or PE-2 yeasts, respectively. Wang et al. described a similar effect of substrate concentration on the asymmetric reduction of 4′-methoxyacetophenone with immobilized *Rhodotorula* sp. AS22.2241 cells in aqueous buffer, where an increase in substrate concentration above 40 mM led to a marked drop in the maximum substrate conversions [44].

3.2. Effect of temperature

The temperature in an enzyme-catalyzed process may increase the reaction rate because a higher temperature accelerates molecular collisions between the enzyme and the substrate, but inactivation of the enzyme at higher temperatures can also occur [49]. To evaluate the influence of temperature on the biohydrogenation of (2*E*)-1,3-diphenyl-2-propen-1-one (**1**) in the water/*n*-hexane biphasic system with yeast cells, the temperature of the reaction was varied from 20 to 50 °C.

When the temperature increased from 20 to 35 °C, the formation of product **10** mediated by the yeast cells increased slightly, and the highest conversions into product (>99%) were achieved at temperatures between 35 and 45 °C (Fig. 2B). The different conversions obtained, especially at 30 °C, may reflect a better performance of the industrial yeast strains PE-2 and CAT-1 at this temperature [36]. Nevertheless, a further increase in temperature led to a drop in the conversion into **10**, especially for PE-2, indicating some loss of the yeast cell activity at higher temperatures.

Similar results were obtained by Matsuda et al. in the asymmetric reduction of acetophenone in aqueous solution using free or immobilized cells of *Geotrichum candidum* NBRC 5767 with yields of 85–89% (ee_p >99%) at temperatures of 30–40 °C [50]. From these results a temperature of 35 °C was considered as appropriate for the biohydrogenation of **1** into its product **10** by whole yeast cells in water/organic solvent biphasic systems.

3.3. Effect of aqueous medium pH

Another important parameter which must be evaluated in biocatalyzed reactions is the pH of the reaction medium, as all enzymes have an optimal pH at which the reaction rate is maximized. Deviations in pH from the optimal value can lead to decreased activity due to changes in the ionization of amino acid residues at the active site of the enzyme, while larger deviations in pH leads to denaturation of the enzyme protein itself. Therefore, pH is among the most significant factors affecting enzyme-catalyzed reactions [47,49,51].

The effect of the pH values of the aqueous medium for the reduction of (2*E*)-1,3-diphenyl-2-propen-1-one (**1**) in a water/*n*-hexane biphasic system was studied in a pH range from 3.5 to 13.0 (Fig. 3A). The conversion into 1,3-diphenyl-1-propanone (**10**) mediated by yeasts was strongly inhibited at acidic pH (pH 3.5), but increased significantly at pH 4.5, and reached maximum values (>99% conversion) at pH 5.5 or higher (Fig. 3A), using any of the three yeast strains. As described previously, deviations in pH from the optimal values can decrease the activity and the conversion degrees into products, but this effect was not observed in this reaction mediated by yeast cells. These results indicating a wide range of stability in relation to pH (especially at basic pH) are of great interest, and might reflect the high stress tolerance that these industrial yeasts have [36]. A buffer with pH 5.5 was considered to be the optimum condition for the biohydrogenation of chalcone **1** into its corresponding saturated ketone **10** mediated by yeast cells.

Lou et al. described a similar pH effect in the biosynthesis of optically active organosilyl alcohol *via* asymmetric reduction of acyl

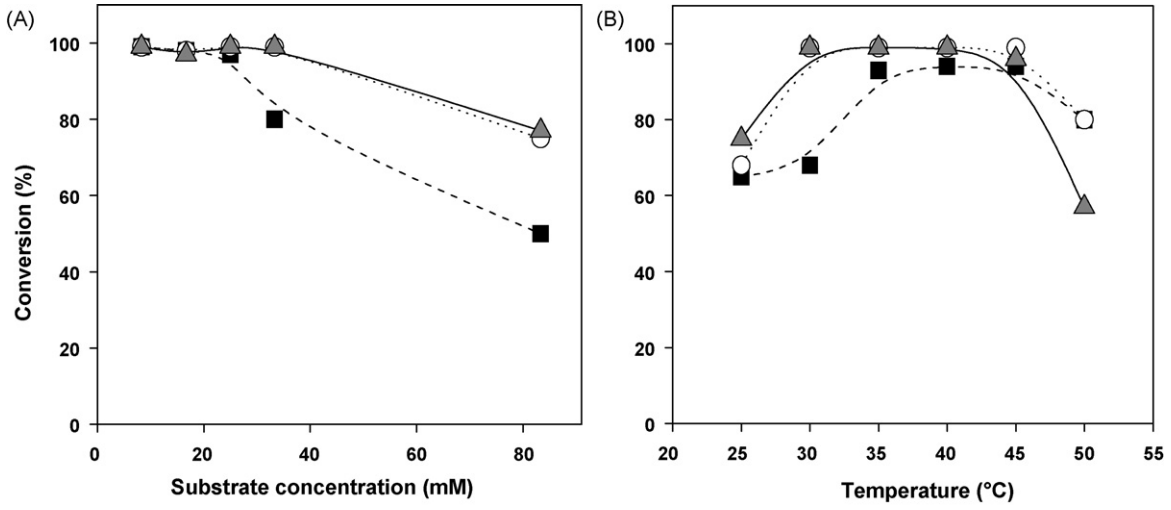


Fig. 2. Influence of substrate concentration (A) and temperature (B) on the biohydrogenation of (2*E*)-1,3-diphenyl-2-propen-1-one in water/*n*-hexane biphasic system mediated by whole yeast cells of BY (■), CAT-1 (○) and PE-2 (▲). Reaction conditions as given in Fig. 1, except for cell concentration (100 g/L), time (3 h), and the indicated substrate concentrations or temperatures. In the case of (A) a pH of 5.5 and temperature of 35 °C were used.

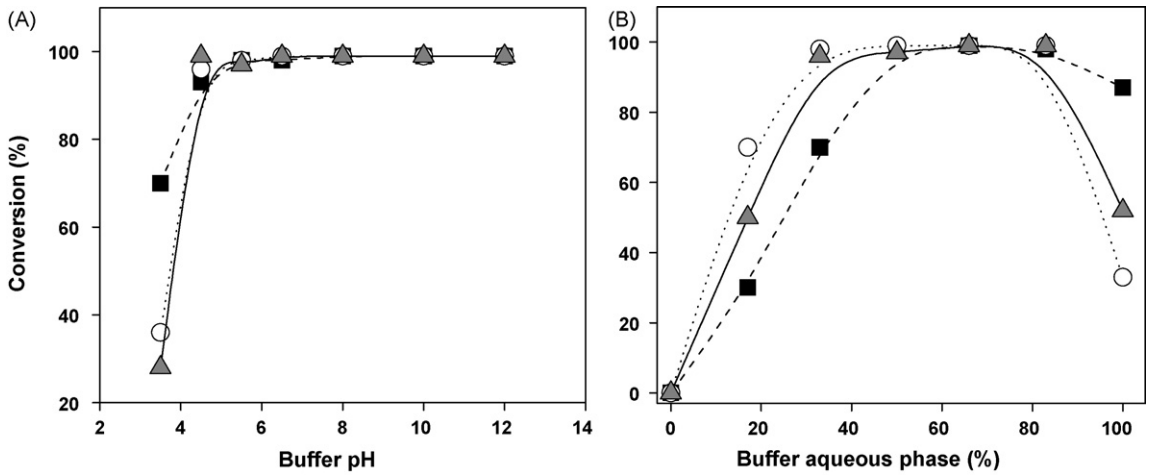


Fig. 3. Influence of buffer pH (A) and of the percentage of volumetric buffer aqueous phase (B) on the reduction of (2*E*)-1,3-diphenyl-2-propen-1-one in water/*n*-hexane biphasic system mediated by whole yeast cells of BY (■), CAT-1 (○) and PE-2 (▲). Reaction conditions as in Fig. 2A but with the indicated buffer pH (A), or volumetric buffer percentage (B), and 1 mmol substrate.

silane catalyzed by immobilized *S. cerevisiae* mutant cells, where high conversions (83–95%) and ee_p (93–96%) were obtained also in a broad pH range (pH 5.5–9.0) [47].

3.4. Effect of volumetric buffer phase ratio (V_{aq}/V_{org})

In an aqueous–organic solvent biphasic system, the volumetric phase ratio influences the interfacial area, which in turn affects biotransformation rates [52]. Generally speaking, enzymes and active cells may be inactivated once in direct contact with organic solvents due to their toxicity [47]. Thus, the effect of the volume ratio of the aqueous phase to the organic phase (V_{aq}/V_{org}) on the biohydrogenation of **1** catalyzed by BY, CAT-1 and PE-2 strains was studied in water/*n*-hexane biphasic system with varying the V_{aq}/V_{org} values maintaining a total volume of 60 mL (Fig. 3B).

The volumetric percentage of the aqueous buffer phase to the organic solvent phase (V_{aq}/V_{org}, %) substantially affected the conversion of **1** into **10**. The conversions increased significantly with an increase in the V_{aq}/V_{org}, reaching higher levels in the range of 50–80% V_{aq}/V_{org} (Fig. 3B). A further rise in the V_{aq}/V_{org} ratio led to a small decrease in the reduction of **1**, possibly owing to the lower substrate solubilization in the aqueous phase. Similar results were obtained by Cruz et al. for the whole-cell bioconversion of β-sitosterol in aqueous–organic two-phase systems [23], and by Bie et al. in the bioconversion of methyltestosterone by *Arthobacter simplex* AS.1.94 [53]. A volumetric phase ratio of 50% (corresponding to 30 mL of each solvent) was considered to be the optimum condition under which a >99% conversion of **1** to **10** was obtained using yeast cells in the water/organic solvent biphasic system.

3.5. Influence of organic solvents

The feasibility of biphasic bioconversion systems is dependent both on the organic phase toxicity and on its ability to act as a reservoir for the substrate and product [28]. The organic solvent mediates substrate and product partitioning between the organic phase and aqueous phase, which is beneficial to maintain a high catalytic activity of the cells present in the aqueous phase, particularly when the solvent has a low toxicity toward the microbe [11,24]. In order to study this effect, a series of organic solvents was chosen covering a wide range of log *P* values, with special emphasis on those which are hydrophobic and are often assumed to be more biocompatible in the biocatalytic process. As is well known, log *P* denotes the hydrophobicity of the organic solvents [28,29].

Fig. 4 shows that the conversion in 1,3-diphenyl-1-propanone (**10**) mediated by yeast cells increased with an increase in the log *P* value of the organic solvent. The highest conversion rates (99%) were achieved using *n*-heptane (log *P* 4.0), but *n*-hexane (log *P* 3.5) or cyclohexane (log *P* 3.2) also proved to be suitable solvents for this reaction, forming **10** with 98% conversion. Using toluene (log *P* 2.5), only moderate conversions were obtained, while using solvents with log *P* values lower than 2, such as chloroform (2.0) or dichloromethane (1.5), the product was not formed. Probably cell membranes are easily destroyed by organic solvents with low log *P* values, and this autolysis of the yeast cells will certainly limit the biocatalytic process [11,24,48,53].

The order of the solvents in which the highest conversions were obtained was as follows: *n*-heptane > *n*-hexane > cyclohexane > toluene. This data is also in agreement with those reported for the baker's yeast reduction of ethyl acetoacetate in organic–aqueous biphasic systems, with complete conversions in 24 h with ee_p of 92.1% in a water/*n*-hexane system [4,48].

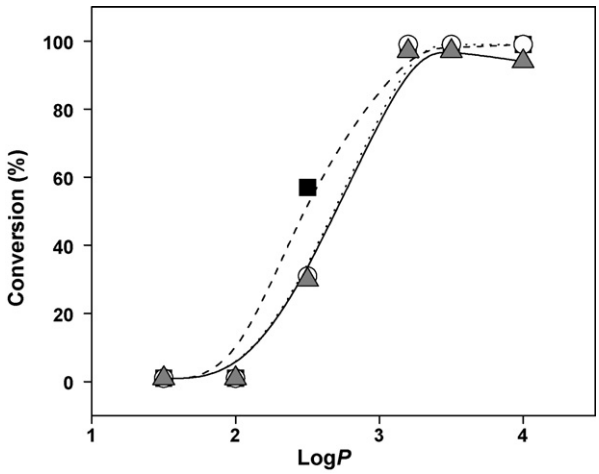
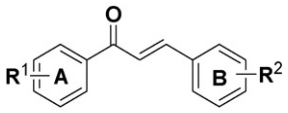


Fig. 4. Reduction of (2*E*)-1,3-diphenyl-2-propen-1-one catalyzed by whole yeast cells of BY (■), CAT-1 (○), or PE-2 (▲) in water/organic solvent biphasic systems containing either dichloromethane (log *P* = 1.5), chloroform (2.0), toluene (2.5), cyclohexane (3.2), *n*-hexane (3.5), or *n*-heptane (4.0) as organic solvents. Reaction conditions as in Fig. 2A except for a substrate concentration of 16.7 mM.

Table 1
Chemoselective biohydrogenation of chalcones with yeasts.[a]



1-9

Chalcones	R_1	R_2	Conversion (%)[b]		
			BY	CAT-1	PE-2
1	H	H	79	92	96
2	4-OCH_3	H	49	56	50
3	3,4-*di*-OCH_3	H	57	30	40
4	3,4-(OCH_2O)	H	74	91	91
5	4-NO_2	H	62	45	47
6	H	4-OCH_3	97	97	94
7	H	3,4-*di*-OCH_3	16	2	8
8	H	3,4-(OCH_2O)	64	49	47
9	H	4-NO_2	94	74	63

[a] Reaction conditions: 16.7 mM substrate, 100 g/L yeasts, 30 mL potassium phosphate/citric acid buffer (0.2 M, 0.1 M pH 5.5), 30 mL *n*-hexane (V_{aq}/V_{org} 1:1%), 35 °C, 1 h.
[b] Determined by chiral-GC.

3.6. *Effect of substituents present in chalcones* ***2–9***

After optimizing all the parameters described above, a series of substituted chalcones (**2–9**) was tested on the reduction catalyzed by BY, CAT-1, and PE-2 yeast cells. These compounds have electron-donating (4-OCH_3, 3,4-*di*-OCH_3, 3,4-[OCH_2O]) or electron-withdrawing (4-NO_2) groups on each phenyl ring. Table 1 summarizes the results obtained for chalcones **1–9** after incubation with whole yeast cells in a water/*n*-hexane biphasic system. It is worth mentioning that in all cases the reaction remained with high chemoselectivity, and formed the corresponding dihydrochalcones **10**–**18** as the single product.

Due to the conjugation present on the chalcones, it is expected that electron-withdrawing groups on ring B could increase the reactivity of the β-carbonyl carbon, and that electron-donating groups should decrease the reactivity in relation to hydride addition, while electron-withdrawing or electron-donating groups on ring A should not interfere with the reaction [54].

However, the results presented in Table 1 show that for chalcones **2**, **3** and **4** (with electron-donating groups) or chalcone **5** (with an electron-withdrawing group) on ring A, the conversions into product were similar or lower than that obtained for chalcone **1**. This data indicates that the presence of different groups on ring A did not have the expected effect on the biohydrogenation reaction mediated by yeast cells in organic–aqueous biphasic systems.

Considering the substituents on ring B, chalcone **6** (with an electron-donating group) and chalcone **9** (with an electron-withdrawing group) showed conversions into the corresponding dihydrochalcones at levels similar to or lower than that obtained for chalcone **1**, which are unexpected results. Finally, for chalcones **7** and **8**, with two strong electron-donating groups, the conversions into product were lower than that for chalcone **1,** an expected result due to the increased electron density of the β-carbonyl carbon. All together, our results showed not only some dependence on conversion degrees in relation to the substituent groups, but also to the use of the different yeast strains. These results are in agreement with those published by Otha et al., as they also observed a small effect on both rings of some substituted chalcones (compounds **2**, **5**, **6** and **9**) when the reaction was catalyzed by *C. equi* IFO3730 in aqueous medium [45].

4. Conclusions

This is the first report related to the use of whole cells of three *S. cerevisiae* industrial yeast strains for the biohydrogenation of 1,3-diaryl-2-propen-1-ones (**1–9**) in water/organic solvent biphasic systems, producing the corresponding saturated ketones (**10–19**). After optimizing the reaction conditions, ketone **10** was obtained with high conversions (>99%). The influence of substituent groups on rings A and B of chalcones **1–9** was, in general, small and no correlation between the donor or withdrawing electron groups was observed. The use of microorganisms as biocatalysts in the biotransformation of chalcones is an important strategy to obtain dihydrochalcones with high chemoselectivity, under mild reaction conditions, using low cost reagents and with low environmental pollution.

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