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Improving the enantioselective bioreduction of aromatic ketones mediated by *Aspergillus terreus* and *Rhizopus oryzae*: the role of glycerol as a co-solvent

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ABSTRACT

The improvement of the enzymatic performance of *Aspergillus terreus* and *Rhizopus oryzae* in enantioselective bioreductions by using glycerol as a co-solvent has been studied. In the most of the bioreductions, glycerol has demonstrated its potential for improved conversions (up to >99%) and enantioselectivities (up to >99%) when compared to reactions in aqueous or other aqueous–organic media (THF, diethyl ether, toluene, DMSO and acetonitrile). Moreover, high isolated yields of the desired chiral alcohols have been obtained on a preparative scale showing the great potential of this green solvent in biocatalysis.

1. Introduction

Biocatalytic asymmetric reactions have been widely used in organic synthesis.[1–3] As the enzymatic reactions are usually carried out in water, the use of organic compounds as solvent or co-solvent has proven to be useful for expanding the applicability of biocatalytic processes to poorly water-soluble organic compounds. Therefore, the presence of a hydrophilic solvent can improve the solubility of organic compounds and facilitates the enzyme–substrate interaction.[4] Several solvent systems have been applied in reactions mediated by purified enzymes or microorganisms.[5–11] In some cases, organic co-solvents have improved the yield and enantioselectivity of enzymatic transformations.[12] Nowadays, the choice of an appropriate solvent for enantioselective reactions has to take into account environment concerns, and green solvents, such as supercritical CO_2 and ionic liquids have been studied with this purpose.[13–15] Another green solvent that has received attention is glycerol, especially because it is a by-product of biodiesel production, which can be obtained from renewable sources. Glycerol is an environmentally friendly, non-toxic, biodegradable solvent. It has already been applied in biocatalytic reactions, and demonstrated its potential for biocatalytic reactions.[16,17] Moreover, the water–glycerol combination leads to a homogenous system and avoids mass transfer limitations, which is common in biphasic systems. Furthermore, glycerol solubilizes several organic compounds, but is immiscible with hydrophobic solvents (e.g., ethyl acetate, diethyl ether). This feature allows easy product recovery by simple extraction with a hydrophobic solvent. Due to these properties, we decided to study the effect of the glycerol as a co-solvent in the bioreduction of haloacetophenones mediated by whole cells of *Aspergillus terreus* SSP 1498 and *Rhyzopus oryzae* CCT 4964. Herein, we report an improvement in the enantioselective bioreduction of haloacetophenones using glycerol as co-solvent.

2. Results and discussion

2.1. Screening of solvents for bioreduction mediated by *A. terreus* and *R. oryzae*

Despite all the relevant properties of glycerol discussed above, we also decided to evaluate the influence of some hydrophobic solvents (toluene, diethyl ether and tetrahydrofuran) and hydrophilic solvents (dimethyl sulfoxide and acetonitrile) in the bioreduction of haloacetophenones by cells of *A. terreus* and *R. oryzae*. The hydrophobic solvents gave biphasic systems that were used for comparison with the bioreductions in glycerol. For this study, we selected 2′-chloroacetophenone **1** as a model substrate and the reactions were performed in different concentrations of organic solvent (Table 1).

As can be seen from the results presented in Table 1, cells of *A. terreus* and *R. oryzae* promoted the reduction of the ketone **1** into the (*S*)-alcohol with good results. For example, the bioreduction catalyzed by cells of *A. terreus* in phosphate buffer solution (PBS) led to (*S*)-**1a** alcohol with low concentration (24%) and enantiomeric excess (65%; Table 1, entry 1). However, by using PBS–glycerol (9:1), the values of concentration and ee of the (*S*)-**1a** alcohol increased to 49% and 92%, respectively (Table 1, entry 2). Similarly, PBS–glycerol (4:1) gave better results (44% concentration, 99% ee) than the reaction using PBS as solvent (Table 1, entry 3). When PBS–DMSO (9:1) was used with cells of *A. terreus*, moderate concentration and excellent enantioselectivity were observed for (*S*)-**1a** alcohol (51% and >99%, respectively; Table 1, entry 4). However, by using PBS–DMSO (4:1) a significant decrease in the concentration (10%) was observed with maintenance of the enantioselectivity (Table 1, entry 5).

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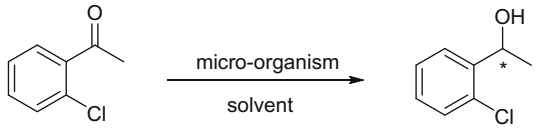
Table 1
Influence of co-solvents in the bioreduction of the 2′-chloroacetophenone[a]



1 **1a**

Entry	Solvent	*A. terreus* SSP 1498		*R. oryzae* CCT 4964	
		c^b (%)	ee^c (%)	c^b (%)	ee^c (%)
1	PBS	24	65 (*S*)	88	94 (*S*)
2	PBS–glycerol (9:1)	49	92 (*S*)	72	94 (*S*)
3	PBS–glycerol (4:1)	44	>99 (*S*)	50	97 (*S*)
4	PBS–DMSO (9:1)	51	>99 (*S*)	27	>99 (*S*)
5	PBS–DMSO (4:1)	10	>99 (*S*)	19	>99 (*S*)
6	PBS–acetonitrile (9:1)	9	>99 (*S*)	–	–
7	PBS–acetonitrile (4:1)	–	–	–	–
8	PBS–toluene (9:1)	4	–	–	–
9	PBS–toluene (4:1)	<1	–	–	–
10	PBS–diethyl ether (9:1)	4	–	–	–
11	PBS–diethyl ether (4:1)	3	–	–	–
12	PBS–THF (9:1)	2	–	–	–
13	PBS–THF (4:1)	2	–	–	–

PBS: Phosphate buffer solution [Na_2HPO_4/KH_2PO_4 buffer (pH 7)].
[a] Reaction conditions: 3.0 g of fungal cells, 20 μL of 2′-chloroacetophenone, 50 mL of solvent, 48 h, 32 °C, 160 rpm.
[b] Concentration of product was determined by chiral GC analysis.
[c] Enantiomeric excess was determined by chiral GC analysis; (–) not determined due to low conversion.

The bioreduction of ketone **1** with cells of *R. oryzae* in PBS showed good concentration and ee of the product (88% and 94%, respectively; Table 1, entry 1). However, the use of PBS–glycerol (4:1) increased the ee to 97% and the concentration was slightly decreased to 50% (Table 1, entry 3). By using PBS–DMSO (9:1 and 4:1) excellent enantioselectivity (>99%) was observed but the product concentration was low (27% and 19%, respectively; Table 1, entries 4 and 5). No significant bioreduction was observed employing *A. terreus* or *R. oryzae* in acetonitrile, toluene, diethyl ether and THF (Table 1, entries 6–13). The reason for such a low conversion can be related to the strong inhibition of the alcohol dehydrogenase involved in the bioreduction. Moreover, the biphasic system obtained by hydrophobic solvents (toluene, diethyl ether and THF) can make the enzyme–substrate interaction difficult, and consequently the bioreduction reaction fails.

As can be seen in Table 1, the bioreduction with *A. terreus* and *R. oryzae* using glycerol or DMSO as co-solvent afforded (*S*)-**1a** alcohol in similar enantioselectivities (entries 2–5). However, it was observed that the use of glycerol as a co-solvent gave higher conversions than the reaction with DMSO. By using glycerol, the concentration range of the product was 44–72% (Table 1, entries 2 and 3), and 10–50% by using DMSO. Based on these results we selected glycerol as the co-solvent to be applied in the following studies. Moreover, the choice of the co-solvent can be reinforced by the growing availability of glycerol in the market, since it is a by-product from biodiesel production.

In general, the use of the glycerol improved the enantioselectivity and conversion in the bioreductions, with the desired chiral alcohols being obtained with higher concentrations, and mainly excellent enantiomeric excesses in comparison to the bioreductions in a phosphate buffer solution (Table 1, entries 2 and 3).

We can associate the successful application of glycerol as a co-solvent in the bioreductions mediated by *A. terreus* and *R. oryzae* to different factors: (i) It was created as a homogeneous system by the glycerol–water combination that avoids the mass transfer limitation, thus increasing the enzyme–substrate interaction; (ii) glycerol has a protein-stabilizing action. Timasheff and Gekko[18] explained the stabilizing-action of glycerol in terms of energy by a model called preferential exclusion that was formulated based on the preferential hydration of proteins. According to this model, in a protein–water–glycerol system, the glycerol is preferentially excluded from the immediate proximity of the protein (unfavourable interaction), and the protein will tend to be preferentially hydrated.[19] Thus, it will stabilize the protein native structure and prevent its denaturation; consequently the enzymatic activity can be maintained.[20] As we know that glycerol can penetrate the cell wall and the cytoplasmic membrane,[21] the same protein-stabilizing action can be occurring with alcohol dehydrogenases from *A. terreus* and *R. oryzae*.

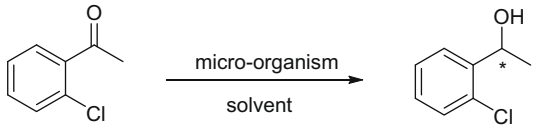
Based on the remarkable enhancement of both conversion and enantioselectivity by the use of glycerol as co-solvent, we decided to apply it in the bioreduction of different haloacetophenones. However, we initially investigated the ideal ratio of PBS and glycerol in order to evaluate its influence on the conversion and enantioselectivity of the bioreduction.

2.2. Study of the ratio of glycerol to aqueous media for bioreduction reactions

To optimize the ratio of glycerol to aqueous media for the bioreduction, we selected 2′-chloroacetophenone **1** as the substrate model and a phosphate buffer solution as the aqueous media (Table 2).

As we can see from the results summarized in Table 2, the concentration of glycerol between 5% (PBS–glycerol 9.5:0.5) to 20% (PBS–glycerol 4:1) gave good values of conversion and ee (Table 2, entries 2–4). It is also noteworthy that the enantiopreference was not affected by the different concentrations of glycerol. In all cases, the formation of alcohol (*S*)-**1a** was observed. Above 30% of glycerol, a gradual and significant decrease in the conversion was observed, especially for the reactions using 100% of glycerol in which no conversion was observed (Table 2, entries 5–8). Based on these results, PBS–glycerol (9:1) and PBS–glycerol (4:1) were selected as the ideal solvent systems for the bioreduction of different haloacetophenones **1**–**9**.

Table 2
Study of the ratio of glycerol to aqueous media in the bioreduction of 2′-chloroacetophenone[a]



1 **1a**

Entry	Solvent—PBS–glycerol (glycerol,%)	*A. terreus* SSP 1498		*R. oryzae* CCT 4964	
		c^b (%)	ee^c (%)	c^b (%)	ee^c (%)
1	0	24	65 (*S*)	88	94 (*S*)
2	5	49	92 (*S*)	75	95 (*S*)
3	10	49	95 (*S*)	72	96 (*S*)
4	20	44	>99 (*S*)	50	97 (*S*)
5	30	14	>99 (*S*)	13	>99 (*S*)
6	40	10	>99 (*S*)	5	—
7	50	7	>99 (*S*)	4	—
8	100	<1	—	<1	—

[a] Reaction conditions: 3.0 g of fungal cells, 20 μL of 2′-chloroacetophenone, 50 mL of solvent [Na_2HPO_4/KH_2PO_4 buffer (pH 7):glycerol], 48 h, 32 °C, 160 rpm.
[b] Concentration of product was determined by chiral GC analysis.
[c] Enantiomeric excess was determined by chiral GC analysis; (—) not determined due to low conversion.

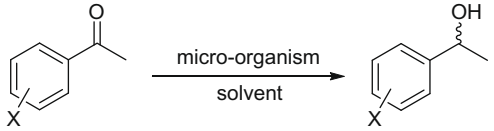
2.3. Bioreduction of chloro, fluoro and bromoacetophenones 1–9

Having in hand the appropriate reaction conditions, they were applied to the bioreduction of chloro-, fluoro- and bromoacetophenones **1**–**9** (Table 3).

2.3.1. Bioreduction of bromoacetophenones

The important influence of glycerol as co-solvent was observed in the reduction of the bromoacetophenones **3**, **6** and **9**. In comparison to the PBS system, in all cases, the use of PBS–glycerol (9:1) improved the conversion of ketones **3**, **6** and **9** into the correspond-

Table 3
Bioreduction of haloacetophenones **1**–**9** in phosphate buffer solution (PBS) and PBS–glycerol system[a]



1-9 **1a-9a**

Entry	X	Solvent system					
		PBS		PBS–Glycerol (9:1)		PBS–Glycerol (4:1)	
		c^b (%)	ee^c (%)	c^b (%)	ee^c (%)	c^b (%)	ee^c (%)
Aspergillus terreus SSP 1498							
1	2′-Chloro **1**	24	65 (*S*)	49	92 (*S*)	44	>99 (*S*)
2	2′-Fluoro **2**	99	89 (*S*)	99	92 (*S*)	99	94 (*S*)
3	2′-Bromo **3**	27	>99 (*S*)	48	>99 (*S*)	35	>99 (*S*)
4	3′-Chloro **4**	72	>99 (*S*)	49	83 (*S*)	64	87 (*S*)
5	3′-Fluoro **5**	98	14 (*S*)	90	47 (*S*)	90	45 (*S*)
6	3′-Bromo **6**	69	87 (*S*)	85	86 (*S*)	78	86 (*S*)
7	4′-Chloro **7**	88	46 (*R*)	87	53 (*R*)	84	31 (*R*)
8	4′-Fluoro **8**	58	61 (*R*)	66	40 (*R*)	48	27 (*R*)
9	4′-Bromo **9**	93	18 (*R*)	87	28 (*R*)	98	17 (*R*)
Rhyzopus oryzae CCT 4964							
10	2′-Chloro **1**	88	94 (*S*)	72	94 (*S*)	50	97 (*S*)
11	2′-Fluoro **2**	90	>99 (*S*)	63	>99 (*S*)	56	>99 (*S*)
12	2′-Bromo **3**	21	>99 (*S*)	29	>99 (*S*)	16	>99 (*S*)
13	3′-Chloro **4**	23	38 (*S*)	34	54 (*S*)	15	51 (*S*)
14	3′-Fluoro **5**	96	39 (*S*)	96	37 (*S*)	88	36 (*S*)
15	3′-Bromo **6**	12	67 (*S*)	19	70 (*S*)	8	58 (*S*)
16	4′-Chloro **7**	20	90 (*S*)	30	93 (*S*)	10	93 (*S*)
17	4′-Fluoro **8**	6	59 (*S*)	3	61 (*S*)	5	64 (*S*)
18	4′-Bromo **9**	30	97 (*S*)	40	98 (*S*)	40	96 (*S*)

PBS: Phosphate buffer solution [Na_2HPO_4/KH_2PO_4 buffer (pH 7)].
[a] Reaction conditions: 3.0 g of fungal cells, 20 μL of haloacetophenone, 50 mL of solvent [PBS–Glycerol], 48 h, 32 °C, 160 rpm.
[b] Concentration of product was determined by chiral GC analysis.
[c] Enantiomeric excess was determined by chiral GC analysis.

ing alcohols. The conversion of 2′-bromoacetophenone **3** into the (*S*)-**3a** alcohol was improved from 27% in PBS to 48%, in reactions catalyzed by *A. terreus* (Table 3, entry 3), and from 21% in PBS to 29% in reactions catalyzed by *R. oryzae* (Table 3, entry 12). For both cases, excellent enantiomeric excesses (up to >99%) were observed.

In the reaction catalyzed by *A. terreus*, the use of PBS–glycerol (9:1) improved the conversion of 3′-bromoacetophenone **6** from 69% in PBS to 85% (Table 3, entry 6). In this case, no alteration was observed in the enantioselectivity and the alcohol (*S*)-**6a** was obtained with 86% ee. In the reaction catalyzed by *R. oryzae* with PBS–glycerol (9:1), the concentration of alcohol (*S*)-**6a** was improved from 12% in PBS to 19% and with 70% ee (Table 3, entry 15).

In the bioreduction of 4′-bromoacetophenone **9** by *A. terreus* in PBS–glycerol (9:1) the ee of alcohol (*R*)-**9a** was improved from 18% in PBS to 28%. In all cases high concentrations of (*R*)-**9a** (up to 98%) were observed (Table 3, entry 18). When the reaction was catalyzed by *R. oryzae,* the concentration of the product was improved from 30% in PBS to 40% PBS–glycerol 10%. For this case, no remarkable change in the ee was observed, and alcohol (*S*)-**9a** was obtained with 98% ee (Table 3, entry 18).

2.3.2. Bioreduction of chloroacetophenones

The enantioselectivity of the bioreduction of 2′-chloroacetophenone **1** using cells of *A. terreus* was strongly influenced by the addition of glycerol. When the reaction was carried out in only PBS, a low concentration of alcohol (*S*)-**1a** (24%) and the ee (65%) were observed. However, the reaction in PBS–glycerol (4:1) improved the concentration of (*S*)-**1a** to 44% and the enantiomeric excess to >99% (Table 3, entry 1). For the reactions catalyzed by cells of *R. oryzae*, an improvement in the ee of the alcohol (*S*)-**1a** from 94% in PBS to 97% in PBS–glycerol (4:1) was observed. Moreover, a decrease in the concentration to 50% by using PBS–glycerol system (4:1) was also observed (Table 3, entry 10).

The bioreduction of 3′-chloroacetophenone **4** by *A. terreus* was not positively affected by glycerol. In this case, the best result was observed in the PBS system where the concentration of the (*S*)-**4a** alcohol was 72% with >99% ee (Table 3, entry 4). On the other hand, in the reactions catalyzed by *R. oryzae*, the addition of glycerol improved the concentration of (*S*)-**4a** from 23% in PBS to 34% in PBS–glycerol (9:1). The ee of the (*S*)-**4a** alcohol was also improved from 38% to 54% by the use of glycerol as co-solvent (Table 3, entry 13).

The use of PBS–glycerol (9:1) was shown to be the best system for reducing 4′-chloroacetophenone **7**. When the ketone **7** was reduced by *A. terreus* an improvement in the ee of (*R*)-**7a** from 46% in PBS to 53% in PBS–glycerol (9:1) with high concentration of the product (87%; Table 3, entry 7) was observed. When *R. oryzae* was used as a biocatalyst, the reduction of **7** in PBS led to (*S*)-**7a** alcohol in 20% concentration and 90% ee. By using PBS–glycerol (9:1), the concentration and ee of (*S*)-**7a** were improved to 30% and 93%, respectively (Table 3, entry 16).

2.3.3. Bioreduction of fluoroacetophenones

The bioreduction of **2** by *A. terreus* using all solvent systems gave alcohol (*S*)-**2a** with excellent concentration (>99%) and ee (up to 94%) (Table 3, entry 2). In the reactions catalyzed by *R. oryzae*, the addition of glycerol decreased the concentration of (*S*)-**2a** from 90% in PBS to 56% in PBS–glycerol (4:1). However, excellent enantiomeric excesses (>99%) were observed for the three solvent systems evaluated (Table 3, entry 11).

3′-Fluoroacetophenone **5** was best reduced by cells of *A. terreus* into (*S*)-**5a** in PBS–glycerol (9:1), in which the ee was improved to 90% while the bioreduction carried out in PBS led to (*S*)-**5a** with low ee (14%; Table 3, entry 5). Moreover, the ee was not affected by glycerol when cells of *R. oryzae* were used in the reduction of **5**. In all cases, low ee was observed (37%). A slight decrease in the concentration of alcohol (*S*)-**5a** (96–88%) was observed when PBS–glycerol (4:1) was used (Table 3, entry 14).

In the reduction of 4′-fluoroacetophenone **8** by *A. terreus*, PBS was the best solvent leading to (*R*)-**8a** with moderate concentration and ee (58% and 61%, respectively). In this case, the use of PBS–glycerol (9:1) system improved the concentration to 66%, but the ee was reduced to 40% (Table 3, entry 8). When PBS–glycerol (4:1) was used both conversion and ee were decreased to 48% and 27%, respectively (Table 3, entry 8). The reductions of **8** by *R. oryzae* occurred with very low concentration of the (*S*)-**8a** alcohol (up to 6%) and moderate ee (60%) (Table 3, entry 17).

In general, the use of PBS–glycerol, especially in a 9:1 ratio was shown to be an excellent system for the reduction of haloacetophenones. Glycerol has demonstrated its potential for the improvement of the enzymatic performance of *A. terreus* and *R. oryzae* in terms of conversion and enantioselectivity.

In order to evaluate the application of the bioreduction in a PBS–glycerol system on a higher scale, we decided to carry out different assays for the reduction of 2′-chloroacetophenone **1**: (i) A set of 10 Erlenmeyer flasks (150 mL) each one containing solvent (PBS–glycerol, 9:1) or (PBS–glycerol, 4:1), ketone **1** and cells of *A. terreus*. (ii) One Erlenmeyer flask (2000 mL) with solvent (PBS–glycerol, 9:1 or PBS–glycerol, 4:1), ketone **1** and fungi cells of *A. terreus*.

Both reaction systems carried out with PBS–glycerol (4:1) led to (*S*)-**1a** with excellent enantioselectivity (>99% ee) and high concentration (90%). The product was obtained in 67% isolated yield from the assay carried out in a set of 10 Erlenmeyer flasks, and 72% from the assay with one flask. Both reaction systems in PBS–glycerol (9:1) showed high enantioselectivity (95% ee) and >99% concentration of the (*S*)-**1a** alcohol. The isolated yield from the assay using a set of 10 Erlenmeyer flasks was 71%, while for the assay with one flask, the yield was 80%. These similar results (high yield and enantioselectivity) showed us that the chiral alcohol production, (*S*)-**1a**, can be easily carried out in a simple one reaction system.

3. Conclusion

In conclusion, we have shown that glycerol, a by-product from biodiesel production, is an excellent option as a co-solvent to improve the bioreduction of halo-acetophenones, in terms of concentration and enantioselectivity of the products. For both fungi, *A. terreus* and *R. oryzae*, bioreduction provided the desired chiral alcohols in high enantiomeric excess (up to >99%) and conversion (up to >99%). Moreover, these data gave us support for further studies using new alcohol dehydrogenases from *A. terreus* and *R. oryzae*.

4. Experimental

4.1. General methods

2′-Bromoacetophenone, 3′-bromoacetophenone, 4′-bromoacetophenone, 2′-chloroacetophenone, 3′-chloroacetophenone, 4′-chloroacetophenone, 2′-fluoroacetophenone, 3′-fluoroacetophenone and 4′-fluoroacetophenone were purchased from Aldrich® and used as supplied. Microorganisms were manipulated in a Vecco laminar flow cabinet, and all incubation experiments were carried out using sterile materials. A Technal TE-421 orbital shaker was employed in the bioreductions. The compound purification was performed by chromatographic column over silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and ethyl acetate as eluent. Column effluents were monitored by TLC on pre-coated Silica Gel 60 F_{254} layers (aluminium-backed: Merck) with mixtures of *n*-hexane and ethyl acetate. Products from the enzyme-catalyzed

reactions were analyzed by a Shimadzu model GC-17A gas chromatograph equipped with a flame ionization detector (FID). A chiral capillary column (Chirasil-Dex CB B-cyclodextrin—25 m × 0.25 mm) was used for determination of the conversion and enantiomeric excesses. Optical rotation values were determined with a Jasco DIP-378 polarimeter using a 1 dm cuvette and the reported values refer to the Na-D line.

4.2. General procedure for the bioreduction reactions

4.2.1. Growth conditions for the fungi cultures

The fungi were grown in Erlenmeyer flasks (2000 mL) containing 1000 mL of culture medium (malt extract—20 g/L) at 32 °C (4 days) in an orbital shaker (160 rpm). After this stage, the cells were filtered off and used for bioreduction assays.

4.2.2. Small scale reactions

Cells of fungi (3 g) were resuspended in 50 mL of the appropriate solvent system (Tables 1 and 2) in an Erlenmeyer flask (150 mL) followed by the addition of the desired haloacetophenone (20 μL; Table 3). The reaction mixture was stirred in an orbital shaker (32 °C; 160 rpm) for 48 h. The bioreductions were analyzed by GC (Section 4.3).

4.2.3. Preparative scale reactions

Assays 1 and 2: In a set of 10 different Erlenmeyer flasks (150 mL) each one containing 50 mL of PBS–glycerol (9:1 or 4:1) were added cells of *A. terreus* SSP 1498 (3 g) and 2′-chloroacetophenone (20 μL). The reaction was carried out in an orbital shaker at 32 °C, 160 rpm for 48 h. After this time the content of the flasks were combined, filtered off and the cells were washed with ethyl acetate (200 mL). The aqueous phase was extracted with ethyl acetate (2 × 200 mL). The organic phases were combined and dried over $MgSO_4$ and filtered. The solvent was removed in vacuum, and the residue was purified by column chromatography on silica gel using a mixture of *n*-hexane and ethyl acetate (9:1) as eluent to afford (*S*)-1-(2-chlorophenyl)ethanol. Isolated yield for bioreduction in PBS–glycerol (4:1) system: 67%; >99% ee; $[\alpha]_D^{22} = -59.8$ (*c* 1.00, $CHCl_3$).

Isolated yield for bioreduction in PBS–glycerol (9:1) system: 71%; 95% ee; $[\alpha]_D^{22} = -59.6$ (*c* 1.00, $CHCl_3$).

Assays 3 and 4: In one Erlenmeyer flask (2000 mL) containing 500 mL of PBS–glycerol (9:1 or 4:1) were added cells of *A. terreus* SSP 1498 (30 g) followed by 2′-chloroacetophenone (200 μL). The reaction was carried out in an orbital shaker at 32 °C, 160 rpm for 48 h. After this time, the content of the flask was filtered and washed with ethyl acetate (200 mL). The aqueous phase was extracted with ethyl acetate (2 × 200 mL). The organic phases were combined and dried over $MgSO_4$ and filtered. The solvent was removed in vacuum and the residue was purified by column chromatography on silica gel using a mixture of *n*-hexane and ethyl acetate (9:1) as eluent to afford (*S*)-1-(2-chlorophenyl)ethanol.

Isolated yield for bioreduction in PBS–glycerol (4:1) system = 72%; >99% ee; $[\alpha]_D^{22} = -60.0$ (*c* 1.00, $CHCl_3$).

Isolated yield for bioreduction in PBS–glycerol (9:1) system = 80%; 95% ee; $[\alpha]_D^{22} = -59.5$ (*c* 1.00, $CHCl_3$).

4.3. Determination of the enzymatic activity of the microorganisms

The reaction progress was monitored after 48 h by collecting 10 mL samples. These samples were extracted by stirring with ethyl acetate (2 mL) followed by centrifugation (6000 rpm, 5 min). The organic phase was analyzed by GC (1 μL) using a chiral capillary column (Chirasil-Dex CB B-cyclodextrin—25 m × 0.25 mm) for determination of the conversion and enantiomeric excesses. The products of the biocatalyzed reactions were compared to a racemic mixture. The preparation of the racemic alcohols **1a**–**9a** was carried out by reduction of the corresponding acetophenones **1**–**9** with sodium borohydride in methanol.

Racemic compounds:[22] GC conditions (carrier gas H_2; 100 kPa; injector 220 °C; detector 220 °C): *(RS)-1-(2-bromophenyl)ethanol* (140 °C, 5 °C/min up to 180 °C; (*R*)-enantiomer 5.11 min; (*S*)-enantiomer 5.88 min); *(RS)-1-(3-bromophenyl)ethanol* (130 °C, 3 °C/min up to 160 °C; (*R*)-enantiomer 7.89 min; (*S*)-enantiomer 8.27 min); *(RS)-1-(4-bromophenyl)ethanol* (110 °C, 3 °C/min up to 150 °C; (*R*)-enantiomer 5.33 min; (*S*)-enantiomer 5.65 min); *(RS)-1-(2-chlorophenyl)ethanol* (125 °C, 5 °C/min up to 150 °C (3 min); (*R*)-enantiomer 5.72 min; (*S*)-enantiomer 6.44 min); *(RS)-1-(3-chlorophenyl)ethanol* (130 °C, 3 °C/min up to 160 °C; (*R*)-enantiomer 6.03 min; (*S*)-enantiomer 6.37 min); *(RS)-1-(4-chlorophenyl)ethanol* (130 °C, 5 °C/min up to 160 °C); (*R*)-enantiomer 5.25 min; (*S*)-enantiomer 5.54 min); *(RS)-1-(2-fluorophenyl)ethanol* (113 °C, 3 °C/min up to 145 °C; (*R*)-enantiomer 6.03 min; (*S*)-enantiomer 6.35 min); *(RS)-1-(3-fluorophenyl)ethanol* (110 °C, (isotherm - 9 min); (*R*)-enantiomer 5.99 min; (*S*)-enantiomer 6.67 min); *(RS)-1-(4-fluorophenyl)ethanol* (110 °C, 3 °C/min up to 180 °C; (*R*)-enantiomer 11.92 min; (*S*)-enantiomer 13.47 min).

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