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Polycyclic Ketone Monooxygenase (PockeMO): A Robust Biocatalyst for the Synthesis of Optically Active Sulfoxides

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Abstract: A recently discovered, moderately thermostable Baeyer-Villiger monooxygenase, polycyclic ketone monooxygenase (PockeMO), from *Thermothelomyces thermophila* has been employed as a biocatalyst in a set of asymmetric sulfoxidations. The enzyme was able to catalyze the oxidation of various alkyl aryl sulfides with good selectivities and moderate to high activities. The biocatalytic performance was able to be further increased by optimizing some reaction parameters, such as the addition of 10% $v\ v^{-1}$ of water miscible solvents or toluene, or by performing the conversion at a relatively high temperature (45 °C). PockeMO was found to display an optimum activity at sulfide concentrations of 50 mM, while it can also function at 200 mM. Taken together, the data show that PockeMO can be used as robust biocatalyst for the synthesis of optically active sulfoxides.

Keywords: biocatalysis; Baeyer-Villiger monooxygenases; chiral sulfoxides; selective oxidations

1. Introduction

Polycyclic ketone monooxygenase (PockeMO) from the thermophilic fungus *Thermothelomyces thermophila* has recently been identified, overexpressed and characterized [1]. This newly discovered Baeyer-Villiger monooxygenase (BVMO) was found to be moderately thermostable, and tolerates several organic cosolvents. Its substrate acceptance profile is somewhat unusual, as it is able to act on relatively bulky and polycyclic ketones. Elucidation of the crystal structure of PockeMO and comparison with other BVMO structures revealed that PockeMO harbors an additional subdomain that enables it to convert bulky substrates. The first characterization study on PockeMO focused on its ability as BVMO to convert ketones. Here, we explored the potential of PockeMO as biocatalyst for the asymmetric oxidation of sulfides.

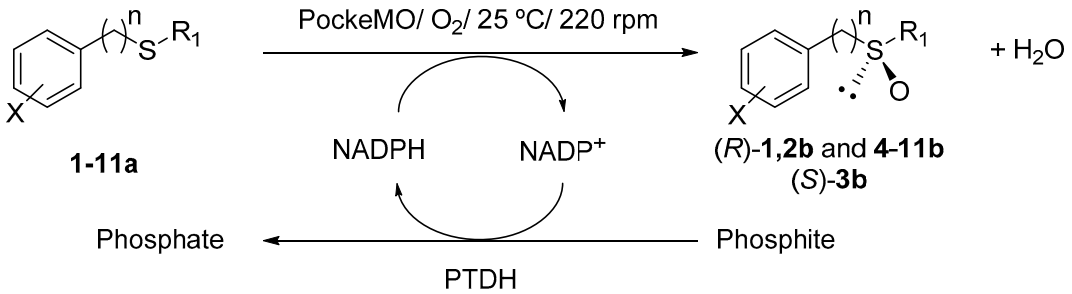
Optically active sulfoxides are very valuable building blocks in organic synthesis, as well as chiral auxiliaries in asymmetric catalysis and compounds with interesting biological properties [2–4]. Several methodologies, employing different catalysts, have been described for their preparation [5,6]. Biocatalytic procedures present some advantages, such as the use of mild and environmentally friendly reaction conditions and oxidants [7,8]. Several types of oxidative enzymes can be used for the synthesis of chiral sulfoxides, which include peroxidases [9–11], dioxygenases [12,13] and different types of monooxygenases [14,15]. BVMOs represent a class of flavin-containing monooxygenases that catalyze the Baeyer-Villiger oxidation of aldehydes and ketones, but they are also able to perform epoxidations and the oxygenation of sulfides and other heteroatom-containing compounds, often with high chemo-, regio- and/or enantioselectivity [16–19]. For these reasons, BVMOs have been intensely studied for their use in the synthesis of valuable compounds in organic and pharmaceutical chemistry.

The most extensively studied BVMO, cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIB 9871, is able to perform the oxidation of numerous alky aryl sulfides, dialkyl sulfides and disulfides, but this catalyst lacks stability in non-conventional reaction media and at high temperatures, and often suffers from substrate inhibition [20–23]. Other recently discovered BVMOs have also been employed in the synthesis of optically active sulfoxides, including 4-hydroxyacetophenone monooxygenases (HAPMO) from *Pseudomonas fluorescens* ACB [24,25], or from *Pseudomonas putida* JD1 [26], ethionamide monooxygenase (EtaA) from *Mycobacterium tuberculosis* [27,28], as well as some BVMOs obtained from *Rhodococcus jostii* RHA1 [29]. Yet, all these BVMOs suffer from low operational stability. Phenylacetone monooxygenase (PAMO) from *Thermobifida fusca* is one of the few known BVMOs that is stable at elevated temperatures [25,30–33], and it tolerates, to some extent, the use of cosolvent; in fact, some cosolvents even improve its performance [28]. Nevertheless, all of the above-mentioned BVMOs have the drawback that their substrate profile is restricted to relatively small substrates. In view of the observation that PockeMO accepts rather bulky ketones, we set out to explore its use as a biocatalyst for the preparation of various optically active sulfoxides.

2. Results and Discussion

2.1. PockeMO-Catalyzed Sulfoxidations

PockeMO was overexpressed and purified as a single fusion protein containing His-tagged phosphite dehydrogenase (PTDH) from *Pseudomonas stutzeri* as N-terminal fusion partner [34], which simplifies the protein purification. This bifunctional redox biocatalyst can be used as a so-called self-sufficient BVMO: the BVMO domain utilizes nicotine adenine dinucleotide phosphate (NADPH) for performing the oxygenation reaction, while the PTDH domain regenerates NADPH at the expense of phosphite, as shown in Scheme 1 [35,36]. As control, the reactions were also performed in the absence of catalyst, but in that case, no significant oxidation was observed. Our first efforts were devoted to establishing the substrate scope of PockeMO in the enzymatic synthesis of optically active sulfoxides. For this reason, we performed the enzymatic oxidation of structurally different sulfides at 25 °C in 50 mM Tris/HCl, pH 8.0. As shown in Table 1, PockeMO readily converts thioanisole (**1a**) into (*R*)-methyl phenyl sulfoxide with complete conversion and an excellent selectivity. By employing other alkyl phenyl sulfides, a decrease in both enzyme activity and selectivity was observed. Ethyl phenyl sulfide (**2a**) was oxidized into ethyl phenyl sulfoxide (*R*)-**2b** with a 76% conversion after 24 h and a moderate optical purity, while the presence of a chlorine atom in the alkyl chain led to a similar selectivity (entry 3 in Table 1), but an even lower conversion (27%). For both compounds, some overoxidation from sulfoxide to sulfone was observed. This is most prominent in the oxidation of 2-chloroethyl phenyl sulfide **3a**, where 81% of the product represented the sulfoxide while also 19% of sulfone was formed.



Scheme 1. PockeMO (Polycyclic ketone monooxygenase) -catalyzed sulfoxidation of a set of prochiral substrates **1–12a** in Tris/HCl 50 mM (pH 8.0) buffer containing NADPH as coenzyme and phosphite as cosubstrate. PTDH: His-tagged phosphite dehydrogenase; NADPH: nicotine adenine dinucleotide phosphate.

Table 1. Enzymatic sulfoxidation of prochiral sulfides **1**–**11a** catalyzed by PockeMO [a].

Sulfide	X	n	R_1	Time (h)	Conversion (%) [b]	% Sulfoxide [c]	*ee* (%) [d]	Configuration
1a	H	0	Methyl	16	≥97	≥97	91	*R*
2a	H	0	Ethyl	24	76	94	72	*R*
3a	H	0	CH_2CH_2Cl	24	27	81	76	*S* [e]
4a	*p*-cyano	0	Methyl	24	43	88	91	*R*
5a	*p*-methoxy	0	Methyl	24	≥97	94	93	*R*
6a	*p*-Cl	0	Methyl	22	78	≥97	88	*R*
7a	m-Cl	0	Methyl	20	≥97	≥97	79	*R*
8a	*o*-Cl	0	Methyl	20	85	95	93	*R*
9a	H	1	Methyl	16	70	82	68	*R*
10a	Phenyl	0	Methyl	24	16	85	83	*R*
11a	H	1	Phenyl	56	15	≥97	83	*R*

[a] For reaction details, see Materials and Methods Section. X, n, and R_1 represent the same groups shown in Scheme 1. [b] Determined by Gas Chromatography/Mass spectroscopy (GC/MS) and refers to the amount of sulfide that is consumed. See Supplementary Material. [c] Represents the percentage of sulfoxide obtained from all the sulfide converted. [d] Determined by High Performance Liquid Chromatography (HPLC), see Supplementary Material. [e] Opposite configuration due to change in priority by CIP rules.

PockeMO efficiently oxidizes a range of *para*-substituted phenyl methyl sulfides. It seems that the electronic nature of the substituent has no effect in terms of selectivity, as chiral sulfoxides (*R*)-**4b**, (*R*)-**5b** and (*R*)-**6b** are obtained with optical purities around 90%. The methyl phenyl sulfide containing a strong electron-withdrawing group, such as *p*-cyano, led to lower conversions after 24 h (43% after 22 h), while a 78% of (*R*)-methyl *p*-chlorophenyl sulfoxide **6b** (88% *ee*) was obtained after 22 h. The *p*-methoxy derivative **5b** was achieved with complete conversion at 24 h. For methyl *p*-cyano and *p*-methoxyphenyl sulfides **4a** and **5a** respectively, minor amounts of sulfones **4c** and **5c** were obtained, while no significant overoxidation was observed with methyl *p*-chlorophenyl sulfide **6a**.

The effect of the chlorine atom position in the aromatic ring was analyzed by performing the oxidation of the prochiral methyl *m*-chloro and *o*-chlorophenyl sulfides **7a** and **8a**. Complete conversion was achieved for (*R*)-methyl *m*-chlorophenyl sulfoxide (**7b**) after 20 h, while the *ortho*-derivative was converted slower, with 85% of sulfide **8a** consumed in 20 h. In contrast, (*R*)-methyl *o*-chlorophenyl sulfoxide (**8b**) was obtained with a very good optical purity (93% *ee*), higher than that achieved for (*R*)-**7b** (79% *ee*).

(*R*)-Benzyl methyl sulfide (**9a**) was also accepted by PockeMO. The enzyme displayed a moderate selectivity (68% *ee*) and 70% conversion after 16 h. The oxidized product mixture contained 85% of (*R*)-benzyl methyl sulfoxide (**9b**), while 15% of sulfone **9c** was formed. The biocatalyst was also able to convert some bulky sulfides, as shown in Table 1 for the oxidation of methyl naphtyl sulfide (**10a**) or benzyl phenyl sulfide (**11a**). Chiral sulfoxide (*R*)-methyl naphthyl sulfoxide **10b** was obtained with 16% conversion after 24 h, while the bulkier sulfide **11a** required 56 h to achieve a similar conversion. For both substrates, the enzyme showed good selectivities (83% *ee*).

Finally, a dialkyl sulfide, cyclohexyl methyl derivative (**12a**), was also tested. The biocatalyst was able to convert all the starting substrate within 24 h, yielding to 82% of (*R*)-cyclohexyl methyl vv sulfoxide with a low optical purity (31% *ee*) and 18% of cyclohexyl methyl sulfone **12c**.

After having established the ability of PockeMO to selectively oxidize various sulfides, we analyzed the effect of different reaction parameters (pH, temperature, sulfide concentration and organic cosolvents) on the activity and selectivity of PockeMO in order to improve its biocatalytic performance.

2.2. Effect of pH and Temperature on PockeMO-Catalyzed Sulfoxidations

The sulfoxidation of **1a** was carried out at 25 °C in Tris/HCl 50 mM buffer at varying pH values ranging from pH 6.5 to 9.0. As can be seen in Figure 1, there is no significant effect of the pH on the optical purity of (*R*)-**1b** (*ee* values around 88–91% for all the pH range), while the enzyme seems to

have an optimal activity at pH 8.0 (complete conversion after 16 h), which is in accordance with the initial enzyme characterization study. pH values below 7.5 led to significant loss in activity.

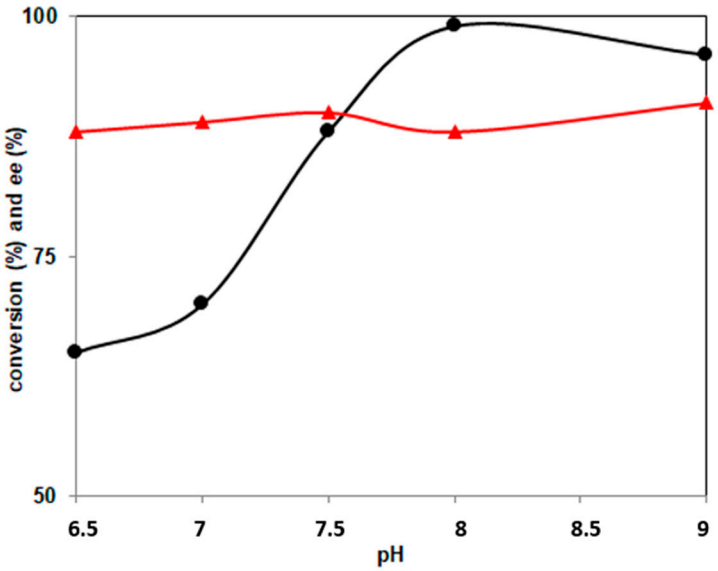


Figure 1. Effect of pH on the activity (●) and selectivity (▲) of PockeMO-catalyzed oxidation of thioanisole to optically active (*R*)-**1b**.

As PockeMO exhibits a considerable degree of thermostability, with the highest activity at 50 °C, those sulfides which were converted poorly (<80%) at 25 °C were also tested at 45 °C. As shown in Table 2, significantly improved conversions were observed, whereas the enantioselectivity remained similar. For example, it was possible to fully oxidize sulfide **2a** in 20 h while at 25 °C the conversion was only 76% after 24 h. The oxidation of bulky sulfides was also significantly accelerated at 45 °C, as shown in entries 5 and 6. This is probably due to the higher enzyme activity at elevated temperatures, while an effect on the solubility may also contribute to higher conversions. For most of the substrates, a higher amount of sulfone was achieved with respect to the oxidations carried out at 25 °C. For instance, a significant amount of sulfones **2c** and **10c** (33% and 50%, respectively), was obtained in the oxidation of sulfides **2a**, and **10a** (entries 1 and 5).

Table 2. Temperature effect in the PockeMO-catalyzed sulfoxidations [a].

Entry	Sulfide	Time (h)	Conversion (%) [b]	% Sulfoxide [c]	*ee* (%) [d]
1	**2a**	20	≥97	67	71
2	**3a**	20	43	80	69
3	**5a**	20	67	90	87
4	**6a**	20	≥97	95	83
5	**10a**	30	77	50	77
6	**11a**	48	27	≥97	80

[a] Reactions were performed at 45 °C in Tris/HCl buffer pH 8.0. [b] Determined by GC/MS and refers to the amount of sulfide that is consumed (Supplementary Material). [c] Represents the percentage of sulfoxide obtained from all the sulfide converted. [d] Determined by HPLC (Supplementary Materials).

2.3. Sulfide Concentration Effect

The influence of the substrate concentration (**1a**) on the activity and selectivity of PockeMO was studied for the sulfoxidation reaction carried out at pH 8.0 and 25 °C (Figure 2). In order to compare the results obtained at different concentrations and times, the space time yield (expressed as mmoles of thioanisole consumed $L^{-1} \cdot h^{-1}$) has been determined. This parameter increased from

10 mM (61.9 mmol $L^{-1}{\cdot}h^{-1}$) to 50 mM (140.6 mmol $L^{-1}{\cdot}h^{-1}$). Higher thioanisole concentrations led to significant lower activities, but it is worth to mention that the enzyme is still able to catalyze the oxidation of 200 mM **1a** with a space time yield of 20.0 mmol $L^{-1}{\cdot}h^{-1}$.

The effect of substrate concentration was studied at several temperatures (see Supplementary Material for complete data). Oxidation of 10 mM **1a** at 45 °C led to complete conversion after 8 h (96% of sulfoxide **1b** and 4% of sulfone **1c**) and was much more efficient when compared with the conversion at 25 °C. Oxidation of 50 mM **1a** at 45 °C afforded the highest space time yield (240 mmol $L^{-1}{\cdot}h^{-1}$). Increasing the substrate concentration led to a decrease in space time yield. Yet, the enzyme still presented a high activity at substrate concentrations further 100–200 mM. PockeMO was also tested at 60 °C and the enzyme was still able to carry out sulfoxidations. When **1a** was oxidized at 10 mM concentration, 85% conversion was observed after 6 h (with 93% of (*R*)-**1b** and a 7% of sulfone **1c** as products) which results in the highest space time yield when compared with the lower temperatures. Increasing the concentration of **1a** led to a similar trend as observed for the other two temperature conditions. However, the space time yields were lower when compared with the conversions at 45 °C. It was gratifying to observe that, except for altering enzyme activity, varying the temperature and/or the substrate concentration did not have a significant effect on the stereoselectivity of the biocatalyst, confirming the robust character of this biocatalyst (see Supplementary Materials).

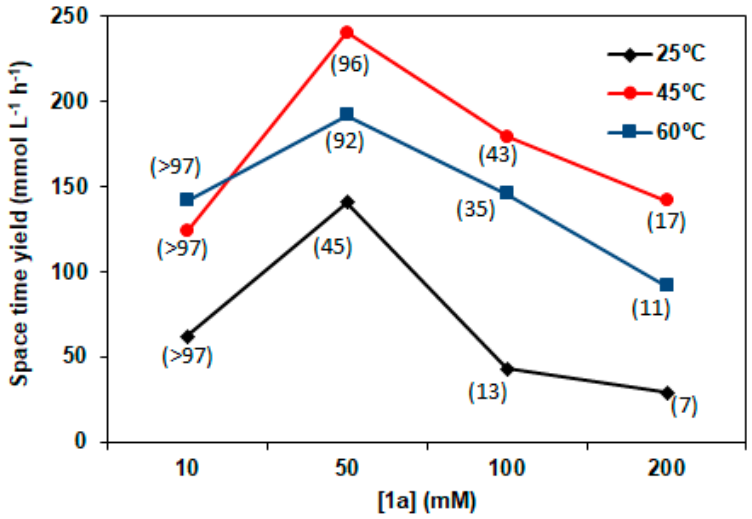


Figure 2. Effect of the thioanisole concentration at different temperatures (25, 45 and 60 °C) in the PockeMO activity, expressed as mmoles of **1a** consumed $L^{-1}{\cdot}h^{-1}$. In brackets, the degree of conversion (%) is indicated.

2.4. Enzymatic Sulfoxidations in the Presence of Cosolvents

As previously reported, PockeMO showed a moderate tolerance to organic cosolvents in the Baeyer-Villiger oxidation of ketones. In addition, certain BVMOs have shown interesting properties in the biocatalyzed sulfoxidation of prochiral sulfides in presence of organic cosolvents [14]. For these reasons, we decided to test the effect of organic cosolvents with different properties, using **2a** as model sulfide substrate, a substrate which was oxidized with good activity and selectivity to (*R*)-**2b** in 50 mM Tris/HCl, pH 8.0. The initial set of reactions were performed at 25 °C using a mixture of Tris/HCl buffer containing 10% $v\,v^{-1}$ of cosolvent (Figure 3). PockeMO displays a higher activity in the presence of hydrophilic solvents such as dimethylsulfoxide, acetonitrile and 1,4-dioxane (>90% conversion for the three solvents). Interestingly, the presence of 10% $v\,v^{-1}$ 1,4-dioxane or dimethylsulfoxide (DMSO) improved the stereoselectivity of the biocatalyst when compared with using just Tris/HCl, as (*R*)-**2b** was recovered with 89% and 80% *ee*, respectively. The use of short alkyl chain alcohols such as ethylenglycol, methanol, ethanol and 2-propanol has a negative effect on the activity of PockeMO, while

the selectivities do not significantly change. In general, the presence of hydrophobic solvents resulted in a significant loss of enzyme activity, with the exception of 10% $v\,v^{-1}$ of 2-methyltetrahydrofuran and toluene, for which conversions of 79% and 89% were obtained. The presence of toluene also results in an increase in the optical purity of the formed sulfoxide (*R*)-**2b** (83%).

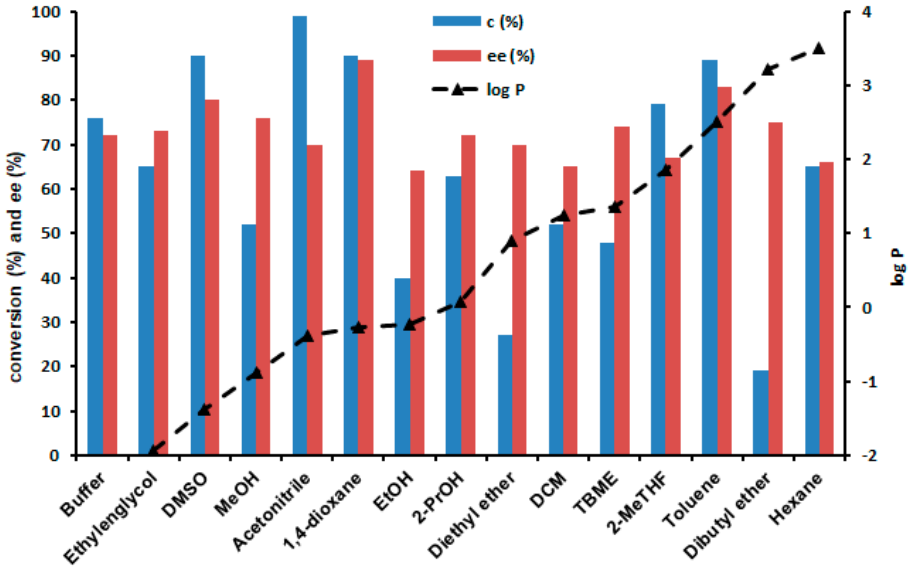


Figure 3. Effect of different organic solvents (10% $v\,v^{-1}$) on the conversion and the stereoselectivity of the enzymatic sulfoxidation of **2a** catalyzed by PockeMO. Reactions were performed at 25 °C for 24 h. log P (▲) values are plotted as reference for the hydrophobicity of the solvent. DMSO: Dimethylsulfoxide; MeOH: Methanol; EtOH: Ethanol; 2-PrOH: 2-Propanol; TBME: *tert*-butyl methyl ether; DCM: Dichloromethane; 2-MeTHF: 2-Methyltetrahydrofuran.

As the presence of both 1,4-dioxane and toluene (10% $v\,v^{-1}$) seems to have a positive effect on PockeMO's performance, the sulfoxidation of ethyl phenyl sulfide was tested with higher amounts of 1,4-dioxane and toluene (Table 3). The use of 30% $v\,v^{-1}$ of 1,4-dioxane (entry 1) led to (*R*)-**2b** with 77% conversion and 77% enantiomeric excess, which is still a better result than those obtained for bioxidation in Tris/HCl buffer. A higher amount of this solvent (entry 2) led to a drastic decrease in conversion, with only 7% of the sulfoxide (with only 39% *ee*) being formed. The presence of 30% $v\,v^{-1}$ of toluene also has a very negative effect on PockeMO performance, with only 11% of chiral sulfoxide obtained, as shown in entry 3.

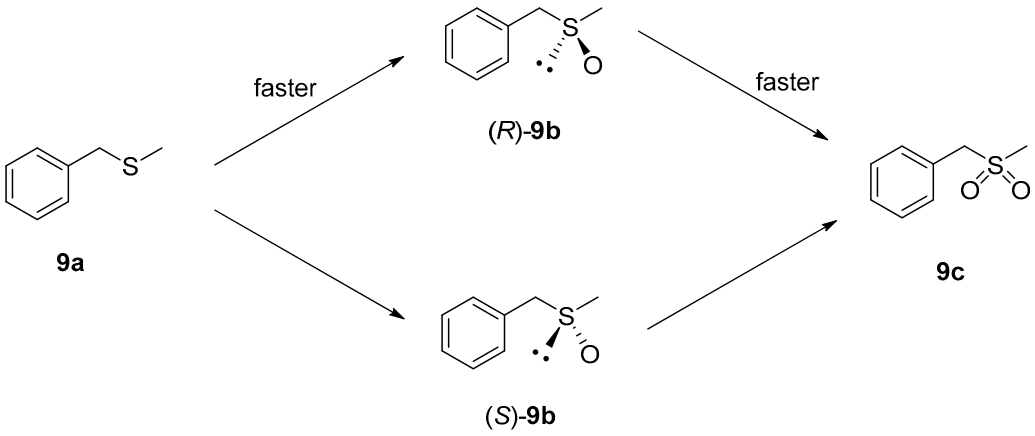
Table 3. Solvent effects in the oxidation of different sulfides catalyzed by PockeMO [a].

Entry	Sulfide	Cosolvent	% Cosolvent	Time (h)	Conversion (%) [b]	% Sulfoxide [c]	*ee* (%) [d]
1	**2a**	1,4-dioxane	30	24	77	95	77
2	**2a**	1,4-dioxane	50	24	7	≥97	39
3	**2a**	toluene	30	24	11	≥97	74
4	**4a**	1,4-dioxane	10	24	88	≥97	95
5	**6a**	1,4-dioxane	10	24	69	≥97	97
6	**9a**	1,4-dioxane	10	24	95	72	31
7	**10a**	1,4-dioxane	10	30	29	≥97	85
8	**11a**	1,4-dioxane	10	56	23	≥97	87
9	**12a**	1,4-dioxane	10	24	≥97	74	33

[a] Reactions were performed at 45 °C in Tris/HCl buffer pH 8.0. [b] Determined by GC/MS. This refers to the amount of sulfide that is consumed in the reaction. See Supplementary Materials. [c] Represents the percentage of sulfoxide obtained from all the sulfide converted. [d] Determined by HPLC, see Supplementary Materials.

The use of 10% *v* v^{-1} 1,4-dioxane was also tested in the oxidation of several other sulfides, with the aim of improving some of the biocatalyzed reactions that led to moderate or low activities and/or selectivities, as shown in Table 3. Thus, the sulfoxidation of **4a** in the presence of 1,4-dioxane led to (*R*)-**4b** with an excellent selectivity and a high conversion (entry 4). Chiral sulfoxide (*R*)-**6b** can be obtained with 97% *ee*, while the conversion for this compound was slightly lower when compared with the oxidation in buffer. A positive effect was observed for the bulky sulfides methyl napththyl sulfide **10a** and benzyl methyl sulfide **11a**, as the (*R*)-sulfoxides are recovered with higher conversions in the presence of 10% *v* v^{-1} 1,4-dioxane. In a similar manner, complete conversion was achieved in the preparation of the alkyl sulfoxide (*R*)-cyclohexyl methyl sulfoxide **12b**. For this compound, a higher amount of sulfone was obtained when using the organic solvent. For sulfoxides (*R*)-**10**–**12b** the organic solvents had no effect on stereoselectivity.

The sulfoxidation of **9a** in 10% *v* v^{-1} 1,4-dioxane after 24 h afforded (*R*)-**9b** with 95% conversion, for which 72% corresponds to the optically active sulfoxide with 31% *ee* (entry 6, Table 3), a much lower optical purity when compared with sulfoxidation carried out in Tris/HCl buffer. When this reaction in presence of 1,4-dioxane was stopped after 12 h, a conversion of 77% was measured (95% sulfoxide), obtaining (*R*)-**9b** with 39% *ee*. This result indicates that the optical purity of the chiral sulfoxide decreased during the conversion. The same experiment was performed for the reaction carried out in buffer. After 12 h, (*R*)-**9b** was obtained with 48% conversion (90% sulfoxide) and 65% *ee*, a similar value to that achieved after 16 h (entry 9, Table 1). In order to establish the reason for this effect, we analyzed the PockeMO-catalyzed oxidation of the racemic benzyl methyl sulfoxide (±)-**9b** in both 50 mM Tris/HCl (pH 8.0) and in Tris/HCl buffer containing 10% *v* v^{-1} 1,4-dioxane. The kinetic resolution performed in absence of the organic solvent led to the formation of 75% of sulfone **9c**. The remaining sulfoxide presented the *S*-configuration and 33% *ee*. The oxidation in presence of solvent yielded a higher conversion (87%). The 13% of remaining (*S*)-**9b** was achieved with 77% *ee*. Thus, the kinetic resolution of (±)-**9b** was faster and more selective in the presence of 1,4-dioxane, but PockeMO mainly oxidizes the *R* enantiomer, the one formed preferentially in the sulfoxidation of **9a**, into benzyl methyl sulfone **9c**, which led to a decrease in the sulfoxide optical purity over time, as shown in Scheme 2.



Scheme 2. PockeMO-catalyzed sulfoxidation of benzyl methyl sulfide **9a** in 50 mM Tris/HCl (pH 8.0) containing 10% *v* v^{-1} 1,4-dioxane.

3. Materials and Methods

3.1. General Materials and Methods

Purified PockeMO was prepared as described previously [1]. Sodium phosphite dibasic pentahydrate, starting sulfides **1a**, **3a** and racemic sulfoxide (±)-**1b** were obtained from Sigma-Aldrich, Steinheim, Germany. Sulfides **2a**, **4a**, **7**–**9a** and sulfoxide (±)-**9b** were purchased from TCI Europe,

Zwijndrecht, Belgium. NADPH and starting compounds **5a** and **10**–**12a** were obtained from Alfa Aesar, Karlsruhe, Germany. Sulfide **6a** was purchased from Acros Organics, Geel, Belgium. Racemic sulfoxides (±)-**2**–**8b** and (±)-**10**–**12b** were obtained by oxidizing the corresponding sulfides in presence of hydrogen peroxide and methanol at room temperature (yields higher than 80%), and exhibited physical and spectral properties in accordance with those reported [24,31].

GC/MS analyses were performed with a GC Hewlett Packard 7890 Series II equipped with a Hewlett Packard 5973 chromatograph MS (Agilent Technologies, Santa Clara, CA, USA) using a HP-5MS cross-linked methyl siloxane column (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2). To monitor levels of conversion, substrates and products were quantified by use of calibration curves. Further data are supplied at the Supplementary Materials. HPLC analyses were performed on a Waters 2695 Instrument equipped with a Waters 996 Photodiode Array Detector. To determine the enantiomeric excesses of sulfoxides **1**–**12b** the following columns from Daicel were employed: Chiralcel OD (25 cm × 0.46 cm) for sulfoxides **1**–**4b** and **9**–**11b**, Chiralcel OB (25 cm × 0.46 cm) for compounds **6**–**8b** and **12b** and Chiralcel OJ-H (25 cm × 0.46 cm) for product **5b**. HPLC complete data are supplied at the Supplementary Material. The sulfoxide configurations were established by comparing the HPLC chromatograms with the ones described in the bibliography [24,31,33].

3.2. General Procedure for the Enzymatic Sulfoxidation of Prochiral Sulfides

Unless otherwise stated, the prochiral sulfides **1**–**12a** (10–200 mM) were dissolved in 1.0 mL Tris/HCl 50 mM (pH 8.0) containing, when indicated, the corresponding organic solvent, NADPH (0.2 mM), sodium phosphite (1.0 equivalent) and PockeMO (1.0 μM). Reactions were stirred at the temperatures selected at 220 rpm for the times established. Once finished, the reactions were extracted with EtOAc (2 × 0.5 mL) and dried onto Na_2SO_4. The samples were directly analyzed by GC/MS and HPLC in order to determine, respectively, the level of conversion, the percentage of chiral sulfoxides **1**–**12b** and sulfones **1**–**12c**, as well as the enantiomeric excesses of sulfoxides (*R*)-**1**,**2b**, (*S*)-**3b** and (*R*)-**4**–**12b**.

4. Conclusions

The thermostable Baeyer-Villiger monooxygenase PockeMO from *Thermothelomyces thermophila* has been employed in the biocatalytic preparation of various optically active sulfoxides. In general, good conversions and high enantioselectivities can be achieved in the preparation of chiral sulfoxides **1**–**12b**. For those compounds that showed a lower activity, the sulfoxidations performed at 45 °C lead to higher conversions with no effect on the enzyme selectivity, while the presence of 10% v v^{-1} of hydrophilic organic solvents such as DMSO, acetonitrile and 1,4-dioxane also has a highly positive effect on the performance of this biocatalyst. PockeMo-catalyzed sulfoxidations can even be carried out at 60 °C. At this temperature, the enzyme shows a higher space time yield than at room temperature, but the highest activities are obtained, in general, at 45 °C. PockeMO seems to also be very robust regarding substrate loading, as the experiments show that it can accept thioanisole concentrations of 200 mM. Overall, the results demonstrate that PockeMO is a valuable oxidative biocatalyst that can be employed for the preparation of optically active sulfoxides by properly choosing the reaction conditions.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/7/10/288/s1. Table S1. Enantiomeric excesses, conversions and space time yields obtained in the PockeMO-catalyzed sulfoxidation of thioanisole at different concentrations and temperatures, Table S2. Determination of conversions and amounts of sulfoxides and sulfones by employing GC, Table S3. Determination of enantiomeric excesses by HPLC.

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Author Contributions: Gonzalo de Gonzalo performed the experiments and Gonzalo de Gonzalo, Maximilian J.L.J. Fürst and Marco W. Fraaije conceived, designed and wrote the paper.

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