Applied Catalysis A: General 441–442 (2012) 42–46

Contents lists available at SciVerse ScienceDirect

Applied Catalysis A: General

journal homepage: www.elsevier.com/locate/apcata

Sequential deracemization of sulfoxides *via* whole-cell resolution and heterogeneous oxidation

Madalina Tudorache[a], Simona Nica[b], Emerich Bartha[b], Iulia Lupan[c], Vasile I. Parvulescu[a,*]

[a] University of Bucharest, Department of Organic Chemistry, Biochemistry and Catalysis, Bd. Regina Elisabeta 4-12, Bucharest 030016, Romania
[b] Center of Organic Chemistry "C.D. Nenitzescu", Bucharest, Romania
[c] Institute for Interdisciplinarity Experimental Research "Babes-Bolyai" University, Molecular Biology Center, Cluj, Romania

ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form 29 June 2012
Accepted 30 June 2012
Available online 21 July 2012

Keywords:
Sequential deracemization
Racemic sulfoxides
Escherichia coli
Enantioselective reduction
Selective heterogeneous oxidation

ABSTRACT

A new concept for the synthesis of asymmetric sulfoxides exploiting sequential deracemization of racemic (*rac*) sulfoxides using a two-stage protocol had been developed. Enantio-pure sulfoxide was obtained using *Escherichia coli* (*E. coli*) cells to catalyze the reduction of (*R*)-sulfoxide to the thioether, and then using a heterogeneous Ta_2O_5–SiO_2 catalyst for catalyzing the oxidation of the ethers to the *rac*-sulfoxide. The performance of *E. coli* depended on the strain and the conditions under which the bacteria growth was carried out (*e.g.* source of carbon, presence of vitamins, and concentration of dimethyl sulfoxide, DMSO). Under optimized conditions, the *E. coli* cells performed the *rac*-sulfoxide resolution for different sufoxides leading to the best enantiomeric excess (ee) of around 62% (*S*)-MTSO (methyl tolyl sulfoxide) with 49% conversion of the *rac*-MTSO. The sequential deracemization process applied for *rac*-MTSO was cyclically performed leading to 97.5% ee of (*S*)-sulfoxide and a 56% yield in (*S*)-sulfoxide after three deracemization cycles of *rac*-MTSO.

1. Introduction

Chiral sulfoxides belong to the class of chiral organosulfur compounds widely used as important intermediates in organic synthesis (*e.g.* asymmetric synthesis) [1] and as bioactive ingredients in the pharmaceutical industry [2]. Their application in synthesis has now become a well-established and reliable strategy (*i.e.* the chiral groups are easy to introduce and remove, and give high asymmetric induction in many reactions). To date chiral sulfoxides have been investigated in a wide range of reactions such as the reduction of β-ketosulfoxides, the Michael addition to α,β-unsaturated sulfoxides, the extension of carbon chains with new C—C bonds, or the Diels–Alder reaction of vinyl derivatives [2]. Optically active sulfoxides also have many industrial applications in the pharmaceutical field, *e.g.* the synthesis of NSAID (an efficient non-steroidal anti-inflammatory drug) [3] and Nexium (esomeprazole – one of the most effective anti-ulcer drugs) [4]. Usually, only one enantiomer (the eutomer) exhibits the desired biological activity [5]. For example, the (*S*)-enantiomer of the β-receptor antagonist propanolol has 100-fold higher activity than the (*R*)-enantiomer [6].

During the last two decades, several chemical approaches have been proposed for the synthesis of enantiopure sulfoxides [7–9]. Optical resolution, asymmetric oxidation of prochiral sulfides, and nucleophilic addition of alkyl or aryl ligands to diastereoisomerically pure chiral sulfinates are representative examples of available methods [10,11]. Biocatalytic approaches for enantio-enriched sulfoxides can be also performed involving the oxidation of prochiral sulfide precursors with isolated enzyme or whole-cell system. Such processes have been developed with high enantioselectivity under environmentally benign reaction conditions [8,9,12]. However they could not be applied to every substrate and there are demands for new systems with better performance.

Asymmetric reduction of racemic sulfoxides by bacterial enzymes is another biological route used for the synthesis of enantio-pure sulfoxides [4,13]. However, this option has received much less attention until recently. Hanlon et al. reported an investigation of three different microbial cells (*e.g. Rhodobacter capsulatus*, *Escherichia coli* and *Proteus vulgaris*) [13] indicating that bacterial (*S*)-oxide reductases, found in anaerobic bacteria, can exhibit activity for the reduction of sulfoxides. The catalytic activity of this reductase enzyme was evaluated both as whole-cell bacteria and in the isolated form. Holt and co-workers [4] demonstrated that microbial deoxygenating systems such as *Citrobacter braakii*, *Klebsiella* sp. and *Serratia* sp. represent alternatives for the synthesis of enantiopure sulfoxides. Also, *Pseudomonas putida* UV4 bacterium demonstrated stereo-differentiating reductase-catalytic

* Corresponding author. Tel.: +40 21 4100241; fax: +40 21 4100241.
E-mail addresses: v_parvulescu@yahoo.com, vasile.parvulescu@g.unibuc.ro (V.I. Parvulescu).

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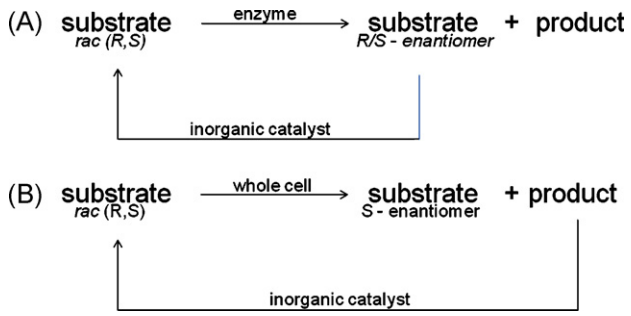
http://dx.doi.org/10.1016/j.apcata.2012.06.050



Scheme 1. DKR (A) *versus* deracemization (B) strategies for the synthesis of chiral molecules (*e.g.* alcohols, amines and sulfoxides).

activity for deoxygenation of *rac*-sulfoxides [14]. In spite of the excellent results, these reports suggest that the use of bacterial enzymes for deoxygenation of sulfoxides in a one-step process allows only partial enantio-purification of the optically-active sulfoxide [4,13].

Dynamic kinetic resolution (DKR) and also deracemization concepts offer a versatile alternative for the enantioselective synthesis of asymmetric compounds (Scheme 1A). These methodologies only been reported in connection with the synthesis of chiral alcohols, amines and amino acids [15–19]. In principle, both concepts (*e.g.* DKR and deracemization) require two steps: racemization followed by a subsequent asymmetric transformation. For such purposes, a tandem application of biocatalytic resolution and inorganic catalyst racemization was reported by several groups as being effective [20–24]. However, despite the chemo-, regio- and enantioselectivity of enzymes, the use of a single (purified) enzyme has several disadvantages such as the high cost of the process (due to the enzyme purification step), poor enzyme stability in the reaction medium, or requirement of additional reagents (*e.g.* coenzymes and activators). In order to circumvent these disadvantages, a whole-cell approach in which the cells express a certain enzyme could be an alternative.

In the present study, we investigated a new concept for the synthesis of asymmetric sulfoxides exploiting sequential deracemization of racemic (*rac*) sulfoxides using a two-step protocol (Scheme 1B). This involves the enantioselective deoxygenation of *rac*-sulfoxides using *E. coli* bacteria combined with the selective heterogeneous-catalytic oxidation of the resulting sulfide to the racemate sulfoxide by a Ta_2O_5–SiO_2 catalyst. Dimethyl sulfoxide (DMSO) reductase contained in the *E. coli* bacteria is responsible for deracemization of *rac*-sulfoxide [13]. A whole-cell system (*E. coli*) is used because DMSO reductase, a membrane-bound enzyme, often loses catalytic capacity when it is isolated from the cell [25]. *E. coli* resolution is efficient in terms of selectivity, but suffers from being limited by a maximum theoretical conversion of 50% (kinetic resolution). The combination of selective heterogeneous oxidation with an enantioselective reduction is a strategy that can increase not only the sulfoxides enantiopurity but also the efficiency of the reaction. To the best of our knowledge this is the first example of a deracemization process using a whole-cell biocatalyst combined with a heterogeneous catalyst. The deracemization protocol described in Scheme 1 involves a transformation process leading back to the sulfide. This differs from the already well-known DKR methodology (Scheme 1B).

2. Experimental

2.1. Chemicals and solutions

E. coli ATTC11303, glycerol, glucose and all of the salts used to prepare the working solutions were purchased from Sigma-Aldrich. *E. coli* top 10, dalfa5HL and Mac1 were purchased from Invitrogen.

MM (minimal medium) were prepared according to Hanlon et al. [13] with the following composition: 48 mM $Na_2HPO_4 \times 2H_2O$, 22 mM KH_2PO_4, 8.5 mM NaCl and 2 g/L yeast. After autoclaving, the trace element solution was added to the medium (1:50 dilution of trace element solution). The stock trace elements solution contained: 64 mg/L H_3BO_3, 18 mg/L $CoCl_2{\cdot}6H_2O$, 4 mg/L $CuCl_2{\cdot}2H_2O$, 340 mg/L $ZnCl_2$, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$ and 1 mg/L Na_2MoO_4. MM solution was enriched with 20 μg/L vitamin B_1 and 40 μg/L vitamin B_6. The carbon source for growth was either glucose or glycerol (2 or 10 g/L, respectively) and the nitrogen source was NH_4Cl (1 g/L). The MM also contained DMSO (dimethyl sulfoxide) at different concentrations (*e.g.* 0.1, 0.2, 0.4, 0.5, 1, 2, 4 and 8 g/L) as the respiratory electron acceptor of *E. coli* bacteria under anaerobic conditions.

Stock solution of phosphate saline buffer (PBS) of 100 mM was prepared by mixing 80 g NaCl, 2 g KCl, 14.3 g $Na_2HPO_4{\cdot}2H_2O$ and 3.43 g KH_2PO_4 in 1 L distilled water. The stock solution was diluted to 25 mM PBS with distilled water and the pH value was adjusted to 7.4 with either NaOH or HCl.

2.2. rac-Sulfoxide synthesis and characterization

Methyl phenyl sulfoxide (MPSO) was used as received from Acros Organics. Absolute configuration of methyl phenyl sulfoxide was assigned by comparison of the sign of specific rotations with pure enantiomers that were isolated by HPLC semi-preparative chromatography. The optical rotation [α] was recorded on a JASCO OR–1590 polarimeter at 589 nm and 25 °C and compared to literature data [26].

Methyl *p*-tolyl sulfoxide (MTSO) and methyl mesityl sulfoxide (MMSO) were prepared *via* the oxidation of the corresponding thioether (*e.g.* methyl *p*-tolyl sulfide and methyl mesityl sulfide). Methyl *p*-tolyl sulfide was purchased from Sigma–Aldrich and mesityl methyl sulfide was prepared by chemical synthesis in-house.

2.2.1. Methyl mesityl sulfide

2,4,6-Trimethylbenzenethiol (10 mmol, 1.55 g) was added to a suspension of NaH (20 mmol, 0.48 g) in benzene (50 mL). The reaction mixture was stirred until no hydrogen evolution could be observed. Then, the benzene was distilled off and the residue re-dissolved in DMSO (15 mL) and treated with methyl iodide (40 mmol, 5.68 g). The resulting solution was stirred overnight at room temperature, then decomposed with water and extracted with diethyl ether. The organic extracts were washed with brine, $NaHCO_3$ and dried over Na_2SO_4. Purification by silica gel chromatography with a mixture of petroleum ether/diethyl ether (3/1, v/v) afforded 1.62 g (97.6%) of a viscous compound. ^{1}H NMR ($CDCl_3$): 6.95 (s, 2H, ar-*H*), 2.53 (s, 6H, *o*-CH_3), 2.27 (s, 3H, *p*-CH_3), 2.22 (s, 3H, SCH_3). Anal. Calcd. C, 72.23; H, 8.49; S, 19.28; Found: C, 72.25; H, 8.46; S, 19.34.

2.2.2. Oxidation of sulfides

A solution of H_2O_2 in acetic acid (65.8 mg/mL H_2O_2) was prepared. One equivalent of H_2O_2 solution was added to a cooled (0–5 °C) solution of sulfide in acetic acid (10 mM sulfide) using a syringe. The resulting mixture was stirred for 20 min and then stored in a refrigerator overnight. Then, the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Crude extract was separated on a silica gel column with a mixture of petroleum ether:diethyl ether (from 25% to 100% diethyl ether) as eluent. Pure sulfoxides were obtained.

All the prepared sulfoxides were characterized based on ^{1}H NMR spectra recorded on a Varian Gemini 300BB NMR spectrometer (TMS internal standard). Chemical shifts (δ) are expressed in ppm,

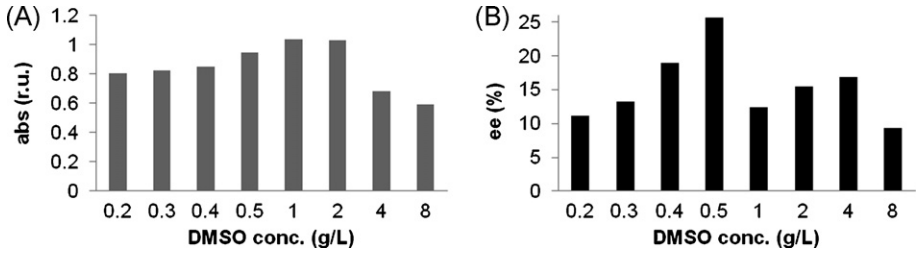


Fig. 1. Influence of DMSO concentration in the culture medium on the *E. coli* growth (A) and sulfoxide enantio-reduction processes (*S*-ee (%)). Conditions: (A) *E. coli* (ACC11303 strain) growing conditions – 2 g/L glucose, 20 μg/L vitamin B_1 and 40 μg/L vitamin B_6 in MM, and (B) deracemization (1.0 g/L *rac*-MPSO and 2 g/L glucose in 25 mM PBS (pH 7.4)).

and *J* values are given in Hz. Elemental analysis was performed on a Perkin Elmer CHN 240B instrument using a silica gel column (70–230 mesh ASTM).

2.2.3. Methyl p-tolyl sulfoxide

This compound was prepared based on the reaction of methyl *p*-tolyl sulfide (10 mmol, 1.38 g) and H_2O_2 in acetic acid (5.16 mL of 65.8 mg/mL solution). After reaction, 1.50 g (97.4% purity) methyl *p*-tolyl sulfoxide was isolated. ^{1}H NMR ($CDCl_3$): 7.54 (d_{AB}, 2H, *J* = 9.6 Hz, ar-*H*), 7.34 (d_{AB}, 2H, *J* = 9.5 Hz, ar-*H*), 2.71 (s, 3H, SO*CH*$_3$), 2.42 (s, 3H, *p*-CH_3). Anal. Calcd. C, 62.30; H, 6.54; O, 10.37; S, 20.79; Found: C, 62.28; H, 6.55; S, 20.74.

2.2.4. Methyl mesityl sulfoxide

This compound was prepared from methyl mesityl sulfide (10 mmol, 1.66 g) and H_2O_2 in acetic acid (5.16 mL of 65.8 mg/mL solution). 1.62 g (89.0% purity) methyl mesityl sulfoxide was isolated after reaction. ^{1}H NMR ($CDCl_3$): 6.85 (s, 2H, ar-*H*), 2.83 (s, 3H, SO*CH*$_3$), 2.55 (s, 6H, *o*-CH_3), 2.27 (s, 3H, p-C*H3*). Anal. Calcd. C, 65.89; H, 7.74; S, 17.59; Found: C, 65.91; H, 7.72; S, 17.51.

2.3. E. coli growth

E. coli cells were grown anaerobically in MM at 37 °C under a gentle stirring. The cultures were grown under inert nitrogen atmosphere in glass bottles equipped for gas bubbling. Nitrogen atmosphere ensured anaerobic conditions for the bacterial growth. The cell harvesting was performed at stationary phase followed by centrifugation, washing and dispersing the *E. coli* cells in 25 mM PBS (pH 7.4).

The active bacterial growth was followed using spectrophotometric analysis of the liquid medium. Samples from the growing medium were taken at different incubation times and analyzed with a UV–Vis detector set up at 610 nm (the absorbance signal increases with bacterial cell population).

2.4. Sequential deracemization of rac-sulfoxide

Deracemization was performed in a glass vessel containing 10 mL PBS (25 mM, pH 7.4), 1.8 mg glucose and 10 mg sulfoxide. The vessel was sealed with a rubber stopper and nitrogen was flushed inside. The reaction mixture was incubated at 37 °C without agitation for 24 h. After reaction, the solution was extracted with diethyl ether (~2 mL) and evaporated to dryness. The residue was re-dissolved in hexane/iso-propanol (8/2).

The enantiomeric excess (ee) of the separated sulfoxides was determined by HPLC analysis (AGILENT 1100/1200 with a S,S-Whelk-O chiral column (4.6 × 250 mm) and a mobile phase of hexane:*iso*-propanol (8/2) with a flow rate of 1 mL/min). The detection of the sulfoxides was performed at 254 ± 4 nm. The elution times for sulfoxides and corresponding thioethers were 18.6 min and 23.3 min for (R)- and (S)-methyl phenyl sulfoxide respectively; 19.9 min and 25.7 min for (R)- and (S)-methyl *p*-tolyl sulfoxide respectively; 25.1 min and 30.3 min for (R)- and (S)-methyl mesityl sulfoxide respectively; 5.6 min for methyl phenyl sulfide; 5.9 min for methyl *p*-tolyl sulfide; and 6.2 min for methyl mesityl sulfide. It has been noticed that the HPLC chromatograms contained only three peaks, *e.g.* (*R*) and (*S*) enantiomer of *rac*-sulfoxide, and the corresponding sulfide.

3. Results and discussion

3.1. Whole-cell resolution of rac-sulfoxide

Bio-resolution of the *rac*-sulfoxide by whole-cell (*E. coli*) reduction has been systematically optimized with respect to the source and growth conditions of the biocatalyst. Thus, different strains of *E. coli* (*e.g. E. coli* ATTC11303, top 10, dalfa5HL and Mac1) were tested for the resolution of *rac*-sulfoxides. The reactions focused on the separation of the (*S*) enantiomer to the detriment of the (*R*) one. Methyl phenyl sulfoxide (MPS) racemate was the first substrate investigated in this context.

Deracemization of *rac*-sulfoxides to sulfides was achieved by dimethyl sulfoxide (DMSO) reductase contained in the *E. coli* bacteria. DMSO reductase was expressed during the anaerobic bacterial growth in MM (minimal medium) enriched with DMSO as an enzyme substrate, and also with additional carbon (glycerol or glucose) and energy (B_1 and B_6 vitamins) sources.

The experimental conditions for bacteria growth were optimized taking into account the composition of the growth medium. No growth of the cultures was detected when only DMSO enriched the MM (*e.g.* a maximum absorbance of only 0.4 for culture suspensions was reached after 24 h – Table 1). Also, no significant differences in growth rate were obtained changing the carbon source (0.77 and 0.60 absorbance for glycerol and glucose, respectively – Table 1). However, a consistent enhancement of the growth rate was observed when B_1 and B_6 vitamins were added, for both carbon sources equally (Table 1).

Fig. 1 shows the effect of the DMSO concentration in the culture medium on the *E. coli* (ATTC11303 strain) growth process (Fig. 1A) and the correlation of growth rate with DMSO reductase

Table 1
Optimization of culture medium content for *E. coli* growth expressing DMSO reductase enzyme. Conditions: *E. coli* ATTC11303 strain, 0.5 g/L DMSO and 24 h incubation time.

DMSO (g/L)	Glycerol (g/L)	Glucose (g/L)	Vitamins		Abs.[a]
			B_1 (μg/L)	B_6 (μg/L)	
0.5	–	–	–	–	0.40
0.5	10	–	–	–	0.77
0.5	–	2	–	–	0.60
0.5	10	–	20	40	1.06
0.5	–	2	20	40	1.03

[a] Abs. – optical absorbance of the culture medium.

Table 2
Screening of *E. coli* in *rac*-MPSO deoxygenation.

	ATTC11303	Mac1	dH5alfa	Top10
Abs.[a]	0.90	1.19	1.04	1.37
ee (%)[b]	25.60	23.60	2.56	3.54

[a] *E. coli* (ATTC11303 strain) (growing conditions – 2 g/L glucose, 20 μg/L vitamin B_1 and 40 μg/L vitamin B_6 in MM; incubation time 8 h).
[b] *rac*-MPSO enantio-reduction (1.0 g/L *rac*-MPSO and 2 g/L glucose in 25 mM PBS (pH 7.4); ee (%) of (*S*)-MPSO).

activity (Fig. 1B). These dependences demonstrate that the *E. coli* (ATTC11303 strain) growth process was influenced by the presence of DMSO in the growth medium. The culture density measured after a standard time increased slowly with DMSO concentration in the range of 0.2–2.0 g/L (Fig. 1A). A higher DMSO concentration (*e.g.* in the range 4–8 g/L DMSO) led to poisoning of the bacteria and thus to an inhibition of the growth process (Fig. 1A). Deracemization of *rac*-MPSO was found to depend on the DMSO concentration in the growth medium (Fig. 1B). The maximum conversion of *rac*-MPSO was achieved when bacteria were treated in a growth medium with 0.5 g/L DMSO (26% ee of (*S*)-MPSO). Thus, 0.5 g/L DMSO was chosen as an optimum DMSO concentration in the growth medium for further experiments. In summary, *E. coli* (ATTC11303 strain) bacteria were cultivated in a culture medium with an optimum composition: 2 g/L glucose, 0.5 g/L DMSO, 20 μg/L B_1 and 40 μg/L B_6 vitamins dissolved in MM [13].

Collections of different strains of *E. coli* (*e.g.* ATTC11303, Mac1, top10 and dalfa5HL) were investigated in terms of DMSO reductase capacity of *rac*-sulfoxides. *Rac*-MPSO was selected again as a model for testing the catalytic capacity of *E. coli* strains. The resulting DMSO reductases showed catalytic activity which varied for the different bacteria strains (Table 2). Thus, the *E. coli* top 10 strain exhibited the highest rate of rate of growth but had a low deracemization activity (1.37 absorbance of *E. coli* culture and 4% ee in (*S*)-MPSO). In contrast, *E. coli* ATTC11303 and Mac1 exhibited high preference for the *R*-MPSO enantiomer leading to high enantioselectivities in (*S*)-MPSO (26 and 24% ee, respectively).

Next, the catalytic activity of DMSO reductase of *E. coli* (ATTC11303) was investigated with respect to different *rac*-sulfoxides. Methyl phenyl sulfoxide (MPSO), methyl *p*-tolyl sulfoxide (MTSO) and methyl mesityl sulfoxide (MMSO) were examined as substrates (Fig. 2). The whole-cell bacteria catalyzed the deracemization of *rac*-sulfoxide converting the (*R*)-enantiomer to the thioether, except MMSO that was not recognized as a DMSO reductase substrate (1% ee (*S*)-MMSO). The investigated strain developed 62% ee of (*S*)-MTSO ($E = 4$) with 38% conversion of *rac*-MTSO in one reduction step of *rac*-MTSO. The ability of the developed cells to performed enantio-conversion of sulfoxide to thioether is confirmed by the data already reported in the literature (*e.g.* 60% ee (*S*)-MTSO for conversion lower than 50%) [4].

3.2. Heterogeneous oxidation of thioethers

Oxidation of the resulting thioethers (sulfides) to sulfoxides was carried out with a silica-incorporated Ta catalyst (15 wt% Ta_2O_5) prepared following a procedure reported elsewhere [27]. The sulfoxidation of thioether was carried out in an ionic liquid ([bmim][NTf_2]) using an aqueous solution of 30 wt% hydrogen peroxide, as the oxidant, at 40 °C, for 15 min with 5 mg catalyst. The reaction occurred with a selectivity of 92% in MPSO for a conversion of 20%, 93% MTSO for a conversion of 22%, and 94% MDMTSO for a conversion of 19%. The difference in the selectivity from 100% was explained by the presence of the corresponding sulfones. The selection of the Ta-based catalyst was made due to the fact that it generates no leaching under the investigated conditions. Titanium and tungsten-based catalysts tend to generate leaching but with an extent correlated with the nature of the support [28,29].

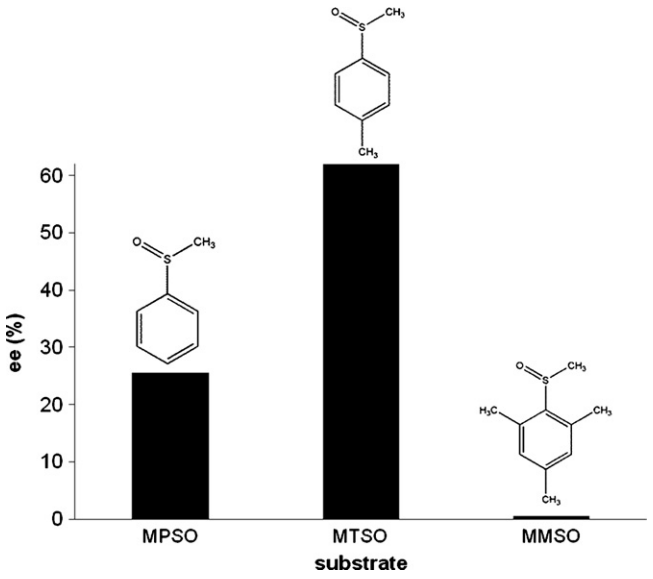


Fig. 2. Testing the catalytic capacity of *E. coli* (ATTC11303 strain) bacteria on enantio-reduction of *rac*-sulfoxides. Conditions: (A) *E. coli* (ATTC11303 strain) (growing conditions – 0.5 g/L DMSO, 2 g/L glucose, 20 μg/L vitamin B_1 and 40 μg/L vitamin B_6 in MM), and (B) deracemization (1.0 g/L *rac*-sulfoxide and 2 g/L glucose in 25 mM PBS (pH 7.4); ee (%) of (*S*)-MPSO).

However, the Ta_2O_5–SiO_2 catalyst is characterized by high selectivity and easy recovery from the reaction mixture [27], which are important properties for our oxidation system. The reason we tolerate such low conversion efficiencies is to preserve the high selectivity. Obviously increased conversions, led to further oxidation of the sulfoxides to sulfones, which is easily achieved [9,30,31].

3.3. Sequential deracemization of sulfoxides

The main drawback of the bio-resolution of *rac*-sulfoxide is the chemical conversion of 49.3% (limited to a maximum of 50%). This limitation was overcome by combining *E. coli* resolution with achiral oxidation of thioether product back to the *rac*-sulfoxide.

Deracemization steps (*e.g.* sulfoxide desoxygenation and thioether oxidation) were performed in different vials due to the incompatibility of the experimental parameters for these two chemical processes (*i.e.* sulfide oxidation requires oxygen precursor, while sulfoxide desoxygenation is perturbed by the presence of oxygen). First, the desoxygenation step of *rac*-MTSO allowed us to reach 62% ee of (*S*)-MTSO ($E = 4$) with 38% conversion of *rac*-MTSO in one reduction step of *rac*-MTSO. The conversion of *rac*-MTSO involved the (*R*)-MTSO deoxyreduction to methyl *p*-tolyl sulfide. No secondary products were detected in the reaction mixture extract (Section 2.4).

The enantioselective reduction was repeated three times with fresh *E. coli* leading to an ee enrichment of 97.3% (*S*)-MTSO ($E = 73$) for 49.3% conversion of *rac*-sulfoxide. Then, the resulting sulfide was purified by TLC technique (alumina-coated plate, 60% EtAc in hexane) [14]. The purified sulfide was subjected to oxidation with 22% conversion of sulfide. Working with *rac*-sulfoxides led to the same selectivities as those obtained using pure substrates. The resulting *rac*-sulfoxide was exposed to a new deracemization cycle. After three cycles, purified (*S*)-MTSO was obtained with 56% yield (98% ee of (*S*)-MTSO). Coupling the conventional catalytic oxidation process with the bio-reduction of *rac*-sulfoxide led a 20% enhancement of the process yield (the yield of the bio-reduction step was

36%, while for the sequential deracemization process the yield was 56%).

4. Conclusions

In conclusion, optically pure sulfoxides ((*S*)-enantiomer) were obtained based on cyclic deracemization with a sequential protocol. Practically, *E. coli* cell catalyzed the reduction of (*R*)-sulfoxide to the thioether and a heterogeneous Ta_2O_5–SiO_2 catalyst catalyzed the oxidation of the ethers to the *rac*-sulfoxide. The resulting *rac*-sulfoxide was then exposed to a new deracemization cycle. This process was repeated until the (R)-enantiomer was eliminated from the reaction phase. The performances of *E. coli* depended on the strain and the conditions in which bacteria growth occurred (*e.g.* source of carbon, presence of vitamins, and concentration of DMSO). Under optimized conditions, the *E. coli* cells performed the *rac*-MTSO resolution leading to an enantiomeric excess of around 97% (*S*)-MTSO for 49% conversion of the *rac*-MTSO. The oxidation of the separated thioether to the sulfoxide occurred with a high selectivity for conversion of 22%. Finally, 98% ee of (*S*)-sulfoxide with 56% yield in (*S*)-sulfoxide was reached after three deracemization cycles for *rac*-MTSO. The sequential deracemization approach is an efficient way to enantio-separate *rac*-sulfoxides allowing to gain an additional 20% yield. A scale-up of the proposed sequential deracemization process will be carried out in the next future.

Acknowledgments

This work was financially supported by the PN II HR YT-96 program, contract no. 91/2010 from CNCSIS.

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