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(54) Title: PROCESS FOR AN ENZYMATIC OXYGENATION BY DIRECT ELECTROCHEMICAL REGENERATION OF THE FAD-DEPENDANT MONOOXYGENASE

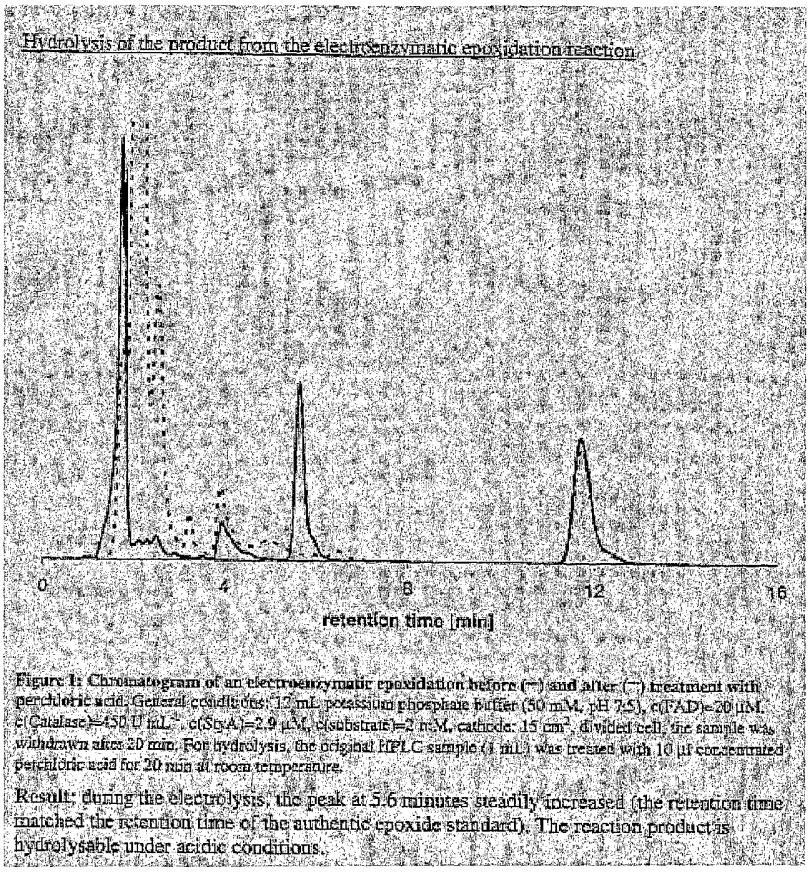

Figure 1: Chromatogram of an electroenzymatic epoxidation before (—) and after (—) treatment with perchloric acid. General conditions: 12 mL potassium phosphate buffer (50 mM, pH 7.5), c(FAD)=20 µM, c(Catalase)=450 U mL^{-1}, c(StyA)=2.9 µM, c(substrate)=2 mM, cathode: 15 cm^2, divided cell, the sample was withdrawn after 20 min. For hydrolysis, the original HPLC sample (1 mL) was treated with 10 µl concentrated perchloric acid for 20 min at room temperature.

Result: during the electrolysis, the peak at 5.6 minutes steadily increased (the retention time matched the retention time of the authentic epoxide standard). The reaction product is hydrolysable under acidic conditions.

(57) Abstract: Process for an enzymatic oxygenation catalyzed by a FAD-dependant monooxygenase and direct electrochemical regeneration of the FAD-dependant monooxygenase.

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Process for an enzymatic oxygenation by direct electrochemical regeneration of the FAD-dependant monooxygenase

Background

Selective oxyfunctionalization of unreactive hydrocarbons still represents one of the most challenging frontiers of synthetic organic chemistry. Especially the delicate balance of reactant-activation and selectivity of the reaction has to be dealt with. 'Classical' chemical oxygen donors such as peroxides, hypochlorites, iodosobenzenes, or dioxiranes [1] lack the selectivity which is required for oxyfunctionalizations of more complex substrates.

Furthermore, most catalytic chemical approaches are not very far developed yet, so that turnover numbers and frequencies as well as the stereodiscrimination of the catalysts tend to be low. [2, 3] Nature on the other hand has developed a versatile toolbox of catalysts meeting exactly the aforementioned criteria:

Monooxygenases catalyze highly diversified oxygenation reactions generally in a very regio- and stereoselective manner at catalyst performances reaching several hundred turnovers per minute. [4] The reactive oxygenating species is generated *in situ* from molecular oxygen at the monooxygenase's active site thereby minimizing undesired side reactions. Thus, monooxygenases are promising catalysts to be used in synthetic organic chemistry. [5-8] In return however, monooxygenases are cofactor-dependent enzymes, which have to be supplied with reducing equivalents for O_2 activation. Generally those reducing equivalents are derived from the costly and instable nicotinamide cofactors (NAD(P)H). [9-11] Furthermore, monooxygenases often are composed of complex multienzyme systems accomplishing the electron transfer from NAD(P)H to the terminal oxygenase. Due to the sophisticated molecular architecture and the NAD(P)H dependency, preparative applications of monooxygenases - with few exceptions - [8, 12-16] have been largely confined to whole-cell approaches using metabolically active microorganisms. [5, 8, 17-19]

Given the complexities of mimicking the native monooxygenase cycle, direct introduction of reducing power into the oxygenation cycle offers the possibility of drastic simplification biocatalytic oxyfunctionalization reactions. Electrochemical reduction is one approach of choice since the reducing power applied can be controlled and the cathode serves as reagent-free source of electrons. In this respect, the class of heme-dependent monooxygenases so far has been the favored subject of research. Electrical communication between the monooxygenase's heme-iron center and the cathode was established either by direct contact, [20, 21] and via artificial [22] or biological redox relays [23-25] mediating the electron transfer.

In contrast to the varied research activities on P450 monooxygenases, similar approaches for the class of flavin-dependent monooxygenases have not been reported yet, which is astonishing insofar, as this enzyme class catalyzes synthetically interesting oxyfunctionalization reactions such as hydroxylations, [12, 26, 27] Baeyer-Villiger oxidations, [28, 29] and epoxidations. [30]

Styrene monooxygenase (StyAB) from *Pseudomonas sp.* VLB120 catalyzes the specific *(S)*-epoxidation of a broad range of styrene derivatives. [31, 32] The enzyme is composed of a FAD-dependent monooxygenase component (StyA) that catalyzes the epoxidation reaction and a NADH-dependent reductase component (StyB) delivering the reducing equivalents from NADH to StyA via $FADH_2$. [33]

Previously, we have shown that StyB is not directly involved in the epoxidation reaction since it can be replaced by chemical reductants without impairment of the stereochemical course or the rate of the reaction. [34] There, *in situ* regeneration of $FADH_2$ was achieved using the organometallic complex $[Cp^*Rh(bpy)(H_2O)]^{2+}$ as transfer hydrogenation catalyst together with formate as stochiometric source of reducing equivalents.

Description of the invention

The following invention relates to a process for an enzymatic oxygenation of an educt E to a product P catalyzed by an FAD-dependant monooxygenase, characterized in that the FAD-dependant monooxygenase is regenerated by direct electrochemical reduction.

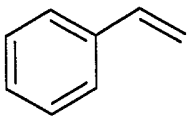
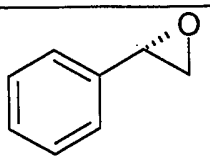
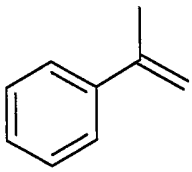
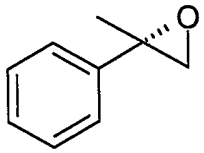
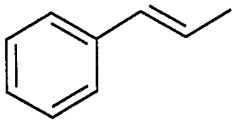
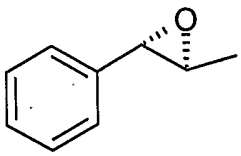
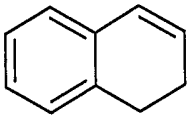
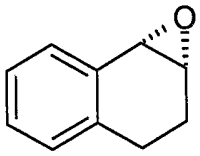
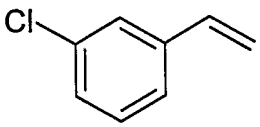
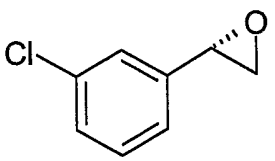
The chemical nature of the educt E can be varied in a broad range as long as an monooxygenase, especially an FAD-dependant monooxygenase is able to accept the educt E as a substrate for oxygenation. Preferred as educt E are compounds substituted styrenes and styrene derivatives, especially preferred are the subtrates mentioned in table 1.

As monooxygenase according to the invention are preferred the styrene monoooxygenase (Sty AB) from Pseudomonas sp. [31, 32] Other preferred enzymes are listed in Fig. 9.

Initial experiments on the electroenzymatic epoxidation were performed with *trans*-β-methyl styrene as substrate. Electrolyses were performed potentiostatically applying a cathode potential of -550 mV vs. $Ag/AgCl_{sat.}$. No product formation was detectable when either StyA or FAD was omitted from the reaction medium. On the other hand, electrolyses in the presence of all reaction components yielded the formation of a hydrolysable, more polar product, which was confirmed to be practically enantiopure

(*1S,2S*)-1-phenylpropylene oxide. [36] Similarly, a broad variety of diversely substituted vinylaromatic compounds could be transformed to the more than 98% optically pure corresponding *(S)* epoxides (Table 1) .

Table 1: Electroenzymatic epoxidation of substituted styrene derivates.

Substrate	Product	Rate [U g^{-1}] [a]	ee-value [%]
		28.1	98.5
		14.6	99.5
		35.5	> 99.9
		58.9	99.2
		27.7 [b]	98.1

[a] general conditions: 10 mL potassium phosphate buffer (50 mM, pH 7.5), T=30°C, c(StyA) = 2.13 µM, c(FAD)= 300 µM, c(catalase)= 480 U mL^{-1}, c(trans-β-methyl styrene) = 2 mM cathode: 14 cm^2.

[b] T = 25°C, activity determined after 15 min.

However, while the stereodiscrimination of the electroenzymatic oxygenation reactions met the values obtained with whole-cells [31, 32] as well as cell-free reactions [34, 35], the epoxidation rate was comparably poor. In initial-rate studies, specific StyA-activities up to 2.1 U mg^{-1} had been determined. [33] Thus, the rates depicted in Table 1 constitute only a fraction (less than 2%) of the catalytic potential of StyA. With the goal of determining the rate-limiting factors of the presented electroenzymatic epoxidation reaction, we further investigated the influence of varying reaction parameters on the rate of the electroenzymatic epoxidation reaction.

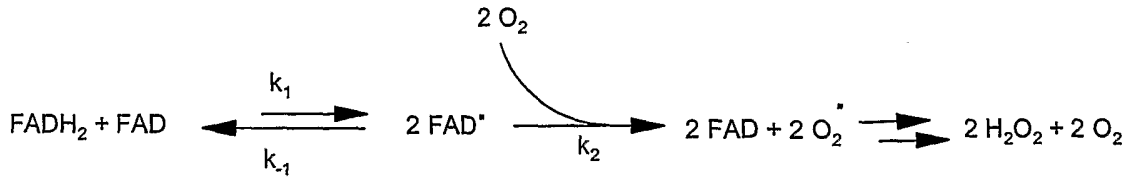
As shown in Fig. 10, the rate of the electroenzymatic epoxidation reaction correlated with the biocatalyst concentration applied. Specific StyA activities of 35.5 ± 2.1 U g^{-1} were observed independent from the biocatalyst concentration. This specific activity was temperature-dependent as increasing of the reaction temperature from e.g. 25°C to 37°C resulted in a 2.5-fold increase of epoxidation activity under otherwise identical conditions. [36] Thus, at a first glance, StyA appeared to be rate-limiting in the electroenzymatic reaction. However, the poor catalytic performance of StyA compared to maximal values suggested yet other factors severely limiting the rate of the electroenzymatic epoxidation reaction.

Lowering the cathode potential from -550 to -650 mV vs. $Ag/AgCl_{sat}$ did not significantly influence the reaction course [36] suggesting that the electron transfer from the cathode to FAD was not rate-limiting for the regeneration of $FADH_2$. As heterogeneous reaction, however, the regeneration of $FADH_2$ may be limited by mass transport to the cathode surface. In fact, we observed that increasing FAD-concentrations up to at least 500 µM resulted in increasing epoxidation rates. [36] These results are contrary to previous findings where a defined optimal FAD concentration between 10 and 20 µM was observed using homogeneous regeneration of $FADH_2$. [33, 34] There, autocatalytic oxidation of $FADH_2$ [37] accounted for the decrease of epoxidation rate at FAD concentrations higher than 20 µM. In the present case, this effect may be overruled by the increased $FADH_2$ generation rate due to the increased availability of FAD at the cathode surface. Provided the latter assumption was correct and cathodic $FADH_2$ regeneration is subject to diffusion limitation, also the cathode surface should affect the regeneration rate. Therefore, the influence of ratio of cathode surface to reaction volume was investigated. As shown in 11, the specific StyA activity (here depicted as turnover frequency [catalytic cycles per minute]) correlated directly with the ratio of cathode areas and reaction volume.

Altogether, these observations suggested that StyA activity in the electroenzymatic epoxidation reaction is limited by the availability of $FADH_2$ for the epoxidation reactions. Since reduced flavins are not stable in the presence of molecular oxygen, [37] we investigated the influence of aeration on the rate of the electroenzymatic epoxidation reaction (Figure 12).

Interestingly, we found that increasing aeration rates drastically accelerated the epoxide formation rate. Without active intake of air a specific StyA activity was in the range of 30 U g^{-1} was determined reaction (Figure 12). Furthermore, only approximately 50 µM of epoxide were overall formed, suggesting that more than 80% of the dissolved oxygen is consumed by reactions other than the enzymatic epoxidation. High aeration rates on the other hand increased the specific StyA activity up to 215 U g^{-1} corresponding to approximately 10% of the maximal StyA activity. This is interesting since studies on the direct reductive regeneration of P450 monooxygenases identified oxidative un-

coupling of the electrochemical regeneration reaction from the enzymatic oxygenation reaction to be overall limiting. [22, 24, 38] For example, Vilker and coworkers found a drastic increase in $P450_{cam}$-driven hydroxylation of camphor if the electrolysis buffer was Ar-purged prior applying the cathode potential and *in situ*-regeneration of O_2 at the anode. This apparent discrepancy may be explained considering the mechanism of $FADH_2$ oxidation [37] as outlined in Scheme 1.



Scheme 1: Predominant mechanisms for the non-StyA related oxidation of $FADH_2$. [37] $k_1=1\times10^6\ M^{-1}\ s^{-1}$; $k_{-1}=5\times10^8\ M^{-1}\ s^{-1}$; $k_2=8\times10^7\ M^{-1}\ s^{-1}$

Accordingly, the formation of the semiquinone radical anion by reversible synproportionation limits the overall rate for the non-enzyme-supported oxidation of reduced flavins. Thus, in our experiments $c(O_2)$ did not influence the rate of the non-enzyme supported re-oxidation of $FADH_2$. On the other hand, molecular oxygen is involved directly in the formation of the catalytically active 4α-peroxoflavin. Provided this is the overall rate-limiting step of the StyA-catalyzed epoxidation reaction, this would sufficiently explain the dependence of the epoxidation rate on the aeration rate. The latter assumption is supported by similar findings with the FAD-dependent *p*-hydroxyphenylacetate-3-hydroxylase where formation of 4α-peroxoflavin was found to be rate-limiting and O_2-dependent. [39] Future experiments will examine the influence of the *in situ* concentration of O_2 on the electroenzymatic reaction more deliberately.

One particular challenge for the preparative application of the new electroenzymatic epoxidation reaction so far is its comparably low long-term stability. Generally, the reactions ceased after 1 to 1.5 h. From the results obtained so far, some qualitative conclusions can be drawn. First, a correlation of the overall reaction time with the total protein content applied can be detected (compare also Fig. 10) and, second, the reaction times decrease with the rate of air intake (Fig. 12) Both observations point towards a low stability of the biocatalyst under the reaction conditions. This low stability of StyA may partially be due to the absorption of StyA to the cathode surface were it is exposed to locally high concentrations of partially reduced oxygen originating from cathodic reduction of O_2. [40] Furthermore, the heterogeneous intake of O_2 brings about the occurrence of shear forces and surface tensions at the liquid-gaseous interface destabilizing the three-dimensional structure of the biocatalyst. Previous studies suggested a beneficial influence of additional 'sacrificial' proteins such as bovine serum albumin (BSA)

[35] also heterogenzation of StyA, e.g. via immobilization to Eupergit C may be viable. Further studies aiming towards increased biocatalyst stability under the conditions are underway.

In conclusion, our study demonstrates for the first time the direct electrochemical regeneration of a flavin-dependent monooxygenase. Driven only by electrical power, optically pure epoxides were synthesized from corresponding vinyl aromatic compounds. Thus, the rather complicated native electron transport chain consisting of 3 polypeptides (StyA, StyB, and a NADH regenerating enzyme) and 2 cofactors (NADH and FAD) could be cut down to the components absolutely necessary for the epoxidation a maximally simple biocatalytic epoxidation reaction. Now, having shown the usefulness of the electroenzymatic approach to simplify such complicated enzyme system it may be extended to other enzymatic oxygenation reactions [41] making synthetically interesting reactions such as oxidative desulphurization [42], specific hydroxylation of aromatic rings [43-45], enantioselective Baeyer-Villiger Reactions, [46] and even selective halogenation reactions [47] feasible using only the isolated monooxygenases and FAD in an electrochemical cell.

Experimental Section

Chemicals were purchased from Fluka (Buchs, Switzerland) in the highest purity available and used without further purification.
StyA was enriched from recombinant *Escherichia coli* JM101 as described previously [35]. The purity of the lyophilized biocatalyst was approximately 70% (as determined by SDS gel-electrophoresis).

Electrolyses were performed in a thermostatted stirred tank reactor. Cylindrical carbon felt served as cathode (working electrode) and the potential was adjusted versus a saturated $Ag/AgCl_{sat.}$ reference electrode. The dimensions of the working electrode are given a macroscopic area (corresponding to an average of 27.1 ± 2.1 mg cm^{-2}). Conditions of either a divided or an undivided cell were chosen. For the divided cell, the Pt-wire counter electrode was placed in a dialysis membrane; otherwise a Pt-foil (Ø1cm) was used. After supplementing the reactor with the reaction components indicated a cathode potential of -550 mV vs. $Ag/AgCl_{sat.}$ was applied. In case of divided cell, O_2 was supplied by heterogeneous intake of air (intake rates were estimated with a Hewlett Packard soap film flowmeter; under the conditions of an undivided cell, O_2 was generated at the counter electrode.

Reaction rates (and enzyme performances calculated thereof) were determined based on the product formation as determined by HPLC using protocols previously reported. [34, 35]

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Claims:

1. Process for an enzymatic oxygenation of an educt E to a product P catalyzed by an FAD-dependant monooxygenase, characterized in that the FAD-dependant monooxygenase is regenerated by direct electrochemical reduction.

2. Process according to claim 1 where the oxygenation reaction is an epoxidation.

3. Process according to claim 1 where the oxygenation reaction is an oxidative desulphurization.

4. Process according to claim 1 where the oxygenation reaction is an enantioselective Baeyer-Villiger reaction.

5. Process according to claim 1 where the oxygenation reaction is a hydroxylation of an aromatic molecule.

6. Process according to claim 1 where the FAD-dependant monooxygenase is 4-hydroxyphenylacetate-monooxygenase

7. Process according to claim 1 where the FAD-dependant monooxygenase is pyrrole-2-carboxylate-monooxygenase.

8. Process according to claim 1 where the FAD-dependant monooxygenase is chlorophenol-4-hydroxylase.

9. Process according to claim 1 where the educt E is a substituted or unsubstituted styrene.

10. Process according to claim 1 where the FAD-dependant monooxygenase is the styrene monooxygenase (Sty AB) from pseudomonos.

Figure 1:

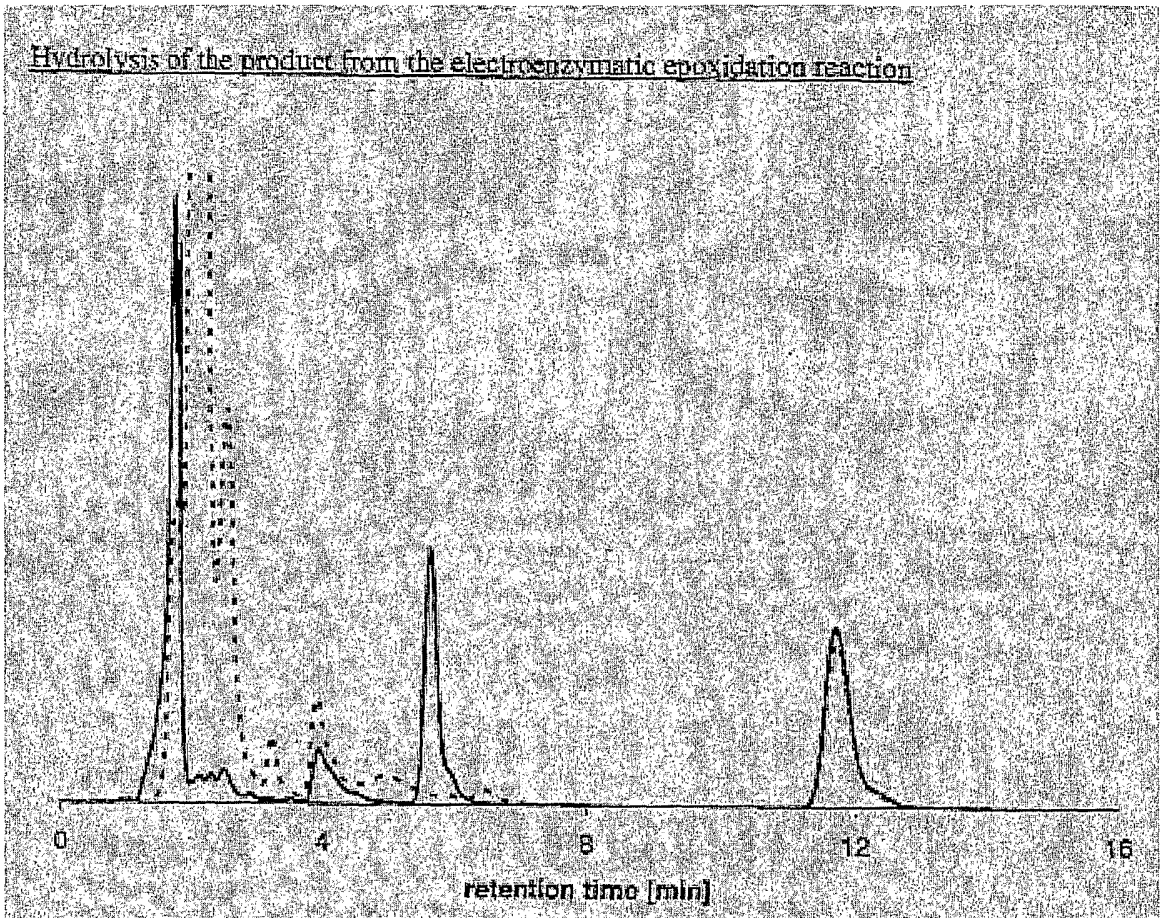

Figure 1: Chromatogram of an electroenzymatic epoxidation before (—) and after (—) treatment with perchloric acid. General conditions: 12 mL potassium phosphate buffer (50 mM, pH 7.5), c(FAD)=20 µM, c(Catalase)=450 U mL^{-1}, c(StyA)=2.9 µM, c(substrate)=2 mM, cathode: 15 cm^2, divided cell, the sample was withdrawn after 20 min. For hydrolysis, the original HPLC sample (1 mL) was treated with 10 µl concentrated perchloric acid for 20 min at room temperature.

Result: during the electrolysis, the peak at 5.6 minutes steadily increased (the retention time matched the retention time of the authentic epoxide standard). The reaction product is hydrolysable under acidic conditions.

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Figure 2:

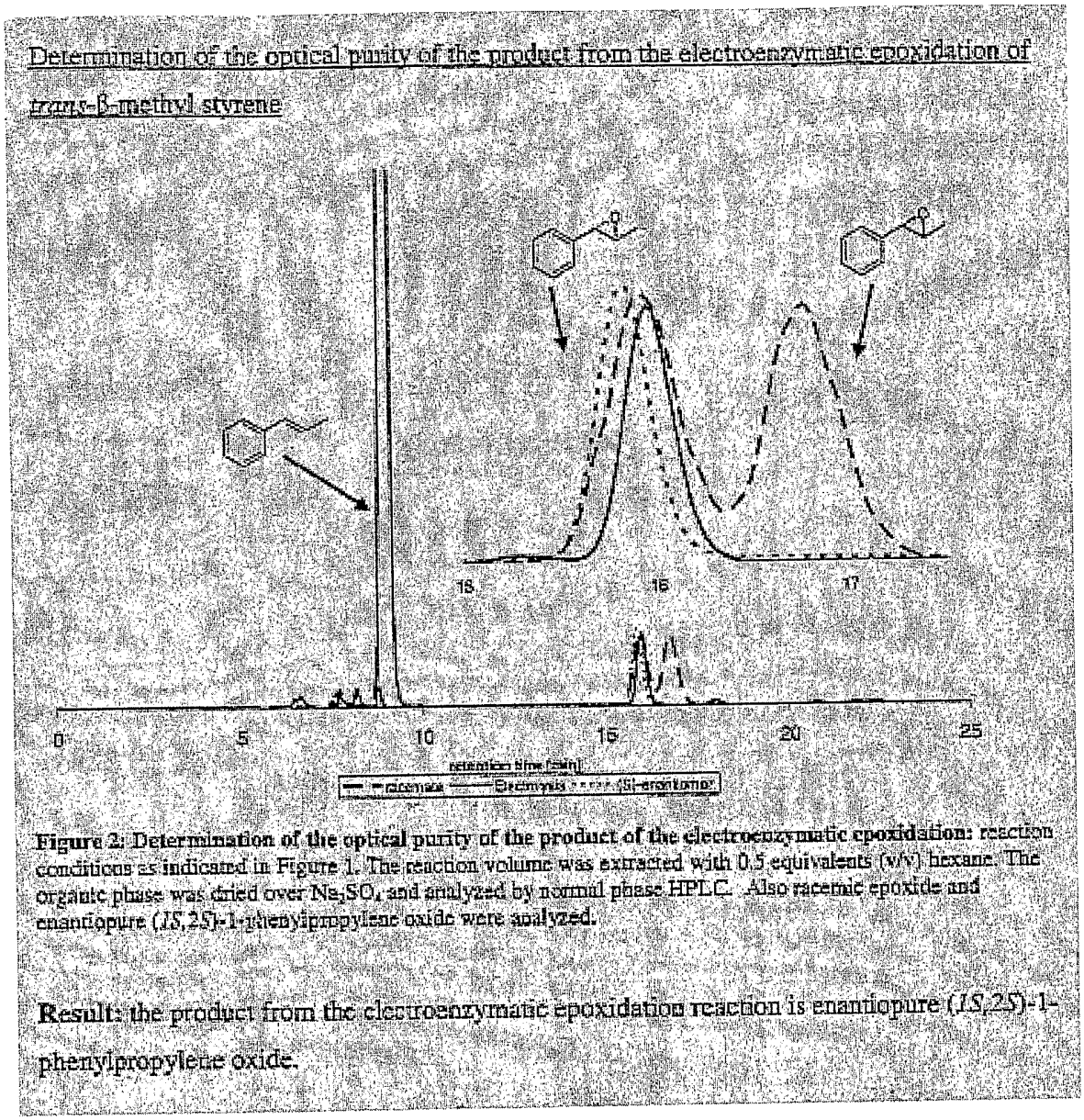

Figure 2: Determination of the optical purity of the product of the electroenzymatic epoxidation: reaction conditions as indicated in Figure 1. The reaction volume was extracted with 0.5 equivalents (v/v) hexane. The organic phase was dried over Na_2SO_4 and analyzed by normal phase HPLC. Also racemic epoxide and enantiopure (*1S,2S*)-1-phenylpropylene oxide were analyzed.

Result: the product from the electroenzymatic epoxidation reaction is enantiopure (*1S,2S*)-1-phenylpropylene oxide.

Figure 3:

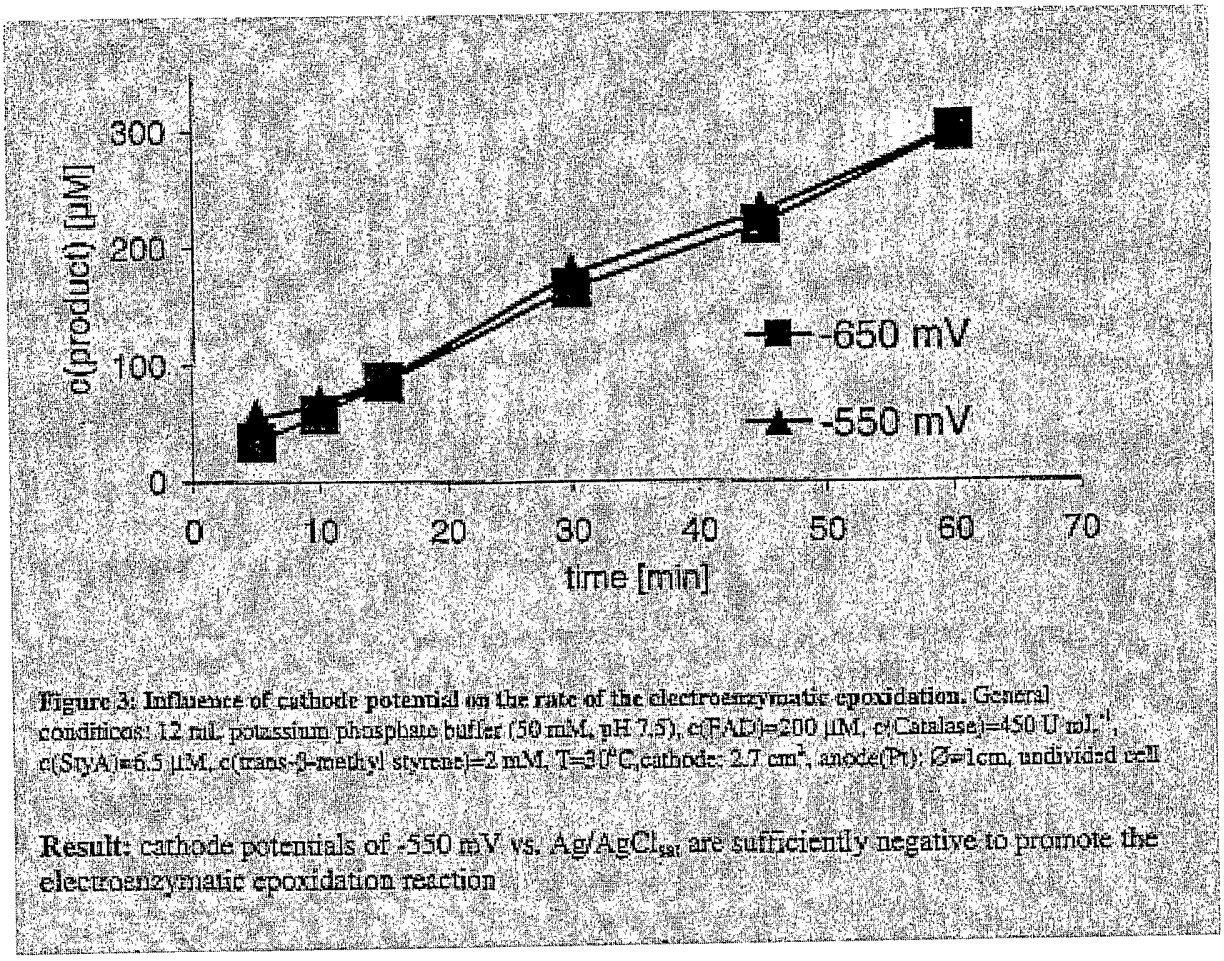

Figure 3: Influence of cathode potential on the rate of the electroenzymatic epoxidation. General conditions: 12 mL potassium phosphate buffer (50 mM, pH 7.5), c(FAD)=200 µM, c(Catalase)=450 U mL^{-1}, c(StyA)=6.5 µM, c(trans-β-methyl styrene)=2 mM, T=30°C, cathode: 2.7 cm^2, anode(Pt): Ø=1cm, undivided cell

Result: cathode potentials of -550 mV vs. $Ag/AgCl_{sat.}$ are sufficiently negative to promote the electroenzymatic epoxidation reaction

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Figure 4:

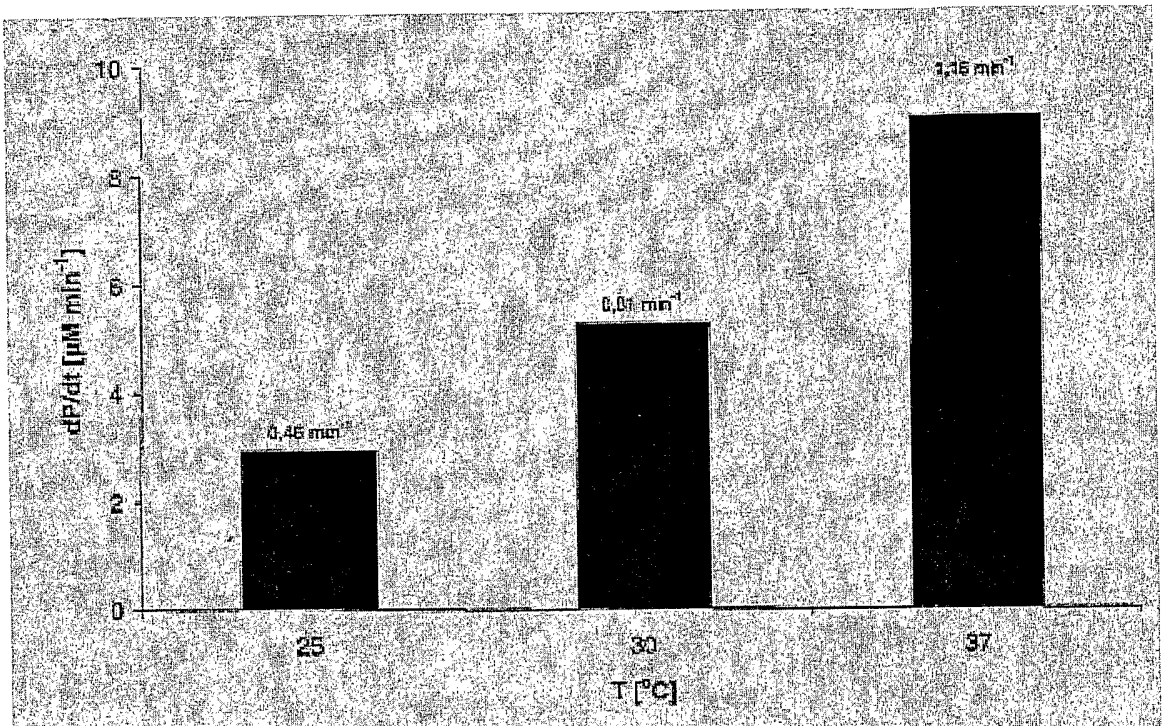

Figure 4: Influence of Temperature on the rate of the electroenzymatic epoxidation of trans-β-methylstyrene. General conditions: 12.9 mL potassium phosphate buffer (50 mM, pH 7.5), c(FAD)=186 µM, c(Catalase)=450 U mL^{-1}, c(StyA)=9.85 µM, c(substrate)=1.9 mM, cathode: 2.7 cm^2, anode= Pt-foil (Ø=1 cm), undivided cell. Values in the graph indicate the calculated StyA-turnover frequencies.

Result: the rate of the electroenzymatic epoxidation reaction is dependent on the reaction temperature.

Figure 5:

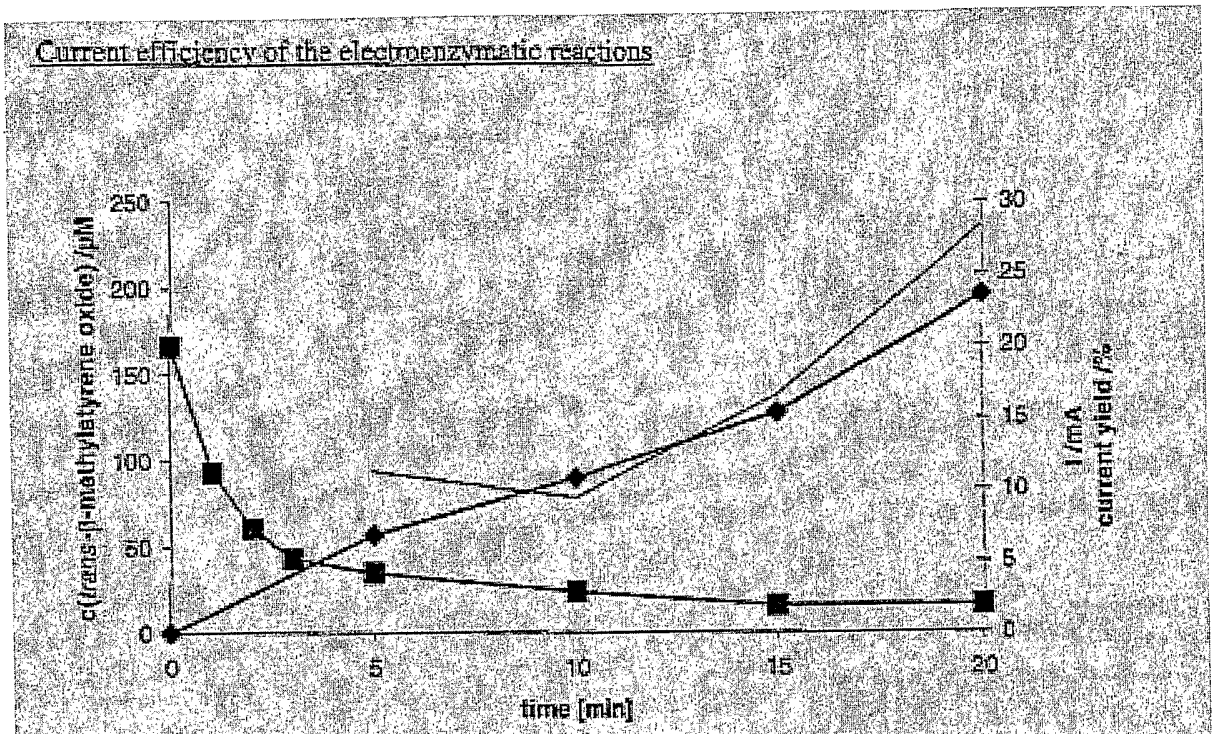

Figure 5: Determination of the current efficiency of a representative electroenzymatic epoxidation. General conditions: 12.9 mL potassium phosphate buffer (50 mM, pH 7.5), T=37°C, c(Catalase)=450 U ml-1, c(trans-β-methyl styrene)=1.8 mM, c(StyA)=9.85 µM, c(FAD)=186µM, cathode: 2.7 cm^2 with in situ generation of O_2 at the anode (undivided cell). (■: electrical current, ◆: product concentration, —: current yield)

Result: Current efficiencies of up to 28% can be achieved under non-optimized conditions for the electroenzymatic epoxidation reaction (between 15 and 20 min the epoxide formation rate was 13.7 µM min^{-1}, during that time the electrical current was approximately 2 mA, this corresponds to a theoretical electrochemical FAD reduction rate of 48.2 µM min^{-1}).

Figure 6:

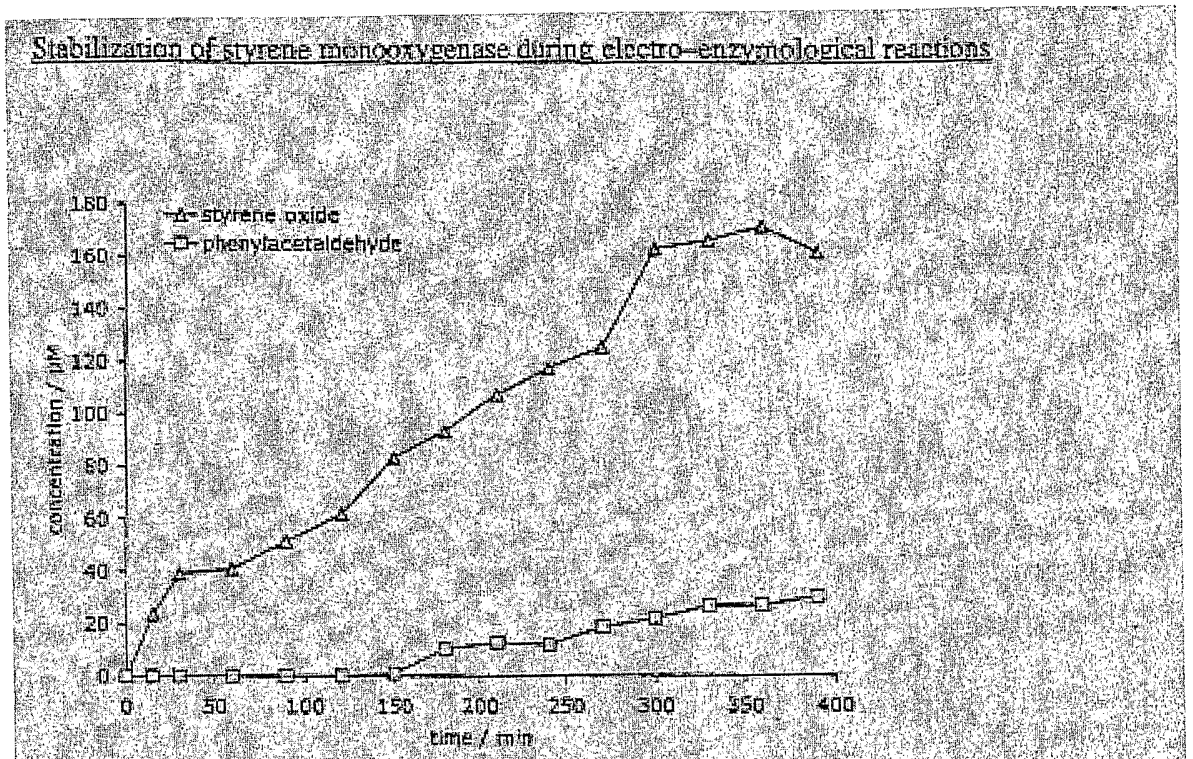

Figure 6: **Long-term StyA stability during the reaction with direct FAD reduction at the cathode.** The reaction solution contained 0.1 g/l StyA, 5 g/l BSA, 25% (wt/wt) sucrose, 500 µM FAD, and 500 U/ml catalase in 200 mM KPi buffer pH 7.5. 2 mM styrene was present at the beginning of the reaction and every 30 min additional 2 mM was added. Stirring speed was 200 rpm at a temperature of 30°C. Reactant concentrations have been measured by HPLC. Average epoxidation activity of 5 U/g for 6 h could be achieved with an initial value of 20 U/g.

Carbon felt: 530 mg; potential applied: -550 mV against Ag/AgCl electrode.

Result: The epoxidation reaction could be prolonged for several hours using BSA and sucrose in the reaction solution.

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Figure 7:

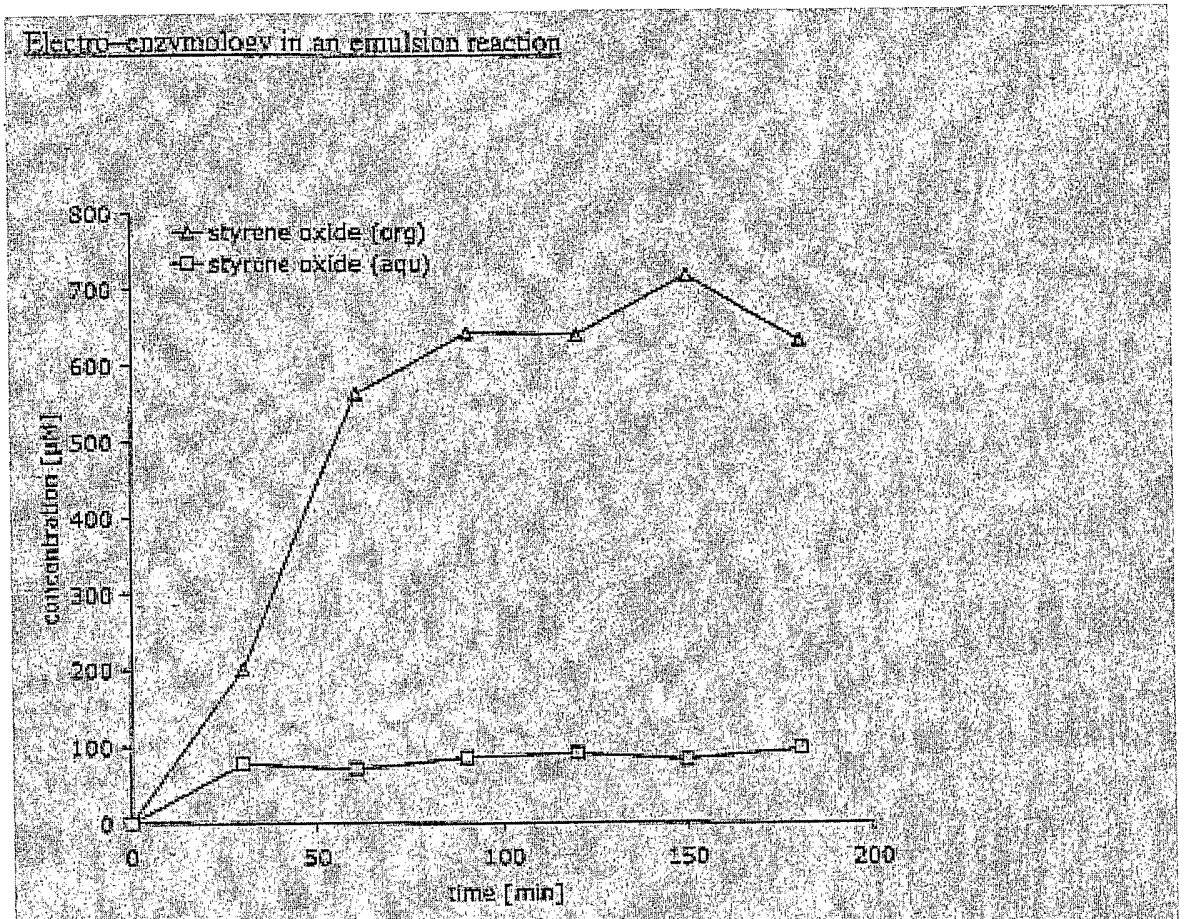

Figure 7: Styrene oxide concentrations during emulsion reactions in the organic and the aqueous phase. The aqueous phase consisted of 0.1 g/l StyA, 5 g/l BSA, 2% (wt/wt) sucrose, 500 U/ml catalase, and 500 µM FAD in 200 mM KPi buffer pH 7.5, and 50 mM substrate (styrene) was added to the organic phase (dodecane). The reaction was performed at 30°C at a phase ratio of 0.5. After 30 min the stirring speed was increased, as at 200 rpm no emulsion has been formed.

Carbon felt: 490 mg; potential applied: –550 mV against Ag/AgCl electrode.

Result: StyA can be used in an emulsion reaction with direct FAD reduction at the cathode. The organic phase allows the application of higher substrate concentrations and the evaporation of the highly volatile reactants can be decreased.

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Figure 8:

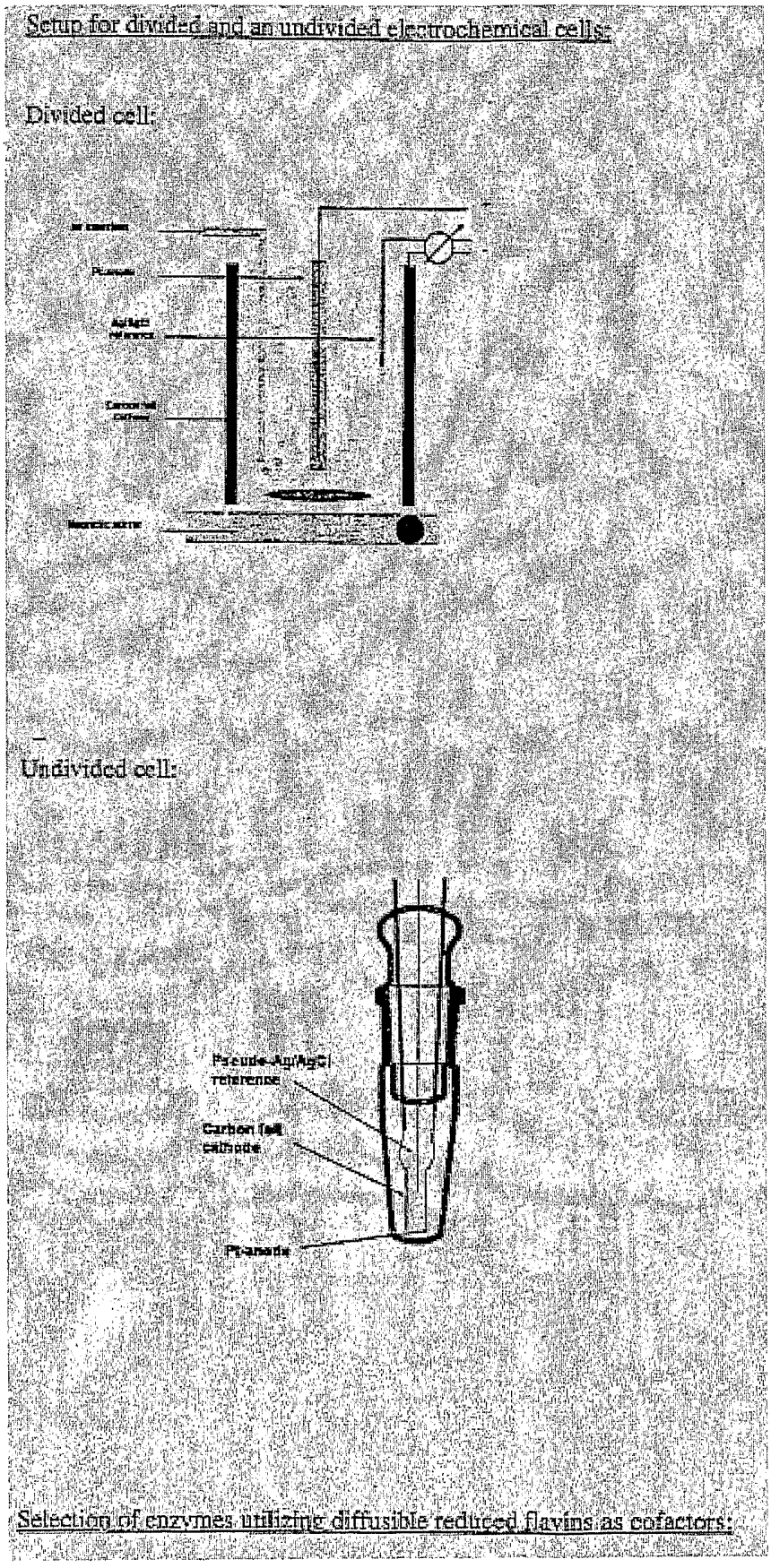
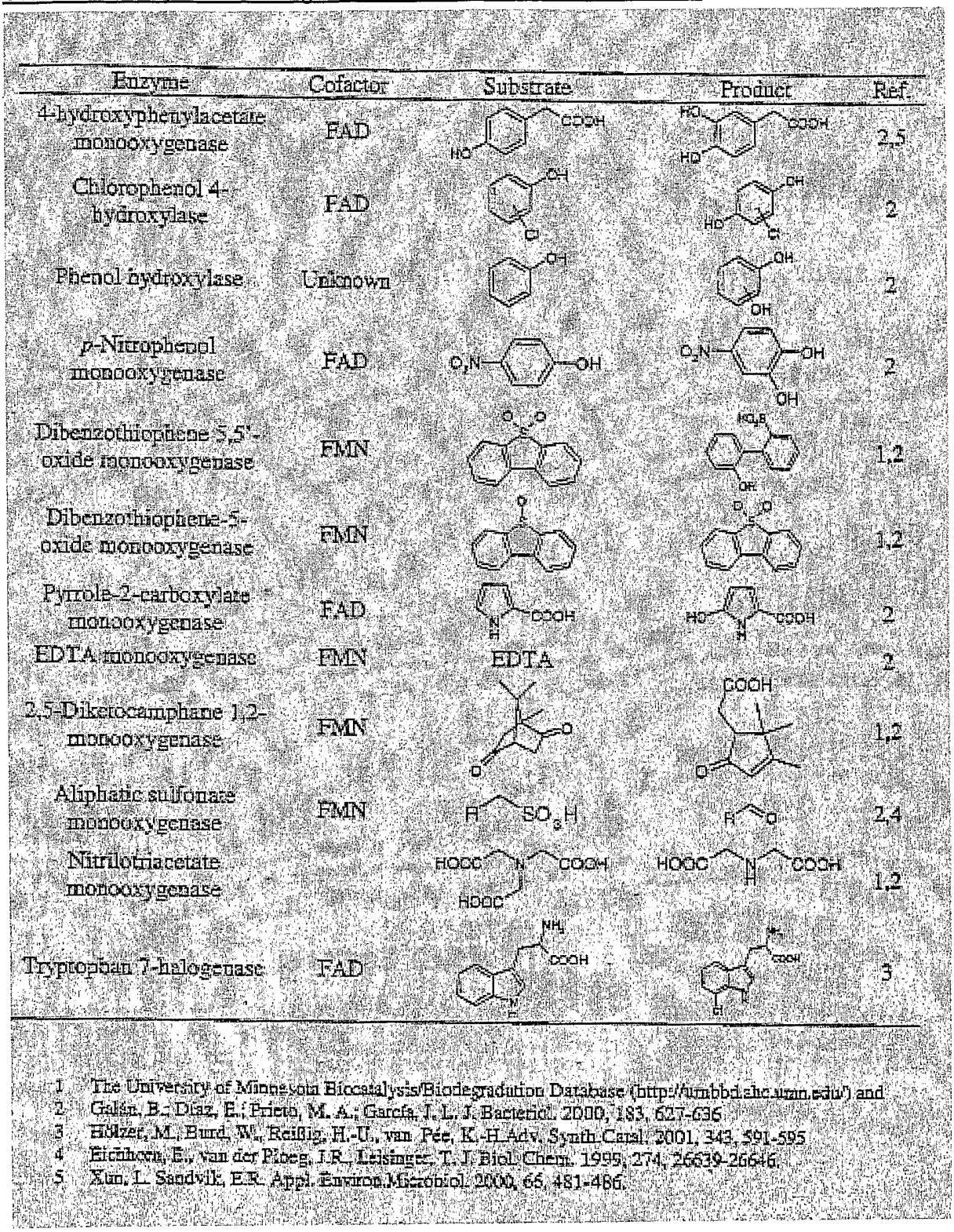

Setup for divided and an undivided electrochemical cells:
Divided cell:
Undivided cell:
Pseudo-Ag/AgCl reference
Carbon foil cathode
Pt-anode
Selection of enzymes utilizing diffusible reduced flavins as cofactors:

Figure 9:

Selection of enzymes untilizing diffusible reduced flavins as cofactors:



Enzyme	Cofactor	Substrate	Product	Ref.
4-hydroxyphenylacetate monooxygenase	FAD			2,5
Chlorophenol 4-hydroxylase	FAD			2
Phenol hydroxylase	Unknown			2
p-Nitrophenol monooxygenase	FAD			2
Dibenzothiophene 5,5'-oxide monooxygenase	FMN			1,2
Dibenzothiophene-5-oxide monooxygenase	FMN			1,2
Pyrrole-2-carboxylate monooxygenase	FAD			2
EDTA monooxygenase	FMN	EDTA		2
2,5-Diketocamphane 1,2-monooxygenase	FMN			1,2
Aliphatic sulfonate monooxygenase	FMN			2,4
Nitrilotriacetate monooxygenase				1,2
Tryptophan 7-halogenase	FAD			3

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2 Galán, B.; Díaz, E.; Prieto, M. A.; García, J. L. J. Bacteriol. 2000, 183, 627-636
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5 Xun, L. Sandvik, E.R. Appl. Environ. Microbiol. 2000, 66, 481-486.

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Figure 10:

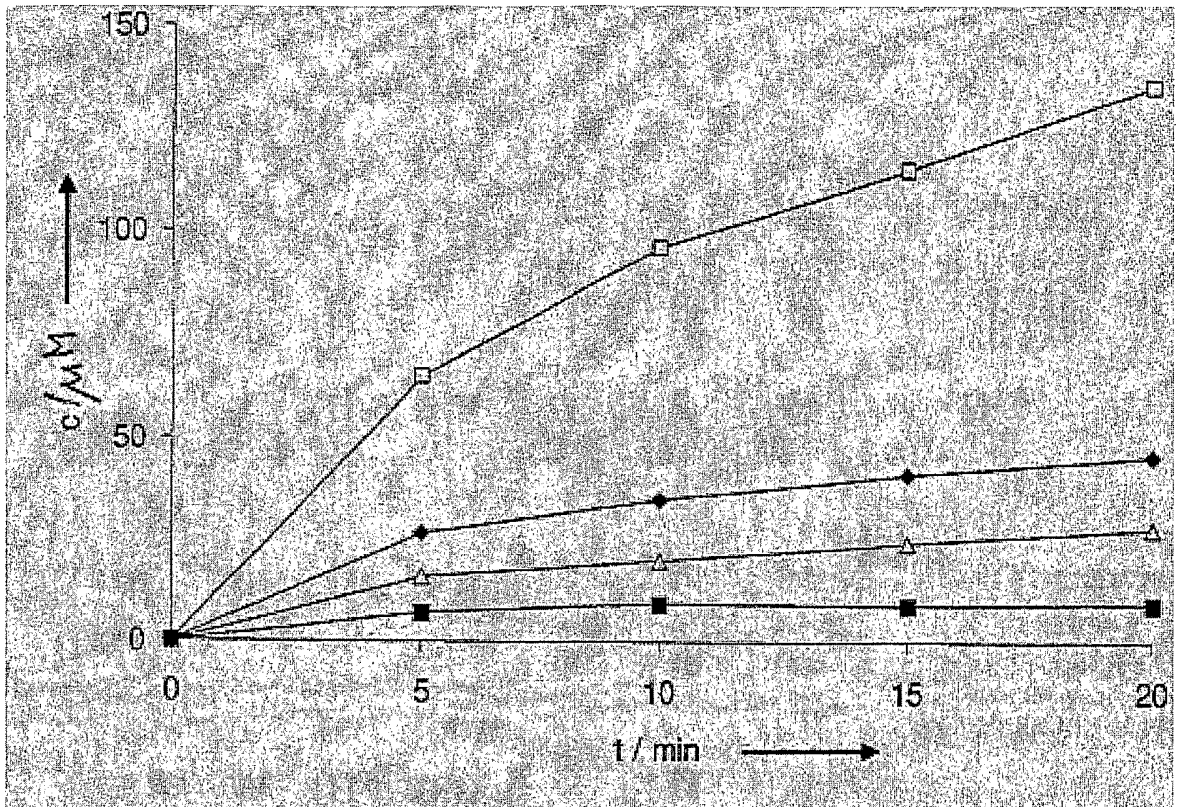

Figure 10: Influence of StyA-concentration on the rate of the electroenzymatic epoxidation of trans-β-methyl styrene. Reaction conditions: 12.9 mL potassium phosphate buffer (50 mM, pH 7.5), T = 30°C, c(FAD)=190 µM, c(catalase) = 460 U mL^{-1}, c(trans-β-methyl styrene) = 2 mM, cathode : 27 cm^2, c(StyA)= 0.84 µM (■), 1.64 µM (△), 3.35 µM (◆), 8.38 µM (□), aeration: ca. 2 ml min^{-1}.

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Figure 11:

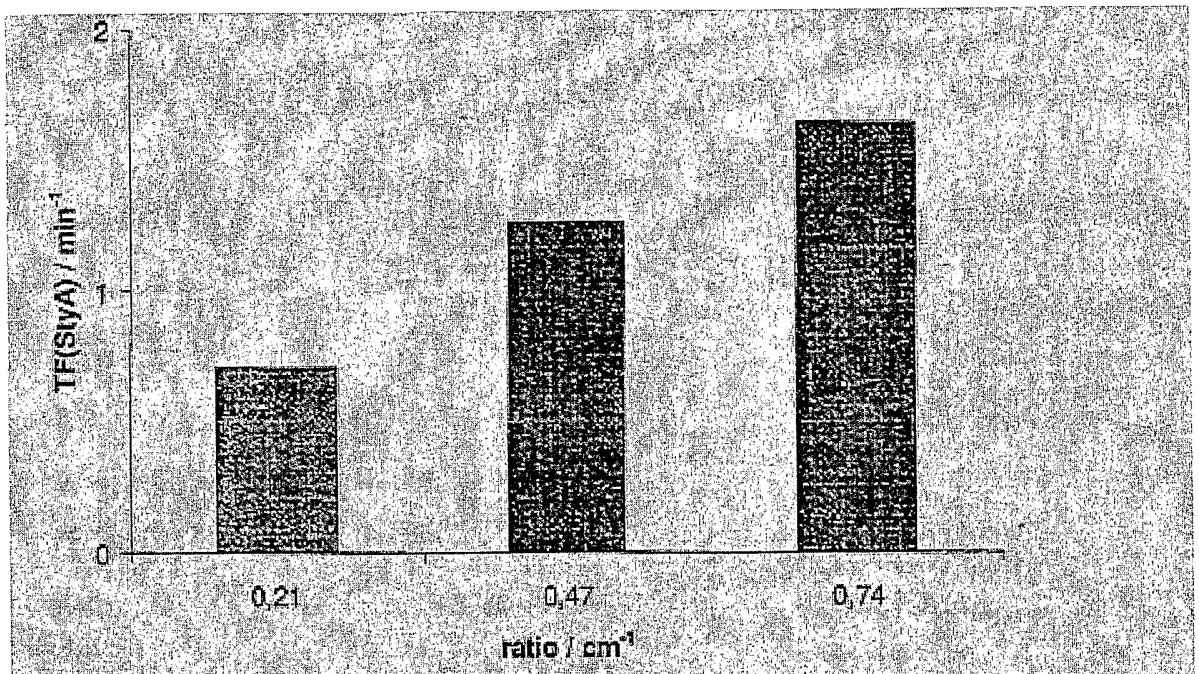

Figure11: Influence of ratio of cathode area over reaction volume on the efficiency of the StyA-catalyzed epoxidation reaction. General conditions: 50 mM potassium phosphate buffer pH 7.5, T=30°C; an undivided electrolysis cell was used, here the reaction buffer was deaerated prior initiation of the electrolysis, due to the undivided conditions. O_2 is *in situ* generated by the anode reaction.

Figure 12:

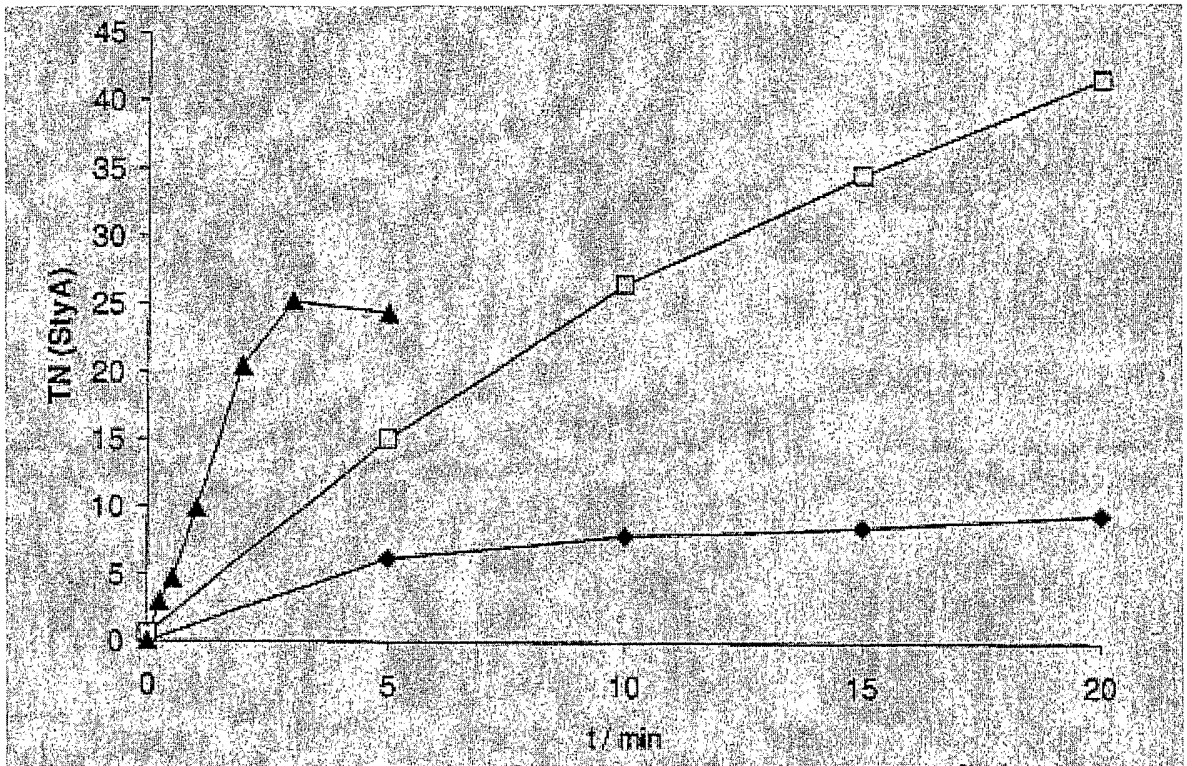

Figure 12 Influence of aeration on the rate of the electroenzymatic epoxidation. Reaction conditions: ◆, □: 12.9 mL potassium phosphate buffer (50 mM, pH 7.5), T = 30°C, c(StyA)=5.1 µM, c(FAD) = 186 µM, c(catalase) = 460 µM, c(trans-β-methyl styrene) = 2 mM, cathode: 27 cm^2, ◆: no external aeration, □: aeration of approximately 5-7 mL min^{-1}; ▲: 21.2 mL potassium phosphate buffer (50 mM, pH 7.5), T = 25°C, c(StyA)=4.9 µM, c(FAD) = 566 µM, c(catalase) = 460 µM, c(trans-β-methyl styrene) = 2 mM, cathode: 20 cm^2.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/005071

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P1/00 C12P7/00 C12N9/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, FSTA, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
16 August 2005	06/09/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	van de Kamp, M

INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/005071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	DRAUZ K ET AL.: "Enzyme Catalysis in Organic Synthesis. A Comprehensive Handbook. Second, Completely Revised and Enlarged Edition. Chapter 16: Oxidation Reactions" February 2002 (2002-02), WILEY-VCH VERLAG GMBH , WEINHEIM , XP002224911 cited in the application page 1065 - page 1280	1-5
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INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/EP2005/005071

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