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Quantitative and Non-Quantitative Assessments of Enzymatic Electrosynthesis: A Case Study of Parameter Requirements

Giovanni Sayoga+,[a] Michael Abt+,[b] Niklas Teetz+,[c, d] Victoria Bueschler+,[a] Andreas Liese,[a] Matthias Franzreb,[b] and Dirk Holtmann*[c, d]

The integration of enzymatic and electrochemical reactions offers a unique opportunity to optimize production processes. Recently, an increasing number of laboratory-scale enzymatic electrosyntheses have shown impressive performance indicators, leading to scientific interest in technical implementation. However, important process parameters are missing in most of the relevant literature. On one hand, this is due to the large variety of relevant performance indicators. On the other hand, enzyme technologists and electrochemists use different parameters to describe a process. In this article, we review the most important performance indicators in electroenzymatic processes and suggest that in order to allow quantitative comparison, these indicators should be reported in all respective publications. In addition to quantitative parameters, non-quantitative assessments often need to be included in a final evaluation. Examples of such parameters are sustainability, contribution to the UN Sustainable Development Goals or interactions with the overall process. We demonstrate the evaluation of processes using hydrogen peroxide-dependent peroxygenases. The strength of the proposed evaluation system lies in its ability to identify weaknesses in a process at an early stage of development. Finally, it can be concluded that all evaluated enzymatic electrosynthesis do not yet meet typical industrial requirements for an enzyme-based process.

1. Introduction

The importance of energy- and material-efficient industrial processes is increasing with the current challenges of a growing world population, unstable availability and costs of raw materials and energy, and environmental requirements. The combination of biotechnology and electrochemistry can contribute to the realization of efficient industrial processes. The general motivation for studying electro-biotechnological processes is to combine the advantages of electrochemistry (high energetic and atomic efficiencies) with the advantages enzymes or whole-cell catalysts (high regio- and enantioselectivity), thus electro-biotechnological processes are considered as one of the emerging techniques to combine biochemical transformations with the storage and utilization of electrical energy from renewable sources.

The applications of electro-biotechnology mainly comprise sensory aspects (biosensors), the conversion of chemical energy into electrical energy in enzymatic or microbial fuel cells and electro-biotechnological production processes. Among these applications, the synthesis of chemicals and energy carriers has gained the greatest interest so far.[1] Electro-biotechnological production processes can be divided in microbial and enzymatic electrosynthesis (MES or EES). In MES, the metabolic pathways of organisms are used to produce complex molecules such as bioplastics or terpenes as well as bulk chemicals such as acetate, methane and isopropanol.[2–8] In contrast, EES addresses mostly single reaction steps or small cascades up to 3 enzymatic reactions. EES has gained prominence because of its use of renewable energy inputs as well as highly specific enzyme biocatalysts and its capability of performing reactions with high yields.[9] Most enzymes used in EES are members of the group of oxidoreductases (Enzyme Commission number 1). Oxidoreductases are enzymes that catalyse the transfer of electrons from one molecule (the reductant or electron donor) to another molecule (the oxidant or electron acceptor). A large number of the oxidoreductases are using oxidised or reduced cofactors (such as nicotinamide adenine dinucleotides or flavins) as electron carriers. In whole-cell catalysts, these cofactors are regenerated intracellularly; when using isolated enzymes, this regeneration must be technologically solved. One option is the use of electrochemical regeneration or substitution processes. Figure 1 schematically shows the different

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[a] *G. Sayoga,+ V. Bueschler,+ Prof. Dr. A. Liese*
Institute of Technical Biocatalysis, Hamburg University of Technology
Denickestraße 15, 21073 Hamburg, Germany

[b] *M. Abt,+ Prof. Dr. M. Franzreb*
Institute of Functional Interfaces, Karlsruhe Institute of Technology
Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

[c] *N. Teetz,+ Prof. Dr. D. Holtmann*
Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen
Wiesenstrasse 14, 35390 Giessen, Germany

[d] *N. Teetz,+ Prof. Dr. D. Holtmann*
Institute of Process Engineering in Life Sciences, Karlsruhe Institute of Technology
Fritz-Haber-Weg 4, 76131 Karlsruhe, Germany
E-mail: dirk.holtmann@kit.edu

[+] *contributed equally*

modes of electron transfer between electrodes and enzymes. The direct electron transfer (DET) is based on the interaction between a redox-active motive of the enzyme and an electrode (Figure 1 A). However, because the redox cofactors are typically deeply embedded in the protein matrix of the enzymes, electrons cannot easily be transferred between the electrode and enzyme.[10] Different types of mediated electron transfer (MET) can be used to overcome this limitation (Figure 1 B–D). Mediators hereby acts as an electron shuttle between electrode and biocatalysts or cofactors.[11] In MET the electron shuttle is used to transfer electrons between the electrode and the natural cofactor. The enzyme reaction is comparable to the natural reaction. A mediated electron transfer between the electrode and an enzyme can be used to drive an enzymatic reaction or to regenerate a cofactor for a second enzymatic reaction. The use of direct electron transfer between the cofactor and the electrode minimizes the complexity of the reaction system caused by the addition of a mediator (Figure 1 E). EES often uses either cofactor-dependent enzymes or

Giovanni Sayoga was born in Tasikmalaya, Indonesia in 1992. He obtained his Bachelor's degree in 2018 and Master's degree in 2020, both in the field of Bioprocess Engineering from the Hamburg University of Technology (TUHH), Germany. Currently, he is pursuing his PhD at the Institute of Technical Biocatalysis of TUHH, under the supervision of Prof. Andreas Liese. His current PhD research revolves around in situ electrochemical generation of H_2O_2 as a co-substrate for biocatalytic reactions.

Michael Abt received his MSc degree in chemical engineering from the Technical University of Munich in 2020, where he investigated key performance indicators for the operation of a high-gradient magnetic separator for protein purification. He is currently working on his PhD project at the Karlsruhe Institute of Technology, under the supervision of Prof. Dr.-Ing. Matthias Franzreb. His research focuses on the development, the exemplary application, and the process characterization of novel electrochemical fluidized bed reactors for electroenzymatic syntheses including gaseous phase.

Niklas Teetz received his M. Sc. degree in molecular biology from the Goethe University of Frankfurt a. M. in 2021 after investigating a paired electrolysis cell combining Kolbe electrosynthesis and biosynthesis via Cupriavidus necator at DECHEMA Research Institute. He started his PhD studies at University of Applied Sciences Mittelhessen at the Institute of Bioprocess Intensification and is currently concluding this work at the Institute of Electrobiotechnology at the Karlsruhe Institute of Technology. His research focuses on reaction and enzyme engineering of unspecific peroxygenases.

Victoria Bueschler completed their Bachelors's and Masters's degrees in Bioprocess Engineering at Hamburg University of Technology (TUHH) in Hamburg, Germany. Focussing on heterogeneous enzyme biocatalysis and inline analytical methods in their Bachelors's and Masters's thesis, they began their Ph.D. research project on an enzymatic bioelectrochemical system in early 2021. Since 2023 they are the research group leader for the Process Analytical Technologies group at the Institute of Technical Biocatalysis at the Hamburg University of Technology.

Matthias Franzreb (1963) received his Ph.D. in chemical engineering from the University of Karlsruhe. Afterwards he entered the Karlsruhe Institute of Technology and started his work on magnetic micro-adsorbents and their application in protein purification and enzyme immobilization. In 2002, he finished his Habilitation and in 2009, he was awarded an Extraordinary Professorship by the Karlsruhe Institute of Technology (KIT). His research focuses on device development and modelling for novel bioseparations and electroenzymatic reactions.

Andreas Liese studied chemistry and carried out his doctoral research at the Research Center Jülich, Germany. He received his Ph.D. from the University of Bonn in 1998. From 1998 to 2003, Liese was an assistant professor at the University of Bonn and head of the Enzyme Group within the Research Center Jülich. After a sabbatical in 2000 at Pfizer Global Research & Development, San Diego, USA, he worked as associate professor at the University of Münster from 2003 to 2004. In 2004 he became a full professor of the Institute of Technical Biocatalysis at the Hamburg University of Technology (TUHH), Germany.

Dirk Holtmann completed his diploma in chemical engineering/biotechnology in 1999. He received his PhD at the Otto-von-Guericke University Magdeburg on the electrochemical measurement of microbial activities. Until September 2019 he was head of the Industrial Biotechnology Group at the DECHEMA Research Institute. After that he was professor for Intensification of Bioprocesses at THM (Gießen, Germany). In 2023 he became full professor for Electrobiotechnology at the Karlsruhe Institute of Technology. His current research activities focus on biocatalysis, bioprocess engineering and bioelectrochemical synthesis.

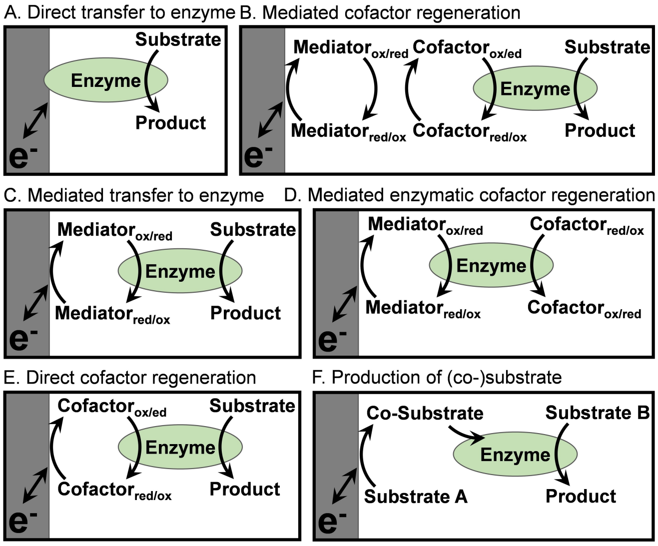


Figure 1. Scheme of electroenzymatic processes.

enzymes whose substrates can be generated electrochemically (especially H_2O_2-dependent enzymes, Figure 1 F).

Interest in enzymatic electrosynthesis has increased significantly in recent years, as evidenced by the growing number of publications in the field. Unfortunately, many of the studies are difficult to compare or evaluate. Proof-of-principle studies often do not report any metrics at all. Other publications use a wide variety of metrics. The aim of this paper is to describe the most important performance indicators and to compare the current examples in the field of EES using these indicators. Finally, different options will be compared based on these indicators and other non-quantitative parameters.

2. Overview of Important Performance Indicators

Understanding and overcoming the limiting factors of electroenzymatic systems is a challenging task that requires a parameter-based approach using standardized performance indicators (PIs). These PIs help to understand the system, enable comparability between different systems and allow benchmarking with more conventional processes. Table 1 provides an overview of the key performance indicators for enzymatic electrosynthesis. The table assumes that the geometry and volume of the reactor and the surface area of the electrodes are known. In addition, we assume that the kinetics of the enzyme reaction of interest have been studied in small, well-mixed batch studies that provide the traditional indicators V_{max}, k_{cat} and K_m. During the electroenzymatic process in the reactor, the applied potential and the resulting current should be monitored. In 3-electrode setups, the cell potential U_{cell} between the anode and cathode should be recorded in addition to the adjusted potential of the working electrode versus the reference electrode. In the following, it is assumed that a cofactor or co-substrate is electrochemically produced at the working electrode and simultaneously consumed by an enzymatic reaction involving an additional substrate in the reactor volume. However, other types of electroenzymatic processes can be treated analogously.

The first section of Table 1 lists the most important general reaction engineering PIs which can be calculated from the experimental data. It is always recommended to report the space-time-yield (STY) and achievable product titre for comparison with traditional processes.[12] The product titre also allows for an estimation of the effort required for product recovery in downstream processing. In case of an electrochemical co-factor regeneration or co-substrate generation, it can be useful to correlate the electroenzymatic STY of the product to the purely electrochemical STY of the intermediate to determine how much of the electrochemical generated substance is used in the combined system.[13] Together with the known amount of applied enzyme the total turnover number (TTN) and the turnover frequency (TOF) can be calculated as two of the key PIs of enzymatic as well as electroenzymatic systems.[14]

Important electrochemical PIs, such as the current efficiency (CE), the current density, and the specific energy consumption per kg of product can be calculated together with the data monitored by the potentiostat. To understand the relation of CE and applied potential, laboratory-scale experimental setups need to include a reference electrode to investigate the resulting overpotential of the electrode configuration. Besides the potential, the CE should always be paired with the current density. In many publications, the current density is referred to the projected electrode area. This is correct in the case of flat electrode types, like polished electrodes, meshes, and plates, when the geometric electrode area is equal to the electrode active surface area.[15] However, to calculate the correct electrode surface of porous electrodes with rougher surfaces such as carbon foam, carbon felt and diffusion electrodes, it is advised to determine the electrochemically active surface area via chronoamperometry or cyclic voltammetry.[15]

Most enzymatic electrosynthesis are adjusted to enzyme performance and suffer from comparable low concentrations and low electrolyte conductivity,[16] which can make it necessary to improve electrolyte-electrode mass transfer and to reduce ohmic resistance.[17] One simple beneficial way of improvement is to increase the surface-to-volume ratio.[18,19] Another often missing PI for reactor characterization is the mass transfer coefficient (k_{film}) at the electrode. For defined electrode geometries k_{film} can be calculated by the respective correlation between the Sherwood, Reynolds, and Schmidt number. In other cases, k_{film} can be estimated from well-defined electrochemical model reactions conducted in the same reactor.[20] The listed indicators deliver a comprehensive overview of the performance indicators of an electroenzymatic process and help to comprehend the system, to determine bottlenecks and to facilitate a scale-up of the process for industrial application.

Table 1. Performance indicators to characterize electroenzymatic processes.

Symbol	Name	Formula	Meaning	Remarks
General reaction engineering performance indicators				
$\sigma_p,\ \dot{q}_{p,v}$	Space time yield (STY), volumetric productivity	$\sigma_p = m_p/(V \cdot \tau)$ $\dot{q}_{p,v} = m_p/(V \cdot t)$	Mass of a product formed per time and volume in batch and continuous process	Reported to compare different reactors. Ideally, use the liquid volume of the compartment with the working electrode for calculation
Y_p	Reaction yield	$Y_p = n_p/n_{r,0} \cdot \lvert v_r/v_p \rvert$ $Y_p = (\dot{n}_{p,out} - \dot{n}_{p,in})/\dot{n}_{r,in} \cdot \lvert v_r/v_p \rvert$	Yield of batch and of continuous electroenzymatic reaction	Specify components (products and reactants: co-factor, co-substrate or substrate) and clarify system boundary as well as process step.
X_r	Conversion	$X_r = 1 - n_r/n_{r,0}$ $X_r = 1 - \dot{n}_{r,out}/\dot{n}_{r,in}$	Conversion of batch and of continuous electroenzymatic reaction	Specify components (products and reactants: co-factor, co-substrate, substrate) and clarify system boundary as well as process step
S_p	Selectivity	$S_p = n_p/\Delta n_r \cdot \lvert v_r/v_p \rvert$ $S_p = (\dot{n}_{p,out} - \dot{n}_{p,in})/(\dot{n}_{r,in} - \dot{n}_{r,out}) \cdot \lvert v_r/v_p \rvert$	Selectivity of batch and of continuous electroenzymatic reaction	Specify components (products and reactants: co-factor, co-substrate or substrate) and clarify system boundary as well as process step.
$\mathrm{E-factor}$	Environmental factor	$\mathrm{E-factor} = c_{waste}/c_p$	Ratio of waste to product	The amount of waste is difficult to quantify, we recommend excluding water and normalize the waste generation to the reactor volume[21,22]
$c_p,\ c_s$	Product titer and substrate loading		Concentration of product/substrate	Declare concentrations to benchmark against conventional processes and to estimate costs[23]
Enzymatic performance indicators				
TTN / $\mathrm{TTN}_{co-factor}$	Total turnover number	$\mathrm{TTN} = n_p/n_{biocat}$ $\mathrm{TTN} = n_{co-factor}/n_{biocat}$	Stability of the biocatalyst under process conditions	Reported for the product and the co-factor
TOF	Turnover frequency	$\mathrm{TOF} = n_p/(n_{biocat} \cdot t)$	Turnover per unit time	Disregards enzyme stability in the process and should be paired with the total turnover number
$t_{0.5}$	Half-life time		Half-life time of enzyme	Gives information on enzyme stability under process conditions (time of the process when the residual activity ofthe enzyme is 50%)
c_{biocat}	Biocatalyst load		Concentration of enzyme	Declare the biocatalyst concentration for comparability and for cost estimation (i.e. ratio of deployed enzyme to product)[23]
ee	Enantiomeric excess (ee)	$\mathrm{ee} = \lvert n_R - n_S \rvert/(n_R + n_S)$	Purity of chiral substance	Highlights enantioselectivity of biocatalyst
Electrochemical performance indicators[24–26]				
Φ_p^e	Current efficiency (CE)	$\Phi_p^e = n_p \cdot z/v_p \cdot F/Q$ $Q = \int_0^t I(t)\mathrm{d}t$	Ratio between the amount of product and total amount of consumed electrons	Important for economic analysis and can be calculated for the electrochemical product or entire electroenzymatic reaction
Φ_G	Energy efficiency	$\Phi_G = (\Delta G \cdot n_p)/(Q \cdot U_{cell})$ $= v_p/Z \cdot (\Delta G \cdot \Phi_p^e)/(F \cdot U_{cell})$ $= \Delta E_{cell,eq}/U_{cell} \cdot \Phi_p^e$	Fraction of applied energy which is thermodynamically stored in the product of the electrochemical reaction	The energy efficiency for the applied electric energy combines voltage and current efficiency of the electrochemical cell
$j,\ j_v$	Current density, volumetric current density	$j = I/A_e$ $j_v = I/V$	Current per active electrode area or volume	Determine correct active electrode area or use liquid volume of compartment with working electrode for calculation
η_{WE}	Overpotential	$\eta_{WE} = E_{WE} - E_{eq,WE}$	Extra potential than thermodynamically expected from the equilibrium potential to drive a reaction	Overpotential at the working electrode
w	Specific electric energy consumption (sEEC)	$w = U_{cell} \cdot Q/m_p$	Electric energy consumption per mass of product	Reports the specific energy consumption of the production process
ETY	Electrode surface time yield	$\mathrm{ETY} = m_p/(A_e \cdot t)$	Mass of product formed per time and electrode surface	Reported to determine scalability

Table 1. continued

Symbol	Name	Formula	Meaning	Remarks
$Y_{p/e}$	Product yield per electron[18]	$Y_{p/e} = F \cdot n_p / Q$	Product yield per electron	Characterization and benchmarking
Reactor performance indicators				
A_e/V	Electrode surface per reactor volume	A_e/V	Ratio of electrode surface to volume	Determine active electrode surface and use liquid volume of compartment with working electrode for calculation
k_{film}	Film mass transfer coefficient	$k_{film} = Sh \cdot D_m / L$ $Sh = f(Re,\ Sc)$	Correlates diffusion rate with convective mass transfer rate	Defines the rate by which the educts can pass the Nernst film which surrounds the electrode

3. Examples of Electroenzymatic Processes

Numerous enzymes have been used for enzymatic electrosynthesis and the four most prominent electroenzymatic processes utilize the unspecific peroxygenase from the fungus *Agrocybe aegerita* (*Aae*UPO) for the hydroxylation of non-activated carbon,[27] chloroperoxidase (CPO) for the oxidation of thioanisole,[28,29] glucose oxidase (GOx) for the oxidation of glucose,[22,30] and formate dehydrogenase (FDH) for the reduction of the greenhouse gas CO_2.[31,32] To overcome the major enzyme instabilities at excess co-substrate H_2O_2 concentration, Lütz and co-workers combined the electrochemical H_2O_2 supply with CPO for the first time. The electrochemical system contained a cylindrical carbon felt working electrode and the oxidation of thioanisole to (*R*)-methylphenylsulfoxide was used as the model reaction system. Using this setup, a productivity up to $30\ \mathrm{g\,L^{-1}\,d^{-1}}$ and a TTN as high as $95{,}000\ \mathrm{mol\,mol^{-1}}$ were reported.[28] In electroenzymatic processes, high selectivity and sustainability are usually only being assumed. Varničić studied both of these aspects in more detail within an electroenzymatic process for the oxidation of the renewable feedstock glucose to gluconic acid in a membrane-less flow reactor using immobilized GOx at the anode and co-immobilized GOx and horseradish peroxidase (HRP) (catalase reaction) at the cathode (Vulcan carbon nanomaterials as support for biocatalysts). With the help of nuclear magnetic resonance (NMR) spectroscopy, a product selectivity of 97% was reported. Additionally, glucose conversion of 80% and E-factor of 9 were obtained.[22] A further distinctive result of the application of an electroenzymatic process was reported by Sokol et al. They mimicked the biological formate hydrogen lyase (FHL) complex and performed a reversible conversion of formate to H_2 and CO_2 under ambient conditions, which is normally realized via mixed-acid fermentation in *Escherichia coli*. It was achieved by employing a semiartificial system consisting of FDH and hydrogenase (H_2ase) (from *Desulfovibrio vulgaris*) immobilised on indium tin oxide (ITO). A TON of $23{,}000\ \mathrm{mol\,mol^{-1}}$ and a TOF of $6.4\ \mathrm{s^{-1}}$ were achieved.[33]

In addition to the already mentioned processes, further recent and noteworthy examples of electroenzymatic processes are presented in Table 2, along with key performance indicators, electron transfer mechanisms, and process conditions used to perform the experiments. Among these examples, peroxidases (e.g., CPO and horseradish peroxidase (HRP)) have been implemented into various electroenzymatic processes such as chlorination, sulfoxidation and demethylation, and within different setups. With such a diverse set of processes, a comparison of the results is challenging even for comparable reaction systems, as different or unrelated parameters and performance indicators are reported. Firstly, this may be due to a broad range of available relevant performance indicators that can be used. Secondly, the background of researchers might be responsible as different indicators to describe a process are used in different academic disciplines. To better facilitate comparisons in the field of electrobiotechnology, a standardized reporting on electroenzymatic processes should be implemented.

The numerous listed examples demonstrate that electrobiotechnology has come a long way since its first inception in 1911.[1,48] Moreover, it is expected that electroenzymatic processes gain even more relevance in the near future. It can also be noted that developed and established processes on a lab scale have been reported in the literature quite progressively.[9] Several processes even show remarkable performance indicators, which is promising for future pilot scale processes.

4. Non-quantitative Parameter

Natural scientists, engineers as well as economists usually decide on the base of performance indicators (see above). However, there are also some evaluation criteria that cannot be directly linked to key indicators. Examples in this context are the contribution of a technology or product to the achievement of the United Nations Sustainable Development Goals (SDGs), the effort required to train employees on new processes, the flexibility of the application, the availability of necessary materials, or the acceptance of new products or processes by customers and the general public. Non-quantitative properties may also include non-detectable fluctuations in raw materials. In electroenzymatic processes, these can be, for example, typical variations in the composition or purity of the natural substrates or in the specific activity or stability of the enzymes. It should be noted that some key figures can only be calculated/estimated on an industrial scale. Our considerations (see below) refer to the early stages of process development.

Table 2. Examples of electroenzymatic processes.[a]

Process types	e^- transfer mechanism	Enzyme, co-factors, substrate and product	Type of working electrode	Process condition	Key performance indicators	Ref.
Hydroxylation	F ($2e^-$)	Enzyme: UPO, co-substrate: H_2O_2, substrate: ethylbenzene, product: 1-phenethyl alcohol	CB GDE, *A*: 2 cm^2	30 mL 0.1 M KP_i pH 7, 900 µL acetone, 500 µL substrate, 50 nM (0.3 U mL^{-1}) UPO, applied currents: −5 to –30 mA cm^{-2}	TTN: 400,000, productivity: 25 g L^{-1} d^{-1}, loss of enzyme activity: 0.33 U mL^{-1} h^{-1}, CE: 78 %, H_2O_2 productivity a).: 225 µM min^{-1} cm^{-2}	[27]
Flow-through reactor, hydroxylation	F ($2e^-$)	Enzyme: UPO, co-substrate: H_2O_2, substrate: 4-ethylbenzoic acid, product: 4-(1-hydroxyethyl)benzoic acid	CB GDE, *A*: 5.5 cm^2	100 mL 100 mM KP_i pH 7, 10 mM substrate, 12.5 nM UPO, flow rate: 40 mL min^{-1}, 250 mL reservoir, applied currents: −10 to −80 mA, room temperature	TTN: 400,000; TOF: 150 s^{-1}, H_2O_2 productivity a).: 32 µM min^{-1} cm^{-2}, kinetic parameter (*e.g.* K_m)	[34]
Flow-through reactor, chlorination	F ($2e^-$)	Enzyme: CPO, co-substrate: H_2O_2, substrate: monochlorodimedone (MCD), product: dichlorodimedone (DMD)	GDE, *A*: 5.5 cm^2	8 mL 100 mM citrate pH 3.5/ 2.8 + 10 mM NaCl, flow rate: 50 mL min^{-1}, 50 mL reservoir, 5–30 nM CPO, applied currents: 5–30 mA, T: 30 °C	TTN: 1,150,000, productivity: 52 g L^{-1} d^{-1}, H_2O_2 productivity a).: 10 µM min^{-1} cm^{-2}, CE: 50 %, A_e/V: 0.11–0.32 cm^2 mL^{-1}, H_2O_2 / CPO productivity: 0.2 µmol H_2O_2 min^{-1} $U_{CPO}{}^{-1}$, ETY: 0.16 g cm^{-2} d^{-1}	[13]
Batch & fed-batch, flow-through packed bed reactor, sulfoxidation	F ($2e^-$)	Enzyme: CPO, co-substrate: H_2O_2, substrate: thioanisole, product: (*R*)-methylphenylsulfoxide	Graphite grains, *d*: 0.6-1 mm, *A*: 1,350 cm^2	Cathode chamber: 40 mL 100 mM NaOAc pH 5 + 50 mM Na_2SO_4 + 10 % (v/v) *t*-butanol, anode chamber: 0.05 M H_2SO_4, 11 U mL^{-1} CPO, total volume: 70–100 mL, flow rate: 100 mL min^{-1}, O_2-saturated, applied potential: 1.95 V	TTN: 145,000, medium conductivity: 13.4 mS cm^{-1}, productivity: 104 g L^{-1} d^{-1}, mass of isolated product: 1.2 g, purity: > 98 %, ee: > 98.5 %, CE: 75 %	[29]
2-compartments reactor, sulfoxidation, 3- electrode configuration	F ($2e^-$)	Enzyme: CPO, co-substrate: H_2O_2, substrate: thioanisole, product: (*R*)-methylphenylsulfoxide	GF (cylindrical), *A*: 74 cm^2	300 mL 0.1 M potassium citrate pH 5 + *t*-BuOH, 6 mmol substrate, O_2-saturated; T: 20 °C, 70 nmol CPO, applied potential: −0.5 V vs Ag/AgCl	TTN: 95,000, productivity: 30 g L^{-1} d^{-1}, ee: 98.5 %, CE: 65.6 %, current: 170 mA, H_2O_2 productivity a).: 0.002 µM min^{-1} cm^{-2}, conversion: 100 %, 23 % CPO residual activity	[28]
H-cell, halogenation, 3- electrode configuration	F ($2e^-$)	Enzyme: CPO, co-substrate: H_2O_2, substrate: 4-pentanoic acid, KBr, product: bromolactone	GDE with oCNT coating, *A*: 25 cm^2	Cathode chamber: 100 mL 100 mM Na citrate pH 5, 100 mM KBr, 50 mM substrate, 100 nM CPO, applied potential: −0.35 V vs Ag/AgCl or 25 nM at −0.25 V vs Ag/AgCl, anode chamber: 100 mL 100 mM Na citrate pH 5	Formation rate: 4.5 mM h^{-1}, CE: 80 %, H_2O_2 productivity a).: 4.7 µM min^{-1} cm^{-2}	[35]
Flow-through reactor, chlorination, sulfoxidation, oxidation	F ($2e^-$)	Enzyme: CPO, co-substrate: H_2O_2, substrate: MCD, thioanisole, indole, product: DMD, methylphenylsulfoxide, oxindole	GDE, *A*: 5.5 cm^2 or 16.5 cm^2	8 mL 0.1 M citrate pH 2.75 + 10 mM NaCl or 0.1 M NaOAc pH 5 + 50 mM Na_sSO_4, reservoir: 50 mL, flow rate: 63 mL min^{-1}, 5 mM substrate, 10–600 nM CPO	TTN: 203,100 (MCD), productivity: 23 g L^{-1} d^{-1} (thioanisole), ETY: 0.87 g cm^{-2} d^{-1} (MCD), CE: 88 %, H_2O_2 productivity a).: 1.5 µM min^{-1} cm^{-2}, initial conversion rate a).: 43.4 mM h^{-1}	[36]
2-compartments reactor, sulfoxidation, 3- electrode configuration	F ($2e^-$)	Enzyme: CPO, co-substrate: H_2O_2, substrate: methyl p-tolyl sulfide, 1-methoxy-4-(methylthio)-benzene (**1**), *N*-MOC–L-methionine methyl ester, product: methyl p-tolyl sulfoxide, methoxypehnylmethylsulfoxide	CF (cylindrical), *A*: 74 cm^2	Cathode chamber: 290 mL 90 vol.–% 100 mM NaOAc + 50 mM Na_2SO_4 pH 5 and 10 vol.–% *tert*-butanol + 6 mmol substrate, T: 20 °C, anode chamber: 10 mL buffer, O_2-	TTN: 64,400 (**1**), isolated yield: 74.3 % (**2**), CPO residual activity, conversion: 83 % (**1**), K_m and V_{max}, ee: 99 % (**1**), enzyme purity: 63 %	[37]

Table 2. continued

Process types	e^- transfer mechanism	Enzyme, co-factors, substrate and product	Type of working electrode	Process condition	Key performance indicators	Ref.
		(**2**), *N*-MOC–L-methionine methyl ester sulfoxide		saturated, 300 μL or 3.5 mL (22,700–36,500 U mL^{-1} stock solution) CPO, applied potential: −0.5 V vs. Ag/AgCl		
Oxidation, 3-electrode configuration	F (2e^-)	Enzyme: CPO, co-substrate: H_2O_2, substrate: cinnamyl alcohol, product: cinnamic aldehyde	Composite film-modified GC comprising chitosan, CPO, DDAB, and Nafion	2 mL 50 mM Phos. pH 4.5, T: 25 °C, O_2-saturated, immo. CPO (2.9×10^{-4} mol L^{-1}) on the electrode surface, 0.05 mmol substrate, applied potential: −0.6 V vs. SCE	analytical yield: 51.8 %, TTN: 80,500, formation rate $^{a)}$: 1.5 mM h^{-1}, turnover rate: 3.2 μmol h^{-1}, current density: 0.87 mA cm^{-2}, e^- transfer rate: 2.3 s^{-1}	[38]
H-cell, demethylation, 3-electrode configuration	F (2e^-)	Enzyme: HRP, co-substrate: H_2O_2, substrate: *N,N*-dimethylaniline, product: *N*-methylaniline, formaldehyde	GC disk, *d*: 5 mm	30 mL (both chamber) 0.2 M Phos. pH 5.5, 0.04 μg mL^{-1} HRP, 2 mM substrate, room temperature, air-saturated, applied current: 0.08 mA	K_m: 0.19 mM, V_{max} 2000 mol min^{-1} mol enzyme^{-1}, H_2O_2 productivity $^{a)}$: 1.3 μM min^{-1} cm^{-2}, formation rate $^{a)}$: 0.1 mM h^{-1}, ratio of formed product to theoretical H_2O_2 generated: 1.75	[39]
Packed-bed flow reactor, 2 compartments, demethylation, 3-electrode configuration	F (2e^-)	Enzyme: HRP, co-substrate: H_2O_2, substrate: *N,N*-dimethylaniline, product: *N*-methylaniline, formaldehyde	GF (*V*: 2 cm^3) & RVC (*V*: 8.2 cm^3)	Working volume, $^{a)}$: 12.29 mL, 0.2 M Phos. pH 5.5, reservoir: 200 mL, flow rate: 50 mL min^{-1}, O_2-saturated, 2 mM substrate, immo. HRP: 5.4 mg mL^{-1}, applied current: 20 mA	CE: 45 %, formation rate $^{a)}$: 7.8 mM h^{-1}, K_m: 0.52 mM (substrate) and 0.034 mM (H_2O_2)	[40]
H-cell flow-reactor, oxidation, 3-electrode configuration	F (2e^-)	Enzyme: HRP, co-substrate: H_2O_2, substrate: 2,4,6-trimethylphenol, product: 3,5-dimethyl-4-hydroxybenzyl alcohol, 3,5-dimethyl-4-hydroxybenzaldehyde, 2,6-dimethylbenzoquinone	RVC disk (60 ppi), *V*: 0.8 cm^3 −1.96 cm^3	15–120 mL 0.1 M pH 7 Phos., 5 mM substrate, 10 U mL^{-1} HRP, O_2-saturated, electrode rotation: 500 rpm, applied potential: −0.5 V vs. SCE, room temperature, reservoir: 500 mL, flow rate: 200 mL min^{-1}	Total yield: 95 %, CE: 62 %, charge: 3.7 F, current: 5 mA cm^{-3}	[41]
H-cell, conversion of CO_2 to methanol, 3-electrode configuration	E (1e^-)	Enzyme: FDH, FalDH, ADH, co-factor: NADH, substrate: CO_2, product: methanol	Rh complex-grafted CF	10 mL 50 mM Tris-HCl pH 7.4 (Serine Glycerol), N_2-saturated, 1 mg FDH, 1 mg FaldDH, 1 mg ADH, and 1 mM NADH, applied potential: 0.62 V vs. Ag/AgCl/KCL (3 M)	Enzyme activity: 384.6 U mL^{-1}, current density: −0.23 mA cm^{-2}, charge transfer resistance: 48 Ω, co-factor yield: 60 %, formation rate: 0.48 mM h^{-1}	[31]
H-cell, conversion of CO_2 to formate, 3- electrode configuration	E (1e^-)	Enzyme: FDH, co-factor: NADH, substrate: CO_2, product: formate	Cu foam, *A*: 2.66 cm^2	Both chamber 15 mL 0.2 M PBS pH 7, 1 mg mL^{-1} FDH (free), 1 mg mL^{-1} cm^{-2} immo. FDH on EPSNF, NAD$^+$: 0.85 (free FDH) & 0.95 mM (immo. FDH), applied potential: −1.1 V vs. Ag/AgCl/KCL (3 M), CO_2 flow rate: 30 mL min^{-1}	Immo. FDH stability: 41 % after 20 d, immo. FDH relative activity: 43 %, reusability of immo. FDH: 8 cycles, immo. efficiency: 57 %, optimum co-factor concentration: 0.45 mM (free FDH) & 0.51 mM (immo. FDH), product concentration: 0.61 mM (free FDH) & 0.31 mM (immo. FDH), yield of co-factor: 96 %	[42]
Semi-continuous, H-cell with *in situ* product removal, conversion of CO_2 to	E (1e^-)	Enzyme: FDH, co-factor: NADH, substrate: CO_2, product: formate	Cu foam, *A*: 2.66 cm^2	Both chamber 20 mL 0.2 M PBS pH 7, 1 mg mL^{-1} cm^{-2} immo. FDH on EPSNF, NAD$^+$: 0.95 mM, applied po-	Regenerated co-factor concentration: 0.5 mM, immo. FDH relative activity: 43 %, immo. FDH activity: 1.6 U mg^{-1}, immo.	[43]

Table 2. continued

Process types	e^- transfer mechanism	Enzyme, co-factors, substrate and product	Type of working electrode	Process condition	Key performance indicators	Ref.
formate, 3- electrode configuration				tential: −1.1 V vs. Ag/AgCl/KCL (3 M), CO_2 flow rate: 30 mL min^{-1}, ethyl acetate as extraction phase, half of the reaction volume was replaced with fresh NAD^+ solution every hour	efficiency: 57 %, loading capacity of immo. FDH: 90 µg cm^{-2}, reusability of immo. FDH: 8 cycles, immo. FDH stability: 41 % after 20 d, product concentration: 0.44 mM	
Oxidation & reversible and interconversion of H_2 and CO_2 into formate, 3-electrode configuration	A (1e$^-$)	Enzyme: FDH, H_2ase, substrate: formate, CO_2, H_2, product: formate, H_2	Macro-mesoporous inverse opal ITO, thickness: 25 µm, *A*: 0.25 cm^2	2 mL 100 mM CO_2/$NaHCO_3$ pH 6.5–6.7, 50 mM KCl, 1 bar CO_2 or 0.4/0.6 bar H_2/CO_2, formate:10 or 20 mM, T: 23 or 25 °C, FDH: 2 µL, 19 µM, H_2ase: 2 µL, 5 µM, co-assembled: FDH: 19 nM, H_2ase: 3.4 nM, scan rate: 5 mV s^{-1}	current density: 0.25 mA cm^{-2}, CE: 81 %, product concentration $^{a)}$: 2.91 mM H_2 & 18 mM formate, formation rate $^{a)}$: 0.12 mM H_2 h^{-1} & 0.67 mM formate h^{-1}, TON: 23,000, TOF: 6.4 s^{-1}	[33]
Flow- and membrane-less reactor, oxidation, 2- and 3-electrode configuration	C (1e$^-$)	Enzyme: GOx, HRP, BOD, mediator: tetrathiafulvalene, O_2, substrate: glucose, product: gluconic acid, side products: D-arabinose, formic acid	Vulcan carbon nanomaterials, *A*: 0.28 cm^2 or 1 cm^2	70 mL 0.1 M Phos. pH 6, 20 mM substrate, flow rates: 2–14 mL min^{-1}, O_2 supply: 500 mL min^{-1}, T: 22 °C, 10 mg mL^{-1} immo. GOx (anode) and 6 mg mL^{-1} and 18 mg mL^{-1} immo. GOx-HRP or 6 mg mL^{-1} and 10 mg mL^{-1} immo. GOx-BOD (cathode), applied cell potential: 0.0 V vs. SCE	Selectivity: 97 %, conversion: 80 %, productivity $^{a)}$: 864 g L^{-1} d^{-1}, product concentration $^{a)}$: 15.8 mM, yield: 75 %, E-factor: 9, atom efficiency: 100 %, CE: 100 % (anode) & 16 % (cathode), current density: 0.6 mA cm^{-2}	[22]
Oxidation, 3-electrode configuration	C (2e$^-$)	Enzyme: GOx, co-substrate: O_2, substrate: glucose, product: gluconic acid, H_2O_2	Polypyrole (film)-GOx-modified Pt, film thickness: 500 nm	2.5 mL 0.1 M Phos. pH 7, 20 mM substrate, 0.565 U GOx, air-saturated, room temperature, applied potential: 0.4 V vs. SCE	Thiele modulus, effectiveness factor, conversion: 62 %, productivity $^{a)}$: 4.8 g L^{-1} d^{-1}, initial conversion rate: 1.6 mM h^{-1}	[44]
Packed-bed reactor, oxidation, 3-electrode configuration	B (1e$^-$)	Enzyme: GDH, co-factor: NADH, mediator: ABTS, methylene blue, substrate: glucose, product: lactone	GC particles, *A*: 24 m^2, m: 7.8 g, splintered: 1000–2000 µm	15 mL 50 mM TRIS/HCl pH 8, 2 U GDH, 10 mM substrate, 0.1 mM NADH, 0.01 mM ABTS, flow rate 2.5 mL min^{-1}, applied potential: 0.7 V vs. Ag/AgCl/KCL (3 M)	TTN of mediator: 1,860, TTN of co-factor: 93, productivity $^{a)}$: 33.6 g L^{-1} d^{-1}, conversion: 93 %, TOF of mediator $^{a)}$: 33.3 s^{-1}, CE: 87 %	[45]
Reduction, 3-electrode configuration	E (1e$^-$)	Enzyme: DSDH, co-factors: NADH, substrate: D-Fructose, product: D-Sorbitol	Multi-layer bioelectrode consisted of Rh-complex and glassy fibre on Bucky-Paper, *A*: 4 cm^2	30 mL 50 mM PBS pH 6.5, 1 mM NADH, 1 mM substrate, 1 mM NADH, immo. DSDH on the electrode, N_2-saturated, applied potential: −0.72 V vs. Ag/AgCl	TTN: 12,000, TTN of-co-factor: 2.61, TOF: 0.19 s^{-1}, TOF of co-factor: 1.3 s^{-1}, CE: 83 %, conversion: 87 %, current density: 0.037 mA cm^{-2}	[46]

Table 2. continued

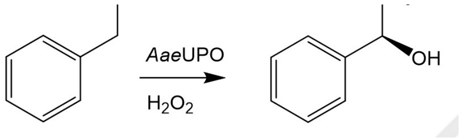
Process types	e^- transfer mechanism	Enzyme, co-factors, substrate and product	Type of working electrode	Process condition	Key performance indicators	Ref.
Hydroxylation, 3- electrode configuration	C ($1e^-$)	Enzyme: cytochrome P450 monooxygenases (P450cin), mediator: CoSep, PSF, SAF, FAD, FMN, substrate: 1,8-cineole, product: 2β-hydroxy-1,8-cineole	Pt, *A*: 2 cm^2	50 mM KP_i pH 7.5, 1 μM CinA, 3 μM CinC, 6 mM substrate, 1500 U mL^{-1} catalase, 5 mM CoSep, 1.2 mM PSF, 1.2 mM SAF, 0.6 mM FAD, 0.6 mM FMN, 0.8 mM potassium ferrocyanide, 0.8 mM ethyl-/methyl viologen, room temperature, applied potential: −0.75 V vs. Ag/AgCl	TTN: 2,600, formation rate: 6.5 nmol (product) - $nmol^{-1}$ (P450) min^{-1} cm^{-2} (electrode), CE: 10%,	[47]

[a] Unless stated otherwise, batch reaction process in a standard one chamber reactor using 2-electrode configuration are described. **e^- transfer mechanism**, each alphabet refers to the electroenzymatic process depicted in Figure 1. **Enzyme**, ADH: alcohol dehydrogenase, BOD: bilirubin oxidase, CPO: chloroperoxidase, DSDH: D-sorbitol dehydrogenase, FalDH: formaldehyde dehydrogenase, FDH: formate dehydrogenase, GDH: glucose dehydrogenase, GOx: glucose oxidase, H_2ase: hydrogenase, HRP: horseradish peroxidase, UPO: unspecific peroxygenase. **Co-factor/ mediator**, ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CoSep: cobalt sepulchrate, FAD: flavin adenine dinucleotide, FMN: flavin mononucleotide, NAD^+: oxidized nicotinamide adenine dinucleotide, NADH: reduced nicotinamide adenine dinucleotide, PSF: phenosafranine, SAF: safranine. **Type of working electrode**, *A*: geometric surface area, CB: carbon black, CF/ GF: carbon/ graphite felt, oCNT: oxidized carbon nanotubes, Cu: copper, *d*: diameter, DDAB: di-dodecylmethylammonium bromide, GC: glassy carbon, GDE: gas diffusion electrode, ITO: indium tin oxide, ppi: pores per linear inch, Pt: platinum, Rh: rhodium, RVC: reticulated vitreous carbon, *V*: volume. **Process condition**, Ag/AgCl: silver/silver chloride, EPSNF: electrospun polystyrene nanofiber, Immo.: immobilized, KBr: potassium bromide, KCl: potassium chloride, KP_i: potassium phosphate, Na_2SO_4: sodium sulfate, NaCl: sodium chloride, NaOAc: sodium acetate, PBS: phosphate-buffered saline, Phos.: Phosphate buffer, SCE: saturated calomel electrode, TRIS/HCl: TRIS-hydrochloride. **Key performance indicator**, A_e/V: ratio of apparent electrode surface area to reaction volume, CE: current efficiency, d: days, ee: enantiomeric excess, ETY: electrode-time yield, K_m: Michaelis-Menten constant, STY: space-time yield, TOF: turnover frequency, TON: turnover number, TTN: total turnover number, V_{max} : maximum reaction rate. [a)] parameter and key performance indicators were recalculated/ converted from the actual reported value and its unit using the available information from the corresponding publication to have the same consistent unit with the other references.

Finally, in the case of electrochemical and electroenzymatic processes, electricity prices can be subject to very strong fluctuations due to political decisions, although they are quantitative values.

5. Case Study: Hydrogen Peroxide-Driven Bio-Catalysis – Using Quantitative and Non-quantitative Parameters To Compare Different Processes

As shown above, both quantitative and non-quantitative parameters must be considered to characterise or to compare processes. The following case study illustrates how these parameters can be applied. Here, we compare different enzymatic processes for the enzyme catalysed synthesis of (*R*)-1-phenylethanol from ethylbenzene with an unspecific peroxygenase (UPO, Scheme 1).



Scheme 1. Hydroxylation of ethylbenzene to (*R*)-1-phenylethanol using an unspecific peroxygenase (UPO)

In general, the chemical oxyfunctionalization of C–H bonds requires elevated temperatures and large amounts of organic solvents. However, there are biocatalysts that allow the conversion of non-activated C–H bonds under mild conditions. A prominent and emerging example is the unspecific peroxygenase from the fungus *Agrocybe aegerita* (*Aae*UPO), which can be exploited for regio- and stereospecific oxyfunctionalisation of various substrates.[49,50] UPOs have emerged as "dream biocatalysts" of great industrial interest because of their tremendous potential to catalyse this oxyfunctionalization. For this reason, and because of the availability of several well-described processes, these enzymes were used in this case study. A major drawback of *Aae*UPO, which prevents its application on a technical scale, is its inactivation at excess concentrations of the co-substrate H_2O_2. Therefore, several approaches to supply hydrogen peroxide at low but sufficient levels have been investigated in combination with the UPO (Figure 2):

- Feeding of H_2O_2: Kluge *et al.* used a combined H_2O_2 and substrate solution feeding.[51,52]
- Immobilised enzymes and feeding of H_2O_2: Hobisch et al. used a covalently immobilized UPO variant in a rotating bed reactor two-liquid-phase system.[53]
- Enzyme cascades: An enzymatic methanol oxidizing cascade was used to provide sufficient hydrogen peroxide concentrations for *Aae*UPO. Here the complete oxidation of 1 equivalent of methanol resulted in the generation of 3 equivalents of hydrogen peroxide.[54]

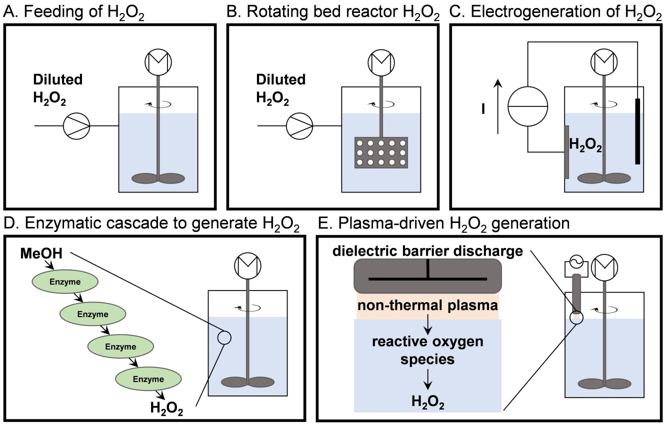


Figure 2. Schematic representation of the different approaches for the hydroxylation of ethylbenzene to (*R*)-1-phenylethanol using the unspecific peroxygenase from the fungus *Agrocybe aegerita* (*Aae*UPO, description in the main text)

- Plasma driven system: The *in-situ* production of H_2O_2 by dielectric barrier discharge plasma was coupled with the peroxygenase reaction.[55]
- Electrochemistry: The electrochemical reduction of molecular oxygen to H_2O_2 using a gas diffusion electrode in combination with the peroxygenase was investigated by Horst et al.[27]

For comparison of these processes, 5 parameters were used as examples. Three of them are of quantitative nature and are listed in Table 1 (final product concentration, space-time yield, total turnover number). For comparison, the highest value is defined to be 100%. All values between 100% and 90% are assigned a value of 5, values between 75 and 90% are assigned a value of 4, values between 50 and 75% are assigned a value of 3, and values between 20 and 50% are assigned a value of 2. Finally, values between 0 and 20% are assigned a value of 1. In addition, two non-quantitative parameters are considered. The "simplicity of the system" means how close a process is to common enzyme technology processes and is therefore related to the estimated investment costs for new technology deployment and staff training. This parameter can also be referred to as the hurdle for implementing an "unusual" technology in an enzyme process. Scalability describes the challenges of transferring laboratory processes to large-scale applications. At this early stage of the process, the variable costs cannot be definitively determined, so they are estimated here (parameter estimated flexible cost to operate). The fewer chemicals required for the process, the better the process is evaluated. Again, the processes were assigned values from 5 (best) to 1 (worst). This assignment was made after extensive discussion among the team of authors; it can be assumed that other teams of experts will come to slightly different conclusions, but most probably the tendency will remain the same (Table 3 and Figure 3).

A number of conclusions can be drawn from this comparison, e.g. (i) there is no perfect procedure yet, (ii) the process in the rotating bed-reactor with immobilized enzymes and feeding of diluted H_2O_2 shows the best overall performance so far and (iii) the electrochemical system needs to be improved with respect to the product concentration and thus the substrate concentration used, as well as the space-time yield.

Although the evaluation of each parameter can be different, as described above, this principle seems to be the only way to compare different processes. Only focusing on single variables such as TTN or productivity or only using quantitative data would lead to an incomplete evaluation. The strength of this evaluation system also lies in its ability to identify weaknesses in a process at an early stage of development. The following two comments are especially important. First, we are comparing published performance indicator, not technologies. In some cases, the technology is still in the early stages of development and significant improvements in performance indicators can be expected. Second, all of the processes described do not yet meet the typical industrial requirements for an enzyme-based process.[23] Here, the following values are given as examples for the production of a prochiral ketone: substrate loading $>160\ \mathrm{g\,L^{-1}}$, reaction time $<10\ \mathrm{h}$, catalyst loading $<1\ \mathrm{g\,L^{-1}}$, isolated yield $>90\%$, and space-time yield/productivity $>16\ \mathrm{g\,L^{-1}\,h^{-1}}$. Processes with comparably high metrics should also be the objective for process development when using UPO as enzyme. Since typical electroenzymatic processes (Table 2) are usually also aimed at the production of bulk chemicals, comparable performance indicators should be realized.

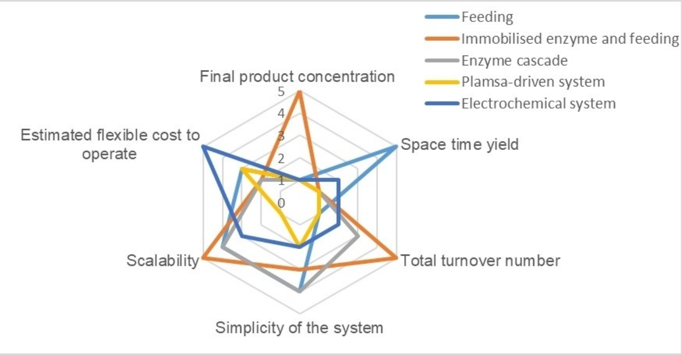


Figure 3. Example of the use of quantitative and non-quantitative parameters to compare different approaches to hydroxylate ethylbenzene to (*R*)-1-phenylethanol using an unspecific peroxygenase.

Table 3. Evaluation of different approaches to provide H_2O_2 for the hydroxylation of ethylbenzene to (*R*)-1-phenylethanol using the unspecific peroxygenase from the fungus *Agrocybe aegerita* (*Aae*UPO).

	Feeding	Immobilized enzyme and feeding	Enzyme cascade	Plasma-driven	Electro-chemical system
Final product concentration	$0.4\ g\,L^{-1}$	$50.6\ g\,L^{-1}$	$3.8\ g\,L^{-1}$	$0.2\ g\,L^{-1}$	$2.2\ g\,L^{-1}$
	0.8 %	100.0 %	7.5 %	0.3 %	4.3 %
	1	5	1	1	1
Productivity	$2.50\ g\,L^{-1}\,h^{-1}$	$0.41\ g\,L^{-1}\,h^{-1}$	$0.03\ g\,L^{-1}\,h^{-1}$	$0.34\ g\,L^{-1}\,h^{-1}$	$0.55\ g\,L^{-1}\,h^{-1}$
	100.0 %	16.4 %	1.3 %	13.5 %	22.0 %
	5	1	1	1	2
Total turnover number	43,000	909,000	468,500	13,787	400,000
	4.7 %	100.0 %	51.5 %	1.5 %	44.0 %
	1	5	3	1	2
Simplicity of the system	4	3	4	2	2
	Installing a pumping system can be regarded as straightforward. Reaction volume increases when diluted solutions are used.	Using a specialized type of reactor and immobilisation plus losses during enzyme immobilisation (not regarded).	For an enzyme technician, the threshold for using an enzyme cascade is quite low. The product isolation can be a challenge.	For the introduction of a completely different technology, there is a high threshold.	For the introduction of a completely different technology, there is a high threshold.
Scalability	4	5	4	1	3
	On a larger scale, there may be problems with heterogeneity.	Scale-up was shown in chemical engineering.	On a larger scale, there may be problems with heterogeneity.	Up-scaling has not been the subject of much research so far.	Up-scaling has been demonstrated in process engineering, not for enzyme processes.
Estimated flexible cost to operate	3	2	2	3	5
	Here, only H_2O_2 has to be added, and the authors have developed a recycling concept for the solvent. However, enzymatic stability is very low.	Enzyme immobilization and high losses during the immobilization process result in high costs.	High costs for the different enzymes and cofactors.	Only small amounts of reagents are required. The energy consumption has not yet been evaluated.	No additional reagents are required and energy efficiency is very high.

6. Summary and Outlook

The integration of enzymatic and electrochemical processes offers a unique opportunity to optimize production processes. Recently, more and more electroenzymatic processes have shown impressive performance indicators at laboratory scale, which has led to scientific interest in their technical implementation. For this transfer of processes from laboratory scale to technical applications, different options for electroenzymatic processes have to be considered on the one hand, but also in contrast to alternative processes. A large number of relevant parameters can be used for this purpose, and it must be decided in each case which parameters are relevant. However, it is important to define benchmarks or key performance indicators for all laboratory investigations - only in this way can promising laboratory results be transferred to applications.

Abbreviations

Symbols and descriptions used in formulas.

A_e	Electrode surface (m^2)
c_i	Concentration with indices *i*: p = product, s = substrate, waste = waste, r = reactants (cofactor, co-substrate or substrate), biocat = biocatalyst ($mol\ L^{-1}$)
D_m	Diffusion coefficient ($m^2\ s^{-1}$)
$\Delta E_{cell,eq}$	Difference of working and counter electrode potentials in a reversible equilibrium (V)
ee	Enantiomeric excess (–)
$E_{eq,WE}$	Equilibrium working electrode potential (V)
E_{WE}	Electrode potential working electrode (V)
ETY	Electrode surface time yield ($kg\,m^{-2}\,s^{-1}$)
F	Faraday constant ($A\,s\,mol^{-1}$)
ΔG	Theoretical Gibbs energy change ($J\,mol^{-1}$)
I	Current (A)
j	Current density ($A\,m^{-2}$)
j_v	Volumetric current density ($A\,m^{-3}$)
k_{cat}	Catalytic rate constant (s^{-1})
k_{film}	Film mass transfer coefficient ($m\ s^{-1}$)
K_m	Michaelis-Menten-constant ($mol\ L^{-1}$)
L	Characteristic length (m)
m_p	Mass of product (kg)

n_i	Mole amount with Indices *i*: p = product, r = reactants (co-factor, co-substrate or substrate), biocat = biocatalyst, R = (R)-enantiomer, S = (S)-enantiomer (mol)
Q	Electric charge (A s)
$\dot{q}_{p,v}$	Volumetric productivity ($kg\,m^{-3}\,s^{-1}$)
Re	Reynolds number (–)
S_r	Selectivity (–)
Sc	Schmidt number (–)
Sh	Sherwood number (–)
t	Time (s)
$t_{0.5}$	Half-life time (s)
TOF	Turnover frequency ($mol_p\ mol_{biocat}{}^{-1}\ s^{-1}$)
TTN/$TTN_{cofactor}$	Total turnover number ($mol_p\ mol_{biocat/cofactor}{}^{-1}$)
U_{cell}	Cell voltage (V)
V	Liquid volume of compartment with working electrode (m^3)
V_{max}	Maximum rate at substrate saturation ($mol\,L^{-1}\,s^{-1}$)
w	Specific electric energy consumption (sEEC) ($J\,kg^{-1}$)
X	Conversion (–)
Y	Reaction yield (–)
$Y_{p/e}$	Product yield per electron[18] (–)
Z	Charge number (–)
σ_p	Space time yield (STY) ($kg\,m^{-3}\,s^{-1}$)
η_{WE}	Overpotential (V)
Φ_G	Energy efficiency (–)
Φ_p^e	Current efficiency (–)
v_i	Stochiometric number with indices *i*: p = product, r = reactants (co-factor, co-substrate or substrate) (–)
τ	Residence time (s)

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: Biocatalysis · Electrobiotechnology · Enzymatic electrosynthesis · Performance indicators · Unspecific peroxidases

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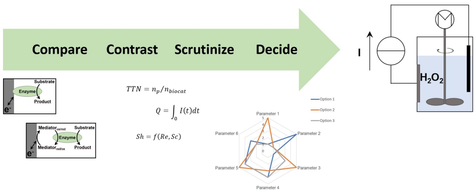
CONCEPT



Compare, contrast, scrutinize and decide: Various process options can be used to bring enzyme processes to industrial application; these include electroenzymatic processes. To evaluate the processes quantitatively, appropriate performance indicators must be determined. In addition, there are non-quantitative variables that need to be considered. This article shows how laboratory processes can be evaluated and how options for action can be identified.

*G. Sayoga, M. Abt, N. Teetz, V. Bueschler, Prof. Dr. A. Liese, Prof. Dr. M. Franzreb, Prof. Dr. D. Holtmann**

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Quantitative and Non-Quantitative Assessments of Enzymatic Electrosynthesis: A Case Study of Parameter Requirements