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GUIDELINES FOR REPORTING OF BIOCATALYTIC REACTIONS

Lucia Gardossi[1], Poul B. Poulsen[2], Antonio Ballesteros[3], Karl Hult[4], Vytas K. Švedas[5], Đurđa Vasić-Rački[6], Giacomo Carrea[7], Anders Magnusson[8], Andreas Schmid[8], Roland Wohlgemuth[9], Peter J. Halling[10]
Members of the scientific committee of the European Federation of Biotechnology Section of Applied Biocatalysis (ESAB)

1 - Dipartimento di Scienze Farmaceutiche, Università di Trieste, Piazzale Europa 1, 34127 Trieste, Italy; 2 - Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark; 3- Department of Biocatalysis, CSIC Institute of Catalysis, Madrid, Spain; 4 - Department of Biochemistry, School of Biotechnology, Royal Institute of Technology (KTH), AlbaNova University Center, SE-106 91 Stockholm, Sweden; 5 - Faculty of Bioengineering and Bioinformatics, Belozersky Institute of Physicochemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia; 6 - Faculty of Chemical Engineering and Technology, University of Zagreb, Savska c.16, 10000 Zagreb, Croatia; 7 - Laboratory of Chemical Biotechnology, Istituto di Chimica del Riconoscimento Molecolare, CNR, via Mario Bianco 9, 20131 Milan, Italy. 8 - Department of Biochemical & Chemical Engineering, TU Dortmund University, Emil Figge Str. 66, D-44227 Dortmund, Germany; 9 - Sigma-Aldrich, Research Specialties, Industriestrasse 25, CH-9470 Buchs, Switzerland; 10 – WestCHEM, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XL, Scotland, UK.

Corresponding Author: Peter J. Halling, e-mail: P.J.Halling@strath.ac.uk; Tel: +44 141 548 2683.

Abstract
Enzymes and whole cells are being increasingly applied in research and industry, but the adoption of biocatalysis relies strongly on useful scientific literature. Unfortunately, too many published papers lack essential information needed to reproduce and understand the results. Here, members of the scientific committee of the European Federation of Biotechnology Section on Applied Biocatalysis (ESAB) provide practical guidelines for reporting experiments. The document embraces the recommendations of the STRENDA initiative (Standards for Reporting Enzymology Data) in the context of pure enzymology and provides further guidelines and explanations on topics of crucial relevance for biocatalysis. In particular, guidelines are given on issues such as the selectivity, specificity, productivity and stability of biocatalysts, as well as on methodological problems related to reactions in multiphase systems. We believe that adoption and use of these guidelines would greatly increase the value and impact of published work in biocatalysis, and hence promote the further growth of applications.

Introduction

Biocatalysts (enzymes and whole cells) are increasingly being exploited for preparative syntheses from laboratory to industrial scale [1-5]. There are a steadily rising number of publications reporting their use. Unfortunately, the value of many of these publications is limited because

essential information about the experiments is not always presented. The scientific committee of the European Federation of Biotechnology Section on Applied Biocatalysis (ESAB) [footnote1] felt that some clear guidelines for the reporting of experiments in biocatalysis might help improve the situation. The initiative was approved at the January 2008 meeting, and a working group established and this document is the result. Because many researchers who are not specialists in the field are now using biocatalysis, we believe it is appropriate to supply detailed notes explaining most of the requirements. For those experienced in biocatalysis, the summary checklists in the tables should be sufficient to prompt checks for accidental omissions. We believe the checklists should also be useful to journal editors and referees, and we would hope they might be incorporated into instructions to authors.

We emphasize that an initiative named STRENDA (Standards for Reporting Enzymology Data) has been pursuing a similar aim for several years, in the context of pure enzymology, and a checklist of items with recommendations can be found at http://www.strenda.org/ [footnote2] Our document incorporates the STRENDA checklist, but adds value by appending additional items and explanations that are specific to applied biocatalysis (see Tables 1 and 2). Therefore, reports of experiments in applied biocatalysis should follow the STRENDA checklist, which also avoids an unnecessary duplication of standards and checklists in overlapping scientific areas. Moreover, a list of some items specific to applied biocatalysis has been included in Table 3. Note that not all items on the checklists will need to be specified for every study in biocatalysis, but they rather act to prompt for information that will be required where necessary for the type of experiments reported or that will be useful to include if available.

It should be noted that this is not the first initiative in the area of guidelines and standardization. Many of the issues examined herein have been discussed in previous literature, which covered also first immobilized biocatalysts in general [6-8] and subsequently immobilized cells [9-11].

This article reflects views that are supported by most of the specialists in the area of biocatalysis. It must be noted that some controversial points still exist even among specialists in the field, notably with regard to the treatment of kinetic model fitting and terms relating to specificity/selectivity. However, with the aim of presenting an authoritative and largely shared viewpoint, we refrain from presenting any controversial recommendations in this document.

The basis of this article is the proposed checklists for biocatalysis in the tables, while the figures provide a schematic summary of some key recommendations. The text below gives more detailed explanations for those less experienced in the field, while the boxes deal in detail with particularly difficult issues, especially sampling procedures and specificity. By pointing out some of the common pitfalls, we also illustrate the need for and value of the checklists.

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footnote 1. www.esabweb.org . The complete list of members of the Scientific Committee of ESAB is: Dietmar Haltrich, Bernd Nidetzki, Erick Vandamme, Wim Soetart, Durda Vasic-Racki, Jiri Damborsky, Vladimir Kren, Sven Pedersen, Lars Pedersen, Poul Poulsen, Niklas Weymann, Philippe Soucaille, Pierre Monsan, Uwe Bornscheuer, Willi Meier, Andreas Schmid, Laszlo Gubicza, Hordur Filipusson, Paul Engel, Sergio Riva, Lucia Gardossi, Gervydas Dienys, Thorleif Anthonsen, Vincent Eijsink, Stanislaw Bielecki, Luis Fonseca, Fernando Garcia, Francisc Peter, Vytas Svedas, Milan Polakovic, Antonio Ballesteros, Vicente Gotor, Karl Hult, Sven Panke, Roland Wohlgemuth, Roger Sheldon, Marcel Wubbolts, Jennifer Littlechild, Peter Halling, Erik Van Hellemond, Hans Tramper, Joaquim Cabral, Juozas Kulys, Fragiskos Kolisis, Patrick Adlercreutz, Josefa Friedrich.

footnote 2. The checklist here reported is Version 1.5, dated October 9th 2008, still current in December 2009 and available at www.strenda.org.

Identity of the biocatalysts

The biocatalysts used must be specified unambiguously, and, where necessary, details of the preparation procedure must be provided (see Table 1 and Figure 1 for overview).

Table 1. Checklist for complete description of a biocatalytic reaction

Topics	Data items from STRENDA checklist	*Our explanations and additions*
Identity of the enzymes	Name of Reaction Catalyst EC-Number Sequence accession number Organism/species & strain Isoenzyme	*Biocatalysts may often be less well characterised than is usual in pure academic enzymology. The only requirement, which is equally important for biocatalysis, is that the catalysts used must be specified unambiguously.*
Additional information on the enzyme	Tissue/organelle Localization Posttranslational modifications	
Preparation	Description Artificial modifications Enzyme or protein purity	*Information about impurities in the biocatalyst* *Additives included deliberately* *Description of immobilized derivative preparation and characterization*
Assay conditions	Measured reaction Assay temperature Assay pressure Assay pH Buffer & concentrations Metal salt(s) & concentrations Other assay components Coupled assay components Substrates & concentration ranges Enzyme/protein concentration Variable components Total assay mixture ionic strength	*pH electrode calibration basis* *If reaction mixture is, or might be, multiphase: agitation conditions.* *If reaction mixture is non-aqueous (e.g. based on organic solvents, ionic liquids, gasses, supercritical fluids), residual water content or water activity must be specified*
Activity	Initial rates of the reaction measured Proportionality between initial velocity and enzyme concentration Specific activity	*A statement of conversion or product concentration at a given time is acceptable only when describing a biocatalytic synthesis – but not for kinetic studies.* *Preferably biocatalyst specific activity – if not, either: rate of concentration change and biocatalyst concentration or rate of amount change and biocatalyst amount* *Basis for biocatalyst amount clear – total mass*

		protein mass, or active enzyme mass. *Reference rate specified if others shown relative* *Rates for new catalyst types compared with best-known alternative*
Methodology	Assay method Type of assay Reaction of stopping procedure Direction of the assay Reactant determined Reaction stoichiometry	*Description of sampling procedure to deal with multiple-phase reaction mixture* *Report observations on phase separation*
Additional information desirable	Total assay mixture ionic strength Free metal-cation concentrations Reaction equilibrium constant *K*	

The first two columns of this table are taken directly from the STRENDA Checklist, Version 1.5, dated 9th October 2008, and were still valid in December 2009. Therein, they appear as "Level 1, List A. Data required for a complete description of an experiment." The last column is new and includes explanations and additions from the viewpoint of applied biocatalysis.

Enzymes:

An enzyme name and source is usually insufficient, as several different preparations with different contents are possible. For example, there are many different preparations of "lipase from *Candida rugosa*", which differ greatly in composition, activity and specificity.

A product code from a manufacturer can be a good option, but it should be taken into account that not all products might be available in the medium to long term and only if they are characterised, will the recipe ever be repeatable (useful information on industrial enzyme applications and technical details for some commercial enzymes can be found at the websites of three of the major enzyme producers: http://www.novozymes.com, http://www.genencor.com and http://www.amano-enzyme.co.jp/english/index.html). When working with commercial enzyme products, one should list: manufacturer with name and address; product number and complete product name; lot number. It must be kept in mind that many commercial enzyme products are crude formulations, which might contain several enzymes, thus causing side-reactions. For non-commercial sources, reference to a fully described preparation procedure is a necessity.

Enzyme names should also include the source organism (animal, plant or microbe). In the case of enzymes now produced after gene transfer to an expression host, this should also be specified, and the name clearly distinguished from that of the original gene source (both organisms should be named if possible).

To enable cross-referencing to further information about the enzyme, it is helpful to include the IUBMB EC number (see http://www.brenda-enzymes.info: a comprehensive enzyme information system with structural and functional data).

Reference to a sequence database accession number is useful, but only where there are reasonable grounds for believing that the sequence of the enzyme studied is identical [12].

When relevant to the study, reference to information about glycosylation and other post-translational modifications of the enzyme used is advisable.

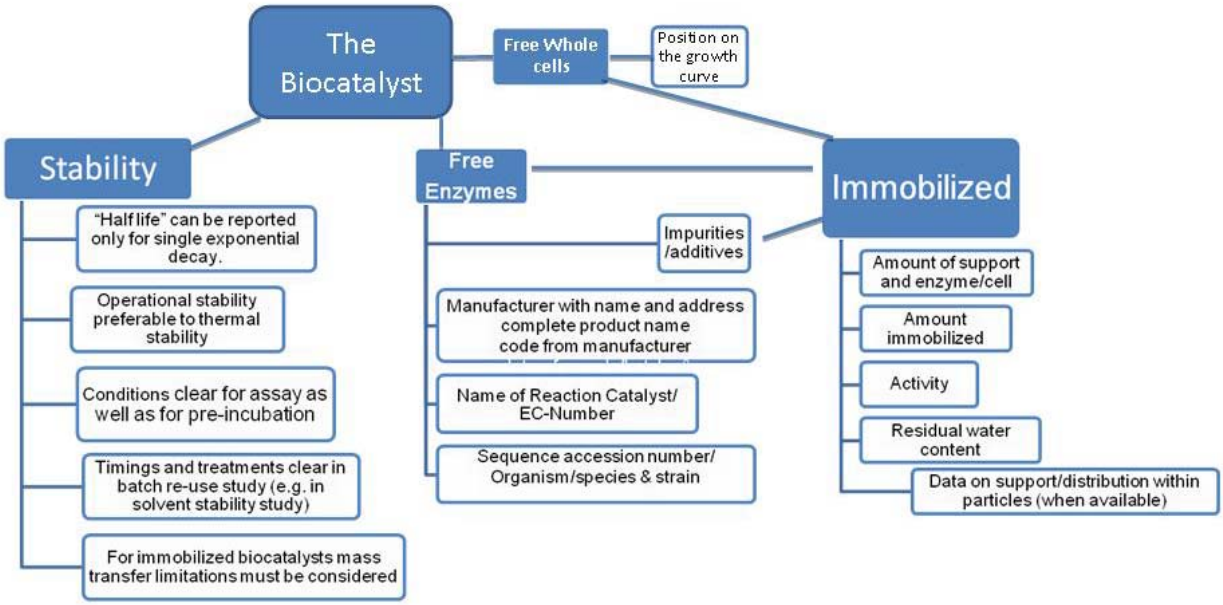

Figure 1. Schematic summary of key information required to characterise a biocatalyst

Whole cells:

The performance of a cell as a biocatalyst can be drastically affected by its position on the growth curve, and this should be described as clearly as possible. This requires many variables to be specified, including: medium, inoculum culture details, aeration/agitation conditions, temperature, control applied during growth (e.g. pH), monitoring used a basis to choose harvest time. It might be necessary to monitor and report cellular levels of enzymes involved in the biotransformation, or in cofactor recycling. In general, specialist microbiological expertise is highly desirable for work with whole cell catalysts.

Immobilized biocatalysts:

The protocol of immobilization must be made clear, together with the results of the characterizations. Information to be specified includes: amounts of support and enzyme or cells contacted for immobilization; estimate of amount immobilised, usually calculated as the difference between amounts added and that remaining un-immobilised; assayed activity of immobilised preparation; distribution of enzyme molecules or cells within the immobilised particles (uniform or not) [13]. For detailed guidelines when using immobilised biocatalysts, previously published recommendations should be followed (see [6-8] for immobilised enzymes and [9–11] for immobilized cells).

Additives and impurities

References to information about impurities in the preparation used are always necessary, as there are examples where useful biocatalytic conversions have been shown to be attributable to enzymes present as impurities, rather than the one for which the preparation is named.

Any additives included deliberately as part of the biocatalyst preparation should be specified fully. In many cases, they may be responsible for observed effects, rather than differences in the enzyme or cell itself. Controls without the additive and without the biocatalyst are thus advisable.

Reporting conditions of assays and reactions

Needless to say, the concentrations of all ingredients present should be specified unambiguously, as biocatalyst behaviour may be affected by any other species present.

Buffer

A common omission is failure to specify the counter-ions present in buffers – e.g. 'Tris buffer' or 'phosphate buffer'. Note that the standard definition regarding the concentration of a buffer refers to the sum of the concentrations of the buffer species in both (or all) ionisation states. Buffers can be correctly described as, for example, '50 mM Tris-Cl pH 8.5' or '20 mM K-phosphate pH 6.5'. If there are both cationic and anionic buffer species present (e.g. a Tris phosphate buffer), it is necessary to specify to which component the stated concentration applies. One reason why complete specification is important is to allow calculation of the ionic strength of the buffer – optionally a value of this can be stated explicitly.

pH

Clearly, the pH should normally be specified. When the medium is not a dilute aqueous solution, to get a true pH, the electrode needs to be calibrated with pH standards in the same medium (if suitable standards are known). More commonly, the electrode will have been calibrated as normal using dilute aqueous standards, in which case this should be stated clearly and the readings referred to as 'apparent pH'. Sometimes pH will be set or read before the medium is in the final form used for reaction (e.g. before heating or adding a co-solvent). Again this must be specified.

Temperature

Similarly, the temperature of course should always be stated. An approximate range is preferable to 'room temperature' – biocatalyst behaviour can be very different at 15 and 30^{o}C. Pressure may be assumed to be atmospheric unless specified otherwise.

Amount of biocatalyst

The biocatalyst concentration in the reaction mixture should be specified. When mass concentrations are given, it must be clear whether these refer to mass of a solid preparation, measured protein content, or estimated content of the active enzyme. An estimate of purity is always useful if available. In whole-cell catalysis, the activity is usually given per gram of cell dry weight (g_{CDW}).

Information on catalytic activity measured by a separate assay is helpful, but the assay needs to be fully described or referenced (following STRENDA guidelines), and results presented in such a way that the amount of activity used in the biocatalytic transformation can be determined (e.g. give assay activity per unit mass, and state mass of preparation used for the biocatalysis application).

Multiphase systems

Many reaction mixtures used for biocatalysis have more than one phase present (e.g. suspended solid biocatalyst, immiscible liquid phases, undissolved substrates or products) [14-19]. In such systems, it is generally necessary to agitate continually during the reaction, and the method used can

affect the observed reaction progress (because agitation can affect mass transfer rates that are limiting). Hence it is desirable to describe the agitation conditions as clearly as possible, although it might not be easy to characterise them clearly in a small-scale reaction vessel. A large number of variables can affect the intensity of agitation in the reaction mixture, and Table 4 details what should be reported. [14].

Table 4. Information needed to characterise agitation conditions.

Vessel shape and dimensions

Fractional filling with liquid

Revolution or reciprocation rate

Stirring?

- Magnetic or overhead?
- Dimensions of stirrer bar/paddle/impeller

Shaking?

- Reciprocal horizontal, reciprocal vertical, rotary, inversion or other?
- Distance of throw

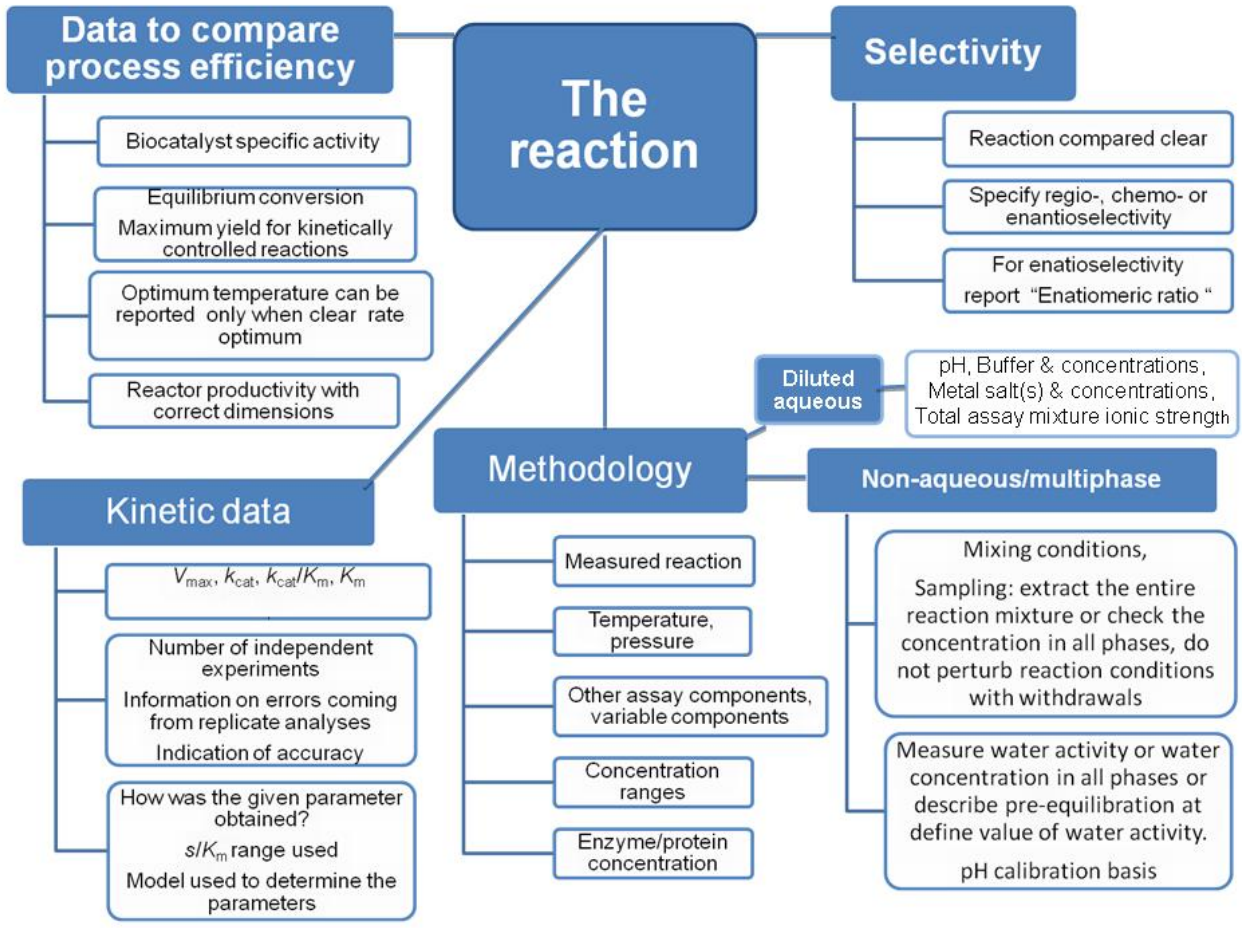

Figure 2. Schematic summary of key data for the characterisation of a biocatalytic reaction

Low-water media

In media of low water content (such as those based on organic liquids or ionic liquids) the exact residual water level usually has an important effect, and sufficient information must be given to allow reproduction of the experiments. It is not sufficient to state the amount of added water, unless this is clearly much greater than that present associated with other ingredients. Possible options are to: (i) describe procedures used to dry or bring to a defined water content all other ingredients; (ii) state measured water content of a defined phase in a multi-phase reaction mixture; (iii) state water activity to which defined parts of the reaction mixture were pre-equilibrated beforehand [20].

If pre-equilibration with drying and/or known water activity agents is used, it is necessary to state the time allowed for equilibration, together with some evidence that this was sufficient (e.g. measured water contents, literature references). Some materials with high water capacity (e.g. polar solvents or very hygroscopic polymers) can take many days to reach an equilibrated value. For further details of methods to control or monitor water levels, see [20].

Further recommendations

Rates of reaction per unit amount of biocatalyst can be lower at low concentration because of inactivation at interfaces, particularly where the reaction mixture has more than one phase [21].

Some enzymes are very sensitive to redox conditions in the reaction mixture. Any measures used to control these should be fully specified (e.g. exclusion of oxygen by means of a specified procedure, addition of reducing agents, redox buffers). The performance in whole-cell biocatalysis might be strongly affected by the oxygen level, which should be reported if measured together with the methods of aeration and agitation.

Monitoring reactions

Reaction progress

If proper kinetic understanding is desired, it is always necessary to make measurements at several time-points so that the reaction progress becomes clearer. Where the aim is merely to describe a useful synthesis, a statement of conversion, yield or product concentration at a single time-point might be useful (reaction time must always be stated). But such measurements should only be used with great caution where the aim is to describe the kinetic behaviour of the biocatalyst. It is not meaningful to estimate ‘activity’ from such a single-time point – this is only valid if it is known that progress is essentially linear over the time-period studied. This requires at the very least some time-course studies to demonstrate that the linear assumption is likely to be correct. The progress of a biocatalytic reaction will not continue linearly indefinitely, but will tend to slow down because of one or more of the following three reasons: (i) decreasing substrate concentrations; (ii) increasing product concentrations; (iii) inactivation of the biocatalyst. Hence, a fixed time-point measurement cannot be simply interpreted. Finally, many biocatalytic reactions approach chemical equilibrium at conversions less than 100% (the ultimate effect of factors (i) and (ii)), and so a conversion measurement can be largely influenced by this rather than through any effect of the biocatalyst at all.

For methodological recommendations for monitoring biocatalyzed reactions, see Box 1; Figure 2 provides a summary.

Box 1: Methodological recommendations for the monitoring of biocatalyzed reactions

Stopping the reaction

Usually the reaction is terminated before analysis of a sample or of the entire reaction mixture. If so, the procedure used to terminate the reaction should be described clearly. It can often be much harder to stop an enzymatic reaction completely than might be believed. Normally, continuation of a reaction should be tested for by varying times between reaction 'termination' and analysis.

Sampling multiphase systems

Where a reaction mixture consists of more than one phase, there are complications in obtaining meaningful samples for monitoring reaction progress. A safe (but more laborious) option is to terminate and extract the entire reaction mixture, using separate reaction vessels to explore different time-points.

It might be possible to withdraw representative samples of a multi-phase reaction mixture, usually while it is under vigorous agitation. However, this always requires careful checks that the samples really are representative, with determination of the relative volumes or masses of different phases by a suitable approach. If a sample of one liquid phase can be removed uncontaminated by others (e.g. after briefly stopping agitation), analysis should give reproducible and meaningful concentrations. However, samples removed according to this procedure will not give complete information about the reaction progress, particularly for compounds mainly distributed into other phases. Furthermore, the total volume removed for analysis must account only for a small fraction of the total volume of this liquid phase, or the behaviour of the reaction mixture as a whole will be perturbed by sample removal. Whichever approach to analysis is adopted, the analytical approaches adopted with multi-phase reaction mixtures require careful description [14]. When separation into multiple phases is likely or observed, it is also useful to report observations of the appearance of the reaction mixture – and in particular ones that suggest separation is occuring, or its absence when it might have been expected.

Reaction rate

Where reaction rates or conversions are reported, it should always be made clear which concentrations were actually measured – starting material disappearance and/or product appearance – and which ones in the case of multiple substrates or products. If more than one concentration is measured, the formula used to estimate a single conversion value should be given.

Where rates of reaction are presented, this should always be done in such a way that the intrinsic activity of the biocatalyst can be identified, independent of the arbitrary choice of scale for the experiments. Ideally, a specific activity should be given, with units of moles of reaction per unit time per unit amount of biocatalyst [22-24].

Rates are often presented as percentages of a selected reference value. This can be useful to show the effects of conditions, but the absolute reference rate should always be specified in line with the guidelines above.

The units for absolute rates should always be carefully checked, including at the proofing stage during publication. There are examples in the literature where values are almost certainly wrong by 1000-fold (e.g. implying that the reaction would be complete before the first sample is analysed). Interchange of 'mmol' and 'μmol' is a common problem, and it can happen accidentally through a font change on a word processor.

Graphical representation of reaction progress

It is normally good practice to include one figure showing actual progress curves, even when most subsequent analysis is in terms of estimated rates.

Equilibrium of reactions

In some cases, it can be considered that the reaction does reach the equilibrium position. If so, it is informative to report this as 'equilibrium conversion' or similar – but only after having specified the tests used to verify that this really is the equilibrium position. Running reactions to approach equilibrium from both directions is the strongest test. Terms such as 'maximum conversion' should not normally be used as this has no clear meaning, and it might depend on the arbitrary choice of when to terminate the reaction.

There is a category of biocatalytic reactions where the concentration of the desired product does pass through a maximum and then decline. An example for this is the industrial synthesis of ampicillin from D-phenylglycine methyl ester and 6-aminopenicillanic acid. In such 'kinetically controlled' reactions, the initial and wanted product is destroyed in a subsequent biocatalytic reaction. In such cases, 'maximum conversion' does have a defined meaning [25,26].

Kinetic parameters

Table 2 summarises recommendations on this topic. The effects of substrate concentration on reaction rate are commonly studied. The data are still often analysed by the Lineweaver–Burk plot. It is generally recognised that this is error prone and must now be considered superseded and obsolete. Views differ on the best alternative, with supporters of: computer-based nonlinear regression of untransformed data direct to the kinetic equation (e.g. Michaelis–Menten) [27,28]; but also linear graphical methods, such as Eadie–Hofstee and Hanes–Wolff [22]. In any case, it is always recommended to examine a plot of the fitted line and the experimental points, to examine for systematic deviations. It is also useful here to plot residuals (differences between the fitted line and the points). An authoritative source on the analysis of biocatalytic kinetic data is [22].

The sequential application of double reciprocal plots to data for multiple-substrate enzymes is particularly dubious – normally the entire data set with varied concentrations of all substrates should be fitted as a whole to an appropriate kinetic equation (see [22]).

Sometimes, authors wish to argue that kinetic data support a particular model. In anything more complicated than the simple Michaelis–Menten one-substrate case, there will usually be several possible model equations that might agree with the data quite well. For example, it is only in certain ranges of substrate concentration that the rates expected for 'Ping-Pong' and general two-substrate models are significantly different. Thus, if authors want to argue that a particular model applies, they should have tested the fit of likely alternatives, and clearly present the arguments for their final choice. It must be noted that the fit will necessarily improve somewhat by adding a new term to the model equation with an additional adjustable parameter, but only a significant improvement in fit justifies the new term. Statistical literature should be consulted about the acceptable grounds for concluding that the extra term should be included to give a more complicated model [28,29].

Because it is often desired to use high substrate concentrations in biocatalysis, substrate inhibition can become an issue. If this is characterised by a K_i value, the kinetic model fitted must be made explicit. Inhibition at high substrate concentrations can sometimes be rather complicated, and it might be better just to show dependence of rate on substrate concentration. For an example, see reference [26,30].

Table 2. Checklist for reporting results from biocatalytic experiments.

Topics	Information required: items from STRENDA list	*Our explanations and additions*
Required data for all functional enzyme descriptions	Number of independent experiments Indication of accuracy Specification whether relative to subunit or oligomeric form	*Any study of biocatalyst performance must distinguish: information on errors coming from replicate analyses, replicate reactions, or statistical analysis of data sets. Never quote just maximum yield or conversion*
Data necessary for reporting kinetic parameters	V_{max} k_{cat} k_{cat}/K_m K_m $S_{0.5}$ Hill coefficient, saturation ratio (R_s) or other coefficients of co-operativity How was the given parameter obtained? s/K_m range used Model used to determine the parameters High-substrate inhibition, if observed, with K_i value	*Clear what model is basis for K_i values*
Data required for reporting inhibition data	Time-dependency and reversibility	
For reversible inhibitors	Type and K_i values	
For tight-binding inhibitors	Association/dissociation rates, inhibition type and K_i values	
For irreversible inhibitors: type	Appropriate kinetic parameters	
Data required for reporting activation data	Appropriate kinetic parameters	
Additional material desirable	Kinetic mechanism Data for cooperative behaviour: model used Time-dependency of enzyme reactions Example of at least one experiment	

	together with raw data

As for table 1, the first two columns were taken from the STRENDA Checklist, Version 1.5, dated 9th October 2008, and also valid in December 2009. Therein, they appear as "Level 1, List B. Description of Enzyme Activity Data". We wish to endorse the available STRENDA checklist, but also added some additional explanations from the viewpoint of applied biocatalysis.

Table 3. Further topics important for biocatalysis but not dealt with in the STRENDA checklist

Further topics	***Information required from the viewpoint of applied biocatalysis***
Specificity and selectivity	*Reactions compared must be indicated clearly* *For resolutions, report E values, not just ee%*
Turnover number	*' Turnover number' not used for* k_{cat}
Reactor productivity	*Reactor productivity must be reported with correct dimensions.* *Reactor dimensions should be described, as well as flow rates through the reactor, if it is a plug flow reactor.*
Optimum temperature	*Optimum temperature can be reported only when clear rate optimum, but application temperature should be always stated*
Biocatalyst stability	*Conditions clearly described for assay as well as for pre-incubation* *Timings and treatments clearly described in batch re-use study* *'Half life' can be reported only for single exponential decay*

Required data for studies of biocatalyst performance and functional properties

Reproducibility

Information about reproducibility should always be given for any biocatalytic studies. There should be statements of the number of replicate analyses of a single sample or reaction mixture as well as replicate reactions under nominally identical conditions.

The results should then be presented with a clearly specified measure of reproducibility. The following are all acceptable: list individual replicate measurements; standard deviation; range; standard error of the mean; confidence intervals. Where a series of measurements is made under similar (but not identical) conditions (e.g. rates with variation of conditions), the errors might appear to be similar (often as a percentage) for the entire set. In such cases, it is acceptable to present an error estimate that applies to the whole series.

As well as the two types of replicate noted above, other possible estimates of errors can come from treatment of a series of measurements: (i) an estimate of error in a single rate value determined from

a series of time-points, based on errors in chemical analysis and fit of points to a line; (ii) an estimate of rate error based on replicate reactions; (iii) an estimate of error in fitted kinetic parameters determined from a statistical method (which should be specified).

It is important to indicate clearly which of these methods is the origin of information offered on reproducibility. In most cases, it is useful to give error estimates based on more than one of these approaches [31].

Units for reporting biocatalyst activity

For ease of comparison of reported activities, it is preferable to use the SI recommended katal (mol s^{-1}) or its submultiples, or the units previously preferred in enzymology – μmol min^{-1}. Hence specific activity should be given in units such as kat kg^{-1} or μmol $min^{-1}mg^{-1}$. It is acceptable to specify a rate of concentration change together with a biocatalyst concentration, or a rate in terms of moles per unit time together with a biocatalyst amount. But other combinations (such as a rate of concentration change combined with a biocatalyst amount) are meaningless because such a rate value will increase as the volume chosen for the experiment is reduced. Similarly meaningless are activities quoted with units such as mM $min^{-1}mg^{-1}$, presumably obtained by dividing a rate of concentration change by an amount of biocatalyst.

One should also be careful to specify the basis on which a mass of biocatalyst is presented. This could be the mass of the entire preparation (for example including an immobilization support), the mass of protein present within it, or even the mass of an active enzyme within it. For some biocatalysts, a wet mass can be given, but a dry mass is always more precisely defined. Determining dry mass of a wet biocatalyst is, however, usually a destructive measurement.

Turnover number

The term ‘turnover number’ should be used with care. This was formerly in widespread use in enzymology for refering to the k_{cat} of the enzyme. In catalysis, it refers to the number of moles of product formed per mole of catalyst over the reaction period. This dimensionless ratio can correctly be referred to as a number, unlike k_{cat}, which has units of $time^{-1}$. We recommend that if ‘turnover number’ is used, this is only with the mole ratio meaning, and this is clarified by giving it explicit units of (mol product) $(mol\ enzyme)^{-1}$. The term ‘total turnover number’ may also be used where the biocatalyst is recovered and re-used in a series of batches. Turnover numbers might also be reported for recycled cofactors, with units now (mol product) $(mol\ cofactor)^{-1}$ [32].

Comparison with different biocatalysts/preparations

When presenting new methods for biocatalyst preparation or use, it is common to make comparison with some alternative – naturally showing that the new method is superior. However, authors often choose to compare with an alternative, which is a particularly poor option, making it easy to demonstrate perceived improvement. Sometimes only relative rates will be presented, so the reader cannot even make other comparisons independently. In fairness, comparisons should whenever possible include the best options known from the literature for comparable applications.

Optimum temperature

Studies of temperature effects are often be summarised by reference to an ‘optimum temperature’. With many biocatalysts, however, this is not a clearly defined value, unlike for example optimum pH. At higher temperatures, the biocatalyst can be progressively inactivated during the reaction period studied, so that the progress curve becomes highly non-linear. If then the conversion reached at a fixed time is studied as a function of temperature, an optimum will be found. But this value will get higher as the arbitrarily chosen reaction time is lowered. If the rate of reaction does show a true optimum, it should be made clear that reaction progress was shown to remain more or less linear even above the optimum. Therefore the term ‘optimal temperature’ is only meaningful if the reaction time is described.

Reporting biocatalyzed synthesis

Where specific or new biocatalytic syntheses are described, it is not acceptable to quote just the maximum yield or conversion from a series of replicate reactions. Some biocatalytic syntheses are rather poorly reproducible because of important, but as yet unknown and uncontrolled, variables. It is only fair that others are made aware of this – and perhaps prompted to identify some of the unknown variables. It is, however, acceptable to quote the maximum along with other information (e.g. a range), as an indication of potential.

Specificity and selectivity

An important advantage of biocatalysis is the possibility of highly specific or selective reactions. Hence the accuracy of information on this aspect as well as on the quantitative evaluation of enantioselectivity is crucial [33-36]. See Box 2 for details.

Box 2: Topics of particular importance for biocatalysis

Specificity and selectivity

The biocatalyst may be making a 'distinction' between two or more possible substrates, or between two or more possible reactions on a single substrate. In any statement about specificity or selectivity, it should be made absolutely clear what comparison is being made. The choice of the word 'selectivity' or 'specificity' does not give clear information here – while some authors assign distinct meanings to them, this is not universally understood, and the definitions used are not exactly the same. By contrast, the prefixes 'chemo-', 'regio-' and 'enantio-' do have clearly defined meanings, and their use might enable the comparison to be made clear.

Quantitative evaluation of enantioselectivity

For enantio-selectivity/specificity, the basic measurements are enantiomeric excesses (ee's) of substrates and/or products. However, in the case of kinetic resolutions and related reactions, ee's will vary through the progress of the reaction. To characterise the biocatalyst, the ratio of specificity constants (E values) should be calculated using the appropriate equation [33,34]. Only E values can sensibly be compared in order to evaluate the performance of the biocatalyst under different conditions. For asymmetric syntheses generating a new chiral centre, product ee values will normally be constant as the reaction proceeds, and do directly characterise the biocatalyst [36].

It is also important to be careful with references to magnitude in relation to specificity/selectivity, including words such as 'high' and 'low'. The specificity constant (k_{cat}/K_m) is a defined value for a given biocatalyst, reaction and conditions, and can be discussed in quantitative terms [23]. Specificity/selectivity can also refer to a ratio of specificity constants, and can be treated quantitatively, provided it is clear which reactions are being compared. But, as a more general description of a biocatalyst, it is clearer to use terms such as 'broad', 'wide' or 'narrow' to reflect a more qualitative summary – indeed, some prefer a term such as 'wide/narrow substrate spectrum' rather than specificity, to avoid confusion with the specificity constant.

Reactor productivity

Reactor productivity is important if the operation of a process at a larger scale is being considered. This is presented as 'Space–Time Yield' or 'Volumetric productivity', defined as the amount of product generated divided by reactor volume and by time [1].

Biocatalyst stability

Methodological recommendations

Studies of stability can sometimes give very misleading results if the regular assays of activity are based on single time-points (see previous paragraphs). For example, if the reaction is already close to equilibrium at the selected time, considerable loss in activity may occur with only limited fall in measured conversion. Another problem is stability studies on immobilised biocatalysts where activity is measured under conditions of strong mass transfer limitation. Again, the intrinsic activity of the catalyst can fall substantially with little change in measured activity – because this is still largely limited by the same mass transfer rate [37].

Operational stability

More relevant to biocatalysis practice are studies of operational stability (i.e. under the conditions of the useful biocatalytic synthesis). This can be studied in a continuous reactor, or by recycling the catalyst in repeated batches [1]. In the latter case, the treatment (e.g. rinsing) of the biocatalyst after recovery can play a crucial role, so these procedures should be specified. Presentation of results must clearly distinguish total time spent under reaction conditions from elapsed time, particularly when the cycle includes an extended storage time between successive batch reactions.

Thermal stability

Biocatalyst stability is commonly studied by exposing the catalyst to certain conditions for a time, then assaying the remaining activity. In such studies, it is essential to make clear the conditions both of the pre-treatment and the subsequent assay. For example, if inactivation at high temperature is studied, it should be specified whether the activity assays were carried out at the preincubation temperature, or after cooling to some standard assay temperature (the latter being more common).

Solvent stability

If inactivation by co-solvents is studied, it must be specified whether samples were diluted into essentially aqueous media before assay, or was the co-solvent still present during assay at a significant concentration, or even that in the pre-incubation medium.

Half-life

The results of stability studies are sometimes summarised by statement of a half-life. This only has a clear and simple meaning in the case of first-order kinetics, which are rarely followed precisely for biocatalyst inactivation (certainly if studies are continued to 10% residual activity, which is necessary to allow clear distinction of different kinetic models). Full plots of activity against time or batch number are preferred. If it is necessary to summarise them, this should be by means of parameters in a defined equation that fits the experimental data acceptably well (e.g. a sum of two or more exponentials).

Concluding remarks

As a discipline, biocatalysis touches upon different fields, ranging from microbiology to chemical engineering, also including chemistry, biochemistry and enzymology. Gaining a comprehensive and detailed knowledge of all aspects of biocatalyst behaviour is a very difficult proposition. From this comes the necessity to provide practical and schematic guidelines for reporting experimental data effectively while, most importantly, enabling other scientists to reproduce the experiments. In addition, standardized reporting creates value for many other scientific disciplines, especially where biocatalysis must be integrated in multi-step syntheses [38].

ESAB intends to promote these guidelines actively among its own members, and we hope they will be widely distributed (and followed!) within the worldwide community of those working on biocatalysis. We will also ask editors of journals and books in the field to incorporate them into instructions to authors.

No doubt these guidelines will be improved after further discussion within the community, and eventually a version might be adopted in an appropriate international forum. One attractive possibility is that the guidelines will form the basis for an electronic data capture and validation tool that automatically ensures any essential and required information is entered.

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