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Abstract

The application of conventional organic solvents is essential in several steps of bioprocesses in order to achieve sufficient economic efficiency. The use of organic solvents is frequently used either to partly or fully replace water in the reaction medium or as a process aid for downstream separation.
Nowadays, manufacturers are increasingly requested to avoid and substitute solvents with hazardous potential. Therefore, the solvent selection must account for potential environmental hazards, health and safety problems, in addition to fulfilling the ideal characteristics for application in a process.
For the first time, criteria including Environment, Health and Safety (EHS), as well as the technical requirements for reaction and separation have been reviewed, collected and integrated in a single organic solvent screening strategy to be used as a guideline for narrowing down the list of solvents to test experimentally. Additionally, we have also included a solvent selection guide based on the methodology developed in the Innovative Medicines Initiative CHEM21 (IMI CHEM21) project and applied specifically to water-immiscible solvents commonly used in bioprocesses.

Keywords: Organic solvents screening, Bioprocesses, Biphasic systems, Downstream processing, *In situ* product removal

1. Introduction

There is currently significant interest in the application of biotechnology to chemical manufacture, driven in part by the need to replace (or at least minimize) existing fossil feedstocks by renewable and sustainable ones. Likewise the chemical industry, and perhaps even more importantly the pharmaceutical industry, needs to use ever cleaner processes, with reduced reagent use and waste generation. For example, while the *E* factor is a measure of the amount of waste produced in a

process (*E* factor = kg waste / kg product) (Sheldon, 2017), it is perhaps more useful to examine the composition of the waste from a given process. This quickly motivates the need to reduce or replace the use of organic solvents, applied primarily for product recovery and purification. For this reason several pharmaceutical companies, academic groups and organisations like the ACS Green Chemistry Institute (GCI) Pharmaceutical Roundtable have successfully driven an agenda of solvent reduction and replacement (Constable *et al.*, 2007; Jessop *et al.*, 2015; Tucker and Faul, 2016). To a large extent this has been focused on chemical synthetic strategies. However, while this serves as a very valuable guidance for today, the range of industrial processes is changing. For example, already today several hundred small-molecule pharmaceutical production processes use one or more bioprocess steps (Buchholz *et al.*, 2012; Meyer *et al.*, 2013; Woodley, 2017). Indeed as industrial interest in cleaner synthesis grows it becomes clear that in the future many more bioprocesses will be implemented in industry (Cue and Zhang, 2009; Sheldon and Woodley, 2017). Even if fermentation and biocatalysis were to replace a significant fraction of the synthetic reactions in the fine chemical and pharmaceutical industry, it remains the case that the products still need to be recovered and purified. The downstream separation can include many potential unit operations which are dependent upon the product (as well as by-product and substrate) properties. Nevertheless, for most biocatalytic reactions and fermentations the product is often toxic (leading to an irreversible loss of activity) or inhibitory (leading to a reversible loss of activity) to the biocatalyst/microorganism at concentrations much lower than are the minimum required to feed a conventional downstream process. This has been the major motivation behind the implementation of *in situ* product removal (ISPR), where inhibitory or toxic products are removed during the reaction (either at the site of the reaction, or else in a recycle loop) (Van Hecke *et al.*, 2014; Woodley *et al.*, 2008; Zou, 2014). Various methods have been proposed including the use of adsorption, pervaporation, perstraction, and crystallization. Extensive reviews have been written on this topic and a number of industrial processes use the technology (Carstensen *et al.*, 2012; Dafoe and Daugulis, 2014; Freeman *et al.*, 1993; Lye and Woodley, 1999; Stark and von Stockar, 2003; Van Hecke *et al.*, 2014; Woodley *et al.*, 2008). Of particular interest is that polymers have been used in many ISPR solutions (Phillips *et al.*, 2013) and can potentially be an effective, safer and cheaper alternative to the use of organic solvents (Dafoe and Daugulis, 2014). Regardless of the type of phase used to recover product it is clear that systematic selection methods are required. On this premise we recognized that one of the most used separation methods (aqueous-organic liquid-liquid extraction) could in particular benefit from a more systematic screening procedure for the organic solvent. In this review, for the first time, the criteria to screen for solvents for a bioprocess are integrated in a single report, accounting for both the technical, as well as EHS requirements which as we have indicated earlier are a prerequisite for industrial implementation. The collection of these criteria forms the basis of a screening procedure in particular focused on biphasic systems in bioprocesses in order to narrow down the number of solvents to be tested experimentally. In this paper in contrast to previous publications (Elgue *et al.*, 2006; Gani, 2006; Zhou *et al.*, 2014), we deliberately restrict ourselves to bioprocesses using enzymes or microorganisms, to manufacture chemical products. We consider this screening procedure essential for the scientific community involved in the early stage development and research of new bioprocesses. Interestingly, this rationale is supported by journals such as ChemSusChem (Kemeling, 2012) which has specifically asked authors to justify their choice of solvents in submitted manuscripts and if possible to consider replacing harmful ones.

2. Use of organic solvents in bioprocesses

Whilst the use of water-miscible organic solvents (e.g. ethanol, dimethyl sulfoxide) to help solubilize poorly-water soluble organic compounds in single phase biocatalytic systems has been

widely reported in the scientific literature, such systems may give only a 10-20% increase in substrate and/or product concentration (Sheldon and Pereira, 2017). Additionally, with only a few exceptions, such polar solvents strip essential water from the biocatalyst resulting in a loss of enzyme stability (Gorman and Dordick, 1992; Kamal *et al.*, 2013; Taher and Al-Zuhair, 2017; Yang *et al.*, 2004). On the other hand, essentially water-immiscible organic solvents (containing only small amounts of water, at concentrations less than saturation) like n-hexane, t-butyl methyl ether etc. can be used for lipase reactions run in a synthetic direction (to avoid hydrolysis)(Bose and Keharia, 2013; Carvalho *et al.*, 2015; Devi *et al.*, 2017). In this paper we will focus on the third case, where water-immiscible solvents are used in a distinct phase from the aqueous phase, to form a two-liquid phase system.
Here the organic solvents are used for substrate supply, or product removal, in order to overcome the low water-solubility of organic compounds and enzyme inhibition by substrate or product. Potentially, the solvent may also be used to overcome an unfavourable equilibrium, although this requires sufficient driving force to be effective. In this way, the application of two-liquid phase systems improves the bioreaction space-time yield (productivity) as well as the product concentration fed to the downstream process, and in some cases the selectivity (Boghigian *et al.*, 2011; Dafoe and Daugulis, 2014; Jung *et al.*, 2013; Mutti and Kroutil, 2012).

2.1 Bioreaction systems

Several considerations are important in aqueous-organic two-phase biocatalytic systems. The organic phase may be deleterious to the biocatalyst in two ways; either by the presence of the interface (Martínez-Aragón *et al.*, 2009; Perez-Rodriguez *et al.*, 2003) or by the amount of organic solvent dissolved in the aqueous phase which may cause biocatalyst inactivation (Bes *et al.*, 1995; Stepankova *et al.*, 2013). Both appear to be important, but in many cases the biocatalyst needs to be kept away from the interface.
Despite the downside described above the introduction of an organic solvent in the bioreaction system presents several advantages such as the dissolution of substrates and products at higher concentrations in the reactor than would otherwise be achievable. This means that the downstream process can be fed at high concentrations, while avoiding inhibitory concentrations of substrate or product in the aqueous reaction environment (Hua and Xu, 2011; Lima-Ramos *et al.*, 2014). Easier product recovery may also result from the fact that the solvent has a low boiling point, facilitating evaporation (Dafoe and Daugulis, 2014). Likewise when designing an *in-situ* product removal (ISPR) process, the mode of contact (direct or indirect) between the biocatalyst and the organic phase which removes the product, should be considered (Stark and von Stockar, 2003; Woodley *et al.*, 2008). A bioreaction system with direct solvent contact can be characterized by the direct exposure of the biocatalyst/cells to the organic solvent [Figure 1 a) and b)]. For a bioreaction system with indirect solvent contact [Figure 1 c) and d)] the biocatalyst is not in contact with the organic solvent (Stark and von Stockar, 2003; Woodley *et al.*, 2008).
In Figure 1, two possibilities for running systems with direct contact are presented: a) corresponds to the exposure of the biocatalyst to organic solvent within the reactor and b) corresponds to the direct contact in a different vessel to the reactor through an external loop. Configuration a) has the advantage that both reaction and product removal take place in the same vessel and therefore the equipment costs are lower. Configuration b) reduces the contact time between the biocatalyst and the organic solvent by introducing an external loop through a separation unit. However, the choice

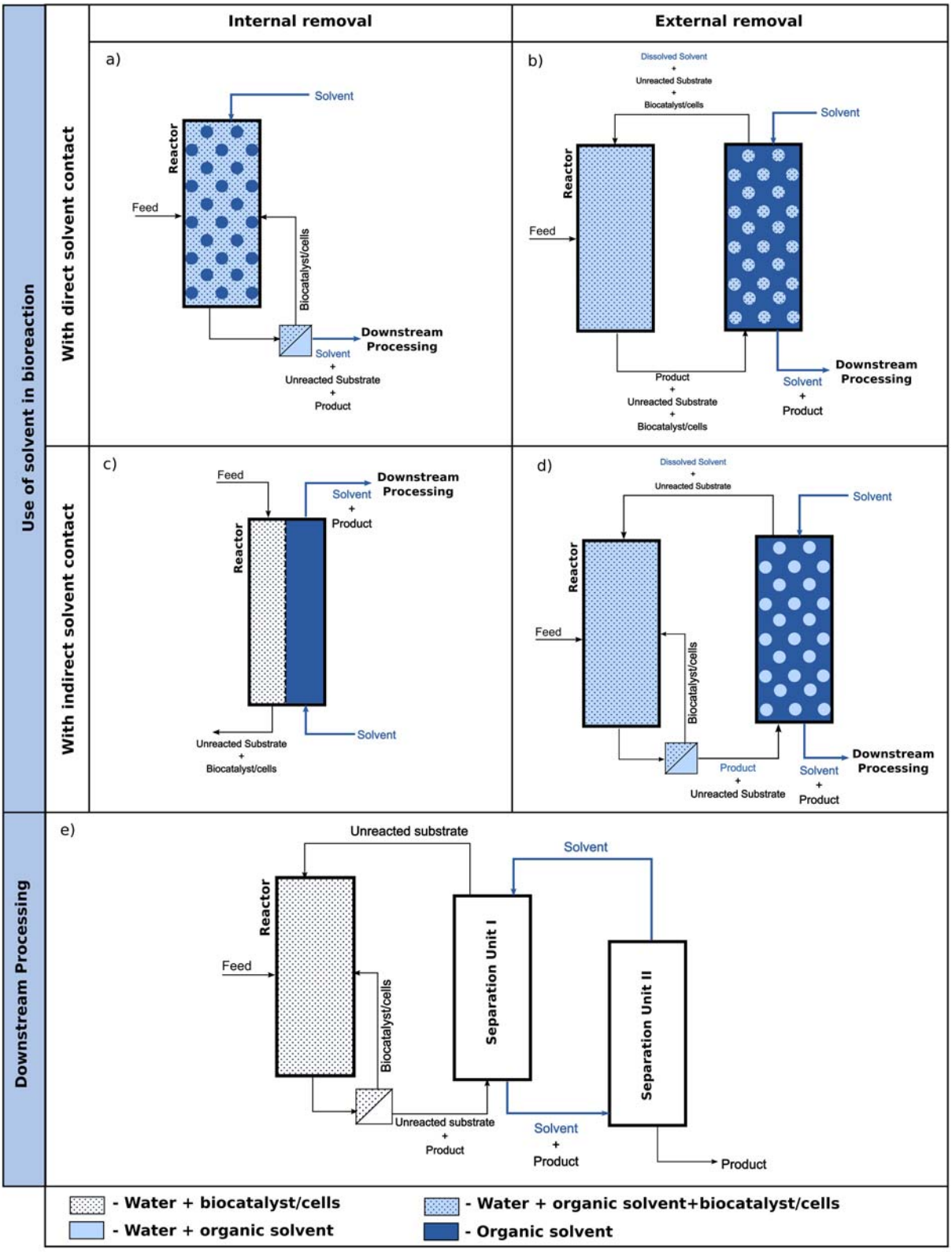

Figure 1 – Three process configurations for a bioreaction in an aqueous-organic two-phase system. Figure adapted from Stark and von Stockar, 2003.

of solvent has to ensure that the solvent does not deactivate the biocatalyst/microorganism and the product has a high enough affinity and solubility.
Additionally, two configurations for indirect contact are presented in Figure 1: c) corresponds to a biphasic reactor with a membrane which separates the two liquid phases and d) corresponds to the separation of the biocatalyst/cells from the reactor medium and use of another vessel for the product removal. In systems such as c) there is usually a physical barrier such as a membrane which prevents the contact of the biocatalyst with the solvent (Stark and von Stockar, 2003; Woodley *et al.*, 2008). In the configuration d), the biocatalyst/microorganism is never in direct contact with the solvent. The biocatalyst/microorganism is separated from the product and is recycled to the reactor. The medium with product dissolved, in its turn, enters a liquid-liquid extraction unit where the product is partitioned to the organic solvent and the medium that exits the vessel is recycled to the reactor.
The choice of solvent for a two liquid-phase system with direct contact is more difficult than for an indirect contact configuration since it must be compatible with the biocatalyst/microorganism and therefore requires a careful study of its toxic effects.

2.2 Downstream processing

Organic solvents play an important role as separation and purification agents for small-molecule chemical products from bioprocesses since they allow easy recovery of organic compounds. The use of water as a solvent may present some challenges for downstream processing such as separation difficulties, and its high specific heat capacity implies high energy consumption in distillation and difficulties rapidly heating and cooling (Adams *et al.*, 2003). Moreover, the solubility of many of the most interesting compounds is often very low in water which implies excessive amounts of water in order to recover small amounts of product, resulting in high costs. When choosing an organic solvent, it should be possible to separate it from the aqueous phase as well as recover the desired products from the solvent as shown in Figure 1 e) (Gu, 2000; Koch, 2015). This should also enable options for recycling the solvent if viable, which could help optimize the economic feasibility of a given process, due to lower overall solvent use. Nowadays, the recycling of solvents is a common practice in industry. Besides the advantages mentioned above, the separation costs for isolating a product from an organic solvent can be much lower when compared to an aqueous system.
The determination of the exact downstream processing conditions depends not only on the nature of the product (solid or liquid) but also on the phase in which the product is primarily soluble. For a two-liquid phase system (i.e. with two immiscible phases), the operation unit mostly used to purify products is liquid-liquid extraction. Concerning energy consumption, liquid-liquid extraction can be more attractive since it is a less energy consuming process compared to distillation and gives a relatively high efficiency for product recovery (Kurzrock and Weuster-Botz, 2010; Stratakos and Koidis, 2016).

3. Overview of criteria to screen solvents for an industrial bioprocess

The list of solvents applicable to industrial processes is extensive and thus, the choice of the optimum solvent can be a significant challenge. Hence, at an early stage of process development, it is necessary to make a screening of solvents for evaluation of their suitability for the industrial process.
Figure 2 shows a screening procedure which is divided in four evaluation categories: (1) environment, health and safety, (2) affinity, recovery and recyclability properties, (3) stability and (4)

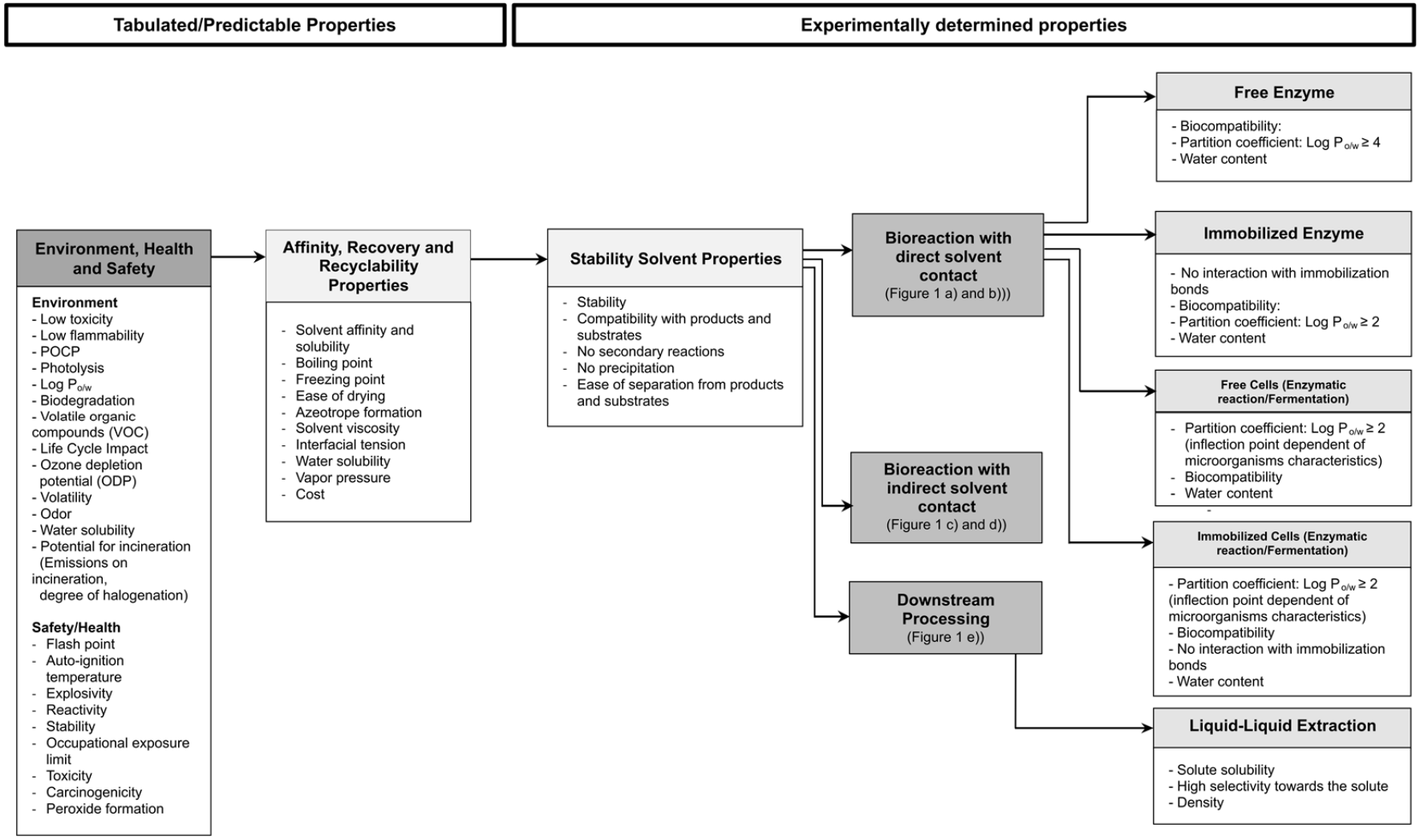

Figure 2 – Overview of criteria for choosing an organic solvent for a bioprocess divided in tabulated properties in scientific literature and experimentally determined properties which are dependent on system and compounds characteristics.

application. The screening is also divided between tabulated properties which are already available in the literature and experimentally determined properties, which are dependent on the characteristics of the system and have to be experimentally investigated in order to evaluate the its performance.

The purpose of the screening procedure is to help narrowing the list of possible solvents to be applied in a bioprocess by evaluating the most important criteria first and eliminating those solvents which do not fulfill the requirements. The methodology starts by evaluating solvents in terms of environment, health and safety issues because this is the greatest concern for process development. Indeed, in order to implement a process it is necessary to fulfill legal and regulatory requirements in this category. Subsequently, solvents are evaluated in terms of recovery and recyclability properties and finally the list is shortened by considering those which fulfill the criteria for application in a given bioprocess.

Ultimately an experimental investigation has to be performed since the solvent is selected according to the specific system under study. Nevertheless, some of the listed properties such as Log $P_{o/w}$ provide a direction for the search.

3.1 Criteria to screen for organic solvents with low hazard environmental, health and safety (EHS) issues

The adequate selection of solvents is dependent on their suitability for a given application. However, considerations regarding solvent recovery, solvent release as well as safety at an industrial site have particular importance. Hence, the primary category to assess is their impact on environmental, safety and health. It is necessary to take several parameters into account such as those quantifying the environmental impact (ecotoxicity, flammability, ozone depletion, incineration potential, etc). Regarding health and safety, some of the parameters are: toxicity & occupational exposure, auto-ignition temperature, boiling point, flash point, explosivity, reactivity and vapor pressure; these are particularly important considerations where a bioprocess is run in the presence of air or oxygen. Solvent selection guides are available, and some institutions and companies have also made studies to evaluate the hazards of the solvents and suggested alternative solvents which could substitute the most hazardous ones (Alfonsi *et al.*, 2008; American Chemical Society (ACS), 2011; Elgue *et al.*, 2006; Henderson *et al.*, 2011; Prat et al., 2016, 2013).

3.2 Criteria to evaluate the recovery strategies and affinity and stability of an organic solvent

When screening for organic solvents for a particular application in a process there are initially several considerations to take into account including the affinity, stability and recovery of the solvent.

The affinity of a given solvent towards a solute is a fundamental property to consider when choosing a solvent since it determines the viability of the solvent application. Even though this property is very specific for the process, it is possible to find data bases with information for specific solute-extractant pairs such as,(Dortmund Data Bank, 2018). In those cases where the information is not tabulated, the ternary phase behavior can be predicted using thermodynamic methods such as NRTL, UNIFAC and UNIFAQ. The successful application of these predictable methods has been widely reported in scientific literature (Abildskov et al., 2001; Brennan et al., 2012; Bruce and Daugulis, 1991; Cheng and Wang, 2010, 2007; Domańska et al., 2015; Ellegaard et al., 2009; Janseen et al., 1993; Malinowski, 2001, 1999; Malinowski and Daugulis, 1994; Modarresi et al., 2008; Priebe and Daugulis, 2018; Scilipoti et al., 2014). The reader should also note that any solvent selected in this way will still need be experimentally tested, not only for affinity but also for emulsion formation and biocompatibility.

When choosing a solvent for a bioprocess it is also necessary to take into consideration parameters such as viscosity, vapor pressure and melting point (Martínez-Aragón *et al.*, 2009; Tzia and Liadakis, 2003). The values of all these parameters should be low enough to ensure ease of handling and storage. For example, highly viscous solvents lead to problems effective liquid-liquid mass transfer. With respect to recovery and recyclability, the boiling point is an important parameter to consider, especially if the separation is done by distillation (Barwick, 1997). There are several other criteria to take into consideration as well, such as the ease of drying and azeotrope formation (Smallwood, 1996; Tzia and Liadakis, 2003). It is relevant to consider that all the factors mentioned above are very important in order to run a process with a solvent and solvent selection can be a delicate balance between the different parameters. The properties above are already tabulated and can be used for screening solvents and reduce the number of solvents to be tested.
The non-precipitation, non-reactivity and chemical stability in the reaction system of the solvent are also important factors to consider (Tzia and Liadakis, 2003). Likewise the solvent should be stable and not interact with the reaction solutes (e.g. substrate(s) and product(s)) and cause secondary reactions. Needless to say, being able to operate the process safely is of paramount importance.
Since most of these properties are dependent on the characteristics of an individual system, experimental work is necessary in order to assess the suitability of the solvent for the process. Therefore, these criteria should be evaluated in the end of the screening process to a very short list of solvents already chosen considering the tabulated properties.

3.3 Criteria for screening organic solvents as part of a reaction medium in two-liquid phase systems with free or immobilized biocatalyst/microorganisms

There are some specific challenges related to the use of solvents in bioreactions. As mentioned earlier, solvents can be damaging to the biocatalyst, causing degradation and inactivation. For an enzymatic reaction in a two-liquid phase system, there are some basic principles that can be followed in order to shorten the list of feasible solvent candidates for initial testing. The solvent should be as apolar as possible. Nevertheless, it should be noted that for such systems the aqueous-organic interface can also have toxic effects on the biocatalyst. The Log $P_{o/w}$ value is the accepted parameter for defining the polarity of a solvent. Hence, Log $P_{o/w}$ is useful for describing the influence of a solvent on enzyme activity. In the scientific literature, high partition coefficients (Log $P_{o/w} > 4$) are considered suitable, whilst those with lower values have frequently been found toxic to biocatalysts (Halling, 1994; Laane *et al.*, 1987; Straathof, 2003).
Solvents with Log $P_{o/w}$ values higher than 4 present a low solubility in water and, practically, the enzyme dissolved in the aqueous phase does not have contact with the solvent and is able to support effective product synthesis. On the other hand polar solvents with low Log $P_{o/w}$ values (Log $P_{o/w} <2$) are more soluble in water and consequently remove the essential water from the enzyme and disrupt its conformation with attendant deactivation (Soo *et al.*, 2003). Several authors have reported the effect of solvents on the performance of enzymes and have shown that enzymes present better activity in media containing solvents with high Log $P_{o/w}$ values (Bemquerer *et al.*, 1994; Koutinas *et al.*, 2018; Lara and Park, 2004; Valivety *et al.*, 1991; Zaks and Klibanov, 1985).
Interestingly, whilst the partition coefficient (Log $P_{o/w}$) is an important parameter to assess the suitability for an organic solvent for soluble enzymes, it has also been found useful for immobilized enzyme systems, although with a more relaxed requirements. For example it has been possible to achieve good enzyme performance in biphasic systems using immiscible organic solvents with lower Log $P_{o/w}$ values (range 1-3) (Chaplin *et al.*, 2001; Reslow *et al.*, 1987).
This indicates that the immobilization of the enzyme results in a shift of the Log $P_{o/w}$-activity curve as shown in Figure 3 (Laane *et al.*, 1986; Mionetto *et al.*, 1994).Consequently, with immobilized

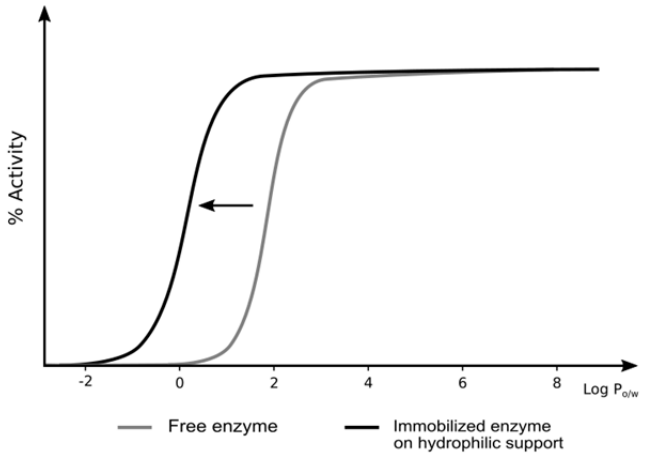

Figure 3-Schematic representation of enzymatic activity for both free enzyme and immobilized enzyme on a hydrophilic support plotted against Log $P_{o/w}$ of the solvent. Figure adapted from Mionetto *et al.* 1994.

enzymes there is a broadening of the solvent polarity range and an increased number of suitable solvent options. In these reaction systems, it is believed that the support retains the water molecules and therefore stabilizes a water layer around the enzyme molecules. The water layer protects the enzyme molecules and therefore makes them more stable even in organic solvents with lower partition coefficients.

From the different studies reported, we can conclude that Log $P_{o/w}$ should only be used as a guideline for screening biocatalyst-compatible solvents. In fact, it is not possible to determine the suitability of the solvent without performing experiments. Some exceptions to the guideline have been reported (Cantarella *et al.*, 1993; Geok *et al.*, 2003; Gonçalves *et al.*, 1997).

Furthermore, the characterization of enzyme performance in organic media has often been reported in an inconsistent manner. So while some authors report the enzyme activity (Mionetto *et al.*, 1994), or specific activity (Norin *et al.*, 1988), other report the residual activity (Geok *et al.*, 2003; Reslow *et al.*, 1987) and others again, the reaction conversion or yield (Chaplin *et al.*, 2001; Koutinas *et al.*, 2018; Lara and Park, 2004). These inconsistencies mean that drawing conclusions about the use of Log $P_{o/w}$ as a parameter for solvent selection is difficult.

For whole-cell biocatalytic systems and fermentation, the relation between cellular activity of different microorganisms against Log $P_{o/w}$ is also represented by a sigmoidal curve, similar to that for soluble enzyme (Laane *et al.*, 1987). Several authors (Bassetti and Tramper, 1994; Cruz *et al.*, 2001; Fragnelli *et al.*, 2012; Neumann *et al.*, 2005; Rojas *et al.*, 2004; Silva *et al.*, 2010) have studied the relationship between cellular activity and Log $P_{o/w}$. From their results, unsurprisingly it is possible to conclude that the inflection points between toxic and non-toxic solvents vary significantly between different microorganisms. Bruce and Daugulis have proposed that the tolerance of the microorganism is dependent on the characteristics of the cellular membrane (L J Bruce and Daugulis, 1991). Whole-cells biocatalysis using organic media has been reviewed and the authors concluded that the inflection point of the sigmoidal curve is in general above the value Log $P_{o/w}$ 2 (Heipieper *et al.*, 2007; León *et al.*, 1998). Nonetheless, it is still necessary to perform experimental screening work for the microorganism of interest. Some authors have engineered the microorganisms, in order to improve microorganisms tolerance to solvents, and in this way have

adapted a specific cell to have tolerance to a specific solvent (Mukhopadhyay, 2015; Volmer *et al.*, 2014; Zhang *et al.*, 2015).

Regarding bioreactions in water-organic solvent two-phase systems with immobilized enzymes or cells, the toxicity of the solvent to the biocatalyst/microorganism is a crucial factor to consider when screening for organic solvents. However, the interaction of the solvents with the immobilization matrix is also an important factor to take into consideration. Immobilization by adsorption is the simplest method and is characterized by reversible surface interactions between enzyme/cells and the support material. The interaction forces can be van der Waals forces, ionic and hydrogen bonding interactions. Since these forces are weak, desorption can occur in the presence of organic solvents (Brena *et al.*, 2013; Dwevedi, 2016). Synthetic polymer resins can be prone to swell with certain classes of solvent. On the other hand, porous silica and porous glass have been shown to be durable and resistant to solvent destruction (Datta *et al.*, 2013). Entrapment, encapsulation and cross-linking are more resistant methods to solvent interactions. In fact, it has been reported that these methods are often used to retain catalytic activity in harsh conditions (temperature and pH extremes and exposure to organic solvents) (Kourkoutas *et al.*, 2004).

3.4 Criteria for screening organic solvents as extraction agents in downstream processes

Crucial criteria to consider when choosing a solvent as an extraction agent are the solubility of the target compound to be extracted, affinity towards this compound and the ease of subsequent phase separation. For instance, when extracting the product with a solvent it is important that the product is highly soluble in the solvent in order to efficiently recover most of the product from the outlet stream of the reactor (Kolář *et al.*, 2002). The ease of separation of the solvent from the aqueous phase is also important, since a complete separation reduces costs. Hence, a large density difference between the extract phase and raffinate phase (from which the components of interest have been removed) allows high capacities particularly in liquid-liquid extraction (Gu, 2000; Koch, 2015). Likewise, the higher the interfacial tension (Gu, 2000; Tzia and Liadakis, 2003), the more readily coalescence of emulsions will occur and the easier phase separation will be.

In some cases, the direct recovery of a product may not be possible using solvents alone and it is necessary to use a reactive liquid-liquid extraction which involves a reversible reaction between the desired chemical compound and the extractant or a host chemical species present in the extractant. Examples include the removal of carboxylic acids (acetic acid (Mahfud *et al.*, 2008), lactic acid (Wasewar *et al.*, 2003, 2002), pyruvic acid (Marti *et al.*, 2011), citric acid (Poposka *et al.*, 1998)) by amines. The extractions involve the complexation reaction of the undissociated acids and amines. The complexation reaction improves the distribution coefficient. The reaction promotes the migration of the product to the organic phase. The choice of the solvent is also important when establishing a reactive liquid-liquid extraction because it has to solvate the amine-acid complex to avoid its precipitation (Yang *et al.*, 2007).

Another solution, in case direct extraction is not possible, is to manipulate other properties such as modifying the pH of the output aqueous solution can be useful for separation. An excellent example of this is the downstream processing for penicillin production. After filtration of the mycelium, the pH of the broth is adjusted to pH 2-2.5 in order to convert penicillin acid carboxylate into penicillanic acid. The acidification of the broth increases the partition coefficient of penicillanic acid (Najafpour, 2007). However, penicillanic acid is unstable in aqueous solution, and this compound is recovered by an organic solvent, e.g. butyl acetate. The decision regarding the pH value to be selected should be a compromise between the partition coefficient and product stability (Wennersten, 2004) and acidification of the broth should be performed in order to minimize product degradation (Hook, 2006).

4. Solvent selection guide for biphasic bioreaction systems

An overview of the criteria to take into account when selecting a solvent for a specific application in a process has been described in the preceding sections. In this section, a selection guide for solvents that are, or could potentially be, used in biphasic biocatalytic/fermentation reactions is described. The evaluation procedure used to rank the different solvents is similar to the CHEM21 solvent selection guide published by Prat *et al.* (Prat *et al.*, 2016).

Although there are many beneficial uses for organic solvents in bioprocesses, the use of solvents presents several environmental, health and safety challenges. When choosing a solvent for the development of a process it is important to take into account the environmental impact of the chosen solvent, and the potential safety and health risks associated with handling and using the given solvent (Clark and Tavener, 2007). Solvents having significant issues should of course be avoided, if at all possible. There are several solvent selection guides in the scientific literature. GlaxoSmithKline (GSK) (Alder *et al.*, 2016), the American Chemical Society, Green Chemistry Institute Pharmaceutical Roundtable (ACS GCIPR) (American Chemical Society (ACS), 2011) and the solvent guide from CHEM21 (Prat *et al.*, 2016) have presented guides with numerical rankings and dividing solvents in categories. Pfizer's (Alfonsi *et al.*, 2008) and Sanofi's (Prat *et al.*, 2013) guides present the evaluation results solely in the form of a color code for each solvent, without a numerical ranking. In addition, the solvent guide from Pfizer presents an overall summarized evaluation for all solvents, rather than divided in categories.

The survey by Prat *et al.* (Prat *et al.*, 2014) presents a summary and a comparison of the Health, Safety and Environment assessments of several solvent guides. The solvent guides considered were Astra Zeneca's, ACS GCIPR's, GSK's 2011 guide (Henderson *et al.*, 2011), Pfizer's and Sanofi's. The main purpose of this survey was to compare the evaluation criteria across the different solvent guides and compare the consistency of solvent evaluation across the various guides.

In the present article a new selection guide for solvents commonly used, or of potential use, as reaction media in biphasic biocatalysis is presented. Some of the included solvents have never been assessed in previous solvent selection guides due to their specific application in biocatalytic reaction systems. Other solvent selection guides focus strongly on solvents used in the main for synthetic organic chemistry applications (Alfonsi *et al.*, 2008; American Chemical Society (ACS), 2011; Henderson *et al.*, 2011; Prat *et al.*, 2016, 2013). An accurate and detailed comparison of all of the required properties of solvents is not an easy or exact task, since the level and quality of data available for each solvent is different. This is especially true for the comparison of older solvents that might have a large amount of data available e.g. substances fully registered under REACH (ECHA 2016), and newer solvents where very little data is available (at least available in the public domain). A key feature of the CHEM21 methodology is that it allows a high level ranking of all solvents where basic physical/safety data and the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) is known (Prat *et al.*, 2016). The solvents in the guide presented here have been classified based on the methodology developed within the CHEM21 project. CHEM 21 is a collaborative project between European universities and companies and aims to develop sustainable biological and chemical alternatives to finite resources and more environmentally friendly processes. The guide presented here is targeted at process chemists and engineers charged with operating bioprocesses. In this guide we also provide some examples from the literature which document the use of the solvents in biocatalytic systems and additionally, the enzymes which have been used. Other useful data such as solubility in water, Log P and CAS number are also included. We hope these data will be useful for looking for greener solvents where similar Log P and/or water solubility values are needed for a successful bioprocess. For large scale processing, solvents which are solid close to ambient temperature can present specific logistical challenges, so solvents with

mp ≥ 10 °C have been marked in the table. The solvents included in the guide were chosen from a literature survey of biphasic bioreactions, or by looking for newer solvents that may have similar properties and could be good candidates for this type of transformation. Generally solvents have been chosen which have 10% or lower solubility in water as a cut off point for a water-immiscible organic solvent. In certain circumstances, solvents such as tetrahydrofuran and acetonitrile that are fully water-miscible can form biphasic mixtures with water (high aqueous solute content), but these were excluded. Data on water-miscible solvents can be found in other published guides (Alfonsi *et al.*, 2008; American Chemical Society (ACS), 2011; Henderson *et al.*, 2011; Prat *et al.*, 2016, 2013). Solvents that are common to this guide and CHEM21 used the data collated for the CHEM21 guide (Prat *et al.*, 2016). The data required to assess the new solvents were obtained from manufacturer's safety data/material safety data sheets – freely available from suppliers, and from the European Chemicals Agency, Registered Substances Data (ECHA-RS), 2016. In the case of the less common solvents and newer solvents, not all of the required data was available or found. In particular, it was difficult to find values for resistivity (the ability to accumulate a static charge). Under the methodology solvents likely to build up a static charge (> 10^8 Ω.m) incrementally add 1 to the safety score. For the additional solvents here, ethers and hydrocarbons were scored as resistive, and the other solvents as non-resistive. Needless to say, before using any solvents at scale, a full assessment needs to be made of all operational and safety hazards, including resistivity. Where air is used for bio oxidation and/or for transformations with living cells, appropriate care needs to be taken to avoid the formation of an explosive head space if a flammable solvent is used. Since the processes under consideration here are all biphasic, the production of aqueous waste streams containing low levels of the organic solvent needs to be considered. Some calculated data on persistence, bioaccumulation and toxicity has been included in the table. Thus the solvent selection guide includes an evaluation of persistence in the environment, bioaccumulation in food chains and toxicity to fish. The persistence, bioaccumulation and toxicity (PBT) evaluation follows the criteria established by New Chemicals Program (EPA (U.S. Environmental Protection Agency), 2012). The persistence evaluation is performed by investigating the half-life of the compound in water and air. In relation to the water criteria, if the compound's half-life is less than 2 months it is considered recommended (green). If the compound's half-life is between 2 and 6 months it is considered problematic (amber). Solvents with a half-life greater than 6 months are considered hazardous (red). Persistence in air is also evaluated by the half-life; compounds with a half-life lower or equal to 2 days are considered harmless, those with a half-life greater than 2 days are considered hazardous (EPA (U.S. Environmental Protection Agency), 2012).

The bioaccumulation criterion corresponds to the bioconcentration factor of a chemical uptake from the surrounding media by an organism living in that media. If the range of bioconcentration factors is less than 1000, the solvent is considered recommended for industrial applications (green). Solvents with bioconcentration factors greater than or equal to 1000 and less than 5000 are considered to be problematic (amber). Solvents with bioconcentration factors higher than 5000 are considered hazardous and not advised to be applied in industrial applications (red) (EPA (U.S. Environmental Protection Agency), 2012).

Toxicity to fish is evaluated by the concentration of the solvent which is chronically toxic to fish, chronic toxicity value (ChV). Solvents with a ChV greater than 10 mg/L and that do not present any toxic risk are considered harmless (green). ChV in the range of concentrations 0.1-10 mg/L present moderate concern and are considered problematic (amber). Solvents with ChV less than 0.1 mg/L are considered hazardous (red) (EPA (U.S. Environmental Protection Agency), 2012).

Table 2 is the compilation of the assessment of the solvents which are commonly applied, or could be applied, in bioreactions as a medium. The guide contains a score for each parameter [1 (good) to 10 (bad)] and is color coded for easy reference. The guide is divided into safety, health and

environmental sections, with an overall recommendation. Scoring is based on physical parameters such as boiling point, auto ignition temperature etc. and GHS statements. Full details of the methodology are given in the CHEM21 publication (Prat *et al.*, 2016). For easy comparison in tabular form, the output is color coded. Green (recommended) indicates that the solvent can be used with few issues (given normal safe operating procedures are in place to deal with issues such as flammability, etc). Yellow (problematic) indicates that there may be some issues, but the solvent should be usable with appropriate mitigation strategies. Red - solvents labeled hazardous or highly hazardous should be replaced or avoided in developing new processes. In the overall ranking column, some solvents have a split ranking. This is due to current industrial thinking and practice that would generally move a solvent into a higher hazard band than that given by the ranking/scoring process.

For newer solvents that are not fully registered in REACH (thus potentially lacking in some data sets), the CHEM21 scoring methodology defaults to 5 (problematic/yellow). This is why solvents such as diethyl succinate and butyl butyrate rank as problematic when compared to very similar structures like ethyl, tert-butyl or isopropyl acetate, which are fully registered. When full datasets are available, these materials may become more harmonized in the guide. The REACH process is generating a lot of data on solvents and the overall picture is constantly changing. Looking into the future, before using any newer solvent, it would be advised to search for any new data or change in REACH status that could change the ranking in the table. It is worth noting that especially in the context of this guide, the methodology scores high boiling solvents (especially > 200 °C, e.g. diethyl succinate b.p. = 218 °C) poorly in the environmental section since these materials will be very energy intensive to purify or recover by distillation.

Lastly, the reader should note that the limits of the CHEM 21 selection algorithm define the assessment of each solvent. There are other solvent selection guides available in the literature and there are some differences in the classification of the solvents (Prat *et al.,* 2014). Moreover, the assessment limits might also change with future legislation. In line with this, we are aware that some solvents which present some toxic and flammable properties (e.g. n-butanol) currently fall into the category of “Recommended” due to the limits of the evaluation. Moreover, azeotrope formation was not considered in the selection guide, although in principle it should also be taken into account when screening for solvents due to separation problems with the recovery of the solvent or waste water treatment.

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Table 1 – Compilation of organic solvent selection guides and potential substitution solvents.

High Level Solvent Guide for Biphasic Biocatalysed Reactions												
Solvent, (CAS N°), mp if ≥ 10 °C	**Solubility in water g litre^{-1}***	**Log P***	**Precedent for use in biphasic bioreaction**	**Reference**	**In published guides****	**Safety Score**	**Health Score**	**Environment Score**	**PBT profile*****			**Overall Ranking using CHEM21 methodology******
									P	**B**	**T**	
Alcohols												
n-Butanol (71-36-3)	63.2	0.79	Dehydrogenase	(Cremonesi *et al.*, 1973)	**P, G, S, C21, RT**	3	4	3				**Recommended**
Isobutanol (78-83-1)	70	0.79	Oxidase	(Zaks, 1988)	**G, S, C21, RT**	3	4	3				**Recommended**
n-Pentanol (71-41-0)	2.03	1.44	Decarboxylase	(Rosche *et al.*, 2004)	**No**	3	2	3				**Recommended**
n-Heptanol (111-70-6)	1.63	2.2	None found		**No**	1	2	5				**Recommended**
tert-Amyl alcohol (75-85-4)	98	0.77	Oxidase	(Zaks, 1988)	**S**	4	2	3				**Recommended**
Isoamyl alcohol (123-51-3)	21.2	1.35	None found		**G, C21**	3	2	3				**Recommended**
1-Octanol (111-87-5)	0.5	3.15	Oxygenase	(Hüsken *et al.*, 2002)	**No**	1	2	5				**Recommended**
Benzyl alcohol (100-51-6)	40	1.05	None found		**G, S, C21, RT**	1	2	7				**Problematic**
1-Dodecanol (112-53-8) mp 22 °C	0.0019	5.13	Reductase	(De Wulf and Thonart, 1989)	**No**	1	5	7				**Problematic**
1-Decanol (112-30-1)	0.021	4.5	Dehydrogenase	(Pinheiro and Cabral, 1992)	**No**	2	2	7				**Problematic**
Esters												
Ethyl acetate (141-78-6)	87.9	0.68	Dehydrogenase	(Cremonesi *et al.*, 1973)	**P, G, S, C21, RT**	5	3	3				**Recommended**
tert-Butyl acetate (540-88-5)	6.7	1.64	ω-Transaminase	(Meadows *et al.*, 2013)	**G**	4	1	3				**Recommended**
n-Butyl acetate (123-86-4)	5.3	2.3	KRED	(Ye *et al.*, 2010)	**G, S, C21, RT**	4	2	3				**Recommended**
Isobutyl acetate (110-19-0)	5.6	2.3	None found		**S, C21, RT**	4	2	3				**Recommended**

n-Propyl acetate (109-60-4)	18.7	1.27	None found		**G**	4	2	3				**Recommended**
Isopropyl acetate (108-21-4)	31.9	1.03	None found		**P, G, S, C21, RT**	4	2	3				**Recommended**
Isoamyl acetate (123-92-2)	2	2.7	None found		**C21**	3	1	5				**Recommended**
n-Butyl butyrate (109-21-7)	0.31	2.83	None found		**No**	3	5	5				**Problematic**
n-Octyl acetate (112-14-1)	0.033	3.81	P450	(Toda *et al.*, 2012)	**G**	1	5	7				**Problematic**
Diethyl succinate (123-25-1)	19	1.26	None found		**G, S, C21**	1	5	7				**Problematic**
Lauryl acetate (112-66-3)	0.00036	5.88	P450	(Garikipati *et al.*, 2009)	**No**	1	5	5				**Problematic**
Ethyl decanoate (106-33-2)	0.00041	5.71	P450	(Tan and Day, 1998)	**No**	1	5	7				**Problematic**
Ethyl oleate (111-62-6)	$6x10^{-7}$	8.51	P450	(Kuhn *et al.*, 2012)	**No**	1	5	7				**Problematic**
FAME-Fatty acid methyl esters (67762-38-3)	0.000023	>6.2	P450	(Schrewe *et al.*, 2014)	**No**	Mixture						**Problematic**
Bis n-butyl phthalate (84-74-2)	0.011	4.46	KRED	(He *et al.*, 2006)	**No**	1	9	7				**Hazardous**
bis(2-ethylhexyl) phthalate (117-81-7)	$3x10^{-6}$	7.86	P450	(Park *et al.*, 2007)	**No**	1	9	7				**Hazardous**
Tricaprylin (538-23-8)	$1.5x10^{-8}$	9.2	Plant cell culture	(Dutta, 1994)	**No**	1	5	7				**Problematic**
Ketones												
Methyl Isobutyl ketone (MIBK) (108-10-1)	14.1	1.9	α-Galactosidase	(Bennett *et al.*, 1992)	**S,G,C21, RT**	4	2	3				**Recommended**
Cyclohexanone (108-94-1)	90	0.86	Imidase	(Ogawa *et al.*, 2000)	**G, S, C21, RT**	3	3	5				**R** / **P**
2-Octanone (111-13-7)	0.9	2.5	KRED	(Kohlmann *et al.*, 2011)	**No**	3	5	5				**Problematic**
2-Undecanone (112-12-9) mp 15 °C	0.04	4.1	Oxidation	(Collins and Daugulis, 1997)	**No**	1	5	7				**Problematic**
Ethers												

Dimethyl ether† (115-10-6)	335	0.07	KRED	(Lu et al., 2004)	**G**	9	2	7				**H**	**HH**
Diethyl ether (60-29-7)	43.1	1.05	Dehydrogenase	(Cremonesi *et al*., 1973)	**P, G, S, C21, RT**	10	3	7				**H**	**HH**
Diisopropyl ether (108-20-3)	3.1	1.52	Enoate reductase	(Hall *et al*., 2012)	**P, G, S, C21, RT**	9	3	5				**Hazardous**	
Dibutyl ether (142-96-1)	0.11	3.35	ω-Transaminase	(Meadows *et al*., 2013)	**S**	5	2	5				**Problematic**	
2-Methyltetrahydrofuran (96-47-9)	140	1.1	Benzaldehyde lyase	(Shanmuganathan *et al*., 2011)	**P, G, S, C21, RT**	6	3	3				**R**	**P**
Cyclopentyl methyl ether (CPME) (5614-37-9)	3.1	1.59	Benzaldehyde lyase	(Wiedner *et al*., 2015)	**G, S, C21, RT**	7	2	5				**Problematic**	
tert-Butyl methyl ether (TBME) (1634-04-4)	41.9	1.23	Hydroxy nitrile Lyase	(Wiedner *et al*., 2015)	**P, G, S, C21, RT**	8	3	5				**Hazardous**	
Ethyl tert-butyl ether (ETBE) (637-92-3)	2.37	1.48	None found		**G, S, C21**	7	3	3				**Problematic**	
tert-Amyl methyl ether (TAME) (994-05-8)	10.7	1.55	None found		**G, C21**	6	2	3				**Recommended**	
Diisoamyl ether (544-01-4)	0.028	5.08	Dehydrogenase	(Hocknull and Lilly, 1990)	**No**	4	2	7				**Problematic**	
Anisole (100-66-3)	1.71	2.11	Lipase	(Wells, 2010)	**G, S, C21, RT**	4	1	5				**P**	**R**
Halogenated													
Dichloromethane (DCM) (75-09-2)	13.2	1.25	Dehydrogenase	(Cremonesi *et al*., 1973)	**P, G, S, C21, RT**	1	7	7				**Hazardous**	
Chloroform (67-66-3)	8.7	1.97	Protease	(Ogino et al., 1995)	**P, G, S, C21, RT**	2	7	5				**P**	**HH**
Carbon tetrachloride (56-23-5)	0.65	2.64	Oxidase	(Liu *et al*., 1996)	**P, G, S, C21, RT**	2	7	10				**H**	**HH**
1,2-Dichloroethane (107-06-2)	7.9	1.45	None found		**P, G , S, C21, RT**	4	10	3				**H**	**HH**
Chlorobenzene (108-90-7)	0.21	2.98	Dehydrogenase	(Cremonesi, 1975)	**G, S, C21, RT**	3	2	7				**Problematic**	
Methoxyperfluorobutane (163702-07-6)	0.01	3.93	Nitrile hydratase	(Zhu *et al*., 2015)	**No**	3	6	5				**Problematic**	
Benzotrifluoride	0.21	3.01	None found		**G, RT**	5	5	7				**Problematic**	

(98-08-8)													
Aromatic hydrocarbons													
Benzene (71-43-2)	1.78	2.1	KRED	(Shi *et al.*, 2008)	**P, G, S, C21, RT**	6	10	3				**H**	**HH**
Toluene (108-88-3)	0.52	2.73	Nitrile hydratase	(Cull *et al.*, 2001)	**P, G, S, C21**	5	6	3				**Problematic**	
Xylene (1330-20-7)	0.16	3.15	Oxidase	(Aono *et al.*, 1994)	**P, G, S, C21, RT**	4	2	5				**Problematic**	
p-Cymene (99-87-6)	0.03	4.1	Lipase	(Paggiola *et al.*, 2014)	**G, S, C21**	4	5	5				**Problematic**	
Tetralin (119-64-2)	0.045	3.78	Reductase	(Ferrante *et al.*, 1995)	**S**	3	6	7				**Problematic**	
Cumene (98-82-8)	0.05	3.55	None found		**S, G**	5	2	7				**Problematic**	
Aliphatic hydrocarbons													
n-Pentane (109-66-0)	0.039	3.45	None found		**P, G, S, C21**	8	3	7				**Hazardous**	
n-Hexane (110-54-3)	0.01	4	KRED	(de Gonzalo *et al.*, 2007)	**P, G, S, C21, RT**	8	7	7				**Hazardous**	
n-Heptane (142-82-5)	0.0024	4.5	Dehalogenase	(Zou, 2014)	**P, G, S, C21, RT**	6	2	7				**Problematic**	
n-Octane (111-65-9)	0.0007	5.15	Nitroreductase	(Meyer *et al.*, 2006)	**S, C21**	5	2	7				**Problematic**	
Isooctane (540-84-1)	0.0022	4.08	Lipoxygenase	(Kermasha *et al.*, 2002)	**G, RT**	6	2	7				**Problematic**	
Cyclohexane (110-82-7)	0.052	3.44	Esterase	(Lee, 1997)	**P, G, S, C21, RT**	6	3	7				**Problematic**	
Methylcyclohexane (108-87-2)	0.014	3.88	None found		**P, G, S, C21, RT**	6	2	7				**Problematic**	
Petroleum ether 60/80 (101316-46-5)	As n-hexane	As n-hexane	KRED	(Pathan *et al.*, 2012)	**G**	Mixture						**P**	**H**
Paraffin oil (8012-95-1)	Insoluble	>4	Oxidase	(Oda *et al.*, 1996)	**No**	Mixture						**P**	**H**
Decane (124-18-5)	0.000083	5.86	Expandase	(Gao and Demain, 2001)	**No**	4	2	5				**Problematic**	
Dodecane (112-40-3)	0.000005	6.98	KRED	(Huang *et al.*, 2005)	**No**	2	2	7				**Problematic**	
Tetradecane	$2.8x10^{-7}$	7.2	Dioxygenase	(Collins *et al.*,	**No**	2	2	7				**Problematic**	

(629-59-4)				1995)								
Hexadecane (544-76-3) mp 18 °C	0.000001	8.20	P450	(Furuhashi, 1986)	**No**	2	2	7				**Problematic**
D-Limonene (5989-27-5)	0.006	4.4	Hydratase	(Savithiry *et al*., 1997)	**S, C21**	4	2	7				**Problematic**
Turpentine (8006-64-2)	0.002 to 0.35	3 to 6	None found		**S, C21**	4	2	7				**Problematic**

* Data from ECHA database [(ECHA-RS), 2016], literature values (sourced from the Reaxys database, Chemspider) or calculated. Values between 20 and 30 °C.

** Solvent listed in other guides P=Pfizer (Alfonsi *et al.*, 2008), G= GSK (Alder *et al*., 2016), S= Sanofi (Prat *et al*., 2013), C21= CHEM21 (Prat *et al*., 2016), RT= ACS GCI Pharmaceutical Roundtable (ACS 2011).

Grey shading indicates scoring is not appropriate due to mixtures, or values cannot be calculated for PBT profiler (Environmental Health Analysis Center, 2012)

*** Calculated environmental fate http://www.pbtprofiler.net/

P = Persistence

B = Bioaccumulation

T = Toxicity to fish

**** Recommendation as an output from the CHEM21 solvent selection methodology (Prat *et al.,* 2016). Where a cell is split, the first column represents the output from the tool. However, for certain solvents, a second column has been added to reflect current industrial practice and thinking.

† solvent used under pressure, the boiling point of dimethyl ether -24 °C at atmospheric pressure.

5. Concluding remarks and future perspectives

This article summarizes water-immiscible solvent applications in bioprocesses and enumerates the different criteria to take into account in order to select a solvent. The criteria have been compiled and organized in a screening procedure which helps to narrow down the number of potentially feasible solvents to be tested experimentally during early stage process development, and to help guide chemists and engineers towards solvents with the best EHS profiles. The most important properties that are necessary to consider when screening organic solvents for a process are related to their environment, health and safety impact, recoverability and stability and their application in the process, as a reaction medium or applied to downstream processing.

Unfortunately, an ideal solvent is not always available from the shortlist of solvent options, and it is not always possible to fulfill all of the requirements. For example solvents with high Log P values are favored for two-liquid phase systems with free, immobilized biocatalysts or whole cells, whereas these are the very solvents which tend to persist in the environment and score poorly in the environmental assessment of the solvent guide. More lipophilic solvents also tend to have higher resistivity and consequently a higher safety score. Therefore, when making the final choice it is necessary to take a decision about the importance of the evaluation categories and to set strategies to overcome the constraints of the unfulfilled requirements. These strategies should still establish a safe and environmentally friendly process with reasonable acceptable costs.

Moreover, sometimes there are also process challenges to overcome such as deactivation of the biocatalyst in the presence of an organic solvent. This can often be overcome by using an indirect solvent contact process. In fact, it is also possible to avoid the contact of the biocatalyst with the solvent by making the extraction outside of the reactor without recirculation of the aqueous phase to the reactor – Figure 1.

The selection of solvents for application in industrial processes has been changing over the past two decades. In fact, today there is a tendency both in industry and in research to choose a solvent taking greater consideration of the environmental impact and also an impact on health, safety and costs aspects. As an example GlaxoSmithKline Pharmaceuticals' most frequently used solvents list has changed towards greener solvents. Solvents such as toluene, dichloromethane and tetrahydrofuran, which were applied greatly in industry in the 90's, are presently being replaced. The three top ranked solvents for industrial application were 2-propanol, ethyl acetate and methanol. The list of the 10 top ranked solvents includes also ethanol, n-heptane, tetrahydrofuran, toluene, dichloromethane, acetic acid and acetonitrile (Constable *et al.*, 2007). Moreover, a survey of solvent usage in development of processes revealed that although there is some room for improvement on substituting solvents of concern, there is already some reduction of chloroform and n-hexane applications. Additionally, this investigation shows that the usage of dipolar aprotic solvents at larger scale (>100 kg scale) is much smaller than in processes at smaller scale (Ashcroft *et al.*, 2015). Another factor driving industry towards more benign solvents is legislation, especially Registration and Evaluation of Chemicals (REACH) in the EU which seeks to limit and eventually remove from use substances with carcinogenic, reprotoxic and mutagenic properties, as well as materials with a high environmental impact (European Chemicals Agency (ECHA), 2016).

The scientific community has focused research to find greener solvents for bioprocesses and these efforts are centered on the application of ionic liquids, deep eutectic solvents and supercritical carbon dioxide (Jessop, 2011). Ionic liquids have been extensively studied by the scientific community as possible reaction media for biocatalysis. Ionic liquids are mixtures of cations and anions which do not pack well and therefore, these mixtures are in liquid phase at room temperature. Several enzymes have been tested having ionic liquids or a mixture of ionic liquid and water as reaction media. From the scientific literature, it is possible to conclude that in ionic liquids

several enzymes present good stereoselectivity, reaction yield, activity and stability (Lou *et al.*, 2005; Lozano *et al.*, 2001). For example, Lozano and coworkers have studied α-chymotrypsin and verified an increase of its half-life and the conversion of the substrate when compared to 1-propanol (Lozano *et al.*, 2001). The implementation of ionic liquids in industrial processes will require more information regarding their toxicity, ecotoxicity and their life cycle impact. Moreover the ecotoxicity of the ionic liquid seems to be related to the branching of the alkyl chain and to hydrophobicity of the cation (Docherty and Kulpa, Jr., 2005). Some of the ionic liquids have EC_{50} (acute toxicity value) values much lower than for example toluene, which means they are more ecotoxic. Another aspect to consider when evaluating the environmental impact of ionic liquids is the environmental impact of their synthesis. The synthesis of an ionic liquid sometimes requires the use of harmful organic solvents (Deetlefs and Seddon, 2010; Zhang *et al.*, 2008). There have also been efforts to decrease the toxicity of ionic liquids. In fact the third generation of ionic liquids has been considered cheaper, sustainable, non-toxic and biodegradable (Domínguez de María and Maugeri, 2011; Fukaya *et al.*, 2007).

Supercritical carbon dioxide ($scCO_2$) is also considered a sustainable solvent since it is non-flammable, has low toxicity, is broadly inert limiting unwanted reactions, and is present in abundance as a by-product of industrial processes like fermentation and thermal cracking. Although $scCO_2$ presents several advantages at safety and process level, it has also some associated disadvantages. Some organic substrates have poor solubility in $scCO_2$, requiring the use of a co-solvent. A process which uses supercritical carbon dioxide requires high pressure equipment and therefore it is necessary to consider carefully the safety aspects. Furthermore, another constraint of the application of $scCO_2$ in a process is the cost of operation and equipment capital cost which is much higher compared to a standard organic solvent since the process has to operate at high pressure (Beckman, 2004). Concerning application in bioprocesses, studies have demonstrated that $scCO_2$ can improve reaction rates and control reaction selectivity by pressure. Many enzymes have been demonstrated to have a high performance in $scCO_2$ compared to organic solvents. Examples include hydrolases, oxygenases and dehydrogenases, and have been reviewed by Wimmer and Zarevúcka, 2010. In addition, lipases seem to have been extensively studied and reported in the scientific literature (Khosravi-Darani and Mozafari, 2009). However, the enzyme is not always stable in a biphasic CO_2/H_2O system due to the dissolution of CO_2 in water which causes the formation of H_2CO_3. Consequently, pH will decrease (2.85) and the enzyme can be deactivated. In addition, carbon dioxide is a Lewis acid and reacts with strong bases and nucleophiles (Beckman, 2004). Therefore, it is necessary to take this fact into account when considering the application of $scCO_2$ in processes in which these compounds are substrates or products.

The solvent for a process can be chosen from several categories of solvents: water, organic solvents, ionic liquids and supercritical fluids. Jessop has consulted top academic experts in green solvents about which solvents they would choose for industrial application, and the choice fell on water, supercritical carbon dioxide and carefully-selected organic solvents (Jessop, 2011).

In conclusion, the choice of a solvent for a bioprocess should comprise a balance between the effects on the environment, effects on human health, safety hazards, biocatalyst/microorganism activity, solubility and selectivity of substrates and/or products and recovery. This balance is important because it is not always possible to find a solvent which fully covers all these criteria. Problems regarding the impact of a solvent on Environment, Health and Safety are increasingly being taken into account in process development when considering the application of a solvent as a reaction medium or as part of downstream processing in new processes. Moreover, in recent years, there has been more focus to substitute the hazardous solvents in already running processes.

6. Acknowledgment

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7. References

Abildskov, J., Gani, R., Rasmussen, P., O'Connell, J.., 2001. Analysis of infinite dilution activity coefficients of solutes in hydrocarbons from UNIFAC. Fluid Phase Equilib. 181, 163–186.

Adams, D.J., Dyson, P.J., Tavener, S.J., 2003. Chemistry in alternative reaction media. John Wiley & Sons, Ltd, Chichester.

Alder, C.M., Hayler, J.D., Henderson, R.K., Redman, A.M., Shukla, L., Shuster, L.E., Sneddon, H.F., 2016. Updating and further expanding GSK's solvent sustainability guide. Green Chem. 18, 3879–3890.

Alfonsi, K., Colberg, J., Dunn, P.J., Fevig, T., Jennings, S., Johnson, T.A., Kleine, H.P., Knight, C., Nagy, M.A., Perry, D.A., Stefaniak, M., 2008. Green chemistry tools to influence a medicinal chemistry and research chemistry based organisation. Green Chem. 10, 31–36.

American Chemical Society (ACS), 2011. ACS GCI Pharmaceutical Roundtable Solvent Selection Guide [WWW Document]. URL http://www.acs.org/content/dam/acsorg/greenchemistry/industriainnovation/roundtable/acs-gci-pr-solvent-selection-guide.pdf (accessed 5.24.16).

Aono, R., Doukyo, N., Kobayashi, H., Horikoshi, K., Nakajima, H., 1994. Oxidative Bioconversion of Cholesterol by Pseudomonas sp. Strain ST-200 in a Water-Organic Solvent Two-Phase System. Appl. Environ. Microbiol. 60, 2518–2523.

Ashcroft, C.P., Dunn, P.J., Hayler, J.D., Wells, A.S., 2015. Survey of Solvent Usage in Papers Published in Organic Process Research & Development 1997–2012. Org. Process Res. Dev. 19, 740–747.

Barwick, V.J., 1997. Strategies for solvent selection — a literature review. TrAC Trends Anal. Chem. 16, 293–309.

Bassetti, L., Tramper, J., 1994. Organic solvent toxicity in Morinda citrifolia cell suspensions. Enzyme Microb. Technol. 16, 642–648.

Beckman, E.J., 2004. Supercritical and near-critical CO2 in green chemical synthesis and processing. J. Supercrit. Fluids 28, 121–191.

Bemquerer, M.P., Adlercreutz, P., Tominaga, M., 1994. Pepsin-catalyzed peptide synthesis in organic media: studies with free and immobilized enzyme. Int. J. Pept. Protein Res. 44, 448–456.

Bennett, C., Dordick, J.S., Hacking, A.J., Cheetham, P.S., 1992. Biocatalytic synthesis of disaccharide high-intensity sweeterner sucralose via a tetrachlororaffinose intermediate. Biotechnol. Bioeng. 39, 211–217.

Bes, M.T., Gomez-Moreno, C., Guisan, J.M., Fernandez-Lafuente, R., 1995. Selective oxidation: stabilisation by multipoint attachment of ferredoxin NADP+ reductase, an interesting cofactor recycling enzyme. J. Mol. Catal. A Chem. 98, 161–169.

Boghigian, B.A., Myint, M., Wu, J., Pfeifer, B.A., 2011. Simultaneous production and partitioning of heterologous polyketide and isoprenoid natural products in an Escherichia coli two-phase bioprocess. J. Ind. Microbiol. Biotechnol. 38, 1809–1820.

Bose, A., Keharia, H., 2013. Production, characterization and applications of organic solvent tolerant lipase by Pseudomonas aeruginosa AAU2. Biocatal. Agric. Biotechnol. 2, 255–266.

Brena, B., González-Pombo, P., Batista-Viera, F., 2013. Immobilization of enzymes: a literature survey. Methods Mol. Biol. 1051, 15–31.

Brennan, T.C.R., Turner, C.D., Krömer, J.O., Nielsen, L.K., 2012. Alleviating monoterpene toxicity using a two-phase extractive fermentation for the bioproduction of jet fuel mixtures in Saccharomyces cerevisiae. Biotechnol. Bioeng. 109, 2513–2522.

Bruce, L.J., Daugulis, A.J., 1991. Solvent selection-strategies for extractive biocatalysis. Biotechnol. Prog. 7, 116–124.

Buchholz, K., Kasche, V., Bornscheuer, U.T., 2012. Biocatalysts and enzyme technology. Wiley-VCH Verlag, Weinheim.

Cantarella, L., Alfani, F., Cantarella, M., 1993. Stability and activity of immobilized hydrolytic enzymes in two-liquid-phase systems: Acid phosphatase, β-glucosidase, and β-fructofuranosidase entrapped in poly(2-hydroxyethyl methacrylate) matrices. Enzyme Microb. Technol. 15, 861–867.

Carstensen, F., Apel, A., Wessling, M., 2012. In situ product recovery: Submerged membranes vs. external loop membranes. J. Memb. Sci. 394, 1–36.

Carvalho, A.C.L. de M., Fonseca, T. de S., Mattos, M.C. de, Oliveira, M. da C.F. de, Lemos, T.L.G. de, Molinari, F., Romano, D., Serra, I., 2015. Recent advances in lipase-mediated preparation of pharmaceuticals and their intermediates. Int. J. Mol. Sci. 16, 29682–29716.

Chaplin, J.A., Budde, C.L., Khmelnitsky, Y.L., 2001. Catalysis by amine oxidases in nonaqueous media. J. Mol. Catal. B Enzym. 13, 69–75.

Cheng, H.-C., Wang, F.-S., 2010. Computer-aided biocompatible solvent design for an integrated extractive fermentation–separation process. Chem. Eng. J. 162, 809–820

Cheng, H.-C., Wang, F.-S., 2007. Trade-off optimal design of a biocompatible solvent for an extractive fermentation process. Chem. Eng. Sci. 62, 4316–4324.

Clark, J.H., Tavener, S.J., 2007. Alternative solvents: Shades of green. Org. Process Res. Dev. 11, 149–155.

Collins, A.M., Woodley, J.M., Liddell, J.M., 1995. Determination of reactor operation for the

488 microbial hydroxylation of toluene in a two-liquid phase process. J. Ind. Microbiol. 14, 382–388.

489 Collins, L.D., Daugulis, A.J., 1997. Biodegradation of phenol at high initial concentrations in two-
490 phase partitioning batch and fed-batch bioreactors. Biotechnol. Bioeng. 55, 155–162.

491 Constable, D.J.C., Jimenez-Gonzalez, C., Henderson, R.K., 2007. Perspective on solvent use in the
492 pharmaceutical industry. Org. Process Res. Dev. 11, 133–137.

493 Cremonesi, P., 1975. Enzymatic preparation of 20 beta-hydroxysteroids in a two-phase system.
494 Biotechnol. Bioeng. 17, 1101–1108.

495 Cremonesi, P., Carrea, G., Sportoletti, G., Antonini, E., 1973. Enzymatic dehydrogenation of
496 steroids by β-hydroxysteroid dehydrogenase in a two-phase system. Arch. Biochem. Biophys. 159,
497 7–10.

498 Cruz, A., Fernandes, P., Cabral, J.M.S., Pinheiro, H.M., 2001. Whole-cell bioconversion of β-
499 sitosterol in aqueous–organic two-phase systems. J. Mol. Catal. B Enzym. 11, 579–585.

500 Cue, B.W., Zhang, J., 2009. Green process chemistry in the pharmaceutical industry. Green Chem.
501 Lett. Rev. 2, 193–211.

502 Cull, S., Woodley, J., Lye, G., 2001. Process selection and characterisation for the biocatalytic
503 hydration of poorly water soluble aromatic dinitriles 19, 131–154.

504 Dafoe, J.T., Daugulis, A.J., 2014. In situ product removal in fermentation systems: improved
505 process performance and rational extractant selection. Biotechnol. Lett. 36, 443–460.

506 Dortmund Bank Data, 2018. Dortmund Data Bank [WWW Document]. URL
507 http://www.ddbst.com/ddb.html (access 3.22.2018)

508 Datta, S., Christena, L.R., Rajaram, Y.R.S., 2013. Enzyme immobilization: an overview on
509 techniques and support materials. 3 Biotech 3, 1–9.

510 De Gonzalo, G., Lavandera, I., Faber, K., Kroutil, W., 2007. Enzymatic reduction of ketones in
511 “micro-aqueous” media catalyzed by ADH-A from Rhodococcus ruber. Org. Lett. 9, 2163–6.

512 De Wulf, O., Thonart, P., 1989. Bioconversion of vanillin to vanillyl alcohol in a two-phase reactor.
513 Appl. Biochem. Biotechnol. 20, 165–180.

514 Deetlefs, M., Seddon, K.R., 2010. Assessing the greenness of some typical laboratory ionic liquid
515 preparations. Green Chem. 12, 17–30.

516 Devi, N.A., Radhika, G.B., Bhargavi, R.J., 2017. Lipase catalyzed transesterification of ethyl
517 butyrate synthesis in n-hexane— a kinetic study. J. Food Sci. Technol. 54, 2871–2877.

518 Docherty, K.M., Kulpa, Jr., C.F., 2005. Toxicity and antimicrobial activity of imidazolium and
519 pyridinium ionic liquids. Green Chem. 7, 185–189.

520 Domańska, U., Okuniewski, M., Okuniewska, P., Paduszyński, K., Turowski, T., 2015. Phase
521 equilibrium and bioproduction of the aroma compound 2-phenylethanol in a biphasic aqueous
522 system. Eur. Food Res. Technol. 240, 1177–1186.

523 Domínguez de María, P., Maugeri, Z., 2011. Ionic liquids in biotransformations: From proof-of-

524 concept to emerging deep-eutectic-solvents. Curr. Opin. Chem. Biol. 15, 220–225.

525 Dutta, A., 1994. Two-phase culture system for plant cells. Ann. N. Y. Acad. Sci. 745, 251–260.

526 Dwevedi, A., 2016. Enzyme Immobilization - Advances in Industry, Agriculture, Medicine, and the
527 Environment. Springer International Publishing, Cham.

528 Elgue, S., Prat, L., Cabassud, M., Cezerac, J., 2006. Optimisation of solvent replacement procedures
529 according to economic and environmental criteria. Chem. Eng. J. 117, 169–177.

530 Ellegaard, M.E., Abildskov, J., O'Connell, J.P., 2009. Method for predicting solubilities of solids in
531 mixed solvents. AIChE J. 55, 1256–1264.

532 Environmental Health Analysis Center, 2012. Persistent, Bioaccumulative, and Toxic (PBT)
533 profiler [WWW Document]. URL http://www.pbtprofiler.net/ (accessed 12.1.16).

534 EPA (U.S. Environmental Protection Agency), 2012. Estimating Persistence, Bioaccumulation, and
535 Toxicity Using the PBT Profiler, in: Sustainable Futures / P2 Framework Manual 2012 EPA-748-
536 B12-001. U.S. Environmental Protection Agency Office of Chemical Safety and Pollution
537 Prevention.

538 European Chemicals Agency (ECHA), 2016. Registration, Evaluation, Authorisation and
539 Restriction of Chemicals (REACH) [WWW Document]. URL
540 http://echa.europa.eu/regulations/reach (accessed 2.9.16).

541 Ferrante, A.A., Augliera, J., Lewis, K., Klibanov, A.M., 1995. Cloning of an organic solvent-
542 resistance gene in Escherichia coli: the unexpected role of alkylhydroperoxide reductase. Proc. Natl.
543 Acad. Sci. 92, 7617–7621.

544 Fragnelli, M.C., Hoyos, P., Romano, D., Gandolfi, R., Alcántara, A.R., Molinari, F., 2012.
545 Enantioselective reduction and deracemisation using the non-conventional yeast Pichia glucozyma
546 in water/organic solvent biphasic systems: preparation of (S)-1,2-diaryl-2-hydroxyethanones
547 (benzoins). Tetrahedron 68, 523–528.

548 Freeman, A., Woodley, J.M., Lilly, M.D., 1993. In Situ Product Removal as a Tool for
549 Bioprocessing. Nat. Biotechnol. 11, 1007–1012.

550 Fukaya, Y., Iizuka, Y., Sekikawa, K., Ohno, H., 2007. Bio ionic liquids: room temperature ionic
551 liquids composed wholly of biomaterials. Green Chem. 9, 1155–1157.

552 Furuhashi, K., 1986. Fermentation process for the production of optically active epoxides. CEER,
553 Chem. Econ. Eng. Rev. 18, 21–26.

554 Gani, R., 2006. A modern approach to solvent selection. Chem. Eng. 113, 30–43.

555 Gao, Q., Demain, A., 2001. Improvement in the bioconversion of penicillin G to
556 deacetoxycephalosporin G by elimination of agitation and addition of decane. Appl. Microbiol.
557 Biotechnol. 57, 511–513.

558 Garikipati, S.V.B.J., McIver, A.M., Peeples, T.L., 2009. Whole-cell biocatalysis for 1-naphthol
559 production in liquid-liquid biphasic systems. Appl. Environ. Microbiol. 75, 6545–52.

560 Geok, L.P., Razak, C.N.A., Abd Rahman, R.N.Z., Basri, M., Salleh, A.B., 2003. Isolation and

561 screening of an extracellular organic solvent-tolerant protease producer. Biochem. Eng. J. 13, 73–
562 77.

563 Gonçalves, A.P.V., Lopes, J.M., Lemos, F., Ramôa Ribeiro, F., Prazeres, D.M.F., Cabral, J.M.S.,
564 Aires-Barros, M.R., 1997. Effect of the immobilization support on the hydrolytic activity of a
565 cutinase from Fusarium solani pisi. Enzyme Microb. Technol. 20, 93–101.

566 Gorman, L.A., Dordick, J.S., 1992. Organic solvents strip water off enzymes. Biotechnol. Bioeng.
567 39, 392–397.

568 Gu, T., 2000. Liquid-liquid partitioning methods for bioseparations. Sep. Sci. Technol. 2, 329–364.

569 Hall, M., Winkler, C.K., Tasnádi, G., Faber, K., 2012. Enoate Reductases for Reduction of Electron
570 Deficient Alkenes, in: Whittal, J., Sutton, P.W. (Eds.), Practical Methods for Biocatalysis and
571 Biotransformation 2. John Wiley & Sons, Ltd, Chichester.

572 Halling, P.J., 1994. Thermodynamic predictions for biocatalysis in nonconventional media: Theory,
573 tests, and recommendations for experimental design and analysis. Enzyme Microb. Technol. 16,
574 178–206.

575 He, J.-Y., Sun, Z.-H., Ruan, W.-Q., Xu, Y., 2006. Biocatalytic synthesis of ethyl (S)-4-chloro-3-
576 hydroxy-butanoate in an aqueous-organic solvent biphasic system using Aureobasidium pullulans
577 CGMCC 1244. Process Biochem. 41, 244–249.

578 Heipieper, H.J., Neumann, G., Cornelissen, S., Meinhardt, F., 2007. Solvent-tolerant bacteria for
579 biotransformations in two-phase fermentation systems. Appl. Microbiol. Biotechnol. 74, 961–973.

580 Henderson, R.K., Jiménez-González, C., Constable, D.J.C., Alston, S.R., Inglis, G.G. a., Fisher, G.,
581 Sherwood, J., Binks, S.P., Curzons, A.D., 2011. Expanding GSK's solvent selection guide –
582 embedding sustainability into solvent selection starting at medicinal chemistry. Green Chem. 13,
583 854–862.

584 Hocknull, M.D., Lilly, M.D., 1990. The use of free and immobilised Arthrobacter simplex in
585 organic solvent/aqueous two-liquid-phase reactors. Appl. Microbiol. Biotechnol. 33, 148–153.

586 Hook, D.H., 2006. Production of antibiotics by fermentation, in: Ratledge, C., Kristiansen, B.
587 (Eds.), Basic Biotechnology. Cambridge University Press, Cambridge.

588 Hua, D., Xu, P., 2011. Recent advances in biotechnological production of 2-phenylethanol.
589 Biotechnol. Adv. 29, 654–660.

590 Huang, Y., Zhang, F., Gong, Y., 2005. A convenient approach to (S)-2-ethylhexan-1-ol mediated by
591 baker's yeast. Tetrahedron Lett. 46, 7217–7219.

592 Hüsken, L.E., Oomes, M., Schroën, K., Tramper, J., de Bont, J.A.M., Beeftink, R., 2002.
593 Membrane-facilitated bioproduction of 3-methylcatechol in an octanol/water two-phase system. J.
594 Biotechnol. 96, 281–289.

595 Janseen, A.E.M., Van der Padt, A., Van Sonsbeek, H.M., Van't Riet, K., 1993. The effect of
596 organic solvents on the equilibrium position of enzymatic acylglycerol synthesis. Biotechnol.
597 Bioeng. 41, 95–103.

598 Jessop, P.G., 2011. Searching for green solvents. Green Chem. 13, 1391–1398.

599 Jessop, P.G., Ahmadpour, F., Buczynski, M.A., Burns, T.J., Green II, N.B., Korwin, R., Long, D.,
600 Massad, S.K., Manley, J.B., Omidbakhsh, N., Pearl, R., Pereira, S., Predale, R.A., Sliva, P.G.,
601 VanderBilt, H., Weller, S., Wolf, M.H., 2015. Opportunities for greener alternatives in chemical
602 formulations. Green Chem. 17, 2664–2678.

603 Jung, D.-H., Choi, W., Choi, K.-Y., Jung, E., Yun, H., Kazlauskas, R.J., Kim, B.-G., 2013.
604 Bioconversion of p-coumaric acid to p-hydroxystyrene using phenolic acid decarboxylase from B.
605 amyloliquefaciens in biphasic reaction system. Appl. Microbiol. Biotechnol. 97, 1501–1511.

606 Kamal, M.Z., Yedavalli, P., Deshmukh, M. V., Rao, N.M., 2013. Lipase in aqueous-polar organic
607 solvents: Activity, structure, and stability. Protein Sci. 22, 904–915.

608 Kemeling, G.M., 2012. Editorial: Solvent Choices and Sustainable Chemistry. ChemSusChem 5,
609 2291–2292.

610 Kermasha, S., Dioum, N., Bisakowski, B., Vega, M., 2002. Biocatalysis by immobilized
611 lipoxygenase in a ternary micellar system. J. Mol. Catal. B Enzym. 20, 305–317.

612 Khosravi-Darani, K., Mozafari, M.R., 2009. Supercritical fluids technology in bioprocess
613 industries: A review. J. Biochem. Technol. 2, 144–152.

614 Koch, J., 2015. Design Principles for Liquid-Liquid Extraction. Chem. Eng. Prog. 111, 22-30.

615 Kohlmann, C., Robertz, N., Leuchs, S., Greiner, L., Na'amnieh, S., 2011. Utilising hardly-water
616 soluble substrates as a second phase enables the straightforward synthesis of chiral alcohols. Green
617 Chem. 13, 3093–3095.

618 Kolář, P., Shen, J.-W., Tsuboi, A., Ishikawa, T., 2002. Solvent selection for pharmaceuticals. Fluid
619 Phase Equilib. 194, 771–782.

620 Kourkoutas, Y., Bekatorou, A., Banat, I.., Marchant, R., Koutinas, A.., 2004. Immobilization
621 technologies and support materials suitable in alcohol beverages production: a review. Food
622 Microbiol. 21, 377–397.

623 Koutinas, M., Yiangou, C., Osório, N.M., Ioannou, K., Canet, A., Valero, F., Ferreira-Dias, S.,
624 2018. Application of commercial and non-commercial immobilized lipases for biocatalytic
625 production of ethyl lactate in organic solvents. Bioresour. Technol. 247, 496–503.

626 Kuhn, D., Julsing, M.K., Heinzle, E., Bühler, B., 2012. Systematic optimization of a biocatalytic
627 two-liquid phase oxyfunctionalization process guided by ecological and economic assessment.
628 Green Chem. 14, 645–653.

629 Kurzrock T., Weuster-Botz D., 2010. Recovery of succinic acid from fermentation broth,
630 Biotechnol. Lett., 32, 331-339.

631 Laane, C., Boeren, S., Hilhorst, R., Veeger, C., 1986. Optimization of biocatalysis in organic media,
632 in: Laane, C., Tramper, J., Lilly, M.D. (Eds.), Biocatalysis in Organic Media. Elsevier Science
633 Publishers B.V., Amsterdam, 65–84.

634 Laane, C., Boeren, S., Vos, K., Veeger, C., 1987. Rules for optimization of biocatalysis in organic

635 solvents. Biotechnol. Bioeng. 30, 81–87.

636 Lara, P.V., Park, E.Y., 2004. Potential application of waste activated bleaching earth on the
637 production of fatty acid alkyl esters using Candida cylindracea lipase in organic solvent system.
638 Enzyme Microb. Technol. 34, 270–277.

639 Lee, Y., 1997. Whole-cell biotransformation in a biphasic aqueous-organic solutions. Preparation of
640 optically active cis-1,3-dibenzyl-2-oxoimidazolidinedicarboxylic acid monoester as a chiral
641 precursor of (+)-biotin. Bull. Korean Chem. Soc. 18, 101–102.

642 León, R., Fernandes, P., Pinheiro, H.M., Cabral, J.M.S., 1998. Whole-cell biocatalysis in organic
643 media. Enzyme Microb. Technol. 23, 483–500.

644 Lima-Ramos, J., Tufvesson, P., Woodley, J.M., 2014. Application of environmental and economic
645 metrics to guide the development of biocatalytic processes. Green Process. Synth. 3, 195–213.

646 Liu, W.-H., Horng, W.-C., Tsai, M.-S., 1996. Bioconversion of cholesterol to cholest-4-en-3-one in
647 aqueous/organic solvent two-phase reactors. Enzyme Microb. Technol. 18, 184–189.

648 Lou, W.Y., Xu, R., Zong, M.H., 2005. Hydroxynitrile lyase catalysis in ionic liquid-containing
649 systems. Biotechnol. Lett. 27, 1387–1390.

650 Lozano, P., de Diego, T., Guegan, J.P., Vaultier, M., Iborra, J.L., 2001. Stabilization of alpha-
651 chymotrypsin by ionic liquids in transesterification reactions. Biotechnol. Bioeng. 75, 563–569.

652 Lu, J., Lazzaroni, J., Hallet, J.P., Bommarius, A.S., Liotta, C.L., Eckert, C.A., 2004. Tunable
653 solvents for homogeneous catalyst recycle. Ind. Eng. Chem. Res. 43, 1586–1590.

654 Lye, G.J., Woodley, J.M., 1999. Application of in situ product-removal techniques to biocatalytic
655 processes. Trends Biotechnol. 17, 395–402.

656 Mahfud F. H. ,van Geel F. P.,Venderbosch R. H., Heeres H. J., 2008. Acetic Acid Recovery from
657 Fast Pyrolysis Oil. An Exploratory Study on Liquid-Liquid Reactive Extraction using Aliphatic
658 Tertiary Amines, Sep. Sci. Technol., 43, 3056-3074

659 Malinowski, J.J., 2001. Two-phase partitioning bioreactors in fermentation technology. Biotechnol.
660 Adv. 19, 525–538.

661 Malinowski, J.J., 1999. Evaluation of liquid extraction potentials for downstream separation of 1,3-
662 propanediol. Biotechnol. Tech. 13, 127–130.

663 Malinowski, J.J., Daugulis, A.J., 1994. Salt effects in extraction of ethanol, 1-butanol and acetone
664 from aqueous solutions. AIChE J. 40, 1459–1465.

665 Marti, M.E., Gurkan, T., Doraiswamy, L.K., 2011. Equilibrium and Kinetic Studies on Reactive
666 Extraction of Pyruvic Acid with Trioctylamine in 1-Octanol. Ind. Eng. Chem. Res. 50, 13518–
667 13525.

668 Martínez-Aragón, M., Burghoff, S., Goetheer, E.L.V., de Haan, A.B., 2009. Guidelines for solvent
669 selection for carrier mediated extraction of proteins. Sep. Purif. Technol. 65, 65–72.

670 Meadows, R.E., Mulholland, K.R., Schürmann, M., Golden, M., Kierkels, H., Meulenbroeks, E.,
671 Mink, D., May, O., Squire, C., Straatman, H., Wells, A.S., 2013. Efficient Synthesis of (S)-1-(5-

672 Fluoropyrimidin-2-yl)ethylamine Using an ω-Transaminase Biocatalyst in a Two-Phase System.
673 Org. Process Res. Dev. 17, 1117–1122.

674 Meyer, D., Bühler, B., Schmid, A., 2006. Process and catalyst design objectives for specific redox
675 biocatalysis. Adv. Appl. Microbiol. 59, 53–91.

676 Meyer, H.-P., Eichhorn, E., Hanlon, S., Lütz, S., Schürmann, M., Wohlgemuth, R., Coppolecchia,
677 R., 2013. The use of enzymes in organic synthesis and the life sciences: perspectives from the Swiss
678 Industrial Biocatalysis Consortium (SIBC). Catal. Sci. Technol. 3, 29–40.

679 Mionetto, N., Marty, J.L., Karube, I., 1994. Acetylcholinesterase in organic solvents for the
680 detection of pesticides: Biosensor application. Biosens. Bioelectron. 9, 463–470.

681 Modarresi, H., Conte, E., Abildskov, J., Gani, R., Crafts, P., 2008. Model-Based Calculation of
682 Solid Solubility for Solvent Selection - a Review. Ind. Eng. Chem. Res. 47, 5234–5242.

683 Mukhopadhyay, A., 2015. Tolerance engineering in bacteria for the production of advanced
684 biofuels and chemicals. Trends Microbiol. 23, 498–508.

685 Mutti, F.G., Kroutil, W., 2012. Asymmetric Bio-amination of Ketones in Organic Solvents. Adv.
686 Synth. Catal. 354, 3409–3413.

687 Najafpour, G., 2007. Production of antibiotics, in: Biochemical Engineering and Biotechnology.
688 Elsevier B. V., Amsterdam.

689 Neumann, G., Kabelitz, N., Zehnsdorf, A., Miltner, A., Lippold, H., Meyer, D., Schmid, A.,
690 Heipieper, H.J., 2005. Prediction of the Adaptability of Pseudomonas putida DOT-T1E to a Second
691 Phase of a Solvent for Economically Sound Two-Phase Biotransformations. Appl. Environ.
692 Microbiol. 71, 6606–6612.

693 Norin, M., Boutelje, J., Holmberg, E., Hult, K., 1988. Lipase immobilized by adsorption. Appl.
694 Microbiol. Biotechnol. 28, 527-530.

695 Oda, S., Kato, A., Matsudomi, N., Ohta, H., 1996. Enantioselective oxidation of racemic citronellol
696 with an interface bioreactor. Biosci. Biotechnol. Biochem. 60, 83–87.

697 Ogawa, J., Soong, C.-L., Ito, M., Segawa, T., Prana, T., Prana, M.S., Shimizu, S., 2000. 3-
698 Carbamoyl-α-picolinic acid production by imidase-catalyzed regioselective hydrolysis of 2,3-
699 pyridinedicarboximide in a water-organic solvent, two-phase system. Appl. Microbiol. Biotechnol.
700 54, 331–334.

701 Ogino, H., Yasui, K., Ishikawa, H., 1995. Organic solvent-tolerant bacterium which secretes an
702 organic solvent-stable proteolytic enzyme. Appl. Environ. Microbiol. 61, 4258–4262.

703 Paggiola, G., Hunt, A.J., McElroy, C.R., Sherwood, J., Clark, J.H., 2014. Biocatalysis in bio-
704 derived solvents: an improved approach for medium optimisation. Green Chem. 16, 2107–2110.

705 Park, J.-B., Bühler, B., Panke, S., Witholt, B., Schmid, A., 2007. Carbon metabolism and product
706 inhibition determine the epoxidation efficiency of solvent-tolerant Pseudomonas sp. strain
707 VLB120DeltaC. Biotechnol. Bioeng. 98, 1219–1229.

708 Pathan, N.B., Rahatgaonkar, R.M., Chorghade, M.S., 2012. Stereoselective bioreduction of

709 chalcone and beta-diketone by Saccharomyces cerevisiae in biphasic solvent system: A mechanistic
710 study. Indian J. Chem. Sect. B-organic Chem. Incl. Med. Chem. 51, 992–1001.

711 Perez-Rodriguez, C., Montano, N., Gonzalez, K., Griebenow, K., 2003. Stabilization of alpha-
712 chymotrypsin at the CH2Cl2/water interface and upon water-in-oil-in-water encapsulation in PLGA
713 microspheres. J. Control. Release 89, 71–85.

714 Phillips, T., Chase, M., Wagner, S., Renzi, C., Powell, M., DeAngelo, J., Michels, P., 2013. Use of
715 in situ solid-phase adsorption in microbial natural product fermentation development. J. Ind.
716 Microbiol. Biotechnol. 40, 411–425.

717 Pinheiro, H.M., Cabral, J.M., 1992. Activity and stability of an entrapped-cell system for the
718 Delta(1)-dehydrogenation of steroids in organic media. Biotechnol. Bioeng. 40, 1123–1127.

719 Poposka, F.A., Nikolovski, K., Tomovska, R., 1998. Kinetics, mechanism and mathematical
720 modelling of extraction of citric acid with isodecanol/n-paraffins solutions of trioctylamine. Chem.
721 Eng. Sci. 53, 3227–3237.

722 Prat, D., Hayler, J., Wells, A., 2014. A Survey of Solvent Selection Guides. Green Chem. 16, 4546–
723 4551.

724 Prat, D., Pardigon, O., Flemming, H.-W., Letestu, S., Ducandas, V., Isnard, P., Guntrum, E., Senac,
725 T., Ruisseau, S., Cruciani, P., Hosek, P., 2013. Sanofi's Solvent Selection Guide: A Step Toward
726 More Sustainable Processes. Org. Process Res. Dev. 17, 1517–1525.

727 Prat, D., Wells, A., Hayler, J., Sneddon, H., McElroy, C.R., Abou-Shehada, S., Dunn, P.J., 2016.
728 CHEM21 selection guide of classical- and less classical-solvents. Green Chem. 18, 288–296.

729 Priebe, X., Daugulis, A.J., 2018. Thermodynamic affinity-based considerations for the rational
730 selection of biphasic systems for microbial flavor and fragrance production. J. Chem. Technol.
731 Biotechnol. 93, 656–666.

732 Reslow, M., Adlercreutz, P., Mattiasson, B., 1987. Organic-solvents for bioorganic synthesis 1.
733 Optimization of parameters for a chymotrypsin catalyzed process. Appl. Microbiol. Biotechnol. 26,
734 1–8.

735 Rojas, A., Duque, E., Schmid, A., Hurtado, A., Ramos, J.-L., Segura, A., 2004. Biotransformation
736 in double-phase systems: physiological responses of Pseudomonas putida DOT-T1E to a double
737 phase made of aliphatic alcohols and biosynthesis of substituted catechols. Appl. Environ.
738 Microbiol. 70, 3637–3643.

739 Rosche, B., Breuer, M., Hauer, B., Rogers, P.L., 2004. Biphasic aqueous/organic biotransformation
740 of acetaldehyde and benzaldehyde by Zymomonas mobilis pyruvate decarboxylase. Biotechnol.
741 Bioeng. 86, 788–794.

742 Savithiry, N., Cheong, T.K., Oriel, P., 1997. Production of α-terpineol from Escherichia coli cells
743 expressing thermostable limonene hydratase. Appl. Biochem. Biotechnol. 63, 213–220.

744 Schrewe, M., Julsing, M.K., Lange, K., Czarnotta, E., Schmid, A., Bühler, B., 2014. Reaction and
745 catalyst engineering to exploit kinetically controlled whole-cell multistep biocatalysis for terminal
746 FAME oxyfunctionalization. Biotechnol. Bioeng. 111, 1820–1830.

747 Scilipoti, J.A., Cismondi, M., Andreatta, A.E., Brignole, E.A., 2014. Selection of Solvents with A-
748 UNIFAC Applied to Detoxification of Aqueous Solutions. Ind. Eng. Chem. Res. 53, 17051–17058.

749 Shanmuganathan, S., Natalia, D., van der Wittenboer, A., Kohlmann, C., Greiner, L., Dominguez de
750 Maria, P., 2011. Enzyme-Catalyzed C-C Bond Formation Using 2-Methyltetrahydrofuran (2-
751 MTHF) as (Co)solvent: Efficient and Bio-Based Alternative to DMSO and MTBE. ChemInform
752 42, 2240–2245.

753 Sheldon, R.A., 2017. The E factor 25 years on: the rise of green chemistry and sustainability. Green
754 Chem. 19, 18–43.

755 Sheldon, R.A., Pereira, P.C., 2017. Biocatalysis engineering: the big picture. Chem. Soc. Rev. 46,
756 2678–2691.

757 Sheldon, R.A., Woodley, J.M., 2017. Role of Biocatalysis in Sustainable Chemistry. Chem. Rev.
758 118, 801-838

759 Shi, Y.-G., Fang, Y., Wu, H.-P., Li, F., 2008. Asymmetric reduction of ethyl 2-oxo-4-
760 phenylbutyrate with baker's yeast in water/organic biphasic system. J. Biotechnol. 35, 1419–1424.

761 Silva, V.D., Stambuk, B.U., Nascimento, M. da G., 2010. Efficient chemoselective
762 biohydrogenation of 1,3-diaryl-2-propen-1-ones catalyzed by *Saccharomyces cerevisiae* yeasts in
763 biphasic system. J. Mol. Catal. B Enzym. 63, 157–163.

764 Smallwood, I.M., 1996. Handbook of organic solvent properties. John Wiley & Sons Inc., New
765 York.

766 Soo, E.L., Salleh, A.B., Basri, M., Rahman, R.N.Z., Kamaruddin, K., 2003. Optimization of the
767 Enzyme-Catalyzed Synthesis of Amino Acid-Based Surfactants from Palm Oil Fractions. J. Biosci.
768 Bioeng. 95, 361–367.

769 Stark, D., von Stockar, U., 2003. In situ product removal (ISPR) in whole cell biotechnology during
770 the last twenty years. Adv. Biochem. Eng. Biotechnol. 80, 149–175.

771 Stepankova, V., Bidmanova, S., Koudelakova, T., Prokop, Z., Chaloupkova, R., Damborsky, J.,
772 2013. Strategies for Stabilization of Enzymes in Organic Solvents. ACS Catal. 3, 2823–2836.

773 Straathof, A.J.J., 2003. Auxiliary phase guidelines for microbial biotransformations of toxic
774 substrate into toxic product. Biotechnol. Prog. 19, 755–762.

775 Stratakos, A.C., Koidis, A., 2016. Methods for Extracting Essential Oils, in: Essential Oils in Food
776 Preservation, Flavor and Safety. Academic Press, pp. 31–38.

777 Taher, H., Al-Zuhair, S., 2017. The use of alternative solvents in enzymatic biodiesel production: a
778 review. Biofuels, Bioprod. Biorefining 11, 168–194.

779 Tan, Q., Day, D.F., 1998. Organic co-solvent effects on the bioconversion of (R)-(+)-limonene to
780 (R)-(+)-α-terpineol. Process Biochem. 33, 755–761.

781 Toda, H., Imae, R., Itoh, N., 2012. Efficient biocatalysis for the production of enantiopure (S)-
782 epoxides using a styrene monooxygenase (SMO) and Leifsonia alcohol dehydrogenase (LSADH)
783 system. Tetrahedron: Asymmetry 23, 1542–1549.

784 Tucker, J.L., Faul, M. M., 2016. Industrial research: Drug companies must adopt green chemistry.
785 Nature 534, 27–29.

786 Tzia, C., Liadakis, G., 2003. Extraction optimization in food engineering. Marcel Dekker, Inc., New
787 York.

788 Valivety, R.H., Johnston, G.A., Suckling, C.J., Halling, P.J., 1991. Solvent effects on biocatalysis in
789 organic-systems - equilibrium position and rates of lipase catalyzed esterification. Biotechnol.
790 Bioeng. 38, 1137–1143.

791 Van Hecke, W., Kaur, G., De Wever, H., 2014. Advances in in-situ product recovery (ISPR) in
792 whole cell biotechnology during the last decade. Biotechnol. Adv. 32, 1245–1255.

793 Volmer, J., Neumann, C., Bühler, B., Schmid, A., 2014. Engineering of Pseudomonas taiwanensis
794 VLB120 for constitutive solvent tolerance and increased specific styrene epoxidation activity. Appl.
795 Environ. Microbiol. 80, 6539–6548.

796 Wasewar, K.L., Heesink, A.B.M., Versteeg, G.F., Pangarkar, V.G., 2002. Equilibria and kinetics for
797 reactive extraction of lactic acid using Alamine 336 in decanol. J. Chem. Technol. Biotechnol. 77,
798 1068–1075.

799 Wasewar, K.L., Pangarkar, V.G., Heesink, A.B.M., Versteeg, G.F., 2003. Intensification of
800 enzymatic conversion of glucose to lactic acid by reactive extraction. Chem. Eng. Sci. 58, 3385–
801 3393.

802 Wells, A., 2010. Biocatalytic Routes to the GPIIb/IIIa Antagonist Lotrafiban, SB 214857, in:
803 Blaser, H.-U., Federsel, H.-J. (Eds.), Asymmetric Catalysis on Industrial Scale: Challenges,
804 Approaches and Solutions. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

805 Wennersten, R., 2004. Extraction of organic compounds, in: Rydberg, J., Cox, M., Musikas, C.,
806 Choppin, G.P. (Eds.), Solvent Extraction Principles and Practice. Marcel Dekker, Inc., New York.

807 Wiedner, R., Kothbauer, B., Pavkov-Keller, T., Gruber-Khadjawi, M., Gruber, K., Schwab, H.,
808 Steiner, K., 2015. Improving the Properties of Bacterial R-Selective Hydroxynitrile Lyases for
809 Industrial Applications. ChemCatChem 7, 325–332.

810 Wimmer, Z., Zarevúcka, M., 2010. A review on the effects of supercritical carbon dioxide on
811 enzyme activity. Int. J. Mol. Sci. 11, 233–253.

812 Woodley, J.M., 2017. Bioprocess intensification for the effective production of chemical products.
813 Comput. Chem. Eng. 105, 297–307.

814 Woodley, J.M., Bisschops, M., Straathof, A.J.J., Ottens, M., 2008. Future directions for in-situ
815 product removal (ISPR). J. Chem. Technol. Biotechnol. 83, 121–123.

816 Yang, L., Dordick, J.S., Garde, S., 2004. Hydration of Enzyme in Nonaqueous Media Is Consistent
817 with Solvent Dependence of Its Activity. Biophys. J. 87, 812–821.

818 Yang, S.-T., Huang, H., Tay, A., Qin, W., De Guzman, L., Nicolas, E.C.S., 2007. Extractive
819 Fermentation for the Production of Carboxylic Acids, in: Bioprocessing for Value-Added Products
820 from Renewable Resources. Elsevier, pp. 421–446.

Ye, Q., Cao, H., Zang, G., Mi, L., Yan, M., Wang, Y., Zhang, Y., Li, X., Li, J., Xu, L., Xiong, J., Ouyang, P., Ying, H., 2010. Biocatalytic synthesis of (S)-4-chloro-3-hydroxybutanoate ethyl ester using a recombinant whole-cell catalyst. Appl. Microbiol. Biotechnol. 88, 1277–1285.

Zaks, A., 1988. The effect of water on enzyme action in organic media. J. Biol. Chem. 263, 8017–8021.

Zaks, A., Klibanov, A. M., 1985. Enzyme-catalyzed processes in organic solvents. Proc. Natl. Acad. Sci. U. S. A. 82, 3192–3196.

Zhang, F., Qian, X., Si, H., Xu, G., Han, R., Ni, Y., 2015. Significantly improved solvent tolerance of Escherichia coli by global transcription machinery engineering. Microb. Cell Fact. 14, 175.

Zhang, Y., Bakshi, B.R., Demessie, E.S., 2008. Life cycle assessment of an ionic liquid versus molecular solvents and their applications. Environ. Sci. Technol. 42, 1724–1730.

Zhou, T., Qi, Z., Sundmacher, K., 2014. Model-based method for the screening of solvents for chemical reactions. Chem. Eng. Sci. 115, 177–185.

Zhu, S., Ma, X., Su, E., Wei, D., 2015. Efficient hydration of 2-amino-2,3-dimethylbutyronitrile to 2-amino-2,3-dimethylbutyramide in a biphasic system via an easily prepared whole-cell biocatalyst. Green Chem. 17, 3992–3999.

Zou, S.-P., 2014. Enhancement of (S)-2,3-dichloro-1-propanol production by recombinant whole-cell biocatalyst in n-heptane–aqueous biphasic system. J. Biotechnol. 188, 42–47.