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Tutorial Review

Sponge-Like Ionic Liquids a new platform for green biocatalytic chemical processes

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To build a green chemical industry it is necessary to develop integrated processes of selective transformation and separation, able to directly provide pure products, including the reuse of all the elements of the reaction system, *e.g.* catalysts, solvents, etc. In recent years, the unsurpassed selectivity of enzymes for chemical reactions, combined with the excellent solvent properties of ionic liquids (ILs), have provided shown an excellent setting for carrying out sustainable chemical transformations. However, implementation of this to industrial chemical processes needs the development of straightforward, cheap and/or sustainable approaches for pure product extraction, including the reuse of ILs. Hydrophobic ILs based on cations with long alkyl side-chains, *e.g.* octadecyldecyltrimethylammonium bis(trifluoromethylsulfonyl)imide, ($[C_{18}tma][NTf_2]$), are temperature switchable ionic liquid/solid phases that behave as sponge-like systems (Sponge-Like Ionic Liquid, SLILs). Based on this newly found property, SLILs have been used to develop straightforward and clean approaches for producing nearly pure synthetic liquid compounds of high added value (*e.g.* flavour esters, biodiesel, etc.) in two steps: a biocatalytic step that occurs as monophasic liquid systems, followed by a product separation step carried out by cooling/centrifugation/filtration of the solid reaction system. SLILs therefore might be considered a new green platform for easy preparation of pure products.

Dedicated to the 75th Anniversary of the Faculty of Chemistry at the University of Murcia (Spain)

1. Toward clean chemical processes.

Sustainability is certainly one of the most fundamental challenges facing the technological development in this century. In the vast field covered by Chemistry and Chemical Technology, this challenge could be illustrated by reference to the list of the so-called principles of green chemistry,[1] and green engineering.[2] The application of these principles is essential for the construction of a clean chemical industry able to operate efficiently, leaving a minimal environmental footprint and minimizing risks.

Nowadays, the art of chemical synthesis has reached a considerable degree of maturity, and it is possible to prepare any target organic molecule regardless of its complexity. However, when analysing many procedures intended for synthetic transformations from a scaling-up perspective of ease for scaling, very few are viable, because key criteria such as product separation and purification, the recovery and reuse of catalysts and reaction media, energy consumption, environmental impact, toxicity of solvents and reagents, etc., were not taken into account at the outset. Thus, for scaling a production system of interest, a complete redesign and optimization of the synthetic methodology is often required, hindering the transfer from research to commerce.[3]

In this context, the preferred use of synthetic (bio)catalytic processes, the replacement of traditional non-aqueous solvents by others with a lower environmental impact, including their recovery and reuse, process intensification, energy savings, etc. are basic principles that must be taken into account when designing clean chemical processes.[4]

Traditional synthetic processes, especially in the field of Fine Chemicals, have generally been associated with the use of stoichiometric amounts of reagents and/or promoters, with the inherent formation of waste and concomitant products.[5] Switching from stoichiometric methodologies to catalytic processes is perceived as a major way to improve the efficiency of the chemical synthetic toolbox, reducing the formation of undesired by-products and waste through greater product selectivity.[6] In this regard, the tremendous potential of enzymes as ‘‘green’’ catalysts cannot be doubted, since they are able to accelerate stereo-, chemo- and regio-selectively chemical transformations. Besides, a great variety (more than 13,000) of enzyme-catalyzed reactions have been successfully demonstrated at the laboratory scale, offering clear advantages for the synthesis of enantiopure fine chemicals against any other kind of catalysts.[7]

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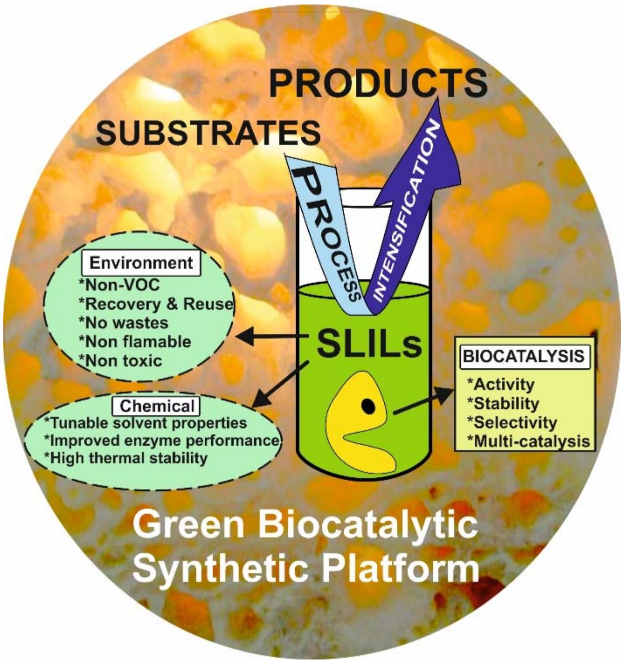

Fig. 1. Schematic representation of key axes for the green synthetic platform based on biocatalysis, sponge-like ionic liquids and process intensification.

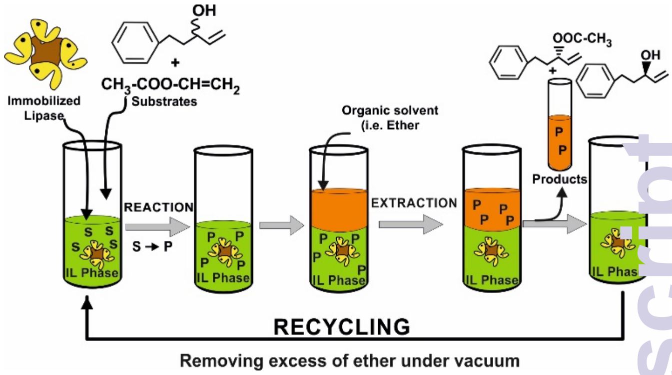

Fig. 2. Scheme of the experimental set-up carried out by Itoh *et al*[21] for lipase-catalysed kinetic resolution of 5-phenyl-1-penten-3-ol in [Bmim][PF$_6$], including product recovery by extraction with ether and recycling of the enzyme/IL system.

On the other hand, the use of solvents is one of the major concerns in the development of environmentally benign processes.[8] Indeed, solvents account for the vast majority of mass wasted in syntheses and processes.[9] Furthermore, many conventional solvents are toxic, flammable, and/or corrosive. Their volatility and solubility have contributed to air, water and land pollution, have increased the risk of workers' exposure, and have led to serious accidents. Moreover, their recovery and reuse, when possible, is often associated with energy-intensive distillation and sometimes cross contamination. During the last decade, chemists have increased their search for safer new environmentally benign solvents or green solvents, which can be recovered/recycled easily and which can allow catalysts to operate efficiently in them. In this respect, ionic liquids (ILs) and supercritical fluids (SCFs) are the non-aqueous green solvents, also named neoteric solvents, which have received most attention worldwide.[10] ILs are a new class of polar liquid solvents and their use has led to a green chemical revolution because of their unique array of physical-chemical properties, headed by their non-volatile character, which makes them suitable for numerous industrial applications.[11] Supercritical fluids (SCFs) are another class of neoteric solvent with unique tuneable properties, which enable them to be applied in green reaction, extraction and fractionation processes. Supercritical carbon dioxide (scCO$_2$) is also widely used and can be regarded as a safe non-toxic solvent.[12]

Moreover, with our increased knowledge of the catalytic and functional properties of enzymes and neoteric solvents, as well as of the physical and chemical properties of reactants and products,[11] it is possible to design sustainable experimental approaches for the transformation and separation pure products.[13] The technology of process intensification is an essential tool for establishing synergies between the different "green tools" that have enabled the development of integrated reaction and separation systems, *e.g.* continuous biocatalytic reactors based on IL / scCO$_2$ biphasic systems.[4a,14]

Through a multidisciplinary approach, this tutorial review aims to illustrate a new synthetic technological platform that will allow to revolutionize the way in which many organic liquid compounds can be obtained by combining different tools that are simultaneously highly efficient and environmentally friendly. This approach revolves around three key axes: (bio)catalysis, Sponge-Like Ionic Liquids (SLILs), and process intensification for straightforward product extraction (Fig.1). The basic idea is to harness the potential of the biocatalytic reactions carried out in certain ILs with sponge-like behaviour, which could then be implemented for developing integrated processes involving clean product separation steps suitable for industrial application.

2. Enzymes, Ionic Liquids and beyond

Enzymes are proteins, polymeric macromolecules based on amino acid units with unique sequences, which show a high level of structural organization maintained by a high number of weak internal interactions (*e.g.* hydrogen bonds, van der Waals, etc.), as well as interactions with other molecules, mainly water as the natural solvent of living systems.[15]

Although enzymes are designed by living systems to work in aqueous solutions, there are numerous potential advantages in employing enzymes in non-aqueous environments. Among of them, the higher solubility of hydrophobic substrates, the insolubility of enzymes facilitating their easy reuse, and the elimination of microbial contamination in reactors, should be mentioned.[10,13,16]

The replacement of water by non-aqueous solvents, as reaction medium for enzyme-catalyzed reactions, is not always a simple answer.[17] As least few clusters of water molecules, presumably bound to the charged groups on the surface of the enzyme molecules, are required for the catalytic function in non-aqueous environments. It was reported that proteins only achieve full biological activity when the surrounding water has approximately the same mass as the protein in question.[18] In this context, hydrophobic solvents typically afford higher enzymatic activity than hydrophilic ones, because the latter have a tendency to strip some of these essential water molecules from the enzyme surface. Water-immiscible ionic liquids (ILs) have been shown as exceptionally suitable reaction media for enzyme-catalyzed reactions,[19] probably due to their hygroscopic character which provides the essential degree of hydration around enzyme molecules (*e.g.* [Bmim][NTf$_2$] is able to absorb up to 1.4% w/w water content),[20] and this feature could be regarded as an additional advantage for biocatalysis.

The most popular approach used for biotransformations in ILs consists of the direct addition of biocatalysts to the reaction medium containing substrates. This can be illustrated by using the pioneering example of Itoh *et al*[21] depicted in Fig. 2, where an immobilized lipase catalysed the kinetic resolution of 5-phenyl-1-penten-3-ol with vinyl acetate in [Bmim][PF$_6$] as reaction medium, leading to an ester yield of > 99 % and E-val > 500. After the reaction was completed, products were extracted by liquid-liquid extraction with diethyl ether in a biphasic system, and the excess of ether in the IL phase was eliminated under vacuum. As the enzyme remained anchored to the IL phase, this approach permits reuse of the enzyme-IL system by addition of fresh substrates, without any loss in activity. The liquid-liquid extraction with organic solvents is the most commonly used approach for the recovery of products after biotransformation in ILs systems.[19,22] This approach has an evident weakness with regards the overall greenness of chemical processes. Consequently, it is necessary to find alternative strategies for product recovery based on sustainable approaches to overcome this limitation.

In 1999, Brennecke's group discovered that ILs (*e.g.* [Bmim][PF$_6$]) and scCO$_2$ form biphasic systems, being the scCO$_2$ is able to extract hydrophobic compounds previously dissolved into the IL phase.[23] This key feature has opened up new opportunities for developing integral green biocatalytic processes in non-aqueous environments. It permits to combine the excellence of ILs for enzyme catalysis with the classical advantages of scCO$_2$ to extract, dissolve and transport chemicals.[4a,10a,14,24]

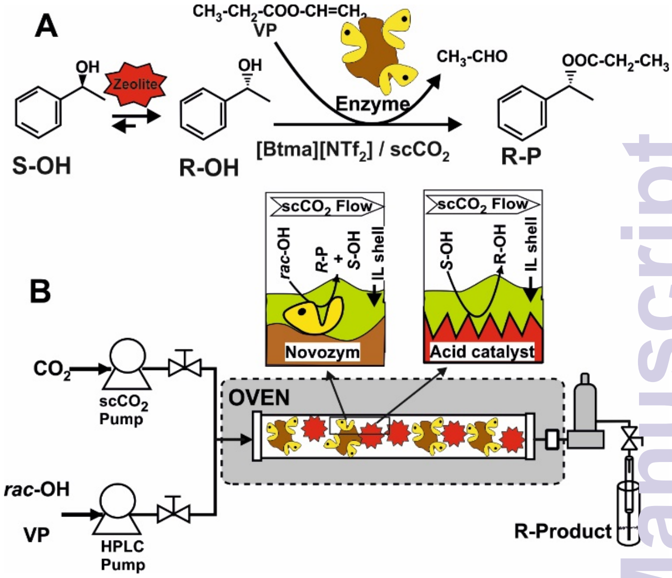

Fig. 3. Scheme of the experimental set-up of Lozano *et al* [26] for the continuous DKR of *rac*-1-phenylethanol (A) carried out by the combined action of immobilized lipase and an acid zeolite chemical catalyst (B), both coated with [Btma][NTf$_2$].

Multiphase biocatalytic systems based on ILs and scCO$_2$ were simultaneously described in 2002 by the groups of Lozano[25a] and Leitner[25b], representing a pioneering operational approach for the development of fully green biocatalytic processes in non-aqueous environments. The scCO$_2$ flow can assist both to transport the substrate(s) to the IL phase containing the biocatalyst, and to extract the product(s) from the IL phase. Subsequently, the products obtained are free from IL, as well as from other organic solvent residues, while CO$_2$ can be recycled by re-compression. Additionally, if the reaction product does not require any further purification, the approach enhances the economic benefit of the process, because the system runs as a black-box able to transform pure substrates into pure products without generating waste. As an example, Fig. 3 shows the continuous DKR process for *rac*-1-phenylethanol in IL/scCO$_2$ biphasic systems, which is catalysed by a cocktail of the immobilized CALB with acid zeolites in a packed bed reactor under scCO$_2$ flow. Both chemical and enzymatic catalysts were previously coated with ILs (*e.g.* [Emim][NTf$_2$], [Btma][NTf$_2$] or [Bmim][PF$_6$]) at a 1:1 (w:w) ratio, to prevent enzyme deactivation by scCO$_2$. The best results (98% product yield, 96% ee) were obtained for [Btma][NTf$_2$], being observed a stable catalytic activity for at least 14 days of continuous operation in scCO$_2$.[26]

A further step towards optimising IL/scCO$_2$ biphasic systems was represented by the immobilization of the ionic liquid species onto solid supports. For instance, using IL-like fragments covalently bonded onto a polymeric support (Supported Ionic Liquid-like Phases or SILLPs).[14,27] The immobilisation of the IL phase facilitates the separation processes and avoids a possible accidental spill to the environment. It reduces the cost of the process since less amount of ILs is employed in the catalytic processes, and enables the development of continuous flow processes, as it was recently reviwed.[4a]

Despite the greenness of technological approaches based on the combination of ILs and supercritical fluids, their industrial application is hindered probably due to the assumption of an excessive cost of high-pressure equipment. The development of fully green chemical processes based on ILs requires the design of new experimental tools allowing a straightforward extraction of products with the highest level of purity possible, and ensuring at the same time the full recovery of the IL for reuse. The inherent advantages of ILs in terms of solvent properties and their ability to provide excellent microenvironments for enzymes during the catalytic action, are not enough to grantee their transference to industrial processes. We need to devise new methodologies that will fulfil the sustainable requirements for product recovery and the reuse of all the elements of the reaction system. Thus, the question to solve is: Can we integrate the use of ILs and their complete separation from the reagents/products with the continuous recovery and reuse of the ILs catalytic phase by an straightforward and cheap methodology?. The answer may lie in the own structure of the ILs.

3. From structure to application as Sponge-like Ionic Liquids

The physicochemical characteristics of ILs depend not only on the coulombic forces between the cation and the anion but also on an extended network of additional interactions (among others H-bonds, π−π or C-H•••π interactions, hydrophobic interactions, etc.).[28] This well-organised network of interactions provides ILs with a nanoscale structure determining their solvent and physical properties (conductivity, viscosity, etc.).[29] These interactions, which define the ILs molecular structure and properties, are highly dependent on the length of the alkyl chain(s) present as well as on the nature of the counterion, and on the substitution pattern.

In this context, according to the unique properties of ILs as neoteric solvents, a new term has recently been coined, *i.e.* sponge-like ILs.[30] This refers to hydrophobic ILs with long alkyl side-chains that behave as temperature switchable ionic liquid/solid phases. It was reported how ILs based on cations with long alkyl side-chains, *e.g.* octadecyltrimethylammonium bis(trifluoromethylsulfonyl) imide $[C_{18}tma][NTf_2]$,[31] 1-ocadecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, ($[C_{18}mim][NTf_2]$,[32] etc., (Fig. 4), are able to dissolve liquid hydrophobic compounds (*e.g.* triolein, geraniol, nerol, citronellol, etc.), forming monophasic liquid systems upon heating above their melting points (see Fig. 5A).[32] It was also observed how then these fully clear liquid solutions, containing both the compound and the IL, became monophasic solid systems after cooling down to room temperature (see Fig. 5B). Furthermore, these solid phases can be separated into two phases by simple centrifugation at a temperature lower than room (see Fig. 5C). The upper liquid phase contained the flavour ester, and bottom solid was the solid IL. The separation can be improved even further by introducing the solid mixture, formed by the hydrophobic compound (*e.g.* anysil acetate) and IL (*e.g.* $[C_{16}tma][NTf_2]$), into a nylon centrifugal filter (0.2 mm pore size). Then, the application of external force at low temperature (*e.g.* 10 min centrifugation, 16,000 rpm, 0ºC) led to the retention of the solid IL phase above the nylon membrane, while the liquid flavour was filtered through the membrane to the bottom of the tube (see Fig.5D), as though wringing out a sponge.[33]

Additionally, to demonstrate the efficiency of this process, both the residual IL content in the liquid phase (using ^{19}F NMR spectroscopy) and the yield of the recovered hydrophobic compound were determined for each of the separation approaches. As an example of the separation by direct centrifugation, the 50/50 (w/w) $[C_{16}tma][NTf_2]$/geranyl acetate mixture can be considered. This mixture, which is liquid at 50ºC and solid at room temperature, could be separated by means of one centrifugation step at 25ºC, but the resulting top liquid phase became solid when incubated in an ice bath, due to the presence of residual IL in the flavour phase. However, by following an iterative centrifugation protocol of four steps (10 min, 14,000 rpm) and lowering the temperature from room (25ºC approx.) to 4ºC, the geranyl acetate can be separated as an upper IL-free liquid phase (confirmed by ^{19}F NMR), while the solid IL remained at the bottom of the tube.[32] It should be noted that the

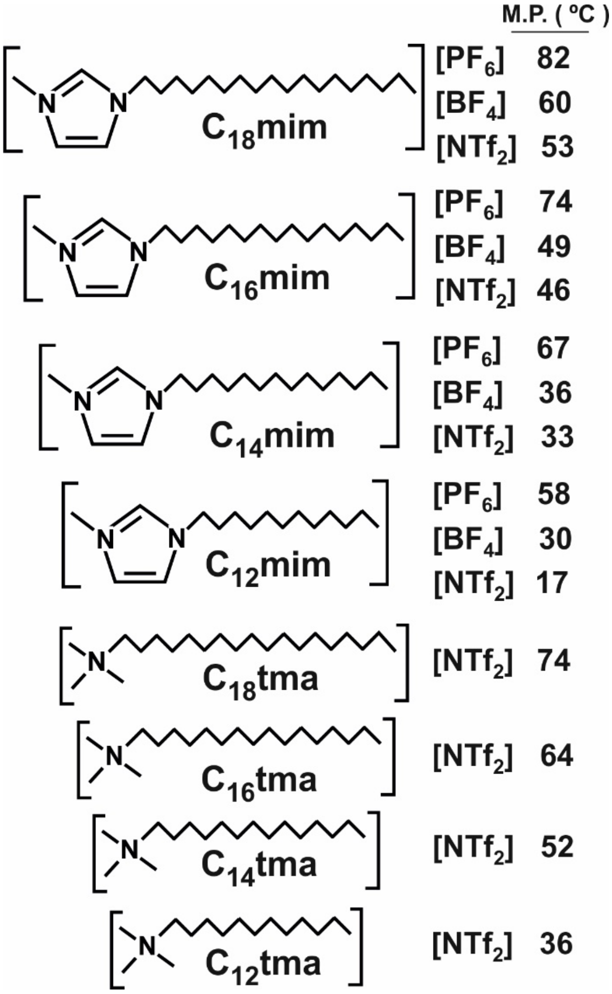

Fig. 4. Structure and melting point of different SLILs used as reaction media for biocatalytic reactions.[30,32,49b]

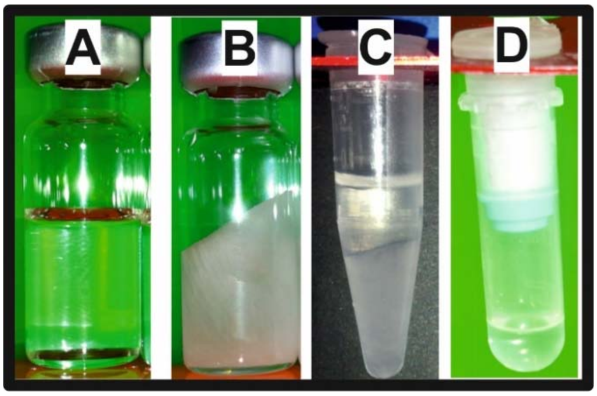

Fig. 5. Phase behaviour of 50/50 (w/w) neryl acetate/$[C_{16}tma][NTf_2]$ mixture at 50ºC (**A**), 25 ºC (**B**), and after four consecutive centrifugation steps at 14,000 rpm (15 mim) at room temperature, 21, 10 and 4ºC (**C**), respectively.[32] Phase behaviour of 30/70 (w/w) anisyl acetate acetate/[C16tma][NTf2] mixture after centrifugation at 16,000 rpm (15 mim) through a nylon filter (0.2mm pore size) (**D**).[33]

yield of the hydrophobic compound recovered by decantation from the [C_{16}tma][NTf_2] solid phase was dependent on the flavour (*i.e.* 51% geranyl acetate, 82% isoamyl acetate, 59% citronellyl acetate, or 60% neryl acetate). However, the use of nylon centrifugal filters improved the separation and provided flavour compound yields of up to 95 % (w/w). [33]

It should be underlined how the components of any monophasic solutions of molecular solvents (*e.g.* 50% v/v ethanol/water, etc.), which become solid though freezing, cannot be separated by centrifugation. Indeed, the separation processes of a homogeneous solution of molecular solvents usually require heating steps (*e.g.* distillation). The unique feature of SLILs permits the extraction of hydrophobic molecules dissolved therein by cooling/centrifugation can be explained as being a function of the solid/liquid structural organization of ILs, and the interaction with the solute induced by these structural characteristics of ILs.

Although many papers have focused on the liquid phase behaviour of ILs with organic compounds,[34] deeper studies on solid/liquid phase transitions of ILs containing dissolved substances should be carried out. In fact, the structural organisation of the ILs may be seen as intermediate between that of a classical solvent and that of a liquid crystal. Although, this degree of organisation is greater and clearly defined for ILs in the solid state, it is also maintained, at least partially, in the liquid phase. Moreover, ILs can preserve, at great extent, this associated structure even when they are mixed with other compounds.[35] Fundamental structural studies evidence that ILs can segregate into polar and non-polar domains over an intermediate length scale of typically 8–20 Å, where the polar domains are formed by the head groups of the cations and anions and of nonpolar domains are formed by the alkyl groups. These domains have been characterised by different experimental techniques as well as by theoretical simulations, which provides good insight into the degree of segregation and the possible morphology of the domains.[36] ILs can be regarded as nano-structured materials, allowing neutral molecules to reside in less polar regions, while ionic or polar species undergo faster diffusion in the more polar ones.[33] Hence, the nanoscale structural heterogeneity could influence not only the ILs properties but also transport properties and availability of reagents and catalysts.

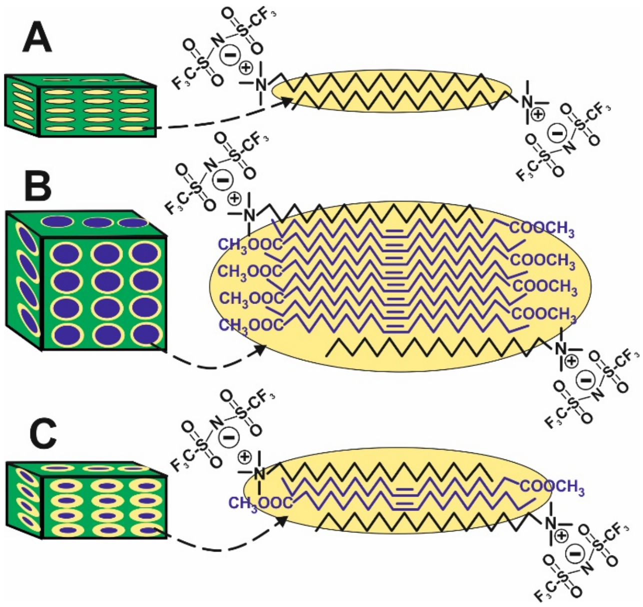

Fig. 6. Schematic representation of the sponge-like IL hypothesis, showing the [C_{18}tma][NTf_2] net as a dry sponge (**A**), a sponge swollen with methyl oleate (**B**), and a “wet” sponge after wringing out by centrifugation (**C**). Reproduced by permission of the Royal Society of Chemistry.[30]

In the same context, Hayes *et al*[37a] studied the local and long-range bulk liquid structures of several primary alkylammonium protic ionic liquids (PILs) (*i.e.* ethylammonium hydrogen sulfate, ethylammonium formate, etc.) by using neutron diffraction and computer simulations. These authors described how the structure of these PILs can arrange into a sponge-like bicontinuous nanostructure consisting of polar and apolar domains where the lengthening of the cation alkyl chain leads to nanostructures in which the polar and apolar domains are better segregated, while changes in the anion have little effect on their structure. It was also reported that pyrrolidinium-based ILs are organized as an intricately nanostructured net, where the IL containing cations with shorter alkyl chain substituents form alternating cation−anion monolayer structures on confinement to a thin film, whereas a cation with a longer alkyl chain substituent leads to bilayer formation.[37b] Other authors reported how 1-alkyl-3-methylimidazolium ILs (*e.g.* butyl, octyl or decyl alkyl chain) can be observed by metastable atom electron spectroscopy, to be organized as a double-layered structure at the surface by using metastable atom electron spectroscopy, which consists of an alkyl chain layer and a polar layer containing anions and imidazolium rings.[37c] Ohno and co-workers reported several interesting poly(IL)s showing temperature-dependent phase behaviour after mixing with pure water, and also with aqueous salts solutions. These poly(IL)s with suitable hydrophobicity undergoes a strongly temperature-sensitive LCST-type phase transition with water, and the resulting hydrated poly(IL) further undergoes a sharp and reversible liquid-to-gel transition at ambient temperatures, being applied for extraction processes.[38]

In agreement with the structural studies above mentioned, these unique features can be schematized according to Fig 6, being a consequence of ability of the well-defined structure of these ILs to be modified by incorporating the non-polar solute in specific regions that become expanded. The use of an external mechanical force would expel the substrate out of these regions. It is known that the large alkyl side chains of cations can lead to smectic mesophase structures induced by solvophobic interactions between the long alkyl chains producing nanoscale heterogeneities or sponge-like structures.[39] Although the interaction between the alkyl chains should be the main driver force, columbic and H-bond interactions could also play a role to define the overall structural self-assembly either in solid or liquid phase.[28] The hydrophobic pockets formed in the smectic mesophase are likely to accommodate the non-polar compounds (*e.g.* methyl oleate). As schematized in Fig. 6A, the hydrophobic interactions between methyl oleate molecules and the alkyl chain of the IL allows the sponge-like structure of the ILs “to be soaked” in the liquid phase (Fig. 6B). The IL-phase is likely to induce the gel formation of the non-polar solute within the non-

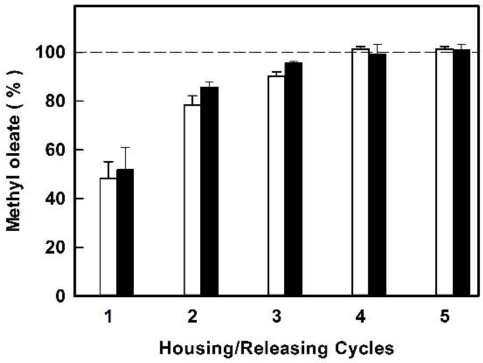

Fig. 7. Methyl oleate (MO) extracted from 55/45 (w/w) MO/[C_{18}tma][NTf_2] (white bars) or [C_{16}tma][NTf_2] (black bars) sponge-like-IL mixtures during five consecutive soak/wring cycles using the cooling/centrifugation protocol with washing-by-water step. After each cycle, the resulting solid IL phase was used to dissolve a new MO sample at 60ºC. Reproduced by permission of the Royal Society of Chemistry.[30]

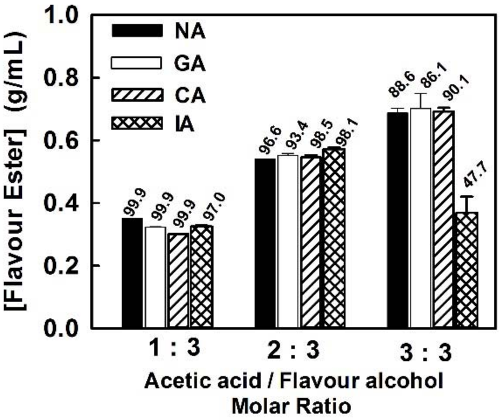

Fig. 8. Flavour ester (NA, neryl acetate; GA, geranyl acetate; CA, citronellyl acetate; IA, isoamyl acetate) concentration in the top phase, as results of the esterification reaction catalysed by immobilized lipase in 60% (w/w) [C_{16}tma][NTf_2] by using different acetic acid/flavour alcohol molar ratio as substrates (reaction product yield in top of bars), then separated by the cooling/centrifugation approach.[32]

polar region of the solid ILs-phase. The thixotropic nature of this gel favours its progressive breakdown on shearing induced by the centrifugation. Thus, the centrifugation force is enough to induce the segregation of the hydrophobic solute from the solid mixture allowed "to wring the soaked sponge" with a partial recovery of the compound leading to a "wet sponge"-like IL (Fig. 6C).[30]

The IL net could be considered as a nano-sponge with holes of variable volume, which are suitable for housing or releasing hydrophobic molecules as a function of their liquid or solid phase, respectively. The use of centrifugal filters in this step improves the efficiency of the sponge wringing, because of the resulting higher product yield with respect to the centrifugation process without a coupled filtration.[31] As this behaviour resembles to a sponge wringing, we have named these systems as sponge-like ILs (SLILs).

The ability of these sponge-like ILs "to soak up" hydrophobic compounds (i.e. methyl oleate) and then to be "wrung out" by the centrifugation protocols was also demonstrated applying consecutive soak/wring cycles to a homogeneous methyl oleate/IL mixture (55/45 w/w).[30] The solid IL phase was iteratively used to dissolve a new methyl oleate sample. The extraction yield was determined at the end of each cycle (Fig. 7). As can be seen for both ([C_{16}tma][NTf_2] and [C_{18}tma][NTf_2] SLILs, the amount of methyl oleate released from the solid IL phase increased for the three initial operation cycles, and then remained constant for the following cycles during which the dissolved methyl oleate was fully extracted. These results reflect a sponge-like behaviour, whereby three initial cycles of "soaking/wringing", provide a "wet sponge"-like IL able to fully release all the new methyl oleate during the centrifugation step.

Additional features also push the excellent suitability of these SLILs for developing selective separation. The insolubility in water of the SLILs, as well as the melting points above room temperature displayed by these compounds, allows their immediate precipitation as solids and their easy full recovery after a possible accident during handling. The reasonable criticism regarding the non-green character of ILs against an environmental contamination produced by an accident are clearly mitigated. Additionally, the non-volatile character of these ILs can be regarded as an important advantage for a final step of products refining, *e.g.* classical distillation process. Furthermore, these ILs presenting a sponge-like behaviour can be used to develop straightforward synthetic protocols for the synthesis of pure products and their separation and isolation.[31] This excellent suitability will be illustrated by two different examples of biocatalytic processes, such as the synthesis of terpene esters,[32] and the synthesis of biodiesel.[30]

4. Biocatalytic production of flavour esters in Sponge-Like Ionic Liquids

Most fragrance compounds are obtained by chemical synthesis, although the demand for natural products is continuously growing. However, natural flavours extracted from plant materials are often too scarce or expensive for industrial use. Hence there is a demand to develop new methods for their production based on "natural" criteria and green approaches suitable for industrial scaling-up.[40] In this context, US and European legislation dictate that "natural" fragrance substances can only be prepared by physical processes (*e.g.* extraction) from natural sources, or by enzymatic or microbial transformation of precursors isolated from nature.[41]

Lipases are among the most important biocatalysts for the synthesis of valuable flavour and fragrance esters by esterification or transesterification approaches in reaction media with a low water content.[42] However, the use of non-natural substrates (*e.g.* acetic anhydride,[43] alkyl vinyl esters,[44] etc.) in transesterification is a clear breakdown in any attempt to obtain "natural" products. The enzyme-catalyzed direct esterification between "natural" substrates could be considered as the best way to obtain "natural" ester products, although the fast enzyme deactivation that occurs as a results of the high carboxylic acid concentration usually needed is a clear drawback for any industrial application.

The suitability of the SLIL [C_{16}tma][NTf_2] as a reaction medium for carrying out the enzymatic synthesis of different flavour esters (*e.g.* neryl acetate, geranyl acetate, citronellyl acetate and isoamyl acetate), by direct esterification of acetic acid and the corresponding alcohol, was studied at 50ºC. All the reactions were assayed at 60% (v/v) SLIL concentration, and at three different acetic acid/flavour alcohol molar ratio (*i.e.* 1/3, 2/3 and 3/3 mol/mol). The obtained product yields range between 90-100% after 4h reaction. The isolation of product esters was performed by a cooling/centrifugation approach allowing the straightforward extraction of the embedded products from the solid SLIL phase. Fig. 8 shows the resulting product concentration in the upper SLIL-free phase for each reaction media, which was practically independent on the nature of the flavour alcohol.[32]

In another example, the synthesis of anisyl acetate, a fragrance ingredient used in many cosmetic and fine fragrance products, was assayed by the direct esterification of acetic acid with anisyl alcohol (at a 1/1 mol/mol acid/alcohol concentration ratio) catalysed by lipase in 70% (w/w) [C_{16}tma][NTf_2]. In this case, the suitability of the SILL reaction system was enhanced by the assistance of MW irradiation (*i.e.* up to 82 % anisyl acetate yield in 1 h reaction), while the product extraction was improved by the use of centrifugal filter (up to 95% extraction yield).[33]

In addition to these excellent features, the key criteria for scaling-up any biocatalytic process for flavour ester production are the operational stability of the enzyme and the recycling of the SLIL. For both reported examples, the remaining solid IL/immobilized lipase system was successfully applied for cyclic protocols of flavour synthesis/product extraction. Noteworthy, the enzyme activity was maintained unchanged upon recycling. The protective effect of hydrophobic ILs, based on [NTf_2] anion and alkyltrimethylammonium or 1-alkyl-3-methylimidazolium cations, has been related to the ability of these ILs to maintain the native structure of proteins even under harsh conditions.[45]

Thus, the structural features of the ILs not only provided and excellent stabilisation medium for enzymatic reaction, but also a simple method for the isolation of the pure products and the recycling and reuse of the catalytic systems (enzyme/SLILs) by centrifugation and/or filtration. The ability of these SLILs to melt at temperatures compatible with enzyme catalysis permitted the development of a simple and easy green method for flavour ester synthesis integrated, at the same time, with clean separation protocols.

5. Biocatalytic production of biodiesel in Sponge-Like Ionic Liquids

Biodiesel is a diesel substitute fuel, composed of fatty acid methyl esters (*e.g.* methyl oleate) and obtained from renewable sources (*e.g.* vegetable oils, fats, etc.) by catalytic transesterification with primary aliphatic alcohols (*e.g.* methanol). The interest in producing biodiesel by though clean and sustainable approaches including biocatalysis is doubtless, because of the improved quality of the final product, as well as the reduction in wastewater production during the separation and purification steps.[46]

The non-miscibility between vegetable oils and methanol is one of the most important limitations for the catalytic production of biodiesel. The presence of two-phase liquid systems reduce process efficiency, and for the case of biocatalysts, resulted in fast and full biocatalyst deactivation as a result of a by the direct contact of the enzyme with the methanol phase.[47] The biocatalytic synthesis of biodiesel in ILs has been reported, by using ILs based on short alkyl chain 1,3-dialkylimidazolium cations (*i.e.* [Bmim][PF_6], Bmim][NTf_2]), etc.).[48] All these ILs were unable to dissolve triglycerides, thus resulting in two-phase reaction media leading to biphasic reaction media with moderate reaction efficiency l (up to 90 % biodiesel yield for 24 h at 60 ºC).[48a]

In accordance with the statement *like-dissolves-like*, ILs based on hydrophobic anions and cations with large alkyl side-chain(s) (*e.g.* [C_{18}mim][NTf_2]) were able to dissolve both vegetable oil (*e.g.* triolein, etc.) and methanol. Thus, they can provide one-phase reaction medium that showed excellent suitability for the biocatalytic synthesis of biodiesel (up to 96% yield in 6 h at 60 ºC for the [C_{18}mim][NTf_2] case).[49] Furthermore, this excellent suitability of SLILs can be extended to the protection of the enzyme against deactivation (*i.e.* up to 1370 days half life time in [C_{18}tma][NTf_2] at 60ºC).[32]

In the search of sustainable synthetic protocols for an industrial application of this biocatalytic reaction system, needs to include a clean approach to extract the synthesized biodiesel and the by-product glycerol, and involve the full recovery and reuse of the SLIL/biocatalytic system should be developed. In an attempt to customize the cooling/centrifugation approach for separating the products from the SLIL in biodiesel synthesis, an initial washing-by-water step on the reaction media as liquid phase before cooling to room temperature was required (see Fig. 9). The semisolid heterogeneous mixtures obtained were then separated by following an iterative cooling/centrifugation protocol (*i.e.* 15,000 rpm 1 h at room temperature, 23 and 15ºC, respectively), which now resulted in three phases: an upper IL-free biodiesel phase, a middle IL-free liquid aqueous phase containing the glycerol, and a bottom solid containing the SLIL (Fig. 9C). The presence of water, a green molecular solvent (non-miscible with the biodiesel and the SLIL), improved the separation of all phases, providing an easy and sustainable way to separate both glycerol and non-reacted methanol from the biodiesel. Once again, the suitability of the proposed methodology for biodiesel synthesis was demonstrated by the. full recovery and reuse of the SLIL/biocatalyst system is depicted in Fig. 9D. Using this approach, an excellent

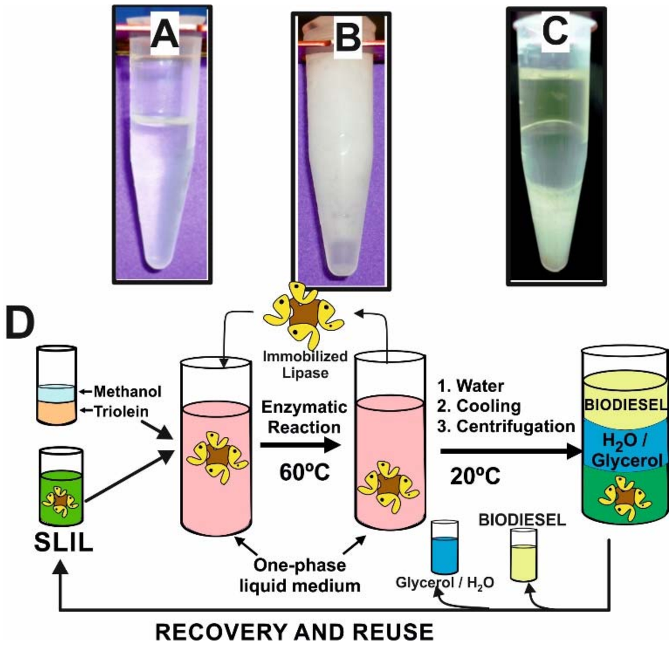

Fig. 9. Phase behaviour of the reaction mixture containing both methyl oleate and glycerol products at 60°C (A), 25ºC after addition of water (B), and after three consecutive centrifugation steps at 15,000 rpm (1 h) at room temperature, 23 and 15ºC, respectively (C). Cyclic protocol for the biocatalytic synthesis and purification of biodiesel in sponge-like IL phases, including the full recovery and reuse of the enzyme/IL system (D).[30]

biocatalytic activity (96-98% biodiesel yield) was obtained, being maintained practically unchanged for 12 consecutive operation cycles. It should be noted that enzyme particles were separated from the reaction mixture after each catalytic cycle, and then washed with a small amount of t-butanol to eliminate the glycerol shell around particles acting as catalyst poison

Conclusions

The development of a network of green chemical processes will depends on the implementation of new integrated reaction / separation systems, which should be simple, effective and sustainable at the same time. Nature provides us with the most powerful toolbox of catalysts for carrying out selective, clean and sustainable transformations. By using enzymes in non-aqueous environments, rather than in their natural aqueous reaction media, their technological applications are greatly enhanced because of the resulting expansion in the repertoire of enzyme-catalysed transformations.

The excellence of hydrophobic ILs to provide appropriate microenvironments for enzyme catalysis, has been supplemented by the phase behaviour of ILs based on cations with long alkyl chains, also named Sponge-Like Ionic Liquids (SLILs). The sponge-like character of these ILs allows the development of easy and green methods for efficiently separating a hydrophobic organic compound (*e.g.* flavours esters) from a homogeneous ionic liquid/organic compound mixture using a simple cooling and centrifugation approach, as opposed to the usual heating step applied in classical separation processes in Chemical Engineering (*e.g.* distillation). All these features has allowed two-step protocols to be developed for producing high added value compounds (*e.g.* flavour esters and biodiesel), with the benefit of: (i) enzyme-catalyzed reactions with a product yield close to 100%, and (ii) clean separation of the reaction products by a cooling/centrifugation method.

The unique ability of these Sponge-Like Ionic Liquids, as temperature switchable liquid/solid phase systems, opens up a new sustainable platform for the chemical synthesis and separation of liquid products. The implementation of all these features in multi-enzymatic and/or multi-chemoenzymatic chemical transformations, mimicking the metabolic pathways found in nature, and the isolation of pure products by means of an easy and clean approach, like that described for SLILs, since is a dream that is becoming closer, since the door to a true green chemical industry is quite open.

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Notes and references

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1 P. T. Anastas and J. C.Warner, *Green Chemistry: Theory and Practice*, Oxford University Press: New York, 1998.

2 P. T. Anastas and J. B. Zimmerman, *Environ. Sci. Technol*, 2003, **37**, 94A-101A

3 a) R. A. Sheldon, *Green Chem*. 2014, *16*, 950-963; b) P. Pollet, E. A. Davey, E. E. Ureña-Benavides, C. A. Ecker and C. L. Liotta, *Green Chem.*, 2014, **16**, 1034-1055; c) R. S. Varma. *Green Chem*. 2014, **16**, 2027-2041.

4 a) E. García-Verdugo, B. Altava, M. I. Burguete, P. Lozano and S. V. Luis, *Green Chem*. 2015, **17**, 2693-2713; b) P. G. Jessop, F. Ahmadpour, M. A. Buczynski, T. J. Burns, N. B. Green II, R. Korwin, D. Long, S. K. Massad, J. B. Manley, N. Omidbakhsh, R. Pearl, S. Pereira, R. A. Predale, P. G. Sliva, H. VanderBilt, S. Weller and M. H. Wolf, *Green Chem*. 2015, **17**, 2664-2678.

5 R. A. Sheldon, *Chem. Commun*. 2008, 3352-3365.

6 R. A. Sheldon, I. Arends and U. Hanefeld, *Green Chemistry and Catalysis*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007.

7 a) P.T. Anastas and R. Crabtree (Eds), *Handbook of Green Chemistry-Green Catalysis: Biocatalysis*, vol.3, Wiley-VCH Verlag GmbH, New York, 2009; b) C. M. Clouthier and J. N. Pelletier. *Chem. Soc. Rev*. 2012, **41**, 1585-1605;

8 a) J. H. Clark and S. J. Tavener, *Org. Proc. Res. Develop*. 2007, **11**, 149-155; b) C. J. Li and B. M. Trost, *Proc. Natl. Acad. Sci. USA*, 2009, **105**, 13197-13202.

9 a) A. D. Curzons, D. J. C. Constable, D. N. Mortimer and V. L. Cunningham, *Green. Chem*., 2001, **3**, 1-6; b) D. J. C. Constable, A. D. Curzons and V. L. Cunningham, *Green. Chem*., 2002, **4**, 521-527; c) E. Buncel, R. Stairs, H. Wilson, *The Role of the Solvent in Chemical Reactions*; Oxford University Press: Oxford, New York, 2003.

10 a) P. Lozano. *Green Chem*. 2010, **12**, 555-569; b) C. J. Li and P. T. Anastas. *Chem. Soc. Rev*. 2012, **41**, 1413-1414

11 a) J. Dupont, R. F.de Souza and P. A. Z. Suarez, *Chem. Rev*. 2002, **102**, 3667-3692; b) P. Wasserscheid and T. Welton, (Eds) *Ionic Liquids in Synthesis*, Wiley-VCH, Weinheim, 2008; c) H. P. Steinruck and P. Wasserscheid, *Cat. Lett*. 2015, **145**, 380-397.

12 W. Leitner. *Acc. Chem. Res*. 2002, *35*, 746-756; W. Leitner and P. G. Jessop (Eds), *Handbook of Green Chemistry-Green Solvents: Supercritical Solvents*, vol.4, Wiley-VCH Verlag GmbH, New York, 2010

13 R.A. Sheldon. *Chem. Soc. Rev*. 2012, **41**, 1437-1451.

14 P. Lozano, E. Garcia-Verdugo, S.V. Luis, M. Pucheault, M. Vaultier, *Curr. Org. Synth*. 2011, **8**, 810-823.

15 a) N. J. Turner, *Nature Chem. Biol*., 2009, 5, 567-573; b) A. S. Bommarius and M. F. Paye, *Chem. Soc. Rev*. 2013, **42**, 6534-6565.

16 a) A. S. Bommarius and B.R. Riebel (Eds), *Biocatalysis*, Wiley-VCH Verlag GmbH & Co. KGaA, 2004; b) Z. Guan, L. Y. Li, Y. H. He, *RSC Adv*, 2015, **5**, 16801-16814.

17 a) A.M. Klibanov. *Nature* 2001, **409**, 241-246; b) H. L. Yu, L Ou, J. H. Xu. *Curr. Org. Synth*. 2010, **19**, 1424-1432; c) M. J. Liszka, M. E. Clark, E. Schneider and D. S. Clark. *Annu. Rev. Chem. Biomol. Eng*. 2012, **3**, 77-102.

18 H. Frauenfelder, G. Chen, J. Berendzen, P. W. Fenimore, H. Jansson, B. H. McMahon, I. R. Stroe, J. Swenson, R. D. Young, *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 5129-5134.

19 a) F. van Rantwijk and R.A. Sheldon. *Chem. Rev*. 2007, **107**, 2757-2785; b) P. Dominguez de Maria, *Angew. Chem. Int. Ed*. 2008, **47**, 6960-6968; c) T. Itoh. *Ionic Liquids - Current State of the Art*. S. Handy (Ed.), InTech, 2015; d) M. Moniruzzaman, N. Kamiya and M. Goto. *Org. Biomolec. Res.* 2010, **8**, 2887-2899; e) M. Naushad, Z. A. Alothman, A. B. Khan and M. Ali. *Int. J. Biol. Macromol*. 2012, **51**, 555-560.

20 P. Bonhote, A. P. Dias, N. Papageorgiou, K. Kalyanasundaram and M. Gra1tzel. *Inorg. Chem*. 1996, **35**, 1168-1178.

21 T. Itoh, E. Akasaki and Y. Nishimura. *Chem. Lett*. 2002, 154-155

22 a) S. Oppermann, F. Stein and U. Kragl. *Appl. Microbiol. Biotechnol*. 2011, **89**, 493–499; b) R. L. Souza, S. P. M. Ventura, C. M. F. Soares, J. A. P. Coutinho and A. S. Lima, *Green Chem*. 2015. DOI: 10.1039/C5GC00262A.

23 L.A. Blanchard, D. Hancu, E.J. Beckman and J.F. Brennecke. *Nature*, 1999, **399**, 28-29;

24 a) S. Keskin, D. Kayrak-Talay, U. Akman and O. Hortaçsu. *J. Supercrit. Fluids*, 2007, **43**, 150-180; b) S. Cantone, U. Hanefeld and A. Basso. *Green Chem*. 2007, **9**, 954–971; c) M. Roth. *J. Chromatogr. A*, 2009, **1216**, 1861-1880.

25 a) P. Lozano, T. De Diego, D. Carrie, M. Vaultier and J.L. Iborra. *Chem. Commun*. 2002, 692-693; b) M.T. Reetz, W. Wiesenhofer, G. Francio and W. Leitner. *Chem. Commun*. 2002, 992-993.

26 P. Lozano, T. De Diego, C. Mira, K. Montague, M. Vaultier and J.L. Iborra. *Green Chem*. 2009, **11**, 538-542.

27 a) P. Lozano, E. Garcia-Verdugo, R. Piamtongkam, N. Karbass, T. De Diego, M.I. Burguete, S.V. Luis and J.L. Iborra. *Adv. Synth. Catal*. 2007, **349**, 1077-1084; b) P. Lozano, E. García-Verdugo, N. Karbass, K. Montague, T. De Diego, M.I. Burguete and S.V. Luis, *Green Chem*. 2010, **12**, 1803-1810; c) P. Lozano, E. García-Verdugo, J. M. Bernal, D.F. Izquierdo, M.I. Burguete, G. Sánchez-Gómez, S.V. Luis. *ChemSusChem*, 2012, **5**, 790-798.

28 a) J. Dupont, *J. Braz. Chem. Soc*. 2004, **15**, 341-350; b) J. Dupont. *Acc. Chem. Res*. 2011, **44**, 1223-1231. c) J. Dupont and M. N. Eberlin, *Curr. Org. Chem*. 2013, **17**, 257-272.

29 J. N. A. Canongia-Lopes and A. A. H. Padua. *J. Phys. Chem. B*, 2006, **110**, 3330-3335.

30 P. Lozano, J.M. Bernal, G. Sánchez-Gómez, G. López-López, M. Vaultier. *Energy Environ. Sci*, 2013, **6**, 1328-1338.

31 a) S. K. Ritter, *Chem. Eng. News*, 2013, **91**, 34–35; b) P Lozano, J. M. Bernal, C. Gomez, E. García-Verdugo, M. I. Burguete, G. Sánchez, M. Vaultier, S. V. Luis. *Catal. Today*, 2015, doi:10.1016/j.cattod. 2014.08.025.

32 P. Lozano, J.M. Bernal and A. Navarro. *Green Chem*. 2012, **14**, 3026 3033.

33 P. Lozano, J. M. Bernal, A. Lajarin, D. Romera, E. Garcia-Verdugo, G. Sanchez-Gomez, M. Pucheault, M. Vaultier, M. I. Burguete, S. V. Luis. *Curr. Green Chem*. 2014, **1**, 145-154.

34 a) A. Chapeaux, L. D. Simoni, T. S. Ronan, M. A. Stadtherr, and J. F. Brennecke, *Green Chem*. 2008, **10**, 1301-1306; b) J. M. Crosthwaite, M. J. Muldoon, S. N. V. K. Aki, E. J. Maginn and J. F. Brennecke, *J. Phys. Chem. B*, 2006, **110**, 9354-9361; c) K. Anderson, H. Rodriguez and K. R. Seddon, *Green Chem*. 2009, **11**, 780-784.

35 H. K. Stassen, R. Ludwig, A. Wulf, and J. Dupont. *Chem. Eur. J*. 2015, **21**, 1-13.

36 a) H. Weingärtner, H. *Angew. Chem. Int. Ed*. 2008, **47**: 654–670; b) S. Chen, S. Zhang, X. Liu, J. Wang, J. Wang, K. Dong, J. Sun and B. Xu *Phys. Chem. Chem. Phys*. 2014, **16**, 5893- 5906

37 a) R. Hayes, S. Imberti, G. G. Warr and R. Atkin, *J. Phys. Chem. C*, 2014, **118**, 13998−14008; b) A. M. Smith, K. R. J. Lovelock, N. N. Gosvami, P. Licence, A. Dolan, T. Welton, S. Perkin, *J. Phys. Chem. Lett.* 2013, **4**, 378-382; c) T. Iwahashi, T. Nishi, H. Yamane, T. Miyamae, K. Kanai, K. Seki, D. Kim, Y. Ouchi, *J. Phys. Chem. C*, 2009, **113**, 19237-19243.

38 Y. Kohno, H. Arai, S. Saita, H. Ohno, *Aust. J. Chem*. 2011, **64**, 1560-1567; Y. Kohno, Y. Deguchi, H. Ohno, *Chem. Commun*. 2012, **48**, 11883-11885; Y. Deguchi, Y. Kohno, H. Ohno, *Chem. Lett*. 2015, **44**, 238-240.

39 T L. Greaves and C J. Drummond, *Chem. Soc. Rev*. 2013, **42**, 1096-1120.

40 S. Serra, C. Fuganti and E. Brenna, *Trends Biotechnol*., 2005, **23**, 193-198.

41 a) *US Code of Federal Regulations*, 1985, 21CFR 101.22, Food an Drug Administration, Washington, D.C. b) *The Council of the European Communities*, 1988, Council Directive 88/388/EEC.

42 a) K. P. Dhake, D. D. Thakare and B. M. Bhanage, *Flavour Frag. J*. 2013, **28**, 71-83; b) R. C. Rodrigues and R. Fernandez-Lafuente, *J. Mol. Catal. B: Enzym*. 2010, **64**, 1-22.

43 a) H. Ghamgui, M. Karra-Chaabouni, S. Bezzine, N. Miled and Y. Gargouri, *Enzyme. Microb. Technol*. 2006, **38**, 788-794; b) M. D.

Romero, L. Calvo, C. Alba and A. Daneshfar, *J. Biotechnol*. 2007, **127**, 269-277.

44 a) P. Lozano, R. Piamtongkam, K. Kohns, T. Diego, M. Vaultier and J. L. Iborra. *Green Chem*. 2007, **9**, 780-784; b) A. Guvenc, N. Kapucu, H. Kapucu, O. Aydogan and U. Mehmetoglu, *Enzyme Microb. Technol*. 2007, **40**, 778-785; c) P. You, E. Su, X. Yang, D. Mao and D. Wei. J. *Mol. Catal. B: Enzym*. 2011, **71**, 152-158.

45 a) P. Lozano, T. De Diego, D. Carrie, M. Vaultier and J. L. Iborra, *Biotechnol. Lett*., 2001, **23**, 1529-1533; b) P. Lozano, T. De Diego, S. Gmouh, M. Vaultier and J. L. Iborra, *Biotechnol. Prog*. 2003, **19**, 380-382; c) T. De Diego, P. Lozano, S. Gmouh, M. Vaultier and J. L. Iborra, *Biomacromolecules*, 2005, **6**, 1457-1464.

46 J. Dupont, P. A. Z. Suarez and M. R. Meneghetti, *Energ. Environ. Sci*. 2009, **2**, 1258-1265; d) R. Luque, J. C. Lovett, B. Datta, J. Clancy, J. M. Campelo and A. A. Romero, *Energ. Environ. Sci*. 2010, **3**, 1706-1721; c) A. Guldhe, B. Singh, T. Mutanda, K. Perrnaul, F. Bux, *Renew. Sust. Energ. Rev*, 2015, **41**, 1447-1464.

47 a) J. M. Bernal, P. Lozano, E. Garcia-Verdugo, M. I. Burguete, G. Sanchez-Gomez, G. Lopez-Lopez, M. Pucheault, M. Vaultier and S. V. Luis, *Molecules*, 2012, **17**, 8696-8719; b) M. Lotti, J. Pleiss, F. Valero, P. Ferrer, Biotechnol. J., 2015, 10, 22-30.

48 a) S. H. Ha, M. N. Lan, S. H. Lee, S. M. Hwang and Y. M. Koo, *Enzyme Microb. Technol*, 2007, **41**, 480-483; b) M. Gamba, A. A. M. Lapis and J. Dupont, *Adv. Synth. Catal*. 2008, **350**, 160-164; c) N. Muhammad, Y. A. Elsheikh, M. I. A. Mutalib, A. A.Bazmi, R. A. Khan, H. Khan, S. Rafiq, Z. Man, I. Khan, J. Ind. Eng. Chem. 21, 1-10.

49 a) P. Lozano, J. M. Bernal, R. Piamtongkam, D. Fetzer and M. Vaultier, *ChemSusChem*, 2010, **3**, 1359-1363; b) P. Lozano, J. M. Bernal and M. Vaultier, M, *Fuel*, 2011, **90**, 3461-3467; c) T. de Diego, A. Manjon, P. Lozano, M. Vaultier and J. L. Iborra. *Green Chem*, 2011, **13**, 444-451.