(Bio) Catalytic Continuous Flow Processes in $scCO_2$ and/or ILs: Towards Sustainable (Bio)Catalytic Synthetic Platforms

Pedro Lozano[1,*] Eduardo Garcia-Verdugo[2,3] Santiago V. Luis[3] Mathieu Pucheault[4] and Michel Vaultier[4]

[1]*Departamento de Bioquímica y Biología Molecular "B" e Inmunología. Facultad de Química. Universidad de Murcia. Murcia. Spain*

[2]*Instituto de Catálisis y Petroleoquímica, CSIC, Campus de la UAM, C/ Marie Curie, 2; Cantoblanco; Madrid; E-28049, Spain*

[3]*Dpto. Química Inorgánica y Orgánica Department, Universidad Jaume I, Avda. Sos Baynat s/n, 12071, Castellon, Spain*

[4]*Laboratoire Chimie et Photonique Moléculaires. UMR-CNRS 6510, Université de Rennes 1, Campus de Beaulieu, Av. General Leclerc, 35042, Rennes, France*

Abstract: This review highlights the tool box for the development of continuous green/sustainable processes aimed at the synthesis of fine chemicals. By combining either chemical and/or biological catalysts with biphasic systems based on neoteric solvents, *e.g.* ionic liquids (ILs) and supercritical carbon dioxide ($scCO_2$), interesting alternatives to organic solvents for designing continuous clean (bio)transformations methods are growing that directly provide pure products. The classical advantages of $scCO_2$ –its ability to extract, dissolve and transport chemicals– are complemented by the high catalytic efficiency of enzymes in ILs. Enzyme behavior in $scCO_2$ and ILs, as well as the phase behavior of ILs/$scCO_2$, are key parameters for carrying out integral green bioprocesses in continuous operation. The preparation, main characteristics and advantages of monolithic microreactors and miniflow reactors for the synthesis of fine chemicals by continuous flow (bio)catalytic processes are underlined. Examples where the use of continuous flow techniques for multi-step synthesis enables multiple reaction steps to be combined into a single continuous operation are provided.

Keywords: Biocatalysis, supercritical fluid, ionic liquids.

1. INTRODUCTION: TOWARDS SUSTAINABLE SYNTHETIC PLATFORMS.

One of the major challenges to be faced by the chemical industry is the development of more efficient and environmentally friendly synthetic processes. In this context, great efforts have been made aiming at process improvement, to fulfil increasing demands for sustainable manufacture and chemical products applications. Reduction and/or elimination of hazardous substances, such as volatile organic solvents (VOS) is one of the main endeavour [1, 2]. Indeed, VOS often account for the vast majority of mass wasted in synthetic processes [3]. Furthermore, many conventional solvents are toxic, flammable, and/or corrosive. Their volatility and solubility contribute to air, water and land pollution, increase the risk of workers' exposure, and can lead to serious accidents. Thus, in an effort to address all those shortcomings, chemists started to search for safer new environmentally benign solvents or green solvents, which could be easily be recovered - recycled and would still allow performing efficient catalysis. These alternative solvents should fulfil a series of environmental, health and safety requirements while contributing to process and chemical performance (see Fig. **1**). In this respect, ionic liquids (ILs) [4], supercritical fluids (SCFs) [5], and the synergetic combination of both neoteric solvents has emerged as a arguable answer. As such, they received an increasing attention in the past few years by both industry and academia as real solvent alternative [6]. The discovery that ILs and $scCO_2$ form biphasic systems was crucial for further developments in non-aqueous multiphase green catalytic synthetic transformations, and lead to processes where most of these requirements, in principle, can be satisfied [7].

Wastes formation in organic synthetic processes is crucial and often related to the traditional use of a stoichiometric amount of reagents, especially in custom synthesis and fine chemicals production [8]. The proper use of catalytic [9] and engineering [10] methodologies can significantly improve energetic and synthetic efficiencies, processes selectivity, while reducing the production of concomitants. In this regard, biocatalysis based processes have enormous potential, since they are able to increase stereo-, chemo- and regio- selectivities in a vast number of chemical transformations [11]. Besides, a great variety (more than 13,000) enzyme-catalyzed reactions have been successfully demonstrated at the laboratory scale, offering clear advantages for enantiopure fine chemicals synthesis when compared to every other catalysts [12]. As a result, chemical industry is exploring its great potential to manufacture both bulk and fine chemicals [13].

In principle, biocatalysts can be considered as ideal green catalysts as they operate highly selectively under mild energy requirements and in water as clean solvent. Nevertheless, this is hampered to some extent by the very low solubility of most organic chemicals in water, as well as by the non inert character of water, which usually leads to undesired side reactions [14]. A wider applicability of the biocatalytic processes is highly desirable. Thus, Chemistry and Biochemistry communities are eager to develop more efficient biocatalytic processes. In this context, neoteric solvents were shown to overcome some of the previously mentioned shortcomings, while satisfying environmental demands.

In the past few years, the substitution of batch processes by flow ones constituted an additional factor which contributed implementing chemical transformation sustainability [15]. Flow processes are related to numerous advantages, including better mass and heat transfer, leading to significant process intensification. These proc-

*Address correspondence to this author at the Departamento de Bioquímica y Biología Molecular "B" e Inmunología. Facultad de Química. Universidad de Murcia. Murcia. Spain Tel: + 34 868 88 73 92; Fax: 34.868.88.41.48; E-mail: plozanor@um.es

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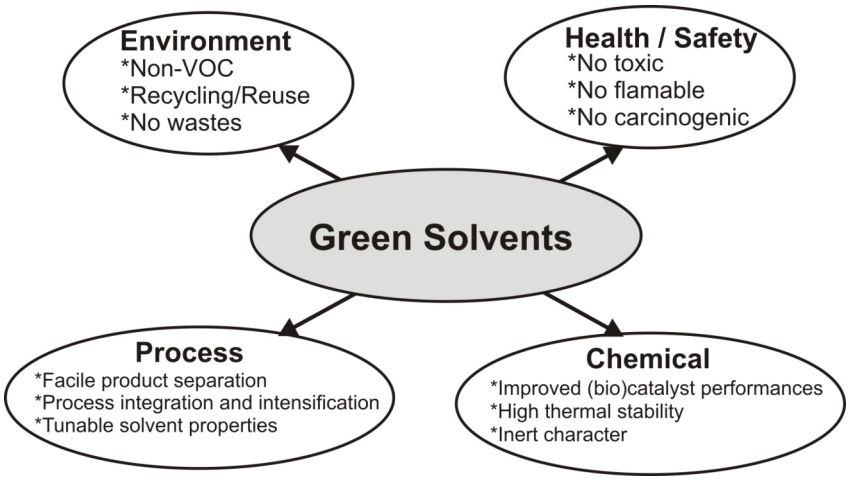

Fig. (1). Essentials requirement for new environmentally benign non aqueous solvents or green solvents.

esses can operate 24 h a day, 7 days a week, and can be optimized easily through fine adjustments of simple parameters such as flow, pressure or temperature. Additionally, flow processes scale-up is facilitated when compared to batch processes, *via* scaling-out or the numbering-up for example.

Most important commercial chemical products, in terms of added value, are chiral particularly in fine chemicals and pharmaceutical industry [16]. Thus, in the next years will arise a large number of applications for the preparation of commercial relevant chiral compounds under flow conditions using highly selective catalysts as biocatalyst [17]. As a matter of fact, recent years have seen the blossoming of new developments in the field of the application of flow processes in complex organic syntheses [18]. Nevertheless, the application of those approaches to asymmetric synthesis has been, until now, much more limited [19]. Regarding biocatalysis, although most examples involve batch processes, many continuous biocatalytic processes are known using conventional solvents. Biocatalytic flow processes under nonconventional conditions present additional advantages. Indeed, flow processes operate above atmospheric pressure, therefore enabling fluids utilization above their boiling point just by including a retention valve at the exit of the reactor. This kind of reactors allow the practical use of superheated solvents [20] and with supercritical fluids, including $scCO_2$ [21]. Considering the excellent compatibility of $scCO_2$ with many ionic liquids (ILs), as well as the efficient stabilization reported for numerous enzymes in ILs [22], enzymes immobilization in an ionic liquid phase opens the way for the simultaneous immobilization, stabilization of enzymes and use in continuous systems. It would provide an highly efficient processes facilitating the separation of the products and long term use of biocatalysts [22, 23].

This review is not intended to be comprehensive [24], but rather to present a general overview of the potential approaches to develop different strategies moving towards more efficient greener biocatalytic synthetic platforms based on the right combination of biocatalysis in neoteric solvents and engineering developments required for continuous flow process (Fig. **2**).

2. (BIO)CATALYTIC CONTINUOUS PROCESSES IN SUPERCRITICAL FLUIDS (SCFS)

A supercritical fluid (SCF) is defined as a state of matter at a pressure and temperature higher than its critical point, but below the pressure required to condense it into a solid. SCFs are characterized by gas like viscosities and solvating properties of a wide range of various organic solvents. Tuning physical properties of these solvents simply by adjusting the pressure and temperature is unique to supercritical systems, which show exceptional abilities for extraction, reaction, fractionation and analysis processes. The key feature of SCFs is the sensitivity of the fluid density to both pressure and temperature, especially in the critical point vicinity. Indeed, owing to these properties, environmentally benign carbon dioxide in its supercritical fluid state ($scCO_2$) has great potential to develop cleaner alternative processes. It allows for the total or partial substitution of most common volatile organic solvents (VOS) [25].

SCFs have been widely used as solvent in different processes [26, 27]. In general, SCFs present a reduced mass-transfer [28], tuneable solvent properties [29], which can lead to considerable changes in the chemo-, regio- and stereoselectivity outcome of the chemical reaction [30]. Furthermore products can be easily freed from solvent traces, which is highly attractive as alternative reaction medium for food and pharmaceutical products. Hence, great number of chemical processes have been developed at both lab and industrial levels using SCF as reaction media [5, 25].

If the appropriated set of conditions are selected, enzymes are catalytically active in SCFs [21, 31]. In general, SCFs with low critical temperature are required as proteins tend to denature at high temperature. Thus CO_2, ethane, propane, butane, SF_6, CHF_3, are typical fluids suitable for biocatalysis under supercritical conditions. The $scCO_2$ is chemically inert, non-toxic, non-flammable, cheap and readily available. With relatively low critical parameters (*e.g*. Pc = 73.8 bar; Tc = 31.0ºC), it remains by far the most popular supercritical solvent for biocatalysis, although some processes have been also assayed in other SCFs [32].

Most biocatalytic processes screening in SCFs are performed in high pressure batch vessels. However, continuous flow processes present a series of practical and technical advantages [17]. Biocatalytic flow processes allow continuously reactor feed with substrates, facilitate mass transfer, an easy control of the experimental conditions (pressure, temperature, flow, etc) and the product isolation and purification. Besides, immobilized enzymes onto solid supports can be used in packed bed reactor leading to a simple continuous enzyme reuse analogous to consecutive cycles without depressurisation requirements [33]. These pressurisation/depressurisation steps, compulsory in batch mode, have a detrimental impact on enzyme activity [22]. In addition to favouring the long-term stability of the biocatalyst, continuous processes avoid downtime, and consequent loss of productivity. Additionally to this loss in enzyme activity, decompression accounts for the single largest energy consumption in a $scCO_2$ process. Thus, performing reactions in series has a considerable advantage over performing the reactions separately because there is no requirement for the SCF to be depressurized between reactions. The productivity is also favoured by instant catalytic ratio between enzyme and substrate given in a flow sys-

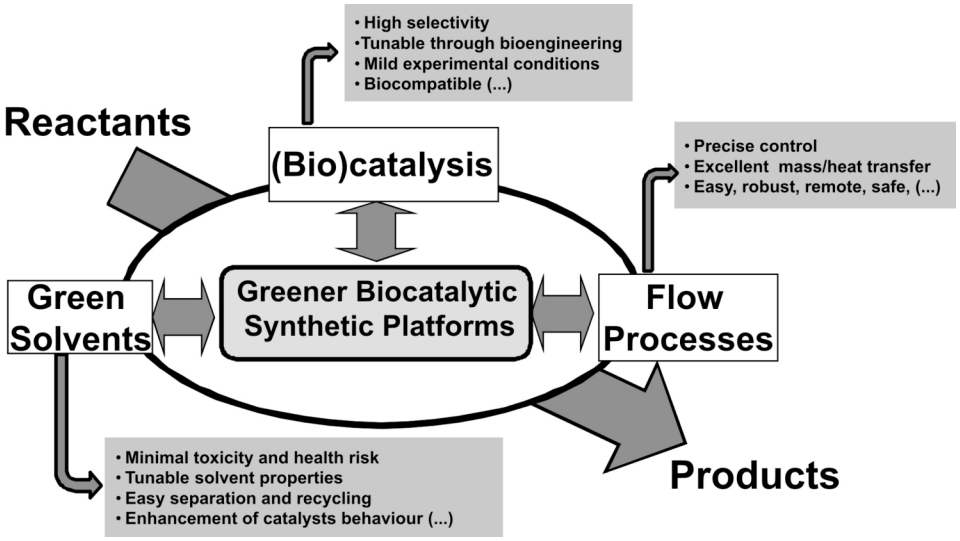

Fig. (2). New synthetic platforms based on the combination of biocatalysis, neoteric solvents and continuous flow processes.

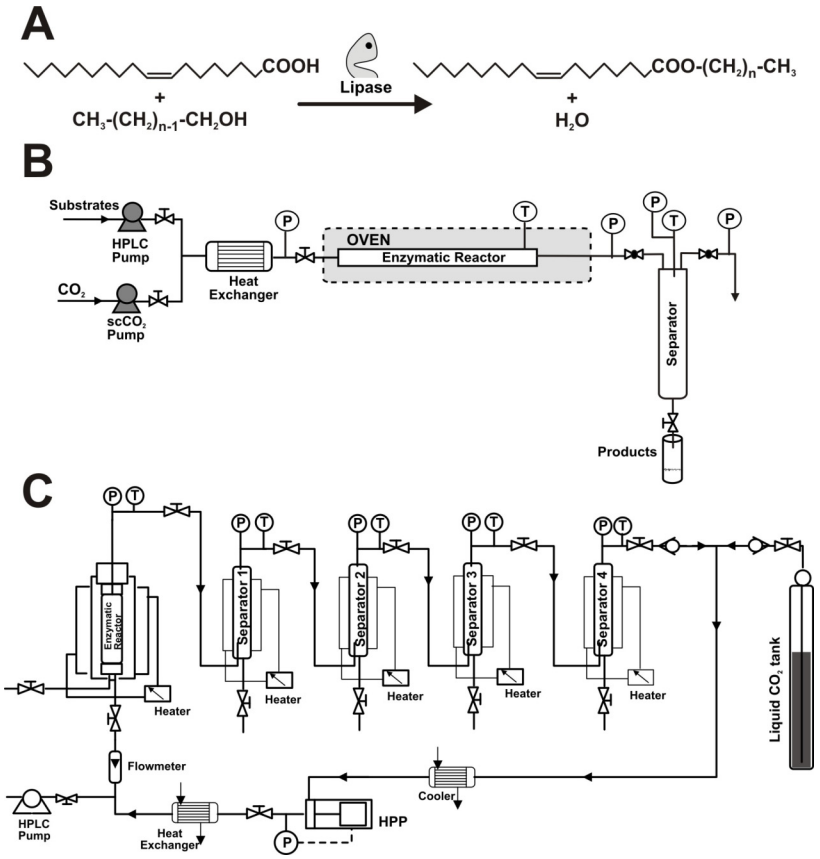

Fig. (3). Continuous flow biocatalytic synthesis of long-chain fatty acid esters. **A**) Chemical reaction scheme. **B**) Continuous flow packed-bed sc-reactor. **C**) Process combining considering the continuous scCO$_2$ biocatalytic reaction and sc-fractionation. HPP, High pressure pump. P, Pressure control. T, Temperature control [35b].

tem, which is significantly higher in fixed-bed continuous reactors than in conventional batches. Furthermore, as some enzymatic reactions are inhibited by products, or side products, constant removal of the inhibitory substance clearly improve the efficiency of the process [34].

In general, temperature, pressure, reaction dilution and flow rates are experimental variables used to optimise a synthetic transformation in a continuous biocatalytic processes. As an example, the production of long-chain fatty acid esters (*e.g.* alkyl oleate) is of great interest for cosmetic, pharmaceutical and lubricant industry. Thus, the esterification between oleic acid and 1-octanol (Fig. **3A**) catalysed by *Rhizomucor miehei* lipase (Lipozyme RM) immobilized on a macroporous anion exchange resin has been studied. In a first example, this reaction was performed in batch and continuous packed-bed reactor using dense CO_2 as solvents (Fig. **3B**), being able to reach up to 93% conversion under the optimised conditions,

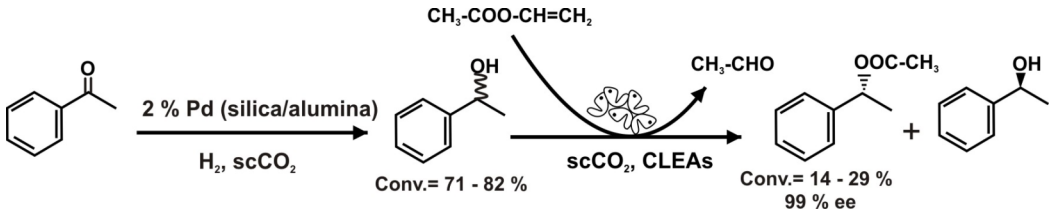

Fig. (4). Pd-catalyzed hydrogenation of acetophenone coupled with CLEAs-catalyzed kinetic resolution (KR) of the resulting *rac*-1-phenylethanol in $scCO_2$ [36].

and the productivity was maintained for long-term period (50 days) without any significant reduction. Noticeably, yields under continuous conditions were 10% higher than those observed in batch processes [35a]. However, the design of such enzymatic reactor under supercritical conditions is key feature, because mass-transfer limitations, environmental conditions (pressure and temperature) and product recovery can easily be controlled to improve results. In this context, (Fig. **3C**) shows a recycling packed bed enzyme-reactor designed at pilot scale for Lipozyme®-catalyzed ethyl oleate synthesis by esterification from oleic acid and ethanol in $scCO_2$. The proposed system was coupled with a series of four high-pressure separator vessels, where a pressure cascade was produced by back-pressure valves, allowing continuous recovery of the liquid product at the bottom of each separator, and recycling of untransformed substrates [35b].

Packed bed reactors containing supported reagents, scavengers and (bio)catalysts can be easily set-up in flow sequential assembling opening the way to produce multistage chemical reactions [15]. This enables the design of cascade multiple-stage chemocatalytic and biocatalytic processes, and easily increases of the complexity and value of synthesized products. In this regard, Poliakoff and co-workers reported a tandem palladium (Pd)-catalysed hydrogenation of acetophenone followed by a lipase-catalyzed kinetic resolution of the resulting *sec*-alcohol in $scCO_2$ [36]. The use of cross-linked enzyme aggregates (CLEAs) was a superior biocatalyst than the classical immobilized enzyme derivative (Novozym 435) (see Fig. **4**). This work clearly shows how performing reactions in series displays a considerable advantage over performing the reactions separately related to the absence of SCF depressurization between reactions. Therefore, the economic productivity of the overall process was increased when a metal-catalyzed reaction was combined with a selective biocatalytic reaction in a multiple-step synthesis in $scCO_2$.

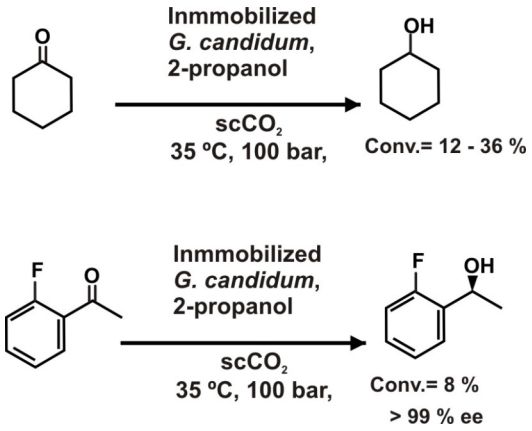

Fig. (5). Biocatalytic reduction of ketones in $scCO_2$ [37].

Biocatalytic transformations induced by cells as biocatalysts are also possible in continuous flow processes involving $scCO_2$. Thus, immobilized resting cells of *G. candidum* were used for the continuous reduction of cyclohehanone under supercritical conditions [37]. The biocatalyst was recycled up to four times with only a slight loss in activity (see Fig. **5**). Recycling was not possible using the corresponding batch system because biocatalysts did not tolerate both repeated depressurization at a very low temperature and separation of the product from the biocatalysts using organic solvents. This method was also applied for the asymmetric reduction of *o*-fluoroacetophenone, achieving excellent enantioselectivity (ee >99%) and a higher space-time yield than the corresponding batch process (0.24 $\mu mol.min^{-1}$ *vs*. 0.13 $\mu mol.min^{-1}$, at 35°C and 100 bar).

As an alternative to packed bed reactors for biocatalytic processes in SCF high-pressure continuous membrane reactors have been also assayed with a variety of assemblies [38]. For example, reactors based on a high-pressure continuous enzymatic flat-shape membrane were applied as separation units to retain the biocatalyst in the system (10,000 Da. MW cut-off) without the need of depressurization. This type of reactor allowed the hydrolysis of octyl oleate in propane and the hydrolysis of sun flower oil in $scCO_2$, catalyzed by non-immobilized lipases from *C. cylindracea* and *A. niger* respectively [39].

Alternatively, tubular membrane reactors can also be considered for designing continuous biocatalytic processes in SCF. Furthermore, these membrane reactors constitute an attempt to combine catalytic conversion, product separation and/or concentration and catalyst recovery into a single operation. As a matter of fact, the hydrolysis of carboxy-methylcellulose in $scCO_2$ was assayed using a high-pressure continuous enzymatic tubular membrane reactor. In this system, cellulase from *Humicola insolens* was covalently attached on a ceramic tubular membrane, which was previously coated with hydrophilic inert polymer [38]. In another example, enzymatic dynamic membranes are formed by depositing water-soluble polymers (*e.g.* gelatine, polyethyleneimine, etc) on a ceramic porous support. This alternative reactor is a porous membrane contactor along and through, which substrates are continuously flowing. It was firstly applied for CALB-catalyzed butyl butyrate synthesis in continuous way under supercritical conditions (Fig. **6**). The reactor was operated in daily cycles (6 h of continuous synthetic process in the selected conditions, and 18 h of storage into the reactor at room temperature), showing an excellent operational behaviour, without practically any loss in activity during the assayed time (half-life time higher than 360 cycles). The better enzymatic activity exhibited by the dynamic membrane in $scCO_2$ with respect to the organic solvents clearly showed the necessity of a relevant selection of experimental conditions set and reactor design in order to avoid the possible adverse effects of CO_2 on enzyme activity [40]. Furthermore, dynamic enzymatic membranes were also used for the lipase-catalysed interesterification between castor oil triglycerides and methyl oleate. The feasibility of this new process was demonstrated on a cross-flow filtration unit operating under $scCO_2$ conditions, showing high activity and stability of immobilized lipases [40b].

3. CONTINUOUS FLOW BIOCATALYSIS IN ILS SYSTEMS

Ionic liquids (ILs) are other exceptionally non-aqueous alternative reaction media for both chemocatalytic [41] and biocatalytic processes [42]. Regarding biocatalysis in ILs a large number of

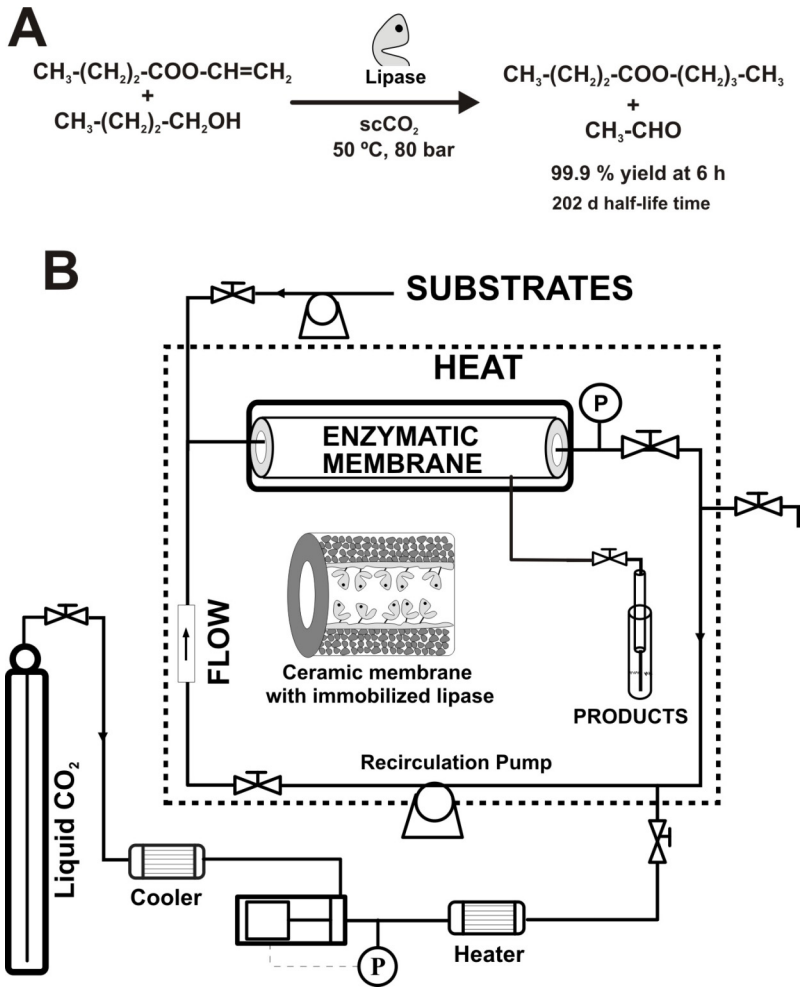

Fig. (6). A) Enzymatic synthesis of butyl butyrate. **B**) Continuous high-pressure membrane reactor with recirculation for enzyme-catalyzed transformations in $scCO_2$. P, Pressure control [44].

enzymes (*e.g.* lipases, proteases, peroxidases, dehydrogenases, glycosidases) have been tested in either monophasic or biphasic liquid systems based on ILs [43], due to their great ability to dissolve both polar and non-polar compounds [44]. However, lipases are by far the most used biocatalysts in water-immiscible ILs. They are used for the synthesis of aliphatic and aromatic esters, chiral esters by kinetic resolution of racemic alcohols, carbohydrate esters, polymers, etc. Additionally, the excellent stability displayed by enzymes in water-immiscible ILs for reuse and under high temperatures has been widely described [45]. Concerning medium engineering for biotransformations in ILs, it has been reported that ILs improves activity (*e.g.* synthesis of aliphatic esters [46a], synthesis of acyl L-canitine) [46b] or selectivity (*e.g.* KR of *rac*-menthol [46c], KR of *rac*-3-phenyllactic acid) [46d] displayed by the enzyme with respect to that observed in organic solvents, although there seem to be no rules for predicting the outcome.

In general, both monophasic and biphasic ILs biocatalytic processes are performed in batch. Despite of excellent catalytic performance, those systems show some limitations regarding biocatalysts reuse and products recovery, which are also key concepts green chemical bioprocess development. The separation of products from enzyme-ILs phase can be facilitated by the inherently low vapour pressure of ILs allowing for easy removal of volatile substances. Thus, products isolation can be carried out by sublimation or distillation [47]. However, such methods are too energetically intensive for application on larger scale. Alternatively, liquid-liquid extraction with organic solvents is the most commonly used approach for products recovery in monophasic batch ILs system. The use of these additional organic solvents must be considered as a clear breakdown regarding process greenness [1, 22].

(Semi)continuous processes based on membrane technology (*e.g.* pervaporation) [48-50] can help to overcome these limitations, allowing products separation from the ionic liquid phase and reuse of the enzyme. For example, the immobilized CALB (Novozym 435) in [Bmim] [PF_6] catalyzed the esterification of acetic acid with ethanol in a membrane reactor. It allowed removing both the ethyl acetate and water produced by a double pervaporation system using hydrophobic and hydrophilic membranes, respectively, in continuous operation for 72 hours without any loss in the enzyme activity (see Fig. **7**).

4. CONTINUOUS FLOW BIOCATALYSIS IN IL/$SCCO_2$ BIPHASIC SYSTEMS

The classical advantages of $scCO_2$ to extract, dissolve and transport chemicals are tarnished in enzymatic processes because of its denaturative effect on enzymes, while ILs have shown themselves to be excellent stabilizing agents of enzymes. In this context, the use of IL/$scCO_2$ biphasic systems as reaction media for enzyme catalysis has opened up new opportunities for integral green processes development of in non-aqueous environments, following the pioneering work of Brennecke's group in 1999 who showed that ILs (*e.g.* [Bmim][PF_6]) and $scCO_2$ form biphasic systems [7a]. Additionally, although $scCO_2$ is highly soluble in the IL phase and is

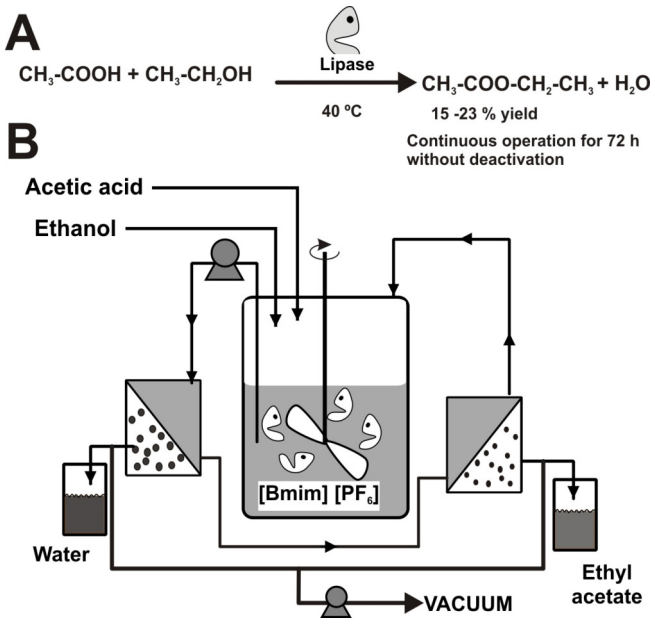

Fig. (7). A) Enzymatic synthesis of ethyl acetate. **B**) Continuous membrane reactor for the enzymatic synthesis of ethyl acetate in the IL [Bmim][PF_6] [49b].

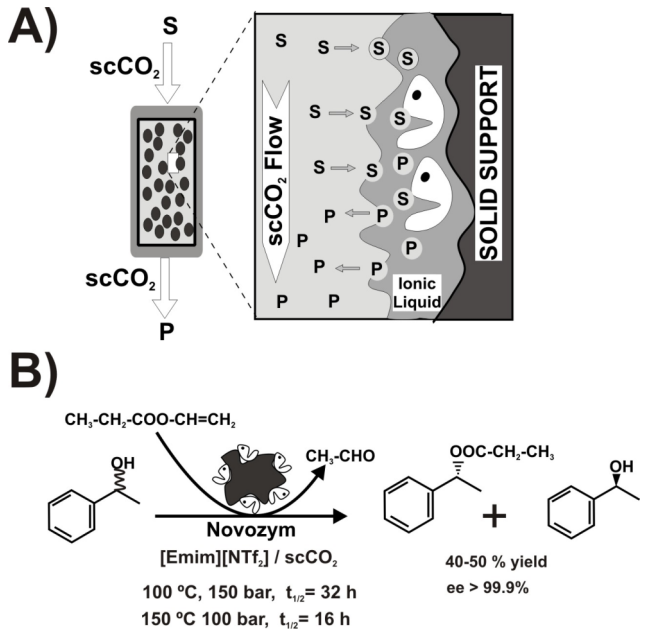

Fig. (8). A) Continuous green enzymatic process working in ILs/$scCO_2$ biphasic system. **B**) KR of *rac*-1-phenylethanol by using vinyl propionate as acyl donor. S, Substrate; P, Product.

able to extract previously dissolved hydrophobic compounds (*e.g.* naphthalene), reversely the same IL cannot be detected in the $scCO_2$ phase [51]. This discovery was crucial for further developments in multiphase green (bio)catalytic processes involving both chemical transformation and extraction steps [52]. Catalysis in multiphase operation offers promising opportunities for developing continuous chemical processes (*e.g.* the catalyst operates in one phase and the product is continuously delivered and extracted in the second phase) [53].

Multiphase systems for biocatalysis based on ILs and $scCO_2$ were originally described in 2002, and were the first operational approach for the development of fully green chemical processes in non-aqueous environments [54]. Using this approach, the $scCO_2$ flow can serve both to transport the substrate to the IL phase containing the biocatalyst, and to extract the product(s) from the IL phase. Subsequently, product(s) are obtained free from IL and from other organic solvent residues by SCF decompression, whereas 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 waste generation. By using this approach, continuous green biphasic biocatalytic systems in non-aqueous environments have been designed by dissolving free enzymes into the IL then adsorbed onto a solid support, or by coating supported enzymes molecules with IL. Biotransformations then occur into an IL phase (catalytic phase), while substrates and products remain largely in the SCF phase (extractive phase) (Fig. **8A**).

The system was firstly tested for two different reactions catalyzed by CALB: aliphatic esters synthesis by transesterification between 1-alkanols and vinyl esters (*e.g.* butyl butyrate from vinyl butyrate and 1-butanol), and the kinetic resolution of *rac*-1-phenylethanol in a wide range of conditions (100-150 bar and 40-100ºC, see Fig **8B**). Under these conditions, the enzyme showed an exceptional level of activity, enantioselectivity (ee> 99.9) and operational stability (*e.g.* the enzyme only lost 15% activity after 11 cycles of 4 h) [54a]. Thus, excellent results can also be obtained for biotransformations in $scCO_2$ using the enzyme coated with ILs even under extreme conditions, such as 100 bar and 150ºC [55]. Further studies on these IL-$scCO_2$ biocatalytic systems attempted to understand mass-transport importance between both neoteric phases. By using two similar ILs with different degrees of hydrophobicity on the cation chain, [Btma][NTf_2] and [$CN(CH_2)_3NMe_3$][NTf_2], six different short chain alkyl esters (*e.g.* from butyl acetate to octyl propionate) were continuously synthesized by using CALB in $scCO_2$ (see Fig. **9**). The Hansen's solubility parameter (δ) was used as criterion to compare substrates, products and ILs hydrophobicity, the latter depending mostly on the main alkyl chain of cations. Similar values of δ for reagents and IL resulted in a clear improvement of productivity, as a consequence of the better mass-transfer phenomena between IL and $scCO_2$ phases [56].

A further step towards green biocatalysis in ILs/$scCO_2$ biphasic systems was the appropriate selection of acyl donor in the CALB-catalyzed kinetic resolution (KR) of *rac*-1-phenylethanol for including the product selective separation step in the full process. By using vinyl laurate as acyl donor, *R*-1-phenylethyl laurate can be selectively separated from unreacted *S*-1-phenylethanol with $scCO_2$ using two different cryo-traps (see Fig. **10**). This process takes advantage of the solubility difference of a compound in $scCO_2$, which depends on both the polarity and vapour pressure. Thus, if the alkyl chain of an ester product is long enough, its low volatility implies a lower solubility in $scCO_2$ than the corresponding alcohol. Using this experimental approach, the introduction of two additional separation chambers connected with cryo-traps in the reactor, and the selection of an appropriate pressure and temperature, resulted in selective separation of the synthetic product and the unreacted alcohol from the reaction mixture (66% yield, ee>99.9%) [57]. Thus, good conditions for reaction and separation were obtained through synergetic combination of continuous process, IL/$scCO_2$ and acylating agent nature.

Two final approaches are worth mentioning which push forward the excellences of IL-$scCO_2$ biphasic systems. Multicatalytic processes and reaction systems with a reduced amount of ILs were developed using supported ionic liquid covalently attached to the support. Integrated multicatalytic processes, whereby one initial substrate is catalytically transformed into one final product by two or more consecutive catalytic steps in the same reaction system, is of great interest for developing future chemical industry [58]. On the other hand, some ILs can display some drawbacks, such as a

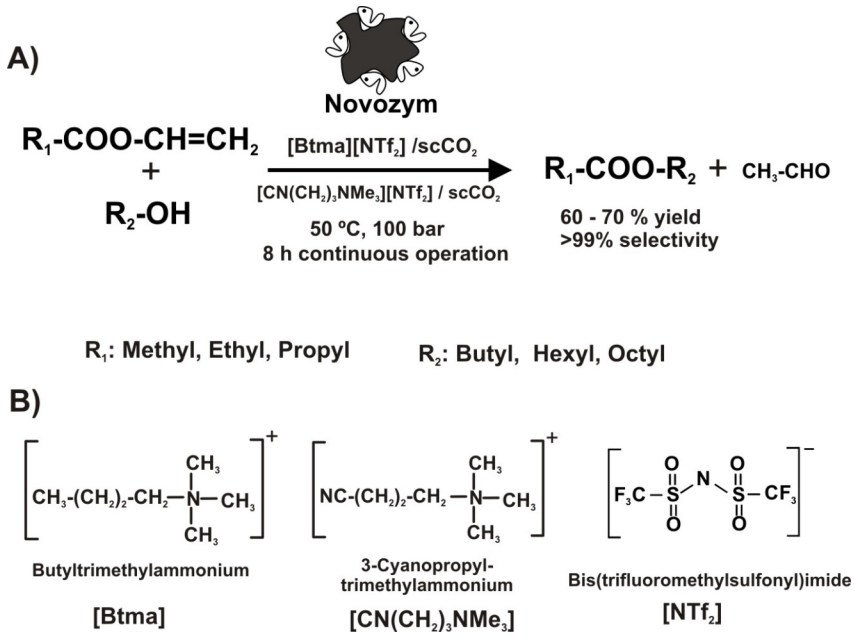

Fig. (9). A) Enzymatic synthesis of alkyl ester in IL/$scCO_2$ biphasic systems. **B**) Structures and names of assayed ILs [56].

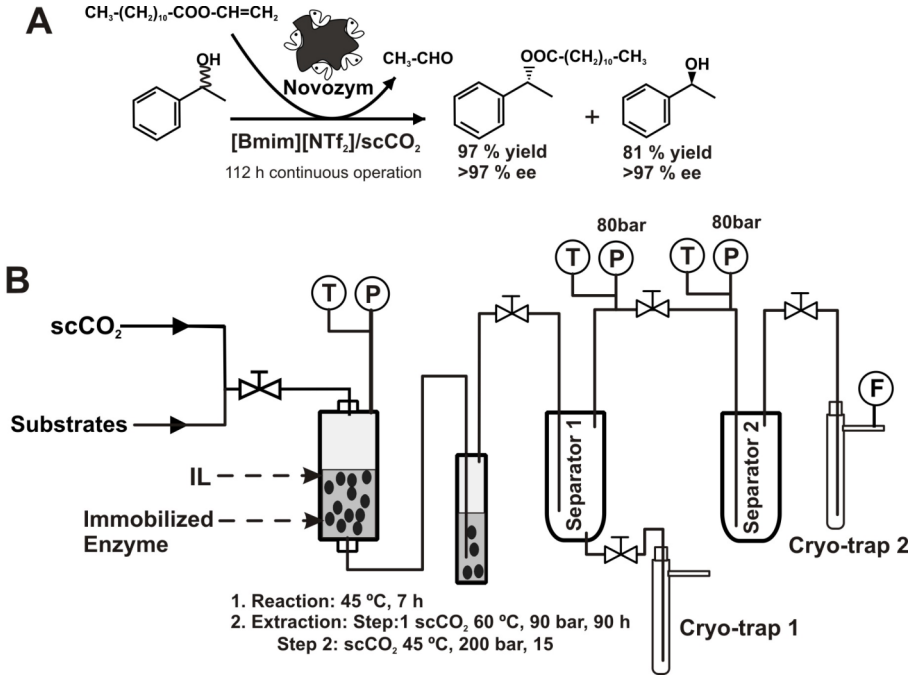

Fig. (10). A) KR of *rac*-1-phenylethanol by using vinyl laurate as acyl donor. **B**) Set-up of a reaction/separation system for continuous-flow combination of enzymatic kinetic resolution and enantiomer separation using an ionic liquid/$scCO_2$ medium. P, Pressure control; T, Temperature control; F, Flow control [57].

lower biodegradability and higher (eco)toxicity, therefore favouring reaction systems based on reduced amounts of ILs [59].

Enzyme-catalyzed kinetic resolution (KR) is probably the most widely used method for separating the two enantiomers of a racemic mixture, the chemical yield of the process being limited to 50%. However, this drawback can be overcome by combining the enzymatic KR with *in situ* racemisation of the undesired enantiomer, using so-called dynamic kinetic resolution (DKR), which theoretically permits to reach up to 100% of one enantiomeric product. For example, the DKR of *rac*-1-phenylethanol was carried out by combining immobilized lipase (Amano PS CI) with a chemical catalyst (either the metal catalyst $[Ru(p\text{-cymene})Cl_2]_2$, or the acid catalyst Nafion®) in a discontinuous way, and without the presence of ILs in $scCO_2$ at 100 bar and 40ºC (see Fig. **11**) [60]. By this approach, the *R*-product yield (70% Ru-catalyst; 85% Nafion) was improved, as compared to the reaction carried out in hexane (30-35% yield), while products enantioselectivities were slightly higher in $scCO_2$ (96% Ru-catalyst; 85% Nafion) than in hexane (91%; 81%). The moderately low enantioselectivities obtained in the case of the acidic Nafion were attributed to the uncontrolled substrates esterification catalysed by this solid acid. A physical separation of the enzyme and the chemical catalyst, along with a continuous flow system would prevent this undesirable side reaction.

The continuous DKR processes of *rac*-1-phenylethanol were first carried out combining immobilized CALB with silica modified

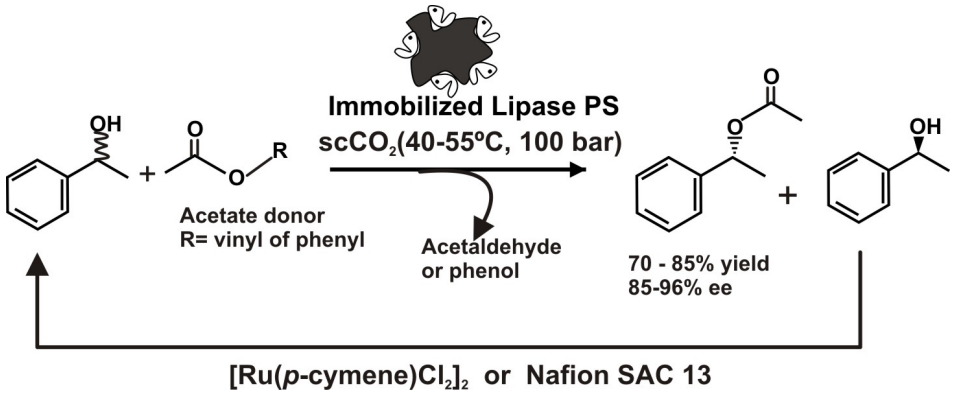

Fig. (11). DKR of *rac*-1-phenylethanol catalyzed by the combined action immobilized *P. Cepacia* lipase and a chemical catalyst (Ru(*p*-cymene)$Cl_2]_2$ metal catalyst or Nafion SAC 13 acid catalyst) [60].

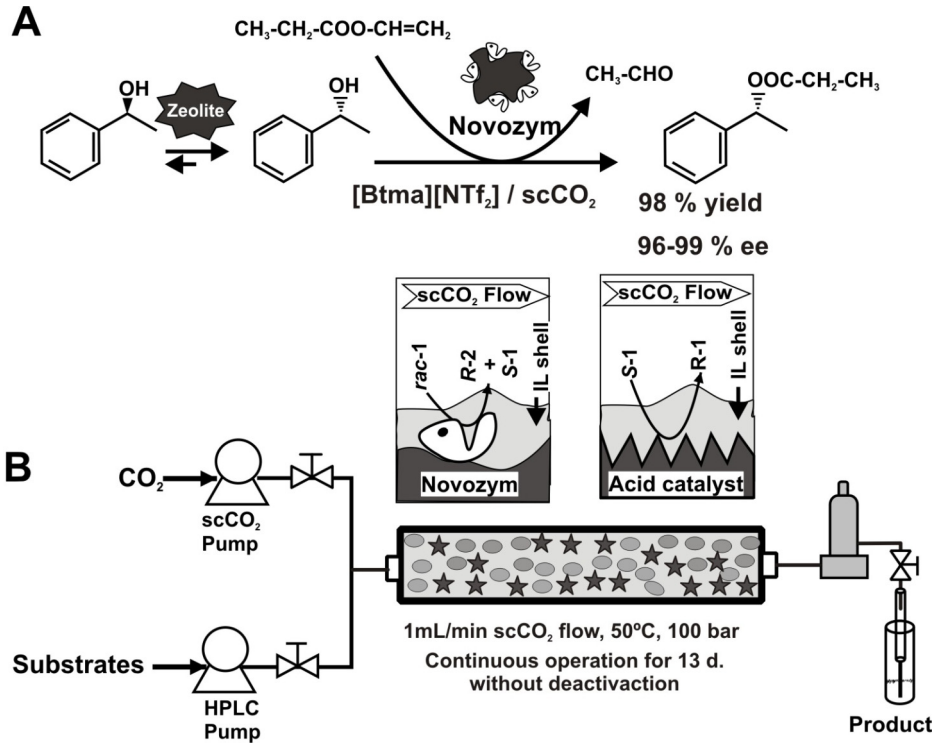

Fig. (12). A). DKR of *sec*-alcohols (*rac*-OH) catalyzed by the combined action immobilized CALB (Novozym 435) and an acid zeolite chemical catalyst. **B**). Set-up of a continuous packed-bed reactor containing both Novozym 435 and acid catalyst coated with [Btma][NTf_2] [62].

with benzenosulfonic acid groups as catalysts in a packed bed reactor under $scCO_2$ at 50ºC and 100 bar. 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 $scCO_2$ [61a]. A simple mixture of both catalysts resulted in a complete loss of activity, probably due to the acid environment around the enzyme particles. However, catalyst particles packed under three different layers, (immobilized enzyme - acid catalyst -immobilized enzyme) physically separated by glass wool, led to encouraging results for the *R*-ester product (76% yield, 91-98% ee) [61a]. For this reactor configuration, the *R*-ester product yield may only tend to 100% if several enzymatic and acid catalyst layers are stacked in the packed bed, according to a dichotomic progression. It is also worth noticing how the presence of the undesired ester *S*-ester and hydrolytic products in the $scCO_2$ flow was enhanced when the acid catalyst particles were assayed without IL coating. The use of weaker solid acids, such as zeolites, as chemical catalyst clearly improved results.

In a further attempt to improve the catalytic system, four different zeolites were assayed as acid catalysts for *in situ* racemization of unreacted alcohol. In all the cases, the acid catalysts were coated with an IL (*e.g.* [Bmim][PF_6], [Bdmim][PF_6], [Odmim][NTf_2], [Toma][NTf_2] or [Btma][NTf_2]). Coated acid catalysts were able to catalyse the *S*-1-phenylethanol racemisation. Their suitability in a continuous DKR of *rac*-1-phenylethanol in combination with immobilized CALB under $scCO_2$ flow was successfully demonstrated (see Fig. **12A** and **B**) [63]. The best results (98% yield, 96% ee) were obtained for a heterogeneous mixture between fajausite-type zeolite (CBV400) particles coated with [Btma][NTf_2] and Novozym particles coated with the same IL. Due to the low acidity of the assayed zeolites, the packaging of the heterogeneous mixture of catalyst particles coated with IL did not result in any activity loss of the immobilized CALB during 14 days of continuous operation in CO_2 under different supercritical conditions. This work clearly demonstrated the exciting potential of multi-catalytic (enzymatic or

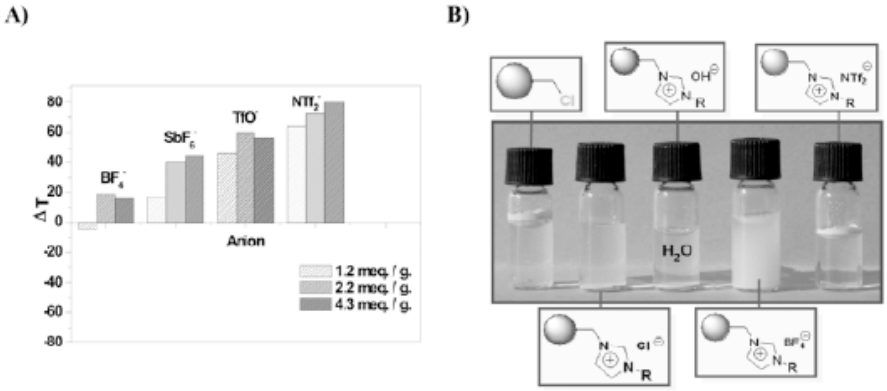

Fig. (13). A) Variation of the thermal stability (DT) of different resins modified with methyl imidazol with respect to the starting gel-type Merrifield resins as a function of degree of ionic liquid-like moieties. **B**) Behaviour of different beads type SILLPs in water (R= butyl) [67].

chemo-enzymatic) systems in ILs/scCO$_2$ for synthesizing optically active pharmaceutical drugs by a sustainable approach.

A further step towards optimised IL/scCO$_2$ biphasic systems arose from the development of the immobilization of the ionic liquid species onto solid supports. The immobilisation facilitates the separation processes and avoids a possible accidental spill in the environment. Besides, it reduces the process cost as a minimal amount of IL is employed in catalytic processes in those biphasic systems [63]. The supported ionic species can be obtained either by adsorbing ILs onto solid supports (*Supported Ionic Liquid Phases* or *SILPs*) or by covalently bonding IL-like fragments on the surface of the solid support (*Supported Ionic Liquid-like Phases* or *SILLPs*) [64].

There are only few examples of biocatalysis using covalently bonded supported ionic liquids. Thus, *C. rugosa* lipase has been

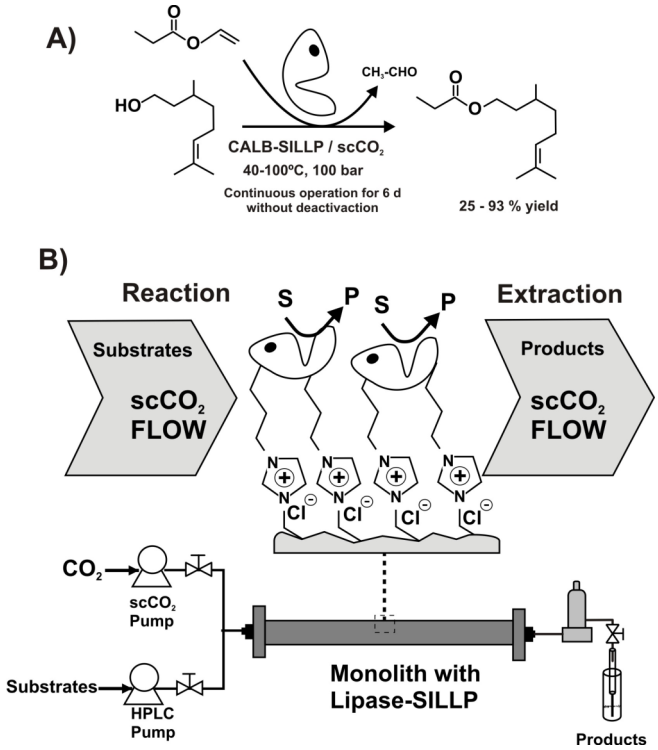

Fig. (14). A) Enzymatic synthesis of citronellyl propionate catalyzed by CALB-SILLP under supercritical conditions. **B**) Reactor set-up with CALB immobilized onto monolith-supported ionic liquid phase (M-SILLP) for continuous operation under flow conditions in scCO$_2$.

immobilised on magnetic nanoparticles coated with supported ILs. Materials based on imidazoliun cations with different chain lengths (C-1, C-4 and C-8) and anions ([Cl], [BF_4] and [PF_6]) were obtained by covalent bonding of ionic liquid-silane moieties on magnetic silica nanoparticles (55 nm diameter), which permits a high lipase loading (about 64 mg /100 mg carrier). Furthermore, the bound lipase activity was 18.3% higher than the native lipase for solvent-free oleic acid esterification with butanol at 30ºC [65]. Recently, horseradish peroxidase was encapsulated in microparticles composed of polymerized ionic liquid. The entrapped enzyme exhibited higher activity than with conventional polyacrylamide microparticles, and was easily recycled by centrifugation from reaction mixtures [66].

The development of covalently Supported Ionic Liquid-Like Phase (SILLP) either by functionalization of the PS-DVB surfaces with IL-like (imidazolium) moieties or by polymerisation of the corresponding functional monomers has open a new way to greatly reduce the amount of ILs and to facilitate its full reuse/recovery in continuous green chemical processes. In this approach, ILs properties are transferred onto the solid phase leading to supported ionic liquid-like phase (SILLP), either in particles or monoliths. A large diversity of SILLPs, varying cation, anion, as well as support nature and loading, have been characterized, including their thermal stability and polarity [67]. Significant changes were found in resin thermal stability upon introduction of the imidazolium subunits onto Merrifield type resins (see Fig. **13A**). In general, it is worth noticing that the polymeric SILLPs thermal stability is increased as compared to that of the initial Merrifield resin. Thermal stability order was $[NTf_2]^- > [TfO]^- > [SbF_6]^- > [BF_4]^-$, showing a similar trend to corresponding molecular ILs [68].

Regarding polarity, Fig. (**13B**) depicts the behaviour of different SILLPs in water to illustrate the change in polarity induced by structural changes in the IL-like moieties. Methyl imidazolium groups have a big impact in the resulting materials polarity, changing from highly hydrophobic resin (SILLPs in floating on top) to very hydrophilic one (SILLPs depositing in the bottom). Counteranion exchange led to a middle situation for the [BF_4] case (SILLPs suspended in water) to again a very hydrophobic one ([NTf_2] floating SILLPs). These polarity changes have also been quantitatively demonstrated by using a ratiometric fluorescence probe such as pyrene, resulting in a SILLPs polarity as compared to the original PS-DVB polymers [67, 69]. The vibrational frequencies for single water molecules associated to ILs have also been used to measure the polarity of SILLPs by the methodology recently described by Ludwig *et al*. [70]. High polarities were observed for polymers with $[Cl]^-$, $[TfO]^-$ and $[NTf_2]^-$) as counteranion, while a lower polarity for polymer with $[SbF_6]^-$ as counterion. Nonetheless, imidazolium alkyl substitution has a lower influence on polarity than the anion.

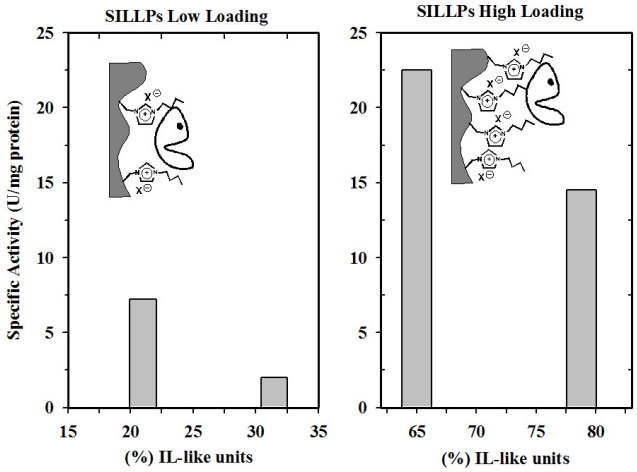

Fig. (15). Influence of loading of SILPs with IL moieties on the specific activity of CALB-SILLPs derivative for the KR of *rac*-phenylethanol with vinyl propianate in hexane at 40°C.

Thus, micropolarities at the SILLPs surface essentially maintain the same bulk ILs polarity. Hence, they might be regarded as "solid solvents" or as nanostructured materials with microenvironments of tuneable polarity able to immobilised and stabilise catalytic species.

SILLPs have successfully been used as supports for metal catalysts [71] and as supported organocatalysts [72]. Furthermore, SILPs can also be used to develop enzymatic catalysed processes in $scCO_2$ (see Fig. **14**). Bioreactors with covalently supported ionic liquid-like phases (SILLP) were prepared as styrene-divinylbenzene based polymeric monoliths, containing imidazolium units with loadings ranging from *ca*. 55 to 40% wt IL per gram of polymer. It resulted in a liquid phase coating of the solid support surface. These SILLPs were able to adsorb CALB, leading to highly efficient and robust heterogeneous biocatalysts. The macroporous monolithic bioreactors were tested as mini-flow systems for the continuous flow transesterification leading to citronellyl propionate in $scCO_2$ at 100 bar and 40-100ºC. The catalytic activity of these mini-flow-bioreactors remained nearly unchanged for seven operational cycles of 5 h each in different supercritical conditions [73].

Alternatively, PS-DVB polymers beads modified with ionic liquid like moieties can be synthesized by grafting butyl imidazole onto a commercial bead-type Merrifield resin leading to the corresponding SILLPs and use for immobilisation and stabilisation of CALB. All the supported enzymes were able to catalyse the selective formation of (*R*)-1-phenylethyl propionate with excellent enantiomeric excess (e.e. above 99.9%) in conventional solvents such hexane. However, the catalytic efficiency of the different immobilised enzymes was clearly dependent of the characteristics of the SILLPs as can be appreciated in Fig. (**14**). Both counterion nature and loading have a dramatic effect on the corresponding biocatalyst specific activity. Similar results were found for highly cross-linked (macroporous) polymers having butylimidazolium choride groups, which present a higher mechanical resistance than gel-type resins. Accordingly they can be more suitable for flow processes allowing for an easy design of a fix-bed reactor to perform the KR under flow conditions. Besides, this set-up facilitates the substitution of a traditional, relatively toxic, solvent (n-hexane) by a non toxic one such as $scCO_2$. Continuous KR of phenylethanol in $scCO_2$ at 50ºC and 10 MPa lead to 50% of conversion in enantiopure product even for an extended period of time, involving up to 6 days of continuous use.

In another example pushing towards the growing of greener synthetic biocatalytic platform, CALB-SILLPs derivatives were combined with solid acids, such as zeolites, to carry out the DKR of the phenylethanol by using a single reactor in continuous way. In this reactor, both the immobilized enzyme and zeolite were mixed together to perform a "one-pot" catalytic system in $scCO_2$ (see Fig. **15**). By using this approach, several "one pot" single columnar minireactors were assembled by loading the corresponding column with a mixture of CALB-SILLP and an acid catalysts (*e.g.* zeolite CP811E-150) [67]. To improve the selectivity of the chemical catalysts, zeolite was coated with a small amount of an IL (*i.e.* [BMIM][PF_6]). However, unlike commercial supported enzyme (for example Novozyme 435), CALB immobilised onto supported ionic liquid like phases (CALB-SILLPs), does not require additional coating with an ionic liquid layer to achieve good activity and stability. The optimisation of the experimental conditions (flow, pressure, temperature, etc.) increased yields of the desired product up to 92% with e.e. >99.9% for the continuous DKR of *rac*-phenylethanol with vinyl propionate in $scCO_2$ [74].

5. CONTINUOUS CATALYTIC PROCESSES IN MICRO-FLOW SYSTEMS

Continuous micro- or mini- flow systems can be quite useful also for homogeneous or heterogeneous transition metal catalysed reactions which employ ILs and (or) $scCO_2$ as the reaction medium. In the case of homogeneous catalysis, catalyst immobilization in the ILs offers attractive advantages in terms of reactivity and selectivity and since this would facilitate the separation of both the catalyst and reaction media from the products. Such a system has been described by Ryu *et al.* [75] for Pd-catalysed Sonogashira and Mizoroki–Heck coupling reactions in a low-viscosity ionic liquid, [Bmim][NTf_2] which involves the reaction, separation of the product and catalyst, and good recycling of the catalyst in a continuous microsystem. In this example, the ionic phase, which contained Pd catalyst, could be continuously recycled to provide an overall yield of 80% (115.3 g, 10 g/h) of the desired product (butyl cinnamate) (see Fig. **16**). Similarly, Pd-catalyzed carbonylation under low pressure using an ionic liquid as a recyclable catalytic phase has also been described using a multiphase microsystem [76]. Superior selectivity and higher yields in both carbonylative Sonogashira coupling and amidation reactions of aryl iodides, as compared to the conventional batch system, have been demonstrated. The results clearly demonstrate that liquid–gas segmented microflow offered excellent selectivity and high yields for carbonylation reactions even when conducted at relatively lower CO_2 pressures.

However the use VOS (*e.g.* hexane) for the isolation of the products and separation and recycling of IL-catalytic phase is a major drawback in terms of sustainability. In this regard and to overcome such problems, supported ionic liquid like phases (SILLPs), where palladium nanoparticles are immobilised and stabilised (PdNPs-SILLPs), can be also considered as efficient alternative to develop more environmentally friendly processes [71b]. The presence of the ILs-like units in the SILLPs plays an important role in the capture and stabilisation of the active Pd species. The PdNPs-SILLPs materials allow the substitution of classical solvents such as DMF by more benign solvents like near-critical ethanol. Thus, monolithic mini-flow continuous reactors were developed for C-C coupling reactions catalysed by supported PdNPs in scEtOH. The presence of IL-like moieties in the polymer helps to stabilise the Pd(0) reducing the leaching. Hence continuous catalytic system is stable during moths without an appreciable reduction on activity.

A continuous-flow catalytic system based on the combination of ionic liquids and $scCO_2$ owing to their complementary properties offers new intriguing possibilities for catalytic synthesis, because of the ability of $scCO_2$ to extract organic substances from an ionic liquid phase without any cross-contamination of the extract with the ionic liquid [7]. This biphasic system was used for the hydrovinylation of styrene catalysed by an immobilised Ni-catalyst in a continuous flow system [77]. The ILs in combination with $scCO_2$ pro-

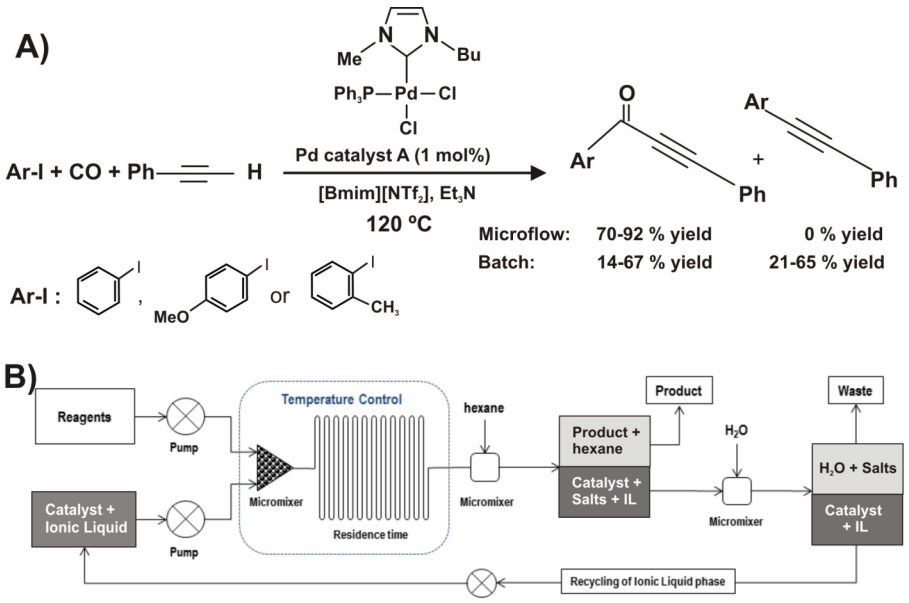

Fig. (16). A) Pd-catalysed Mizoroki–Heck coupling reactions in IL. **B**) Schematic drawing of the automated microflow apparatus, CPC CYTOS lab system and flow workup processes for Mizoroki-Heck reaction in ionic liquids [75].

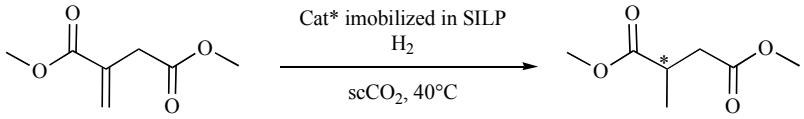

Fig. (17). Enantioselective hydrogenation of dimethyl itaconate by using 1-naphthyl-QUINAPHOS immobilized in [EMIM][NTf$_2$] [80].

vides an attractive approach to environmentally benign processes where product separation from the catalyst is an easy operation. Another advantage of using such a mixture resides in the decrease of the ionic catalyst solution viscosity, thus facilitating mass transfer during the catalytic reaction. Finally, the use of scCO$_2$ as the mobile phase enables a reactor design that is very similar to a classical fixed-bed reactor.

Most processes involving heterogeneous catalysis are batch processes. One important reason for that is that the efficient immobilisation of a heterogeneous catalyst in a fixed bed reactor avoiding leaching of the precious catalyst during continuous operation is an arduous task. An interesting continuous Rh-catalysed hydroformylation of 1-octene in the biphasic system [BMIM][PF$_6$]/scCO$_2$ has been developed by Cole-Hamilton and co-workers [78]. This special phase behaviour has been successfully applied to other continuous catalytic reactions such as Ni-catalysed enantioselective hydrovinylation.

The SILP concept has also been applied for the continuous Rh-catalysed hydroformylation of propene in a gas-phase reaction [79]. In this case, the SILP- catalyst systems, consisting of [Rh(acac)(CO)$_2$] and an IL, *i.e.* [BMIM][PF$_6$] or [BMIM][n-C$_8$H$_{17}$OSO$_3$], were prepared just by impregnation of silica gel 100. Three different ligands were tested for the gas-phase propene hydroformylation in a continuous fixed-bed reactor. The SILP system maintained an excellent level of activity up to 60 h. Moreover, the SILP system showed very stable selectivity with n/iso isomeric ratio remaining as high as 21 after 60 h.

Recently, the use of scCO$_2$ as mobile phase over SILP catalysts has also been used for the asymmetric hydrogenation of dimethyl itaconate with chiral organometallic catalysts, allowing mild reaction conditions and making the use of organic solvents or additional purification steps unnecessary (Fig. **17**). The commercially available (S_a,R_c)-1-naphthyl-QUINAPHOS and the ionic liquid [EMIM][NTf$_2$] (EMIM=1-ethyl-3-methylimidazolium) gave the best results for the SILP-catalyst system [80].

Effective catalyst immobilization with integrated product separation was achieved in an efficient flow process at high space–time-yields. The amounts of valuable catalysts and ILs that are required are very small, rendering such processes economically and environmentally attractive. The modularity of the SILP–scCO$_2$ combination offers a great potential for further optimization on both the molecular and the engineering level to further increase productivity and catalyst stability.

CONCLUSIONS

To develop a clean and sustainable chemical industry, the transfer of the exquisite catalytic efficiency shown by enzymes in Nature to chemical processes is an important challenge. Enzymes constitute the most important toolbox for green organic synthesis, and the interest for application at an industrial scale is beyond doubt. Furthermore, the technological applications of enzymes are enhanced in non-aqueous environments, because of the resulting expansion in the repertoire of enzyme-catalyzed transformations to contribute for the development of green highly selective synthesis of chiral products of high commercial value.

A further improvement in this area comes from the combination of (bio)catalysis with the use of neoteric solvents, such as scCO$_2$ and ILs, and the synergetic combination of both. These systems can contributed not only to the stabilization and easy recycling of the biocatalysts, but also in the combination with systems under flow conditions to further processes improvements. Many different approaches can be used for this purpose, but the preparation of columnar reactors is becoming the most usual alternative. The main advantages associated to the use of continuous flow conditions

involve a higher mechanical and long term stability of the supported catalysts, as well as the achievement of much higher space time yields and productivities. Similar improvements apply to both biocatalysis and chemocatalysts, and the immobilization on supported ionic liquids (SILPS or SILLPs) offer new opportunities for practical applications. A critical key to successfully implement of all these methodologies in chemical manufacturing is the integration of several catalytic steps in multi-step organic syntheses and downstream processing without the need to isolate intermediates, mimicking the metabolic pathways found in Nature. Thus, continuous flow multi-enzymatic and/or multi-chemoenzymatic green chemical processes in multiphase systems for synthesizing pharmaceutical drugs should be developed in the near future.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*, Oxford University Press, New York, **1998**. b) Horvath, I.; Anastas, P. T. Introduction: Green chemistry. *Chem. Rev*. **2007**, *107*, 2167-2168; c). Anastas, P. T.; Eghbali, N. Green chemistry: principles and practice. *Chem. Soc. Rev*. **2010**, *39*, 301-312.

[2] a) Clark, J. H.; Tavener, S. J. Alternative solvents: Shades of green. *Org. Proc. Res. Develop*. **2007**, *11*, 149-155. b) Li, C. J.; Trost, B. M. Green chemistry for chemical synthesis. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13197-13202.

[3] Curzons, A. D.; Constable, D. J. C.; Mortimer, D. N.; Cunningham, V. L. So you think your process is green, how do you know? Using principles of sustainability to determine what is green - a corporate perspective. *Green. Chem*. **2001**, *3*, 1-6. b) Constable, D. J. C.; Curzonsand, A. D.; Cunningham, V. L. Metrics to 'green' chemistry - which are the best? *Green. Chem*. **2002**, *4*, 521-527.

[4] Wasserscheid, P.; Welton, T. *Ionic Liquids in Synthesis*; Wiley-VCH Weinheim, **2008**. b) Plechkova, N. V.; Seddon, K. R. Applications of ionic liquids in the chemical industry. *Chem. Soc. Rev*. **2008**, *37*, 123-150.

[5] Jessop, P. J.; Leitner, W. *Chemical Synthesis Using Supercritical Fluids*; Wiley-VCH, Weinheim, **1999**. b) Beckman, E. J. Supercritical and near-critical CO_2 in green chemical synthesis and processing. *J. Supercrit. Fluids.* **2004**, *28*, 121-191.

[6] DeSimone, J. M. Practical approaches to green solvents. *Science* **2002**, *297*, 799-803. b) Anastas, P. T.; Leitner, W.; Jessop, P. G. C.; Li, J.; Wasserscheid, P.A. S. *Handbook of Green Chemistry - Green Solvents*, Wiley-VCH Verlag GmbH, New York, **2010**.

[7] Blanchard, L. A.; Hancu, D.; Beckman, E. J.; Brennecke, J. F. Green processing using ionic liquids and CO_2. *Nature*, **1999**, *399*, 28-29. b) Keskin, S.; Kayrak-Talay, D.; Akman, U.; Hortaçsu, Ö. A review of ionic liquids towards supercritical fluid applications. *J. Supercrit. Fluids*. **2007**, *43*, 150-180.

[8] Sheldon, R. A. E-factors, green chemistry and catalysis: an odyssey. *Chem. Commun*. **2008**, *29*, 3352-3365.

[9] a) Anastas, P. T.; Kirchhoff, M. M.; Williamson. T. C. Catalysis as a foundational pillar of green chemistry. *Appl. Catal. A*, **2001**, *221*, 3-13. b) Sheldon, R. A.; Arends, I.; Hanefeld, U. *Green Chemistry and Catalysis*; Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2007**. c) Rothenberg, G. *Catalysis, Concepts and Green Chemistry*, Wiley-VCH Verlag GmbH, Weinheim, **2008**.

[10] Anastas, P. T.; Heine, L. G.; Williamson, T. C.; *Green Engineering*; Oxford University Press, Oxford, **2000**. b) Anastas, P. T.; Heine, L. G.; Williamson, T. C. *Green Chemical Syntheses and Processes*; ACS Publications, **2001**. c) Clark, J. H.; Macquarrie, D. *Handbook of Green Chemistry & Technology*, Blackwell Science Ltd, Oxford, **2002**. d) Lankey, R. L.; Anastas, P. T. *Advancing Sustainability Through Green Chemistry and Engineering*, ed. ACS Publications, **2005**.

[11] Silverman, R. B. *The Organic Chemistry of Enzyme-Catalyzed Reactions*; Academic Press, New York, **2002**. b) Bommarius, A. S.; Riebel, B. R. *Biocatalysis*, Wiley-VCH Verlag GmbH & Co. KGaA, **2004**. c) Anastas, P. T.; Crabtree, R. *Handbook of Green Chemistry-Green Catalysis: Biocatalysis*; *3*, Wiley-VCH Verlag GmbH, New York, **2009**.

[12] Gotor, V.; Alfonso, I.; García-Urdiales, E. *Asymmetric Organic Synthesis with Enzymes;* Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2008**. b) Sureshkumar, M.; Lee, C. K. Biocatalytic reactions in hydrophobic ionic liquids. *J. Mol. Catal. B: Enzym*. **2009**, *60*, 1-12. c) Hudlicky, T.; Reed, J. W. Applications of biotransformations and biocatalysis to complexity generation in organic synthesis. *Chem. Soc. Rev*. **2009**, *38*, 3117-3132.

[13] Roberts, S. M.; Casy, G.; Nielsen, M.-B.; Phythian, S.; Todd, C.; Wiggins, K. *Biocatalysts for Fine Chemicals Synthesis*; J. Wiley & Sons, **1999**; b) Liese, A.; Seelbach, K.; Wandrey, C. *Industrial Biotransformations-A Comprehensive Handbook*; Willey-VCH, Weinheim, **2006.**

[14] Klibanov, A. M. Improving enzymes by using them in organic solvents. *Nature* **2001**, *409*, 241-246.

[15] Luis, S. V.; Garcia-Verdugo, E. *Chemical Reactions and Processes under Flow Conditions*; RSC, Green Chemistry Series**, 2009**.

[16] a) Grayson, I. Extending the chemist's chiral toolbox. *Chemistry Today/Chimica Oggi*, **2006**, *24*, 3-4. b) Carey, J. S.; Laffan D.; Thomson, C.; Williams, M.T. Analysis of the reactions used for the preparation of drug candidate molecules. *Org. Biomol. Chem*. **2006**, *4*, 2337-2347.

[17] Rao, N. N.; Lütz, S.; Würges, K.; Mino, D. Continuous biocatalytic processes. *Org. Process Res. Dev*. **2009,** *13,* 607-616.

[18] a) Venturoni F.; Nikbin N.; Ley S. V.; Baxendale I. R. The application of flow microreactors to the preparation of a family of casein kinase I inhibitors. *Org. Biomol. Chem*. **2010**, *8*, 1798-1806. b) Hopkin M. D.; Baxendale I. R.; Ley S. V. A flow-based synthesis of Imatinib: the API of Gleevec. *Chem. Commun*. **2010**, *46*, 2450-2452. c) Baxendale I. R.; Schou S. C.; Sedelmeier J.; Ley S. V. Multi-step synthesis by using modular flow reactors: the preparation of Yne-ones and their ose in heterocycle synthesis. *Chem. Eur. J*. **2010**, *16*, 89-94. d) Baxendale I. R.; Ley S. V.; Mansfield A. C.; Smith C. D. Multistep synthesis using modular flow reactors: Bestmann-Ohira reagent for the formation of alkynes and triazoles. *Angew. Chem. Int. Ed*. **2009**, *48*, 4017-4021.

[19] Mak X. Y.; Laurino P.; Seeberger P. H. Asymmetric reactions in continuous flow. *Beilstein J. Org. Chem*. **2009**, 5, No. 19. doi:10.3762/bjoc.5.19.

[20] Razzaq, T.; Glasnov, T. N.; Kappe, C. O. Continuous-flow microreactor chemistry under high-temperature/pressure conditions. *Eur. J. Org. Chem*. **2009**, *9*, 1321-1325.

[21] a) Hobbs, H. R.; Thomas, N. R. Biocatalysis in supercritical fluids, in fluorous solvents, and under solvent-free conditions. *Chem. Rev*. **2007**, *107*, 2786-2820. b) Habulin, M.; Primozic, M.; Knez, Z. Supercritical fluids as solvents for enzymatic reactions. *Acta Chim. Slov*. **2007**, *54*, 667.

[22] Lozano, P. Enzymes in neoteric solvents: From one-phase to multiphase systems. *Green Chem*. **2010**, *12*, 555-569.

[23] Moniruzzaman M.; Kamiya N.; Goto M. Activation and stabilization of enzymes in ionic liquids. *Org. Biomol. Chem*. **2010**, *8*, 2887-2899.

[24] For excellent recently published reviews see references 21, 22, 23.

[25] a) Brunner, G. (Ed.). *Supercritical Fluids as Solvents and Reaction Media*, Elsevier BV, **2004**. b) DeSimone J. M.; Tumas, W.; Eds. *Green chemistry using liquid and supercritical carbon dioxide*; Oxford University Press, **2003**. c) Jessop, P. G.; Ikariya, T.; Noyori, R. Homogeneous catalysis in supercritical fluids. *Chem. Rev.* **1999**, *99*, 475-493. d) Rayner, C. M. The potential of carbon dioxide in synthetic organic chemistry. *Org. Proc. Res. Develop.* **2007**, *11*, 121-132. e) Kruse, A.; Vogel, H. Heterogeneous catalysis in supercritical media - I. Carbon dioxide. *Chem. Eng. Technol*. **2008**, *31*, 23-32.

[26] McHugh, M. A.; Krukonis, V. J. *Supercritical Fluid Extraction Principles and Practice,* 2nd ed., Butterworth–Heinemann, London, **1994**. b) Mukhopadhyay, M. *Natural Extracts Using Supercritical Carbon Dioxide*; CRC Press, London, **2000**.

[27] Kendall, J. L.; Canelas, D. A.; Young, J. L.; De Simone, J. M. Polymerizations in supercritical carbon dioxide. *Chem. Rev*. **1999**, *99*, 543-563. b) Cansell, F.; Aymonier, C. Design of functional nanostructured materials using supercritical fluids. *J. Supercrit. Fluids* **2009**, *47*, 508-516.

[28] Baiker, A. Supercritical fluids in heterogeneous catalysis. *Chem. Rev*. **1999**, *99*, 453-473. b) Ciriminna, R.; Carraro, M. L.; Campestrini, S.; Pagliaro, M. Heterogeneous catalysis for fine chemicals in dense phase carbon dioxide. *Adv. Synth. Catal*. **2008**, *350*, 221226.

[29] Bourne, R. A.; Stevens, J. G.; Ke, J.; Poliakoff, M. Maximising opportunities in supercritical chemistry: the continuous conversion of levulinic acid to gamma-valerolactone in CO_2. *Chem. Commun*. **2007**, *44*, 4632-4634.

[30] a) Pereda, S.; Brignole, E. A.; Bottini, S. B. Advances in phase equilibrium engineering of supercritical reactors. *J. Supercrit. Fluids* **2009**, *47*, 336-343. b) da Ponte, M.N. Phase equilibrium-controlled chemical reaction kinetics in high pressure carbon dioxide. *J. Supercrit. Fluids* **2009**, *47*, 344350.

[31] For review on enzyme catalysis in SCFs, see: a) Mesiano, A. J.; Beckman, E. J.; Russel, A. J. Supercritical biocatalysis. *Chem. Rev*. **1999**, *99*, 623-633. b) Knez, Z. Enzymatic reactions in dense gases. *J. Supercrit. Fluids* **2009**, *47*, 357-372. c) Eisenmenger, M. J.; Reyes de Corcuera, J. I. High pressure enhancement of enzymes: A review. *Enzyme Microb. Technol*. **2010**, *45*, 331-347.

[32] Casiraghi, L.; Greiner, L.; Leitner, W.; Eggert, T.; Jaeger K. E. Enzyme stability under supercritical conditions. *J. Biotechnol*. **2007**, *131*, 115-116.

[33] Keim, W. Multiphase catalysis and its potential in catalytic processes: the story of biphasic homogeneous catalysis. *Green Chem*. **2003**, *5*, 105-111.

[34] Jeong, S.; Hwang, B. Y.; Kim, J.; Kim, B. G. Lipase-catalyzed reaction in the packed-bed reactor with continuous extraction column to overcome a product inhibition. *J. Mol. Catal. B: Enzym*. **2000**, *10*, 597-604.

[35] a) Laudani, C. G.; Habulin, M.; Knez, Z.; Porta, G. D.; Reverchon, E. Immobilized lipase-mediated long-chain fatty acid esterification in dense carbon dioxide: bench-scale packed-bed reactor study. *J. Supercrit. Fluids* **2007**, *41*, 74-81; b) Marty, A.; Combes, D.; Condoret, J. S. Continuous reaction-separation process for enzymatic esterification in supercritical carbon dioxide. *Biotechnol. Bioeng*. **1994**, *43*, 497-504.

[36] Hobbs, H. R.; Kondor, B.; Stephenson, P.; Sheldon, R. A.; Thomas, N. R.; Poliakoff, M. Continuous kinetic resolution catalysed by cross-linked enzyme aggregates, 'CLEAs', in supercritical CO_2. *Green Chem*. **2006**, *8*, 816-821.

[37] Matsuda, T.; Watanabe, K.; Kamitanaka, T.; Harada, T.; Nakamura, K. Biocatalytic reduction of ketones by a semi-continuous flow process using supercritical carbon dioxide. *Chem. Commun*. **2003**, (10), 1198-1199.

[38] a) Habulin, M.; Primozic, M.; Knez, Z. Enzymatic reactions in high-pressure membrane reactors. *Ind. Eng. Chem. Res*. **2005**, *44*, 9619-9625. b) Primozic, M.; Paljevac, M.; Habulin, M.; Knez, Z. Hydrolase-catalyzed reactions in membrane reactors at atmospheric and high pressure. *Desalination* **2009**, *241,* 14-21.

[39] Habulin, M.; Knez, Z. High-pressure enzymatic hydrolysis of oil. *Eur. J. Lipid Sci. Technol*. **2002**, *104*, 381-386.

[40] a) Lozano, P.; Villora, G.; Gomez, D.; Gayo, A. B.; Sanchez-Conesa, J. A.; Rubio, M.; Iborra, J. L. Membrane reactor with immobilized *Candida antarctica* lipase B for ester synthesis in supercritical carbon dioxide. *J. Supercrit. Fluids* **2004**, *29*, 121-128; b) Pomier, E.; Galy, J.; Paolucci-Jeanjean, D.; Pina, M.; Sarrade, S.; Rios. G. M. A new reactor design combining enzyme, membrane and scCO$_2$: application to castor oil modification. *J. Membr. Sci.* **2005**, *249*, 127-132.

[41] Olivier-Bourbigou, H.; Magna, L.; Morvan, D. Ionic liquids and catalysis: Recent progress from knowledge to applications *Appl. Cat. A: General* **2010**, *373*, 1-56.

[42] Pioneering works using ILs in biotransformations: a) Madeira Lau, R.; van Rantwijk, F.; Seddon, K. R.; Sheldon, R. A. Lipase-catalyzed reactions in ionic liquids. *Org. Lett.* **2000**, *2*, 4189-4191. b) Erbeldinger, M.; Mesiano, A. J.; Russell, A. J. Enzymatic catalysis of formation of Z-aspartame in ionic liquid an alternative to enzymatic catalysis in organic solvents. *Biotechnol. Prog*. **2000**, *16*, 1129-1131. c) Lozano, P.; De Diego, T.; Carrié, D.; Vaultier, M.; Iborra, J. L. Over-stabilization of *Candida antarctica* lipase B by ionic liquids in ester synthesis. *Biotechnol. Lett*. **2001**, *23*, 1529-1533. d) Itoh, T.; Akasaki, E.; Kudo, K.; Shikarami, S. Lipase-catalyzed enantioselective acylation in the ionic liquid solvent system: Reaction of enzyme anchored to the solvent. *Chem. Lett*. **2001**, *3*, 262-263.

[43] Moniruzzaman, M.; Nakashima, K.; Kamiya, N.; Goto, M. Recent advances of enzymatic reactions in ionic liquids. *Biochem. Eng. J*. **2010**, *48*, 295-314.

[44] a) Yang, Z.; Pan, W. Ionic liquids: Green solvents for nonaqueous biocatalysis. *Enzyme Microb. Technol*. **2005**, *37*, 19-28. b) van Rantwijk, F.; Sheldon, R. A. Biocatalysis in ionic liquids. *Chem. Rev*. **2007**, *107*, 2757-2785. c) Durand, J.; Teuma, E.; Gómez, M. Ionic liquids as a medium for enantioselective catalysis. *C. R. Chimie* **2007**, *10*, 152-177. d) Harjani, J. R.; Naik, P. U.; Nara, S. J.; Salunkhe, M. M. Enzyme mediated reactions in ionic liquids. *Curr. Org. Synth*. **2007**, *4*, 354-369. e) Roosen, C.; Muller, P.; Greiner, L. Ionic liquids in biotechnology: Applications and perspectives for biotransformations. *Appl. Microbiol. Biotechnol*. **2008**, *81*, 607-614. f) Sureshkumar, M.; Lee, C. K. Biocatalytic reactions in hydrophobic ionic liquids. *J. Mol. Catal. B: Enzym.* **2009**, *60*, 1-12.

[45] De Diego, T.; Lozano, P.; Gmouh, S.; Vaultier, M.; Iborra, J. L. Fluorescence and CD spectroscopic analysis of the α-chymotrypsin stabilization by the ionic liquid, 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]amide. *Biotechnol. Bioeng*. **2004**, *88*, 916-924. b) De Diego, T.; Lozano, P.; Gmouh, S.; Vaultier, M.; Iborra, J.L. Understanding structure-Stability relationships of *Candida antartica* lipase B in ionic liquids. *Biomacromolecules* **2005**, *6*, 1457-1464. c) Lozano, P.; De Diego, T.; Gmouh, S.; Vaultier, M.; Iborra, J. L. Dynamic structure-function relationships in enzyme stabilization by ionic liquids. *Biocatal. Biotransf.* **2005**, *23*, 169-176. d) Feher, E.; Major, B.; Belafi-Bako, K.; Gubicza, L. On the background of enhanced stability and reusability of enzymes in ionic liquids. *Biochem. Soc. Trans*. **2007**, *35*, 1624-1627. e) Shan, H. X.; Li, Z. J.; Li, M.; Ren, G. X.; Fang, Y. J. Improved activity and stability of *pseudomonas capaci* lipase in a novel biocompatible ionic liquid, 1 isobutyl-3-methylimidazolium hexafluorophosphate. *J. Chem. Technol. Biotechnol*. **2008**, *83*, 886-891. f) Micaelo, N. M.; Soares, C. M. Protein structure and dynamics in ionic liquids. Insights from molecular dynamics simulation studies. *J. Phys. Chem. B* **2008**, *112*, 2566-2572. g) Page, T. A.; Kraut, N. D.; Page, P. M.; Baker, G. A.; Bright, F. V. Dynamics of loop 1 of domain I in human serum albumin when dissolved in ionic liquids. *J. Phys. Chem. B* **2009**, *113*, 12825-12830.

[46] Lozano, P.; De Diego, T.; Carrié, D.; Vaultier, M.; Iborra, J.L. Enzymatic ester synthesis in ionic liquids. *J. Mol Catal. B: Enzym*. **2003**, *21*, 9-13. b) Tian, J. Q.; Wang, Q.; Zhang, Z. Y. Lipase-catalyzed acylation of l-carnitine with conjugated linoleic acid in [Bmim][PF$_6$] ionic liquid. *Eur. Food Res. Technol.* **2009**, *229*, 357-363. c) Zhang, D. H.; Bai, S.; Ren, M. Y.; Sun, Y. Optimization of lipase-catalyzed enantioselective esterification of (±)-menthol in ionic liquid. *Food Chem.* **2008**, *109*, 72-80. d) Banoth, L.; Singh, M.; Tekewe, A.; Banerjee, U. C. Increased enantioselectivity of lipase in the transesterification of dl-(±)-3-phenyllactic acid in ionic liquids. *Biocatal. Biotransf.* **2009**, *27*, 263-270.

[47] Itoh, T.; Akasaki, E.; Y. Nishimura, Y. Efficient lipase-catalyzed enantioselective acylation under reduced pressure conditions in an ionic liquid solvent system. *Chem. Lett*. **2002**, 154-155.

[48] *Pervaporation is a selective membrane technique using hydrophilic or hydrophobic nonporous selective membranes, which are able to break an azeotropic mixture. It is considered as safe and clean technology, because it has low waste emission, energy consumption*, Smitha, B.; Suhanya, D.; Sridhar, S.; Ramakrishna, M. J. Separation of organic-organic mixtures by pervaporation-A review. *J. Membr. Sci*. **2004**, *241*, 1-21.

[49] a) Feher, E.; Illeova, V.; Kelemen-Horvath, I.; Belafi-Bako, K.; Polakovic, M.; Gubicza, L. Enzymatic production of isoamyl acetate in an ionic liquid-alcohol biphasic system. *J. Mol. Catal. B: Enzym*. **2008**, *50*, 28-32. b) Gubicza, L.; Belafi-Bako, K.; Feher, E.; Frater, T. Waste-free process for continuous flow enzymatic esterification using a double pervaporation system. *Green Chem*. **2008**, *10*, 1284-1287.

[50] Feher, E.; Major, B.; Belafi-Bako, K.; Gubicza, L. Semi-continuous enzymatic production and membrane assisted separation of isoamyl acetate in alcohol - ionic liquid biphasic system. *Desalination* **2009**, *241*, 8-13.

[51] Cadena, C.; Anthony, J. L.; Shah, J. K.; Morrow, T. I.; Brennecke, J. F.; Maginn, E. J. Why is CO_2 so soluble in imidazolium-based ionic liquids?. *J. Am. Chem. Soc*. **2004**, *126*, 5300-5308.

[52] Arai, M.; Fujita, S.; Shirai. M. Multiphase catalytic reactions in/under dense phase CO_2. *J. Supercrit. Fluids* **2009**, *47*, 351-356.

[53] Leitner, W.; Hölscher M. *Topics Organometallic Chem.* vol. 23, *Regulated Systems for Multiphase Catalysis,* Springer Berlin, Heidelberg, **2008**.

[54] a) Lozano, P.; De Diego, T.; Carrié, D.; Vaultier, M.; Iborra, J. L. Continuous green biocatalytic processes using ionic liquids and supercritical carbon dioxide. *Chem. Commum*. **2002**, (7), 692-693. b) Reetz, M. T.; Wiesenhofer, W.; Francio, G.; Leitner, W. Biocatalysis in ionic liquids: Batchwise and continuous flow processes using supercritical carbon dioxide as the mobile phase. *Chem. Commun*. **2002**, (9), 992-993.

[55] Lozano, P.; De Diego, T.; Carrié, D.; Vaultier, M.; Iborra, J. L. Lipase catalysis in ionic liquids and supercritical carbon dioxide at 150°C. *Biotechnol. Prog*. **2003**, *19*, 380-382.

[56] Lozano, P.; De Diego, T.; Gmouh, S.; Vaultier, M.; Iborra, J. L. Criteria to design green enzymatic processes in ionic liquid/supercritical carbon dioxide systems. *Biotechnol. Prog*. **2004**, *20*, 661-669.

[57] Reetz, M.T.; Wiesenhofer, W.; Francio, G.; Leitner, W. Continuous flow enzymatic kinetic resolution and enantiomer separation using ionic liquid/supercritical carbon dioxide Media. *Adv. Synth. Catal.* **2003**, *345*, 1221-1228.

[58] Garcia-Junceda, E. *Multi-Steps Enzyme Catalysis: Biotransformation and Chemoenzymatic Synthesis*. Wiley-VCH, Weinheim, **2008**.

[59] Torrecilla, J. S.; Garcia, J.; Rojo, E.; Rodriguez, F. Estimation of toxicity of ionic liquids in Leukemia Rat Cell Line and Acetylcholinesterase enzyme by principal component analysis, neural networks and multiple lineal regressions. *J. Hazard. Mat*. **2009**, *164*, 182-194.

[60] Benaissi, K.; Poliakoff, M.; Thomas, N. R. Dynamic kinetic resolution of *rac*-1-phenylethanol in supercritical carbon dioxide. *Green Chem*. **2009**, *11*, 617-621

[61] Lozano, P.; De Diego, T.; Larnicol, M.; Vaultier, M.; Iborra, J. L. Chemoenzymatic dynamic kinetic resolution of *rac*-1-phenylethanol in ionic liquids and ionic liquids/supercritical carbon dioxide systems. *Biotechnol. Lett*. **2006**, *28*, 1559-1565.

[62] Lozano, P.; De Diego, T.; Mira, C.; Montague, K.; Vaultier, M.; Iborra, J. L. Long term continuous chemoenzymatic dynamic kinetic resolution of *rac*-1-phenylethanol using ionic liquids and supercritical carbon dioxide. *Green Chem*. **2009**, *11*, 538-542.

[63] a) Valkenberg, M. H.; de Castro, C.; Holderich, W. H. Immobilisation of ionic liquids on solid supports. *Green Chem*. **2002**, *4*, 88-93. b) Mehnert, C. P. Supported ionic liquid catalysis. *Chem. Eur. J.* **2004**, 11, 50-56. c) Riisager, A.; Fehrmann, R.; Haumann, M.; Wasserscheid, P. Supported ionic liquids: Versatile reaction and separation media. *Top. Catal*. **2006**, *40*, 91-102. d) Gu, Y.; Li, G. Ionic liquids-based catalysis with solids: State of the art. *Adv. Synth. Catal*. **2009**, *351*, 817-847.

[64] Kim, D. W.; Chi, D. Y. Polymer-Supported Ionic Liquids: Imidazolium Salts as Catalysts for Nucleophilic Substitution Reactions Including Fluorinations. *Angew. Chem. Int. Ed*. **2004**, *43*, 483-485; b) Kim, D. W.; Hong, D. J.; Jang, K. S.; Chi, D. Y. Structural modification of polymer-supported ionic liquids as catalysts for nucleophilic substitution reactions including fluorination. *Adv. Syn. Cat*. **2006**, *348*, 1719-1727; c) Kim, D. W.; Jeong, H. J.; Lim, S. T.; Sohn, M. H.; Chi, D. Y. Facile nucleophilic fluorination by synergistic effect between polymer-supported ionic liquid catalyst and tert-alcohol reaction media system. *Tetrahedron* **2008**, *64*, 4209- 4214.

[65] Jiang, Y. Y.; Guo, C.; Xia, H. S.; Mahmood, I.; Liu, C. Z.; Liu, H. Z. Magnetic nanoparticles supported ionic liquids for lipase immobilization: Enzyme activity in catalyzing esterification. *J. Mol. Catal. B: Enzym*. **2009**, *58*, 103-109.

[66] Nakashima, K.; Kamiya, N.; Koda, D.; Maruyamac, T.; Goto, M. Enzyme encapsulation in microparticles composed of polymerized ionic liquids for highly active and reusable biocatalysts. *Org. Biomol. Chem*. **2009**, *7*, 2353-2358.

[67] Karbass N. *PhD.* Reacciones de Heck en EtOH cuasi- critico catalizadas por partículas de Pd(0) inmovilizadas en líquidos iónicos soportados *Thesis*, University Jaume I, Spain, **2009**

[68] Huddleston, A. E. V. J. G.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Rogers, R. D. Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. *Green Chem*. **2001**, *3*, 156-164.

[69] Burguete, M. I.; Galindo, F.; Garcia-Verdugo, E.; Karbass, N.; Luis, S. V. Polymer supported ionic liquid phases (SILPs) versus ionic liquids (ILs): How much do they look alike. *Chem. Commun*. **2007**, 3086-3088.

[70] a) Köddermann, T.; Wertz, C.; Heintz, A.; Ludwig, R. The association of water in ionic liquids: A reliable measure of polarity. *Angew. Chem. Int. Ed*. **2006**, *45*, 3697-3702. b) Wulf, A.; Köddermann, T.; Wertz, C.; Heintz, A.; Ludwig, R. Water vibrational bands as a polarity indicator in ionic liquids. *Z. Phys. Chem.* **2006**, *220*, 1361-1376.

[71] Altava, B.; Burguete, M. I.; Garcia-Verdugo, E.; Karbass, N.; Luis, S. V.; Puzary, A.; Sans, V. Palladium N-methylimidazolium supported complexes as efficient catalysts for the Heck reaction. *Tetrahedron Lett*. **2006**, *47*, 2311-2314. b) Karbass, N.; Sans, V.; Garcia-Verdugo, E.; Burguete, M. I.; Luis, S. V. Pd(0) supported onto monolithic polymers containing IL-like moieties. Continuous flow catalysis for the Heck reaction in near-critical EtOH. *Chem. Commun*. **2006**, (29), 3095-3097. c) Burguete, M. I.; García-Verdugo, E.; Garcia-Villar, I.; Gelat, F.; Licence, P.; Luis, S. V.; Sans, V. Pd catalysts immobilized onto gel-supported ionic liquid-like phases (g-SILLPs): A remarkable effect of the nature of the support. *J. Catal*. **2010**, *269*, 150-160.

[72] Burguete, M. I.; Erythropel, H.; Garcia-Verdugo, E.; Luis, S. V.; Sans, V. Base supported ionic liquid-like phases as catalysts for the batch and continuous-flow Henry reaction. *Green Chem*. **2008**, *10*, 401-407.

[73] Lozano, P.; Garcia-Verdugo, E.; Piamtongkam, R.; Karbass, N.; De Diego, T.; Burguete, M. I.; Luis, S. V.; Iborra, J. L. Bioreactors based on monolith-supported ionic liquid phase for enzyme catalysis in supercritical carbon dioxide. *Adv. Synth. Catal*. **2007**, *349*, 1077-1084.

[74] Lozano, P.; García-Verdugo, E.; Karbass, N.; Montague, K.; De Diego, T.; Burguete, M. I.; Luis. S. V. Supported Ionic Liquid-Like Phases (SILLPs) for enzymatic processes: Continuous KR and DKR in SILLP-scCO_2 systems. *Green Chem*. **2010**, *12*, 1803-1810.

[75] a) Fukuyama, T.; Shinmen, M.; Nishitani, S.; Sato, M.; Ryu, I. A copper-free Sonogashira coupling reaction in ionic liquids and its application to a microflow system for efficient catalyst recycling. *Org. Lett*. **2002**, *4*, 1691-1694; b) Liu, S.; Fukuyama, T.; Sato, M.; Ryu, I. Continuous microflow synthesis of butyl cinnamate by a Mizoroki-Heck reaction using a low-viscosity ionic liquid as the recycling reaction medium. *Org. Process Res. Dev*. **2004**, *8*, 477-481.

[76] Rahman, T.; Fukuyama, T.; Kamata, N.; Sato, M.; Ryu, I. Low pressure Pd-catalyzed carbonylation in an ionic liquid using a multiphase microflow system. *Chem. Comm*. **2006**, (21), 2236-2238.

[77] Bösmann, A.; Francio, G.; Janssen, E.; Solinas, M.; Leitner, W.; Wasserscheid, P. Activation, tuning, and immobilization of homogeneous catalysts in an ionic liquid/compressed CO_2 continuous-flow system. *Angew. Chem. Int. Ed*. **2001**, *40*, 2697-2699.

[78] a) Sellin, M. F.; Webb, P. B.; Cole-Hamilton, D. J. Continuous flow homogeneous catalysis: Hydroformylation of alkenes in supercritical fluid-ionic liquid biphasic mixtures. *Chem. Comm*. **2001**, *8*, 781-782; b) Cole-Hamilton, D. J. Homogeneous catalysis - New approaches to catalyst separation, recovery, and recycling. *Science* **2003**, *299*,1702-1706; c) Webb, P. B.; Sellin, M. F.; Kunene, T. E.; Williamson, S.; Slawin, A. M. Z.; Cole-Hamilton, D. J. Continuous Flow Hydroformylation of Alkenes in Supercritical Fluid-Ionic Liquid Biphasic Systems. *J. Am. Chem. Soc.* **2003**, *125*, 15577-15588.

[79] Riisager, A.; Wasserscheid, P.; van Hal, R.; Fehrmann, R. Continuous fixed-bed gas-phase hydroformylation using supported ionic liquid-phase (SILP) Rh catalysts. *J. Catal.* **2003**, *219*, 452-455; b) Riisager, A.; Fehrmann, R.; Flicker, S.; van Hal, R.; Haumann, M.; Wasserscheid, P. Very stable and highly regioselective supported ionic-liquid-phase (SILP) catalysis: Continuous-flow fixed-bed hydroformylation of propene. *Angew. Chem. Int. Ed*. **2005**, *44*, 815-819.

[80] Hintermair, U.; Höfener, T.; Pullmann, T.; Francio, G.; Leitner, W. Continuous enantioselective hydrogenation with a molecular catalyst in supported ionic liquid phase under supercritical CO_2 flow. *Chem. Cat. Chem*. **2010**, *2*, 150-154.