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Recovery of 2-phenylethanol from aqueous solutions of biosynthesis using ionic liquids

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ABSTRACT

2-Phenylethanol (PEA) is a very important natural aroma compound with a rose-like odor and its production is one of the most challenging, yet rewarding, activities in fragrance biotechnology. PEA constitutes an important commercial flavor compound for the food, cosmetic and perfume industries. The main aim of this study was to review of all physicochemical data obtained on ternary liquid-liquid equilibrium (LLE) and to determine the best ionic liquid (IL) for a biphasic *in situ* product removal at temperature $T = 303.15$ K in bioproduction of PEA using *Saccharomyces cerevisiae* AM1-d strain and to compare results with popular oleic acid. The comparison with oleic acid, which is traditionally used to extract PEA is necessary to show improvements. Ten ionic liquids (ILs) and one Deep Eutectic Solvent (DES) (Choline chloride + malonic acid, 1:1) were tested in our pioneer work for their influence on yeast growth, concentration of PEA and partition coefficient between phases in comparison with oleic acid in order to compare this results with literature. From our first experience of biosynthesis with yeast the best results were obtained with 1-butyl-1-methylpyrrolidinium bis{(trifluoromethyl)sulfonyl}imide, [BMPyr][NTf_2]. In this work six new ILs was tested in bioconversion process, namely: 1-hexyl-3-methylimidazolium tetracyanoborate, [HMIM][TCB], 1-decyl-3-methylimidazolium tetracyanoborate, [DMIM][TCB], 1-butyl-1-methylpyrrolidinium tetracyanoborate, [BMPyr][TCB], 1-allyl-3-methylimidazolium bis{(trifluoromethyl)sulfonyl}imide, [AMIM][NTf_2], *N*-triethyl-*N*-octylammonium bis{(trifluoromethyl)sulfonyl}imide, [N_{2228}][NTf_2], and 1-hexyl-1-methyl pyrrolidinium bis{(trifluoromethyl)sulfonyl}imide, [HMPyr][NTf_2]. The best results obtained in comparison with oleic acid in terms of extraction capability, biocompatibility and overall performance in the bioprocess were obtained for three of them: [BMPyr][TCB], [AMIM][NTf_2] and [HMPyr][NTf_2]. The careful analysis was made for the best ILs, such as the organic-water ratio, or concentration of PEA after 24 h and 48 h of extraction. The maximum yield of extraction of PEA was found to be (16.46 ± 0.23 g·L^{-1}) after 48 h using [HMPyr][NTf_2]. The process of re-extraction of PEA from ILs was further discussed. These new very optimistic results show a promising method for extraction of PEA and have a good prospective in the industrial application.

1. Introduction

During the past decade biotechnological production of fragrance row materials have become increasingly attractive, especially these being from natural sources and accepted by the European and U.S. food agencies. 2-Phenylethanol (PEA) is an important commercial flavor compound with rose-like aroma for the food, cosmetic and perfume industries [1–5]. It is used in soft drinks, candy, ice cream, pudding, chewing gum, cookies and many others. During the last decade we worked in our Faculty on chemical synthesis of PEA [6,7]. However, according to new directives of European Parliament, the fragrance materials must originate from natural sources [8]. The natural PEA might be extracted from plant material, like rose pedals or produced biotechnologically by microorganisms. Due to the consumer preference in industry arises demand for natural PEA obtained from biosynthesis [2–5]. The latter route seems to be a challenging goal, since it has not been yet applied on an industrial scale. The most promising producers of PEA are yeasts, which can produce PEA by *de novo* biosynthesis and biotransformation of $_L$-phenylalanine (*L*-Phe). In particular, *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* seem to be the best

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Nomenclature

C	concentration of PEA, $g{\cdot}L^{-1}$
$\Delta_{fus}H$	molar enthalpy of fusion (kJ mol^{-1})
S	selectivity
T	equilibrium temperature (K)
x	mole fraction

Greek letters

β	distribution ratio

Superscripts

i	component
1,2,3	type of component
I	water – rich phase
II	ionic liquid – rich phase

Subscripts

1	ionic liquid, or secondary solvent
2	PEA
3	water, or IL in re-extraction
PEA	2-phenylethanol

candidates for this application, since they are able to produce up to about 3–4 $g{\cdot}L^{-1}$ in batch or fed-batch cultures [3,9–11]. It has been reported that the achievement of higher concentration of PEA in broth is impossible because of PEA's cytotoxicity. Therefore, there are attempts to combine the PEA production with the simultaneous removal of toxic product from broth by extraction process. Recent reports have been showing that the addition of organic phase into a culture might be useful for that application. However, it is difficult to find out an appropriate solvent, as it must, except possessing the good physico-chemical properties for PEA extraction, be a biocompatible compound, not influencing both the growth of yeast cells and PEA production [2]. So far, the organic compounds as oleic acid, oleyl alcohol, miglyol, dibutylsebacate, isopropyl myristate and polypropylene glycol 1200 have been successfully employed for the continuous removal of PEA from yeast cultures [9]. For example, the use of oleic acid as an organic phase in two-phase fed-batch culture of *S. cerevisiae* Giv2009 increased threefold the overall concentration of PEA to 12.6 $g{\cdot}L^{-1}$, because the titer of PEA in oleic acid was 24.0 $g{\cdot}L^{-1}$[3]. In the case of the application of polypropylene glycol 1200 for *in situ* product removal (ISPR) in two-phase culture of *K. marxianus* CBS600 the PEA overall concentration of 10.2 $g{\cdot}L^{-1}$was reached [12]. In case of a fed-batch process, successful product removal using microcapsules with an extractive solvent has been achieved [5,12–14].

Recent literature reports have also shown that the use of microcapsules with an extractive solvent for ISPR likewise enhance the PEA productivity [5,13,14]. Microcapsules show excellent monodispersity and spherical morphology with extremely narrow size distribution. Different organic solvents were tested for the extraction of PEA in binary solid-liquid, or liquid-liquid phase equilibrium (LLE), between which the best was 1-decanol [15].

This work proposes to study a particular type of biosynthesis of PEA with fast extraction of PEA "*in situ*" with ionic liquids (ILs). The first information about ILs used for the enhancement of PEA production in a biphasic system specified 1-butyl-3-methylimidazolium bis{(trifluoromethyl)sulfonyl}imide, [BMIM][NTf_2], 1-methyl-1-propylpiperidinium bis(trifluoromethylsulfonyl)imide, [PMPip][NTf_2] and *N*-methyl-*N*-trioctylammonium bis{(trifluoromethyl)sulfonyl}imide, [N_{1888}][NTf_2] as the best entrainers in this process [16]. Whereas the $[PF_6]^-$ and $[BF_4]^-$-based ILs were not successful in this application [16].

Previously, we presented large number of the LLE data in ternary systems {IL + PEA + water}. This analysis was made for choosing the best IL from the physico-chemical point of view in PEA extraction. The ILs used by us were: *N*-octylisoquinolinium bis{(trifluomethyl)sulfonyl}imide, [C_8iQuin][NTf_2] [17], 1-hexyl-1-methyl-pyrrolidinium bis{(trifluomethyl)sulfonyl}imide, [C_6C_1Pyr][NTf_2], 1-hexyl-1,4-diaza[2.2.2]bicyclooctanium bis{(trifluomethyl)sulfonyl}imide, [C_6DBCO][NTf_2], 1-(2-methoxyethyl)-3-methylimidazolium bis{(trifluomethyl)sulfonyl}imide, [COC_2C_1IM][NTf_2], and 1-(2-methoxyethyl)-1-methylpyrrolidinium bis{(trifluomethyl)sulfonyl}imide, [COC_2C_1Pyr][NTf_2] [18], 1-hexyl-3-methylimidazolium tetracyanoborate, [HMIM][TCB], 1-decyl-3-methylimidazolium tetracyanoborate, [DMIM][TCB], and 1-butyl-1-methylpyrrolidinium tetracyanoborate [BMPYR][TCB] [19], 1-hexyl-1-methylmorpholinium bis{(trifluoromethyl)sulfonyl}imide, [HMMOR][NTf_2], 1-allyl-3-methylimidazolium bis{(trifluoromethyl)sulfonyl}imide, [AMIM][NTf_2], and diethylmethylsulfonium bis{(trifluoromethyl)sulfonyl}imide, [S_{221}][NTf_2] [20] and many of ammonium ionic liquids such as *N*-butyl-*N*-trimethylammonium bis{(trifluoromethyl)sulfonyl}imide, [N_{1114}][NTf_2], (2-hydroxyethyl)-*N*-trimethylammonium bis{(trifluoromethyl)sulfonyl}imide, [N_{1112OH}][NTf_2], *N*-*N*-diethyl-*N*-methyl-*N*-(2-methoxyethyl)ammonium bis{(trifluoromethyl)sulfonyl}imide, [$N_{2212OCH3}$][NTf_2], *N*-methyl-*N*-trioctylammonium bis{(trifluoromethyl)sulfonyl}imide, [N_{1888}][NTf_2], and *N*-triethyl-*N*-octylammonium bis{(trifluoromethyl)sulfonyl}imide, [N_{2228}][NTf_2] [21]. Recently, the 1-butyl-1-methylpyrrolidinium bis{(trifluoromethyl)sulfonyl}imide [BMPyr][NTf_2] as well as DES composed of ([BMPyr][NTf_2] + 2-methyl-2-butanol) and (1-hexyl-1-methylpyrrolidinium bis{(trifluoromethyl)sulfonyl}imide [HMPyr][NTf_2] + 2-methyl-2-butanol) were tested by us [22].

The structure type of cation is the key factor for solvation properties of the IL and its extraction potential. The hydrophobic anion $[NTf_2]^-$ is responsible for the immiscibility region in the binary system (IL + water). The second hydrophobic anion proposed by us for the extraction process was bis{(trifluomethyl)sulfonyl}imide, $[FSI]^-$ [23]. The following ILs were measured in ternary LLE {IL + PEA + water}: 1-hexyl-1-methylmorpholinium bis(fluorosulfonyl)imide, [HMMOR][FSI], *N*-octylisoquinolinium bis(fluorosulfonyl)imide, [OiQuin][FSI], 1-butyl-1-methylpyrrolidinium bis(fluorosulfonyl)imide, [BMPyr][FSI] and *N*-triethyl-*N*-octylammonium bis(fluorosulfonyl)imide, [N_{2228}][FSI] [23]. The results of these ternary LLE systems, showing immiscibility in liquid phase at temperature T = 308.15 K, revealed different extracting properties of PEA from the aqueous phase (see Table 1, [17–23]). However, the IL used in bioprocess has to show high extractive selectivity and capacity but the biocompatibility with the yeast is the most important.

Most data in the literature have focused on modifying yeast or fermentation aspects or extracting liquid. Different biotransformation methods and organic phases were proposed for this production, such as enzymes [24], or polymers in binary solid-liquid system [25], or microbial strains [26]. Regarding ILs as an extracting media, their thermophysical and physico-chemical properties and phase equilibrium properties in binary and ternary systems were widely presented by us [14,17–23,27–30]. The best chosen ILs were tested in a biotransformation of L-Phe to PEA by *S. cerevisiae* AM1-d strain, at T = 303 K in our laboratory [31]. The percentage of bioconversion of L-Phe was developed for [BMPyr][NTf_2], 1-hexyl-1-methylpiperidinium bis{(trifluoromethyl)sulfonyl}imide, [HMPip][NTf_2], [C_8iQuin][NTf_2],