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DES as versatile solvents for continuous flow enzymatic kinetic resolution of rac-menthol

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PURPOSE OF THE ABSTRACT
The separation of racemic mixtures and obtention of pure enantiomeric compounds has a significant importance, especially in the pharmaceutical industry. It is common that in a racemic mixture, only one of the enantiomers has the desired effect or properties, and the remaining enantiomer is regarded as impurity or in some cases may even have a toxic effect. It is therefore very important to develop optimized strategies for racemic separation/resolution, having a low cost and high efficiency, and also to be as sustainable as they can.
In this work, the model racemic mixture under study was racemic menthol, widely used as an aroma and flavor in food industry, but that also finds uses as anesthetic and permeation enhancer in pharmaceutical industry. In rac-menthol, only the (-)-menthol enantiomer has the desired properties, hence the needed racemic resolution. This can be achieved by means of the kinetic resolution, using enzymes as catalysts (enzymatic kinetic resolution- EKR)[1] which usually uses a lipase, such as candida rugosa lipase. The lipase catalyzes the selective esterification of one of the menthol enantiomers, yielding the corresponding ester and the unreacted enantiomer.
The choice of solvent for this separation can play an important role, since it greatly affects the enzymatic activity and the efficiency of the separation. Although lipases are known to show good activity in organic solvents and ionic liquids, they have recently been used with deep eutectic solvents (DES)[2,3]. The use of DES in biocatalytic processes has several advantages, all mainly related with the tunability and high sustainability of DES. DES can be composed of hydrogen bond donor and hydrogen bond acceptor species, which form a mixture with a melting point lower that that of the original components[4,5]. They can be formed by different compounds such as quaternary ammonium salts, organic acids, alcohols or even aminoacids. Their applications are now vast and biocatalysis is no exception.
Since it is possible to obtain DES composed of menthol, and also (-).menthol[6?8], a strategy has been developed in our previous work, where the menthol-based DES has the dual role of reaction solvent and also substrate source. Once the enzyme is added to this DES, the reaction and the separation are made possible. In this case, DES composed of rac-menthol and lauric acid are used, and (-)-menthyl laurate is formed. The optimization of the reaction conditions has been carried out in batch mode, varying parameters such as substrate molar ratio, reaction temperature, water activity, among other parameters.
In this work, we report the use of this DES system of solvent/substrate source for this reaction, in a continuous flow mode. This work goes further, by allowing for enzyme reutilization, and a more efficient reaction, while studying the influence of some process parameters, such as flow rate and amount water activity of the DES. Enzyme immobilization through several different methods such as sol-gel entrapment or covalent immobilization were tested. Also, when working in continuous flow mode, the flow rate choice assumes great importance, so this was also under study. The reusability of the enzyme was also evaluated.
This work shows the versatile role that DES can have in lipase catalyzed reactions, and demonstrates that the upscaling of this process is feasible. The resolution of racemic menthol using DES as reaction/separation

strategy, can open doors for its application in the separation of other racemic compounds such as therapeutic compounds used in industry.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

eutectic solvents | racemic mixture | biocatalysis | continuous flow process

BIBLIOGRAPHY