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CONTINUOUS BIOCATALYSED REDOX REACTIONS FOR THE ECOFRIENDLY SYNTHESIS OF PHARMACEUTICALLY RELEVANT COMPOUNDS

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PURPOSE OF THE ABSTRACT

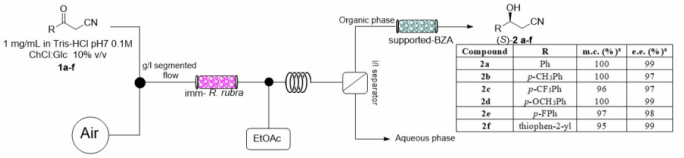
Biocatalysis and flow chemistry are ideal partners for accessing novel chemical spaces and define sustainable and efficient synthetic tools with high level of intensification [1]. Biocatalytic redox reactions are very attractive since they often occur with high stereo- and regio-selectivity under mild and environmentally friendly conditions. The aim of our work was to develop greener and scalable routes for the synthesis of high value chemicals, using both whole cells and isolated enzymes. Moreover, new solvent systems such as Natural Deep Eutectic Solvent (NADES) have been investigated as green co-solvents in enzymatic transformations. The processes have been designed integrating in-line work ups and purification procedures to reduce manual handling, downstream processes and costs. For this purpose, meso reactors were employed in order to demonstrate that with a biocatalytic approach was also possible to obtain, in some cases, gram amounts of pure final compound improving the total productivity of the system. Two studies will be described:

i) Enantioselective reduction of β-ketonitriles
Immobilised whole cells of Rhodotorula rubra MIM 147, a widespred yeast genus, have been exploited in a fully automated system for the enantioselective synthesis of β-hydroxynitriles, starting from β-ketonitriles (Scheme 1) [2]. We selected a natural deep eutectic solvent made by choline chloride/glucose with a dual purpose as a co-solvent and as an internal source of glucose, fundamental for the cofactor regeneration. With the aim of developing a fully automated procedure for the obtainment of a key building block for the synthesis of the antidepressant drug duloxetine, i.e., (S)-3-hydroxy-3-(thiophen-2-yl)propanenitrile, an in-line purification procedure has been developed (scheme 1, compound 2f). An in-line extraction of the desired product was performed adding a flow stream of ethyl acetate followed by a liquid/liquid separation. The organic stream with any unreacted substrate and the desired product was further purified by flowing it through a column packed with polymer supported benzylamine (PS-BZA), allowing the recovery of the pure final product 2f after simple solvent evaporation. The optimised protocol allowed the isolation of the desired product in 80 minutes of residence time with >90% conversion and >99% e.e. and resulted to be versatile for the enantioselective reduction of different β-ketonitriles [3].

ii) Oxidation of tyrosol to hydroxytyrosol
Tyrosol (Ty) and hydroxytyrosol (HTy) are valuable dietary phenolic compounds present in olive oil and wine and possess a range of biological effects [4]. The availability of gram amounts of pure Ty, HTy, their metabolites and derivatives are highly appealing for a deep biological evaluation. Furthermore, it must be considered that the price of HTy compared to the cost of Ty is more than 1000 higher. In this context, we performed the oxidation of easily accessible Ty using a free tyrosinase from Agaricus bisporus in presence of oxygen and ascorbic acid. The

aqueous flow stream was then extracted in-line with ethyl acetate and the aqueous layer, containing the biocatalyst and the excess of ascorbic acid, was recirculated to improve the overall efficiency. An in-line purification for the collection of pure HTy was also designed (Scheme 2). Then, a bioreactor packed with an immobilised acyltransferase from Mycobacterium smegmatis (imm-MsAcT) was used to produce more lipophilic acetate derivatives [5]. With this modular set up, HTy was obtained with an isolated yield of 75%, whereas the acetate metabolites showed yields up to 80% in 10 minutes [6].

FIGURES



Compound	R	m.c. (%)[a]	e.e. (%)[a]
2a	Ph	100	99
2b	*p*-CH_3Ph	100	97
2c	*p*-CF_3Ph	96	97
2d	*p*-OCH_3Ph	100	99
2e	*p*-FPh	97	98
2f	thiophen-2-yl	95	99

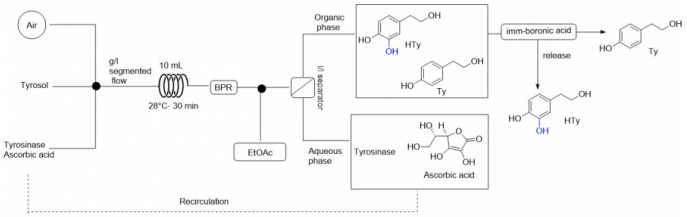


FIGURE 1

Scheme 1

FIGURE 2

Scheme 2

Optimised flow reactor configuration for the bio-oxidation of Ty to HTy and in-line purification. The solutions of Ty and tyrosinase with ascorbic acid were prepared in sodium phosphate buffer 0.1 M, pH 7.0. BPR: 40 psi

KEYWORDS

Flow chemistry | Deep eutectic solvents | Redox reactions | Green ehemistry

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