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Editorial

Special Issue on "Biocatalysis, Enzyme and Process Engineering"

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Biocatalysis entails the use of enzymes, which are catalytically active proteins, to speed up reactions so that products can be obtained swiftly and accurately. Together with high selectivity and catalytic efficiency, which can occasionally be close to the diffusion limit, enzymes operate under mild temperature and pH conditions. Moreover, some enzymes are active and stable in non-aqueous environments, e.g., organic or ionic liquid systems, making these enzymes particularly suitable for synthetic reactions [1,2].

Enzymes may be the cornerstones of efficient and clean processes, contributing to energy savings and negligible by-products and waste. This latter feature is particularly highlighted when synthetic enzymatic cascades are considered, where the product of one reaction is the substrate for the ensuing one and so forth, with no need for the purification of intermediates. Cascade reactions also allow reactions to be completed in cases where the chemical equilibrium is unfavourable, since the second reaction uses the product from the first reaction as a substrate. Moreover, if the enzymes involved in the cascade operate under similar pH and temperature ranges, the one-pot synthesis approach can be implemented [2,3].

Enzymes have thus been used to transform (un)natural substrates into marketable compounds, from bulk chemicals and related low-valued products to speciality chemicals and (bio)pharmaceuticals [1,4]. Accordingly, biocatalytic steps have been introduced into the various processes of interest for agro-based, chemical, detergent, food, and pharmaceutical industries, and their potential for further application is far from exhausted. Still, despite some promising developments in recent years, including economic and environmental incentives, the application of enzymes in commercial processes is still vastly inferior to that observed at the laboratory scale [1,5]. This can be associated with some of the weaknesses of biocatalysis, namely the limited range of enzyme stability to harsh operational conditions, such as high ionic strength, low and high pH values, and high temperature, in a given chemical, large-scale process. Nevertheless, this can be overcome, or at least mitigated, through enzyme immobilization [1–3,5]. The Special Issue on "Biocatalysis, Enzyme, and Process Engineering" showcases several works that provide representative examples of the novel applications of biocatalysis, as well as developments that can contribute to bringing biocatalysis closer to large scale processes.

The paper by Hoa and co-workers [6] features the application of commercial enzymes (hydrolases), e.g., Alcalase®, Pectinex®, Termamyl SC®, to produce a liquid formulation of fresh and easily available ingredients from food markets to be used as enteral nutrition (tube feeding to deliver nutrition directly to the stomach/small intestine for individuals unable to take in nutrition via the mouth). The novel formulation aims to provide an affordable alternative to standard commercial formulas that are often in shortage in developing

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countries, while meeting the requirements of quality and safety. The authors were able to establish that the enzyme-processed material led to a formulation with a caloric density of 1 kcal/mL, oligomeric and monomeric proteins, and 14.8 g of dietary fibres per L, which was considered nutritionally adequate, and with ~90% in vivo digestibility. Short-and medium-chained peptides released during enzymatic hydrolysis evidenced bioactivity, which may improve digestibility and bring several health benefits. The formulation also displayed low viscosity, which eases administration through small diameter tubes, and the absence of refined components is likely to minimize the risk of intolerance by the patients.

Hydrolases, e.g., amylases, cellulases, and pectinases, have been largely used for fruit juice processing and, in recent years, ultrasonication has been evaluated for the extraction of biomolecules from plants, aiming to improve juice yield and quality while decreasing extraction time. Reddy and co-workers combined the two approaches, enzymatic and ultrasonication, for juice extraction from mango pulp and evaluated juice yield and physical-chemical properties [7]. The authors were able to establish that mango treatment with pectinase assisted by ultrasonication maximized juice yield (~94%, w_{juice}/w_{pulp}), compared to enzyme mixture (amylase, cellulase, pectinase) assisted by ultrasonication (~80%). The synergism of enzymatic and ultrasound processing was highlighted as isolated pectinase, enzyme mixture, and ultrasonication treatment led to juice yields of ~86%, ~60%, and ~55%, respectively, whereas a control (extraction with no treatment) led to a juice yield of only ~35%. The results also emphasise the relevance of treatment with pectinase as compared to amylase and cellulase in mango processing, which was ascribed to a high titre of pectin and a low titre of cellulose and starch in mango pulp. Combining ultrasonication and pectinase treatment also allowed a decrease by half the duration of the extraction procedure, as compared to the use of isolated pectinase, and a decrease in enzyme loading from 0.20% to 0.15% with no loss in juice yield. Moreover, treatment with pectinase assisted by ultrasonication enabled a significant reduction in juice viscosity (~6 cP) as compared to the control (~25 cP), leading to the highest clarity (~79% transmittance), total soluble sugars (~17 °Brix), and total polyphenols (~810 mg/L). Overall, the authors concluded that pectinase treatment assisted by ultrasonication markedly increased the quality of mango syrup and could be implemented as an alternative to conventional methods of juice extraction from mango pulp.

Polymers are ubiquitous in our society and most of those that are present in our daily life are synthetic plastics, durable in use but also durable in waste. The latter feature has a marked negative environmental impact; thus, research targeting the synthesis biodegradable polymers has received considerable attention in recent years. In this context, Pfluck and co-workers assessed several methods of producing a biodegradable polyester, more specifically, a poly(octamethylene suberate) (POS), through polycondensation using commercially available lipases [8]. The authors selected *Pseudozyma antarctica* (currently *Moesziomyces antarcticus*) lipase B as a biocatalyst, since it displayed the highest catalytic efficiency in miniemulsion systems, both in free and (particularly) in immobilized form (acrylic adsorption). The immobilized lipase was used for POS synthesis in different media, namely, organic, water and miniemulsion (oil in water, over 80% water). The latter emerged as the most promising, as it led to the highest polymer molecular weight (7800 g/mol) with 98% conversion under incubation at 45 °C for 48 h. Moreover, the immobilized lipase was reused in seven consecutive 72 h cycles at 60 °C in a stirred batch reactor, with stable conversion being observed. The bioconversion system developed was deemed a promising candidate for the green production of biodegradable polymers, given its high enzymatic stability and activity and environmentally friendly nature. The work also provided relevant suggestions for the scale-up of the process, namely involving the type of stirring system, which should be carefully considered.

Adding value to waste materials was triggered by growing environmental concerns and an acknowledged need for sustainable production processes. Within this framework, Mota and co-workers developed processes to valorise coffee industry residues, e.g., spent coffee grounds (SCG) and silverskin (CS) as sources of oil and both of oil and biomass,

respectively [9]. The fatty acid profile of the oils from both SCG and CS was quite similar. Oil from the former was used as a substrate in acidolysis and interesterification reactions using commercial lipases, in an immobilized form, as a catalyst to produce low-calorie structured lipids, e.g., triacylglycerols (TAG), for applications in the food and pharmaceutical industries. Biochar obtained from CS by pyrolysis was also used for lipase immobilization. Among the different immobilized lipases assayed for new TAG synthesis, Lipozyme TL IM, a commercial formulation of *Thermomyces lanuginosus* lipase, proved to be the most efficient catalyst, particularly for the interesterification reaction. However, it displayed poorer physical and mechanical stability than the runner-up, Lipozyme RM IM, a commercial formulation of *Rhyzopus miehei* lipase. The latter was thus selected by the authors for further work, on account of the lower risk of biocatalyst particle disintegration, which negatively impacts biocatalyst catalytic activity, and thermal and operational stability. Using mixtures composed of SCG and either caprylic acid or capric acid (or their ethyl esters) as substrates, the authors reported ~90% TAG conversion values in under 7 h of reaction at 50 °C, and new TAG yields of 75% and 61% in acidolysis and interesterification reactions, respectively. The residual activity of ~40% and ~70% was observed after 10 repeated batch runs for acidolysis and interesterification, respectively; again suggesting the biocatalyst selected favoured interesterification over acidolysis.

Enzyme immobilisation is a relatively simple and straightforward way of improving enzyme performance, e.g., by enhanced stability and a wider mode of operation. Growing interest is being given to nanomaterials, namely of magnetic nature, as these have been shown to present appealing features for enzyme immobilization, e.g., biocompatibility, chemical stability, ease of recovery, and high surface area. Bi and co-workers developed a new biocatalyst based on magnetic Fe_3O_4 nanoparticles coated with polydopamine, which is rich in amino groups, to which *T. lanuginosus* lipase was coupled [10]. The reaction system selected involved the hydrolysis of *p*-nitrophenyl palmitate. The best compromise between enzyme loading and activity recovery was observed at 178.4 mg/g and 85.5%, respectively. Higher enzyme loadings led to a decrease in activity recovery, possibly due to intermolecular steric hindrance and concomitant diffusion restrictions. The optimal coupling time was established at 4 h, corresponding to 161.5 mg/g carrier loading and 88.4% activity recovery. The authors established that slightly alkaline conditions ($7.5 < pH < 8.5$) favoured both enzyme loading and activity recovery, suggesting that the relatively high pH leads to a charge distribution that favours carrier–enzyme interaction. The temperature also influenced the immobilization process: both activity recovery and enzyme loading increased as temperature initially increased from 10 °C to 25 °C but decreased thereafter. This is a typical phenomenon observed in chemisorption. The optimum pH for enzyme activity shifted from 7.5 to 8.0 after immobilization and the activity of the immobilized enzyme was less susceptible to the extreme values of pH than that of the free enzymes. The optimum temperature for activity remained unaltered after immobilization, but again, the activity of the immobilized enzyme was less susceptible to extreme temperatures than that of the free enzymes. These phenomena were attributed to charge interactions between carrier and enzyme molecule that contribute to conformational integrity and adjustability. Immobilization also enhanced pH and thermal stability. Such behaviour was attributed to the increased rigidity of spatial conformation of the enzyme due to immobilization, which minimizes unfolding under aggressive conditions. The same may underlie the higher solvent tolerance of the immobilized enzymes as compared with the free types, which can be advantageously used in a synthetic reaction. Finally, the immobilized enzyme displayed encouraging operational stability, as it retained ~70% of its initial activity after eight consecutive batch runs.

Flow chemistry entails the use of channels (pipes or tubes), through which a stream is continuously fed. Hence, the reaction occurs in a continuous stream instead of a traditional flask. Flow chemistry provides a unique environment characterised by high heat and mass transfer, easy scaling, the precise control of reaction conditions, and high surface to volume ratios that can be advantageously used for either single and multi-enzyme immobilization,

the latter featuring cascade reactions, as reviewed by Fernandes and de Carvalho [11]. The review discusses the different types of reactors and enzyme immobilization methods, delves into the particular requirements and constraints when multiple enzymes are involved, and presents illustrative examples of immobilized multi-enzyme cascades in flow reactors. The critical engineering aspects of flow reactors are also presented in the review, as they are paramount for their characterization and modelling. The use of whole cells to carry out multi-step reactions in flow chemistry is also debated and illustrated with several examples. Flow chemistry is also shown to provide appealing conditions to integrate chemical reactions and biocatalysis, and yet, the same challenges are extant. Finally, the challenges that occur when scaling these systems is envisaged are presented.

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