



## Editorial Biocatalytic Process Optimization

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Biocatalysis refers to the use of microorganisms and enzymes in chemical reactions, has become increasingly popular and is frequently used in industrial applications due to the high efficiency and selectivity of biocatalysts. Enzymes are effective and precise biocatalysts as they are enantioselective, with mild reaction conditions, and are important tools in green chemistry. Biocatalysis is widely used in the pharmaceutical, food, cosmetic, and textile industries. Biocatalytic processes include enzyme production, biocatalytic process development, biotransformation, enzyme engineering, immobilization, and the recycling of biocatalysts. Factors affecting biocatalytic reactions include substrate concentration, product concentration, enzyme or microorganism stability, inhibitors, temperature, and pH. As such, the optimization of biocatalytic processes is an important issue.

Active compounds in natural products usually contain glycosides, which can be cleaved by glycoside hydrolases to increase biological activity. The cocktail enzyme cellulase has been used in the deglycosylation of piceid to produce resveratrol [1]. Rha et al. [2] tested several commercial food-grade enzymes for producing flavonol aglycones from green tea extracts via deglycosylation. Tannin acyl hydrolase and glycoside hydrolase activities in Plantase-CF (a multi-functional food-grade enzyme from *Aspergillus niger* with the ability of cellulolytic hydrolysis of carbohydrates) trigger the degalloylation of catechins and produce flavonol aglycones from green tea extracts. Optimal conditions for producing flavonol aglycones are pH 4.0 and 50 °C. Biocatalysis can prevent catechin degradation, unlike hydrochloride treatment where 70% (w/w) of catechins disappear.

Glycoside hydrolases are enzymes that catalyze the hydrolysis of the glycosidic bonds in glycosides and have many applications, including the production of agaro-oligosaccharides, glucose, xylose, and xylobiose. Jiang et al. [3] reported a novel technique for producing agarotriose and agaropentaose from agaro-oligosaccharide using  $\beta$ -agarase. The results showed that 1.950 U/mL  $\beta$ -agarase AgWH50B was optimal for the preparation of agarotriose from the hydrolysis of agaroheptaose or agarononose, while 0.1900 U/mL  $\beta$ -agarase DagA was optimal for the preparation of agarotriose and agaropentaose from the hydrolysis of agaroheptaose or agarononose. In this study, the authors obtained value-added oligosaccharides from agarose by using different agarolytic enzymes or varying the enzyme amount. Aksenov et al. [4] used cellulolytic enzymes (mainly cellulases and xylanase) from recombinant *Penicillium verruculosum* to hydrolyze hardwood and softwood pulp, though the lignin content and drying process decreased the bioconversion of pulp to glucose. It was determined that fiber morphology, differing xylan and mannan content, and hemicellulose localization in kraft fibers deeply affected the enzymatic hydrolysis of bleached pulp. At a concentration of 10%, never-dried bleached kraft pulp demonstrated highly efficient bioconversion, resulting in a concentration of more than 50 g/L sugar.

Enzymatic biofuel cells rely on enzymes rather than conventional noble metal catalysts. Commonly used redox enzymes include glucose dehydrogenase, glucose oxidase (GOx), lacase (LAc), fructose dehydrogenase, and alcohol dehydrogenase [5]. Bojang and Wu [6] established the use of GOx/LAc modified electrodes as bioanodes and biocathodes for biofuel cells. Electrochemical analysis methods including cyclic voltammetry, the Nicholson method, the Randles–Sevcik equation, and electrochemical impedance spectroscopy were used to evaluate the performance of prepared electrodes. Following testing, the optimal bioanode and biocathode were determined to be a carbon paper–GOx–mediator–carbon nanotube with a current density of 800  $\mu$ A/cm<sup>2</sup> and a carbon paper–Lac–mediator–carbon nanotube with a current density of 600  $\mu$ A/cm<sup>2</sup>, respectively. The construction and use of enzyme electrodes can be applied to biofuel cells, bioreactors, biosensors, and micro-reactors.

The disaccharide trehalose, a natural biostructure stabilizer that accumulates in the cytoplasm under stress conditions, is present in a wide variety of organisms, including bacteria, yeast, fungi, insects, invertebrates, and lower and higher plants [7]. Sampedro et al. [8] studied the effect of trehalose on enzyme reactions using Kramers' theory. The role of trehalose was reviewed and the molecular interactions of trehalose–water–enzymes/proteins were described in detail, supported by recent in vitro and in silico experimental results. Importantly, the concept of coupling the enzyme's structural dynamics to medium viscosity, as described by Kramers' theory, is the central thesis of this paper and the focus is on enzyme catalysis. As such, the application of Kramers' theory is reinforced by relating the rate of inactivation, unfolding, and folding of enzymes to trehalose viscosity. The recently observed effects of trehalose viscosity on DNA and RNA folding is mentioned as a corollary.

Proteases belong to the hydrolase class of enzymes and hydrolyze proteins into smaller polypeptides or single amino acids. Protein hydrolysates have many biological functions and demonstrate antioxidant activity [9,10]. Zhang et al. [11] used pepsin, trypsin, dispase, papain, and bromelin to digest *Pleurotus ostreatus* protein extract (POPE). The antioxidant activity of the protein hydrolysates resulting from five different proteases were compared. The results showed that POPEP (POPE hydrolyzed by pepsin), with a molecular weight of 3–5 kDa, had the strongest antioxidant activity. Excessive free radicals or reactive oxygen species (ROS) are harmful to the human body since these components may destroy the normal functions of cells, tissues, and organs [12]. Superoxide dismutase, glutathione peroxide, and catalase can remove free radicals to reduce the risk of oxidative damage during periods of increased ROS. Mice pretreated with POPEP (3–5 kDa) showed significantly increased superoxide dismutase and glutathione peroxide enzyme activity in the liver, demonstrating that POPEP could protect the liver from oxidative damage.

Sadauskiene et al. [13] reported on the effects of long-term supplementation with aluminum (Al) or selenium (Se) on antioxidant enzyme activity in the brains and livers of mice. The results showed that 8 weeks of exposure to Se caused a statistically significant increase in superoxide dismutase, catalase, and glutathione reductase activities in the brain and/or liver, but the changes were dose-dependent. Exposure to Al caused a statistically significant increase in glutathione reductase activity in both organs.

Protein hydrolysates containing bioactive peptides can be used to formulate nutraceuticals or functional ingredients in food. Cheng et al. [14] used three commercial proteases (alcalase, bromelain, and papain) to obtain eel protein hydrolysates (EPHs) from whole eels (*Anguilla marmorata*). The emulsion activity index (EAI) and emulsion stability index (ESI) of each EPH was determined to test the product stability. The EPH obtained from the treatment with alcalase showed optimal EAI and ESI and demonstrated antioxidant activity. The results indicated that alcalase-hydrolyzed EPH had good emulsifying properties and solubility, making it useful in food processing.

The use of biocatalysis or biotransformation to produce pharmaceutical components has become a hot topic in biotechnology research. There are two main types of biocatalysts: whole cells and free enzymes. Both use enzymes to complete the reaction but in the former, the enzyme remains within the microorganism whereas for the latter, the enzyme has been separated and purified. When producing drugs and their intermediates, the most significant difference between biocatalysis and traditional chemical methods is that the former is very effective in the asymmetric synthesis of chiral compounds. In this Special Issue, several studies used biocatalysis to synthesize special compounds. Calycosin-7-O- $\beta$ -D-glucoside is an isoflavonoid glucoside and one of the principal components of *Radix astragali*, a well known medicinal and edible herb cited in European, Japanese, and Chinese literature. Hu et al. [15] used uridine diphosphate-dependent glucosyltransferase to glucosylate the C7 hydroxyl group of calycosin and synthesize calycosin-7-O- $\beta$ -D-glucoside. Optimal conditions for batch production were determined, including the temperature, pH, and the concentrations of dimethyl sulfoxide, uridine diphosphate, sucrose, and calycosin. Eggerichs et al. [16] used styrene and indole monooxygenase to activate double bonds via chiral epoxidation. The reaction conditions were successfully optimized for two flavins containing two-component monooxygenases during the conversion of large hydrophobic styrene derivatives in the presence of organic cosolvents.

Nguyen et al. [17] used Bacillus megaterium CYP102A1 monooxygenase for the regioselective hydroxylation of naringin dihydrochalcone to produce neoeriocitrindihydrochalcone. Kinetic parameters were used to compare the efficiency of dihydrochalcone hydroxylation by different CYP102A1 mutations. The indolizine core is present in many biologically active compounds and can be considered the scaffolding in the preparation of new pharmaceuticals. Indolizines have been synthesized by lipases from *Candida antarctica* [18]. Botezatu et al. [19] used whole cells to catalyze a multicomponent reaction of activated alkynes,  $\alpha$ -bromo-carbonyl reagents and 4,4'-bipyridine in the synthesis of bis-indolizines. Several yeast strains were tested to evaluate the effect of the reactants on their physiological activity. The optimal strain was Yarrowia lipolytica, which is an effective biocatalyst in cycloaddition reactions and can be used to synthesize indolizines. In recent years, there has been a widespread use of immobilized lipase to catalyze specific reactions in the production of valuable molecules, such as nutraceutical and pharmaceutical compounds [20,21]. Chung et al. [22] used a surface-display system for the expression of lipase A in an E. coli expression system. It was reported that lipase A activity was low at lower shaking rates due to the limited amount of dissolved oxygen, while higher shaking rates increased shear stress, leading to a decrease in the specific activity. This phenomenon was confirmed using kinetic studies and it was established that cultivating lipase A at a moderate shaking speed optimized hydrolysis.

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) ethyl esters are medicines used in the treatment of arteriosclerosis and hyperlipidemia. Kuo et al. [23] studied the lipase-catalyzed synthesis of DHA + EPA ethyl esters via the acidolysis of ethyl acetate with DHA + EPA concentrates. Lipase-catalyzed acidolysis has the advantage of not only synthesizing DHA + EPA ethyl ester efficiently, but also allowing for the easy recovery of the product. Moreover, a response surface methodology (RSM) approach for the evaluation of the kinetic model was successful integrated with the rate equation to simulate the performance of the batch reactor. The integral equation showed a good predictive relationship between the simulated and experimental results. Conversion yields of 88%-94% were obtained for 100-400 mM DHA + EPA concentrate at a constant enzyme activity of 200 U, substrate ratio of 1:1 (DHA + EPA: EA), and reaction time of 300 min. Rychlicka et al. [24] developed a biotechnological method of synthesizing 3,4-dimethoxycinnamoylated phospholipids via the interesterification of egg-yolk phosphatidylcholine with the ethyl ester of 3,4-dimethoxycinnamic acid. RSM and a Box–Behnken design were used to evaluate reaction conditions. The optimal incorporation of 3,4-dimethoxycinnamic acid into phospholipids reached 21 mol%. Moreover, 3,4-dimethoxycinnamoylated lysophosphatidylcholine and 3,4-dimethoxycinnamoylated phosphatidylcholine were obtained in isolated yields of 27.5% and 3.5% (w/w), respectively. Huang et al. [25] developed a biocatalytic process for synthesizing rose-flavored ester-2-phenylethyl acetate using a packed-bed bioreactor system. The synthesis process was performed in a solvent free system, which is an environmentally friendly process. The optimization of the synthesis reaction was carried out by a three-level-three-factor Box-Behnken design and RSM. This continuous process can be applied to the environmentally friendly production of natural flavor compounds, such as rose aromatic esters. Grabner et al. [26] developed a continuous process for the synthesis of a statin side chain precursor using a deoxyribose-5-phosphate aldolase-catalyzed stereoselective aldol addition reaction. A series of substrates was tested but only acetaldehyde and chloroacetaldehyde gave reasonable results. An experimental design was used to optimize pH value, temperature, and flow

conditions. The immobilization alginate was chosen and the reaction rates of both alginate beads and the alginate-luffa matrix were tested. The optimized flow process (0.1 mL/min, 0.25 M of chloroacetaldehyde, and 0.5 M of acetaldehyde) produced 4.5 g of product per day in a bench-top reactor. Du et al. [27] developed a continuous-flow procedure for the synthesis of  $\beta$ -amino acid esters via the lipase-catalyzed Michael reactions of various aromatic amines with acrylates. Seventeen  $\beta$ -amino acid esters were rapidly synthesized by lipase TL IM from *Thermomyces lanuginosus* in continuous-flow microreactors. Optimal reaction parameters were determined, including the reaction medium, temperature, enzyme, substrate molar ratio, residence time/flow rate, and substrate structure. The salient features of this study are the green reaction conditions (using methanol as reaction medium), short residence time (30 min), and high yield. Several articles in this Special Issue have demonstrated that a bioreactor with immobilized enzymes is suitable for use in biocatalysis to synthesize active pharmaceutical ingredients, drug precursors, and value-added chemicals. The benefits of continuous flow biocatalysis, including improved reaction rates, in-line product removal and purification, better mixing, improved control, and improved enzyme stability, ultimately minimize labor and reduce production costs. The increased demand for greener and more cost-effective processes will drive the rapid expansion of continuous flow biocatalysis in the next few years.

In conclusion, this Special Issue shows that an optimized biocatalysis process can provide an environmentally friendly, clean, highly efficient, low cost, and renewable process for the synthesis and production of valuable products. With further development and improvements, more biocatalysis processes may be applied in the future.

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