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Process intensification of immobilized lipase catalysis by microwave irradiation in the synthesis of 4-chloro-2-methylphenoxyacetic acid (MCPA) esters

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ABSTRACT

4-Chloro-2-methylphenoxyacetic acid (MCPA) is a selective systemic herbicide which is absorbed by leaves and roots. MCPA esters are preferred due to their low water solubility and environmental friendliness. Esterification of MCPA with n-butanol was investigated as a model reaction using immobilized enzymes under the influence of microwave irradiation. Different immobilized enzymes such as Novozym 435, Lipozyme TL IM, Lipozyme RM IM and Lipase AYS Amano were studied under microwave irradiation amongst which Novozym 435 (immobilized *Candida antarctica* lipase B) was the best catalyst. Effects of various parameters were systematically studied on rates and conversion. Under microwave irradiation, the initial rates were observed to increase up to 2-fold. Under optimized conditions of 0.1 mmol MCPA and 0.3 mmol n-butanol in 15 mL 1,4-dioxane as solvent, Novozym 435 showed a conversion of 83% at 60 °C in 6 h. Based on initial rate and progress curve data, the reaction was shown to follow the Ping Pong bi–bi mechanism with inhibition by MCPA and n-butanol. Esterification of MCPA was also studied with different alcohols such as isopropyl alcohol, n-pentanol, n-hexanol, benzyl alcohol and 2-ethyl-1-hexanol.

1. Introduction

Phenoxyacetic acids such as 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-DCPA) and 4-chlorophenoxyacetic acid (4-CPA) are extensively used as herbicides and hormone mimics. MCPA is a selective herbicide widely used in controlling weed growth [1]. At 15 mg kg^{-1} concentration of MCPA, adult rats, rabbits and their offspring showed no change in their body weight [2]. However, toxicological assays in rats at higher concentration showed teratogenic effect for 4-chloro-2-methylphenoxyacetic acid ethyl ester (MCPEE) [3] while 2,4-dichlorophenoxyacetic acid (2,4-DCPA) induced myotonia in experimental dogs [4]. Four forms of herbicides related to MCPA are MCPA acid, MCPA sodium salt, MCPA dimethylamine salt (DMAS) and MCPA ester. MCPA ester attracts most attention among four forms because of its low water solubility and environmental friendliness [1]. 2,4-Dichlorophenoxyacetic acid (2,4-DCPA) and 4-chloro-2-methylphenoxyacetic acid (MCPA) were reported to show bacterial degradation [5]. Esterification reaction of 4-chloro-2-methylphenoxyacetic acid (MCPA) was reported in the earlier literature using sulphuric acid [1]. There are several disadvantages of using homogeneous acid catalysis, which include costly materials of construction, neutralization of acidic waste leading to pollution and presence of impurities in the final ester. Therefore, the most appropriate process for organic transformations will be the one which avoids homogeneous liquid acids and is eco-friendly and economical. Several general routes of ester preparation have been investigated. Heterogeneous solid acids as catalysts could be used using high temperature [6,7]. In contrast to solid acids, biocatalysts allow synthesis of esters to be performed at moderate temperatures [8,9].

The exploitation of enzymes as catalysts in chemical synthesis has been explored in recent years. Lipases and esterases are the most commonly used biocatalysts; amongst them lipases have been reported to catalyze many reactions in organic solvents which include esterification, interesterification, transesterification, hydrolysis, amidation, thio-esterification, trans-thioesterification and epoxidation [8–17]. Dehydration reaction of polyanhydrides and glycols was reported using *Candida antarctica* lipase with toluene as solvent [18]. An efficient biodiesel production from

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soybean oil was obtained using a recombinant *Pichia pastoris* displaying *Rhizomucor miehei* lipase (RML) on the cell surface in an isooctane system [19]. Ethyl butyrate was synthesized using a recombinant *Rhizopus oryzae* lipase immobilized on different supports such as Eupergit®CM, EP100 and octadecyl sepabeads [20]. 4-Ethyl-(2-(1,3-dioxo-1,3-dihydro-2-isoindoylyl))-phenoxy acetic acid (LASSBio 482), an anti-asthma drug, was synthesized by selective hydrolysis of its methyl ester using Lipozyme RM IM [21]. Lipase catalyzed reactions were reported to kinetically proceed via Ping Pong bi–bi mechanism, ternary complex ordered bi–bi mechanism or ternary complex random bi–bi mechanism. In some cases, it involves inhibition by either substrate or product or both [16,17,22–26]. A ping–pong bi–bi mechanism was proposed for *C. antarctica* lipase B catalyzed resolution of a tertiary alcohol, citalopram intermediate (diol) [25]. Ping Pong bi–bi mechanisms with 1-butanol inhibition in esterification of oleic acid [16] and inhibition by both acid and alcohol in transesterification of alkyl butyrates to geranyl butyrate [26] were reported. Transesterification of ethyl cinnamate using Novozym 435 was found to proceed via ternary complex ordered bi–bi mechanism with inhibition by citronellol [9].

Microwave irradiation is established as an efficient heating source for a variety of chemical reactions, where high yields and reaction selectivity can be achieved in short reaction time [27–35]. Microwave irradiation results in an instantaneous localized superheating which is achieved due to dipole rotation or ionic conduction [29]. Hence it was thought desirable to employ microwave irradiation to intensify the reaction rates and conversions. Kinetic modelling for enzymatic esterification of MCPA with different alcohols under microwave irradiation has not been reported so far. Kinetics and mechanism for the lipase catalyzed esterification of MCPA and n-butanol was investigated under microwave irradiation to propose a suitable model. The effect of various parameters on conversion and rate of reaction were studied systematically.

2. Materials and methods

2.1. Enzyme and chemicals

The Lipozyme RM IM, Lipozyme TL IM and Novozym 435 were obtained as gift samples from Novo Nordisk, Denmark. Lipase AYS Amano was a gift sample from Amano Enzyme Inc., Japan. Lipozyme RM IM is *R. miehei* lipase immobilized on anionic exchange resin (activity of 30 U g^{-1}, based on tristearin assay) whereas Lipozyme TL IM is *Thermomyces lanuginosus* immobilized on silica. Novozym 435 is *C. antarctica* lipase B (CALB) immobilized on a macroporous polyacrylic resin beads (bead size 0.3–0.6 mm, bulk density 0.430 g cm^{-3}, water content 3%, activity of 7000 PLU g^{-1}). Lipase AYS Amano is *Candida rugosa* lipase in the form of lyophilized powder (activity 30,000 U g^{-1}).

All chemicals used in the study were AR grade, purchased from renowned companies and used with no further purification: 4-chloro-2-methylphenoxyacetic acid (MCPA) (Sigma Aldrich, India), n-butanol, n-pentanol, n-hexanol, isopropyl alcohol, benzyl alcohol, 2-ethyl-1-hexanol, acetonitrile, diisopropyl ether, diisobutyl ether, tetrahydrofuran and 1,4-dioxane (S.D. Fine Chemicals Pvt. Ltd., Mumbai, India). Solvents used for HPLC analysis were obtained from Thomas Baker (Mumbai, India).

2.2. Analytical method

Reaction progress was monitored by periodic withdrawal of clear liquid samples from the reaction mixture which were analyzed by high performance liquid chromatography (HPLC). The HPLC analysis was carried out on Agilent 1260 infinity HPLC-system (pumps G1311C, auto-sampler G1329B, diode-array-detector G1315D) using ZORBAX-RP C-18 column (4.6 mm × 250 mm; 5 μm, Agilent Industries, USA) under the following conditions: Samples (10 μL) were injected via auto-sampler. Column temperature was maintained at 25 °C. The isocratic elution was performed using mobile phase consisting of acetonitrile and water (70:30, v/v) at the flow rate of 1.0 mL min^{-1}. A DAD detector was used at a wavelength of 220 nm. The formation of product was confirmed by LC–MS analysis (Finnigan™ LCQ™ Advantage MAX, Thermo Electron Corporation, USA).

2.3. Experimental set-up and procedure

2.3.1. Conventional heating

The experimental set up used for conventional heating studies was the same as reported before [9]. A typical reaction procedure for lipase catalyzed synthesis of MCPA ester contained 0.1 mmol MCPA and 0.3 mmol n-butanol, diluted to 15 mL with 1,4-dioxane as a solvent. The reaction mixture was agitated at 60 °C for 15 min at a speed of 300 rpm. The reaction was initiated by adding a known amount of lipase. Samples were collected at regular intervals, filtered to eliminate particulate matter, if any, and analyzed by HPLC. All experimental data are average of triplicate values within a standard deviation (SD) of ±2%.

2.3.2. Microwave reactor

Microwave reactor (Discover, CEM-SP 1245 model) set up used in this work was the same as used before [9]. The CEM Discover microwave reactor could be used to conduct experiments up to microwave power of 300 W. The experiments were carried at a constant temperature. A constant microwave irradiation was provided (30–40 W). Experimental conditions maintained for microwave irradiation studies were the same as mentioned for conventional heating, unless stated otherwise. All experimental data are average of triplicate values within a standard deviation (SD) of ±2%.

2.3.3. Enzyme kinetics

Different process parameters were studied to elucidate the kinetics of lipase catalyzed esterification of MCPA with n-butanol. Concentrations of substrates were systematically varied over a wide range to study their effect on rate of reaction with Novozym 435 loading of 4 mg cm^{-3}. In one set of experiments, n-butanol (B) concentration was varied from 0.1 to 0.4 mmol at a fixed quantity of MCPA (A) (0.05–0.3 mmol) while in another set, different MCPA (A) concentrations were employed from 0.05 to 0.3 mmol at a fixed quantity of n-butanol (ranging from 0.1 mmol to 0.4 mmol). The concentration profiles were used to calculate initial rates of reaction.

3. Results and discussion

Scheme 1 depicts the reaction.

3.1. Conventional heating versus microwave irradiation

Enzymatic esterification of MCPA and n-butanol was studied under both conventional heating and microwave irradiation as a model reaction (Fig. 1). A 2-fold increase in initial rate was obtained under microwave irradiation vis-à-vis conventional heating which resulted in reduced time of reaction showing process intensification. This suggested that microwave capturing nature of the reactants (MCPA and n-butanol) was contributing to the higher reaction rate. We have reported similar effect for enzymatic transformation under microwave irradiation for different reactions [9,31,34]. Enzyme under microwave irradiation may behave differently to some extent. This is due to conformational modification in