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Ultrasound-assisted lipase catalyzed hydrolysis of aspirin methyl ester

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ABSTRACT

The ultrasound-assisted hydrolysis of aspirin methyl ester (AME) was investigated using immobilized *Candida antarctica* lipase B (CALB) (1%) in the presence of solvents like triolein, chloroform ($CHCl_3$) and dichloromethane (DCM). The effect of ultrasound and the role of water on the conversion rates have also been investigated. Proton nuclear magnetic resonance spectroscopic (^{1}H NMR) was chosen to calculate hydrolysis convertion rates.

We observed that lipase-ultrasound assisted hydrolysis of AME in the presence of triolein and water showed the highest hydrolysis conversion rate (65.3%). Herein low water amount played an important role as a nucleophile being crucial for the hydrolysis yields obtained. Lipase activity was affected by the conjugated action of ultrasound and solvents (35.75% of decrease), however not disturbing its hydrolytic efficiency. It was demonstrated that lipase is able to hydrolyse AME to methyl 2-hydroxy benzoate (methyl salicylate), which applications include fragrance agents in food, beverages and cosmetics, or analgesic agent in liniments.

1. Introduction

Aspirin methyl ester (AME) (methyl 2-acetoxybenzoate) developed and patented by Thorpe in 1918, is one of the safest, simplest and least expensive anti-inflammatory aspirin prodrugs [1]. AME was synthesized by temporarily masking the functional group of aspirin, carboxylic acid, which was found to have lower gastric ulcerogenic activity as compared to aspirin [2,3]. AME can be chemical or enzymatically hydrolysed to methyl 2-hydroxy benzoate. This compound, also known as methyl salicylate, can be used as a fragrance in food, cosmetics, toiletries, having also application as analgesic agent in liniments [4]. Chemical catalysis can be applied for AME hydrolysis however leading to non-specific byproducts and requiring high reaction temperature and pressure. As reported by others, enzyme catalyzed hydrolysis has been presented has an alternative to the chemical route since it is carried out under mild reaction conditions, minimizing the formation of undesirable by-products. Desai et al. reported the chemoselective hydrolysis of methyl 2-acetoxybenzoate (AME) through batch and fix reactor using free and entrapped esterase in K-carrageenan beads [5]. The immobilized catalysts generally possess specificity, higher catalytic activity and greater thermal stability. The environmental impact is reduced since immobilization allows the reuse of the catalysts, making them eco-friendly and less expensive [6]. Among the variety of enzymes with industrial applications, lipases have shown a vast potential in biotechnological and industrial scenarios. Lipases from different sources are widely used in different biochemical reactions such as esterification, transesterification, hydrolysis, alcoholysis, aminolysis, acidolysis, etc [7–10]. The hydrolysis of esters in the presence of water is a chemical reaction in which bond cleavage is affected by water to produce acid and alcohol, where water acts as nucleophile [11,12].

Among all lipases, Lipase B from *Candida antarctica* (CALB) is widely used as biocatalyst due to its high stereoselectivity, high activity, broad substrate specificity, high thermostability and conformational stability in hydrophilic and hydrophobic environments [13–17]. Nevertheless, low reaction rates have been observed which imply high processing costs. Several attemptes have been made to improve enzyme reaction

Abbreviations: AME, aspirin methyl ester; CALB, lipase B from *Candida antarctica*; $CHCl_3$, chloroform; DCM, dichloromethane; ^{1}H NMR, proton nuclear magnetic resonance ^{1}H NMR; WB, water bath; US, ultrasound

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rates using ultrasound, microwave, ionic liquids and supercritical fluids. Ultrasound mainly contributes to alter the temperature and pressure of the microenvironment as a result of the cavitational effect [18]. Mixing, shearing and mass transfer increase by ultrasound effect lead to high hydrolsysis conversion rates, improving strongly the enzymatic reactions [19–21]. Enzyme-ultrasound assisted reactions present several advantages compared with conventional methods namely a decrease of the reaction time, a reduction of the amount of reagents used, a higher yield, and chemo-, region and stereoselectivities of reactions that normally would not occur under normal conditions [18,22–25].

The addition of solvents which can act as a suitable reaction medium for enzyme catalyzed reactions is another way to overcome lipase hydrolysis limitations. Together with ultrasounds, it will improve the mass transfer and ensure sufficient mixing and emulsification of the two immiscible layers [26].

In the present work, we investigated for the first time the hydrolysis of aspirin methyl ester with immobilized lipase B from *Candida antarctica* (CALB) in the presence of different solvents such as triolein, chloroform ($CHCl_3$) and dichloromethane (DCM). The effect of ultrasound and solvents as well as the role of water in the hydrolysis reactions were studied and compared with their effect in the conventional approach using a shaker bath with controlled temperature. The hydrolysis reaction conversion rates were analyzed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The effect of ultrasound on enzyme activity was evaluated and compared with the effect of a conventional shaker water bath.

2. Materials and methods

2.1. Materials

Fermase CALB™ 10,000, a commercial *Candida Antarctica* lipase B (CALB) immobilized on glycidyl methacrylate-ter-divinylbenzene-ter-ethylene glycol dimethacrylate (particle size of 150–300 μm, pore volume of 1.32 cm^3/g, bulk density of 0.54 g/cm^3 and an activity of 8000 propyl laurate units) was obtained as a gift sample from Fermenta Biotech Ltd., Mumbai, India. Aspirin methyl ester (AME) (purity 98% +) was obtained from TCI Development Co., Ltd., Shanghai, China. Glycerol trioleate (Triolein) (purity 98%+), chloroform ($CHCl_3$) (AR, purity 99%+), dichloromethane (DCM) (AR, purity 99.5%+), phenolphthalein, oleic acid (AR), 2,2,4-trimethyl pentane (isooctane) (90% +), ethanol (AR, 99.7%+) and n-butanol (AR, 99%+) were received from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

The experimental setup involved the use of a thermostatized water bath with orbital oscillation (WB) (model STD-134L, Standard Groups Co., Ltd., Shanghai, China) and an ultrasonic bath (US) (SK5210HP, Kudos Ultrasonic Instrument Co., Ltd., Shanghai, China) (frequency 53 kHz and a power of 100 W).

2.2. Characterization of sonochemical cavitation reactor

2.2.1. Dosimetric characterization

The dosimetric characterization of ultrasonic bath SK5210HP (53 kHz, 100 W) followed the methodology referred by other authors [27,28]. The hydroxyl radicals produced by cavitation were quantified by conversion of terephthalic acid to 2-hydroxyterephthalic acid [27]. Solutions of 0.3 mM terephthalic acid (TA) were prepared in 0.1 M sodium phosphate buffer (pH 7.4) and submitted to cavitation during 1 h at 60 °C. The fluorescence of sonicated TA solutions were monitored by a Multi-mode Microplate Reader Synergy™ Mx and Gen5™ purchased from Biotek Instruments, Inc. (USA) using a wavelength scan confirming peak emission at 425 nm from an excitation wavelength at 315 nm. The calibration curve was plotted using standard TA solutions (0–50 mM) and 0.1 M sodium hydroxide. The hydroxyl radicals were measured at different points of the reactor considering their geometry and transducers positioning [29].

2.2.2. Calorimetric characterization

The calorimetric characterization of the ultrasonic bath was based on the energy measurement during time for several power inputs as it was published previously by other workers [27,30,31]. The measurements were performed using a Pico Technology TC-08 Analogue to Digital converter connected to a computer. The TCs were placed in the same positions used to perform the dosimetric characterization (see Section 2.2.1). The energy profile was followed using distilled water for 1 h and the corresponding calorific power was determined following equation:

$$E = (\Delta T_{ave} \times m \times C_p)/1000$$

where E is the calculated energy (kJ) to raise the water temperature; Δ_{ave} is equal to the difference of the final and initial temperature (K); m is the mass of the water (kg) and C_P is the heat capacity of water (4186 J kg^{-1} K^{-1}); the change in temperature (ΔT) was calculated for each TCs positions and the final value was obtained by the mean of the five temperature sensors.

2.3. Lipase-catalyzed hydrolysis of aspirin methyl ester in the presence of different solvents (triolein, chloroform and dichloromethane)

Firstly, 500 mg of AME were dissolved in 5 mL of different solvents (triolein, $CHCl_3$, or DCM) (see Table S1 of support information). Then, for each reaction, 1% (v/v) of distilled water was added to the system.

For the conventional approach, 1% (w/v) of immobilized CALB was added to the reaction mixtures water bath (100 rpm) at 35 °C for 12 h. For the ultrasound assisted approach a combination of water bath and ultrasound was applied to study the influence of ultrasound on the hydrolysis of aspirin methyl ester (see Table S.2). For this, the reaction was firstly carried out in a water bath for 4 h, then transferred to the ultrasonic bath for 0.5 h, transferred again to the water bath for 4 h, and then to the ultrasonic bath for 0.5 h, and finally to the water bath for 3 h. In all stages the reaction was carried out at 35 °C. The temperature was kept at 35 °C by placing the sample at the center of the ultrasonic bath, where the amount of hydroxyl radicals is higher and the temperature is less susceptible to changes. The total reaction time in ultrasonic bath operating at frequency of 53 kHz, 100 W power rating and incubated in a and duty cycle of 50% (5 min ON/5 min OFF) was observed to be 1 h.

Furthermore, to study the role of water on the enzymatic hydrolysis, batches of hydrolysis reaction without water were also performed for each set of reactions under the same reaction conditions as described above. The lipase activity was measured before hydrolysis and at the end of processing (see details in Scheme 1 of support information).

2.4. NMR characterization

The ^{1}H NMR spectra of the reaction mixtures were obtained by dissolving the products in 500 μL deuterated chloroform ($CDCl_3$). The spectra were recorded using a Bruker avance III 400 NMR spectrometer (Bruker Corporation, Germany), 400 MHz at 25 °C.

2.5. Lipase activity assay

To determine the enzyme activity of the immobilized lipase, 200 mg of vacuum dried enzyme was added to a vial containing a mixture of 0.32 mL oleic acid, 0.27 mL dry n-butanol in 3 mL dry isooctane and 0.05 mL distilled water. The flask was kept at 30 °C for 60 min with a shaking speed of 250 rpm. The reaction was stopped by addition of 10 mL methanol and immediately titrated against 0.05 M alcoholic NaOH and phenolphthalein indicator [32,33]. One unit of enzyme activity is defined as 1 mol of oleic acid consumed in reaction per min per mg of lipase.

$$\text{Enzyme activity}(Ea) = \frac{V \times M \times 100}{E \times t}$$

where V represents the difference in volume in mL of NaOH between the blank and samples which is a measure of oleic acid consumed during the reaction. M represents the molarity of NaOH in M. E represents the amount of enzyme employed in mg. t represents the time of reaction in min.

3. Results and discussion

3.1. Hydrolysis conversion rates of aspirin methyl ester (AME)

AME (methyl 2-acetoxybenzoate) was successfully hydrolyzed to methyl 2-hydroxybenzoate and acetic acid using CALB in the presence of different solvents. The ultrasound-assisted hydrolysis of AME catalyzed by immobilized CALB was performed and compared with the conventional method using a water bath. The effect of ultrasound and the solvents as well as the role of water on the enzymatic hydrolysis of AME were evaluated.

The ^{1}H NMR technique showed up to be the most appropriated method to evaluate the reaction conversion rates due to its high accuracy. The products (methyl 2-hydroxy benzoate and acetic acid) formation was determined through the ^{1}H NMR spectra which have shown the shift in the position of aromatic protons of AME during hydrolysis reaction. The ^{1}H NMR spectra of AME and the corresponding products of hydrolysis (methyl 2-hydroxy benzoate and acetic acid) are shown in Figs. 1 and 2.

In Fig. 2, the peaks observed in the range of 7–8.1 ppm correspond to the aromatic protons of AME which are shown as signs i, j, k and m, whereas for the aromatic protons of the product the shift in the range of 6.8–7.9 ppm are indicated as signs i′, j′, k′ and m′. The hydrolysis conversion rates were calculated using the equation in Scheme 1.

The data also confirm the selective cleavage of the acetic acid unit. This is established by the intensity decrease of the peak n and by the appearance of a small peak at 10.7 ppm corresponding to the OH.

The conversion rates for all the reaction batches are given in Table 1 (the ^{1}H NMR spectra of AME and correspondent hydrolysis products for all remaining reaction batches are given in supplementary data: Fig. S1–S13).

3.1.1. Comparative effect of different solvents on the hydrolysis conversion rate of aspirin methyl ester (AME)

The performance of hydrolysis reactions using organic solvents as reaction medium under the influence of ultrasound can synergistically enhance the mass transfer phenomenum and increase conversion rates. Ultrasound at low intensities enhance the medium motion, upholding the reagent flux to the active site of the enzyme; a more volatile solvent enhances the cavitational effect, being able to favor the reaction. Though, the intensity and irradiation time can cause the inactivation of the enzyme through cavitational collapse [20,34,35].

The influence of solvents such as triolein, $CHCl_3$ and DCM on the hydrolysis conversion rates of AME with immobilized lipase was investigated and the results are shown in Table 1. The solvents were selected considering different parameters such as polarity, solubility of substrate and hydrophobicity [36]. Solvents may directly affect the activity, stability and specificity of enzymes [37]. Mostly in lipase catalyzed reactions, high enzymatic activity and reaction rates are observed using solvents with low polarity [38].

Candida antarctica lipase B (CalB), is one of the most commonly used biocatalysts, which is frequently designated as a typical lipase lacking interfacial activation. It appears in a closed and an open conformation. In the closed conformation, an amphiphilic α-helix, lid, secludes the active site from the medium. In the open form lid gets displaced and hydrophobic residues exposed around the active site to the medium. This exposed hydrophobic area is energetically unfavorable in the

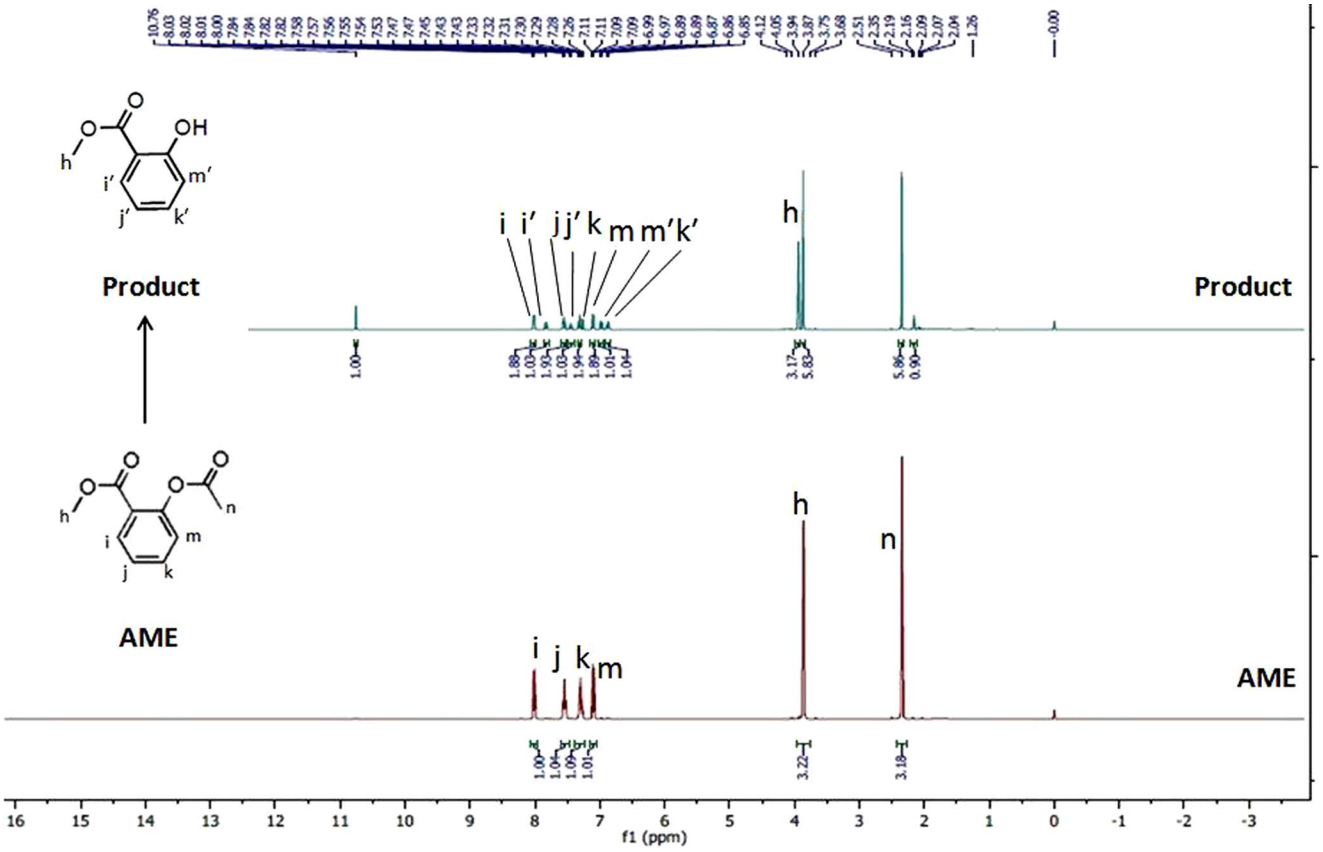


Fig. 1. ^{1}H NMR full spectra of aspirin methyl ester (AME) and the product of AME hydrolysis in the presence of chloroform at 35 °C/12 h in WB/US ($CDCl_3$, 400 MHz, 25 °C).

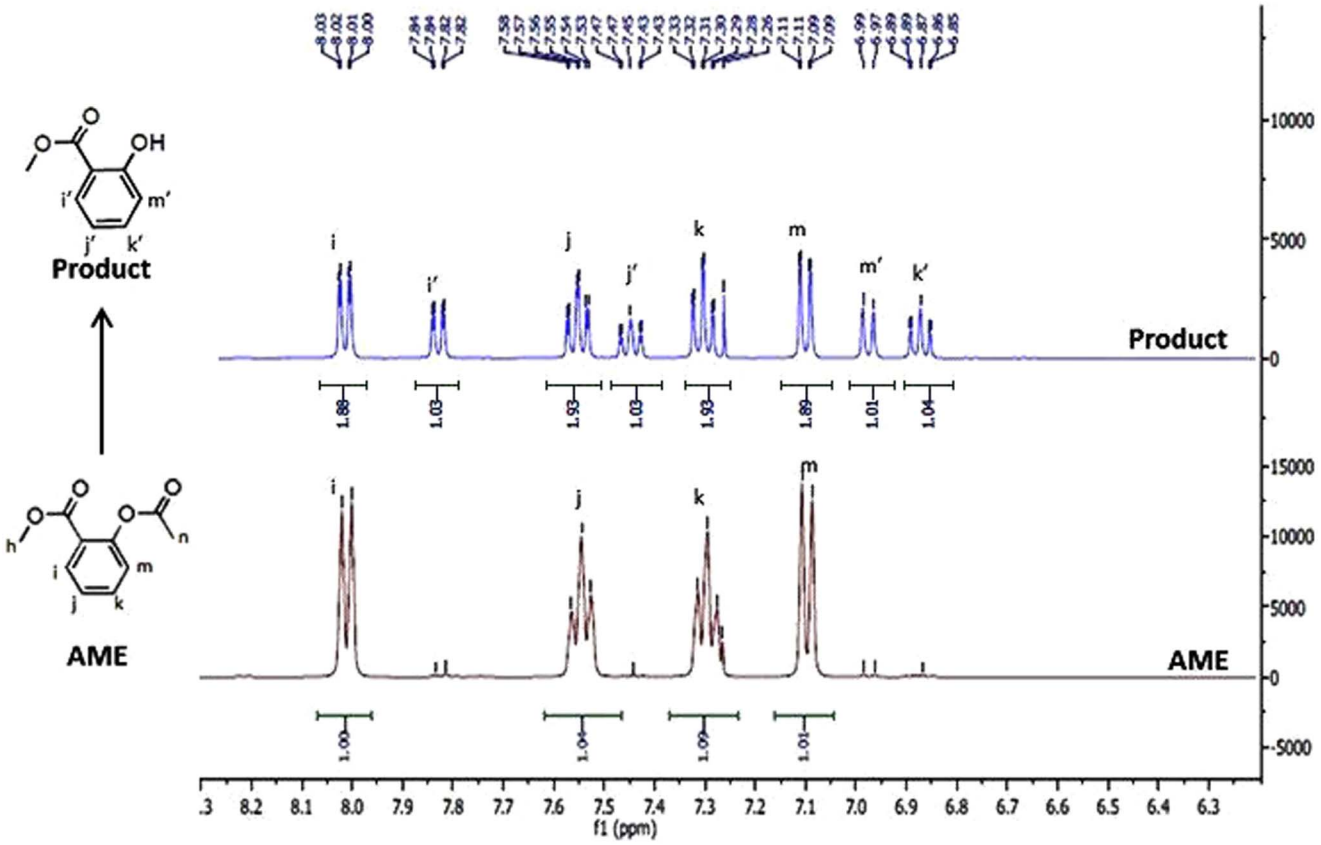


Fig. 2. ^{1}H NMR enlarged spectra (δ = 6.2–8.3 ppm) of aspirin methyl ester (AME) and the product of AME hydrolysis in the presence of chloroform at 35 °C/12 h in WB/US, ($CDCl_3$, 400 MHz, 25 °C).

absence of a hydrophobic interface, resulting to stabilization of the closed conformation. Upon binding to the oil-water interface, opens the lid and the exposed hydrophobic surface area helps to the interaction between the enzyme and its substrate [39]. CALB is therefore less active towards large triglycerides and can be used for the hydrolysis of simple esters containing sensitive functional groups or smaller triglycerides [16]. Moreover, researchers have also reported the effects of high amount of water in lipase catalyzed reactions. Yadav and Manjula Devi showed that 0.12 mol of water were required for the hydrolysis of tetrahydrofurfuryl in the presence of heptane [40]. Similarly, Sharma et al. reported that a high amount of water (1:3 (w/w) oil to water ratio) was required to achieve hydrolysis of tuna fish oil in the presence of *iso*-octane [41]. In our study low amount of water was added to the system including CALB for the hydrolysis of AME in the presence of triolein, being triolein hardly hydrolysed by the enzyme. From the results obtained it can be depicted that for ultrasound-assisted reactions in the presence of water, the hydrolysis conversion rates were higher for all the solvents tested than those observed using a WB, being remarkably greater (65.26%) for triolein. This solvent seems to be more friendly to the enzyme during processing as chloroform or DCM. During sonication, the process of bubble formation, growth and collapse is intimately dependent on factors, including the nature of the solvent, the solvent viscosity, surface tension, vapor pressure, gas solubility, and type of active intermediates or radicals formed (see solvents properties in Table S1) [42]. When applied in the presence of organic solvents, ultrasound processing tends to decrease their viscosity with time [43]. In the case of triolein, the high viscosity and surface tension may influence the ultrasound performance, being the bubble formation and collapse a more gentle process during hydrolysis. This organic solvent, compared to the others, does not loose its enzyme protective behavior, being responsible for an increase of the conversion rate. The viscosity of chloroform and dichloromethane when subjected to ultrasound can

Table 1
Hydrolysis conversion rates (%) of aspirin methyl ester (AME) from NMR data.

Solvents	Without water		With water	
	WB (%)	WB + US (%)	WB (%)	WB + US (%)
Triolein	36.6 ± 0.1	32.1 ± 0.1	58.9 ± 0.1	65.3 ± 0.1
Chloroform	35.0 ± 0.1	36.5 ± 0.1	15.0 ± 0.1	17.7 ± 0.1
Dichloromethane	21.7 ± 0.1	22.0 ± 0.1	16.9 ± 0.1	20.9 ± 0.1

$$\textit{Conversion rate (\%)} = \frac{\textit{Peak area of new position}}{\textit{Sum of peak area of new position and Peak area of original position}} \times 100$$

Note:
- Peaks at original positions represent the aromatic peaks of aspirin methyl ester as reactant in 1H NMR spectra.
- Peaks at new positions represent the aromatic peaks of hydrolysis product from aspirin methyl ester in 1H NMR spectra.

Scheme 1. Calculation of the hydrolysis conversion rates of aspirin methyl ester (AME).

undergo values which are no longer ideal for enzyme protection, thus ultrasound can have a more negative effect against enzyme. The presence of organic solvents would determine ultrasound behavior and enhance the mass transfer phenomena [18].

We could also observe that in absence of water the AME hydrolysis by lipase did not show significant increase when assisted by ultrasound, probably due to a decreased effect of solvent polarity on the enzyme during hydrolysis.

3.1.2. Effect of ultrasound on the hydrolysis conversion of aspirin methyl ester (AME)

To investigate the possible accelerative effects of ultrasound on the hydrolysis of AME in the presence and absence of water using different solvents such as triolein, $CHCl_3$ and DCM, the comparative studies of hydrolysis of AME were carried out. From Table 1, it can be clearly seen that higher conversion rates were obtained for ultrasound assisted reactions of AME in the presence of water for the different solvents tested. The conversion rates increased by 10.87%, 18.26% and 23.29% for ultrasound-assisted reactions in the presence of water using triolein, $CHCl_3$ and DCM, respectively, when compared to WB reactions. This can be explained by the cavitation energy which is thought to accelerate the reaction rates by increasing the movement of liquid molecules, and thus the substrate's access to the active site is increased as well as the mass transfer [44]. At the same time, in the medium frequency equipment used, the irradiation of water with ultrasound leads to the breakdown, or sonolysis, of the liquid resulting in the formation of hydroxyl and hydrogen radicals ([$OH^{\cdot}$]15mM; E = 8 kJ; data not shown). Cavitation (growth and explosive collapse of microscopic bubbles) can generate "hot spots" i.e. localized high temperature and shock waves producing high pressure capable of breaking chemical bonds [36]. At the same time and due to the high temperature and pressure inside the bubbles in the strong collapse, water vapor is dissociated and chemical products such as $OH^{\cdot}$, $O^{\cdot}$ and $H^{\cdot}$, as well as H_2O_2 are created and are responsible for the hydrolysis improvements.

In addition, the physical effects like micro-turbulence, microstreaming and micro-emulsion formation were also generated by cavitation effect, leading to high conversion rates. All these effects contributed to a better mixing of the reaction mass and thus helping to speed up the reactions [21,45,46].

It must be remarked that ultrasound-assisted hydrolysis must not be carried out for prolonged periods since the exposure of enzyme to it would lead to inactivation of the catalyst and the temperature of the reaction may uncontrollably shoot up, which may result in charring of the substrate [47]. We also observed that the conversion rate was significantly enhanced for the reactions sonicated twice for 0.5 h with duty cycle of 50% during 12 h of continuous process. The highest conversion rate achieved was 65.3% for ultrasound assisted enzymatic hydrolysis of AME in the presence of water using triolein as solvent.

3.1.3. Role of water on the enzymatic ultrasound assisted hydrolysis of AME in the presence of organic solvents

Water plays an important role as nucleophile in enzymatic hydrolysis reaction. The effect of water on the conversion rate of lipase catalyzed hydrolysis reaction of AME was studied using different solvents with and without ultrasound assistance, as depicted in Table 1. Blending the solvents with water seems to affect both the apparent conversion rate and the reaction stability as a function of water content [48]. Enzyme catalyzed hydrolysis with low water environment in suitable solvents supports to enhance enzyme activity [49].

The AME hydrolysis in the presence of water (1%, w/v) using triolein, an hydrophobic (non polar) solvent assisted by ultrasound was confirmed to have the highest conversion rate in comparison with the conversions using moderately hydrophobic $CHCl_3$ and DCM. Enzymes need a certain level of water in their structures in order to maintain their natural conformation, allowing them to deliver their full functionality. Moreover, water as a modifier of the solvent and up to a certain level can modify the solvent properties such as polarity and the solubility of the reactants and the products. Depending on the type of the reaction, water can act as a substrate (e.g., in hydrolysis) or as product (e.g., in esterolysis) of the enzymatic reaction, affecting the enzyme turnover in various ways [50].

In our experiments, a better solubility of the AME substrate was obtained applying a mixed aqueous–organic system. A microemulsion of triolein and water is formed which act as reaction medium for hydrolysis; the substrate gets dissolved into the oil phase and the aqueous phase hosts the enzyme [51]. This allows enzyme protection and thus enhances hydrolysis conversion rates. The intentional use of a low water amount relies on the fact that the presence of water molecules in excess can cause inactivation, because the possible enhancement of the kinetic energy in the reaction medium, provoked by ultrasonic waves, may induce enzyme conformational changes. The use of solvents or biphasic systems works as a protective mechanism, because the water molecules find themselves around the enzyme, and they are not easily misplaced by the ultrasound [18].

The trends observed when mixing water with organic solvents like $CHCl_3$ and DCM under ultrasound, show lower hydrolysis conversion rates when compared with the ones obtained in the absence of water. The reaction batches in absence of water using $CHCl_3$ and dichloromethane give higher conversion rate when compared with the batches in the presence of water. This may be due to the stripping out of water from the enzyme by these two solvents, essential for the enzymatic reaction, which decreased the enzyme activity and hydrolysis conversion rates [52].

3.2. Effect of ultrasound on lipase activity

The effect of ultrasound on enzymes depends on parameters such as energy (potency and frequency) and exposure time to irradiation. Studies on the effect of ultrasound on the enantio and regioselectivity of lipase showed that the enzymes kept the enantioselectivities, despite the fact that the porcine lipase has showed a slightly inferior enantioselectivity with the sonication treatment [53,54]. Xiao et al. [55] and Chen et al. [56] found that the ultrasound did not amend the regioselectivity of lipase. Other authors also observed the positive effects of ultrasound in lipase-catalyzes reactions [53,57]. Several other authors have postulated that enzymatic activity in non-aqueous solvents may be diminished due to diffusional limitations on the substrates. This phenomenon, common in heterogeneous systems including immobilized catalysts, leads to the under-utilization of enzymatic power and thus reduces the enzyme activity. Theoretically, it was showed that such mass transfer limitations may decelerate the enzymatic catalysis in organic solvents [58,59].

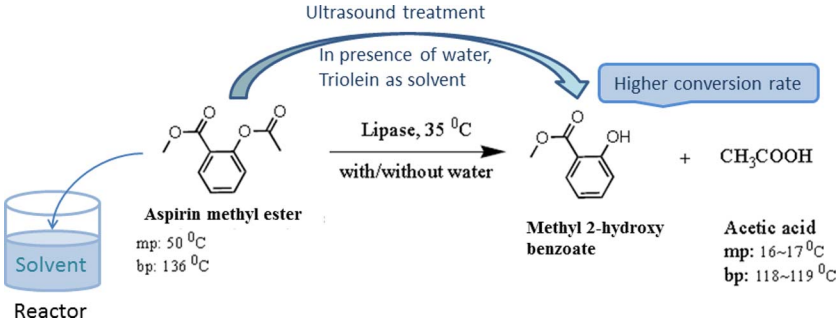
We also observed that after hydrolysis, lipase activity decreased around 37% compared with the initial enzyme activity. The different cycles of ultrasound and the time of exposure can be considered responsible for this behavior observed.

3.3. Proposed methodology for AME hydrolysis

Considering all the results obtained one can propose a methodology for the enzyme-ultrasound assisted hydrolysis of AME in the presence of water using triolein as solvent (Scheme 2).

4. Conclusions

We studied the selectivity of a lipase from *Candida antarctica* to hydrolyze aspirin methyl ester in the presence of different solvents like triolein, chloroform and dichloromethane. Very surprisingly and under the reaction conditions shown triolein was not hydrolysed by CALB with/without water or with/without ultrasound. The highest hydrolysis rates were obtained when triolein was used as solvent in the presence of water. Based on the data achieved a methodology for AME hydrolysis



Scheme 2. Hydrolysis reaction of aspirin methyl ester (AME).

was set up: 1% of CALB, 5 mL of triolein, 1% H_2O (v/v) alternating 100 rpm in WB with US at 53 kHz, 100 W, 50% duty cycle, 35 °C for 12 h.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ultsonch.2017.08.004.

References

[1] K.D. Rainsford, Aspirin and Related Drugs, CRC Press, 2016.
[2] K.D. Rainsford, M.W. Whitehouse, Anti-inflammatory/anti-pyretic salicylic acid ester with low gastric ulcerogenic activity, Agents Actions 10 (1980) 451–456.
[3] H. Bundgaard, N. Nielsen, A. Buur, Aspirin prodrugs: synthesis and hydrolysis of 2-acetoxybenzoate esters of various N-(hydroxyalkyl) amides, Int. J. Pharm. 44 (1988) 151–158.
[4] A. Lapczynski, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia, A.M. Api, Fragrance material review on methyl salicylate, Food Chem. Toxicol. 45 (2007) S428–S452.
[5] P.D. Desai, A.M. Dave, S. Devi, Chemoselective hydrolysis of methyl 2-acetoxybenzoate using free and entrapped esterase in K-carrageenan beads, J. Appl. Polym. Sci. 108 (2008) 2617–2622.
[6] M.M. R., L.I. Z., S.O. S., V.V. B., Heterogeneous base-catalyzed methanolysis of vegetable oils: State of art, Hem. Ind. 64 (2010) 63-80.
[7] F. Hasan, A.A. Shah, A. Hameed, Industrial applications of microbial lipases, Enzyme Microb. Technol. 39 (2006) 235–251.
[8] V. Jyoti, K. Avneet, Yeast lipases: enzyme purification, biochemical properties and gene cloning, Electron. J. Biotechnol. 9 (2006).
[9] Z.-Y. Shu, H. Jiang, R.-F. Lin, Y.-M. Jiang, L. Lin, J.-Z. Huang, Technical methods to improve yield, activity and stability in the development of microbial lipases, J. Mol. Catal. B: Enzyme 62 (2010) 1–8.
[10] H. Treichel, D. de Oliveira, M.A. Mazutti, M. Di Luccio, J.V. Oliveira, A review on microbial lipases production, Food Bioprocess Technol. 3 (2010) 182–196.
[11] S.K. Dahod, P. Siuta-Mangano, Carbon tetrachloride-promoted stereo selective hydrolysis of methyl-2-chloropropionate by lipase, Biotechnol. Bioeng. 30 (1987) 995–999.
[12] A. Charusheela, L. Arvind, Enzyme catalyzed hydrolysis of esters using reversibly soluble polymer conjugated lipases, Enzyme Microb. Technol. 30 (2002) 19–25.
[13] V. Gotor-Fernández, E. Busto, V. Gotor, *Candida antarctica* lipase B: an ideal biocatalyst for the preparation of nitrogenated organic compounds, Adv. Synth. Catal. 348 (2006) 797–812.
[14] C. Li, T. Tan, H. Zhang, W. Feng, Analysis of the conformational stability and activity of *Candida antarctica* lipase B in organic solvents: insight from molecular dynamics and quantum mechanics/simulations, J. Biol. Chem. 285 (2010) 28434–28441.
[15] V. Ferrario, C. Ebert, L. Knapic, D. Fattor, A. Basso, P. Spizzo, L. Gardossi, Conformational changes of lipases in aqueous media: a comparative computational study and experimental implications, Adv. Synth. Catal. 353 (2011) 2466–2480.
[16] E.M. Anderson, K.M. Larsson, O. Kirk, One biocatalyst-many applications: the use of *Candida antarctica* B-lipase in organic synthesis, Biocatal. Biotransform. 16 (1998) 181–204.
[17] J. Uppenberg, N. Oehrner, M. Norin, K. Hult, G.J. Kleywegt, S. Patkar, V. Waagen, T. Anthonsen, T.A. Jones, Crystallographic and molecular-modeling studies of lipase B from Candida antarctica reveal a stereospecificity pocket for secondary alcohols, Biochemistry 34 (1995) 16838–16851.
[18] L.A. Lerin, R.A. Loss, D. Remonatto, M.C. Zenevicz, M. Balen, V.O. Netto, J.L. Ninow, C.M. Trentin, J.V. Oliveira, D. de Oliveira, A review on lipase-catalyzed reactions in ultrasound-assisted systems, Bioprocess Biosyst. Eng. 37 (2014) 2381–2394.
[19] X. Zhao, S.R. Bansode, A. Ribeiro, A.S. Abreu, C. Oliveira, P. Parpot, P.R. Gogate, V.K. Rathod, A. Cavaco-Paulo, Ultrasound enhances lipase-catalyzed synthesis of poly (ethylene glutarate), Ultrason. Sonochem. 31 (2016) 506–511.
[20] C. Leonelli, T.J. Mason, Microwave and ultrasonic processing: now a realistic option for industry, Chem. Eng. Process. Process Intensif. 49 (2010) 885–900.
[21] I. Babicz, S.G.F. Leite, R.O.M.A. de Souza, O.A.C. Antunes, Lipase-catalyzed diacylglycerol production under sonochemical irradiation, Ultrason. Sonochem. 17 (2010) 4–6.
[22] P.R. Gogate, A.M. Kabadi, A review of applications of cavitation in biochemical engineering/biotechnology, Biochem. Eng. J. 44 (2009) 60–72.
[23] A.B. Martins, N.G. Graebin, A.S.G. Lorenzoni, R. Fernandez-Lafuente, M.A.Z. Ayub, R.C. Rodrigues, Rapid and high yields of synthesis of butyl acetate catalyzed by Novozym 435: reaction optimization by response surface methodology, Process Biochem. 46 (2011) 2311–2316.
[24] J.S. Alves, C. Garcia-Galan, D. Danelli, N. Paludo, O. Barbosa, R.C. Rodrigues, R. Fernandez-Lafuente, Use of lecitase-ultra immobilized on styrene-divinylbenzene beads as catalyst of esterification reactions: effects of ultrasounds, Catal. Today 255 (2015) 27–32.
[25] J.S. Alves, C. Garcia-Galan, M.F. Schein, A.M. Silva, O. Barbosa, M.A.Z. Ayub, R. Fernandez-Lafuente, R.C. Rodrigues, Combined effects of ultrasound and immobilization protocol on butyl acetate synthesis catalyzed by CALB, Molecules 19 (2014) 9562–9576.
[26] C. Stavarache, M. Vinatoru, R. Nishimura, Y. Maeda, Fatty acids methyl esters from vegetable oil by means of ultrasonic energy, Ultrason. Sonochem. 12 (2005) 367–372.
[27] C. Little, M. El-Sharif, M.J. Hepher, The effect of solution level on calorific and dosimetric results in a 70 kHz tower type sonochemical reactor, Ultrason. Sonochem. 14 (2007) 375–379.
[28] G.J. Price, F.A. Duck, M. Digby, W. Holland, T. Berryman, Measurement of radical production as a result of cavitation in medical ultrasound fields, Ultrason. Sonochem. 4 (1997) 165–171.
[29] Ragaini et al., Ultrason. Sonochem. 2012, 19(4), 872–876; S. Perincek, A.E. Uzgur, K. Duran, A. Dogan, A.E. Korlu, I.M. Bahtiyari, Design parameter investigation of industrial size ultrasound textile treatment bath, Ultrason. Sonochem. 16 (2009) 184–189.
[30] R. Silva, H. Ferreira, C. Little, A. Cavaco-Paulo, Effect of ultrasound parameters for unilamellar liposome preparation, Ultrason. Sonochem. 17 (2010) 628–632.
[31] I. Gonçalves, M. Martins, A. Loureiro, A. Gomes, A. Cavaco-Paulo, C. Silva, Sonochemical and hydrodinamic cavitation reactors for laccase/hydrogen peroxide cotton bleaching, Ultrason. Sonochem. 21 (2014) 774–781.
[32] N.R. Sonare, V.K. Rathod, Transesterification of used sunflower oil using immobilized enzyme, J. Mol. Catal. B: Enzyme 66 (2010) 142–147.
[33] N. Gharat, V.K. Rathod, Ultrasound assisted enzyme catalyzed transesterification of waste cooking oil with dimethyl carbonate, Ultrason. Sonochem. 20 (2013) 900–905.
[34] L. Lerin, R. Loss, M. Zenevicz, M. Balen, V. Netto, J. Ninow, C. Trentin, J. Oliveira, D. Oliveira, A review on lipase-catalized reactions in ultrasound-assisted systems, Bioprocess Biosystems Eng. 37 (2014) 2381–2394.
[35] J.V. Sinisterra, Application of ultrasound to biotechnology: an overview, Ultrason. Sonochem. 30 (1992) 180–184.

[36] K. Suslick, G.J. Price, Applications of ultrasound to materials chemistry, Annuak Rev. Mater. Sci. 29 (1999) 295–326.
[37] S. Sattari, F. Vahabzadeh, H.K. Aghtaei, Performance of loofa-immobilized Rhizopus oryzae in the enzymatic production of biodiesel, Braz. J. Chem. Eng. 32 (2015) 367–376.
[38] L. Batistella, M.K. Ustra, A. Richetti, S.B.C. Pergher, H. Treichel, J.V. Oliveira, L. Lerin, D. de Oliveira, Assessment of two immobilized lipases activity and stability to low temperatures in organic solvents under ultrasound-assisted irradiation, Bioprocess Biosyst. Eng. 35 (2012) 351–358.
[39] C. Laane, S. Boeren, K. Vos, C. Veeger, Rules for optimization of biocatalysis in organic solvents, Biotechnol. Bioeng. 102 (2009) 1–8.
[40] T. Zisis, P.L. Freddolino, P. Turunen, M.C.F. van Teeseling, A.E. Rowan, K.G. Blank, Interfacial activation of *Candida antarctica* lipase B: combined evidence from experiment and simulation, Biochemistry. 54 (2015) 5969–5979.
[41] G.D. Yadav, K.M. Devi, Kinetics of hydrolysis of tetrahydrofurfuryl butyrate in a three phase system containing immobilized lipase from *Candida antarctica*, Biochem. Eng. J. 17 (2004) 57–63.
[42] A. Sharma, S.P. Chaurasia, A.K. Dalai, Non-selective hydrolysis of tuna fish oil for producing free fatty acids containing docosahexaenoic acid, Can. J. Chem. Eng. 92 (2014) 344–354.
[43] Q. Cheng, S. Debnath, E. Gregan, H.J. Byrne, Ultrasound-assisted SWNTs dispersion: effects of sonication parameters and solvent properties, J. Phys. Chem. C. 114 (2010) 8821–8827.
[44] A.M. Doust, M. Rahimi, M. Feyzi, Effects of solvent addition and ultrasound waves on viscosity reduction of residue fuel oil, Chem. Eng. Process. Process Intensif. 95 (2015) 353–361.
[45] R.S. Malani, S. Khanna, V.S. Moholkar, Sonoenzymatic decolourization of an azo dye employing immobilized horse radish peroxidase (HRP): a mechanistic study, J. Hazard. Mater. 256–257 (2013) 90–97.
[46] S. Khanna, A. Goyal, V.S. Moholkar, Mechanistic investigation of ultrasonic enhancement of glycerol bioconversion by immobilized Clostridium pasteurianum on silica support, Biotechnol. Bioeng. 110 (2013) 1637–1645.
[47] G.L. Maddikeri, A.B. Pandit, P.R. Gogate, Ultrasound assisted interesterification of waste cooking oil and methyl acetate for biodiesel and triacetin production, Fuel Process. Technol. 116 (2013) 241–249.
[48] S.R. Bansode, V.K. Rathod, Ultrasound assisted lipase catalysed synthesis of isoamyl butyrate, Process Biochem. 49 (2014) 1297–1303.
[49] A.M. Gumel, M.S.M. Annuar, T. Heidelberg, Enzymatic synthesis of 6-O-glucosyl-poly(3-hydroxyalkanoate) in organic solvents and their binary mixture, Int. J. Biol. Macromol. 55 (2013) 127–136.
[50] J.S. Dordick, Enzymatic catalysis in monophasic organic solvents, Enzyme Microb. Technol. 11 (1989) 194–211.
[51] K. Rezaei, E. Jenab, F. Temelli, Effects of water on enzyme performance with an emphasis on the reactions in supercritical fluids, Crit. Rev. Biotechnol. 27 (2007) 183–195.
[52] A.K. Steudle, M. Subinya, B.M. Nestl, C. Stubenrauch, Hydrolysis of hydrophobic esters in a bicontinuous microemulsion catalysed by lipase B from *Candida antarctica*, Chem. Eur. J. 21 (2015) 2691–2700.
[53] R.Z. Kazandjian, J.S. Dordick, A.M. Klibanov, Enzymatic analyses in organic solvents, Biotechnol. Bioeng. 28 (1986) 417–421.
[54] F. Wang, H. Zhang, J. Wang, G. Chen, X. Fang, Z. Wang, L. Wang, Ultrasound irradiation promoted enzymatic transesterification of (R/S)-1-chloro-3-(1-naphthyloxy)-2-propanol, Molecules 17 (2012) 10864–10874.
[55] G. Lin, H.-C. Liu, Ultrasound-promoted lipase-catalyzed reactions, Tetrahedron Lett. 36 (1995) 6067–6068.
[56] Y.-M. Xiao, Q. Wu, Y. Cai, X.-F. Lin, Ultrasound-accelerated enzymatic synthesis of sugar esters in nonaqueous solvents, Carbohydr. Res. 340 (2005) 2097–2103.
[57] Z.-G. Chen, M.-H. Zong, Z.-X. Gu, Y.-B. Han, Effect of ultrasound on enzymatic acylation of konjac glucomannan, Bioprocess Biosyst. Eng. 31 (2008) 351–356.
[58] B. Kwiatkowska, J. Bennett, J. Akunna, G.M. Walker, D.H. Bremner, Stimulation of bioprocesses by ultrasound, Biotechnol. Adv. 29 (2011) 768–780.
[59] S. Kamat, E.J. Beckman, A.J. Russell, Role of diffusion in nonaqueous enzymology. 1. Theory, Enzyme Microb. Technol. 14 (1992) 265–271.