University "Politehnica" of Bucharest
Faculty of Applied Chemistry and Materials Science
Department of Bioresources and Polymer Science

Nr. Decizie Senat_____din_____

PHD THESIS

US and / or MW intensification of esterification and transesterification processes using enzyme or resin catalysts

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Thesis Abstract

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Content

Keywords: esterification reaction; transesterification reaction; enzymatic cathalisis; enzymes; ultrasound assisted; ultrasound intensification; process intensification

Note: In this document, the notation of chapters, subchapters and figures and tables are the same as those in the thesis.

Part I- LITERATURE STUDY

A. Esterification Reaction

1. Esters—uses in the food industry and perfume composition

Esters are one of the most important classes of organic compounds. Organic esters are used as solvents, fragrances and precursors in a variety of industries. In particular, aliphatic esters are used as flavors in the food industry and aromatic esters are used in perfumes [1]. Esters synthesized from short-chain acids and alcohols are widely used as flavors and odorants in the food industry, the medicines industry and the cosmetic industry [2]. Odorants are used in deodorants, depilatory creams, soaps and cleansing creams, and the flavors are used in lipsticks, lip balms, lip glosses and toothpaste [3]. Short-chain acids of carbon atoms, such as acetic acid, butyric acid and propionic acid, together with alcohols such as butanol, ethanol, isoamilic alcohol, geraniol, citronellool and 1-methanol, were used for the synthesis of flavours and odorants [4].

Among these products, isoamyl esters (especially isoamyl acetate) are used in the food industry (74,000 kg/year) due to the strong banana flavour [2].

2. Enzymatic Esterification

Natural esters extracted from plants and used as flavors are often either insufficient or too expensive for commercial use. For many industrial products, flavors are obtained by chemical synthesis and are not considered natural products. For this reason, their market value is lower than that of esters obtained from natural sources [5]. However, these esters are considered "green" when they are obtained by synthesis mediated by lipases. Biotransformations catalyzed by lipases in non-apathetic environments began to take on importance due to their remarkable properties, such as improved thermostability, regio, stereo and substrate specificity, milder reaction conditions and the need for a smaller amount of energy. Lipases were used for direct esterification and transesterification reactions in organic solvents for the production of glycerin esters, aliphatic alcohols and terpene alcohols [6]. However, the esterification of short-chain acids with alcohols did not receive much attention. Moreover, substrates with low molecular weight apparently have a lower affinity for these enzymes, compared to long-chain substrates, since these molecules have an inhibitory effect on the enzyme. Industrial applications of lipases have good potential for obtaining a number of commercially important esters [7]. In order to obtain the maximum possible conversion, it is necessary to optimize the parameters that affects the biocatalyzed synthesis of esters. An important impact in the enzymatic activity and in the conversion obtained in the synthesis of esters are the operating parameters, temperature, the amount of enzyme, the molar alcohol-acid ratio [8]. These parameters also affect the total cost of the process. For this reason, it is useful to discuss these variables and give some indications based on the literature and research papers in this field.

3. Esterification with Ion Exchange Resins

The annual demand for isoamyl acetate is more than 90,000 kg [9]. To date, several synthetic pathways and catalysts have been used to obtain these esters. Ion-exchange resins, as heterogeneous catalysts, were used to catalyse various reactions such as esterification, transesterification, alkylation, acylation and other transformations [10, 11].

The use of heterogeneous solid acid catalysts can overcome the disadvantages of mineral acids, such as: corrosion of equipment, low selectivity, contamination of the product and recycling costs. Recently, Duque-Bernal [12] reported an expression of reaction speed for the esterification between acetic acid and isoamyl alcohol in the absence of catalysts. They have shown that there is an autocatalytic effect of acetic acid on the reaction rate, and the equilibrium constant can be considered independent of temperature. Previously, Teo [13] and Singh [14] studied the kinetics of this esterification reaction in heterogeneous catalysis using the Purolite CT-175 ion exchange resin. Therefore, ion-exchange resin-type catalysts have gained popularity because they have advantages: they are environmentally friendly, non-corrosive, have good selectivity and thermal stability [15]. The use of ion exchange resins (IER) as catalysts has distinct advantages [81] over homogeneous catalysis:

(a) the purity of the products is higher because the by-reactions can be completely eliminated or are significantly reduced;

(b) the catalyst can be easily removed from the reaction mixture by filtration;

(c) the corrosive medium caused by the discharge of waste containing acid is disposed of.

4. Enzymatic Transesterification

Biodiesel production has gained importance for its ability to replace fossil fuels. The environmental problems caused by exhaust emissions through the use of fossil fuels are part of the reasons why the use of biodiesel, which has proven to be environmentally friendly, is encouraged. Biodiesel is a mixture of mono-alkyl esters obtained from vegetable oils such as soybean oil, rapeseed oil, palm oil, sunflower oil, corn oil, peanut oil, canola oil and cotton oil [16]. Apart from vegetable oils, biodiesel can also be produced from other sources such as animal fats (seaweed, lard), waste food oils, and algae [17].

The transesterification process can be carried out using various catalysts such as liquid or solid inorganic ones (acids or alkalis) or biocatalysts (enzymes). In the alkaline process, sodium hydroxide (NaOH) or potassium (KOH) is used as a catalyst along with methanol or ethanol. This process is the most effective and least corrosive of all processes and the reaction speed is quite high, even at a low temperature of 60 ºC. If contamination with acid or water occurs, soap is formed, making it difficult to separate [18-20]. Another conventional way of producing biodiesel is to use an acid catalyst instead of a base. Any mineral acid can be used to catalysize the process; the most common acids used are sulfuric acid and sulfonic acids. Although the yield is high, the acids, being corrosive, can cause damage to the equipment [21].

It has recently been found that enzymes such as lipases can be used to catalyse the transesterification process by immobilizing them on a suitable support. The advantage of immobilized enzymes is that the enzyme can be reused without separation. Also, the operating temperature of the process is low (50 ºC) compared to other techniques. The disadvantages include enzyme inhibiting effects due to the presence of methanol and the fact that enzymes are expensive [22]. The production of biodiesel using a biocatalyst eliminates the disadvantages of the alkaline process by obtaining a very high purity product with fewer auxiliary operations [61]. The enzyme that catalyzes the reaction of obtaining biodiesel is a lipase, and this can be obtained from microorganisms such as *Mucor miehei*, *Rhizopus oryzae*, *Candida antarctica*, *Pseudomonas cepacia*. Various alcohols have been tried for the transesterification process including methanol, ethanol, isopropanol and butanol. The advantages of enzymatic transesterification are the easy purification of the by-product, mild operating conditions, high purity of the product and applicability of the process to raw materials with a high content of free fatty acids (FFA) [67-70]. The disadvantages are the high cost of enzymes and a long reaction time. Since immobilised enzymes can be reused, they have been used as a solution to reduce costs [67].

Part II - EXPERIMENTAL STUDY

A. Esterification in the Presence of Enzymes-Methods of Intensifying the Process

Research objectives

The main objective of this research was the study of the influence of ultrasound on enzymatic reactions. Due to the novelty of this topic, fundamental research is able to open up new possibilities for applied research.

The main objectives of the study are divided into:

1. Study of the enzymatic esterification processfor obtaining short-chain esters with uses in food and cosmetics.
2. The use of ultrasound for the intensification of the enzymatic esterification process.

The objective of the experimental research is to study the synthesis of isoamyl acetate (banana flavor) by the direct esterification of acetic acid with isoamyl alcohol, in the presence of enzymes and solvents as adjuvants in the reaction mass. Such a system avoids separation problems, toxicity, flammability of organic solvents and has the advantage of reducing the cost of the final product and allows the product to be recovered without further complex purification or evaporation steps. This type of esterification is advantageous when obtaining esters with uses in food or cosmetics.

A1. Enzymatic Esterification in Discontinuous Regime

Materials and Methods

Materials

Lipozyme 435 from *Candida Antarctica* [23], immobilized on a macroporous acrylic resin was kindly supplied by Novozymes A/S (Denmark). All chemicals: *i*-amyl alcohol (Merck), acetic acid (Merck), *i*-amyl acetate—standard for GC analysis (Aldrich) were of analytical quality.

Procedure for Esterification of Acetic Acid with Isoamyl Alcohol

Acetic acid (50 mmol), isoamyl alcohol (100 mmol) and enzyme were heated to 50 °C. The progress of the reaction was monitored by collecting samples at different time intervals and the conversion of acetic acid was determined by acidity index and by chromatographic analysis. The reaction took place in a 200 mL reactor, equipped with mechanical agitation and reflux refrigerant. The reaction mass temperature was measured using an optical fiber. The constant reaction conditions were: acetic acid molar ratio: 1:2 isoamyl alcohol, temperature of 50 °C. The experiments in the presence of ultrasound were performed in an ultrasonic bath (Ultrasonic cleaning bath COLIBRI L488, volume 200 mL, 22W, frequency 66–80 kHz, STIMIN LTD, Italy) provided with heating system and with a system of variation of ultrasonic power (Figure A1.2).

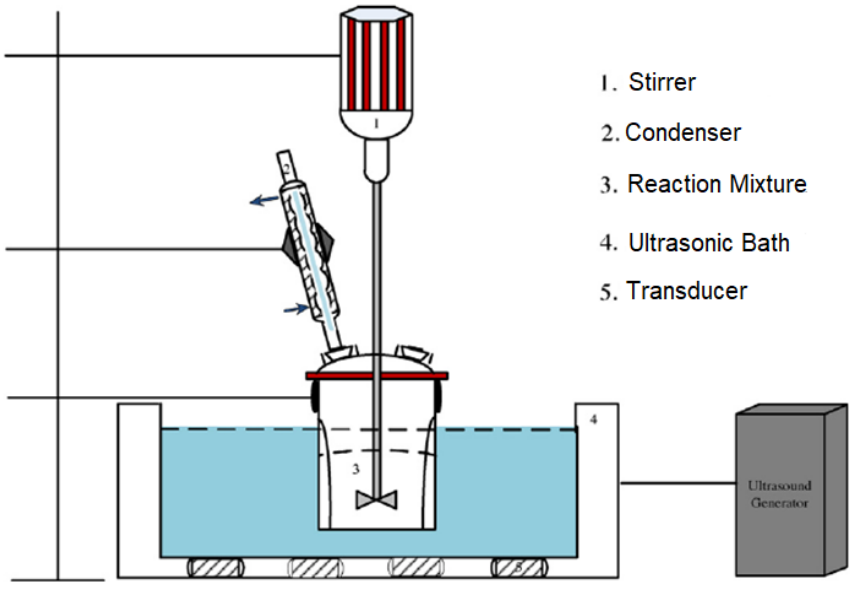


Figure A1.2: Scheme of the ultrasonic intensification plant of the esterification reaction of isoamyl alcohol with acetic acid

Results and Discussions

Influence of Different Types of Enzymes On The Enzymatic Esterification of Acetic Acid with i-Amyl Alcohol

At this stage of the experiments, the influence of the different lipases on the esterification process was pursued. For this, various enzymes were used: *Candida Antarctic lipase* immobilized on an acrylic resin (CALB), Lipozyme 435 and a mixture of enzymes containing lipases. These lipases were used in the process of esterification of acetic acid. Experimental studies were performed at a temperature of 50 °C, a molar alcohol *i*-amyl ratio: acetic acid of 2:1, with maximum mechanical agitation. The ester concentrations obtained in the reaction mass relative to gram of the enzyme used are plotted in Figure A1.4.

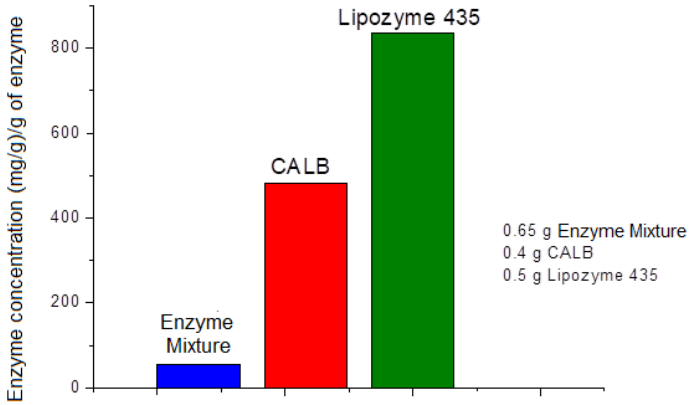


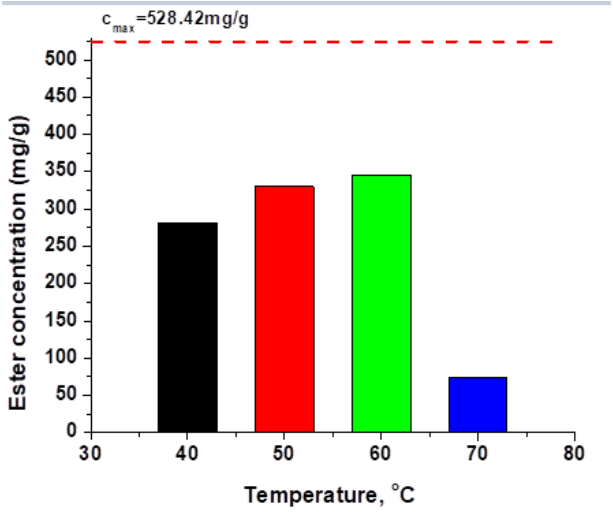
Figure A1.4: Concentration of *i*-amyl acetate in the enzymatic esterification process when using various lipases

From the analysis of the resulting data it is observed that the best results were obtained for the enzyme Lypozyme 435. This enzyme also has the advantage that it is an enzyme immobilized on a solid support and thus can be reused [23].

Study of the Enzymatic Esterification Process by the Conventional Method in the Presence of Lipase Lipozyme 435

- Influence of Reaction Temperature on Enzymatic Esterification of Acetic Acid with *i*-Amyl Alcohol

Reaction temperature is an important factor for enzymatic catalyzed reactions, since the activity of lipase is temperature dependent. The following experiments were conducted in the presence of the enzyme Lipozyme 435. The experimental conditions were: temperature between 40–70 °C, a molar ratio i-amyl alcohol : acetic acid of 2:1, with mild mechanical agitation. The variation in the concentration of *i*-amyl acetate in the process of enzymatic esterification as a function of temperature is shown in Figure A1.7.



FigureA1.7: Variation of ester concentration in the process of enzymatic esterification as a function of temperature, (reaction conditions: molar ratio alcohol : acid 2:1, enzyme cantity 40 mg/g $_{mixture}$, reaction time 2h)

From Figure A1.7 it can be seen that an increase in temperature from 40 to 50 °C led to an increase in the concentration of *i*-amyl acetate. At a temperature higher than 60 °C, the phenomenon of denaturation of the enzyme may occur, which would lead to an increase in the reaction time and a decrease in the conversion of the acid, respectively. Based on these results, subsequent experiments were conducted at 50 °C to avoid denaturation of the enzyme.

Intensification of the Enzymatic Esterification Process with Ultrasounds

The study of the influence of ultrasound on the esterification process was carried out by conducting ultrasonic experiments at different power settings. The ultrasonic bath was not equipped with a power control system and we adjusted the power using a variable voltage autotransformer. We used 100, 150 and 200 V and determined the ultrasonic power by a calorimetric method [23]. The values determined for the ultrasonic power at different voltages were 3, 6 and 8.5 mW/cm$^{3.}$ Testing of the influence of ultrasonic power on the ester efficiency was performed at different temperatures of 40 °C, 50 °C and 60 °C respectively. The other parameters remained constant, a molar ratio of alcohol *i*-amyl: acetic acid of 2:1, amount of Lipozyme 435—50 mg$_{enzyme}$/g$_{mixture\ of\ reaction}$ and mechanical agitation. The variation in the concentration of esters in the ultrasonically assisted enzymatic esterification process for different temperatures is shown in Figure A1.9.

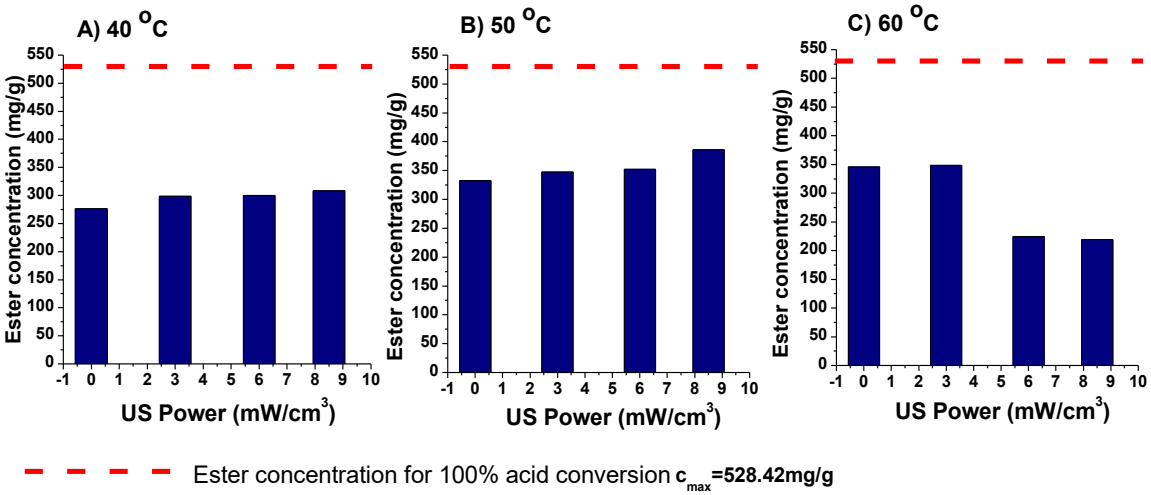


Figure A1.9: Influence of the ultrasonic power on the concentration of *i*-amyl acetate in the enzymatic esterification process for different temperatures (A) 40 °C, B) 50 °C and C) 60 ° (reaction conditions: enzymatic load 50 mg / g and molar ratio alcohol : acid of 2: 1).

Partial Conclusions

The ultrasound-assisted enzymatic synthesis of *i*-amyl acetate was performed using the enzyme Lipozyme 435 in solvent-free system in a stirring reactor. In order to obtain a maximum amount of ester, the effect of various parameters (amount of enzyme, molar ratio alcohol-to-acid, ultrasound power and temperature) on the synthesis of *i-amyl* acetate by enzymatic catalysis was studied. The best result was obtained using 50 mg/g Lipozyme 435, molar ratio of *i*-amyl alcohol:acetic acid 2:1 and a temperature of 50 °C. The most important parameter studied at this stage is the power ultrasound, which correlated with the reaction temperature was 8.5 mW/cm^3. It has been observed that an increase in the ultrasonic power at low temperatures leads to an increase in the concentration of esters in the final reaction mass. At higher temperatures (> 60 °C) the increase in ultrasonic power deactivates the enzyme and the concentration of esters obtained decreases.

A2. Enzymatic Esterification in A "Loop" Type Reactor. Ultrasound Applied with Ultrasound Bath

Materials and Methods

Procedure for Esterification of Acetic Acid with Iso-Amyl Alcohol

Acetic acid, isoamyl alcohol and enzyme (Lipozyme 435) were heated to the proposed temperature. The progress of the reaction was monitored by collecting samples at different time intervals and the concentration of ester was analyzed by the chromatographic method. The intensification of the ultrasonic enzymatic esterification reaction was achieved with the plant shown in Figure A2.1.

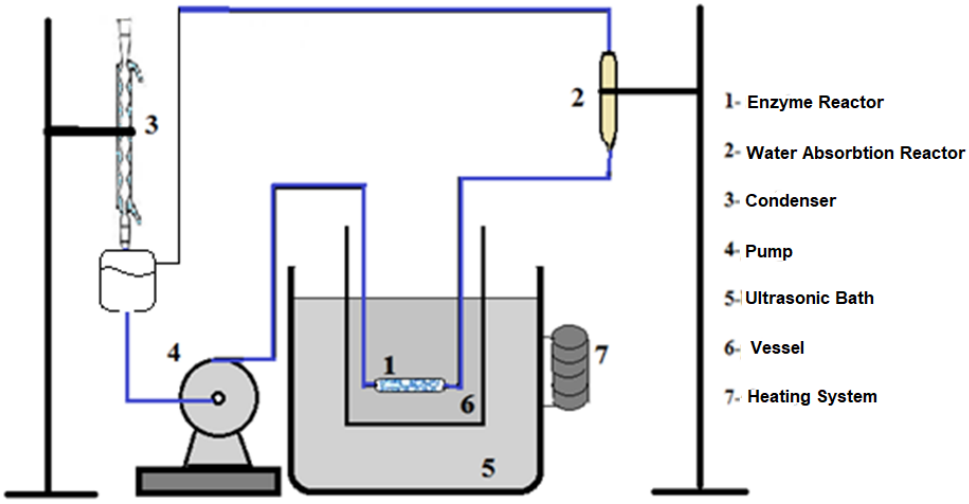


Figure A2.1: Experimental plant for ultrasonic intensification of enzymatic esterification reactions

Procedure for Intensifying the Enzymatic Esterification Reaction

The enzymatic esterification reaction was intensified using the previously presented installation. Since the ultrasound bath used does not have an ultrasonic power adjustment system, to reduce the power, an autotransformer was used with which the supply voltage of the device was reduced. The power of the acoustic waves was determined by the calorimetric method. For intensification, different ultrasound powers were used, from 3W/kg to 13.8W/kg. For each experiment the molar acid:alcohol ratio was 1:2, the mass of the enzyme is 0.8 g, with a reaction volume of 3.9 mL. All analyses were carried out in duplicate.

Results and Discussions

At this stage, the effect of the ultrasonic power on the esterification reaction of the *i*-amyl alcohol with acetic acid in the presence of the immobilized enzyme Lipozyme 435 in continuous regime and the influence of different flow rates of reactants on the ester concentration were monitored.

Intensification of Enzymatic Esterification in The Ultrasonic Bath

In order to intensify the enzymatic reaction of esterification between acetic acid and *i*-amyl alcohol with ultrasounds, experiments were conducted in the plant shown in Figure A2.1. Various ultrasonic powers have been applied to find the optimal value for which the enzyme is activated and not denatured. Since in the specialized literature [24] it is mentioned that better results for the intensification of ultrasonic enzymatic reactions are obtained when the ultrasound bath is operated in ultrasound pulses, in the following experiments the same plant in Figure A2.1 was used, to which was added a control system of the operating regime of the ultrasonic bath. Thus the ultrasound bath worked in pulses.

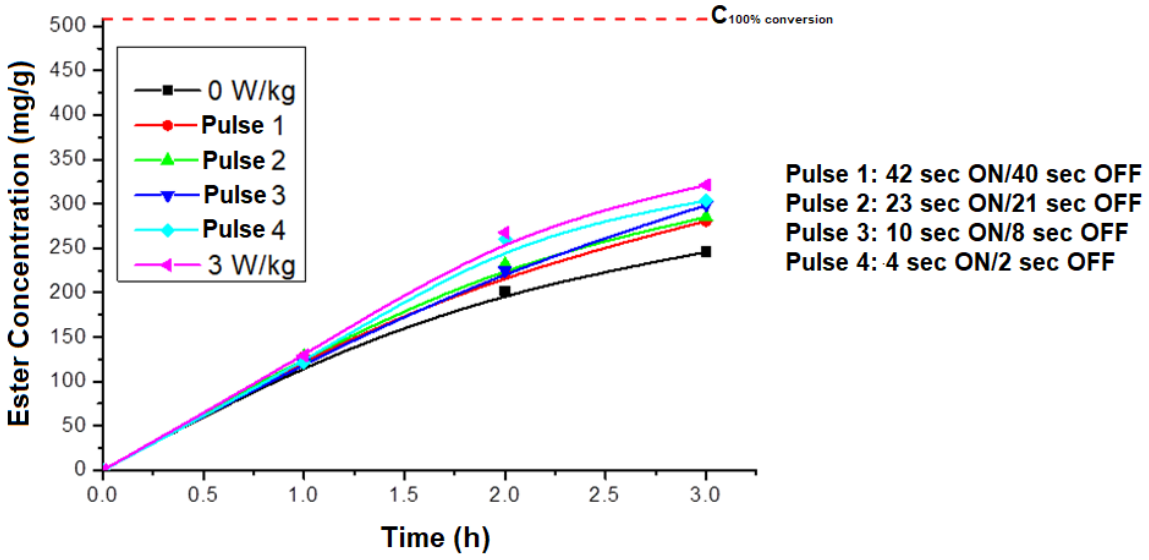


Figure A2.10 : The evolution in time of the concentration of *i*-amyl acetate, at various us powers applied, with 35% more water removal agent from the system, ultrasound applied in pulses, at a US power of 3 W / kg in OFF mode

The best results were obtained when using pulses of 4 seconds ON/2 seconds OFF.

Partial Conclusions

The ultrasound-assisted enzymatic synthesis of *i*-amyl acetate was performed using the enzyme Lipozyme 435 in solvent-free system in a "loop" reactor immersed in an ultrasonic bath using an intermediate vessel. The "loop" reactor allows to put in contact the mixture of reaction with the enzyme at high ratios enzyme : reaction mixture. At the same time, a sufficiently large volume of reaction can be processed, which is stored in a tank and continuously pumped through the reactor. For the highlighting of the effect of the ultrasonic power, several configurations of the ultrasonic bath were used, for each configuration the ultrasonic power was determined by calorimetry and then the concentration of the ester. For the application of ultrasound on a continuous basis, the best ultrasonic power was established as that of 11.3 W/kg. When applying ultrasound in on / off mode, no better values were obtained than when applying ultrasound continuously. It was also highlighted the deactivation in time of the enzyme used successively in the reaction, without being reactivated.

A3. Enzymatic Esterification in A Reactor Type "Loop" - Ultrasound Applied with Ultrasonic Probe

The use of a "loop" reactor immersed in the U.S. bath for the intensification of the enzymatic esterification process did not allow obtaining better results than those obtained at the classical stirring reactor. For this reason, the research was continued by using a loop

reactor and the process was intensify using a US probe that can be positioned at different distances from the reactor.

The focus was to highlight the effect on the formation of free radicals and subsequently on the intensification of mass transfer. In this way, a reactor configuration was obtained in which a favorable effect on the mass transfer was obtained but with reduced formation of active species. Once this configuration was established, the process of enzymatic esterification was studied.

Materials and Methods

Materials

Acetic acid (purity 99.99%) was purchased from Chemical Company CHIMOPAR SRL. Iso-amyl alcohol (≥98%) was supplied by Sigma-Aldrich. The enzyme used, Lipozyme 435, was a *Mucor miehei* lipase immobilized on a macroporous anionic support. The water was removed using molecular sieves (type 3 Grace Davison SYLOBEAD MS 564 C). *p*-Nitrophenol purchased from Sigma–Aldrich, for use in spectrophotometry, sodium hydroxide from Sigma–Aldrich, absolute purity, hydrochloric acid from Chimopar of 1 N concentration. The ultrasonic probe used was a probe 750 W Ultrasonic Processor type, produced by SONICS. The equipment used allows the application of ultrasound with adjustable amplitudes, the user being able to program either the amount of energy or the period of exposure to ultrasound. The control over the working parameters allows a better follow-up and reproducibility of the degradation process by acoustic cavitation of *p*-nitrophenol.

Methods

The esterification reaction was performed in a loop configuration (flow rate of 0.16 mL/min and different volumes of stock solution) at different reaction temperatures (30 to 50 °C) with an acid-to-alcohol molar ratio of 1:2 molar (Figure A3.1) [32]. The cantity of the enzyme used was 0.2 g. Ultrasounds were introduced through an ultrasonic probe-type system (Vibracell VCX-750), using different pulses and amplitudes of ultrasound. The water was removed from the system by passing the reaction mixture through a column, which contains molecular sieves (2.3 g molecular sieves, with a water retention capacity of 22% wt., sufficient to retain all the water formed during esterification). The reaction was carried out for two hours, in a loop system, the samples were taken and analyzed in duplicate. The concentration of isoamil acetate formed by the reaction mixture was determined by gas chromatography.

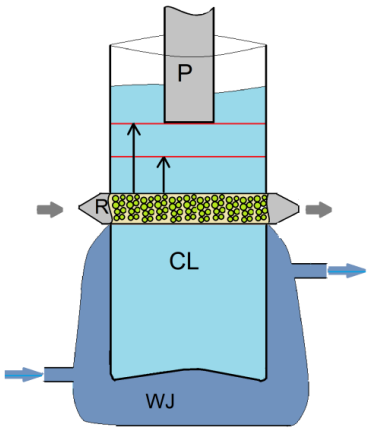


Figure A3.2: Reactor cell design. In which: P—Ultrasound probe; R—Enzyme reactor (1 mL by volume, containing 0.2 g of immobilized enzyme); CL—coupling liquid (water 120 mL volume); WJ—water heating/cooling jacket connected to a thermostat system; T-thermostating system

To overcome the fact that the esterification reactions are reversible, an excess of alcohol was used and the water from the system was continuously removed using molecular sieves, the scheme shown in Figure A3.3.

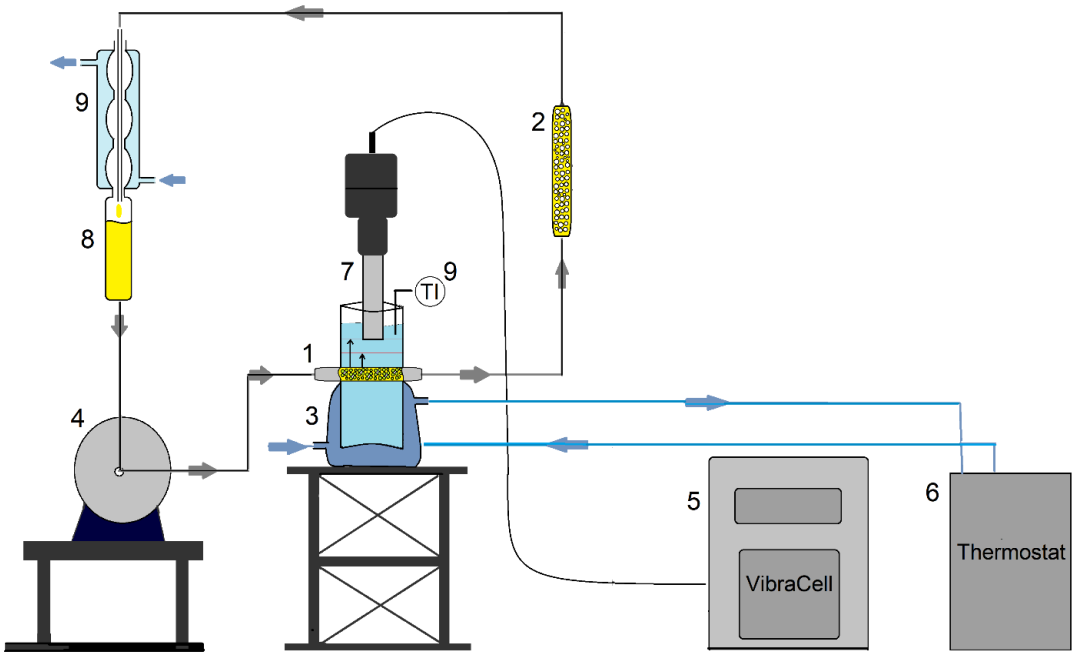


Figure A3.3: Schematic representation of the complete enzyme esterification system of acetic acid with isoamyl alcohol in the presence of ultrasound. 1-Reactor with the enzyme Lipozyme 435; 2-Molecular sieve reactor;3- Cooling heating jacket;4- Pump;5- US control panel;6- Thermostat;7- Ultrasonic generator device (probe type); 8- Stock solution;9- Thermocouple

Results and Discussions

In this study are described the effects of ultrasound on the esterification reaction catalyzed by enzymes to obtain acetate of isoamyl. The reactor was characterized in terms of temperature, ultrasonic power, operating cycle, probe distance from the enzymatic reactor and the specific amount of immobilized enzyme used. We graphically represented, in Figure A3.10, the concentration of PNP depending on the energy emitted by the sonication probe, per unit of volume, thus obtaining the efficiency of ultrasound, in the two reactors, at different reaction temperatures. At lower temperatures of 30 °C, the efficiency of ultrasound is better than at higher temperatures.

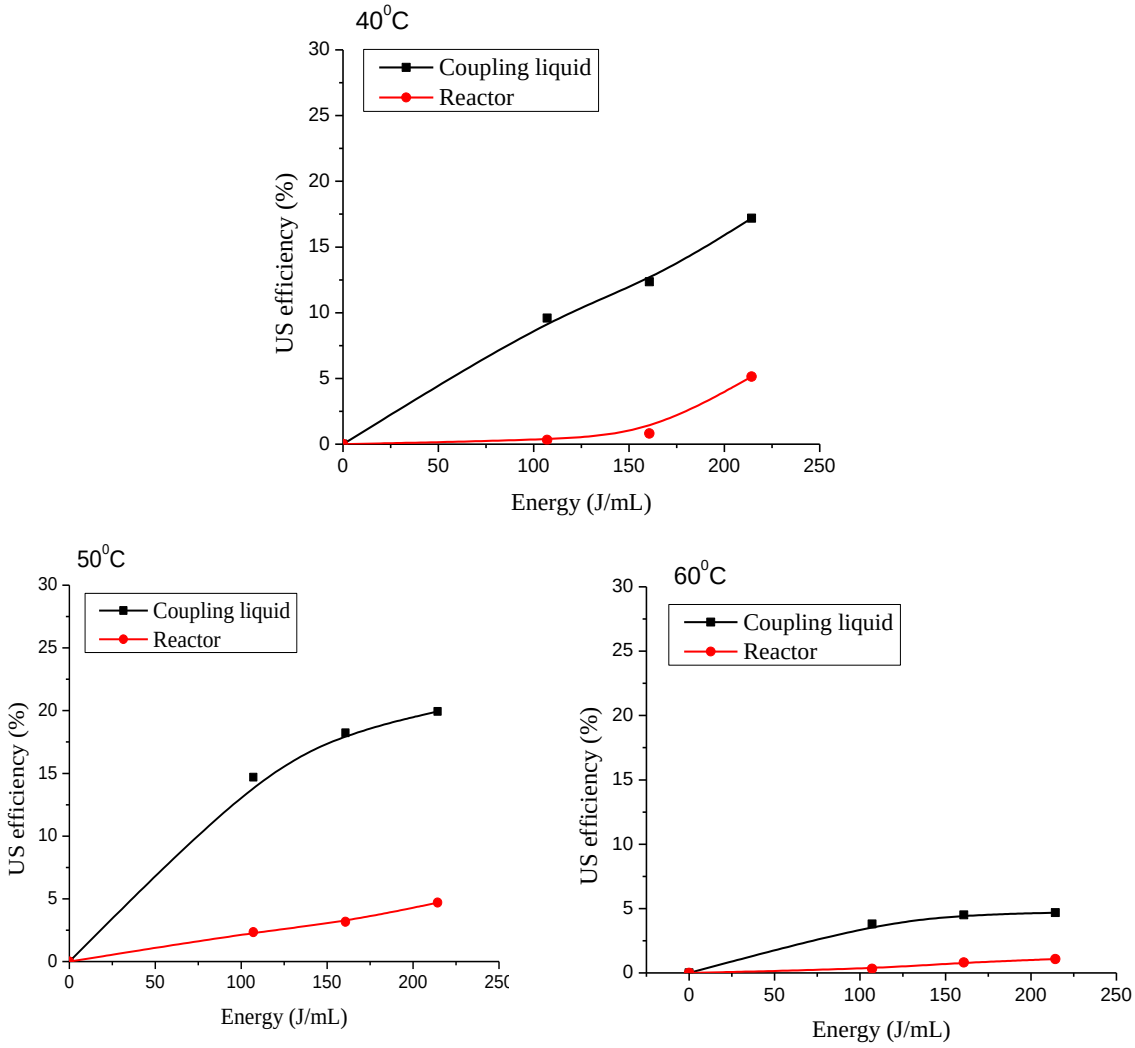


Figure A3.10: Efficiency of ultrasound at different temperatures (20% Ampl, 3on/3off) in both reactor R (1 mL) and coupling liquid (120 mL). Probe-ultrasonic reactor distance = 4.5 cm

Prolonged exposure of the enzymatic catalyst to continuous ultrasound can cause deactivation, but it can be improved with the application of a duty cycle to the ultrasonic

source, i.e. consecutive start-up and shut-down periods [24]. For this reaction, ultrasounds were applied with an operating cycle of 3 seconds on and 3 seconds off.

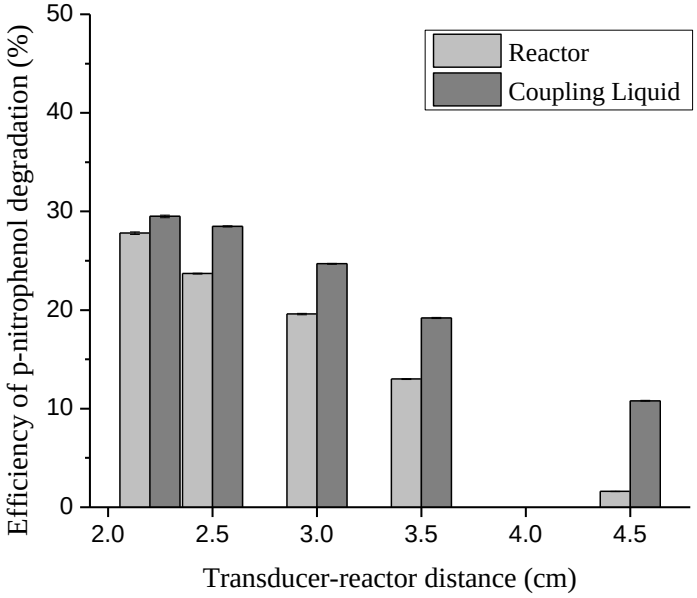


Figure A3.11: Degradation efficiency of *p*-nitrophenol as a function of the distance between the ultrasonic probe and the reactor in the reactor and in the coupling liquid at 50 °C with an ultrasonic energy of 107 J (20% ampl, 3on/3off).

The data presented in Figure A3.11 shows that the slightest degradation of *p*-nitrophenol is obtained at a distance of 4.5 cm of the probe from the enzymatic reactor. This suggests that 4.5 cm is the distance that generates a minimum number of cavitation bubble collapse events, which would provide the optimal geometric arrangement of the reactor for minimal degradation of the enzyme. The effect of the probe distance is almost certainly related to the wavelength of ultrasound in water at a frequency of 20 kHz which is around 7.42 cm [25]. Although the distance of 4.5 cm is not exactly half the wavelength, this is the optimal distance to achieve the activation of the enzyme.

Mass Transfer Effects

To study the intensification of mass transfer in the reactor, a model consisting of a colored salt and a solvent was chosen in which the salt has a solubility limited to the test temperature. The temperature range has been chosen in such a way that it also corresponds to the temperature at which the esterification reaction is being studied.

As a salt, the hydrated nickel sulphate (green color) was chosen, and as a solvent a 50% water-ethanol mixture was chosen. A quantity of salt and solvent was added to the reactor so determined that, under optimal conditions, about 25–30% of the salt in the solvent would be dissolved. The dissolved salt concentration was determined colorimetrically, after the end of ultrasonic treatment. All determinations were carried out in triplicate and an experiment was also carried out in which the salt dissolved naturally, without the influence of ultrasound.

The results in Figure A3.11 indicate that there is an optimum temperature at which salt dissolves in the highest quantity. This fact is consistent with the literature data indicating that the effect of ultrasound in water (water is used as a coupling liquid) decreases when the temperature exceeds a certain value [26].

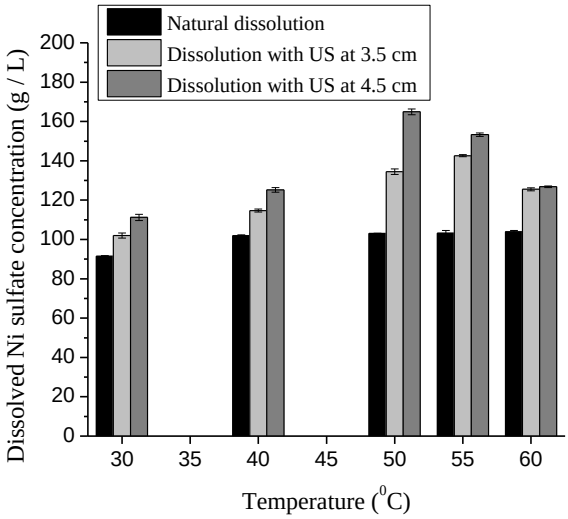


Figure A3.11:Concentration of Ni sulfate dissolved at various temperatures: natural (conventional) and ultrasonic dissolving (sonication probe at a distance of 3.5 cm and 4.5 cm respectively from reactor), 20% amplitude, US power of 7W, 3 on/3 off, for 30 min.

The results indicate that at all temperatures used ultrasound improves mass transfer, but optimal mass transfer occurs at 50 °C for the distance of 4.5 cm of the probe from the reactor wall. In the absence of ultrasound, changes in the concentration of dissolved nickel sulfate are very low, regardless of what temperature, indicates a low level of mass transfer. At 50 °C, the dissolution of nickel sulfate increases with increasing distance of the ultrasonic probe from the reactor (see Figure A3.11). As the distance increases, the sound pressure decreases and there will be a competition between an increasing mass transfer due to the length of the reactor exposed to ultrasound and a decrease in mass transfer due to a reduction in the ultrasonic power with the distance. As a result, the effect of ultrasound on mass transfer reaches its optimal value at a distance of about 5 cm.

Study of the Esterification Reaction

From the previously obtained data in terms of cavitation intensity and mass transfer, the optimal parameters for the reactor were chosen: 4.5 cm probe-reactor distance at a temperature of 50 °C. The reactor was installed in a loop system for esterification with a constant flow rate of 0.16 mL/min and alcohol-acid molar ratio (2:1).

The Effect of US Power

Ultrasound was appliede at three different vibration amplitudes: 20%, 25% and 30% (Figure A3.13). After an hour, the maximum ester concentration was obtained at the lowest amplitude (20%). The overall decrease in ester yield with increased ultrasonic power is the result of competition between the negative effect of cavitation, which stresses the enzyme, and any positive effect resulting from improved mass transfer due to US. The red line in the graphs represents the ester concentration for the 100% conversion.

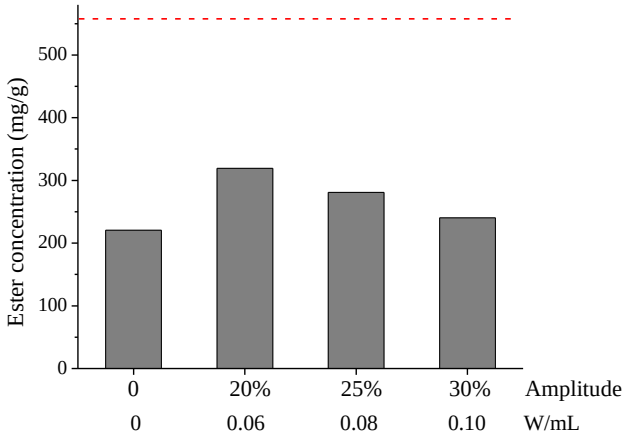


Figure A3.13: Effect of US power on esterification. Reaction time 1 h, reaction temperature 50 °C, 0.047 genzyme/$g_{mixture.}$ The lower values are for the density of the ultrasonic power in the coupling liquid.

The Effect of Temperature

It is known that the effect of ultrasound is better at lower temperatures [27]. At higher temperatures, bubbles are formed in greater quantity, but their collapse is less violent (due to the damping effect of vapors entering the bubble), therefore, the improvement of mass transfer caused by cavitation is reduced. On the other hand, the effect of temperature on enzymatic reactions is positive in the range of 30–50 °C. At lower temperatures, the reaction speed is too low, and at higher temperatures there is a significant decrease in the reaction speed in the second hour compared to the reaction speed of the first hour (Figure A3.15). The preferred temperature range is between 40 and 50 °C.

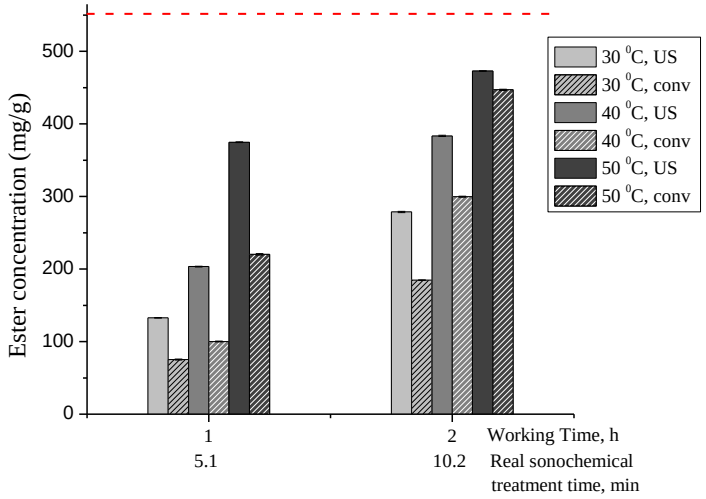


Figure A3.15: Ester concentration function of time for conventional reaction and US intensified esterification at different reaction temperatures: 30 °C, 40 °C, 50 °C, 0.047 g $_{\text{enzyme mixture}}$/g, pulse: 3on/3off

Partial Conclusions

It was designed, built and developed a loop reactor suitable for studying the intensification of their enzymatic reactions,using as a model the esterification of acetic acid with isoamyl alcohol. The reactor configuration has been optimised in order to achieve maximum intensification of mass transfer under the conditions of a minimum formation of active species. Using optimal configuration, the influence of ultrasound (amplitude, operation cycle and sonication time) and temperature on the enzymatic esterification process was studied. Much better values were obtained (450 mg $_{\text{ester}}$/g $_{\text{reaction mixture)}}$ than in the case of the "loop" reactor operated in the ultrasound bath (321 mg $_{\text{ester}}$/g reaction $_{\text{mixture)}}$ or inthe case of the discontinuous stirring reactor (386 mg $_{\text{ester}}$/g $_{\text{reaction mixture}}$).

B. Esterification with Acid Resins—Methods to Intensify the Process

Research Objectives

The main objective of this research is to intensify the esterification reaction of acetic acid with isoamyl alcohol using ion-exchange resins as catalysts. The detailed objectives are:

- Influence of ultrasound on the esterification process in the heterogeneous phase compared to conventional esterification
- The influence of ultrasound on the esterification process in the heterogeneous phase in a "loop" reactor, the effect of amplitude and of the application of US in pulses will be highlighted

B.1. Esterification with Acid Resins - Intensification with US

Materials and Methods

Materials

All chemicals used for esterification: *amyl iso*alcohol(Merck), acetic acid (Merck), isoamyl acetate - standard for GC analysis (Aldrich) were of analytical quality and were used in the form in which they were supplied. The resin used as a catalyst was purchased from Aldrich. Dowex 50W (X8) 50–100 mesh is a strongly acidic styrene-divinylbenzene cationic resin (Table B1.1). The catalyst was used after being dried at a temperature of 100 °C for 2 hours. Drying at much higher temperatures can lead to the loss of the active centers of the catalyst.

Methods of Esterification of Acetic Acid with Isoamyl Alcohol

Esterification Procedure

Conventional reactions of esterification of acetic acid with isoamyl alcohol were performed using a 1 mL loop reactor located in a thermostat vessel and subject to the effect of US. The ultrasound-assisted esterification reaction was performed in a loop reactor [25] described in our previous work [28] at 50 °C with a flow rate of 0.16 mL/min and an acid-to-alcohol molar ratio of 1:2. The reagents are mixed, heated to working temperature and pass through the fixed resin layer in the reactor. Sonication was performed using a probe system of US (Vibracell VCX-750), with a function setting at different amplitudes, continuously or in pulses. The reaction was carried out for two hours and samples were taken at certain periods of time in duplicate. The concentration of isoamyl acetate was determined by gas chromatographic analysis (GC analysis).

Results and Discussions

Conventional Esterification Reactions vs Ultrasonics

The ion-exchange acid resin, Dowex 50 (X8) was selected as a solid catalyst. The composition and structure of an ion-exchange resin are important factors in establishing its catalytic effectiveness. The catalytic activity for this catalyst was tested in conventional and ultrasonic reactions between acetic acid and isoamyl alcohol, and the variation in isoamyl acetate concentration over time is shown in Figure B1.3. It has been observed that the application of ultrasound leads to the obtaining of the ester at a concentration of approximately 30% in 2 hours. Therefore, the use of ultrasound to increase the conversion and reduce the process time for the isoamyl acetate ester is a fairly effective method as shown in Figure B1.3.

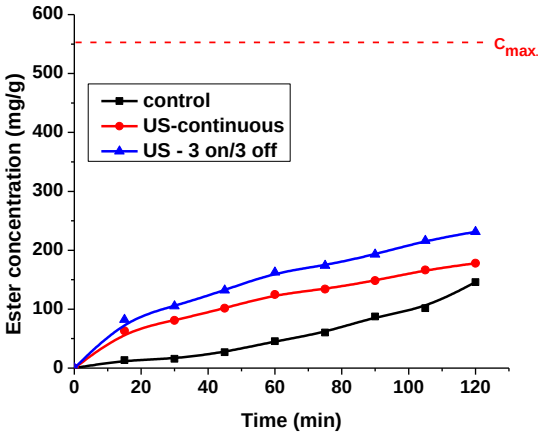


Figure B1.3: Comparison between conventional and ultrasonic esterification (50 °C, sonication probe at 4.5 cm from the reactor, 20% amplitude, catalyst load 0.067 g/mL

The most important phenomenon when using ultrasound is cavitation. The formation of microjets and the effect of increasing the mass transfer lead to an increase in the speed of the process. Cavitation causes local increase in temperature and pressure generating shock waves and microscopic turbulence. Consequently, due to the cavitation generated in the ultrasonic system, these effects enhance the mass transfer and diffusion of the reactant molecules on the active catalytic sites. In addition, the microjets formed when the bubble burst occurs help to renew the interface. All these effects are responsible for improving the concentration of the ester [29].

Influence of Ultrasonic Power

An important factor influencing the properties of cavitation is the amount of energy that is transmitted into the reaction fluid. Studies of the dynamics of bubbles show that their distribution according to size, maximum service life, the number of bubbles and the pressure of collapse are complex functions, which affect the rate of power dissipation. Also, the increase in temperature in the volume of the liquid is dependent on the rate of power dissipation, which leads to changes in the solubility of gases and vapor pressure that affect the ease of generating the phenomenon of cavitation, as well as the final intensity of collapse [30]. To study the effect of the ultrasound power, experiments ranging the amplitude from 20 to 35% were conducted to find the best power required to achieve efficient cavitation for esterification. Figure B1.4 shows the results of experiments catalyzed by Dowex 50 resin (X8).

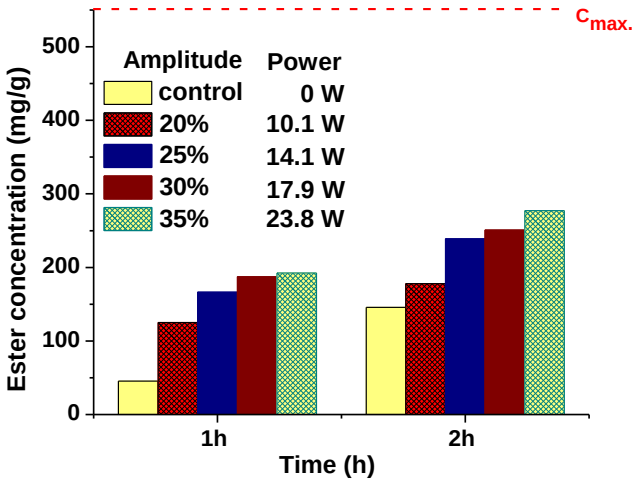


Figure B1.4: Effect of ultrasonic amplitude on ester concentration (50 °C, sonication probe at 4.5 cm from reactor, catalyst load 0.067 g/ml, pulse 3s on/3 off).

Partial Conclusions

The ultrasonic synthesis of isoamyl acetate by the reaction between acetic acid and isoamyl alcohol using as catalyst an ion exchange resin in acid form in the presence of ultrasonic irradiation was successfully performed in a "loop" reactor. The highest ester concentration (300 mg $_{\text{ester/g reaction mixture)}}$ was reached at an ultrasonic amplitude of 35% and at a working cycle of 50%. The results show a favorable perspective of the ultrasonic technique to improve the process efficiency and reduce the reaction time when using dowex 50 (X8) – the process speed intensified with the US is about 2 times higher than that of the conventional process.

C. Enzymatic Transesterification—Methods of Process Intensification

Research Objectives

The main objective of this research is the characterization of the enzymatic synthesis reactor of the biodisel in the presence of ultrasound – with the use of various ultrasonic application equipment. These equipments were: Vibracell, MMM clamp-on, Rheus bath. Within this objective, the following activities were carried out:

- calorimetric determination of the absorbed power,
- determination of free radicals formed by tracking the degradation efficiencyof PNP and
- visualization of the cavitation effect by the aluminum foil method for various ultrasonic equipment.

Materials and Methods

Materials Used

The refined sunflower oil was purchased from CARREFOUR Romania SA Bucharest. The absolute ethyl alcohol, of 99.5% purity, was purchased from CHIMREACTIV SRL, and the immobilized lipase used as a biocatalyst, Lipozyme 435 from Novozyme, Denmark. The standard for GC analysis of biodiesel formed is composed of methyl-heptadecanoate and heptane, heptane 99% purity from Honeywell, and methyl heptadecanoate, 99% (GC) procured from Sigma-Aldrich.

Methods

M1. Conventional Transesterification

Sunflower oil (52.5 mL) along with anhydrous ethyl alcohol (17.5 mL) and the enzyme Lipozyme 435 (immobilized on the support) were introduced into the thermostated reactor at 50°C. Molar ratio intake of sunflower oil: ethyl alcohol was 1:6. The reaction took place in a 100 mL reactor, provided with mechanical agitation.

The progress of the reaction was monitored by collecting samples at different intervals at different intervals and the concentration of esters of fatty acids formed was analyzed at the GC.

C1. Enzymatic Transesterification—Discontinuous Process-MMM Clamp-On

Materials and Methods

For the use of MMM clamp-on,the sunflower oil (15 mL) was used, together with anhydrous ethyl alcohol (5 mL) and the enzyme Lipozyme 435 (0.4 g, immobilized on the support). The reactor was thermostated at 50 °C (inside the metal pipe was inserted a glass pipe – which is the reactor itself; and in the space between the glass pipe and the metal one circulates the thermostating liquid. The reactor has a volume of 20 mL and is provided with mechanical agitation. Different types of mechanical agitators were tested to determine the optimal agitation to obtain a suitable percentage concentration of FAEE. The progress of the reaction was monitored by collecting samples at different time intervals and the concentration of esters of trained fatty acids was analyzed at the GC. Experiments were carried out with conventional heating, but also with ultrasound.

Results and Discussions

Figure C1.8 sums up the results obtained for each fluid both in the glass reactor and in its absence. The power consumed from the power grid of electric current and compared with the absorbed ultrasound power was also recorded. The loss of energy is observed as it is transferred from the metal pipe to the liquid inside the glass reactor. It is the water that absorbs the greatest amount of energy, while the oil absorbs the least vibrational energy.

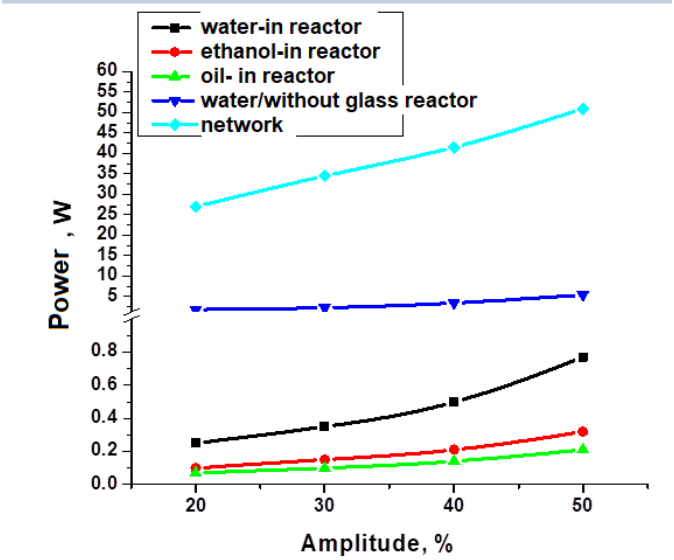


Figure C1.8: Ultrasound power as a function of amplitude for different fluids

Quantification of Active Species Generated by Ultrasound by the Method of Conversion of p-Nitro Phenol to p-Nitro Catehol

The phenomenon of cavitation leads to the generation of active species. Their presence leads to the conversion of *p*-nitrophenol to *p*-nitro-catehol. In order to confirm the formation of free radicals, and to be able to quantify their effect, experiments were performed in acoustic field, following the concentration of *p*-nitrophenol over time, at different amplitudes of acoustic waves. In Figure C1.10 we note that the conversion efficiency of *p*-nitrophenol increases over time and is higher at high amplitude, 40%, results consistent with data from the specialized literature.

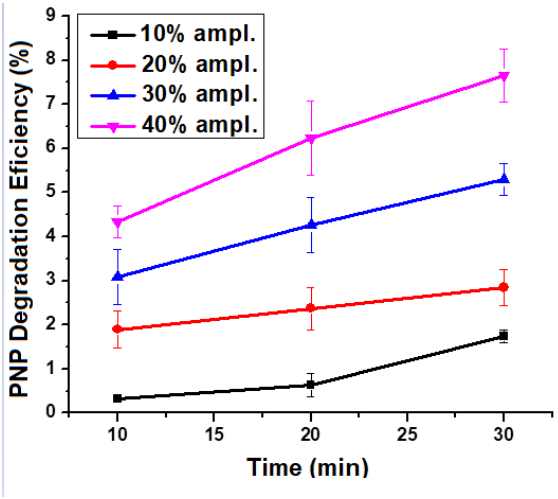


Figure C1.10: Efficiency of converting PNP over time according to the amplitude of the applied acoustic waves

Influence of ultrasound application for 20 min (enzyme activation) on enzymatic transesterification of sunflower oil with ethyl alcohol

In order to achieve a better activation of the enzyme, to reach higher transesterification conversions, we conducted experiments, with the help of the MMM clamp-on equipment, applying the ultrasound at 10% amplitude, for 20 minutes. After the 20 minutes of sonication, the experiment continued at conventional for another 100 minutes.

From Figure C1.13 it appears that an ultrasonic treatment of only 20 minutes leads to the activation of the enzyme Lipozyme 435. Thus we observe a significant increase in the concentration of FAEE obtained with ultrasound versus the conventional method.

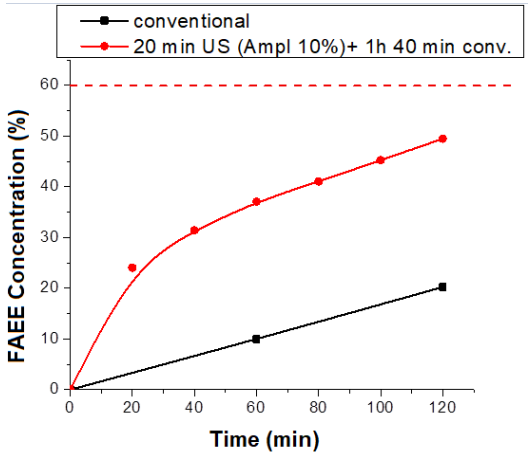


Figure C1.13: Concentration of FAEE over time, reaction temperature of 50 °C, molar ratio 1:6, 0.4g $_{enzyme,}$to conventional heating and ultrasound (10% Ampl)

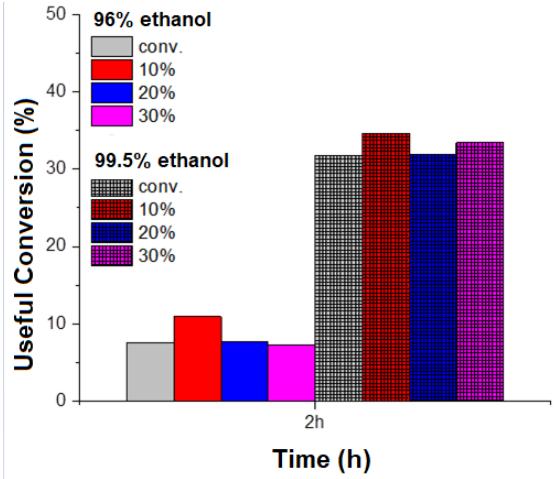


Figure C1.14: Useful conversion after two hours of reaction, using the MMM clamp-on plant, at different amplitudes, using as a reactant either ethanol 96% or absolute ethanol (0.5% water content); (sound power density: for 20% ampl. 0.0125 W/mL; for 30% ampl. 0.0175 W/mL)

In Figure C1.15 it is highlighted how the useful conversion of FAEE increased from 10% to 35% with the decrease in water content from 4% to 0.5% at 2 hours. The active site of *Candida antarctic lipase* is covered by an amphiphilic peptide loop (such as a cap or

flap), which prevents substrates from approaching the active site. The reaction usually takes place at the oil-water interface and an essential amount of water contributes not only to the formation of the interfaith area, but also helps the cover to open the conformation and increases the polarity and structural flexibility of the active site of the Candida *antarctic*lipase , helping toactivate lipase [31]. However, when the water content has increased to 4%, the conversion of FAEE decreases, since too much water damages the lipase facilitating its aggregation, diminishing the diffusion of the substrate and, ultimately, decreasing the efficiency of lipase.

Partial Conclusions

At this stage, the power of ultrasound was determined by the calorimetric method for the ultrasonic MMM Clampon reactor in which the generated acoustic waves are transmitted along a metal pipe. All determinations were carried out at least 5 times. The determinations were made for different amplitudes: 20%, 30%, 40% and 50%. Various configurations of enzymatic transesterification installation with continuous operation (with and without cooling serpentine and with different modes of supply with reactants before and after the pumping system) have been proposed. The power of ultrasonically absorbed is higher for distilled water and lower for ethanol, and much lower for oil. The power generated by ultrasound is dependent on the vapour pressure of the fluid used, which is why the power absorbed by ethanol is lower than in the case of water. The way in which acoustic waves are propagated in the fluid is also a function of the viscosity of the fluid used, the oil being a fluid with a high viscosity, absorbs a much lower ultrasonic power. As for the enzyme-catalyzed transesterification response, the influence of water in ethanol has been highlighted. At a low concentration of water (0.5%) much better results are obtained than in the case of using ethanol with 4% water. The intensifying effect of the ultrasonic process was highly noticeable when ultrasound was applied for a limited period (20 minutes) followed by a conventional reaction period of 100 minutes. In this way, a significant increase in the reaction rate and the conversion of oil into ethyl esters was obtained. Thus oil conversion increased to 60.19% – activation with US 20 minutes, compared to 31.2% – conventional.

C2. Enzymatic Transesterification -"Loop" Type Reactor

The best results were obtained by applying ultrasound at an amplitude of 25%, the concentration of FAEE increasing from 5% to almost 25%. An FAEE concentration of 40% was obtained by applying ultrasound at the amplitude of 25% for 20 minutes, using ethyl alcohol with low water content (0.5%). These results demonstrate the activation of the enzyme following the application of ultrasound over short periods of time.

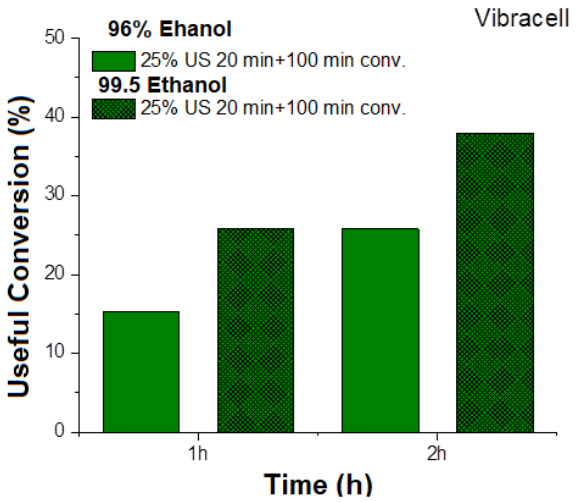


Figure C2.4: Useful conversion over time, using the instalation in continuous mode, applying ultrasound with the probe Vibracell, at different amplitudes, using as a reactant either ethanol 96% or absolute ethanol, applying ultrasound with an amplitude of 25% for 20 minutes, then the reaction went to conventional for 100 minutes (ultrasound power density for 25% ampl. 0.1 W/mL)

Partial Conclusions

When using ultrasound the increase in US amplitude led to an increase in the amount of ester formed, but above certain values the enzyme can be deactivated.

The use of ethyl alcohol(0.5%) leads to better results (37% conversion) than the use of absolute ethyl alcohol (25% conversion).

The best results were obtained in the enzymatic transesterification process by combining the two methods, namely an activation of the enzyme with ultrasound for a period of 20 minutes, followed by conventional transesterification for 100 minutes. This way we get a conversion of 37% compared to only 19.8% when applying US continuously or only 6% to the conventional method.

C3. Enzymatic Transesterification-Rheus Bath

Materials and Methods

Methods

Sunflower oil (22.5 mL) together with anhydrous ethyl alcohol (7.5 mL) and the enzyme Lipozyme 435 (immobilized on support 0.02g/mL) were introduced into the thermostated reactor at 50 °C. Progress of the reaction was tracked by collecting samples at different time intervals and the concentration of esters formed was analyzed at GC. The reaction took place in a 30 mL reactor, provided with mechanical agitation. The intensification of the enzymatic transesterification reaction was achieved using the Sars Rheus ultrasound generator equipment. The ultrasonic equipment is provided with an

ultrasonic bath with cooling system; an ultrasonic intensity control system (equipment with adjustable voltage/ by regulating the voltage from the network we can control the applied sound power) and a system for applying ultrasound in pulses (20 on/10 off). The voltage from the electrical network varied between 80V, 100V and 120V. The constant reaction conditions were: molar ratio sunflower oil : ethyl alcohol of 1:6, temperature of 50 °C.

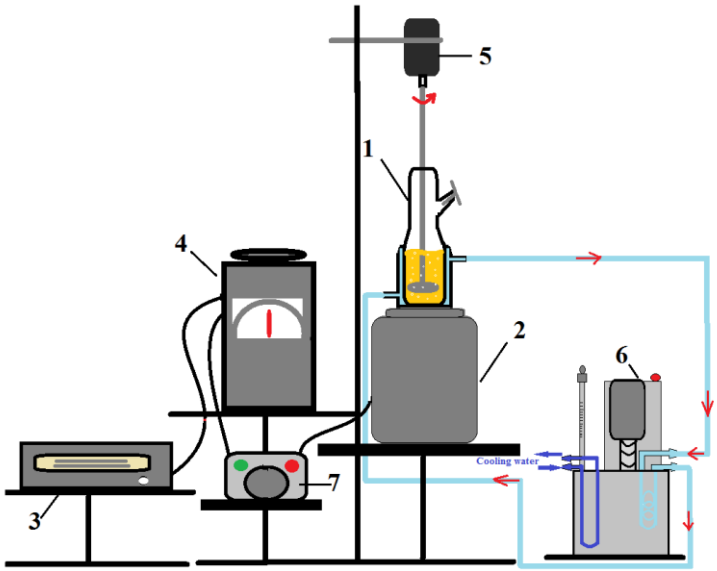


Figure C3.2: Transesterification instalation with discontinuous operation (1 - Transesterification reactor; 2- RHEUS ultrasound bath; 3 - Control system for ultrasound bath; 4 - Power supply energy control system from the mains; 5 - Stirring system; 6 - Thermostat; 7 - Control system for the sonication cycle)

Results and Discussions

Influence of the type of sonication (continuously or in pulses) on the concentration of biodiesel obtained using Rheus bath

In the ultrasonically assisted enzymatic transesterification reactions, an important factor is the ultrasonic power. For the RHEUS ultrasonic bath, the ultrasonic power was previously determined for the *n*-alkane coupling fluid by the calorimetric method and using the *p*-nitrophenol degradation method. Thus, the influence of the sonication type (continuous or in pulses 20 sec on/10 sec off) on the conversion into biodisel at the application of different ultrasonic powers was traced. At an interval of 1 hour, samples were taken and analyzed. The obtained results are plotted in Figure C3.14.

Following the enzymatic transesterification reactions at different ultrasonic powers, it was determined that the optimum power for the enzymatic transesterification reactions for obtaining the ethyl esters of the fatty acids corresponds to the application of ultrasound in pulses at low powers. In processes with continuous sonication, the ultrasonic powers are higher and the enzyme deactivation likely occurs, thus leading to lower conversions. An oil conversion of 60% was obtained when using low-water ethanol (0.5%) and ultrasound was

applied for 30 min, the reaction continuing for another 100 min without applying ultrasound (Figure C3.15).

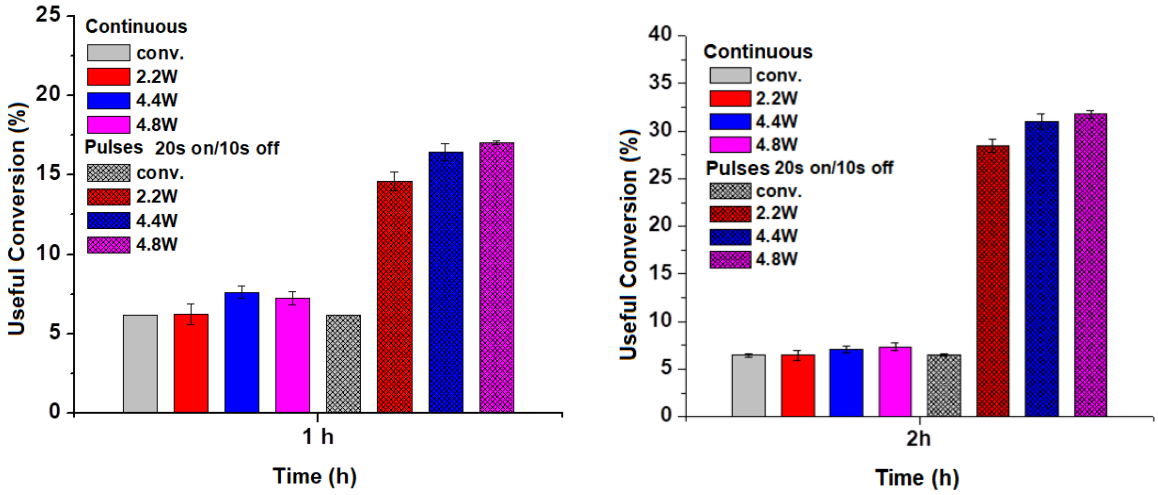


Figure C3.14: Useful conversion after 1 hour and 2 hours of reaction, ultrasounds were applied with Rheus bath at different acoustic powers, using 96% ethanol as a reactant, ultrasound was applied continuously or in pulses of 20s on/10s off

Partial Conclusions

At this stage, the determination of the ultrasonic power for Rheus bath equipment was studied. The ultrasonic power was determined by two methods: the calorimetric method and the method of degradation of *p*-nitrophenol in the presence of ultrasound. The measurements were performed both in the ultrasonic bath (for three coupling liquids: distilled water, *n*-alkanes and polyethylene glycol PEG 200) and in the reactor in the enzymatic transesterification glass (for ethanol, 96%). It has been observed that when applying ultrasound in Rheus bath the highest ultrasonic power is obtained for the polyethylene glycol coupling fluid PEG 200, and the lowest for *n*-alkanes. PEG 200 absorbs the greatest amount of energy, while *n*-alkanes absorb the least vibrational energy. In order to confirm the formation of free radicals, and to be able to quantify their effect, experiments were performed in acoustic field, following the concentration of *p*-nitrophenol over time, at different amplitudes of acoustic waves. One can observe the different behavior of the three solvents used as a coupling liquid in the transfer of ultrasonic energy that can cause the degradation of *p*-nitrophenol. The greatest decrease in the concentration of *p*-nitrophenol was obtained for water, and the slightest decrease for polyethylene glycol.

After calibrating the reactor and choosing the best coupling liquid, experiments were made for the transesterification of the sunflower oil with ethanol. The influence of water concentration in ethanol was highlighted (the best results were obtained at a low water concentration of only 0.5%); of the applied ultrasonic power and pulses (better results were obtained when applying pulses in 20s ON and 10 s Off, compared to continuous application) and the mode of application of ultrasound – the best results being obtained at a continuous

application for 20 minutes followed by the unseating of the reaction for another 100 minutes conventionally. In the best conditions the useful oil conversion was 59%.

D. General Conclusions

In the research on the intensification of enzymatic reactions with the help of US were studied two general processes:

I. ***The esterification process for obtaining isoamyl acetate.***
Heterogeneous esterification reactions were performed using two types of solid catalysts: enzymes deposited on a support and acidic ion exchange resin.

II. ***The transesterification process for obtaining ethyl esters of fatty acids (FAEE).***
Heterogeneous transesterification reactions were achieved using solid catalysts: enzymes deposited on the support.

I. The esterification process for the production of isoamyl acetate

a) Enzymatic esterification

It was aimed at intensifying the process of esterification of acetic acid with isoamyl alcohol catalyzed by the enzyme deposited on the support - Lipozyme 435. The study was performed using different conditions to determine the effect of operational parameters on the production of isoamyl acetate. The best ultrasonication conditions have been determined for improving the activity, stability and reuse of the enzyme.

Two types of distinct reactors were used: a discontinuous reactor with stirring and a "Loop" reactor.

For the discontinuous reactor, the process intensification was performed with the help of ultrasound baths. In order to obtain a maximum amount of ester, the effect of various parameters (amount of enzyme, molar alcohol-acid ratio, power ultrasound and temperature) on the synthesis of *i-amyl* acetate by enzymatic catalysis was studied. The best result was obtained using 50 mg/g Lipozyme 435, molar ratio of *i*-amyl alcohol:acetic acid 2:1 and a temperature of 50 °C. The most important parameter studied at this stage is the power ultrasound, which correlated with the reaction temperature was 8.5 mW/cm^3. It has been observed that an increase in the ultrasonic power at low temperatures leads to an increase in the concentration of esters in the final reaction mass. At higher temperatures (> 60 °C) the increase in ultrasonic power deactivates the enzyme and the concentration of esters obtained decreases.

In order to increase the efficiency of using the enzyme, a loop reactor was used. In the reactor there is 0.8 g of enzyme and the free volume of the reactor is 0.8 mL, in this way a very good ratio is ensured between the amount of enzyme relative to the volume of the reactant. In order to treat a larger volume of reaction, the reaction mixture is stored in a vessel from where it is continuously pumped through the reactor. In this way the amount of enzyme is reduced compared to that used in the reactor with discontinuous stirring. The loop reactor was used in an ultrasound bath or in a special construction where ultrasound is provided by a Vibracell-type probe. For each configuration of the "loop" reactor used in the ultrasonic

bath, the ultrasonic power density was determined calorimetrically. The influence of various parameters on the speed of the esterification process were welded: the liquid flow, the power density, the on/off application cycle of ultrasound. For the application of ultrasound on a continuous basis, the best ultrasonic power was established as that of 11.3 W/kg. When applying ultrasound in on / off mode, no better values were obtained than when applying ultrasound continuously. It was also highlighted the deactivation in time of the enzyme used successively in the reaction, without being reactivated.

The use of a "loop" reactor immersed in the US bath for the intensification of the enzymatic esterification process did not allow obtaining better results than those obtained at the classical stirring reactor. For this reason, the research was continued by using a "loop" reactor and the process was intensely intense by using a US probe that can be positioned at different reactor distances.

It was aimed at highlighting the US effect on the formation of free radicals and subsequently on the intensification of mass transfer. In this way, a reactor configuration was obtained in which to obtain a favorable effect on the mass transfer but with reduced formation of active species. Once this configuration was obtained, the process of enzymatic esterification was studied.

In the optimal configuration was performed the experimental program for the study of the influence of ultrasound (amplitude, operating cycle and sonication time) and temperature on the enzymatic esterification process. Much better values were obtained (450 mg $_{ester}$/g $_{reaction\ mixture}$) than in the case of the "loop" reactor operated in the ultrasonic bath (321 mg $_{ester/g\ reaction\ mixture}$) or in the case of the discontinuous stirring reactor (386 mg $_{ester/g\ \ reaction\ mixture}$) although the actual sonication time was of the order of minutes.

In the next stage of the experimental research, the esterification of acetic acid with isoamilic alcohol using ion-exchange resins as catalysts was studied and the influence of different parameters on the continuous process of esterification assisted by ultrasound was monitored. The process of esterification in heterogeneous catalysis of acetic acid with isoamyl alcohol in the presence of ion exchange resins assisted by utrasounds was carried out using different conditions to determine the best parameters to obtain the highest ester concentrations. For this we studied the influence of the amplitude of ultrasound and of the type of continuous sonication or in pulses on the concentration of ester obtained in the esterification process by conventional method or assisted by ultrasound.

The highest ester concentration (300 mg ester/g reaction mixture) was reached at an ultrasonic amplitude of 35% and at a working cycle of 50%. The results show a favorable perspective of the ultrasonic technique to improve the process efficiency and reduce the reaction time when using the Dowex 50 (X8) – the process speed intensified with the US is approx. 2 times larger than the conventional process

II. The transesterification process for obtaining fatty acid ethyl esters (FAEE)

At this stage the enzymatic transesterification reaction of the sunflower vegetable oil with ethyl alcohol using the enzyme immobilized on the support of Lipozyme 435 was studied. Experiments were conducted by two methods: conventional method and ultrasound assisted

method. By conventional method, the influence of the amount of enzyme and the use of different types of agitation systems were studied. It is worth noting that using an amount of 0.4 g of the enzyme, a fairly large amount of esters is obtained (very close to that obtained after 6 hours). Shortening the transesterification time can compensate for the increase in the amount of enzyme from the point of view of the cost of the process.

The ultrasound-assisted reaction took place using three different acoustic wave generation equipment: a Rheus ultrasound bath, a probe-type equipment - Vibracell and an equipment in which the generated acoustic waves are transmitted along a pipe - MMM clamp-on. The increase in the amount of the enzyme led to an increase in the concentration of the esters obtained. When using ultrasound the increase in US amplitude led to an increase in the amount of ester formed, but above certain values the enzyme can be deactivated.

The ultrasonic power is higher for distilled water and lower for ethanol, and much lower for oil. The power generated by ultrasound is dependent on the vapour pressure of the fluid used, which is why the power absorbed by ethanol is lower than in the case of water. The way in which acoustic waves are propagated in the fluid is also a function of the viscosity of the fluid used, the oil being a fluid with a high viscosity, absorbs a much lower ultrasonic power.

The ultrasonic process intensifying effect was highly noticeable when ultrasound was applied for a limited period (20 minutes) followed by a conventional reaction period of 100 minutes. In this way it was obtained a significant increase in the reaction speed and of the conversion of the oil into ethyl esters. Thus oil conversion increased to 60.19% – activation with US 20 minutes, compared to 31.2% – conventional.

After calibrating the reactor and choosing the best coupling liquid, experiments were made for the transesterification of the sunflower oil with ethanol. The influence of the water concentration in ethanol has been highlighted (the best results have been obtained at a low waterconcentration of only 0.5%); of the applied ultrasonic power and pulses applied (better results were obtained when applying in pulses 20s ON and 10 s Off, compared to continuous application) and the way of applying ultrasound – the best results being obtained at a continuous application for 20 minutes followed by the reaction for another 100 minutes conventionally. In the best conditions the useful oil conversion was 59%.

E. Own Contributions

The elements of originality that are distinguished as a result of this research are:

- ✓ The optimal conditions for the intensification of enzymatic esterification processes with the help of ultrasound were determined
- ✓ The optimal conditions for the intensification of the esterification processes catalyzed by acidic resins with the help of ultrasound were determined
- ✓ The optimal conditions for the intensification of enzymatic transesterification processes with the help of ultrasound were determined
- ✓ An ultrasonic plant for the study of enzymatic reactions was developed and patented

F. Future Research Directions

Taking into account the above mentioned, it can be concluded that the present work has achieved its objectives.

The experimental study can be continued especially in the direction of scaling up to industrial scale the intensification of esterification and transesterification reactions catalyzed by ultrasound or acid resins with ultrasound and / or microwaves.

Further, studies can be performed for the activation of enzymes with the help of ultrasound in the pre-treatment stage of the catalyst.

The effect of ultrasound on a wider spectrum of enzyme-type catalysts and for various types of reactions can be studied.

G. Publication List

Published articles:

1. Calinescu, I., **A. Vartolomei**, I. A. Gavrila, M. Vinatoru and T. J. Mason (2019). "A reactor designed for the ultrasonic stimulation of enzymatic esterification." Ultrasonics Sonochemestry 54: 32-38; WOS:000466997500004. https://doi.org/10.1016/j.ultsonch.2019.02.018; ***(IF = 6.75)***

2. **Vartolomei, A**., I. Calinescu, M. Vinatoru and A. Gavrila (2019). "Intensification of the Enzymatic Esterification Process by Ultrasounds." Revista De Chimie 70(1): 41-44; WOS:000460428100009. ***(IF = 1.755)***

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Patents:

1. I.Calinescu, M.Vinatoru, A.Gavrila, **A. Vartolomei**, N.Ignat, INSTALAŢIE CU ULTRASUNETE DESTINATĂ STUDIULUI REACŢIILOR ENZIMATICE brevet nr. 133341; 30 Decembrie 2021.

Participation in conferences:

1: **Vartolomei**, I., Calinescu, I-A., Gavrila, M., Vinatoru, T. J., Mason,A reactor designed for the ultrasonic stimulation of enzymatic esterification, *4th International Caparica Conference on Ultrasonic-based Applications: from analysis to synthesis 2020, 01–04 iunie, 2020, Costa de Caparica, Portugalia*

2: Adina Ionuta Gavrila, **Anamaria Vartolomei**, Mircea Vinatoru, Ioan Calinescu,The effect of ultrasounds on enzymatic transesterification, *4th International Caparica Conference on Ultrasonic-based Applications: from analysis to synthesis 2020, 01–04 iunie, 2020, Costa de Caparica, Portugalia*

3: **A. Vartolomei**, I. Calinescu, A.I. Gavrila, M. Vinatoru, Activation with ultrasounds of Lipozyme 435 for esterification and transesterification reactions, *Chemisty Conference for Young Scientists - ChemCYS 2020, 19–21 februarie, 2020, Blankenberge, Belgia*

4: Adina Ionuta Gavrila, **Anamaria Vartolomei**, Ioan Calinescu, Mircea Vinatoru, Biodiesel Synthesis by Ultrasound Assisted Enzymatic Transesterification, *International Chemical Engineering & Catalysis Conference (Chemical Engineering & Catalysis-2019), 12 - 13 decembrie 2019, Londra, Marea Britanie*

5: **Anamaria Vartolomei**, Ioan Calinescu, Mircea Vinatoru, Adina I. Gavrila, Lypozyme 435 esterification activity improvement using short term treatments with ultrasounds, *21st Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), 4 - 7 septembrie 2019, Constanta-Mamaia, ROMANIA*

6: **Anamaria Vartolomei**, Ioan Calinescu, Adina-Ionuta Gavrila, Mircea Vinatoru, Optimisation of the ultrasonic assisted lipase catalysed reaction of acetic acid with isoamyl alcohol, *International Symposium of Chemical Engineering and Materials SICHEM 2018, 6 – 7 September, Bucharest, Romania*

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