Analysis of Process Intensification in Enzyme Catalyzed Reactions Using Ultrasound

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M. Ajmal

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Gutachter:	Prof. Dr.-Ing. Georg Fieg Prof. Dr. Dr. h.c. Frerich Keil
Prüfungsvorsitzender:	Prof. Dr. rer. nat. Rudolf Müller
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Contents

List of Symbols

A_S	Particle surface area	[m^2]
A_t	Transducer cross sectional area	[m^2]
A	residual enzyme activity	[%]
Ca	Carberry number	[-]
C_S	Surface concentration	[mol/m^3]
C_{OAC}	Bulk concentration	[mol/m^3]
C_{L0}	Lactose concentration at t=0	[g/L]
C_L	Lactose concentration at t	[g/L]
C_{OAC}	Bulk concentration	[mol/m^3]
C_D	Drag Coefficient	[-]
c	Speed of sound in medium	[m/s]
c_p	Compressional speed of sound in particle	[m/s]
c_p	Specific heat capacity	[J/mol/K]
D	Impeller diameter	[m]
D	Diffusion coefficient	[m^2/s]
D_{eff}	Effective diffusion coefficient	[m^2/s]
d_P	Particle diameter	[m]
F_D	Drag force	[N]
F_g	Gravity force	[N]
F_{aco}	Acoustophoretic force	[N]
g	Gravity constant	[m/s^2]
I	Intensity of sound source	[W/m^2]
I_{US}	Rated Intensity of sound source	[W/m^2]
k_{ls}	Liquid solid mass transfer coefficient	[m/s]
k	Kinetic constant	[1/min]
k_D	Degradation coefficient	[1/min]
k_{Pa}	degradation coefficient dependent on acoustic power	[1/W]

k_{Pc}	constant dependent on acoustic power	[g/l/W]
m_p	Particle mass	[kg]
m	mass of reaction medium	[kg]
n	Reaction order	[-]
N	Impeller rpm	[1/s]
p	Acoustic pressure	[Pa]
p_{in}	Incident external pressure field	[Pa]
P_t	Power of transducer	[W]
P_{US}	Rated power of transducer	[W]
p_t	Pressure from transducer	[Pa]
R	Reflection coefficient	[-]
$Re_{impeller}$	Impeller Reynolds number	[-]
Re_P	Particle Reynolds number	[-]
$(r_v)_{obs}$	Observed reaction rate	[mol/m^3/s]
Sc	Schmidt number	[-]
Sh	Sherwood number	[-]
T	Temperature	[K]
T	time	[min]
u	Fluid velocity	[m/s]
U^{rad}	Radiation potential	[J]
v	Particle velocity	[m/s]
v_{in}	Incident velocity	[m/s]
V_p	Volume of particles	[m^3]
v_s	Slip Velocity	[m/s]
v_{in}	Incident particle velocity amplitude	[m/s]
W	Power	[W]
x	Conversion	[-]
$Z = \rho * c$	Specific acoustic Impedance of medium	[Rayl]

Za	Specific acoustic Impedance of air	[Rayl]

Greek Symbols

α	ratio of the specific activity of the final state to the initial state	[-]
ρ	Medium density	[kg/m^3]
ρ_p	Particle density	[kg/m^3]
μ	Viscosity of medium	[kg/m/s]
ω	Angular frequency	[rad/s]
f	Frequency	[Hz]
τ_p	Particle velocity response time	[s]
τ	Tortuosity	[-]
ε	Porosity	[-]
δ_{bl}	boundary layer thickness	[m]
ν	kinematic viscosity	[m^2/s]
k	1st order rate constant	[1/min]
η	Effectiveness factor	[-]

Abbreviations

B.C	Boundary Condition
CALB	Candida Antarctica Lipase B
DOF	Degrees of freedom
F	Free enzyme
Im	Immobilized
kHz	kilohertz

MHz	megahertz
OAC	Oleic Acid
US	Ultrasound
3-D	3-Dimensional
2-D	2-Dimensional

1 Introduction

Enzymes are biological catalysts in the form of protein which catalyze chemical reactions. They are being actively investigated for their application in chemical process industry. The key advantages associated with enzymes include their high specificity and ability of functioning at milder operating conditions. High specificity helps to minimize or eliminate side product formation [1]. Capability to function at milder operating conditions enables considerable energy efficiency and safety of the process. They also contribute to sustainable development as due to protein nature they are biodegradable and are isolated from microorganisms which are fermented using primarily renewable resources. One of the best examples is the industrial production of acryl amide (20,000 tons/year) from Nitto Chemicals Japan using a nitrile hydratase enzyme. The conventional process operates at temperatures of 80-140°C and always produces acrylic acid as by product. The new process based on enzymes operates at 10 °C and produces acryl amide in 100% yield with no acrylic acid by product [2]. Enzymes can be used in free state but for industrial application they are bound on some porous carrier material and are termed as immobilized enzymes. This facilitates separation of enzyme from product and they can be utilized for entire period of their life time thus leading to reduction of production cost substantially [3].

Tufvesson et al. have recently reported an economic analysis showing that lipase-catalyzed production of chemicals shows an impact of 35% on the total manufacturing cost, thereby making the enzymatic production processes quite expensive [4]. Processes based on enzymes can be made competitive either by decreasing cost of enzyme or by increasing the activity of enzymes so that time required for reaction completion decreases thereby increasing the reactor throughput. Activity of enzymes can be increased by increasing temperature but there is usually a maximum limit of temperature for enzymes (60°C) [3,5]. This means beyond this temperature enzyme activity and hence the reaction rate cannot be increased by thermal energy as it will denature the enzyme. Therefore, to further intensify the rate of enzyme catalyzed reactions energy in some other form should be used. One of the many possibilities for intensifying the enzyme catalyzed reactions is the application of ultrasound (US) as reported in the literature [6,7]. However, published research has so far focused merely on certain aspects of the phenomenon, is scattered and lacks process engineering perspective. The realization of an industrial scale sonicated enzyme catalyzed reactor requires considerable

work in a systematic manner. Therefore, objective of present study was to systematically investigate the phenomenon from process engineering viewpoint.

1.1 Literature Survey

In order to determine the state of art a comprehensive literature survey was carried out at start of the work. Focus was to review different ultrasonic reactors employed by different research groups, potential for activation/deactivation of enzymes from US, clarification of underlying mechanisms and influence of different operating parameters. Findings of this literature survey are summarized in the following pages.

1.1.1 Equipment used for Sonication of Enzyme Catalyzed Reactions in Labs

Studying the effects of US on chemical reactions is termed as sonochemistry. For studying the effect of US on chemical reactions an ultrasonic reactor is used. Although a large variety of ultrasonic reactors are used in sonochemistry but current survey is limited to the ultrasonic reactors used for studying the effects of US on intensification of enzyme catalyzed reactions. The two widely used ultrasonic sources include but not limited to ultrasonic cleaning bath and ultrasonic probe. Ultrasonic cleaning bath (Figure 1.1) is by far the most widely used source of ultrasonic irradiation in the chemical laboratory. Although it is possible to use the bath itself as a reaction vessel but this is seldom done because of the problems associated with corrosion of the bath walls and containment of any evolved vapors and gases. The normal usage, therefore, involves filling of reaction contents in a beaker/flask and this is immersed into the bath. The beaker can be positioned inside bath at a place where the ultrasonic radiation is strongest. This means reaction contents are sonicated indirectly. The reaction vessel does not need any special adaptation and an inert atmosphere or pressure can readily be maintained throughout the reaction time. Temperature control in commercial cleaning baths is generally poor and so the system may require additional thermostatic control [8].

As mentioned earlier reaction contents are held in a glass beaker/flask in ultrasonic bath. Impedance of glass beakers is very high in comparison to liquid medium used in ultrasonic baths e.g. water. The entrance of ultrasonic energy into the beaker/flask (containing reaction contents) can be determined according to Eq. 1.1.

$$\text{Entrance} = 1 - R \tag{1.1}$$

Here R is reflection coefficient which defines the fraction of US reflected from a surface and can be determined from Eq. 1.2.

$$R = \frac{Z_{Glass} - Z_{Water}}{Z_{Glass} + Z_{Water}} = 0.79 \tag{1.2}$$

where [9]

$$Z_{Glass} = \rho c = 13 \times 10^6 \quad \text{[Rayl]}$$

$$Z_{Water} = \rho c = 1.5 \times 10^6 \text{ [Rayl]}$$

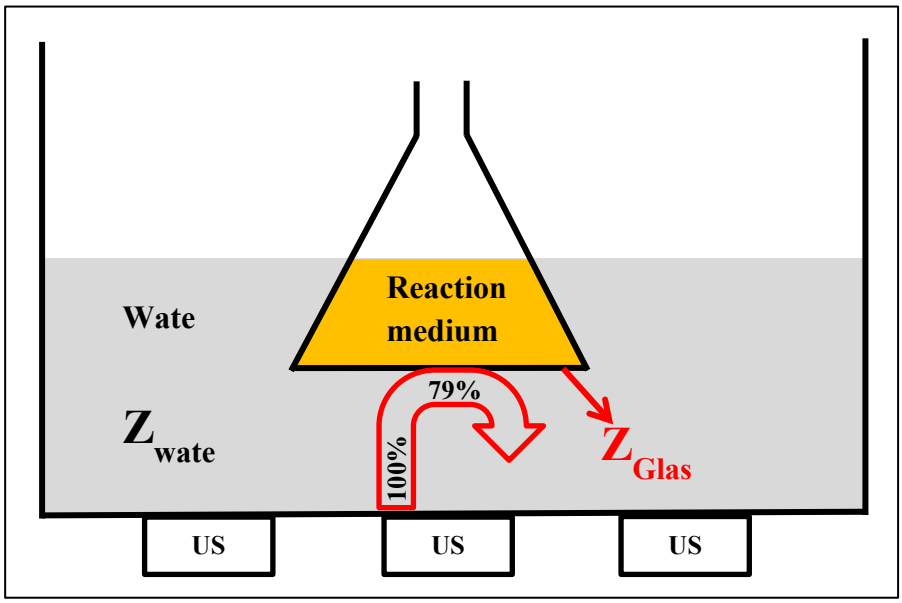


Figure 1.1. Use of ultrasonic cleaning bath in sonochemistry

This means entrance is 0.21 i.e.79% of ultrasonic energy is reflected back from the surface of beaker. Therefore, actual amount of ultrasonic energy going into reaction medium is far lower (1 - 5 [W/cm^2]) [8]. But this phenomenon of US reflection hasn't been discussed in previous studies. Reflection of US can be minimized by using a beaker/flask made from a material having impedance similar/close to the fluid used in ultrasonic bath. Povedano et al [10] have said that non reproducible performance of US cleaning baths and the decline of power with the working time should be taken into account while discussing the results. However, this problem has not been tested or discussed in any of the published results.

The second type of ultrasonic reactor used is the ultrasonic probe system. Unlike ultrasonic cleaning bath the ultrasonic probe (Figure 1.2) allows acoustic energy to be introduced directly into the reaction medium which eliminates the reflection of ultrasonic waves happening in case of ultrasonic bath. The intensity of such systems is controllable and the maximum can be several hundred W/m^2. The probe system is more expensive than the bath. It can be slightly less convenient in use as special seals are needed if the horn is to be used in reactions which involve reflux, inert atmospheres or pressures above (or below) ambient [8]. Kadkhodaee et al have shown that the effect of the sonotrode tip on the reaction rate is dependent on the reaction site and can increase the rate of the reactions in which the reactants are volatile enough to diffuse into the bubbles [11]. Work reported by Lin et al concludes that under probe-ultrasonic conditions, stereoselectivities decrease for porcine pancreatic lipase (PPL) catalyzed hydrolysis of (R)-1, 2, 3, 4-tetrahydro-1-naphthylbutyrate racemate [12].

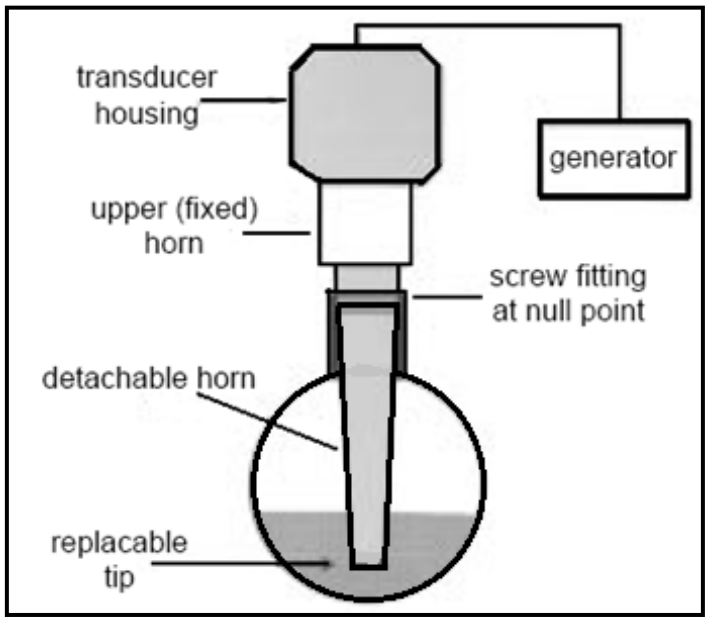


Figure 1.2. Ultrasonic probe systems in sonochemistry [8]

Yasuda et.al [13] has compared the intensification for probe/horn and cup horn type experimental arrangements (Figure 1.3). Cup horn type arrangements resembles ultrasonic bath. Only difference being that in cup horn arrangements reaction contents are sonicated directly. They have shown that probe horn type arrangements are more effective than cup horn type but there has been no discussion about the reasons for this difference in performance.

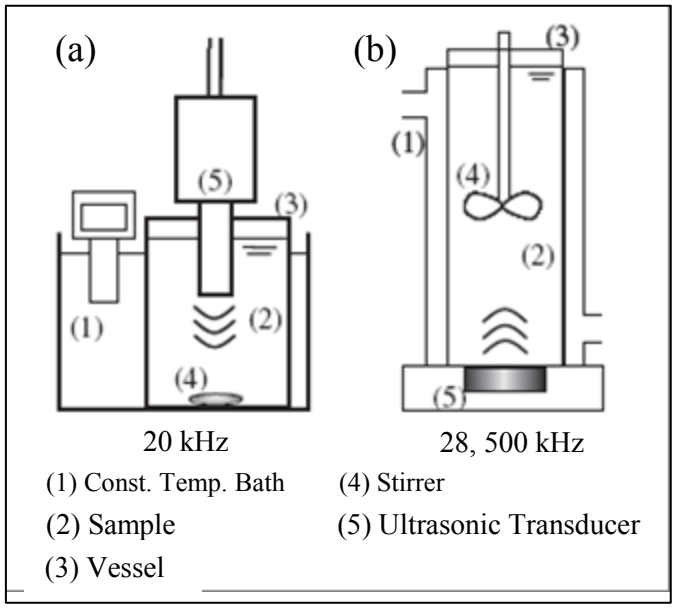


Figure 1.3. Experimental setup used by Yasuda a) probe horn type, b) cup horn type [13]

Besides ultrasonic cleaning bath and probe system there are some examples of specialized arrangements as well. The experimental setup used by Chetverikova et al [14] for studying the influence of ultrasound on intensification of four different enzymes (creatine kinase, lactate dehydrogenase, hexokinase, and pyruvate kinase) is shown in Figure 1.4. The sample chamber consists of an open plastic trough having a cubical lower portion (2 x 2 x 2 cm) and a conical upper portion. The pH electrodes are positioned in conical portion to avoid their protrusion into the acoustic beam. The ultrasonic field is applied by a transducer externally and sample is positioned in the near field of transducer. The sound beam after passing through the sample is reflected into a rubber absorber. Ultrasound at a frequency of 0.88 MHz was generated by means of a commercially available physiotherapy device (UZT-102) and ultrasonic intensity was varied between 0.1 and 1 W/cm^2. Due to applied frequency and intensity it is unlikely that cavitation will have been produced in reaction medium.

Sakakibara et al carried out hydrolysis of sucrose using invertase in an arrangement shown in Figure 1.5. The reactions were carried out in a 400 ml cylindrical glass reactor (7 cm in diameter, 11 cm in height). The sonicator (Type US-150V; Cho-onpa Kogyo Co. Ltd. Japan) consisted of an ultrasonic generator and a barium titanate transducer (6 cm in diameter). A transducer at 815 ± 5 kHz was used for all experiments. To decrease power loss and reflection of the ultrasonic beam, the transducer was positioned near bottom of the reactor, and the bottom was made from 10 μm thick polyethylene film in order to minimize reflection of US.

The reactor and transducer were immersed in a temperature-controlled water bath, the temperature of which was kept at 25 ± 0.01^{o}C [15]. Here reaction was sonicated indirectly.

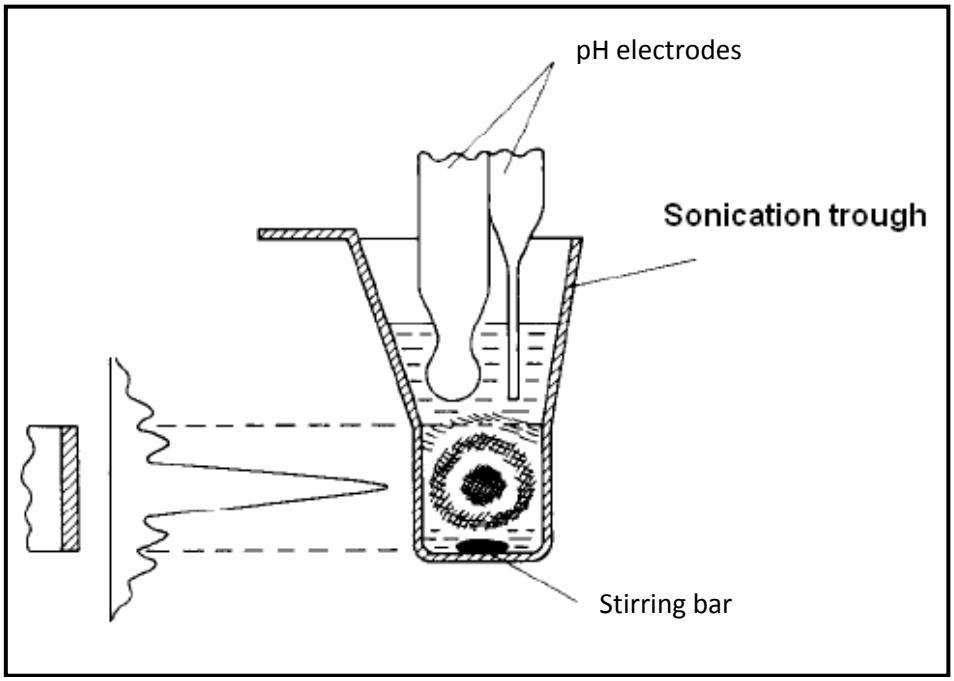


Figure 1.4. Experimental setup used by Chetverikova et al [14]

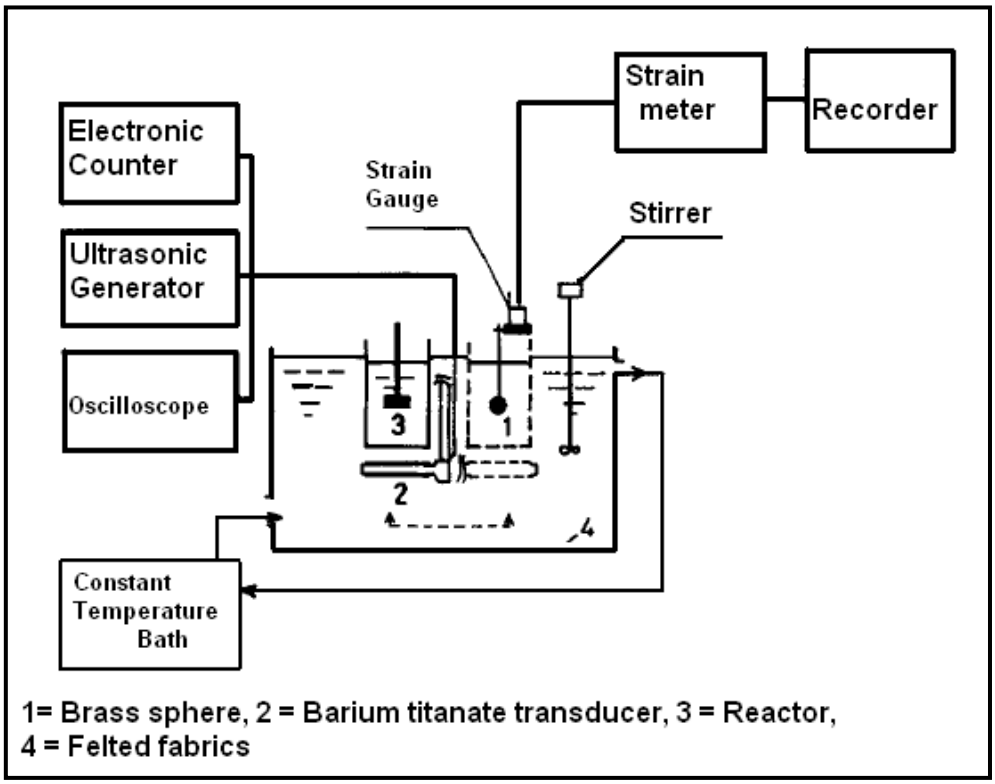


Figure 1.5. Experimental setup used by Sakakibara et al [15]

Figure 1.6 shows the experimental arrangement used by Schmidt et al [16] for studying the effects of ultrasound on intensification of immobilized Glucoamylase using starch and maltose as substrates. It consists of a cylindrically shaped polymethacrylate flow cuvette of ≈ 1 ml volume faced by two 36 μm polyester sheets (2) as acoustically transparent windows. The carrier material was held in place by plugs of glass wool and formed a loosely packed bed ≈ 4 mm thick in the reactor volume (1), the packing fraction being high enough to prevent settling or motion of the beads in the acoustic field. The cell was immersed in a thermostated water bath kept at 37±0.2°C. The temperature within the cell near the outflow was monitored by means of a thermistor (5). The sound field of 7.6 MHz and 5 $kW/m^2$was produced by five circular ceramic transducers, each 20 mm in diameter and having a different resonant frequency. The transducer surface was positioned parallel to the sound windows at a distance of 20 mm. A two component silicone rubber absorber was arranged behind the cell to avoid standing waves. A hydrophone was positioned outside the acoustic beam but near the cuvette and connected to a selective micro voltmeter. No cavitation was observed owing to very high frequency and lower amplitude.

In conclusion it can be said that for an ultrasonic reactor it is important to specify if the applied US caused cavitation or not. The results obtained with a given ultrasonic reactor should be interpreted accordingly.

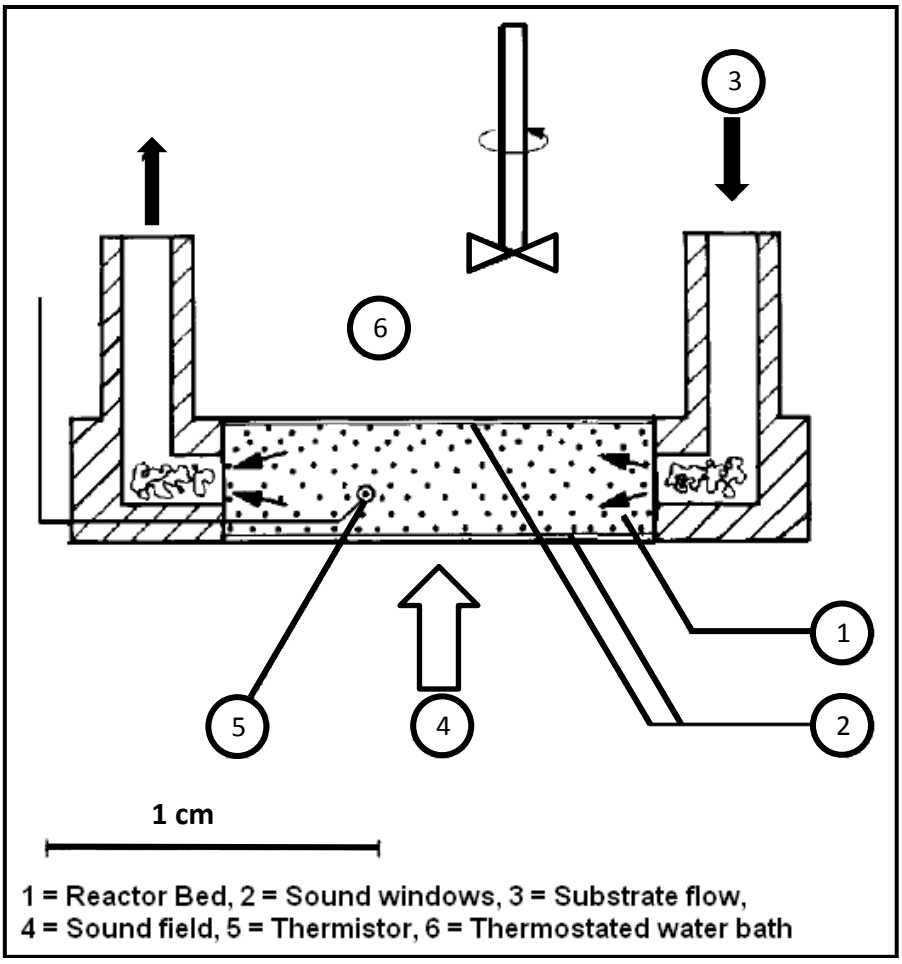


Figure 1.6. Experimental setup used by Schmidt et al [16]

1.1.2 Effect of Ultrasound on Enzyme Catalyzed Reactions

In order to study the intensification of enzyme catalyzed reactions from US a variety of reaction systems have been employed. Intensification from US has been reported for both free as well as immobilized forms of enzyme. Comparison of reaction rate with free and immobilized enzymes in Table 1.1 shows that intensification effect of US on reactions catalyzed with free enzymes is more pronounced than immobilized. Exception is the case of Subhedar [17], where use of US gave 7 fold faster reaction rates. The probable explanation can be that in the studied reaction there involved phase transfer resistance, which was reduced by use of US. So this 7 fold improvement in reaction rate cannot be attributed to the improvement of enzyme activity. This points to the fact that while studying the intensification for multiphase reaction systems it is important to identify the significance of mass transfer limitation. Otherwise, it is difficult to identify

if the observed improvement is coming from enhanced mass transfer rate or improved enzyme activity only or both. Enhanced reaction rate with immobilized enzymes has been attributed to improvement of mass transfer. Although in free enzymes there are no influences of mass transfer (arising from immobilization) but intensification effect is still observed.

Ishimori has measured intensification of 20% with free enzyme but has not given an explanation of it. Schmidt et.al [16] are of the opinion that this intensification with free enzymes can be an increased rate of dissociation of the multimeric enzyme into its more active monomers in the presence of US [6]. Lin has not clearly mentioned that whether the system used was single phase or multiphase. However, he is also of the opinion that the enhancement is probably the result of the increase in usable surface area for catalysis. However, this aspect needs a further discussion looking to its immense importance. In the following section a detailed discussion is being done which involves:

- Mechanism involved in ultrasonic intensification of enzyme catalyzed reactions
- Influence of operating parameters
- Denaturing/deactivation of enzymes due to US

Table 1.1 Comparison of the intensification obtained with different enzymes as reported in the literature

Year/Author	Reaction System					Equipment	Ultrasound		Results
	Substrate	Enzyme	Temp [°C]	pH	Reaction Volume		Frequency	Intensity	
1981/Ishimori et al. [7]	Casein	α-Chymotrypsin	35	8.0	2 ml	US-Bath	20 kHz	10-15 W	200% * [Im] 20% * [F]
	Acetyl tyrosine Ethyl Ester [ATEE]		35		2.5 ml	US-Bath			No effect [Im]
1986/Schmidt et al. [16]	Starch Hydrolysis	Glucoamylase	37±0.2	4.5	1 ml	Figure 1.6	7.6 MHz	5 K W m $^{-2}$	300% * [Im]
	Maltose Hydrolysis		37±0.2	4.5	1 ml				200% * [Im]
1995/Lin et al. [12]	Hydrolysis of 1,2,3,4-tetrahydro-1-naphthyl butyrate (racemate-1) with water	Porcine pancreatic lipase (PPL)	33	NA	NA	US-Bath	NA	375 W	7 fold [F]
	Acylation of 1,2,3,4-tetrahydro-1-naphthyl butyrate (racemate-2) with vinyl acetate		33	NA	NA				83 fold [F]
2006/Talukder [18]	hydrolysis of olive oil in isooctane	Chromobacterium viscosum lipase	25	8.0	5.002 ml	US-Bath	25.8-36.5 kHz	106 W	175% * [F]
2008/Lee [19]	Synthesis of sugar ester using ionic liquids	Novozym 435	40	NA	NA	US-Bath	47 kHz	185 W	260% * [Im]

* Relative to the reaction rate in absence of ultrasound

[Im] Immobilized Enzyme -:- [F] Free enzyme

NA = Not available

Table 1.1(continued) **Comparison of the intensification obtained with different enzymes as reported in the literature**

Year/Author	Reaction System					Equipment	Ultrasound		Results
	Substrate	Enzyme	Temp [°C]	pH	Reaction Volume		Frequency	Intensity	
2014/ Jadhav et al. [20]	Tributyrin Hydrolysis	CALB L (Lipase)	25	7	100	Sonic probe	20 kHz	12.22 W/cm^2	2 fold * [Im]
2015/Bhasarkar et al. [21]	Dibenzothiophene	Horseradish peroxidase	35	7	20 ml	US-Bath	35 kHz	0.78W/cm^{-2}	2 fold * [F]
2015/Singh et al. [22]	Glucose	Novozyme 188	33	NA	25	US-Bath	35 kHz	1.48W/cm^{-2}	4 fold [F]
2015/Subhedar et al. [17]	lignocellulosic biomass (Newspaper)	Cellulase	30	NA	50 ml	US-Bath	25kHz	200 W	7 fold * [Im]
2015/Tomke et al. [23]	Cinnamyl alcohol	Novozym 435	40	NA	15 ml	US-Bath	25,40kHz	0.81 W/cm^2	7 fold * [Im]

* Relative to the reaction rate in absence of ultrasound

[Im] Immobilized Enzyme -:- [F] Free enzyme

NA = Not available

1.1.3 Mechanisms Involved in Ultrasonic intensification of Enzyme Catalyzed Reactions

Very little is known about the actual effects of ultrasound in enzyme catalyzed reactions because contradictory results of activation/inactivation of enzyme upon ultrasonication have been reported [24]. The main factors possibly contributing for such an intensification can be categorized as

- **Thermal factor**: due to the enormous temperatures achieved from cavitation
- **Mechanical factor:** (shear forces) created by micro-streaming and shock wave
- **Chemical factor:** due to free radicals generated by sonolysis.

Each of these factors is being discussed in the following paragraphs individually

a) Thermal factor

With regard to the thermal effects nearly everyone is agreed that such effects do not have a major contribution towards the overall intensification effect. The thermal effects can be subdivided into two categories i.e. bulk temperature rise of the reaction medium (due to dissipation of ultrasonic energy) and temperature rise at micro level because of the imploding cavitation bubbles. In the results presented in Table 1.1 a constant temperature was maintained during sonication but the positive effects of ultrasound on reaction rate were still observable. Therefore, bulk temperature increase cannot be regarded as the main cause of observed intensification. Bulk increase in temperature will decrease cavitation threshold (minimum amount of acoustic pressure required to produce cavitation). However, at higher temperature the rise in vapor pressure of the solvent trapped inside the cavitation bubble will provide the cushioning effect during implosion of cavitation bubbles. This will make the implosion effect milder. The localized temperature rise resulting from cavitation is said to be around 5000 K [10]. But the fast cooling in the order of 10^{10} K/s (<100 ns) [25] gives an indication that the contribution of such an effect is unlikely. Lin et al [12] have also excluded the effect of localized temperature rise by saying that higher temperatures, if prevail, would only result in the denaturation of enzymes as enzymes cannot “survive” at such high temperatures.

b) Mechanical factor

Enhancement of mass transport processes has mostly been agreed to be the major cause of the intensification resulting from ultrasonication. Ishimori et al [7] have concluded that ultrasound can be used for the intensification of reactions with immobilized enzyme where the diffusion of substrate into carrier is a rate determining step. They employed free and immobilized α-Chymotrypsin for casein substrate under ultrasonic radiation (20 kHz, 10-15 W). Intensification effect was observed, however, this effect was more pronounced for immobilized enzyme. For immobilized enzyme with US reaction was 2 - 2.2 times faster than with magnetic stirring. They attributed this faster reaction rate to increased diffusion of substrate through carrier. Same immobilized enzyme systems did not show any activity enhancement for ATEE substrate (N-acetyl-L-tyrosine ethyl ester). Therefore, they concluded that diffusion of substrate through carrier was not a rate determining step in second case. However, they did not investigate diffusion limitation. Intensification effect was observed with free enzyme also. They were of the opinion that for free enzyme the enhancement of reaction rate by US may be due to the increase in collisions between enzyme and substrate [18]. However, looking the larger wavelengths of US used (20 kHz) in comparison to size of enzyme molecule (nano meters) it is not possible that there can be a direct interaction [14].

Schmidt et al. [26] have also made similar observations. The system employed was hydrolysis of starch and maltose using immobilized Glucoamylase as enzyme (0.15-0.2 mm and 0.6 - 0.8 mm). Ultrasound applied had frequency 7.6 MHz. Equipment used by Schmidt is shown in Figure 1.6. The intensification effect was observed and there are two aspects of this. Intensification was higher for larger substrate molecules (starch) compared to smaller substrate molecules (maltose). Similarly for larger carrier particles (0.6-0.8mm) intensification effect was higher when compared with smaller ones. This was explained on the basis of increased mass transport resulting from sonication. They excluded the possibility that these improved results are because of the structural alteration at the enzyme and carrier level. The velocity of sound in water is approximately 1500 m/s, the corresponding acoustic wave lengths are about l0 to 0.01 cm (1-11 MHz frequency), so there is no direct coupling of the acoustic field with enzyme molecules [27]. Chetverikova et al [14] are also of the opinion that a direct

interaction between ultrasound and the catalytic functioning of individual enzyme molecules is unlikely to be the primary step in any acousto-biological interaction, and that this primary interaction appears to be occurring at a higher level of organizational complexity [14].

Contradictory to this Bhasarkar and Jadhav are of the opinion that US does cause a conformational change at enzyme level. Bhasarkar [21] investigated the influence of US on intensification of horseradish peroxidase for desulfurization of liquid fuels. Ultrasonic bath with 35 kHz frequency and 35 W power input was used. They stated that intense micro-convection generated by cavitation bubbles in the form of micro-turbulence velocity and shock waves cause conformational changes and unfolding of the secondary structure of the enzyme molecule, which leads to faster reaction rate. Bhasarkar stated that in absence of cavitation microstreaming produced by US causes a conformational change of enzyme structure. Jadhave and Gogate [20] studied the effect of US on intensification with immobilized Lipase (CALB L) for hydrolysis of tributyrin at 20 kHz and 200 W. They are also of the opinion that a change in the structural conformation of the enzyme as a result of sonication is responsible for observed intensification effect. A part of this conformational change is permanent i.e. enzyme does not go back to its native structure once US is turned off. This means sonicated enzyme should retain a part of its enhanced activity. Unlike Jadhav, Frydenberg et.al [20, 28] observed that there was no change in secondary structure of enzyme before and after sonication. They also observed that enzyme with higher content of β-sheets is more stable in US. A similar conclusion was made by Chetverikova [14].

Summing up the discussion regarding significance of mechanical factor it can be concluded that in the literature a variety of reactions have been studied. Moreover the type of enzymes studied is also diverse which are employed in both immobilized as well as free forms. This is further complicated by the use of varying parameter settings from different writers. Therefore, it is difficult to determine if the observed intensification is only mass transfer related or change of enzyme structure also plays role.

c) Chemical factor

Ultrasonic cavitation has been shown to produce radicals in solution for example hydroxyl and hydroperoxyl groups in water as a result of thermal dissociation of vapor molecules. It was thought that the produced radicals might be responsible for intensification phenomenon. However, Bhasarkar [21] stated that radical produced during cavitation may interact directly with the substrate molecules to produce the reaction intermediate, which ultimately speeds up the reaction. According to them the produced radicals do not activate enzyme molecules as such by direct interaction. It is concluded that direct interaction of produced radicals with enzyme will only cause the denaturing of enzyme molecules [12, 27, 29, 30]. Mechanism of enzyme denaturing as a result of radicals produced from cavitation will be discussed in detail in section1.1.5.

1.1.4 Influence of Operating Parameters

The important parameters that can influence the intensification phenomenon in enzyme catalyzed reactions are power and frequency of applied ultrasound, temperature of the reaction medium and reactor volume. The optimum values of these parameters are system specific. A good example can be the proteolytic reaction studied by Ishimori et al [7]. With increase of ultrasonic power up to 10W reaction rate increased. An increase beyond 10 W caused a gradual decrease of rate of reaction. At 20W reaction was slower than the one measured in absence of ultrasound. This was explained on the basis of enzyme partial denaturation due to higher ultrasound intensity. Talukder et al [18] made similar observations where hydrolysis of olive oil catalyzed by chromo bacterium viscosum lipase (EC 3.1.1.3) in a water/isooctane two-phase system was carried out. The reaction was carried out in 20 ml glass veil and the reaction volume was 5 ml. Figure 1.7 shows the comparison of reaction rate for stirred and sonicated systems at 25^{o}C. Over 181 W or below 40 W the reaction rate was slower than those in the stirred system. At an optimal power of 106 W reaction rate was respectively 175% and 190% of that in stirred systems. Another explanation for this lowering of reaction rate at higher power inputs is given by Kadkhodaee [11]. Kadkhodaee states that the rise of acoustic intensity increases the density of the cloud of bubbles in the vicinity of the emitter which can reduce the amount of ultrasonic energy transmitted into medium thereby reducing the ultrasonic effect. Therefore, slower reaction rate from higher ultrasonic powers cannot be attributed to inactivation of enzymes only.

Khan [31] and Tomke [23] studied the effect of 25 kHz and 40 kHz frequency on reaction catalyzed by Lipozyme-435 (immobilized). They observed that at lower frequency intensification effect is higher. Tomke attributed this to formation of smaller and less violent bubbles at higher frequency. They reported that enzyme lost 6% of its activity after 8 times use. They attributed this loss of activity to detachment of small amount of enzyme during filtering and washing but not US. However, it is surprising that stronger cavitation at lower frequency did not cause any damage to the enzyme carrier particles. Since they used glass beaker as reactor therefore, it is likely that the cavitation was not produced in the reaction medium.

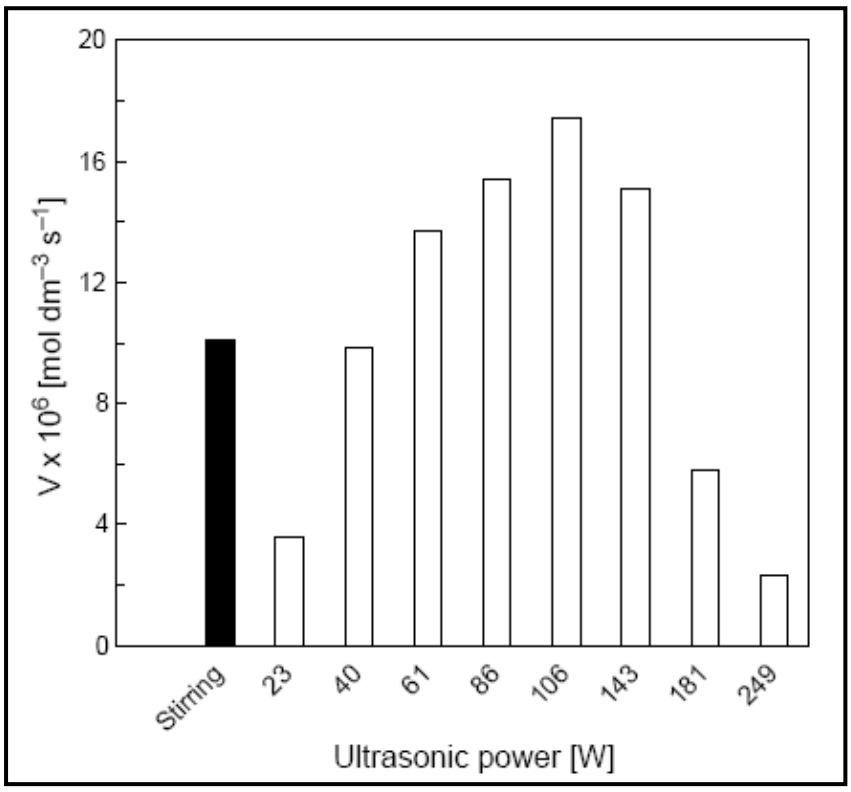


Figure 1.7. Effect of ultrasonic power on enzyme reaction rate at 25^{o}C [18]

A recent review by Povedano [10] has concluded that role of frequency has been so far poorly described. An example in this regard can be the dependency of frequency and amplitude i.e. by varying frequency amplitude also varies. An important point raised by Povedano is that especial care should be devoted to the nomenclature related to US and the way to express the variables and parameters involved in this type of energy.

Summarizing it can be said that while interpreting the results obtained at varying parameters of US their interdependence should be considered. An important aspect in this regard can be that how the variation of US related parameters effect the cavitation phenomenon which is likely to play a major role in defining the behavior of ultrasonic reactor. Further, it also needs to be investigated if the decrease in performance at higher intensities is related to enzyme deactivation or a result of decrease in ultrasonic energy going into reaction medium.

1.1.5 Denaturing/Deactivation of Enzymes from US

In the reviewed publications no discussion was found regarding the detachment of enzymes from carrier material due to sonication. Ishimori et al have demonstrated that for immobilized enzymes the functionality was hardly affected during repeated use of four times (Figure 1.8) [7]. However, the activity of free enzymes decreased. With regard to the effect of irradiation duration Sakakibara et al [15] have shown important observations. 250 ml of sucrose solution was hydrolyzed using 0.1 mg invertase. A 15% inactivation of the enzyme after sonication time of 4 h was observed at the maximum intensity applied (8.5×10^3 W/m^2), whereas only a negligible loss of activity occurred at low intensity.

Dunn and Macleod [32] examined effects of non-cavitating ultrasound on five selected enzymes (free enzyme) α-chymotrypsin, trypsin, aldolase, lactate dehydrogenase, and ribonuclease in aqueous solution. The applied ultrasound was at levels sufficient to cause extensive structural and functional damage in tissues (75W/cm^2 - 1000 W/cm^2 at 1 – 27 MHz). In one set of experiments enzyme solutions were irradiated using US and then analyzed for any changes in their structure/activity. In second set of experiments the enzyme catalyzed reactions were continuously irradiated with US and simultaneously monitored spectrophotometrically to observe any changes in structure. In both cases no negative effects of US were found on tested enzymes. Since for the same enzymes there had been reports in the literature that they got denatured by a cavitating ultrasound. From this they concluded that the inactivation of enzymes is not due to direct interaction of ultrasound with enzymes but it is the cavitation that causes damage to the enzymes. Therefore, they suggested that cavitation is a necessary condition for ultrasonic denaturation of these five enzymes.

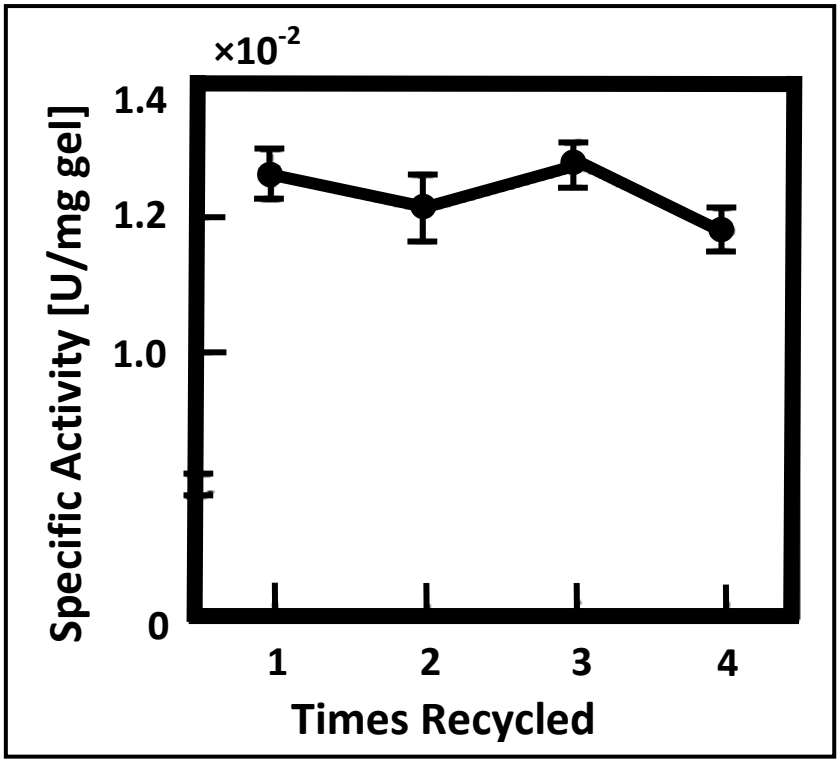


Figure 1.8. Reusability of enzyme after ultrasonication [7]

Cavitation can cause enzyme inactivation through the following three mechanisms, which can act alone or combined [30].

- **Thermal deactivation mechanism:** The first one is purely thermal due to the enormous temperatures achieved during cavitation. However, as discussed earlier such effects don't prevail due to rapid dissipation of heat (10^{10} K/s).

- **Mechanical deactivation mechanism:** Second one is due to the mechanical shear forces created by micro streaming and shock waves. In Figure 1.9 it is shown that when cavitation bubbles are formed at or near to solid surface the bubble collapse will be asymmetrical. As a result of this a liquid jet will be formed targeted at the surface with speeds in excess of 100 m/s [8]. The mechanical effect of this is equivalent to high pressure jetting and is the reason why ultrasound is so effective in cleaning. Patidar et al are also of the opinion that shock wave generated by cavitation bubbles effect the enzyme adversely [33]. Kadkhodaee [11] is of the opinion that free radicals

and the shear forces arising from pulsation and collapse of bubbles are the main causes of protein denaturation and enzyme inactivation.

- **Chemical deactivation mechanism:** The third is due to the formation of free radicals (such as OH^-) by sonolysis. The penetration of these radicals in an enzyme active center results in the enzyme being inactivated due to destruction of certain functional groups important for catalytic activity [29]. Gogate et al have also reported that cavitation produces hydroxyl and hydrogen radicals by virtue of the pyrolysis of water. The observed behavior can be attributed to the reaction of hydroxyl or hydrogen radicals formed during ultrasonication with the protein backbone, which is a probable reason for protein denaturation. This phenomenon can subsequently lead to enzyme aggregation, thus obstructing the active sites and at the same time decreasing protein stability [20]. Riesz et al [26] have shown that inactivation of alcohol dehydrogenase and lysozyme by ultrasonic cavitation at 20 kHz decreased with increasing protein concentration and was markedly inhibited by 2-mercaptoethanol, which appeared to act as a scavenger of free radicals or as a sulfhydryl protective reagent.

Above mentioned effects may act alone or in combination.

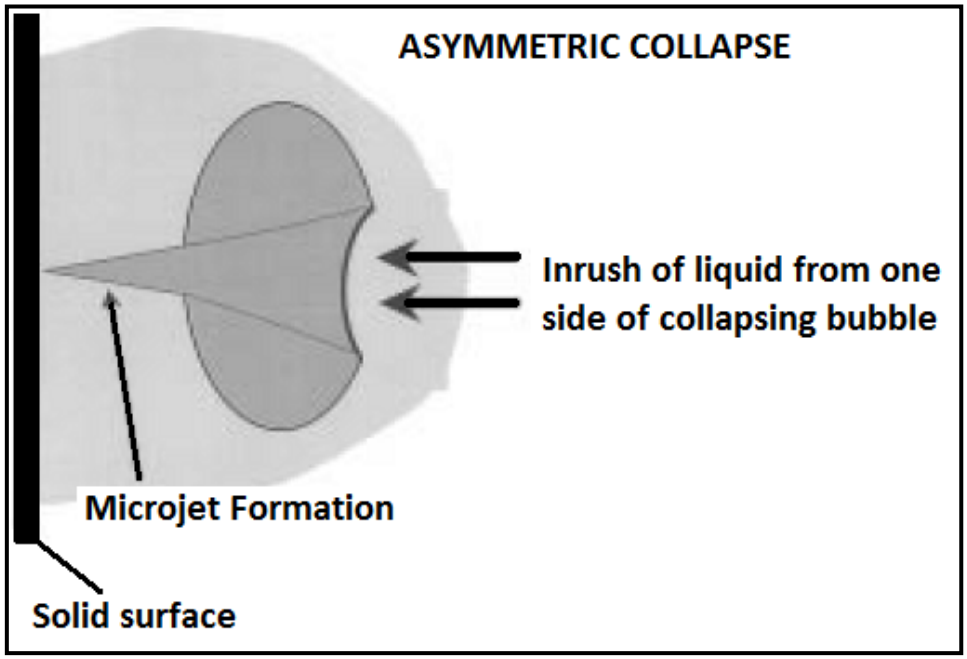


Figure 1.9. Asymmetric collapse of cavitating bubble near solid surface [8]

1.1.6 Modeling Studies

Modeling of an enzyme catalyzed reaction is an important aspect as it can greatly facilitate in analyzing the performance of newly proposed ultrasonic reactors before going into experimental phase. There have been very few attempts regarding the development of a complete model for enzyme catalyzed ultrasonic reactors such as Sener [34]. Sener has studied the galactosidase catalyzed hydrolysis of milk lactose. 20W was found to be the optimum power input to the system. To predict the effect of processing time on lactose hydrolysis under ultrasonic irradiation, the data of residual lactose concentration versus processing time at acoustic power of 20 W were evaluated, by a zero order kinetic expression Eq. 1.3.

$$\frac{d[C_L]}{dt} = -k \qquad (C_L) = (C_{L0} - kt) \tag{1.3}$$

Inactivation of enzyme as a function of time for 20W applied power was expressed by the following equation

$$A = (100 - \alpha)\ \exp(-k_D t) + \alpha \tag{1.4}$$

The effect of ultrasonic power on residual lactose concentration and enzyme activity was evaluated by the following equations.

$$\begin{aligned} &\frac{d(C_L)}{dP} = -k_{Pc}, \qquad (C_L) = (C_L)_{(P_{US}=20W)} + k_{Pc}P_{US}) \\ &\frac{d(A)}{dP} = -k_{Pa}, \qquad (A) = (A)_{(P_{US}=20W)} - k_{Pa}P_{US} \end{aligned} \tag{1.5}$$

where

(C_L), (C_{L0}) = g Lactose L^{-1}

k, k_D = min^{-1}

k_{Pc} = g Lactose L^{-1} W^{-1}

k_{Pa} = W^{-1}

Looking the simplified modeling approach adopted by Sener it is obvious that such a model has very limited scope of application it cannot be used for understanding the behavior of an ultrasonic reactor as it does not account for distribution of ultrasonic field inside reaction medium. Moreover it also does not consider that how the applied US interacts with the enzyme carrier particles. Therefore, there is need for developing a mathematical model of an ultrasonic reactor.

In conclusion it can be said that the use of US for intensification of enzyme catalyzed reactions can have advantages but the published research has merely focused on certain aspects. It is not systematic and lacks process engineering perspective. For immobilized enzymes intensification effect has been attributed to the improvement of mass transfer but there has been no further investigation regarding quantification of the mass transfer resistance in studied systems. Therefore, it is very difficult to attribute the observed improvement to enhancement of mass transfer only. This aspect needs to be investigated in detail as it is vital in understanding of the phenomenon. On the other hand, in free enzymes there are no mass transfer related effects (arising from immobilization) but intensification phenomenon is still observed. This leads to the fact that intensification effect is not mass transfer related only. Intensification with free enzymes has been attributed to the conformational changes in the secondary structure of enzyme from US. But there are also reports claiming that a direct interaction of US with enzyme molecules isn't possible as the used US has much larger wavelength than size of enzyme molecule. Therefore, there is confusion regarding effects of US on structure of enzyme. Moreover it is also not clear if the change in structure of enzyme is permanent or reversible i.e. does enzyme restore its native structure once sonication is turned off. As different enzymes have been used with different reaction systems and ultrasonic reactors, therefore, it is difficult to compare the obtained results. It is also important to clarify the role of non-cavitating and cavitating US.

Due to these ambiguities there is not clarity about the real mechanism of the intensification phenomenon observed with US. Therefore, it is difficult to determine that under what conditions use of US will be effective. To fill afore mentioned gaps and to enhance the understanding of underlying phenomenon a research concept is developed. According to this concept intensification effect of US on reactions involving enzymes is to be investigated in a detailed and systematic manner.

1.2 Research Objectives

In context of present study, intensification of enzyme catalyzed reactions using US is to be studied with an objective for industrial scale realization of the phenomenon. To achieve this objective it is necessary to determine that whether the intensification observed with US is a result of improved enzyme activity or only the enhancement of mass transfer/mixing or both. Moreover, it is also important to understand any possibility of direct interaction of US with enzyme molecules. When US is applied to a reaction medium it may or may not cause cavitation i.e. it is possible to sonicate the medium both under non-cavitating and cavitating conditions. It is obvious that cavitation produces its special effects (hot spots) which cannot be observed under non-cavitating sonication. Therefore, it is critical to study that what type of US can produce intensification effects i.e. non-cavitating and cavitating. With this information it will be possible to determine the conditions necessary for observing the intensification effect. To efficiently utilize the ultrasonic energy in reactor it is also important to study the effect of relevant influencing parameters. Post sonication stability of enzyme also needs to be tested. Once this phenomenon is understood then in the next step a concept for large scale realization of the phenomenon needs to be developed.

1.3 Research Methodology

To achieve afore mentioned objectives a structured research concept is developed. Looking the research objectives activities are classified into two main parts. The two main parts are

- Fundamental understanding of the phenomenon
- Development of a concept for large scale processing.

Each part consists of a number of steps which are organized in a manner that output of one step forms the basis for the next step as depicted in Figure 1.10. First step in gaining a fundamental understanding of the phenomenon is the selection of example enzyme and reaction system. For this selection it is necessary that the selected enzyme/reaction is important from industrial view point. The gained knowledge thus will be beneficial not only for new processes but also for existing processes. Since experiments are to be done in lab,

therefore, it is equally important that the substances involved in selected reaction should be of nontoxic and nonflammable nature in order to avoid the requirements of special safety arrangements in lab. As mentioned in introduction, immobilized form of enzymes is more convenient in use as enzymes can be easily separated from product once the reaction is completed. In this way it is possible to reuse them for multiple production cycles. In this context main motive here is to look for intensification potential with immobilized enzymes. However, to understand the underlying mechanism intensification tests should be made with both free and immobilized enzymes. For this it is necessary that in both cases enzyme is of same origin so that results are directly comparable. According to Figure 1.10 intensification studies need to be done with stirring, non-cavitating and cavitating US. After fundamental understanding comes the large scale application of the phenomenon where a concept for industrial use needs to be developed and tested.

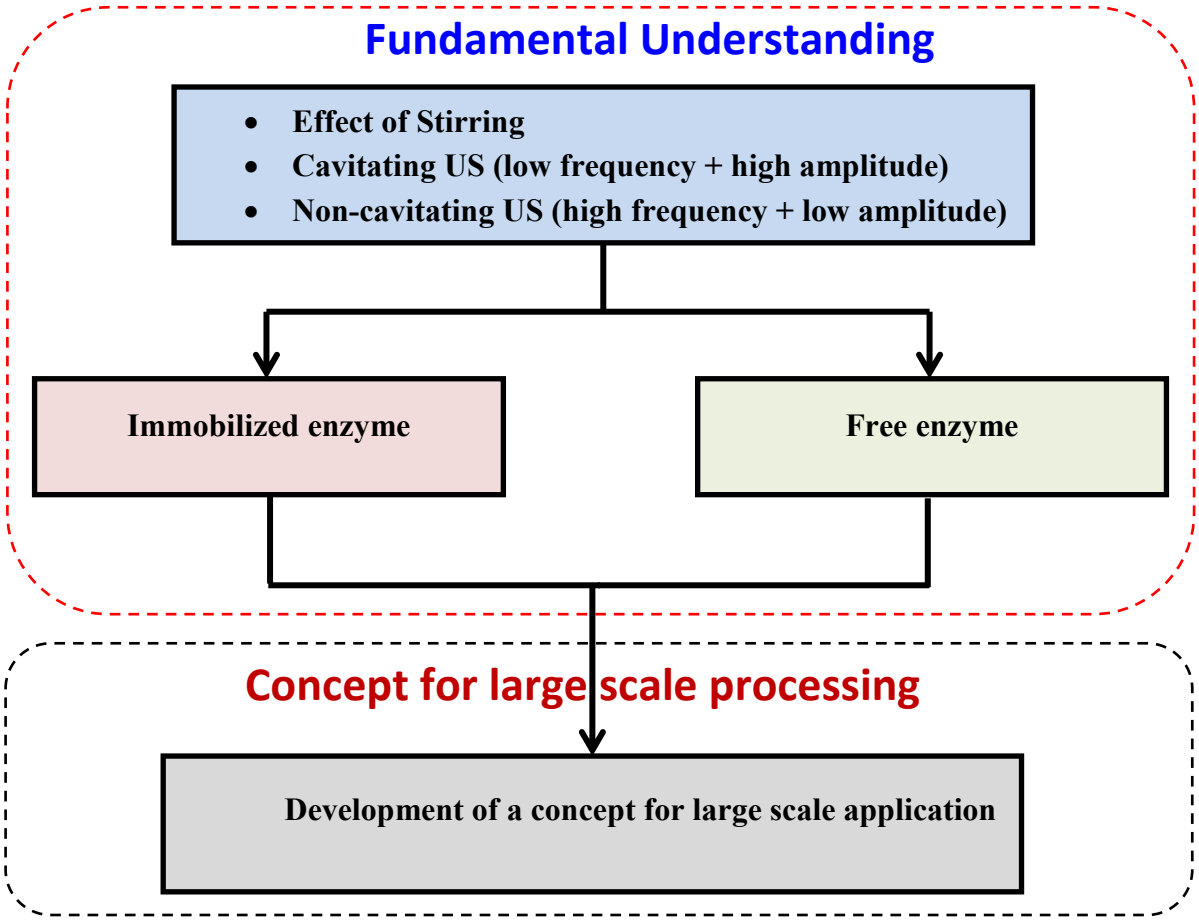


Figure 1.10. Schematic representation of the research steps

In line with previous discussion Lipozyme-435 (immobilized enzyme) and Lipozyme CALB L (free enzyme) are selected as example enzyme. In Lipozyme-435, Lipozyme CALB L is present in immobilized form. This means in both cases selected enzyme is of same origin and therefore, the results obtained are directly comparable. As esterification has continued to be of central importance in both organic synthesis and industry [35] therefore, esterification of oleic acid with n-hexanol is selected as example reaction. Substances involved are nontoxic and nonflammable under experimental conditions used in lab.

In part 1 of research methodology (Figure 1.10) emphasis is on the fundamental understanding of the phenomenon. For fundamental understanding it is necessary to investigate and compare the effect of stirring, non-cavitating and cavitating US on selected reaction. Two different reactors are employed for this purpose. In both of these reactors US is directly introduced into reaction medium so that any disadvantages arising from indirect sonication of reaction (Figure 1.1) are eliminated. One reactor is capable of operating at higher frequencies (206 and 616 kHz) and low power input and therefore, does not produce cavitation in reaction medium. The second reactor is capable of operating at low frequency (24 kHz) and high power input and therefore, produces cavitation. For stirring experiments magnetic stirring was used. Since prime interest is in exploring the possibility of intensification with immobilized enzymes this type of enzymes are tested first. However, to completely understand the involved mechanism tests with free enzymes are also done.

Once the mechanism of intensification from US is understood then in part 2 a reactor concept for large scale application of the phenomenon is tested. While developing the concept for large scale application it is equally important to look the ways for energy efficient operation of such setups. Reusability of enzyme after sonication cycle also needs to be tested so as to test the economic viability of this new development. Research methodology in terms of involved steps is also given in Table 1.2.

Table 1.2 Research methodology

Fundamental Understanding		**Concept for large scale processing**
Intensification Tests with immobilized enzymes (Lipozyme-435)	**Intensification tests with free enzymes (Lipozyme CALB L)**	**Test of flow through reactor**
Effect of stirring and related parameters Effect of non-cavitating US Effect of cavitating US Concepts for energy efficient operation of the process Stability of enzyme after sonication		Effect of circulation rate on flow through reactor performance
Comparison of intensification tests for immobilized and free enzyme		

It is important to mention that development of a mathematical model is also included as the development of the model can greatly facilitate in predicting and analyzing the performance of an ultrasonic reactor for a given application [36]. This model has two parts. In first part distribution of acoustic pressure field inside reaction medium is simulated. In second part effect of acoustic pressure field on trajectories of enzyme carrier particle is simulated. Hydrodynamic information thus obtained can then be combined with intrinsic kinetics of the reaction to predict reactor performance.

2 Theoretical Background

2.1 Enzyme Catalysis

The Swedish chemist Jöns Jacob Berzelius coined the term catalysis (1835) to describe the property of certain substances to accelerate chemical reactions. To characterize the biological molecules that can catalyze chemical reactions Wilhelm Kühne proposed the term “enzyme” in 1876 which literally means “in yeast”[37]. The nineteen-sixties witnessed two major breakthroughs that had a major impact on the enzyme industry: the commercialization of Glucoamylase which catalyzes the production of glucose from starch with much greater efficiency than that of the chemical procedure of acid hydrolysis, and the launch of the first enzyme-containing detergents [38]. Northrop et al established the proteinaceous nature of enzymes in 1930 [37].

The unique functions of enzymes as catalytically active proteins are a result of their complex three-dimensional structures and the active site integrated therein. This enables a highly specific recognition of specific substrates, leading to excellent selectivities. These unique properties of enzymes to stereo selectively recognize substrates were found by Fischer at the end of the 19th century [39]. Since enzymes are (almost always) proteins; hence the chemically reactive groups that act upon the substrate are derived mainly from the natural amino acids. The identity and arrangement of these amino acids within the enzyme active site define the active site topology with respect to stereochemistry, hydrophobicity, and electrostatic character. Together these properties define what molecules may bind in the active site and undergo catalysis. The active site structure has evolved to bind the substrate molecule in such a way as to induce strains and perturbations that convert the substrate to its transition state structure. This transition state is greatly stabilized when bound to the enzyme; its stability under normal solution conditions is much less. Since attainment of the transition state structure is the main energetic barrier to the progress of any chemical reaction, and the stabilization of the transition state by enzymes results in significant acceleration of the reaction rate [1]. It is well known that enzymes are categorized according to the compounds they act upon [40] such as

Proteases: Break down proteins

Cellulases: Break down cellulose

Amylases: Break down starch into simple sugars

Lipases: Split fats (lipids) into glycerol and fatty acids

The selected example enzyme for present study i.e. CALB L (Candida Antarctica Lipase B) belongs to the lipase category of enzymes. Three dimensional structure of CALB L is shown in Figure 2.2 along with indication of active site [41, 42]. CALB L consists of 317 amino acids and is isolated from the yeast Candida Antarctica (isolated from hyper saline lake in Antarctica). Underlying mechanism for functioning of lipase enzymes is given briefly in the following paragraphs which will be of help in interpreting the experimental data. Lipases can be divided into two general structural classes [38]

- Active site covered by movable lid
- Active site permanently open

Lipases with active sites covered by lid occur in alternate open/close conformational states. In the closed conformation the lid covers the enzyme active site, making it inaccessible to the substrate molecules, whereas transition to the open conformation opens the lid to expose the active site (Figure 2.1). Lids are amphipathic structures: in the closed conformation their hydrophilic side faces the solvent and the hydrophobic face is directed towards the active site. As the enzyme shifts to the open conformation, the hydrophobic face becomes exposed and contributes to the formation of a larger hydrophobic surface and the substrate binding region. Studies by several groups have pointed to the lid as being a major molecular determinant of lipase activity and selectivity [38]. A minimum amount of water is required for the catalytic activity of the lipase i.e. to bring it to open/active conformation. In most cases lipase preparations with residual water content of approximately 1% in anhydrous organic solvents are employed [43].

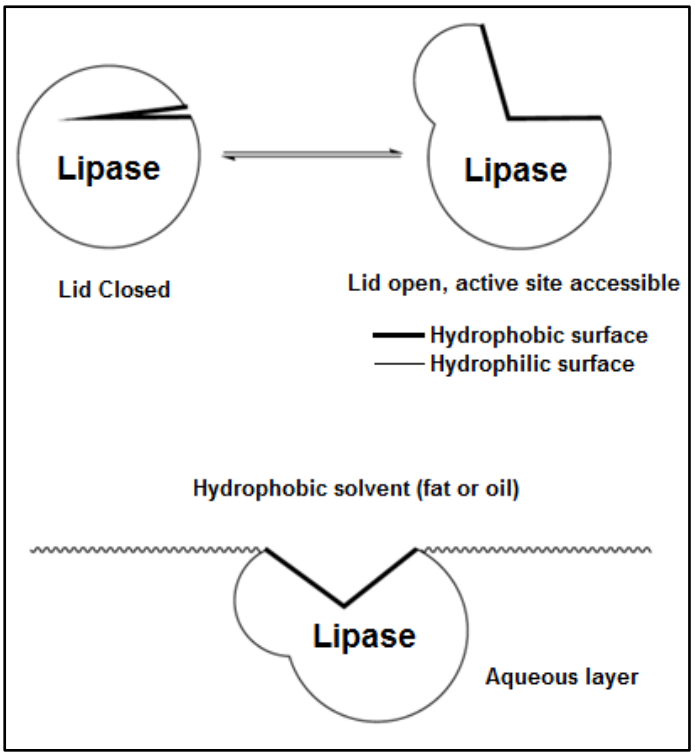


Figure 2.1. Scheme of lipase activation [43]

It is possible to immobilize lipase in an irreversible lid-open conformation and thus active both in aqueous medium and organic solvents [44].

In structural class of lipases with active site permanently open active site is not covered by lid. Properties of CALB L have led scientists to believe that CALB L does not have a real lid covering the active site and hence displays a limited form of interfacial activation. Furthermore, a short helix (α5) in close proximity to the active site has some mobility and probably still allows for some conformational changes [45]. However, there are also reports that CALB L does have a lid on it [46]. Therefore, there are contradictory remarks regarding presence/absence of lid in lipase.

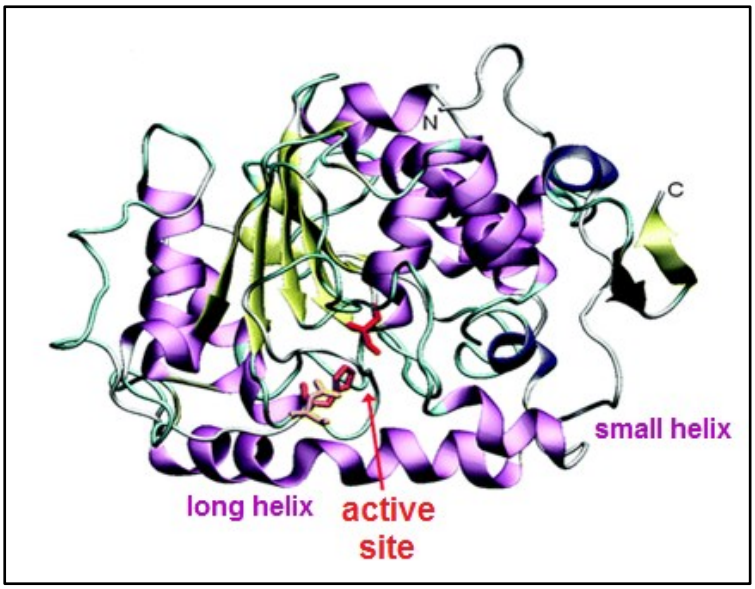


Figure 2.2. Crystal structure of the lipase CALB L [42]

Both free and immobilized forms of CALB L are available from Novozym A/S Denmark. The free form of CALB L is available (Figure 2.3 right) with name "Lipozyme CALB L". Lipozyme CALB L (6%) is dissolved in liquid solution containing mainly water, sorbitol and glycerol with trace amounts of sodium benzoate and potassium sorbate. Sorbitol and glycerol act as stabilizers to prevent enzyme denaturation while benzoate is added to prevent microbial growth [47]. 85% of this 6% protein is catalytically active [41].

The immobilized form of CALB L is available with name Lipozyme-435. In Lipozyme-435 (Figure 2.3 left) CALB L is immobilized on a macro porous week anion-exchange acrylic resin (Lewatit) [38] and has a pore size about 100 nm which is 10 times larger than the size of the CALB L molecule [44]. The particle size of Lipozyme-435 beads is 0.3 - 0.9 mm [3]. Laszlo et.al have reported that Lipozyme-435 has around 10 mass percent of CALB L on it and 35 to 50% of it is catalytically active [41]. Immobilization enables easy handling and separation of enzyme from product for subsequent reuse.

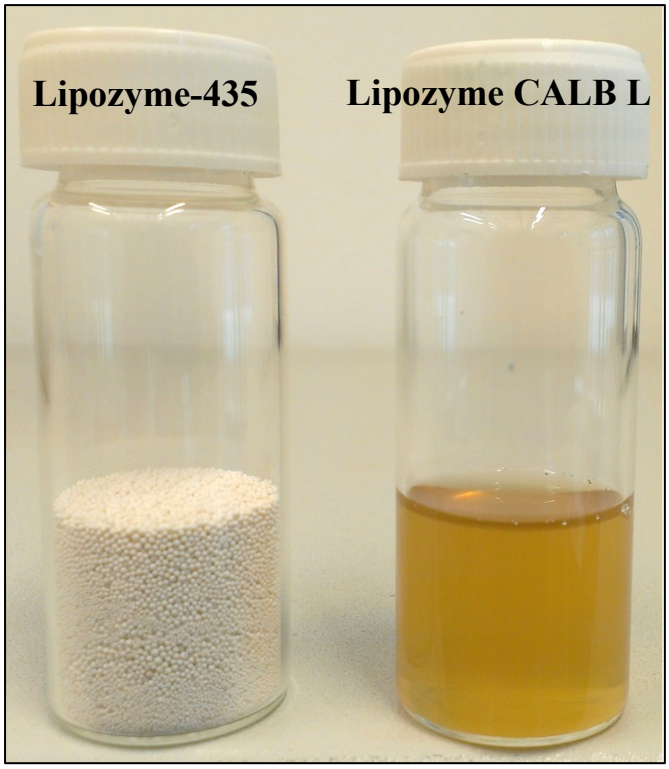


Figure 2.3. Enzyme Samples

2.2 Sonochemistry and Ultrasound

As mentioned earlier sonochemistry deals with short lived, localized field of high pressure and high temperature produced through ultrasonic cavitation [40]. Ultrasound is defined as sound above frequency of 20 kHz, which human beings cannot hear [48]. In order to produce real effects of the sound, energy must be generated within the liquid itself because the transfer of sound energy from air into a liquid is not an efficient process [49]. It is customary to divide ultrasound into two regions [50]

- Power ultrasound (up to 100 kHz)
- Diagnostic ultrasound (1-10 MHz)

Power US is used in sonochemistry. In fact, the range available for sonochemistry has been extended to 2 MHz with the development of high power equipment capable of generating cavitation within liquid systems at these higher frequencies. For the majority of chemists an interest in power US springs from the fact that it provides a form of energy for the modification of chemical reactivity which is different from that normally used e.g. heat, light and pressure [49].

The chemical and physical effects of US cannot result from the direct interaction of sound waves with matter as US has wavelengths much larger than molecular dimensions. Power ultrasound promotes and enhances chemical and physical changes through acoustic cavitation. Cavitation is a rapid formation, growth, and violent collapse of bubbles. Cavitation is produced when the negative pressure during rarefaction cycle exceeds attractive intermolecular forces or tensile strength of the liquid. In order to produce cavitation a minimum threshold of negative pressure should be reached. The threshold of ultrasonic pressure required to produce cavitation for different liquids is different as shown Table 2.1 .

Table 2.1 Threshold of sound pressure for various liquids at atmospheric pressure [51]

Liquid	Sound Velocity [m/s]	Sound Pressure [kPa]
Water [52]	1483	280×10^3
Olive oil [48]	1431	366×10^3
Corn oil	1463	309×10^3
Castor oil	1477	395×10^3
Linseed oil	1468	239×10^3

Dynamics of the cavitation bubbles is a complex phenomenon, largely influenced by the local environment and intensity of applied US. Cavitation is of two types' i.e. stable cavitation and transient cavitation. Stable cavitation is produced at low intensities. In stable cavitation bubbles oscillate gently around some equilibrium size and their mean life time may be longer than a cycle of the sound pressure. Surface oscillations and micro streaming stem from stable cavitaions and in addition stable bubbles often evolve into transient ones over time due to mass or heat transfer, resulting in bubble growth. Transient cavitation is produced at high intensity. Unlike stable cavitation, transient cavitation bubbles generally exist for less than one cycle and will collapse violently releasing enough kinetic energy to drive chemical reactions. During cavitation collapse, the surrounding liquid will quickly quench a short-lived, localized entity (hotspot) with temperatures in the range of 4500-5000 K and pressures exceeding 1000 bar. This event occurs with a lifetime of a few microseconds and cooling rates of about 10^{10} K^{-1}. Cavitation collapse under heterogeneous conditions, such as near a liquid-solid interface, is essentially different and other side effects appear. Collapse is now asymmetrical and an inrush of liquid from one side of the bubble gives rise to a violent liquid jet targeted at the surface (Figure 2.4). The net effects are surface cleaning, the destruction of boundary laver, and concomitant mass and heat transfer improvements.

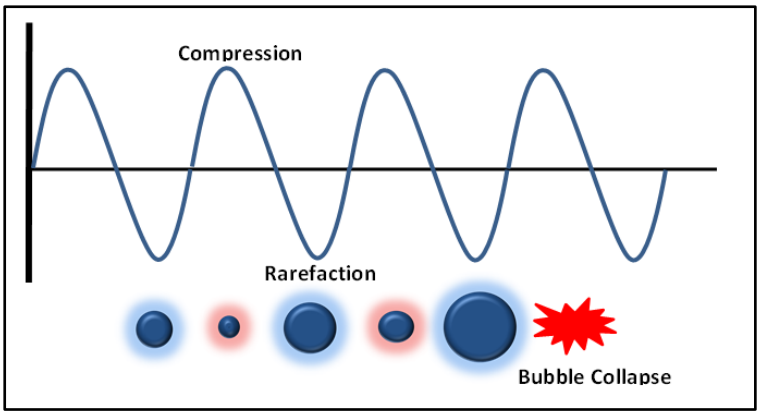


Figure 2.4. Collapse of cavitating bubble

Bubble collapse on the surface of a particle forces it into rapid motion and collision with vicinal solid matter. Overall, such effects account for dispersion, erosion, and size reduction, which represent driving forces in the activation of solid reagents and catalysts. Likewise, in heterogeneous liquid-liquid systems the powerful disruption of the interface will cause efficient mixing and fine emulsions. As a result, the presence of a catalyst is often unnecessary when phase-transfer reactions are conducted under sonication [50]. Important parameters influencing cavitation are shown in Table 2.2.

Table 2.2 Parameters Effecting Cavitation [40/50]

Parameter	Influence on Cavitation
Frequency	At higher frequencies more power is required to make a liquid cavitate as the rarefaction phase shortens. At higher frequency more cavitation bubbles are produced but they are smaller in size and therefore, have a lower impact upon implosion
Intensity	An increase in intensity will also increase the sonochemical effects
Temperature	An increase in temperature will raise the vapor pressure and cavitation will be easier, though a less violent collapse (owing to higher vapor pressure)
External Pressure	Increasing the external pressure raises the threshold of pressure required to initiate cavitation. This means cavitation can be avoided by increasing hydrostatic pressure on medium; however, cavitation produced at higher pressure would give more violent effect

2.3 Modeling of Ultrasonic Reactor

Knowing the kinetic behavior is primary task in describing ultrasonic intensification of enzyme catalyzed reactions, but knowledge regarding acoustical behavior of the employed reactor is equally important. Acoustical behavior concerns with the dispersion pattern of acoustic pressure inside reactor and is termed as acoustic pressure field. Knowing the dispersion pattern of acoustic pressure field in reactor is of prime importance as it governs the reactor performance. Therefore, development of a mathematical model capable of predicting the acoustic pressure field inside reactor is also included in this work. For modeling and simulation of ultrasonic reactors use of COMSOL multiphysics has been reported in a number of publications [53, 47, and 36] and reviewed by Tudela [55]. However, this has limited only to the prediction of acoustic pressure field. Model developed in context of present work is not only capable of predicting acoustic pressure field but can also predict enzyme particle trajectories resulting from ultrasound. This information can be combined with intrinsic kinetics of the considered reaction in order to predict the performance of a sonicated enzyme catalyzed reactor. Mathematical model is capable of predicting acoustic pressure field as a function of influencing parameters such as frequency, power, reactor geometry, position of source, material of construction of reactor, reaction medium etc. Model can also predict the combined effect of ultrasound and stirring on particle trajectories. The hydrodynamic information thus obtained can be combined with intrinsic kinetics of the reaction to predict the performance of an ultrasonic reactor. Experimental data shows that cavitation damages the immobilized enzyme particles and should be avoided. Due to this fact cavitation phenomenon is not considered in the model. Therefore, the developed model is used to simulate enzyme particle trajectories in high frequency reactor (as no cavitation is produced at high frequency). The developed model can also be used for simulating particle trajectories resulting from US only without considering stirring effect [5].

In context of present study following concept for reactor modeling is followed.

Acoustic is simulated in frequency domain (time-independent), while stirring and particle tracing is time-dependent. Simulations are calculated in two steps. First acoustic pressure field is calculated (independent of particle movement). In second step stirring effect is modeled in combination with particle tracing model where acoustic pressure field and drag force are used to calculate the movement of Lipozyme-435 particles.

1. **Modeling of Acoustic Field Pressure:** Simulates the dispersion of ultrasound in reaction medium.
2. **Modeling Stirring Effect:** Simulates the effect of stirrer on reaction medium which ultimately governs particle movement through drag force.
3. **Modeling of Particle Trajectories:** Simulates how particles are affected by the acoustophoretic force (resulting from acoustic pressure field).

In the following sections a detailed explanation about each model will be given.

2.3.1 Modeling of Acoustic Field Pressure

The governing equation for propagation of sound inside a medium is the Helmholtz wave equation given by [53, 47]

$$\nabla\left(\frac{1}{\rho}\nabla p\right)-\frac{\omega^2}{\rho c^2}p=0 \tag{2.1}$$

Defining these properties is tantamount to defining the reaction medium of interest. The above mentioned equation is in time-harmonic formulation. Sound wave travels in harmonic manner, so the time dependence can be taken out of the equation. Using this equation, sound field is described and solved by the pressure p. The pressure represents the acoustic variations (or excess pressure) to the ambient steady state pressure. Acoustic pressure gives the acoustophoretic force (F_{aco}) for particle movement.

Boundary conditions for Acoustics Modeling [56]

- **<u>Impedance Boundary (Reactor top):</u>** This type of boundary assumes that incident wave is partially reflected and partially transmitted i.e. $-1 < R < 1$. This is determined from reflection coefficient R which is written as:

$$\mathbf{R} = \frac{\text{Reflected Pressure}}{\text{Incident Pressure}} = \frac{Z_2 - Z_1}{Z_2 + Z_1} \text{ [-]} \qquad (2.2)$$

- **Sound Hard Boundary (Reactor walls):** This type of boundary assumes that incident sound wave is perfectly reflected back and is in phase with the incident one (R = 1). Wall of reactor employed is made of glass. Yasui et.al. Have simulated the acoustic pressure field in ultrasonic reactor using different wall boundaries (rigid and thin/thick glass) [57]. They have shown that reactor with thicker glass wall acts as a rigid boundary. For the present study a reactor with thick walls has been taken in order to ensure sufficient strength while doing experiments under higher pressure. Therefore, use of sound hard boundary (rigid boundary [58]) is justified in this case.

- **Pressure Boundary** is to define the ultrasound source (transducer) in the liquid medium. It is a Dirichlet Boundary Condition, where the pressure on the boundary is set to be the pressure caused by ultrasonic transducer. Pressure is calculated from ultrasound intensity with following equation [59]

$$I = \frac{p^2}{2 \rho c} \qquad (2.3)$$

$$p = \sqrt{(2 I \rho c)}$$

Intensity mentioned above was measured experimentally according to the calorimetric method. Details of the experimental procedure are given in Appendix A.

2.3.2 Modeling Stirring Effect (CFD)

With ultrasonic reactor at high frequency it has been observed that ultrasound alone is not sufficient to bring the catalyst particles into suspension. Therefore, it is necessary to use a stirrer. The stirrer rpm is kept low in order to minimize disturbance of acoustic field from US. The impeller Reynold number can be calculated according to the following equation [60]

$$\mathrm{Re}_{\mathrm{impeller}} = \frac{\rho\, \mathrm{N}\, \mathrm{D}^2}{\mu} \tag{2.4}$$

The calculated value of Reynolds number for present system comes out to be 343, which indicates that flow is laminar. For laminar flow conditions the governing equations are [61]

$$\rho \frac{\partial \boldsymbol{u}}{\partial t} + \rho(\boldsymbol{u}\, \nabla)\boldsymbol{u} = \nabla.\left[-pI + \mu(\nabla \boldsymbol{u} + (\nabla \boldsymbol{u})^T) - \frac{2}{3}\mu(\nabla\, \boldsymbol{u})I\right] + F\ ; \tag{2.5}$$

$$\rho \frac{\partial \boldsymbol{u}}{\partial t} + \nabla(\rho\, \boldsymbol{u}) = 0 \tag{2.5A}$$

The velocity calculated from above equation is used to calculate drag force caused by movement of stirrer.

Boundary conditions for CFD [61]

- **Wall boundary:** This condition is chosen for walls of the reactor which assumes fluid velocity as u=0 (no slip).

- **Flow Continuity Pair:** In simulation the domain of interest is divided into rotating and stationary domain. The rotating domain lies around the stirrer. The boundary between rotating and stationary domains in reactor is set as continuity pair. This allows flow continuity, where the fluid momentum is transferred from and to either side of the boundary (Dirichlet Boundary Condition) u = u.

- **Pressure Point Constraints**: It is a point (Dirichlet Boundary Condition) where p=0. With this constraint, the system is defined as a batch flow, without inlet or outlet flow to and out of the system.

2.3.3 Modeling of Particle Trajectories

The movement of catalyst particles in reactor is a result of both stirring as well as acoustic field (Figure 2.5). The corresponding forces are drag and acoustophoretic force. According to Newton's law of motion the net force on an object is equal to the derivative of linear momentum. For the present case Newton's law is formulated as

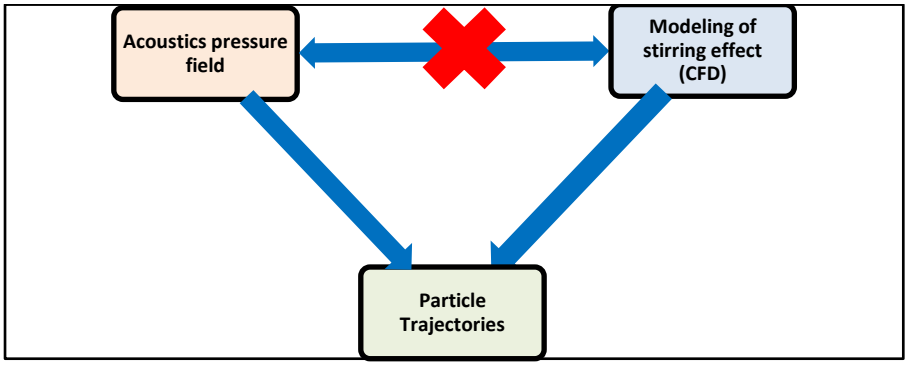


Figure 2.5. Coupling of the forces on catalyst particles

$$\frac{\mathrm{d}}{\mathrm{dt}}(\mathrm{m_p}\mathbf{v}) = \mathrm{F_g} + \mathrm{F_D} + \mathrm{F_{aco}} \tag{2.6}$$

The gravity force (F_g) is defined according to Eq. 2.7,

$$\mathrm{F_g} = \mathrm{m_p g}\frac{(\rho_\mathrm{p} - \rho)}{\rho_\mathrm{p}} \tag{2.7}$$

The drag force (F_D) is defined in Eq. 2.8 as

$$F_D = \frac{1}{\tau_p} m_p(\mathbf{u} - \mathbf{v}) \tag{2.8}$$

The fluid velocity ($\mathbf{u}$) is calculated from Eq. 2.5. In this equation τ_p is the velocity response time. According to Schiller-Naumann, it is defined as

$$\tau_p = \frac{4\,\rho_p\,d_p^2}{3\,\mu\,C_D\,Re}$$

with

$$C_D = \frac{24}{Re}(1 + 0.15\,Re^{0.637}) \tag{2.9}$$

$$Re = \frac{\rho\|\mathbf{u}-\mathbf{v}\|d_p}{\mu}$$

The acoustophoretic force (F_{aco}) is defined in Eq 2.10, where ρ and care density and speed of sound in medium, and c_p is compressional speed of sound in the particle, given as 3000 m/s.

$$F_{aco} = -\nabla U^{rad}$$

$$U^{rad} = V_p\left(f_1\frac{1}{2\rho c^2}(p_{in}{}^2) - f_2\frac{3}{4}\rho_p(v_{in}{}^2)\right) \tag{2.10}$$

$$f_1 = 1 - \frac{\rho c^2}{\rho_p c_p{}^2}; \qquad f_2 = \frac{2(\rho_p - \rho)}{2\rho_p + \rho} \qquad v_{in} = \frac{-1}{i\omega\rho}\nabla p$$

The acoustophoretic force is a special subclass of the CFD forces, which are the acoustic radiation forces on small particles. This implies that the equations are valid for particles of diameter smaller than the acoustic wavelength and larger than acoustic boundary layer thickness defined as [62-64]

$$\delta_{bl} = \sqrt{\frac{\nu}{\omega}} = 0.01 \text{ mm } (10\ \mu\text{m}) \tag{2.11}$$

Calculated values of boundary layer thickness at different frequencies are

f [kHz]	δ_{bl} [mm]
24	0.01
206.3	0.0034
616	0.002

Average particle diameter for Lipozyme-435 is approx. 0.6 mm [3]. From comparison of particle size with wavelength and boundary layer thickness it is obvious that the necessary condition for applicability of above mentioned equations for acoustophoretic force is fulfilled.

Boundary conditions for Particle Tracing Module

- **Wall boundary:** Wall boundary condition defines, what happens to the particles upon coming into contact with wall of reactor. For present study a bounce condition is used which means all particles are bounced back in the direction they come from.

In particle tracing simulations initial position of the particles is taken to be randomly distributed; with initial particle velocity $v_0 = 0$ for every direction (this condition is assumed only for starting point in simulation). It is also assumed that there are no interactions between particles.

Following the trajectory of each particle manually is time consuming. To accelerate this process particle image velocimetry (PIV) tools are utilized. Principles of PIV are described in next section.

2.4 Particle Image Velocimetry

To validate results of simulation catalyst particle velocities were measured using PIV. In PIV particle movement is captured by a high-speed camera (motion pro Y4, Integrated Design Tools, Inc. Figure 2.6). The camera is capable of capturing up to 4000 frames per second (fps). The principle of PIV is described in Figure 2.7. Particle position in frame 30 and 34 is noted. From the distance travelled by particle and time interval between two frames it is possible to predict velocity and direction of particle movement. In present work images are analyzed using a Particle Image Velocimetry program in MATLAB, called PIVlab. The program detects the changes in position of particles between two consecutive images. From the distance traveled by the particle, the program determines the speed of particles in the region of interest (ROI).

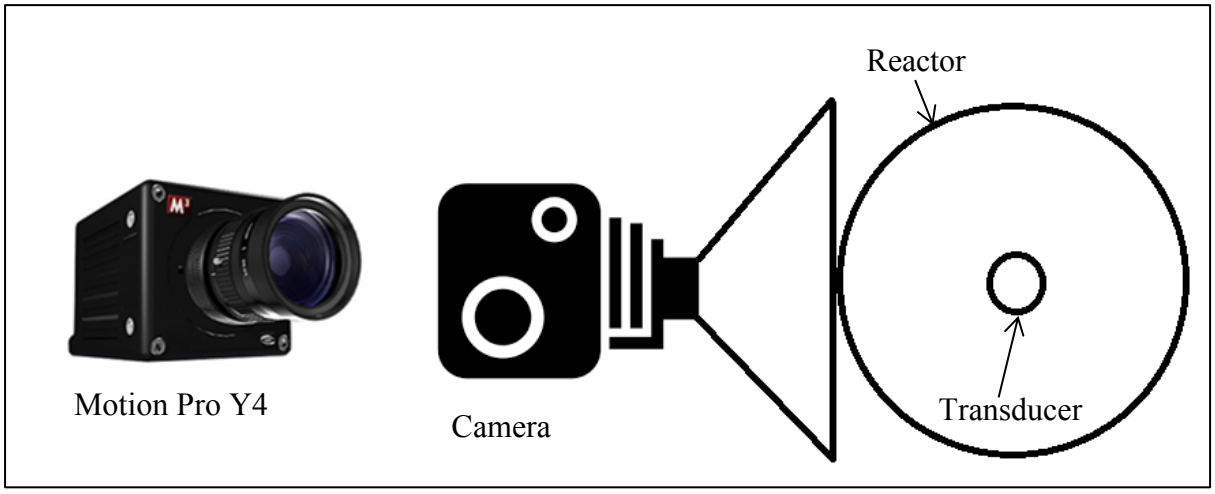


Figure 2.6. Setup for Particle Image Velocimetry measurements (IDT (UK) Ltd)

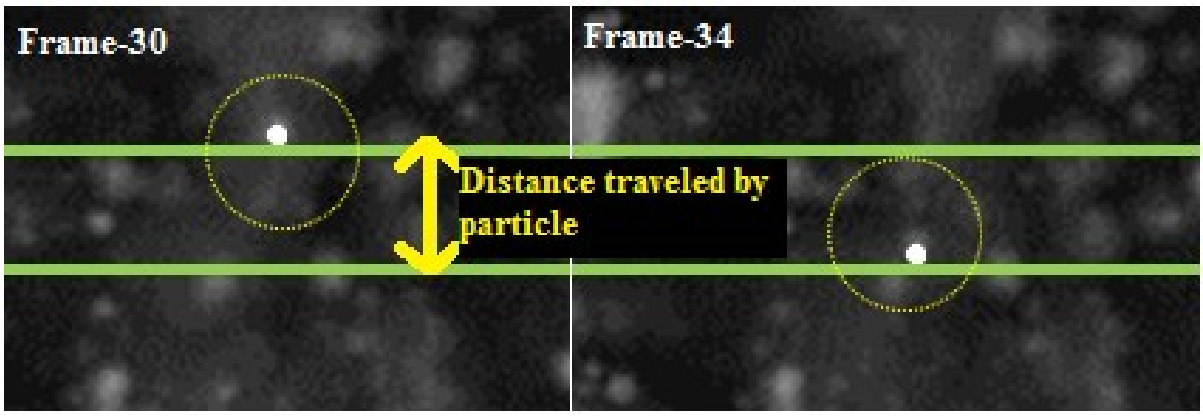


Figure 2.7. Particle movement between two frames

3 Experimental Setup

3.1 Example Reaction

As mentioned earlier Lipozyme-435 and Lipozyme CALB L are used as catalysts. The advantage of selecting these enzymes is that both are commercially available and processes based on them are well established. Therefore, from the results of study not only new but also existing processes can benefit. Esterification of oleic acid with n-hexanol is chosen as the example reaction system. The products of esterification are hexyl (9Z)-is octadec-9-enoate and water. The progress of the reaction can be monitored by acid base titration. The balanced reaction equation can be written as

$$\underset{\text{oleic acid}}{C_{17}H_{33}COOH} + \underset{\text{1-Hexanol}}{C_6H_{14}O} \xleftrightarrow{\text{Lipozyme 435}} \underset{\text{Hexyl(9Z)-is octadec-9-oneate}}{C_{24}H_{46}O_2} + H_2O$$

Oleic acid (Edenor PK 1805) was a gift from BASF SE Germany (formerly Cognis Germany). 1-Hexanol was a gift from Sasol Germany and was with 99.7% purity. Sodium hydroxide, ethanol and acetone were purchased from Carl Roth Germany. Lipozyme-435 was also a gift from Novozymes A/S Denmark. All chemicals were used as received without any further processing or purification.

3.2 Experimental Procedure and Analytics

Reaction volume for all the investigations has been kept constant at 300 ml (except where mentioned) in order to eliminate any influences arising from variation of volume. This reaction volume is significantly larger in comparison to the previous studies [16,7,19] conducted with sonicated enzyme reactions. After filling reactants into the reactor heating/cooling was turned on to achieve the required reaction temperature. Upon reaching the desired reaction temperature two zero samples were taken before addition of enzyme in

every experiment. For the rest of the experiment samples were taken from the reactor at predefined intervals of time and were analyzed by titrating against 0.1 molar NaOH solution. Thymolphthalein was used as indicator. To minimize the sampling errors and to ensure reproducibility of results a sampling volume of 1ml was selected. The samples were collected using Eppendorf pipette and were weighed in a balance to determine errors/variations in sampling amount. In order to quench the rest activity of enzymes, samples were collected in equivolume mixture of ethanol and acetone.

3.3 Ultrasonic Reactors Used

To investigate the effect of different operating parameters on enzyme catalyzed reactions three different reactor configurations were used and are shown in Figure 3.1. These reactors are equipped with ultrasonic sources at different positions and are capable of operating at different ultrasonic intensities and frequencies. The motive for using different reactors was to have the capability of testing the catalytic function of enzyme under stirring (Figure 3.1a), cavitating and non-cavitating US (Figure 3.1b and c). In the following detailed specifications are given for each reactor. As sound is a form of energy and whenever applied to a medium causes the rise in temperature. To eliminate this thermal effect of US on chemical reactions, all the reactors were equipped with a cooling/heating jacket. The temperature of the reaction was monitored by a thermocouple and maintained at required value by circulating water in the reactor jacket. A water bath Julabo (F-12 ED) was used for this purpose.

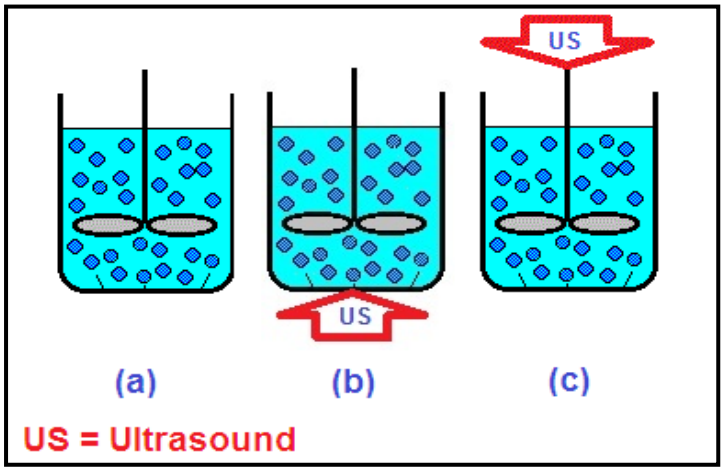


Figure 3.1. Reactor configurations to be studied

3.3.1 High Frequency Reactor (Reactor A, 206.3/616 kHz)

Ultrasonic reactor A consisted of an ultrasonic generator (LVG 60), transducer (USW51) and a glass reactor (with heating/cooling jacket) from L-3 Communications ELAC Nautik GmbH, Germany. There are two transducers capable of operating at different frequencies and power inputs and can be fitted at the bottom of glass reactor (configuration b in Figure 3.1). Through the valves provided in the reactor jacket, it was possible to connect it to cooling/heating bath for maintaining required temperature. Experimental setup for reactor A is shown in Figure 3.2 and specifications of the generator and transducers are given in Table 3.1.

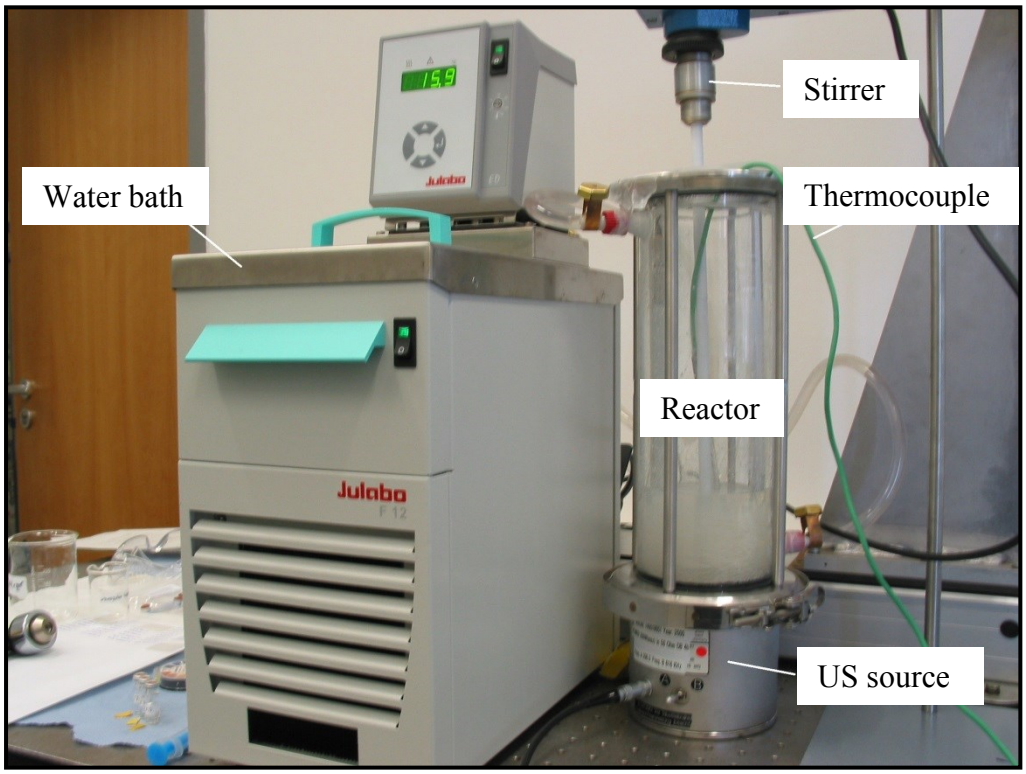


Figure 3.2. Reactor A with USW51 transducer from ELAC Nautik GmbH

Table 3.1. Specifications of reactor A (USW51)

Equipment	Frequency [kHz]	US intensity [W/cm^2]
Transducer-1 USW 51	42.1	4
Transducer-2 USW 51	206.3	4
	616	8

According to Table 3.1 transducer-1 can be operated at a constant frequency of 42.1 kHz however, intensity can be varied from 0 - 100 Watt. Similarly, transducer-2 can operate at two different frequencies i.e. 206.3 and 616 kHz and variable intensity. Due to high frequency and low power it was possible to sonicate the reaction medium without cavitating it.

3.3.2 Low Frequency Reactor (Reactor B 24 kHz)

Reactor B was capable of operating at a constant frequency of 24 kHz and ultrasonic intensity could be varied from 2.4-105W/cm^2 (depending upon sonotrode used). Due to low frequency and higher power density this transducer was capable of producing strong cavitation in the medium. In UP400s ultrasonic generator and transducer are integrated into one assembly. For transfer of US into reaction medium, sonotrodes (horns) were attached to the transducer. The specifications for the sonotrodes used are given in Table 3.2. All experiments were done using sonotrode H14. Sonotrode H40 was used only one time as it was not possible to achieve lower amplitude of 2.4 μm with sonotrode H14. The transducer assembly can be mounted on a stand to sonicate the reaction medium (Figure 3.3 left). To hold the reaction contents a glass reactor from NORMAG Labor- und Prozesstechnik GmbH was employed (Figure 3.3 right). The glass reactor had a heating/cooling jacket around it to maintain the required temperature. The contents of the reactor were also agitated with a magnetic stirrer Heidolph RZR 2000 (when required).

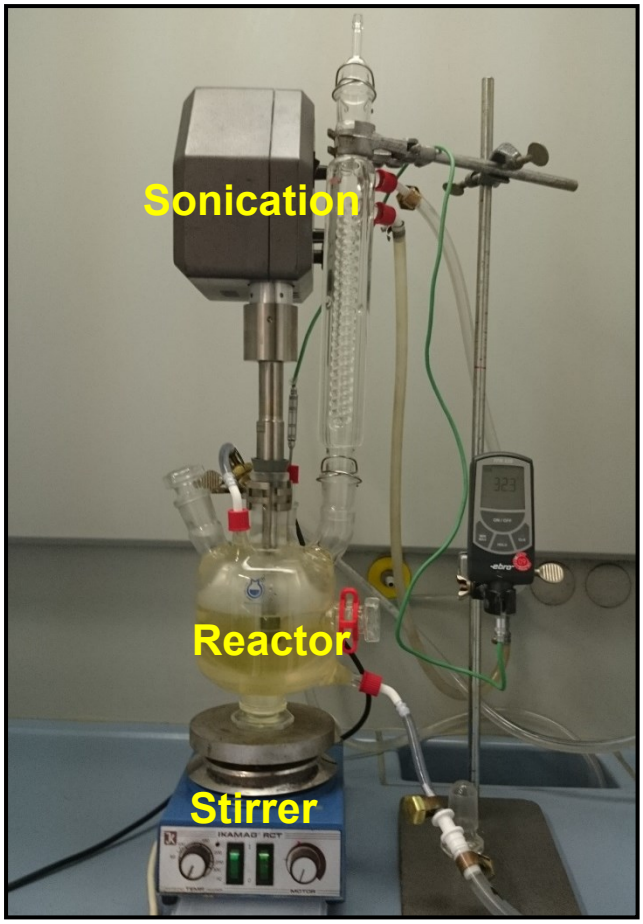


Figure 3.3. Reactor B with UP400S sonotrode from Hielscher Ultrasonics GmbH AG, Germany

Table 3.2. Specifications of reactor B (UP400S)

Frequency [kHz]	Sonotrode	Diameter [mm]	Amplitude [µm]	US Intensity [W/cm^2]
24±1	H14	14	25-125	21-105*
	H40	40	2.4-12	2.4-12

***Amplitude could be varied from 25 to 124µm in incremental steps**

4 Results of Simulation

Description of the ultrasonic reactors to be employed for intensification studies was given in the last section. At this stage before starting experimental work it is appropriate to do simulation of the reactors for selected reaction system and enzymes. The simulation results can be used in analysis and discussion of the experimental data which will be helpful in clarifying the underlying phenomenon.

4.1 Model of an Agitated Ultrasonic Reactor

Governing equations and relevant theory for simulation of reactor A were discussed in section 2.3. These equations will be used to simulate reactor A and B. Before solving the governing equations geometry and meshing of the domain are need to be drawn in COMSOL.

4.1.1 Geometry, Domain, Boundary Conditions and Meshing

3-D geometry of reactor including stirrer is drawn in Figure 4.1a. Reactor domain is divided into two sub domains i.e. rotating and static as shown in Figure 4.1b. Domain of the reactor consists of a certain material. Inserting these material specific properties into model equations is tantamount to defining the domain material. Domain for present work is an equimolar mixture of oleic acid and 1-hexanol at 40 °C. The properties used are given in Table 4.1.

Table 4.1. Required inputs for simulation

Input variables	Symbol	Equation	Value	Unit
Diameter of enzyme particles	d_p	-	0.6	mm
Density of enzyme particles	ρ_p	-	893	kg/m^3
Density of medium	ρ	-	865	kg/m^3
Viscosity of medium	μ	-	18.76	mPas
Speed of sound in medium	c	-	1379.57	m/s
Speed of sound in air	c_a	-	343	m/s
Driving frequency	f	-	206.3	kHz

Power of transducer	P_t	-	0-200	Watt
Effectiveness factor*	η	-	0.20	-
Sound intensity	I	$I = \frac{P_t}{A_t}$	-	W/m^2
Pressure of transducer	p_t	$\sqrt{(2\, I\, \rho\, c)}$	-	Pa
Impedance of air	Za	$Z_a = \rho_a c_a$	411.6	Rayl

*See Appendix A

Next step is to specify the boundary conditions. Figure 4.2 represents the cut off plane from 3D geometry of the reactor.

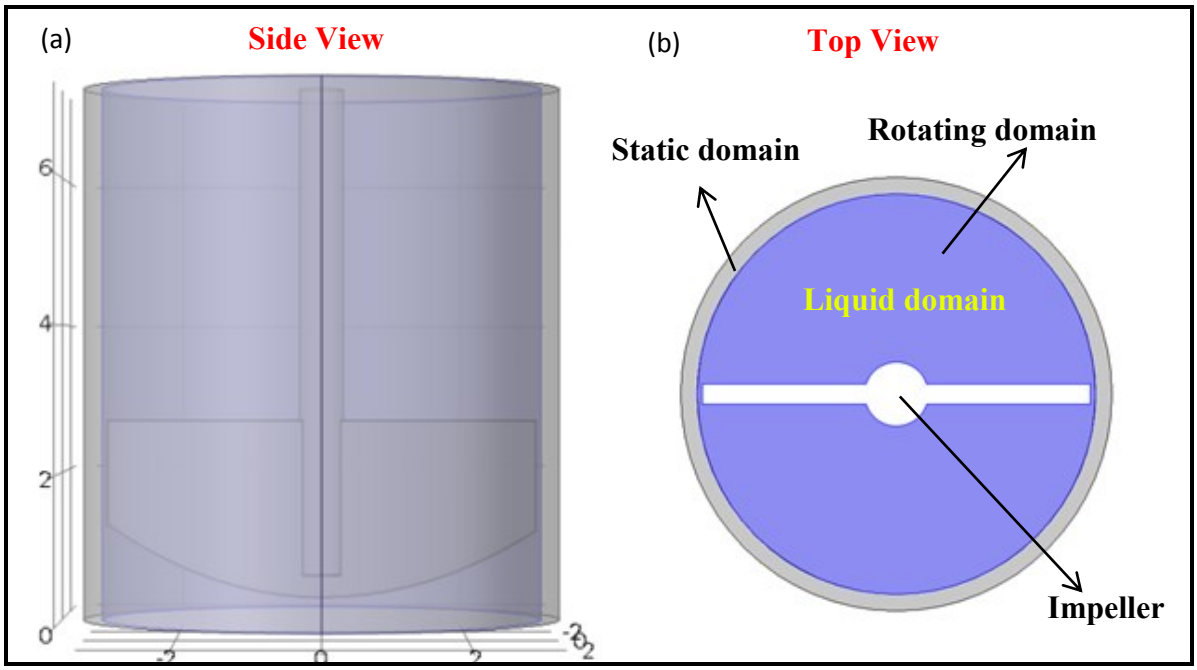


Figure 4.1. 3-D geometry of reactor with stirrer and US source at bottom

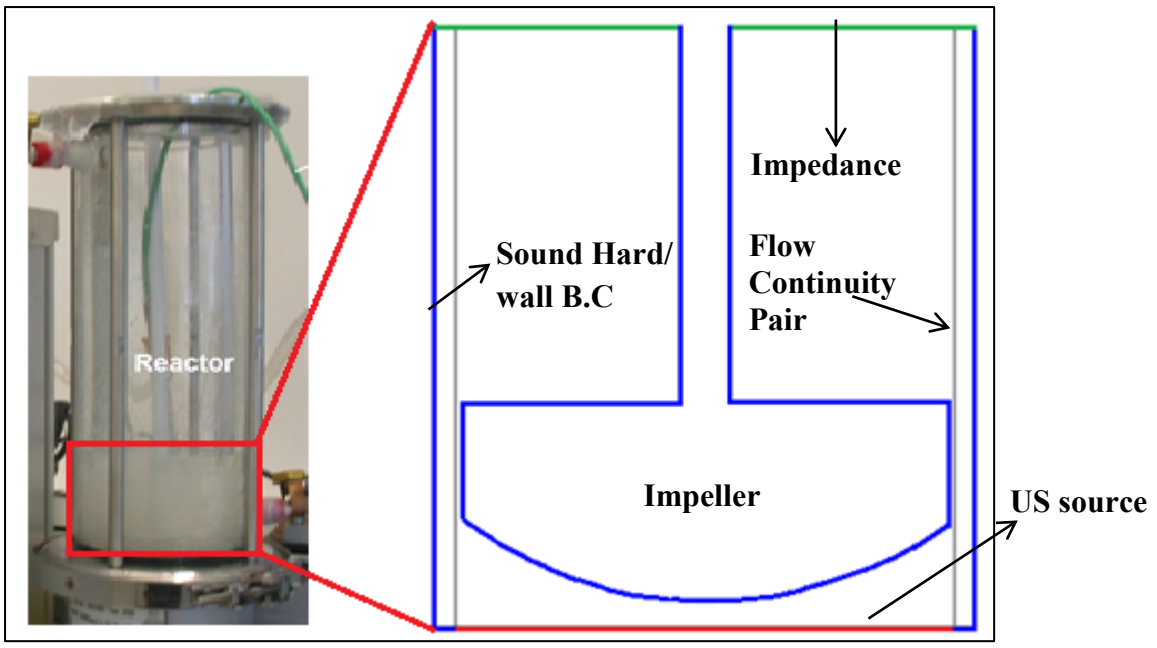


Figure 4.2. Boundaries for simulation of reactor

4.1.2 Meshing

After selecting relevant equations and drawing geometry next step is the meshing of domain. In meshing, entire domain is divided into smaller elements. In CFD meshing is an important step as quality of mesh greatly affects the quality of simulation results. Mesh quality is dependent upon degrees of freedom (DOF). DOF is a product of number of nodes and dependent variables. Therefore, for a given physics problem higher DOFs means higher number of nodes which means more number of elements. Elements and nodes in a mesh are described in Figure 4.3. For acoustic problems required DOF is dependent upon the frequency. At higher frequencies required DOF is also higher which ultimately requires more computational resources. Typically 10-12 DOF per wavelength are recommended for acoustic problems [65].

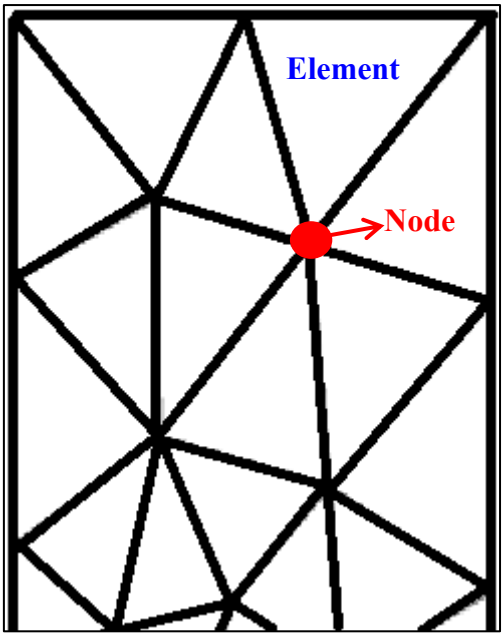


Figure 4.3. Depiction of elements and nodes in a mesh

In order to determine optimum DOF for present case a convergence analysis was carried out (Appendix B). In this analysis simulation was run at different DOFs for a constant ultrasonic power input. Maximum acoustic pressure corresponding to each DOF was plotted in the form of a graph as shown in Figure 4.4. Since the power input is kept constant and variation of acoustic pressure is coming from variation of DOFs (in other words from variation of mesh only). This variation shows that simulation is not converged. Therefore, DOF was increased step by step to a point where no more variation of maximum acoustic pressure was observed. This point was regarded as the minimum number of DOFs required for convergence. Two curves corresponding to 206.3 and 616 kHz are shown. As expected 616 kHz frequency requires much higher DOFs (marked with red circle) for convergence in comparison to 206.3 kHz (marked with blue circle). In the study higher value of DOF was used.

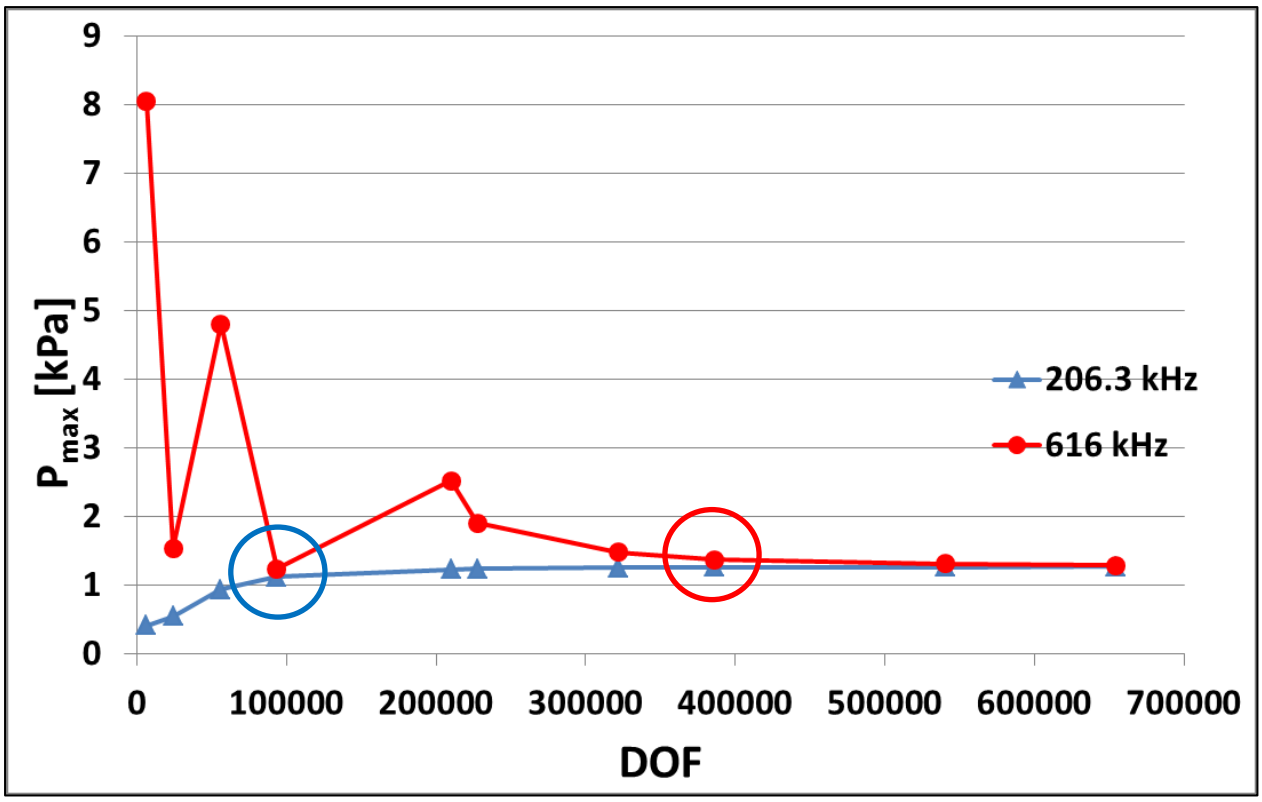


Figure 4.4. Convergence analysis for acoustic problem

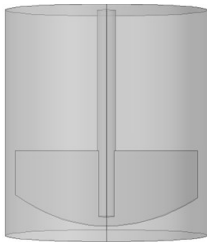
4.1.3 Results and Discussion

For agitated ultrasonic batch reactor there are two aspects of simulation:

1. Analysis of the **acoustic pressure field** created by ultrasound. This field affects the movement of particles in reactor through acoustophoretic force.
2. Analysis of **particle trajectories** resulting from **acoustic pressure field** and **stirring**.

Parameters for simulation are given in Table 4.2. For 2-D simulations two different frequencies (206.3, 616 kHz) at 2 and 6 W/cm^2 are simulated.

Table 4.2. Independent variables in simulation models

Reactor	Independent Variables
	Reactor diameter (d) = 6.5 cm Ultrasonic transducer : USW51 Power : 50W / 150W Frequency : 206.3kHz / 616kHz Transducer diameter : 6.5 cm Stirring: 120 rpm

4.1.4 Analysis of Acoustic Pressure Field

Acoustic pressure field defines how the ultrasound spreads inside the reaction medium. It is the most important aspect while discussing the performance of an ultrasonic reactor. In Figure 4.5 and Figure 4.6 effect of power and frequency on acoustic pressure field for reactor A is shown in 2-D. According to these figures when power is increased at a given frequency, the acoustic pressure also increases, however, this increase in power doesn't change the acoustic pressure field pattern. This phenomenon is observed for both frequencies.

For reactor A acoustic field changes strongly when frequency is changed from 206.3 to 616 kHz. An interesting phenomenon is observed at 206.3 kHz i.e. the presence of spots with high and low pressure points. This is indication of standing waves. Standing waves are produced due to superposition of incident and reflected waves. When enzyme particles are present in such a medium they tend to orientate along acoustic field. Due to high and low pressure spots particle clumps will be formed. Increasing the sound intensity in such cases would result in even higher pressures in these spots which will lead to more densely packed clumps of particles. This might lead to decrease in surface area of enzyme particles, which might results lower conversion. This phenomenon was also observed physically in lab by applying acoustic field to enzyme particles suspension at 206.3 kHz as shown in Figure 4.7. It would be interesting to study that how the clump formation effects the reaction performance. This will

be discussed in detail in chapter 5. On the other hand at 616 kHz standing waves are not observed. This means at 616 kHz particles would be distributed more evenly [51].

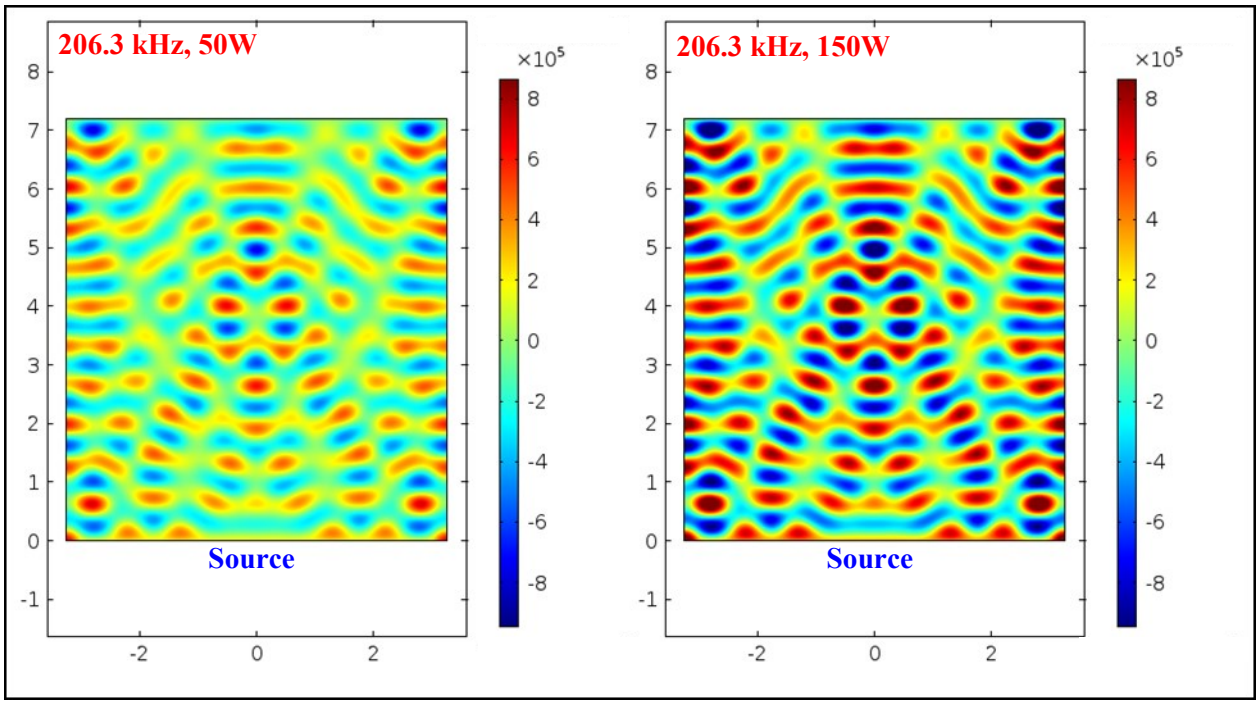


Figure 4.5. Effect of power on acoustic field at 206.3 kHz

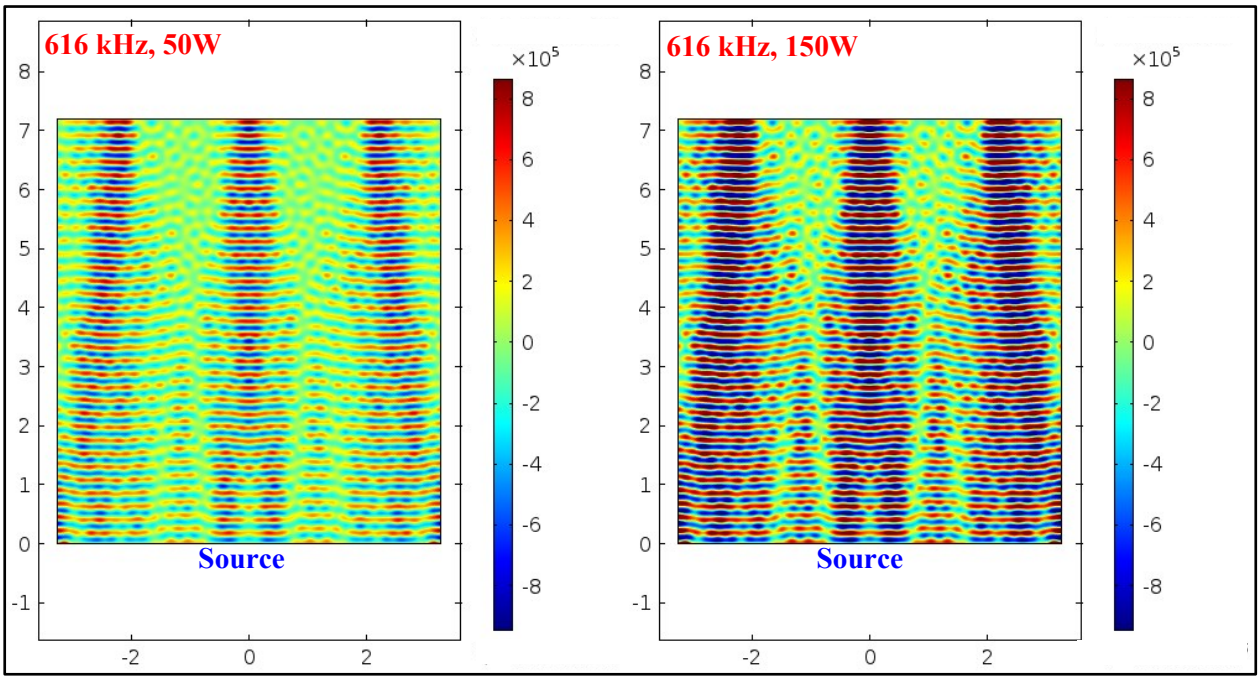


Figure 4.6. Effect of power on acoustic field at 616 kHz

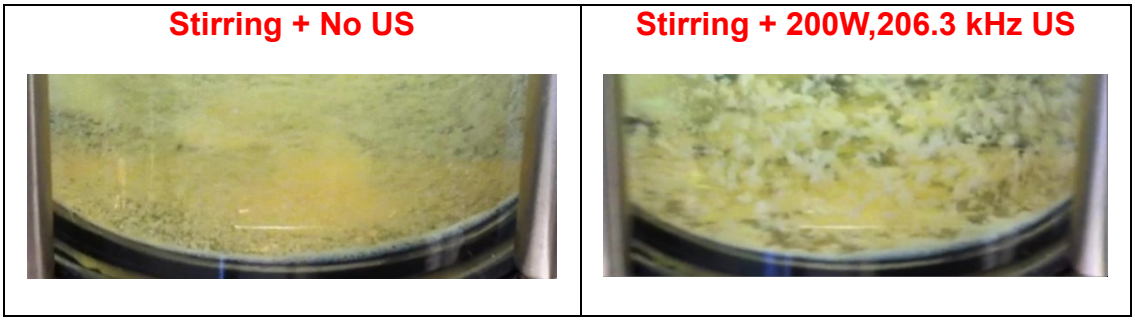


Figure 4.7. Clumping of enzyme particles due to standing waves at 206.3 kHz

4.1.5 Analysis of Particle Trajectories

In reactor A US alone was not sufficient to keep enzyme particles in suspension. Therefore, to bring particles into suspension a stirrer was used (Figure 3.2). The stirrer rpm was kept low in order to minimize disturbance to acoustic field. In COMSOL it was not possible to do a 2-dimensional simulation with stirrer (for reactor orientation used in Figure 4.5 and Figure 4.6). Therefore, a 3-dimensional simulation was prepared based on the specification of example reactor. Two different configurations are simulated based on this concept:

1. In first configuration only the effect of stirring is simulated
2. In second configuration combined effect of stirring and ultrasound is simulated.

The results of simulation with stirrer are presented in Figure 4.8 (a-d). Figure 4.8a shows the particles position at time 0. The initial state of particles assumed here is of random distribution. This is a default setting in COMSOL for particle tracing module. As the time progresses particles start moving due to stirring. Particles near to stirrer start moving first (Figure 4.8b). In Figure 4.8c more particles are influenced by stirring effect. In Figure 4.8d this effect is spread throughout reactor volume. Particles near to stirrer have higher velocities in comparison to the ones away from stirrer.

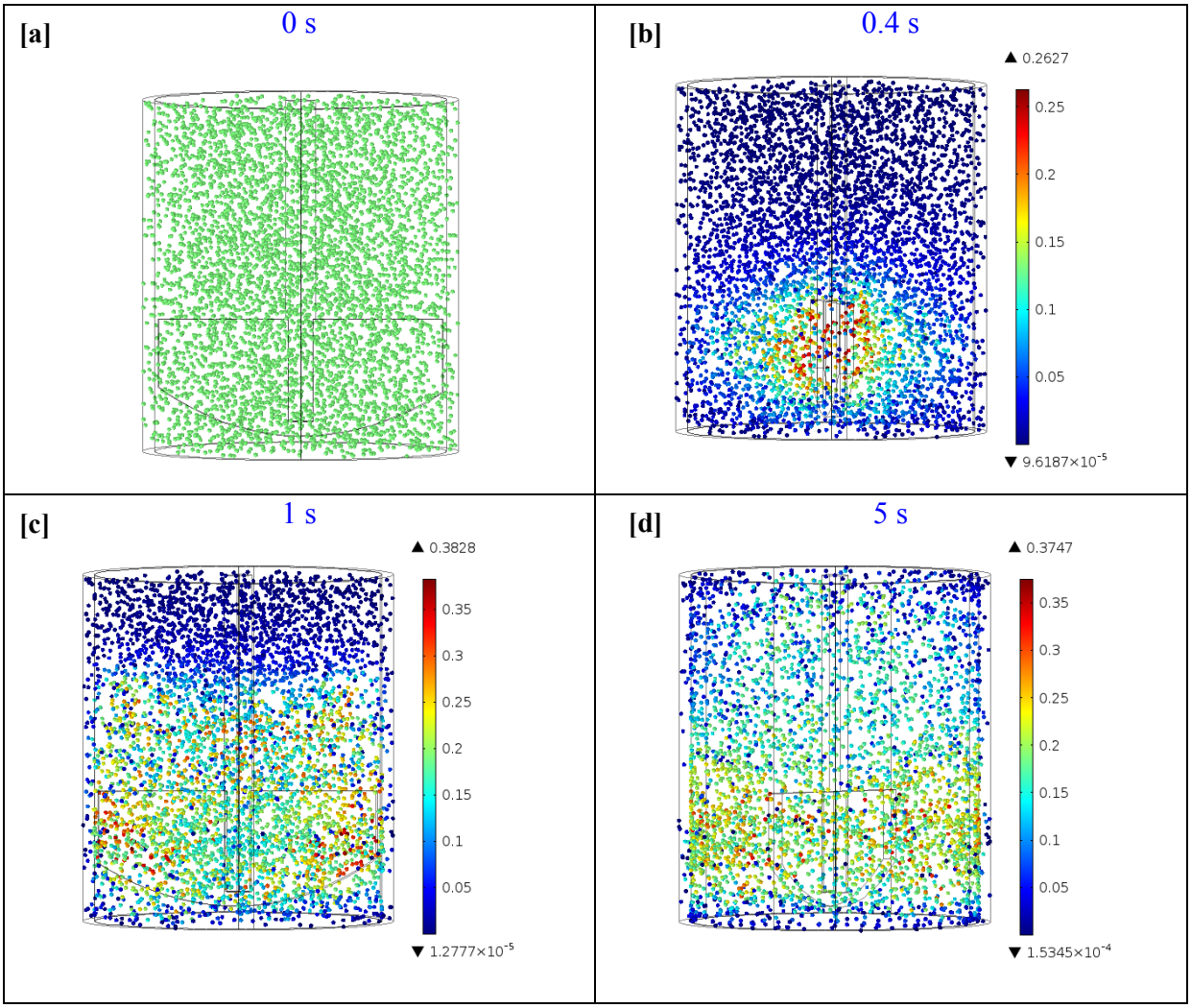


Figure 4.8. Stirring of catalyst particles in reactor A at 150 rpm without US

Simulation of stirring effect on catalyst particles paved the path for simulating the combined effect of ultrasound and stirring on particles trajectories. Acoustic pressure field for 206.3 kHz was shown in former Figure 4.5. In Figure 4.9 a comparison of particle trajectories for the case of stirring and US at 5 s is shown. In case of stirring particles are randomly dispersed due to stirrer (Figure 4.9a). However, in case of ultrasound two regions can be distinguished inside reactor (Figure 4.9b). In the upper region of reactor (marked with red rectangle) acoustophoretic force dominates the drag force as particles have tendency to orientate along acoustic field. Acoustic field restricts the movement of particles as it tries to keep them along acoustic field pattern while flow generated by stirrer exerts force on particles along radial direction. In region near to stirrer particles are dispersed due to stirring i.e. the drag force is

dominating the acoustophoretic force in this region. Kojima et.al [66] is of the opinion that these axial and radial forces bring higher turbulence in ultrasonic reactor.

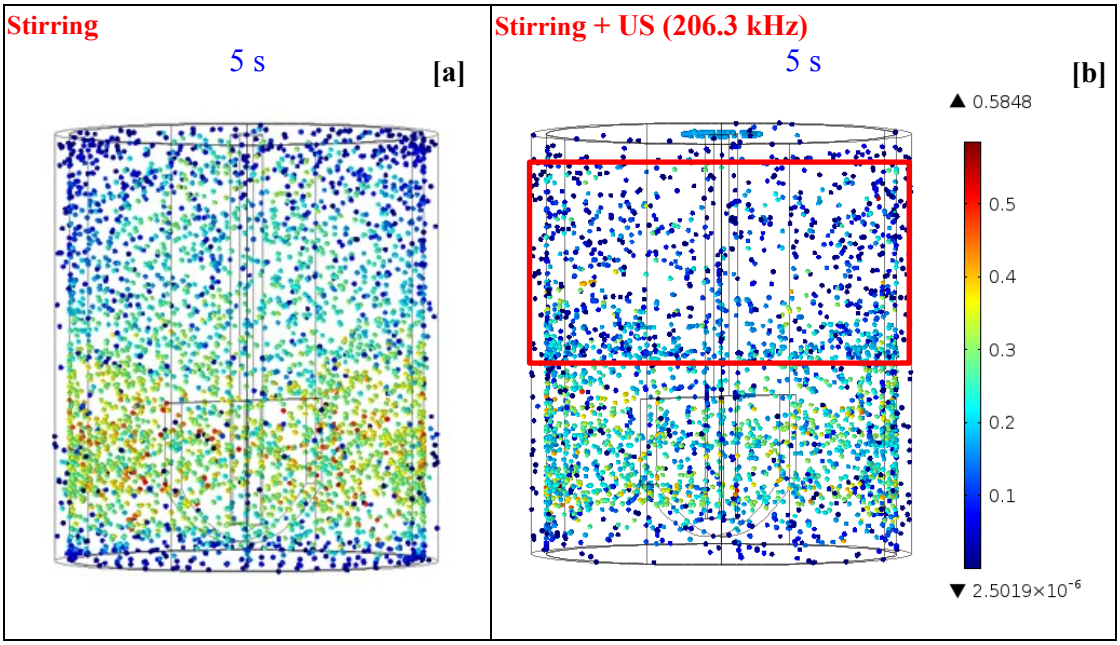


Figure 4.9. Effect of power on acoustic field at 206.3 kHz

To validate the model, particle velocities predicted by simulation are compared against the one measured experimentally (using PIVLab). Figure 4.10 shows the comparison between simulation and experimental velocities. A good agreement between simulation and experimental data is found. This proves the correctness of the modeling approach adopted. All experimental data related to particle trajectories are given in Appendix C.

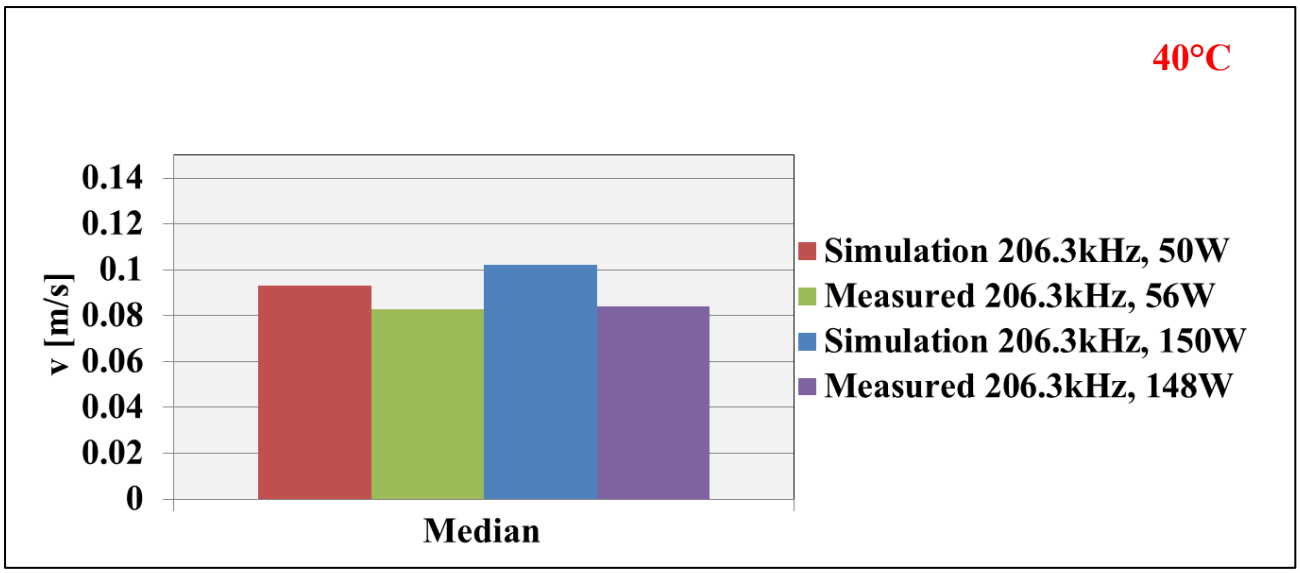


Figure 4.10. Comparison between simulation results and experimental data in reactor A

An important parameter for case of suspended particles is the slip velocity between liquid medium and catalyst particles. Slip velocity influences the external mass transfer resistance directly (from bulk medium to solid catalyst surface). Higher slip velocity will result in higher mass transfer rate and thereby helping to overcome mass transfer resistance. As external mass transfer is dependent on slip velocity, therefore, it is interesting to compare the slip velocity for configurations with stirring and stirring coupled with US. Since the developed modeling approach has correctly predicted the particle trajectories, therefore, it is hoped that the calculated slip velocity shall also be correct. In Figure 4.11 a comparison of slip velocity for aforementioned configurations in form of a histogram is shown. With a combination of stirring and US, there are more particles with higher slip velocity in comparison to stirring alone. Average slip velocity for the case of stirring is 0.042 m/s while for the case of US it is around 0.08 m/s. This higher slip velocity is basically responsible for higher mass transfer rate [67].

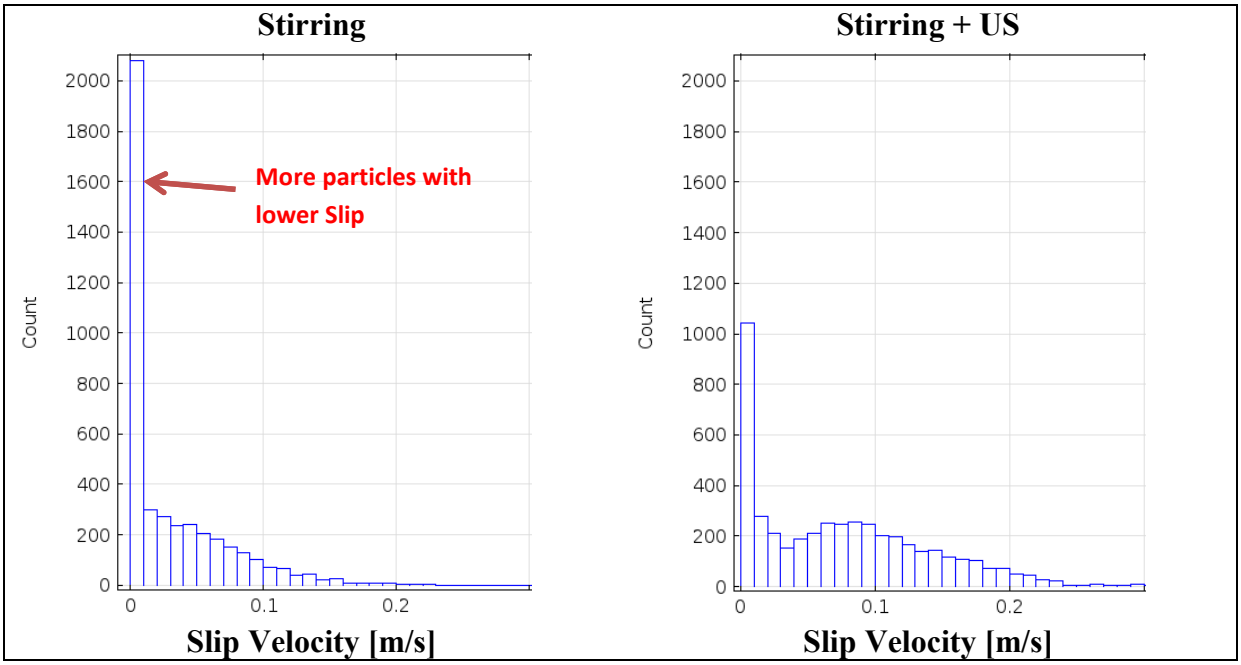


Figure 4.11. Comparison of slip velocity with stirring and US

A possible explanation for higher slip velocity with US can be given based on the acoustophoretic force on catalyst particles. In case of stirring catalyst particles tend to move along bulk liquid flow and, therefore, face less slip. However, in case of ultrasound acoustophoretic force on the particles tends to orientate them along acoustic field (regions of higher and lower pressure). This introduces a restriction on particle movement along liquid and thereby causing higher slip.

This slip velocity can be used to calculate mass transfer coefficient. In order to predict the effect of mass transfer coefficient on reaction performance, intrinsic kinetic equation of the reaction is required. The rate equation to represent the intrinsic kinetics of the studied reaction is to be developed. Development of such rate equation is discussed in next section.

4.2 Kinetic Modeling

Previous studies from the Institute of Process and Plant Engineering have shown that enzyme catalyzed reactions can be evaluated satisfactorily using power law kinetics [68]. Therefore,

for kinetic evaluation power law kinetics with 1st order was used. Rate constant was determined according to Eq. 4.1.

$$k = \frac{\ln(1-x)}{t} \tag{4.1}$$

Here k [1/min] is the first order rate constant while x and t are conversion and time [s], respectively. The data fitting for one experiment is shown in Figure 4.12. The experiment was done for Lipozyme-435 at 40°C using magnetic stirring at 150 rpm with equimolar mixture of oleic acid and n-hexanol. The reaction volume was 300 ml. As can be seen the measured data fits the straight line very well which proves that the reaction under study can be very well described using a first order reaction rate equation.

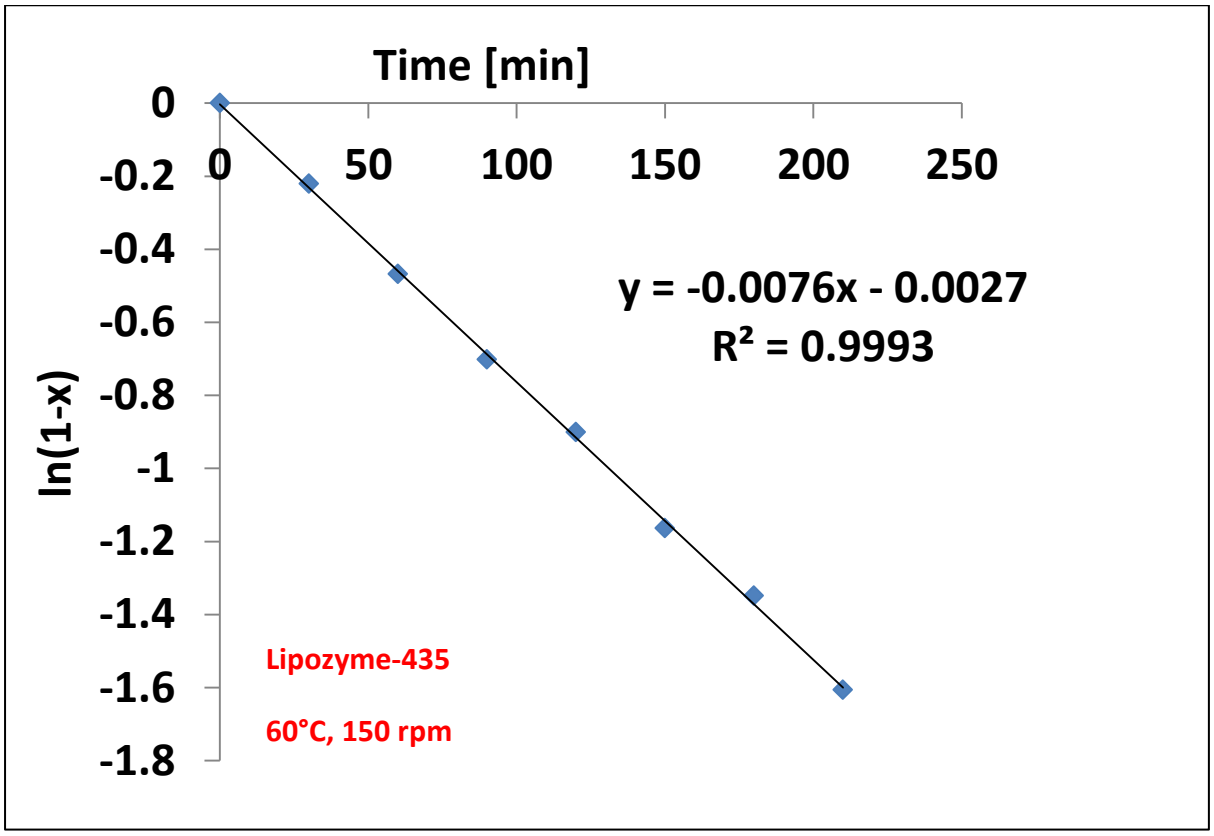


Figure 4.12. Kinetic evaluation of data according to 1st order kinetic

According to the scheme presented in Figure 4.13 intrinsic kinetic can be coupled to the mass transfer coefficient for predicting the performance of an ultrasonic reactor. The slip velocity for mass transfer coefficient comes from simulation. Correlations for calculation of mass transfer coefficient are given in section 5.4.

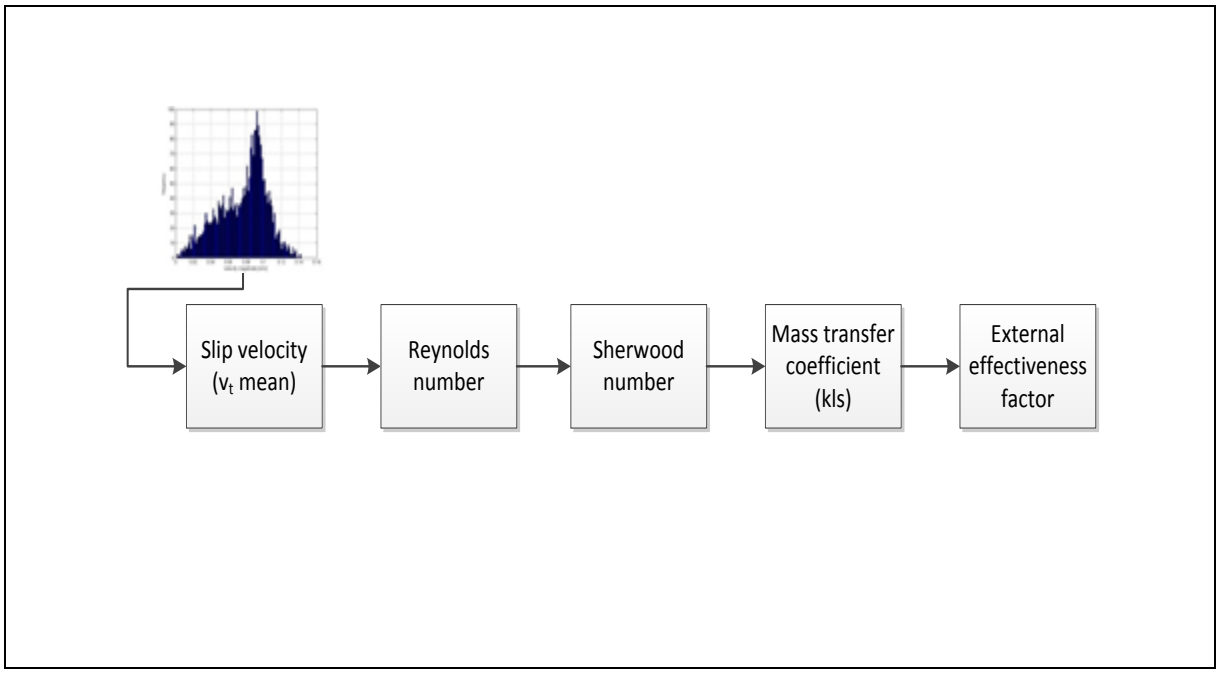


Figure 4.13. Calculation of mass transfer coefficient

5 Analysis of Intensification with Immobilized Enzymes

According to discussion in section 1.2, for understanding the phenomenon of intensification from US it is important that behavior of both immobilized and free enzymes is studied. While doing so it is necessary that in both cases enzyme should be of same origin. In this way the results shall be directly comparable which will help in understanding the underlying mechanisms of the phenomenon.

According to research methodology given in Table 1.2 intensification tests for immobilized enzymes are to be done in first place. In this chapter effect of US on intensification with immobilized enzymes is investigated in detail by taking Lipozyme-435 as example enzyme. The effect of stirring and US on free enzyme shall be investigated systematically in the next chapter.

5.1 Effect of Stirring and Related Parameters on Reaction with Immobilized Enzymes

Immobilized enzymes can be suspended in a reaction medium by stirring. Therefore, behavior of Lipozyme-435 in studied reaction under magnetic stirring is considered as base case. The behavior of Lipozyme-435 measured under US shall be bench marked against this base case to clearly identify the benefits of US.

To bring enzyme particles into complete suspension proper stirring speed (rpm) is necessary. This is required to ensure maximum contact with substrate/reactants. Therefore, reaction was run at three different rpms. These results are presented in Figure 5.1 which shows that increasing stirring speed from 50 to 150 rpm improved the reaction rate but beyond 150 rpm no further improvement was observed. This means at 150 rpm complete suspension of enzymes was already achieved. Therefore, 150 rpm was selected as standard stirrer speed for further experiments.

In introduction it was mentioned that activity of enzyme can be increased by increasing the reaction temperature. Therefore, the effect of temperature on activity of Lipozyme-435 under

stirring is studied as a first step and results are shown in Figure 5.2. As expected, enzyme activity increases by increasing temperature. Progress of 60°C curve shows that enzyme remained active throughout reaction time. This means activity of studied enzyme can be increased safely by increasing temperature up to 60 °C without using US. Therefore, use of US would be logical only if it intensifies the reaction rate beyond 60 °C stirring curve.

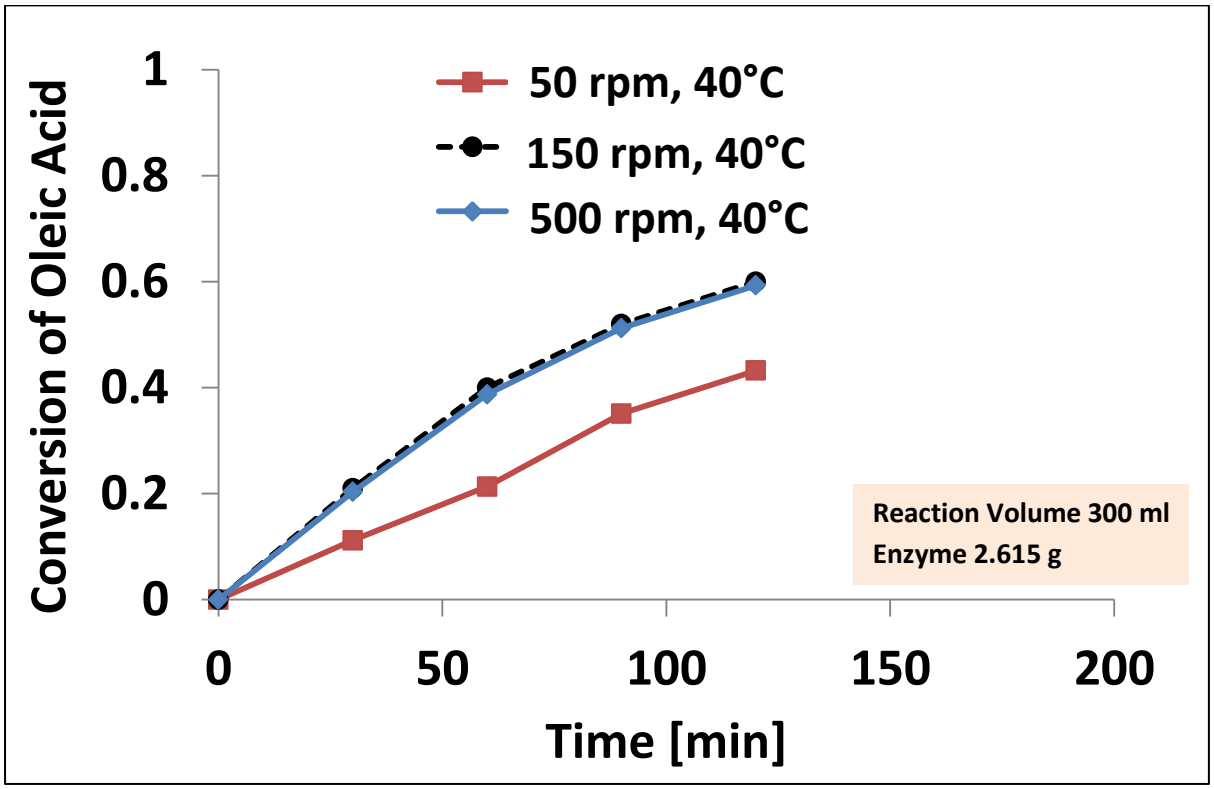


Figure 5.1. Effect of rpm on reaction rate with Lipozyme-435

After studying the effect of stirring and temperature on the reaction rate it is also necessary to determine the equilibrium conversion of the reaction as a function of temperature. The values of equilibrium conversion define the maximum achievable conversion for the studied reaction and are therefore, very important for the analysis of the studied phenomenon. With this perspective equilibrium conversions were determined experimentally at different temperatures. In equilibrium experiments equimolar mixture of oleic acid and n-hexanol was filled into reactor and was heated to required temperature. Once the required temperature was achieved two zero samples were taken from reactor before adding enzyme. The zero samples were titrated against 0.1 molar NaOH solution to determine the start concentration of oleic acid. After addition of enzyme the reaction was allowed to run for 72 hours. After 72 hours samples were taken from the reactor every 30 minute to determine the conversion of oleic

acid. When consecutively three identical values of conversion were obtained the reaction was stopped and the final conversion obtained was recorded as equilibrium conversion.

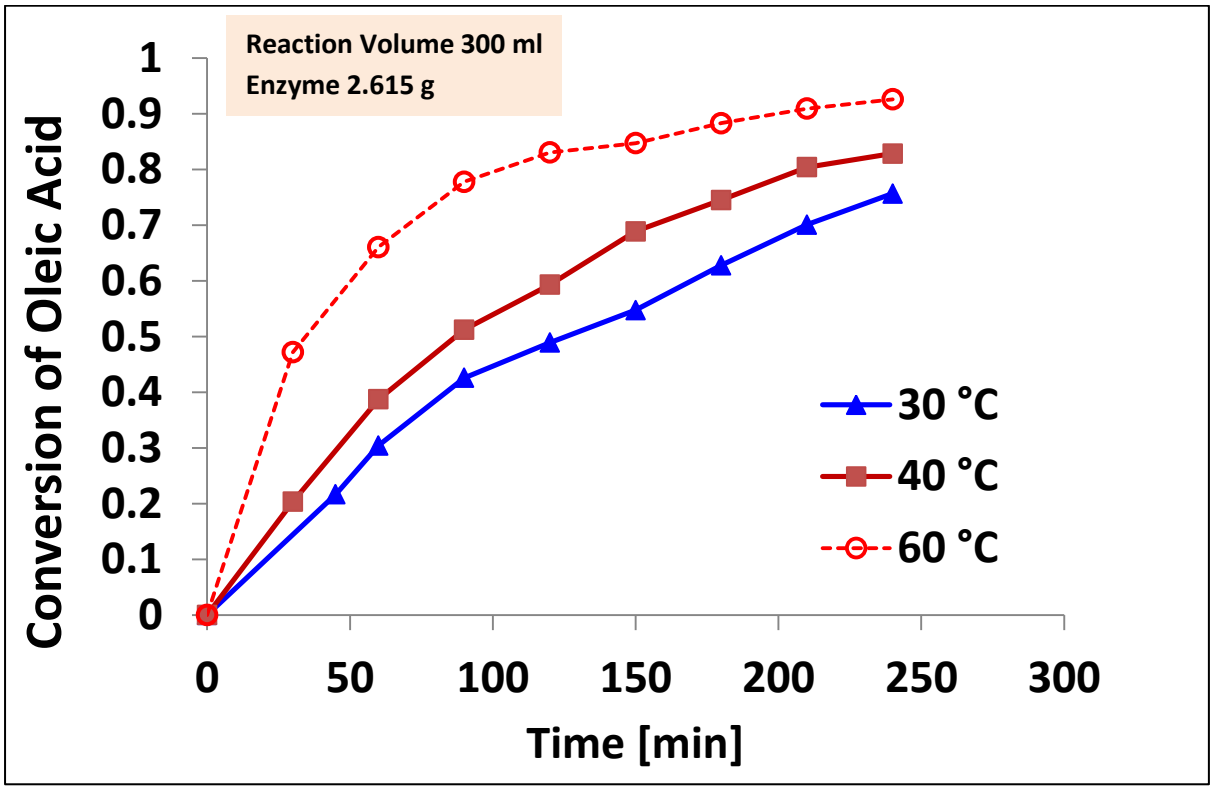


Figure 5.2. Effect of temperature on reaction rate for immobilized enzymes at 150 rpm

In Table 5.1 equilibrium conversion obtained at different temperatures is given. Temperature dependence of rate constant according to Arrhenius equation can be written as

$$k = 3.36 \cdot e^{-\frac{15967}{8.3145T}} \qquad [1/\text{min}] \qquad (5.1)$$

Further details regarding Eq. 5.1 can be found in Appendix D.

Table 5.1. Experimentally determined equilibrium conversion of oleic acid

Temperature [°C]	Percentage conversion
30	87
40	92
60	95

With completion of tests under stirring it was now possible to study the effect of US on Lipozyme-435. As discussed in section 2.2 by varying the frequency and amplitude reaction medium can be sonicated both under non-cavitating and cavitating conditions. Effect of non-cavitating US is tested first.

5.2 Effect of non-Cavitating Ultrasound on Intensification with Immobilized Enzymes

Using reactor A it is possible to sonicate the reaction under non-cavitating conditions as it can operate at higher frequencies with low intensity. In reactor A stirring was kept at 150 rpm to keep particles suspended. Lower rpm was used to minimise the distortion of ultrasonic field as in this reactor primary objective was to study the effect of US. Due to high frequencies and lower power input no cavitation was observed in the reaction medium.The experiment was according to the procedure described in chapter 3. The results (time course of oleic acid conversion) are shown in Figure 5.3. From the comparison it is obvious that use of non-cavitating US did not bring any intensification in comparison to stirring. This leads to the conclusion that for the studied reaction non-cavitating US does not intensify the studied reaction. Therefore, in this case stirring alone is sufficient to achieve the maximum reaction rate. For 206.3 kHz frequency formation of particle clumps was observed due to standing waves (Figure 4.7). Due to agglomeration of particles it was assumed that lower conversion would be observed in this case. However, results in Figure 5.3 show that reaction rate is not lowered due to agglomeration of particles. This can be attributed to higher slip velocity in this case [69].

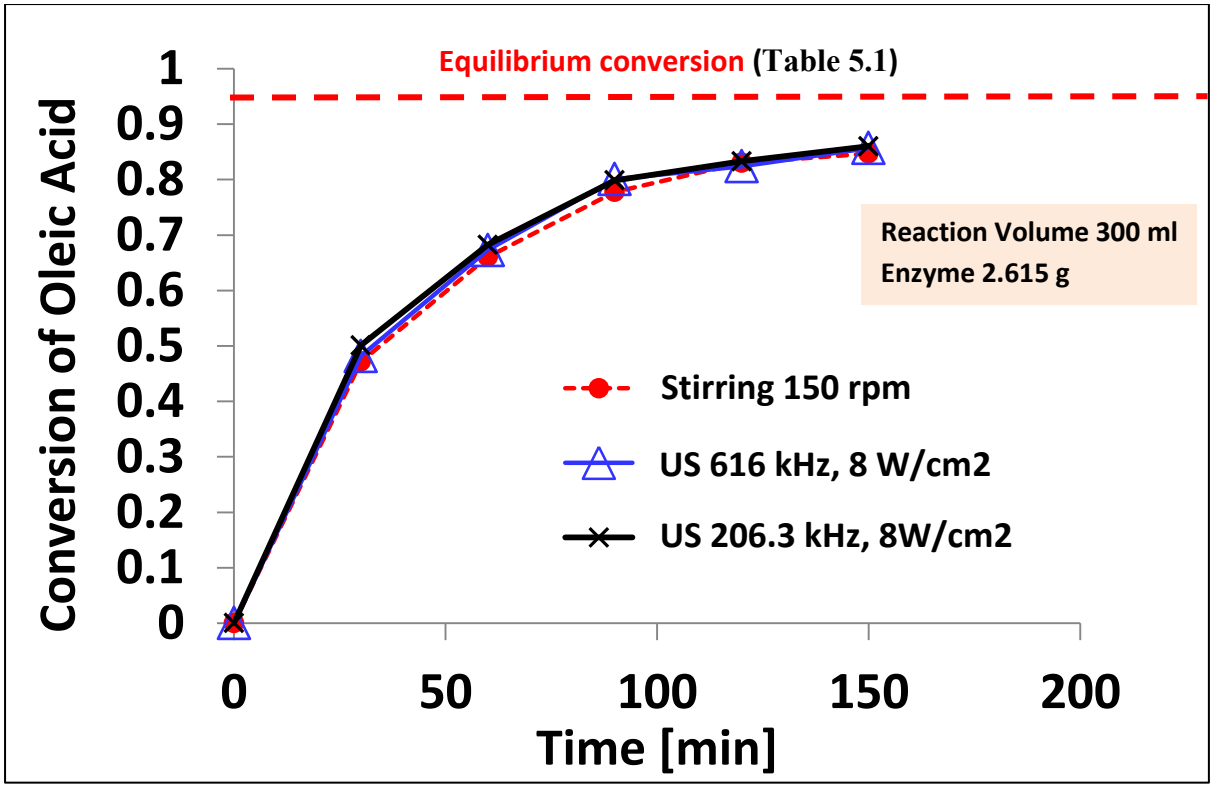


Figure 5.3. Effect of non-cavitating US on intensification of immobilized enzyme [60°C]

5.3 Effect of Cavitating Ultrasound on Intensification with Immobilized Enzymes

Third component of intensification tests for immobilized enzymes is to study the effect of cavitating US (Table 1.1). For this purpose reactor B was used. Reactor B operated at lower frequency of 24 kHz and higher intensity (Table 3.2). Sonotrode H14 was used. Lowest possible amplitude for sonotrode H14 was 12 µm. Therefore, reaction medium was sonicated at this amplitude which corresponds to an intensity of 21 W/cm^2. It is important to mention that the lowest intensity in reactor B is almost three times higher than maximum possible intensity with reactor A (Table 3.1) while frequency is 25 times lower. As application of US alone was sufficient to keep enzyme particles suspended in reactor B, therefore, it was not necessary to use stirring. With cavitating US initial experiments are done at 40 °C with a view to minimize or eliminate the possible detrimental effects of cavitation on enzyme.

5.3.1 Investigation of Sonotrode Position

Sonotrode in reactor B can be positioned anywhere inside reactor. The position of sonotrode in reactor is likely to effect the dispersion of acoustice pressure field in reactor and is, therefore, expected to have influence on performance of the reactor. In the first step before doing experiments developed model was used to simulate the acoutic pressure field and its effect on particle trajectories resulting from variation of sonotrode position. For horizontal variation two positions i.e center and off-center were simulated (the vertical position was kept in the middle).

Center position **Off-center position**

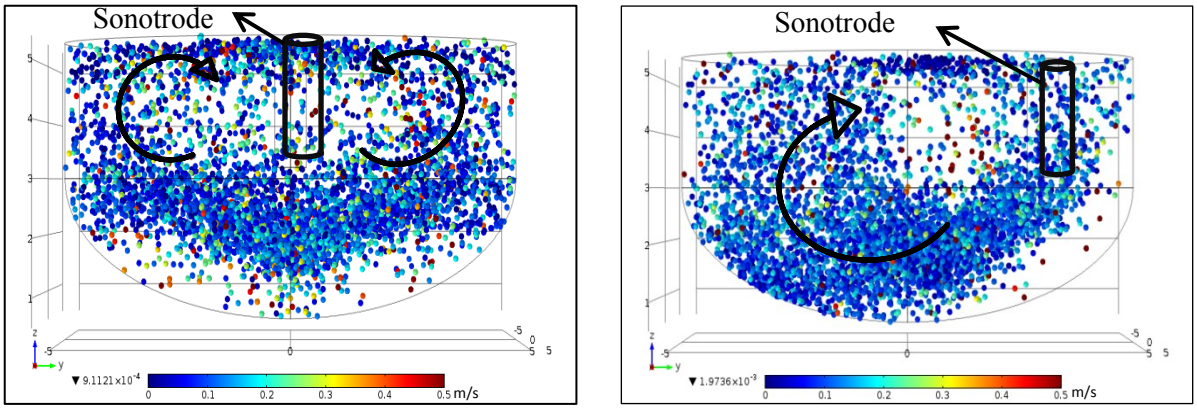


Figure 5.4. Simulation for the effect of sonotrode position on particle trajectories in reactor B at 40°C

For the center position of sonotrode in Figure 5.4 the particles are distributed symmetrically on both side of the transducer and are not settled on the reactor base. Particles trace a circular motion along the the path marked by arrows (Figure 5.4). However, in off-center position particles move away from sonotrode and tend to settle at the base. The motion traced by particles is marked with arrow. From the comparison it can be concluded that center position of the sonotrode should result in a better performance.

In the second step findings from the simulation results were then validated experimentally. For the validation of the simulation results two experiments were done with center and off-center positions of the sonotrodes. As can be seen in Figure 5.5, positioning in the centre (horizontally and vertically) gave the best results as in this case US is equally distributed into

reaction medium. Off-centre positioning of the sonotrode near to the wall gave lower reaction rate owing to unven distribution of US. In off center position enzyme particles start accumulating on the opposite side of the transducer. This diminishes the contact area of enzyme with reactants. This is in agreement with simulation results presented in Figure 5.4.

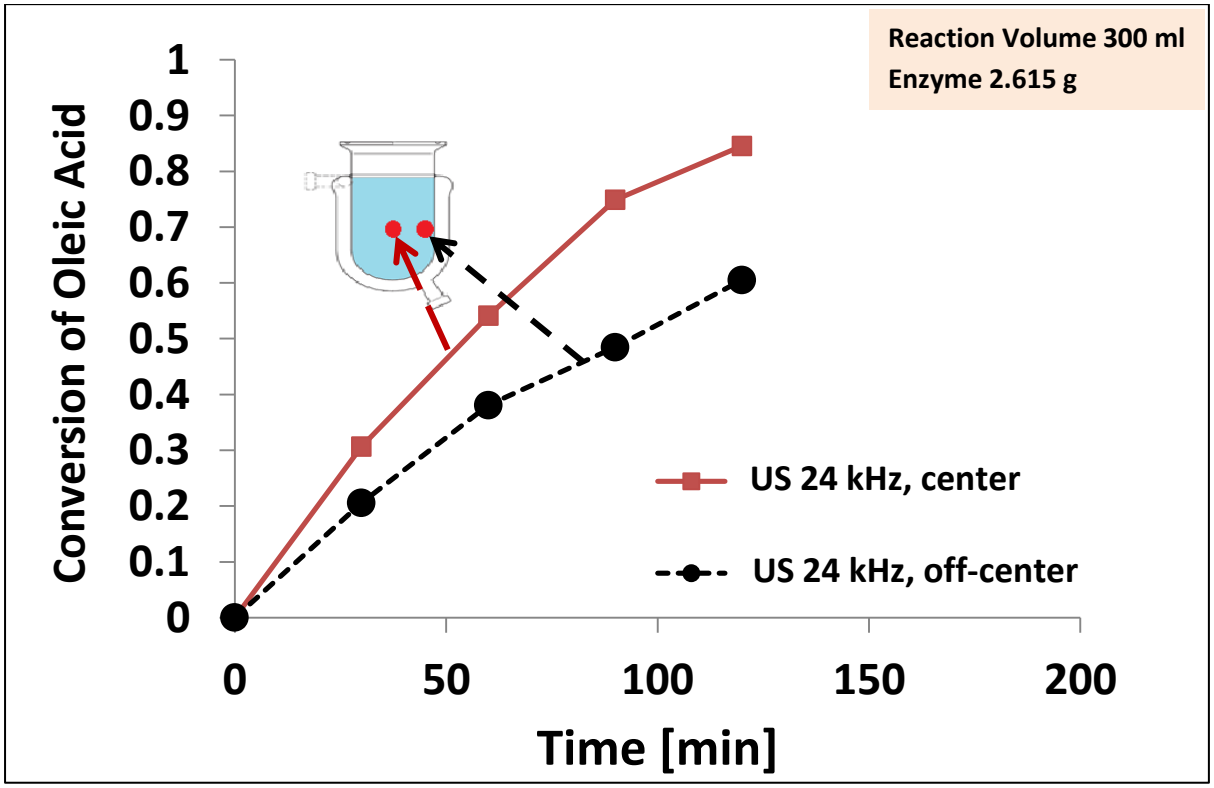


Figure 5.5. Effect of US position on oleic acid conversion at 40 °C

Results obtained with vertical variation of sonotrode position are shown in Figure 5.6 (horizontally the transducer was kept in center). As expected here again the middle position gives the best results. These findings confirm that selection of a suitable sonotrode position inside reactor is an important influencing parameter and should be considered while designing an ultrasonic reactor. These findings prove the significance of simulation results as the hydrodynamics information obtained from simulation results (Figure 5.4) gave a clear indication of the reaction behaviour.

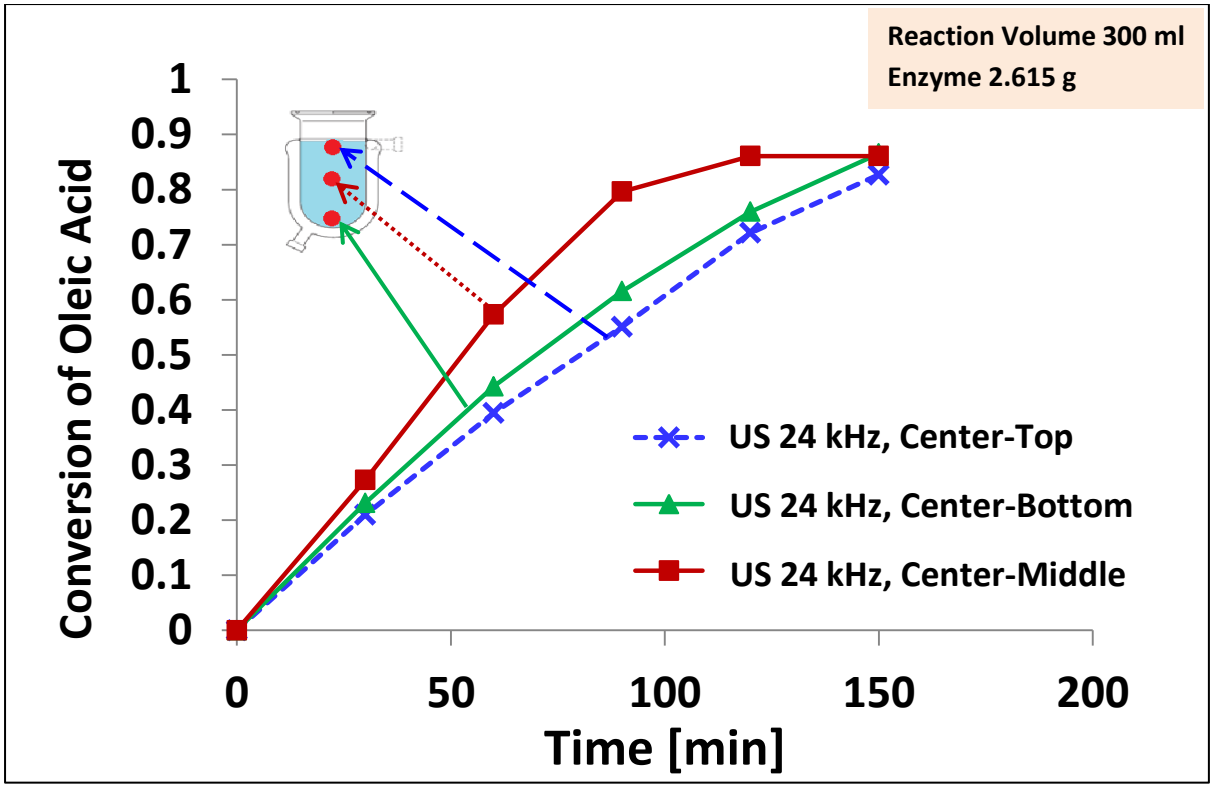


Figure 5.6. Effect of transducer position on oleic acid conversion at 40 °C

A major point in sonochemistry is reproducibility [25, 63]. It is of prime importance for scale up and transferability of the results to industrial scale. In the published literature this aspect has been so far largely overlooked. Therefore, in the present work special emphasis has been put to ensure the reproducibility of results. Moreover, if the data is not reproducible, the results obtained will lead to false conclusions. It also helps in establishing the reliability of analysis technique. In Figure 5.7 data from two experiments is presented as example. The process parameters were identical in both cases as mentioned in Figure 5.7. From Figure 5.7 it is obvious that the results are highly reproducible.

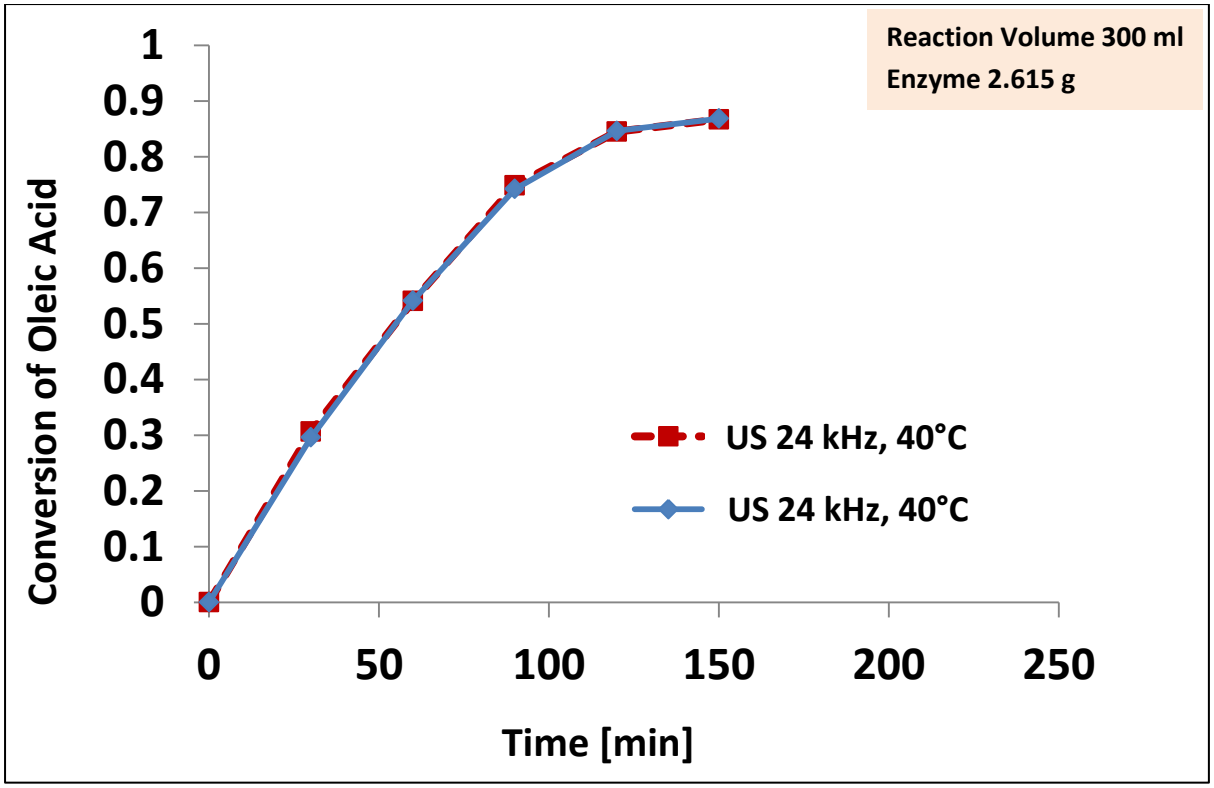


Figure 5.7. Reproducibility of results with cavitating US and immobilized enzymes

The results obtained for the variation of sonotrode position establish that with employed sonotrode the best reactor performance is obtained when it is placed in the middle of the reaction medium. Therefore, in next experiments sonotrode position was fixed in the middle of reaction medium. In Figure 5.8 a comparison of results with cavitating US and stirring for 40°C is made. This comparison shows that the reaction is running faster in case of cavitation. The enhancement of reaction rate in this case ($k_{US}/k_{stirring} = 2.2$) confirms the results literature data presented in Table. 1.1. This enhanced reaction rate is found only with cavitating US. This tells that cavitation is necessary for obtaining the positive benefits of US as with non cavitating US no improvement in reaction rate was obtained. However, this positive effect is accompanied with the damaging of enzyme carrier particles. This damaging of the particles is depicted in Figure 5.9. It is obvious that carrier particles got completely damaged due to cavitation. This results in a big disadvantage as the enzyme can not be reused in subsequent production cycles thus causing a big economic loss. This negative effect of cavitating US on enzyme carrier particles has not been reported in the literature before.

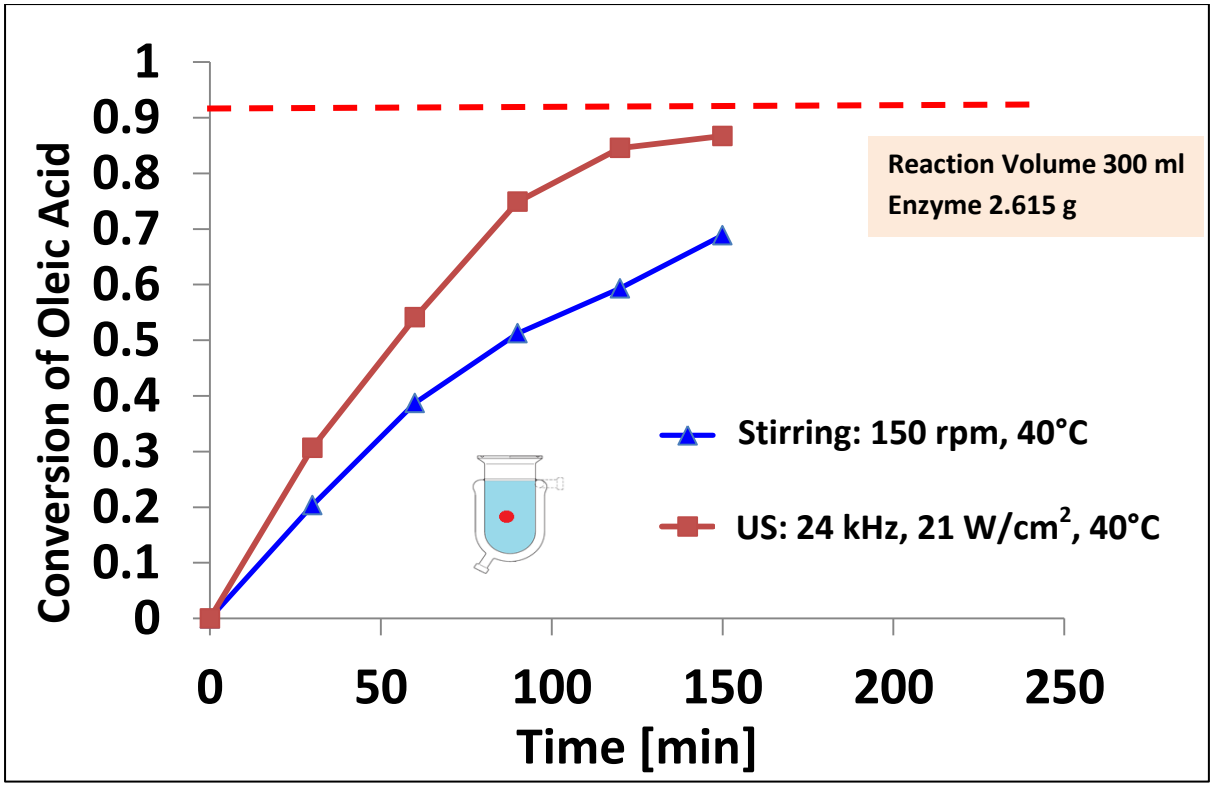


Figure 5.8. Effect of cavitating US on intensification of immobilized enzyme

Figure 5.9. Effect of cavitating US on immobilized enzyme

Unlike cavitation stirring did not damage the enzyme particles (Figure 5.9 left side). They can be reused for subsequent production cycles. Figure 5.10 shows a comparison of 40°C cavitation experiment with 60°C stirring experiment. The intensification effect from cavitation is not better than stirring at 60°C (red dashed curve). This means reaction rate can be increased by thermal energy without damaging the enzyme carrier particles. Moreover, US

consumed around 150 W of electrical power while stirrer consumes only 30 W at 150 rpm. This means ultrasonic reactor consumes much higher energy than stirring reactor. An indication about this fact has been reported by Gogate [20] also. Based on this fact it can be said that for studied enzyme and reaction application of US is not beneficial from economic viewpoint.

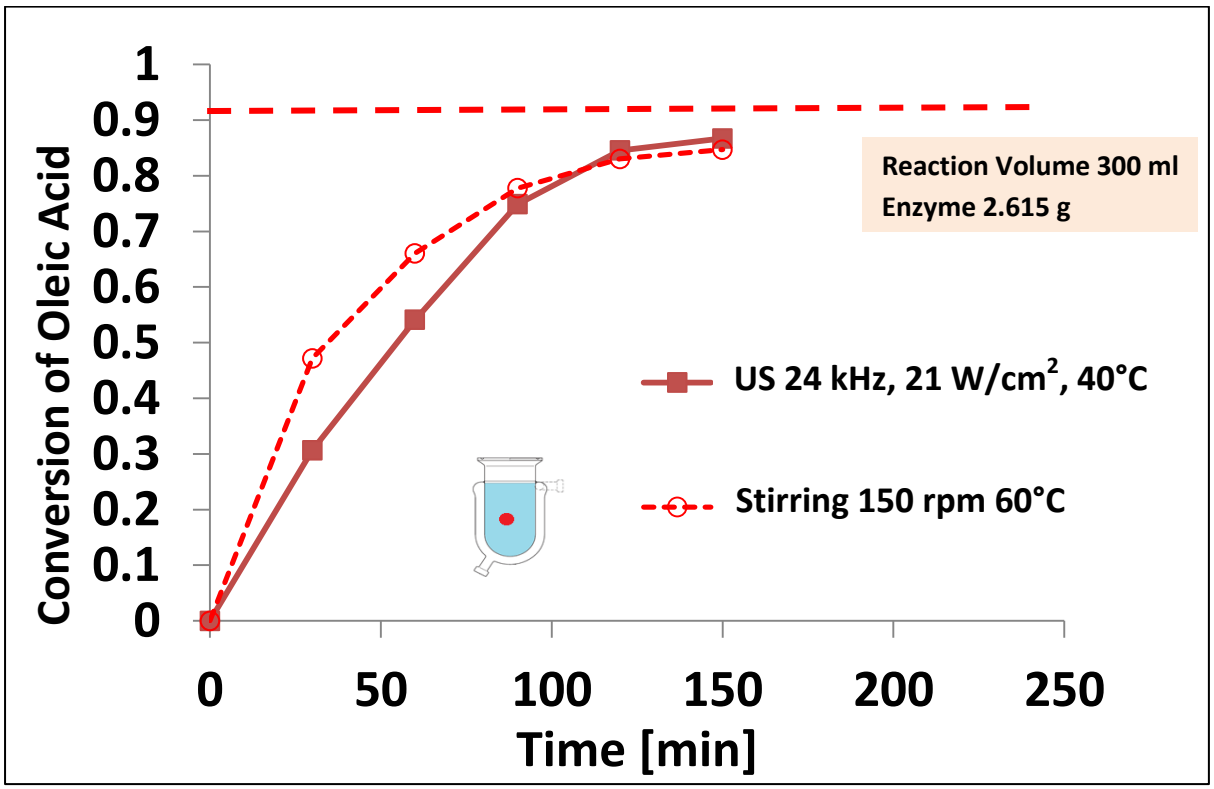


Figure 5.10. Comparison of immobilized enzyme intensification with stirring and US [40°C]

The results obtained with non-cavitating and cavitating US help in clarifying the observations made by Yasuda et.al. in section 1.1.1. Yasuda had concluded that cup horn systems give lower performance. However, the results obtained in present work have shown that it is the absence of cavitation (owing to higher frequency of 500 kHz) that is responsible for lower performance and not the cup horn type arrangement itself.

Based on the experimental and simulation results the following conclusions can be drawn

1. Ultrasound under non-cavitating conditions does not show any intensification effect for the studied example reaction.

2. The intensification of the studied reaction is accompanied by damage of carrier particles

3. In case of cavitating US position of the sonotrode inside reactor is an important factor to be considered as confirmed by simulation and experimental results.

4. Under cavitating conditions US intensifies the rate of the studied reaction.

5. Since US damaged the enzyme particles therefore, it is not possible to say that this intensification is as such a result of improved enzyme activity.

In this context comes the question regarding the mechanism of the observed intensification from cavitating US. Possible explanations that might help in understanding of this fact can be

- Improvement of the external mass transfer resistance
- Increased surface area of the carrier particles due to particle breakage from cavitation. In other words the reduction of internal mass transfer resistance
- Improvement in activity of enzyme itself

In order to test the validity of afore mentioned clarifications the significance of the external and internal mass transfer resistance is being analyzed in the next section.

5.4 Analysis of Mass Transfer Resistance for Example Reaction

In the first step mass transfer related experiments are done in absence of ultrasound using only stirring. As at 60°C the rate of reaction is the highest, therefore, it is appropriate to test the significance of mass transfer resistance at 60°C. Effect of both external and internal mass transfer resistances is tested by using two methods. For testing the external mass transfer

resistance effect two methods are used. In one method the effect of enzyme loading on the first order rate constant is determined and in the second method the carberry number is calculated. To test the significance of internal mass transfer resistance Weisz-Prater criterion is employed.

5.4.1 Analysis of External Mass Transfer Resistance

a) Effect of Catalyst Loading

Drauz has stated that for immobilized enzymes existence of external mass transfer resistance can be tested by measuring the effect of catalyst loading on reaction rate [71-74]. Figure 5.11 shows the effect of catalyst loading on first order rate constant at 60°C. The rate constant increased linearly with increasing the amount of enzyme from 1 to 3 gram. Enzyme quantity used in intensification studies was kept at 1% of the total reaction mass in order to be able to complete a given experiment in a defined interval of time due to practical reasons. Rate constant corresponding to 1% enzyme quantity i.e. 2.615 gram enzyme lies on the straight line in Figure 5.11. This finding confirms that with the selected enzyme amount reaction is not influenced by external mass transfer resistance. Probably this is the reason, behind ineffectiveness of non-cavitating ultrasound in producing intensification effect as micro streaming from US acts only on the external surface of the catalyst to enhance the turbulence.

a) Carberry number

Another method to test the significance of external mass transfer resistance is the calculation of Carberry number. Carberry number is defined as the ratio of observed reaction rate to the rate of mass transfer [75] i.e.

$$Ca = \frac{\text{Observed Reation Rate}}{\text{Rate of Mass Transfer}} \tag{5.2}$$

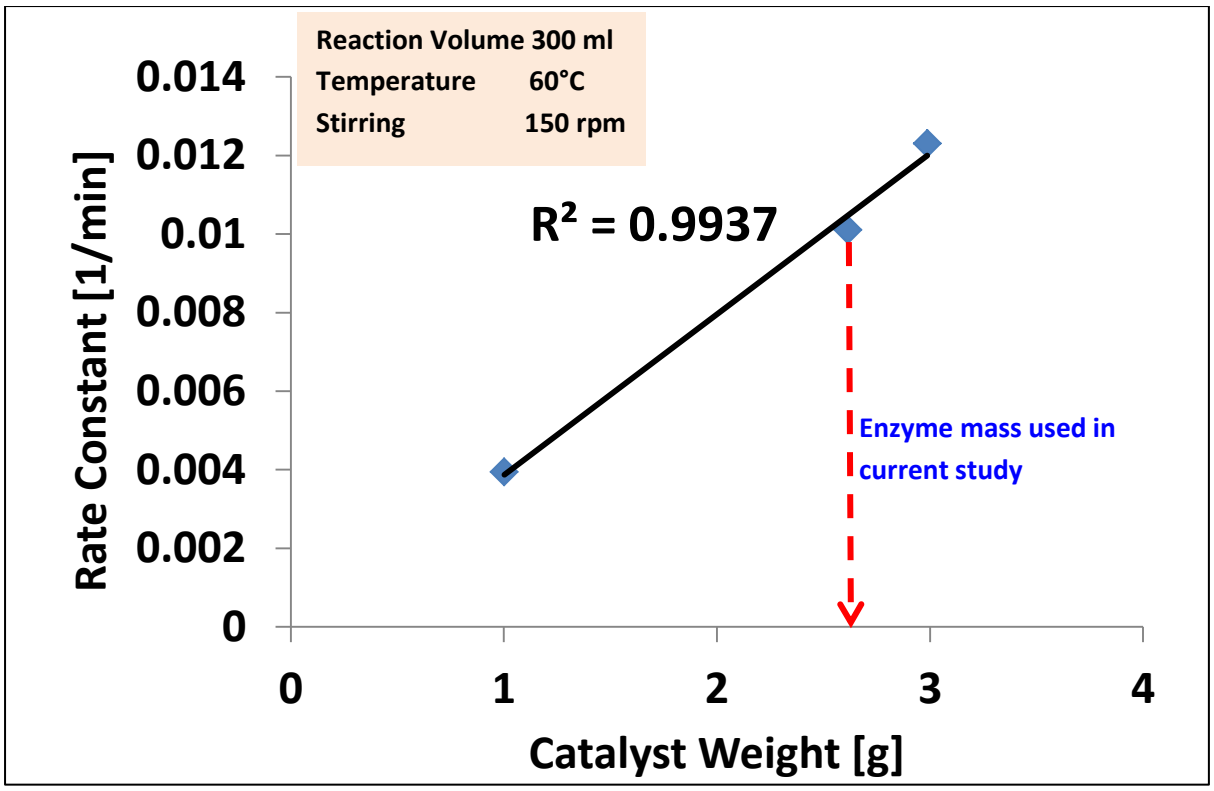


Figure 5.11. Effect of enzyme loading on the 1st order rate constant for the studied reaction

Using Carberry number effectiveness factor can be calculated as [76]

$$\eta = n(1 - Ca) \qquad (5.3)$$

Small values of η correspond to kinetic regime, whereas values close to one correspond to transfer limited regime [65/77].

The advantage in using the Carberry number is that it relies on observed reaction rate rather than intrinsic one. Observed rate was calculated from first order rate constant determined according to the procedure defined in Figure 4.12. Mass transfer coefficient was calculated using Eq. 5.4

$$k_{ls} = \frac{Sh\, D}{d_p} \tag{5.4}$$

Sherwood number was calculated using Eq.5.5 as

$$Sh = 2 + 0.6\ (Sc)^{1/3} (Re_p)^{1/2} \tag{5.5}$$

Schmidt (Sc) and Reynolds (Re) number were calculated according to following equations

$$Sc = \frac{\mu}{\rho D} \tag{5.6}$$

$$Re_p = \frac{d \rho v_s}{\mu} \tag{5.7}$$

Reynolds number involved here is particle Reynolds number and requires the slip velocity between particles and fluid. This slip velocity comes from the simulation results of section 4.1.5. Using the above mentioned equations the calculation of Carberry number is shown in Table 5.2. The calculated value of Carberry number comes out to be 0.054 (with stirring only), which corresponds to an effectiveness factor of 0.95 (Table 5.2). 95 percent effectiveness of mass transfer corresponds to negligible mass transfer resistance. This means in the studied reaction influence of external mass transfer resistance can be neglected under the conditions used in experiment.

Table 5.2 Calculation of Carberry number*

C_{OAC}[mol/l]	k [1/s]	$(r_v)_{obs}$ [mol/m^3/s]	Sc	v_s [m/s]	Re_P	Sh	k_{ls} [m/s]	Ca	η
2092.5	0.0002	0.4185	14045	0.025	3.4	46.9	3.75E-5	0,055	0.95

*Properties used for the calculation of Carberry number are given in Appendix F

Second aspect of the mass transfer related effects is the testing of internal mass transfer resistance and will be discussed in the next section.

5.4.2 Analysis of Internal Mass Transfer Resistance

Although looking the pore size for Lipozyme-435 (315-1000 nm) it is unlikely that US can play any role in improving the internal mass transfer rate but for the sake of clarity this effect is also evaluated. Weisz-Prater criterion is widely used for determining the existence of the internal mass transfer resistance [75, 78, 79]. According to this criteria, reaction is free from internal mass transfer limitation if

$$R_p^{\,2}\,\frac{(\mathrm{r_V})_{\mathrm{obs}}}{\mathrm{D_{eff}}\,C_{OAC}} < 1 \tag{5.8}$$

Diffusivity in Eq. (5.8) is calculated according to Wilke-Chang equation [80]. When reaction is not limited by external mass transfer, surface concentration is equal to bulk concentration. Using data from Table 5.2 the calculated value of Weisz-Prater modulus for oleic acid n-hexanol esterification using an average particle size of 0.6 mm is 0.45. This means the criterion for internal mass transfer resistance according to Eq. 5.8 is fulfilled and the influence of internal mass transfer can be neglected.

Based on the previous discussion it can be concluded that oleic acid-hexanol esterification reaction is neither limited by external nor by internal mass transfer resistance.

Summing up the analysis of mass transfer resistance it can be said that the intensification observed for the case of cavitating US cannot be explained on the basis of the improvement in mass transfer rate. The other possibility for this intensification can be the improved activity of enzyme molecule as stated by Jadhav [20] and Bhasarkar [21]. For this it is necessary to test the effect of US on free enzyme, so that all effects arising from immobilization can be eliminated. This will be the topic of discussion in next chapter.

6 Analysis of Intensification with Free Enzymes

Tests regarding intensification effect with immobilized enzymes were discussed in the last chapter. According to the proposed research methodology in Table 1.2 tests with free enzyme shall be discussed in this chapter. As mentioned in section 2.1 Lipozyme CALB L was selected as example enzyme. Use of free enzyme will help in studying the behavior of enzyme under US in absence of any influences arising from immobilization. Comparison of data from immobilized and free enzymes might help in determining the conditions under which application of US is effective in intensifying the enzyme catalyzed reaction. This can also help in understanding the mechanistic aspects of the underlying phenomenon. As in the literature there are some reports that optimal temperature for functioning of Lipozyme CALB L is 40°C [10] therefore, initial tests are conducted at 40°C in order to avoid the possible damage to enzymes.

6.1 Effect of Stirring on Reaction Rate with Free Enzyme

For Lipozyme-435 test results under sonication were compared against stirring. This helped in clearly identifying the net positive effect brought by US. In a similar manner Lipozyme CALB L tests under stirring are taken as the base case. Reactor B was used for tests with stirring. Reaction volume was kept constant at 300 ml (254.3 g). Enzyme amount was kept constant at 1.4 weight percent of total reactants weight (except where mentioned). It is important to mention here that enzyme amount corresponds to enzyme solution obtained from Novozymes and not the active lipase.

In Figure 6.1 effect of stirring on reaction with of free enzyme is shown. In stirring experiment reaction mixture was fed into reactor and stirring was turned on at 500 rpm. Higher rpm was selected to ensure proper mixing of CALB L solution as at lower stirring speed problems regarding settling of CALB L solution inside reactor were observed. Figure 6.1 shows that with stirring no conversion of oleic acid was observed even after a reaction time of 300 minutes. Looking this inactivity of enzyme a second experiment was performed where cavitating US was introduced into reaction medium to activate the enzyme. As can be seen by dashed curve in Figure 6.1 reaction did not proceed even with US. At 230 minutes 3.5 g of

water was added (as enzyme amount is also 3.5 g) with a view to activate enzyme (as discussed in section 2.1). When the next sample was taken after 20 minutes of water addition a conversion of 17% was obtained. Using 600 kHz US (where no cavitation was observed), reaction did not took place even upon addition of water therefore, non-cavitating US was not used in further investigations.

Looking the role of water in activating enzyme it was necessary to repeat the experiments with initial addition of water.

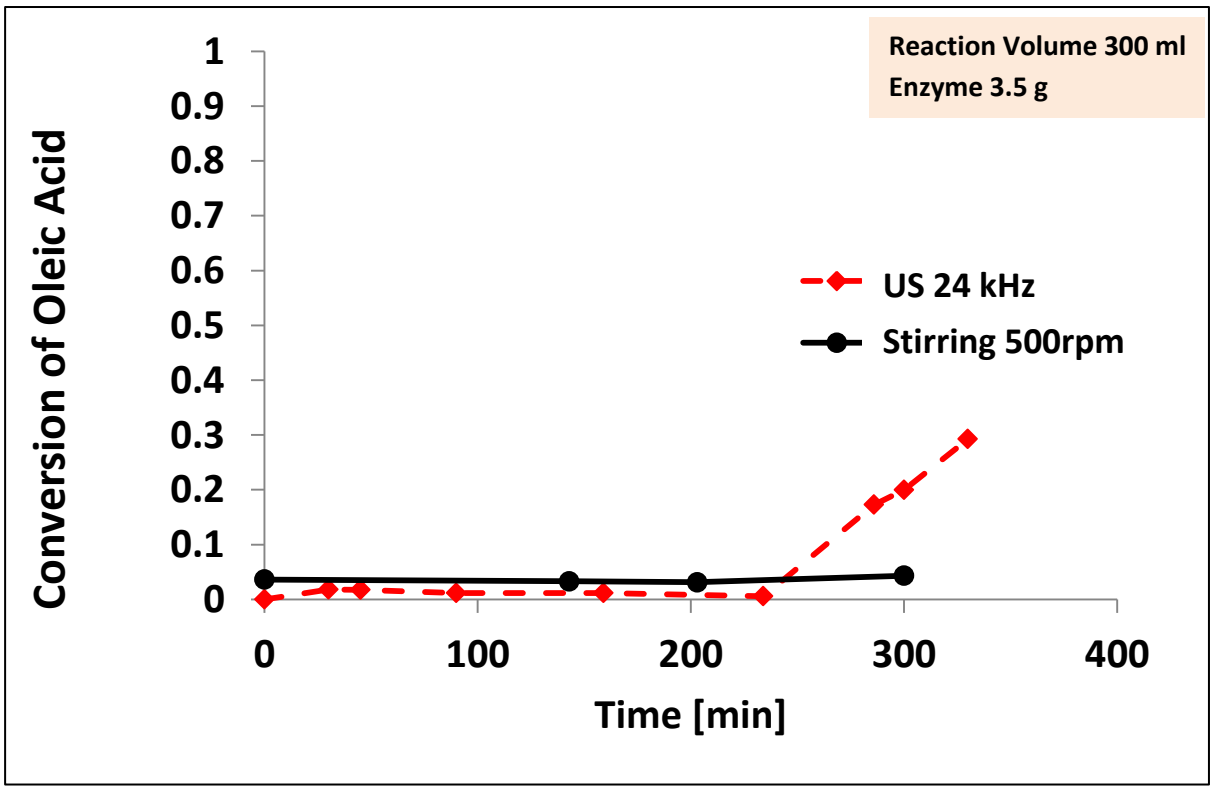


Figure 6.1. Effect of US and stirring on intensification of free enzyme at 40 °C

This result leads to the fact that water present in original Lipozyme CALB L sample (see enzyme concentration in section 2.1) was not sufficient to activate it. Therefore, it needs to be added externally. Looking the necessity of external water addition it was necessary to retest the effect of stirring on free enzyme functioning with added water. From Figure 6.2 it is obvious that rate of reaction with Lipozyme CALB L is very slow although water was added to activate the enzyme. Even after 5 hours of reaction time conversion is below 10%. Unlike Lipozyme CALB L, Lipozyme-435 shows very high activity under stirring and without any external

addition of water. In section 2.1 it was mentioned that lipase needs water to transform into active conformation (lid open) and it is possible to immobilize this enzyme on carrier material in active conformation (lid open). This result confirms the fact that in Lipozyme-435 the enzyme is already in active conformation and therefore, shows higher reaction rate.

The activity of Lipozyme CALB L measured with stirring will be used for comparison against tests from US.

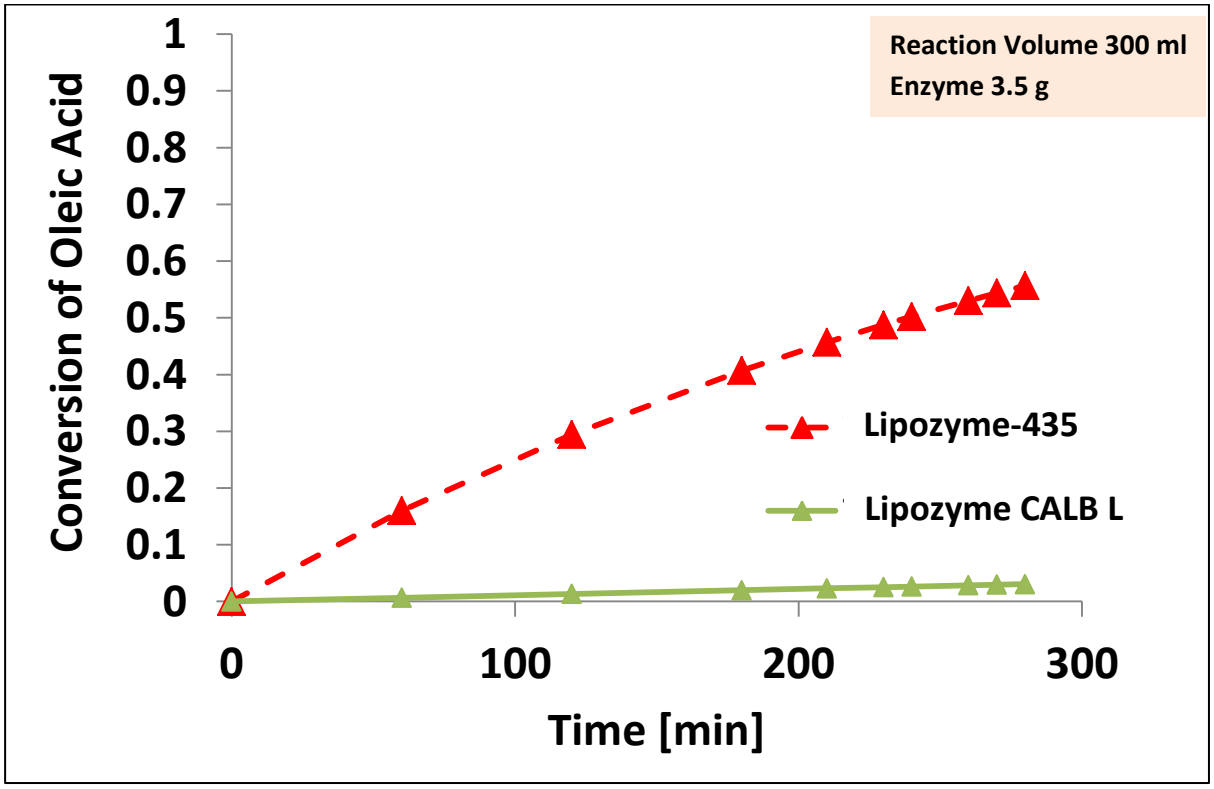


Figure 6.2. Comparison of reaction rate with free and immobilized enzyme under stirring at 40°C

6.2 Effect of Cavitating US on Intensification

Results in Figure 6.1 showed that US makes enzyme active upon addition of water only. Therefore, tests with cavitating US were done with water addition at the start of reaction. In this initial experiment 3.5 g of water was added just to prove the effect but later on detailed and systematic experiments were done in order to determine the minimum amount of water

required to activate the enzyme. Reaction was sonicated with sonotrode H14 at an amplitude of 25 um (lowest possible amplitude with H14) and 21 W/cm^2 intensity.

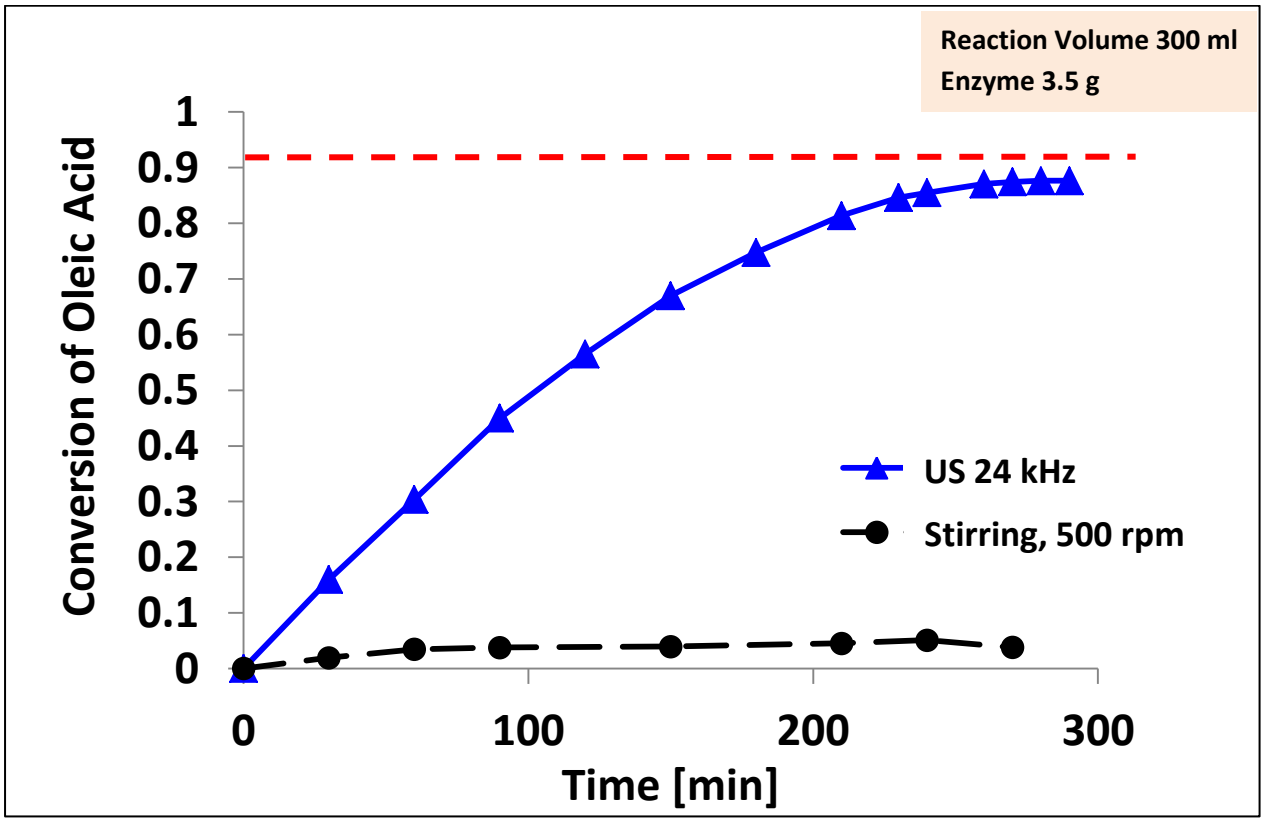


Figure 6.3 Effect of cavitating US on Lipozyme CALB L at 40°C

Figure 6.3 compares the reaction behavior with CALB L observed under stirring and cavitating US. From the comparison it is obvious that in comparison to stirring cavitating US remarkably intensified the reaction. This remarkable intensification effect from US has not been reported in any literature before and was first observed during experiments in context of a master thesis supervised by author [Nr. 7, P 128]. As this is an important finding therefore, it is necessary to test its reproducibility. Figure 6.4 shows that this intensification obtained with US is highly reproducible. This observation leads to the fact that US alone does not cause activation of the native enzyme molecules rather it is the addition of water that activates enzyme. This means for the studied reaction US activates enzyme through a secondary effect i.e. by dispersion of water. This is contradictory to the observations made by Bhasarkar [21] and Jadhav [20] where they claimed that US activates the enzyme by causing a conformational change in secondary structure of enzyme. Though the enzyme and the reaction studied was different than used in present work.

Once it is observed that cavitating US improves reaction rate with Lipozyme CALB L, it is now important to investigate the observed phenomenon in detail. Important aspects to be investigated are the underlying mechanism of the observed phenomenon, role of cavitating US, effect of temperature, amplitude, pulse US and post sonication stability/reusability of enzyme. Aforementioned aspects are investigated in detail and systematic manner in the following.

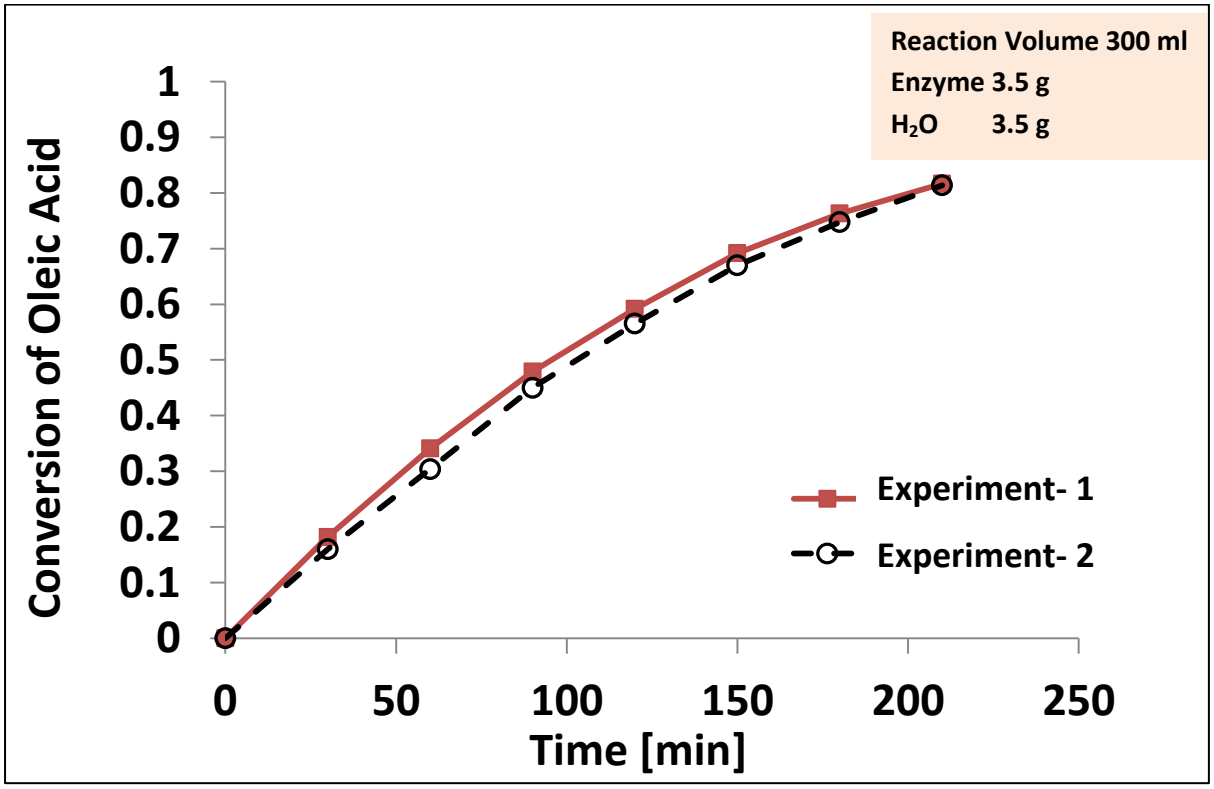


Figure 6.4. Reproducibility of results for reaction with free enzymes at 40°C and 24 kHz

6.3 Effect of Water Addition on Reaction Intensification

In the previous experiments amount of water added was merely based on a rough judgment i.e. 3.5 g of enzyme will require 3.5 g water as the prime objective was merely to observe the influence of water addition on intensification of enzymes. But it is important to test the effect of water quantity on observed intensification in order to determine the optimal water amount required to activate enzyme. Polaina et al. [38] have also reported that a critical amount of

water is necessary for maintaining the active conformation of lipase enzyme. Too much water can hinder the esterification reaction. Therefore, it was decided to experimentally determine the minimum amount of water required for proper functioning of the studied enzyme.

In Figure 6.5 effect of water on reaction progress is shown. The mass of water given in legend belongs to the water mixed with free enzyme before adding to the reaction mixture. With 1g of water addition the reaction is slower in comparison to 1.5 g water addition. However, when water amount is increased further to 2 g no improvement is observed. From this it can be concluded that for 3.5 g Lipozyme CALB L the minimum amount of water required is between 1 and 1.5 g. This 1-1.5 g is in addition to the water already present in enzyme sample provided by Novozymes A/S (40%). Therefore, it can be said that minimum amount of water required is 0.7-0.8 g/g enzyme (enzyme here means enzyme mixture from Novozymes A/S). In order to show that the minimum amount of water required is dependent on the amount of enzyme used, another experiment was done. In this experiment enzyme amount was doubled i.e. around 7 g. To ensure that the results from both experimental sets are comparable substrate to enzyme ratio was kept constant (substrate amount was also doubled i.e. 600 ml (508.6 g)). Figure 6.6 shows that 1.5 g water is not sufficient to activate all enzymes in this case. Adding 3 g water for 7 gm enzyme gave same conversion as with 3.5 gm enzyme. Therefore, it can be concluded that amount of water required is proportional to the number of enzyme molecules present (in other words number of active sites present). Therefore, the amount of water required to activate enzyme remains 0.7-0.8 g/g enzyme. For the following experiments water to enzyme ratio was kept constant at 0.8 g/g enzyme.

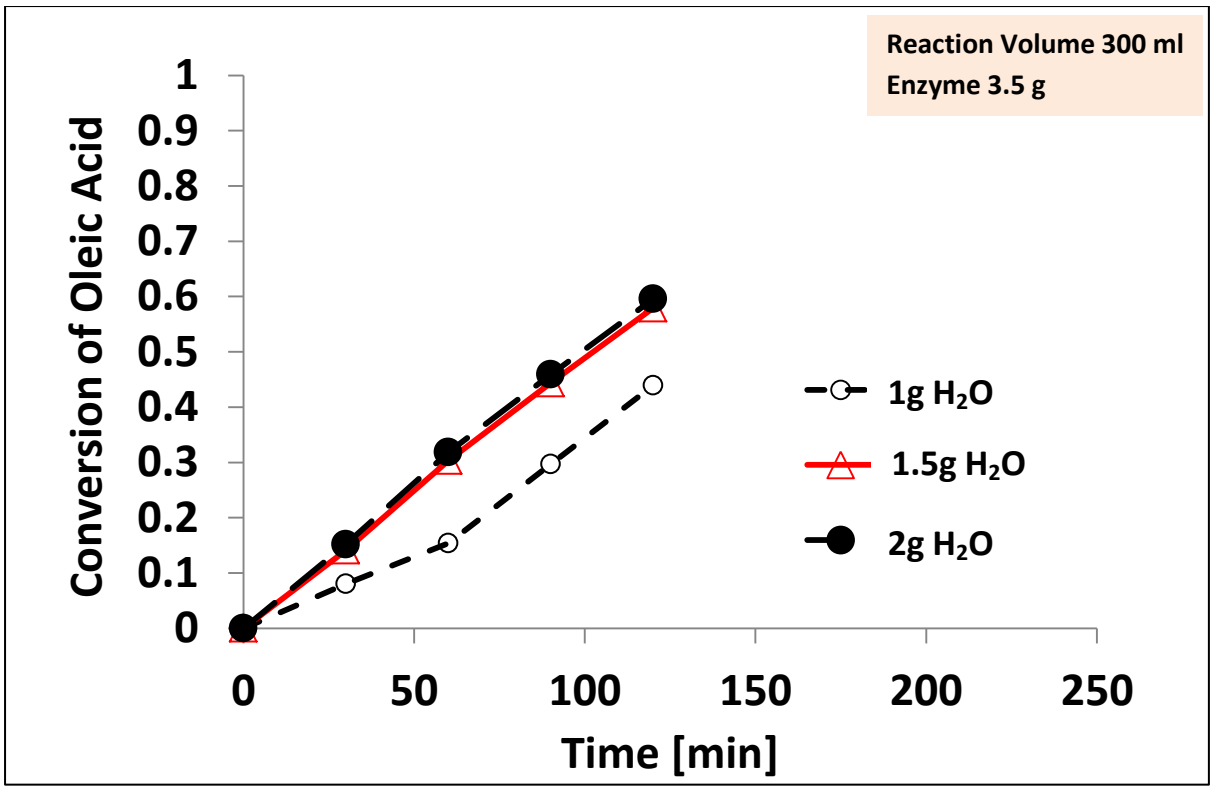


Figure 6.5. Effect of water addition on reaction intensification with Lipozyme CALB L at 40°C and 24 kHz US

It is important to mention that in the studied esterification reaction water is itself produced during reaction (as it is one of the reaction products). Therefore, it is not necessary to add more water during the reaction. However, for other reaction types where water is not produced (e.g. transesterification), it might be necessary to add more water during reaction in order to make up any losses of water from evaporation. This is necessary to maintain the active configuration of enzymes.

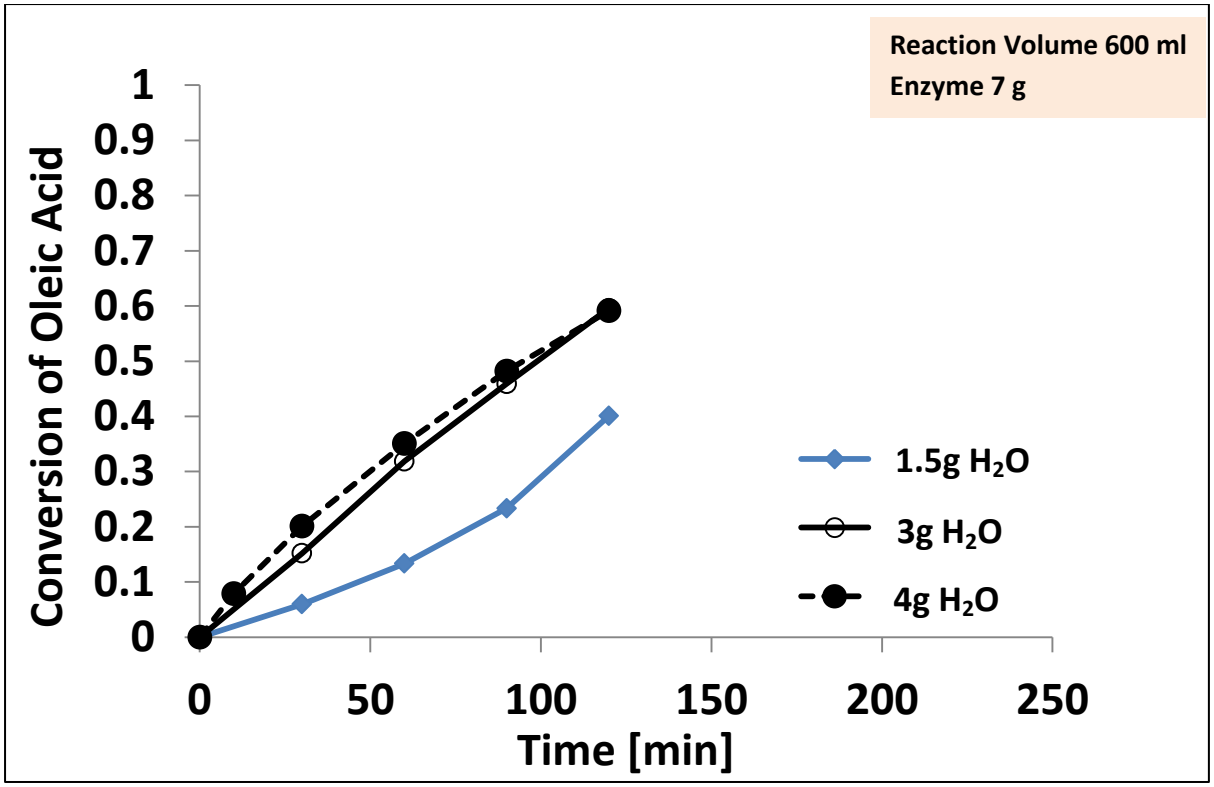


Figure 6.6. Dependence of water requirement on enzyme amount 40°C and 24 kHz US

As Cao has mentioned that lipases can be immobilized on carrier material in active conformation i.e. lid open and, therefore, presence of water is not necessary [81]. In order to test this fact two more experiments were done where water was added to Lipozyme-435 catalyzed reaction. The results are shown in Figure 6.7. The dashed curve represents reaction with immobilized enzyme under cavitating US with no addition of water. The solid curve represents the experiment with same conditions but with addition of 3ml water. It can be seen that unlike free enzyme addition of water in immobilized enzyme did not bring the corresponding intensification effect as observed with free enzymes. In fact the reaction rate was lower with water addition. As water is also being produced by reaction therefore, added water hinders the reaction by shifting equilibrium towards reactant side.

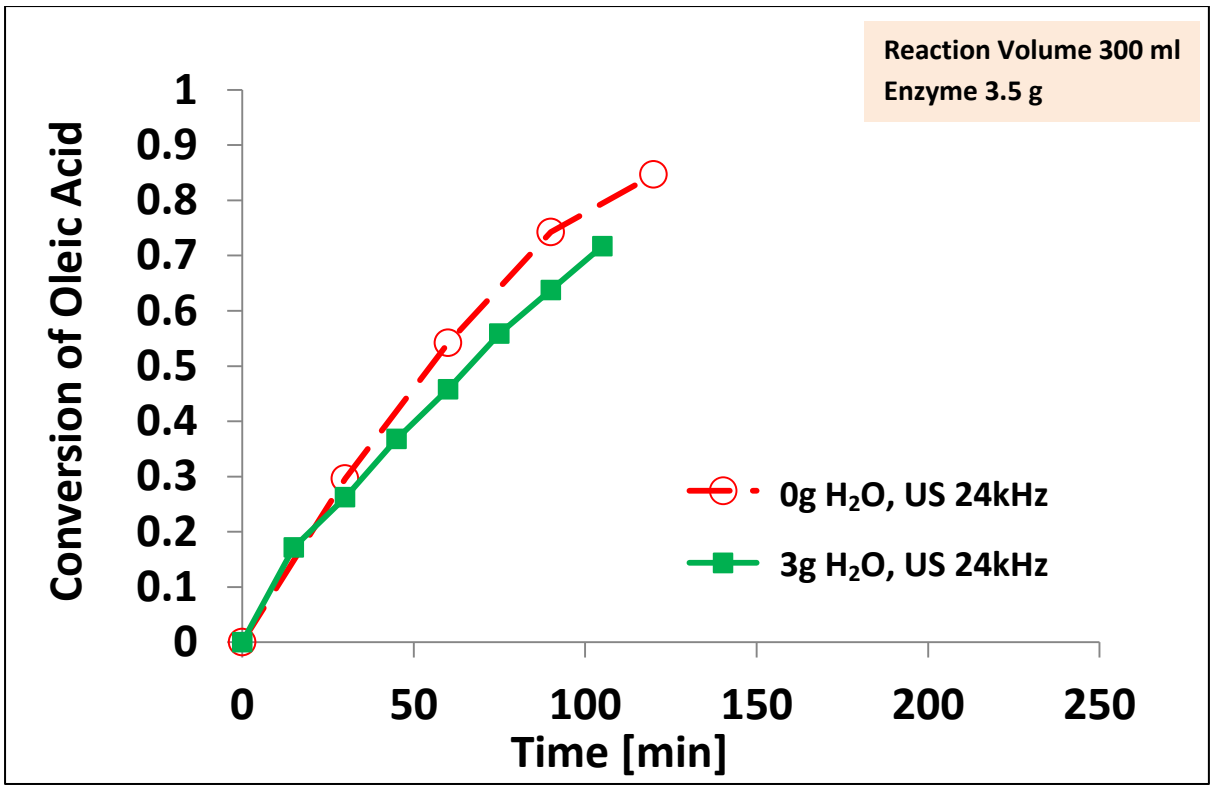


Figure 6.7. Effect of water addition on immobilized enzyme at 40°C

In section 2.1 it was discussed that there are contradictory remarks regarding the presence of a lid in Lipozyme CALB L. However, the findings of this study favor the concept that active site in Lipozyme CALB L is covered by lid (or at least presence of water is vital for functioning of Lipozyme CALB L).

6.4 Role of Cavitation in the Reaction Intensification

After clarifying the role of water the next aspect to be studied is the role of cavitation in the intensification of the esterification reaction with Lipozyme CALB L. There are two motives of this test

1. First motive is to determine if cavitation is necessary for maintaining observed intensification?

2. Second motive is to test if it is possible to use US only in the starting phase of the reaction to activate enzyme. Once the enzymes are activated and reaction starts, US should be turned off. This will help in saving the energy required by transducers.

With these motives an experiment was performed using both ultrasound and stirring. The setup was arranged in a manner that ultrasound and stirring can be turned on/off independent of each other. The results for this experiment are given in Figure 6.8. The dashed curve represents experiment with continuous sonication while solid curve represents the experiment where either ultrasound or stirring was kept active at a given time. Figure 6.8 shows that as soon as ultrasound is turned off and stirring is turned on the conversion curve get flatter indicating that the reaction gets markedly slower. Upon turning on ultrasound again, the conversion curve becomes steeper again indicating that reaction is running faster. From these observations it is obvious that presence of cavitation is necessary for obtaining intensification effect in studied reaction and enzyme.

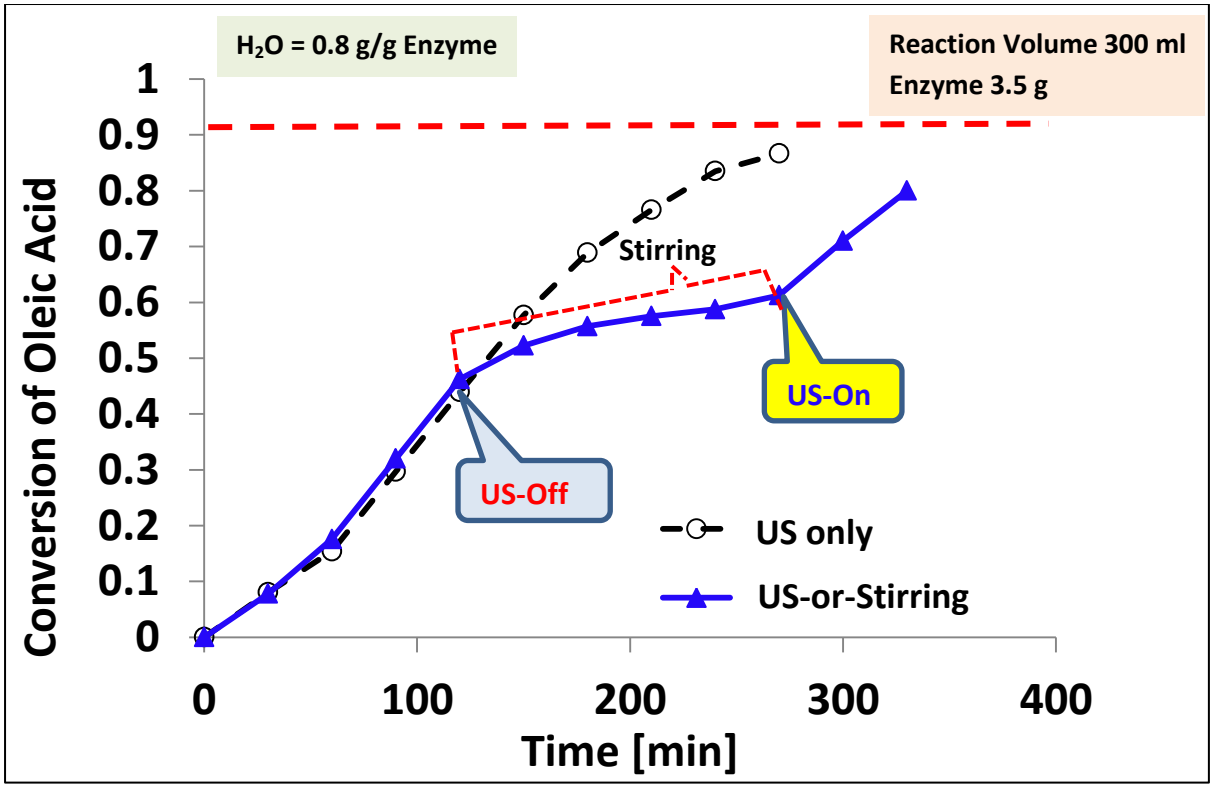


Figure 6.8. Role of cavitation in Intensification at 40°C with 24 kHz US

This important observation leads to the following facts

- It is the presence of cavitation that is necessary to intensify the reaction. Rapidly imploding cavitation bubbles create intense turbulence which might be helping to ensure the dispersion of water to enzyme active sites, thus enabling them to function at their full activity. It is important to mention that the active conformation of enzyme is achieved only upon addition of water and not from US itself (Figure 6.2).

- It is observed that upon turning off US conversion curve gets flatter; this indicates that the configuration of most of enzymes changes back to inactive configuration. This means that conformational change brought about by US is not permanent and that enzymes turn back to their original conformation upon turning off the US. This means for the studied enzyme the conformational change is reversible. This result is contradictory to the results of Jadhav [20].

- As water is one of the reaction products in the studied esterification reaction therefore, buildup of excess water near enzyme active site might be hindering the reaction. Cavitation probably will be helping to drive away the excess buildup of water near the enzyme active site thus helping to drive the reaction in the forward direction. This factor is further favored by the fact that in Lipozyme-435 CALB L is already in active conformation but intensification effect is still observed. Therefore, elimination of excess water buildup near enzyme active site can be considered as a possible phenomenon

6.5 Effect of Temperature on the Reaction Intensification

With Lipozyme-435 it was shown that using stirring it was possible to achieve a reaction rate equivalent to US by raising the reaction temperature only. With this background it was necessary to test if it is the case with Lipozym CALB L also? With this perspective effect of temperature on reaction rate with Lipozyme CALB L was tested using optimum quantity of water (determined in section 6.3). As shown in Figure 6.9 reaction performance under stirring at 60 °C is again very poor when compared with US (40°C). This leads to the conclusion that

for Lipozyme CALB L in studied reaction it is not possible to achieve higher reaction rate by increasing temperature only. Use of cavitating US is necessary to intensify the reaction. Figure 6.10 shows that increase in temperature improves intensification observed with ultrasound. Unlike reported in literature [82,11] enzyme maintained its functioning throughout the reaction even at higher temperature under cavitating ultrasound. However, determining the number of cycles for which enzymes can be reused without loss of catalytic activity is important from process economics viewpoint. This will be discussed in section 6.7. This increase of enzyme activity by temperature is attributed to the conformation change in enzymes structure. Due to thermal energy enzyme structure gets looser making enzyme active site easily accessible to substrate [76/83].

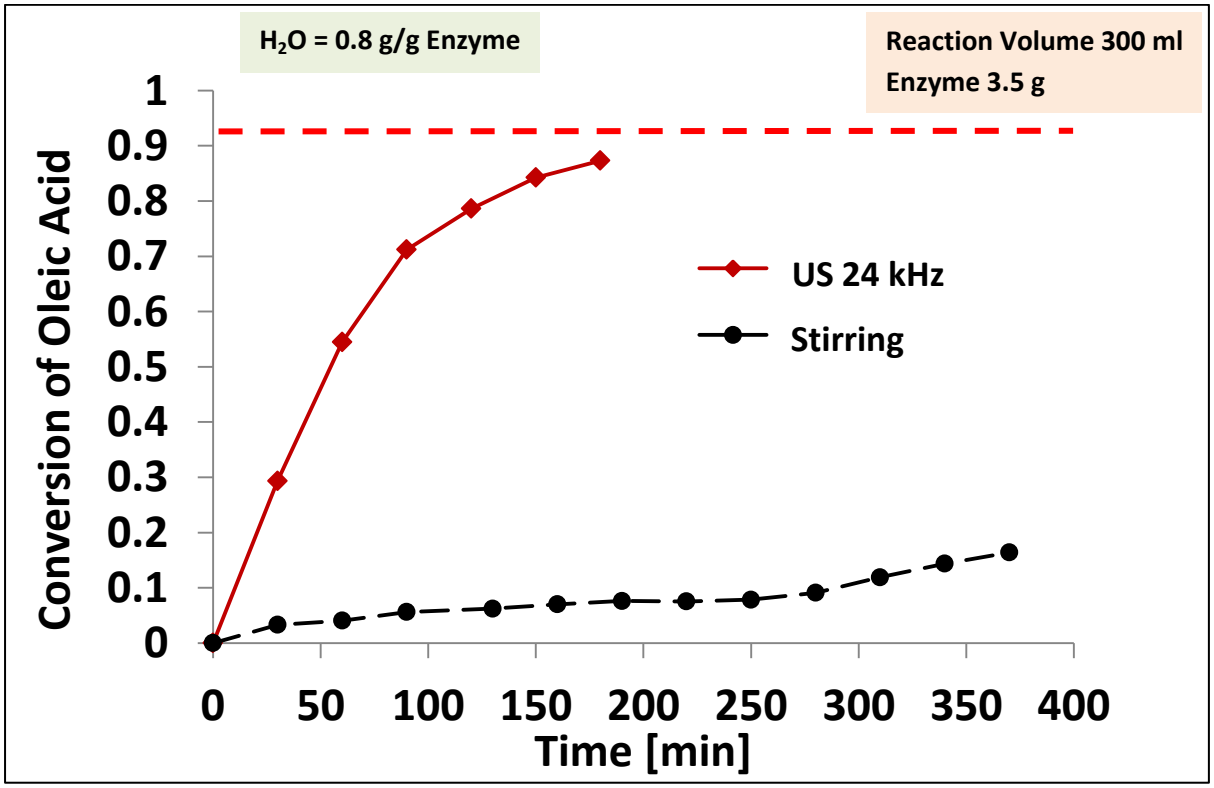


Figure 6.9. Comparison of CALB L functioning with US and with stirring at 60°C

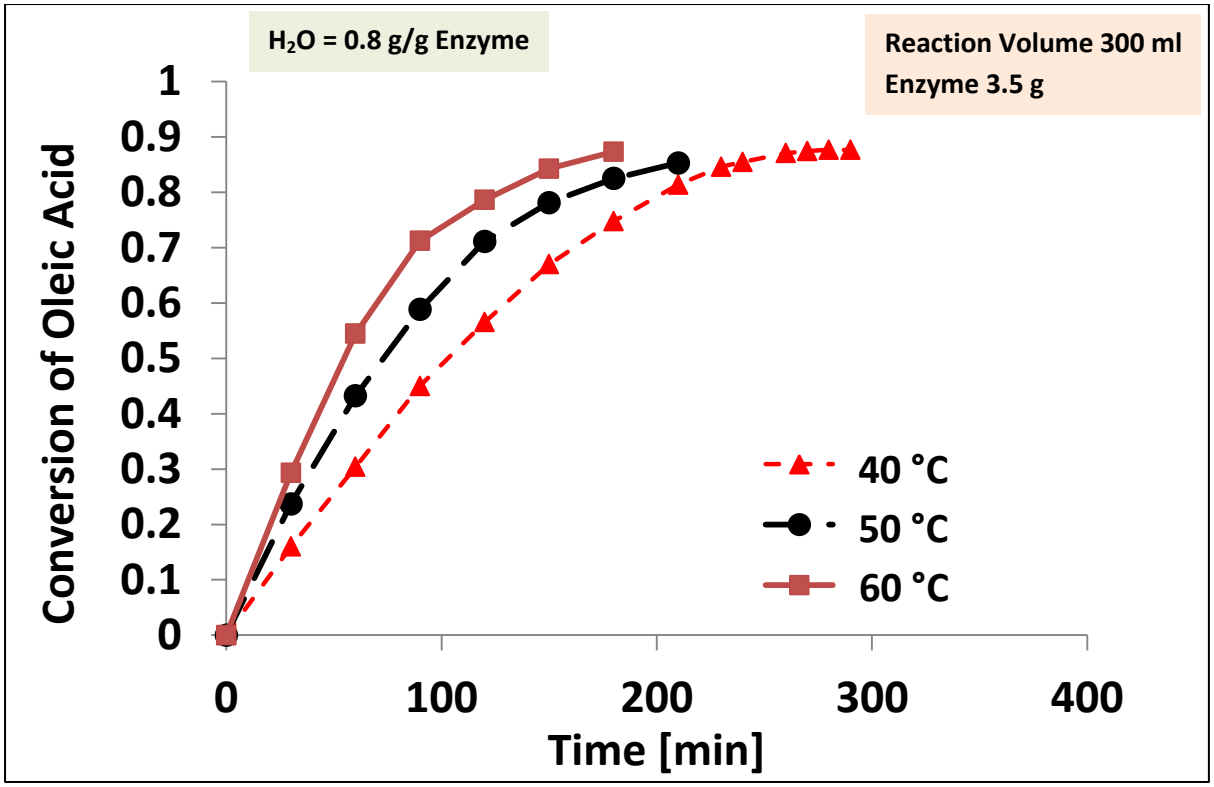


Figure 6.10. Effect of temperature on CALB L functioning with 24 kHz US

6.6 Effect of Amplitude on the Reaction Intensification

After testing the influence of temperature, next parameter to be tested is the amplitude. In section 2.2 it was mentioned that in order to create cavitation minimum amplitude is required to be achieved. Increasing amplitude beyond threshold value would create stronger cavitation effect and would also require more power. There will be an upper limit in the vibrational amplitude above which the transducer will suffer mechanical fracture but before this occurs there will be a reduction in the vibrational energy that a transducer can transfer to the liquid. The generation and collapse of cavitation bubbles is the source of energy for intensification of reaction but if a large number of cavitation bubbles are formed in front of the emitting surface of the transducer these can act as a barrier to the transfer of acoustic energy and dampen the power transmission to the bulk of the reaction medium. When the emitting surface is driven at higher amplitudes the physical motion of the surface travels too fast for reaction medium to remain in contact with it so a gap is generated between transducer and liquid and the majority of the acoustic energy is lost. This is termed decoupling. For this

reason there will be a maximum amount of energy that can be transmitted efficiently into the liquid medium because of cavitation bubble shielding and ‘‘decoupling’’. Generally, for any sonochemical process, there will be an optimum power for maximum effect. This will depend on a range of conditions but will mean that power optimization can lead to a considerable saving in the overall economics of the process [84].

For all previous experiments in this study amplitude was kept constant at 25 µm. However, it would be interesting to observe the effect of varying amplitude on reactor performance as operating at lower amplitudes would help in saving energy. As minimum possible amplitude with H 14 sonotrode was 25 µm. For sonication at even lower amplitudes another transducer (H40) was used. With this sonotrode it was possible to sonicate the reaction medium at amplitudes as lower as 2.4 µm. In Figure 6.11 effect of amplitude on reactor performance is shown. When amplitude is decreased from 25 µm to 7.2 µm reaction behavior remains unchanged. This means, operating the reactor at amplitudes beyond 7.2 µm is wastage of energy. With further decrease in amplitude to 2.4 µm a slight decrease in performance is observed. This means amplitude of 2.4 µm is already creating enough cavitation to keep enzyme active. A further decrease in amplitude was not possible due to limitation of the device.

Energy consumption at different amplitudes is calculated from equation given in Appendix H. Energy comparison for tested amplitudes is given in Table 6.1. In this table, 25 µm is taken as reference case and therefore, set to 100% energy consumption. It is obvious that by decreasing amplitude the energy consumption also decreases. Energy consumption at 7.2 µm is 17% less than 25 µm. Likewise by operating at 2.4 µm amplitude energy consumption is 20% less with minimal loss of performance. This proves the fact that operating an ultrasonic reactor at amplitude higher than required is merely wastage of energy as this does not provide any advantages with regard to the reaction intensification.

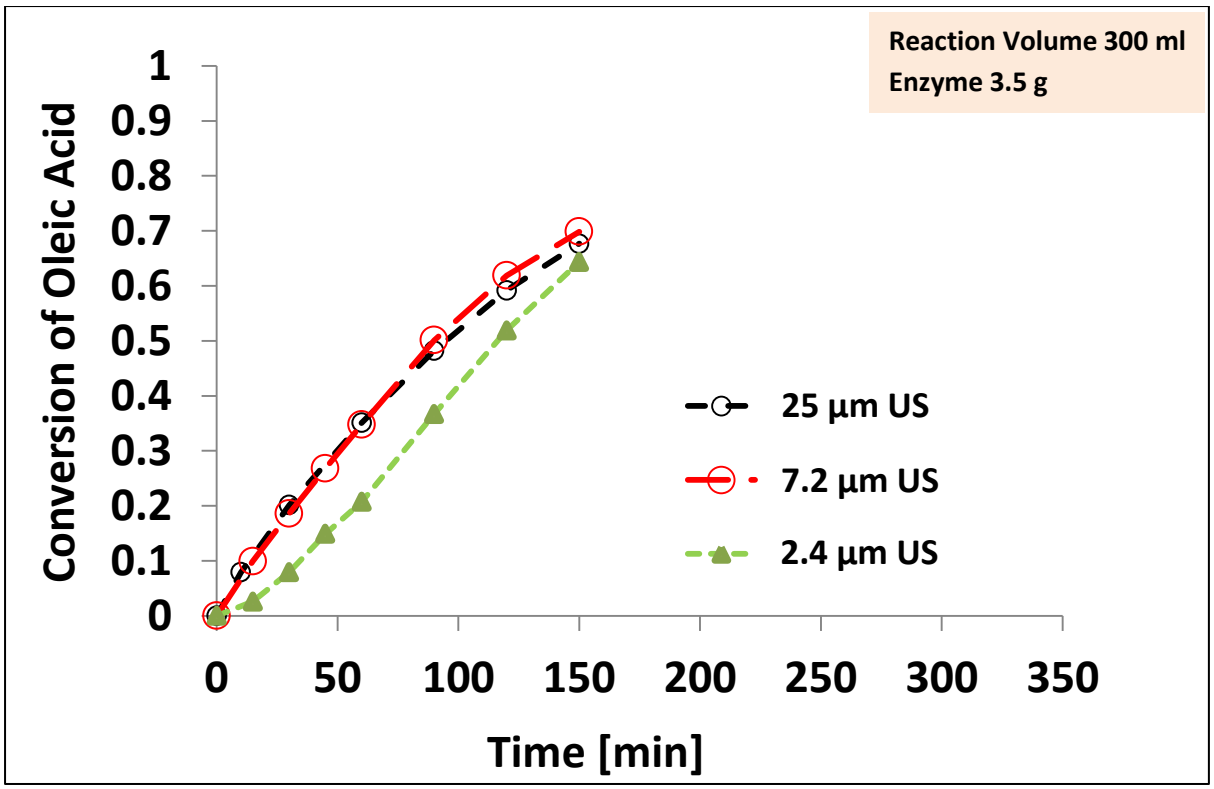


Figure 6.11. Effect of amplitude on reactor performance

Table 6.1 Effect of amplitude on power consumption

Amplitude [µm]	Power [W*]	Energy Consumption [%]
25	150	100
7.2	125	83
2.4	119	79

*Electrical power calculated by measuring current and voltage

6.7 Effect of Pulsed US on the Reaction Intensification

Another important parameter that can influence the energy efficiency of an ultrasonic reactor is the use of continuous and pulse US. In case of pulsed US it is possible to turn-on or turn-off the US at defined intervals. Using pulsed US it is possible to save energy. Ultrasonic

generator in reactor B has the capability of generating pulsed US. The pulse cycle can be selected between 0 – 1 through a switch. If the switch is positioned at 0.3 this means during 1 second time US shall be on for 0.3 seconds and off for 0.7 seconds. In other words for 70% of the time US shall be off. This means 70% saving of energy. Positioning switch at 1 means continuous sonication.

Tests with pulsed US are compared with continuous sonication in Figure 6.12. It is important to mention that for continuous sonication (cycle 1) no stirring was used. For US with cycle 0.3 reaction rate decreased drastically (practically no reaction). But with the cycle 0.3 when stirring was introduced the reaction rate increased. This means at cycle 0.3 US contribution towards bulk mixing becomes negligible and therefore, use of stirring is necessary. Ultrasonic pulse creates some cavitation which speeds up reaction while stirring helps in dispersing the formed products and brings fresh substrates to cavitation zone. Another explanation given by Tsuge [85] is that stirring helps in dispersing the cavitation bubbles away from transducer and therefore, the effect of sonication is widespread. This also leads to the fact that main contribution of US in intensifying the reaction is brought through cavitation and not the enhanced mixing further supporting the observation made in section 6.4.

Table 6.2 shows a comparison of first order rate constant and corresponding energy consumption for continuous and pulse US. It is obvious that for cycle of 0.3, energy consumption is reduced by 68% (compared to continuous sonication). However, corresponding decrease in rate constant is around 32%. Pulse US with cycle 0.3 consumes 45 W at 25 μm. Magnetic stirring on the other hand consumes 30 W of electrical energy. The rate of reaction in case of stirring was extremely slow, this means stirring will have to run for longer time to achieve equilibrium and, therefore, total energy consumed by stirring will be higher than US. This clearly demonstrates the benefits of using pulsed ultrasound to save energy. Amplitude used was 25 μm. Therefore, if amplitude is decreased to 2.4 μm energy consumption would further decrease by 20%. This will be equivalent to 36 W instead of 45 W.

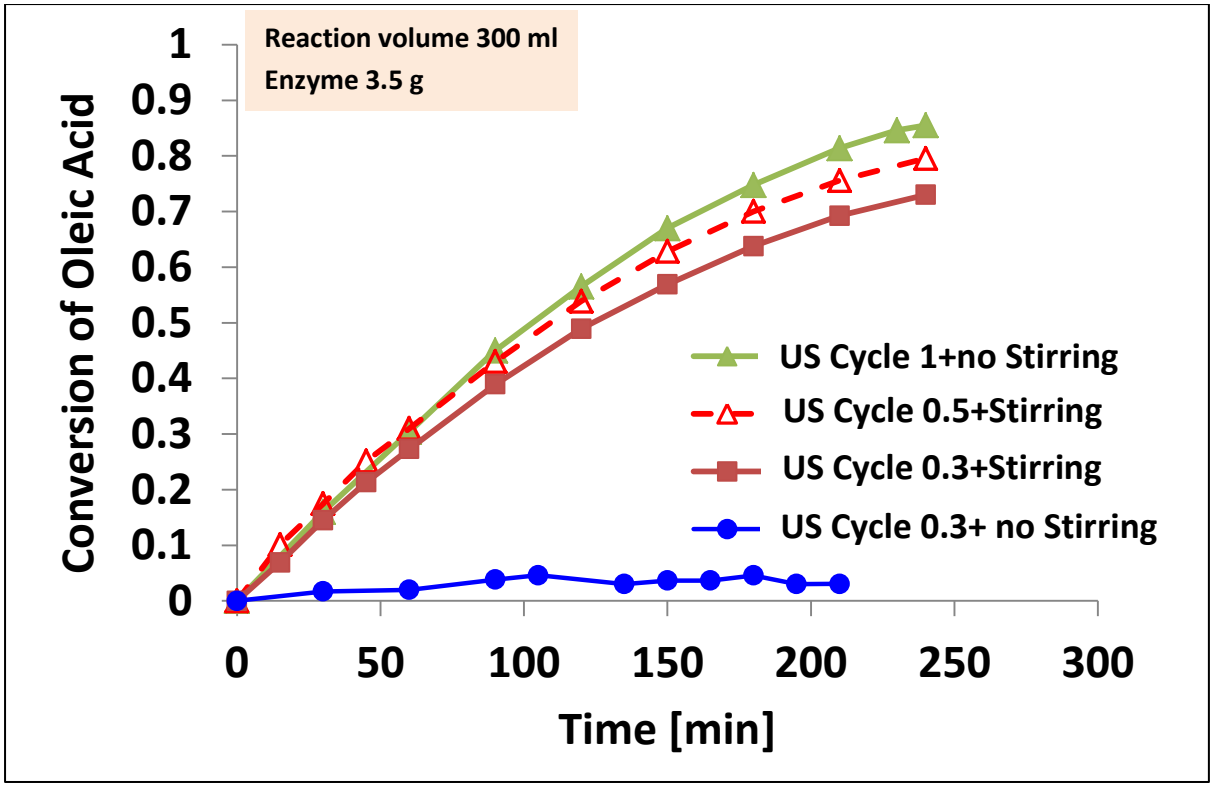


Figure 6.12. Effect of Pulsed US on reactor performance at 40°C

Table 6.2 Comparison of rate constant and energy consumption for pulse US

Cycle	Rate Constant k [L/(mole min)]	Ratio of rate constants $\frac{k_{cycle}}{k_{cycle1}}$	Energy Consumption [W*]
1	0.0084	100	150
0.5	0.0067	80	75
0.3	0.0057	68	45

*Electrical power calculated by measuring current and voltage

The experiments with varying amplitude and pulse US lead to the fact that it is not important to feed more and more power for a better reactor performance. But important is that in which for this energy is being consumed.

6.8 Stability of Free Enzyme against Cavitating US

In case of Lipozyme-435 cavitating US damaged carrier particles and therefore, it was not possible to reuse enzymes. For Lipozym CALB L it has been demonstrated that cavitation greatly improves the reaction rate but it is also necessary to test the stability of this enzyme against cavitating US. From Table 2.1 it is obvious that water has lower threshold of cavitation in comparison to oleic acid. Therefore, for a given ultrasonic intensity cavitation produced in water would be more severe in comparison to reaction under study. Therefore, if enzyme can "survive" sonication in water it is likely that it will retain its functioning in reaction system under study. The advantage of using water for present study was that at the end of sonication water could be easily evaporated in lab under vacuum to get concentrated enzyme for intensification tests.

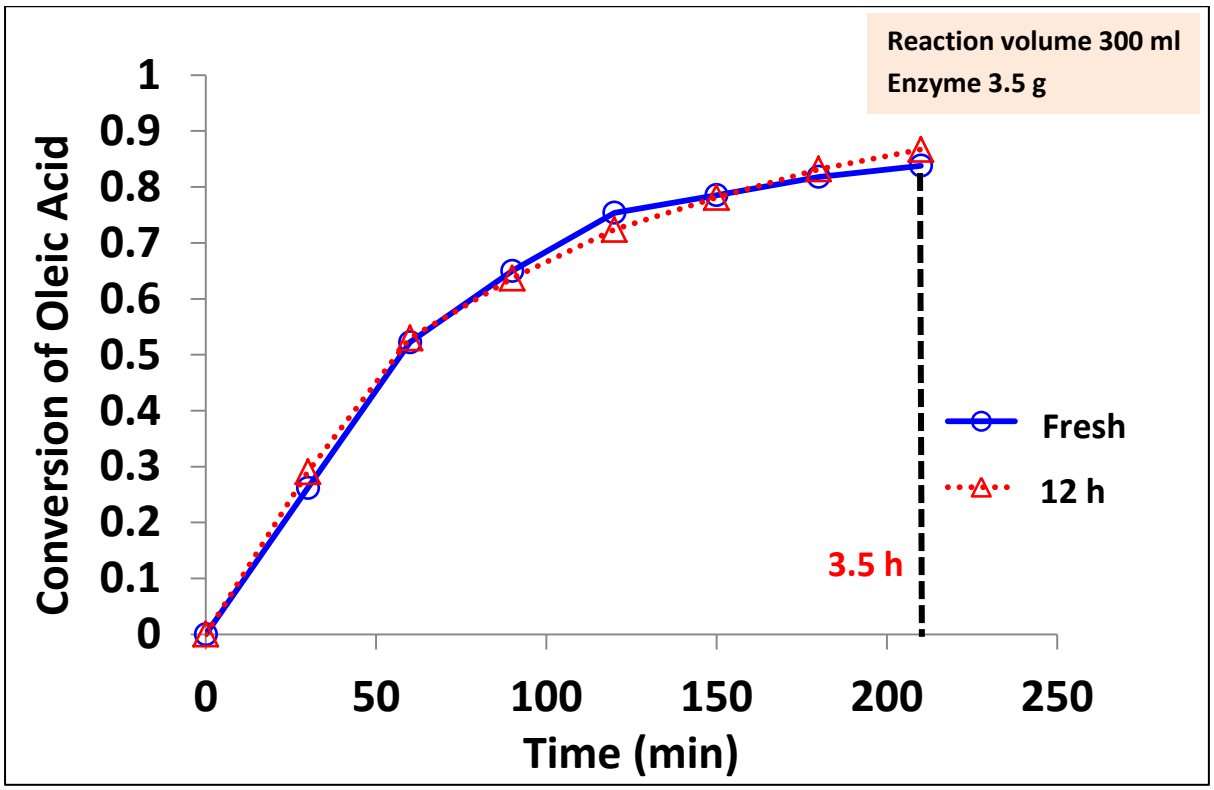


Figure 6.13. Stability of Lipozyme CALB L against sonication at 40°C

Based on the aforementioned concept two samples of Lipozyme CALB L were prepared in water. For both samples volume was kept 300 ml (which is equivalent to reaction volume).

One sample was pre sonicated at 40 °C while the second one at 60 °C. For sonication in water same amplitude and intensity of US was used as for intensification tests in example reaction (25 µm and 21 W/cm^2). For both samples sonication time was kept constant at 12 hours. After 12 hours of pre sonication water was evaporated from both samples at 40 °C under vacuum (in order to avoid damaging of enzyme). After evaporation functioning of this "pre-sonicated" enzyme was tested. The results for 40 °C pre sonicated enzyme are shown in Figure 6.13 (see also Appendix G). The results of this experiment (dotted curve) are compared with experiment where fresh enzyme was employed. From the comparison it is obvious that the enzyme retained its activity even after 12 hours of sonication. If we follow the curve for sonicated enzyme it is obvious that enzyme did not show any deactivation during entire reaction. This means enzyme retained its activity for 15.5 hours (12+3.5) which is equivalent to 4 production cycles (for studied reaction).

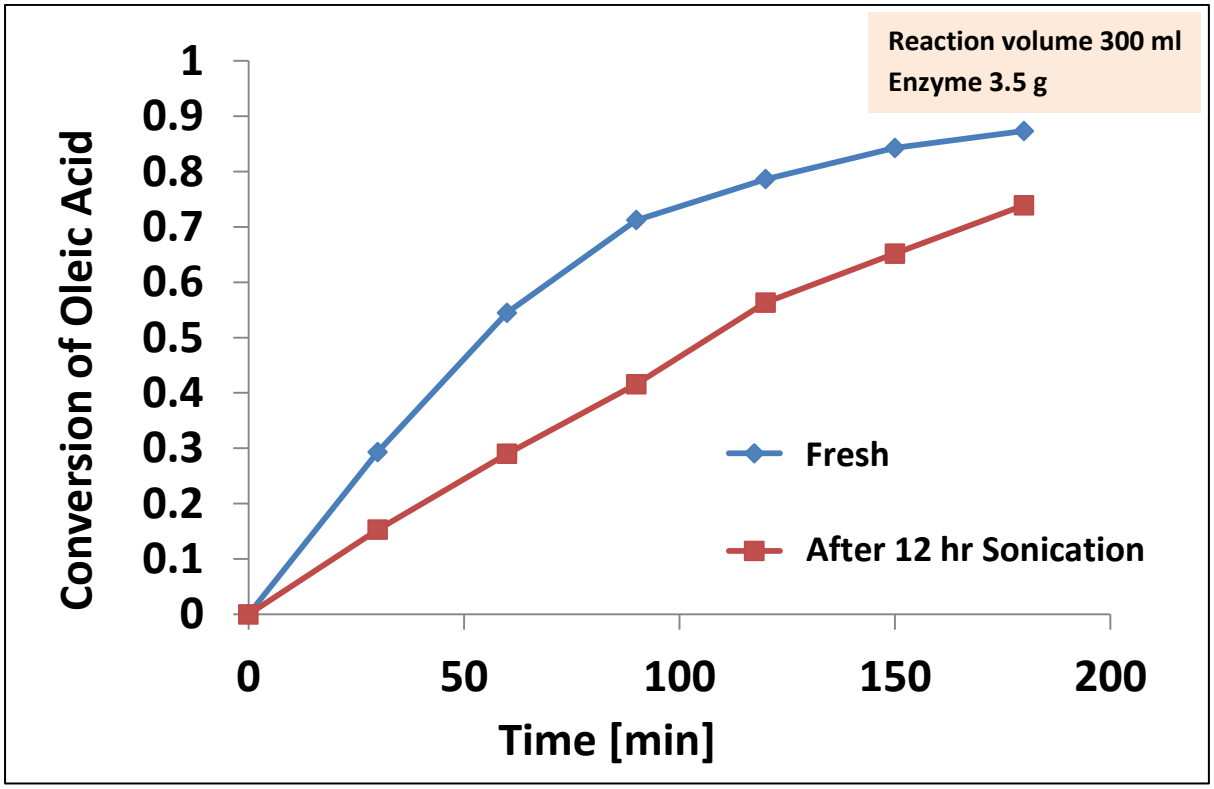


Figure 6.14. Stability of Lipozyme CALB L against sonication at 60°C

Results of stability tests for enzyme that was pre sonicated at 60 °C are shown in Figure 6.14. It can be seen that sonication at 60 °C damaged the enzyme and it lost half of its activity

(Table 6.3) after 12 hour of sonication. From the results it is obvious that for Lipozyme CALB L the preferable temperature in ultrasonic reactor is 40 °C. Possible reasons for this deactivation are already discussed in section 1.1.5. Possible solutions for minimizing the damage to enzyme can be the use of lower amplitude as at lower amplitude cavitation bubbles shall be less detrimental (section 2.2).

Table 6.3 Comparison of rate constants for different sonication times at 60°C

Sonication Time [hour]	Rate Constant [L/(mole min)]	Rate Constant $\frac{k_{sonicated\ enzyme}}{k_{fresh\ enzyme}}$
0	0.018	1
12	0.0075	0.42

6.9 Comparison of Reaction Intensification with Immobilized and Free Enzyme

Tests with immobilized enzyme showed that Lipozyme-435 resulted higher reaction rate with stirring. CALB L on the other hand gave very slow reaction rate under stirring. With CALB L reaction rate was remarkably higher when cavitating US was used. Therefore, it would be interesting to compare the reaction behavior with Lipozyme-435 using stirring to the one with CALB L under cavitating US on the basis of unit quantity of enzyme used. The results for this comparison are shown in Figure 6.15. For both experiments 1 g of enzyme was used. From the comparison it is obvious that CALB L shows significantly higher reaction rate than Lipozyme-435 for the same quantity of enzyme used. This means ultrasound intensifies Lipozyme CALB L catalyzed reaction which is not achievable while using stirring.

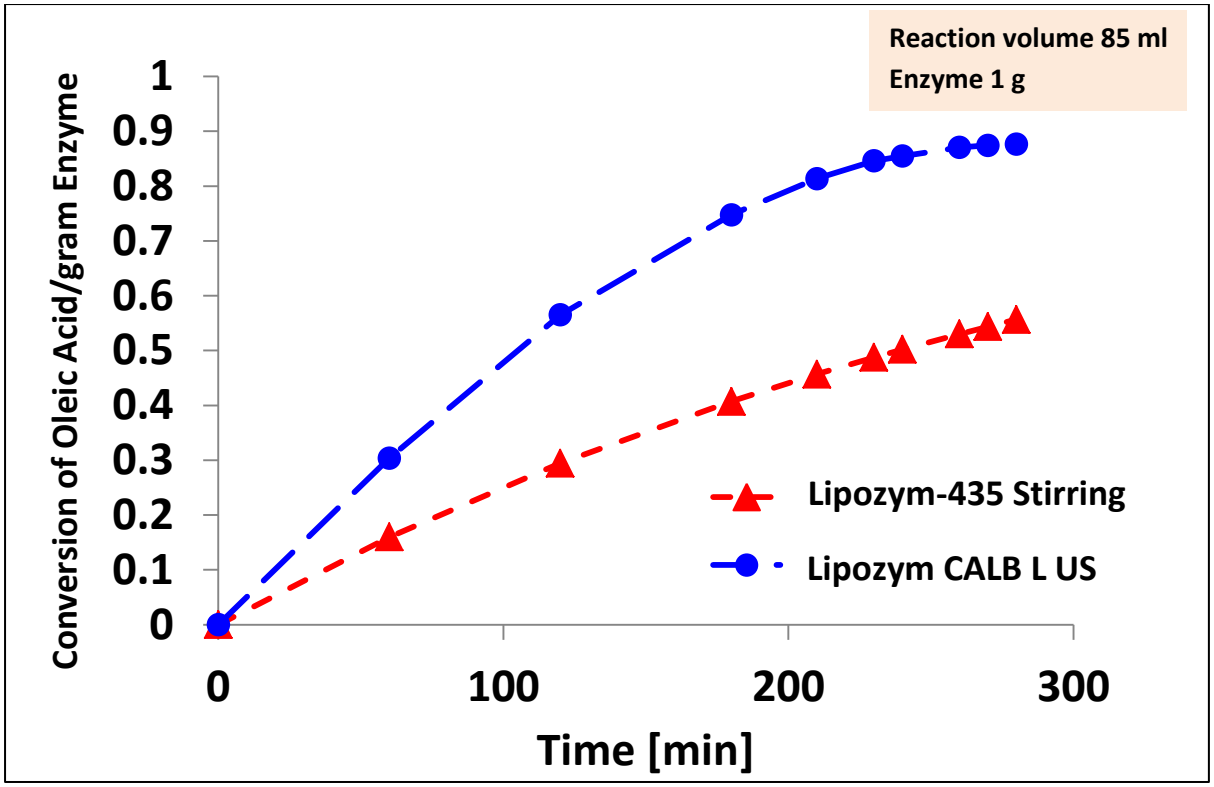


Figure 6.15. Comparison of Lipozyme-435 and Lipozyme CALB L activities

Effect of cavitating US on Lipozyme CALB L catalyzed esterification was studied and important observations made in this chapter can be summarized now. With cavitating US CALB L resulted remarkably higher reaction rate. It was observed that addition of water is necessary for functioning of enzyme and US alone does not activate enzyme. The amount of water required is dependent on the quantity of enzyme used. A minimum ratio of water to enzyme is 0.43 ml water per gram enzyme. On the other hand under stirring conditions same enzyme gave very slow reaction rate even upon addition of water. Addition of water for immobilized enzymes did not improve the reaction rate which led to the conclusion that in immobilized form enzymes are already in active conformation (lid open). Presence of cavitation is found to be necessary throughout reaction to maintain intensification phenomenon. Stability tests for CALB L have shown that sonication at 40 °C did not damage the enzyme up to 15 hours. However, at 60 °C deactivation of CALB L was observed after a sonication time of 12 hours. Therefore, at used amplitude (25 µm) 40°C is preferable reaction temperature. Therefore, preferable operating temperature for tested enzyme is 40 °C (during sonication).

It is possible to save energy by sonicating reaction medium at lower amplitude using pulsed US. For the same amount of enzyme sample used CALB L produced higher reaction rate with US than Lipozyme-435. Comparison of tests with immobilized and free enzymes has helped in determining the conditions under which application of US is effective in intensifying the reaction. With completion of these tests first step of research methodology (section 1.2) is accomplished. With this knowledge in hand it is now possible to proceed to the second step of research methodology i.e. to develop a reactor concept for large scale application of discovered phenomenon. This will be the topic of next chapter.

7 Sonicated Enzyme Reactors for Large Scale Processing

As per research methodology depicted in Table 1.2, a concept for large scale application of the discovered phenomenon is to be developed and tested. In present chapter a concept for this large scale application is presented.

7.1 Concept for Sonication of Large Scale Reactors

Tests with Lipozyme CALB L have shown that presence of cavitation is necessary to obtain intensification effect from US. Pankaj and Ashok Kumar have reported that in low frequency reactors cavitation is concentrated near the transducer [86]. This means using a single ultrasonic source it is not possible to sonicate the entire reactor volume in large scale applications. One possibility of using US for large scale applications can be the use of so-called flow through reactor arrangement as shown in Figure 7.1 (also called Harwell ultrasonic flow reactor). The advantage of using this arrangement is that the sonication chamber is situated outside the reactor. Therefore, it can be easily integrated to the existing processes. More than one sonication chambers can be added in parallel/series in order to cope with higher throughputs.

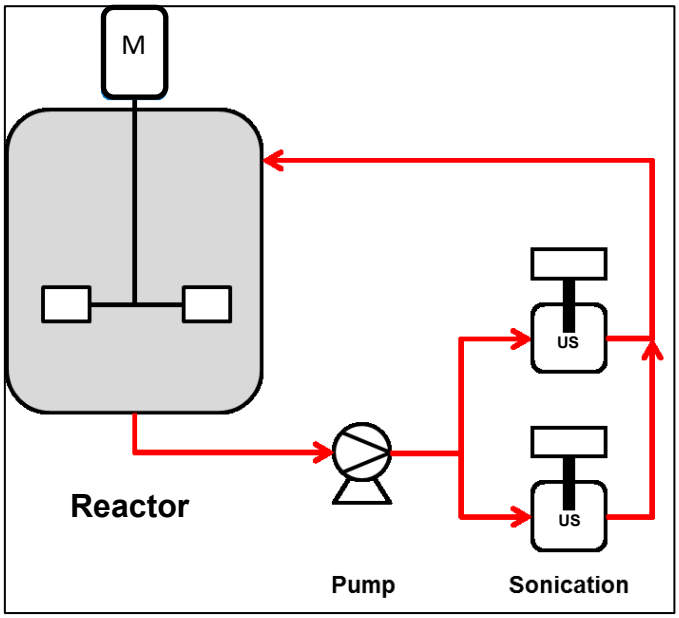


Figure 7.1. Schematic of flow through ultrasonic reactor

To demonstrate the concept and arrangement for large scale application of the phenomenon a flow through reactor was constructed in lab using sonotrode from reactor B (Figure 7.2). It consisted of a sonication chamber, stirred reactor and a pump. Liquid feed is pumped continuously through sonicated chamber. The temperature in reactor and sonication chamber/s was maintained by circulating cooling water through jacket of reactor and sonication chamber. The pump was capable of providing flow ranging from 200-500 ml/min. The performance of flow through reactor is compared with the batch reactor (reactor B). Since for flow through arrangement reaction volume was 6oo ml, therefore, one experiment was done in reactor B using 600 ml reaction volume. The conversion curve thus obtained is shown in Figure 7.3 and served as a reference.

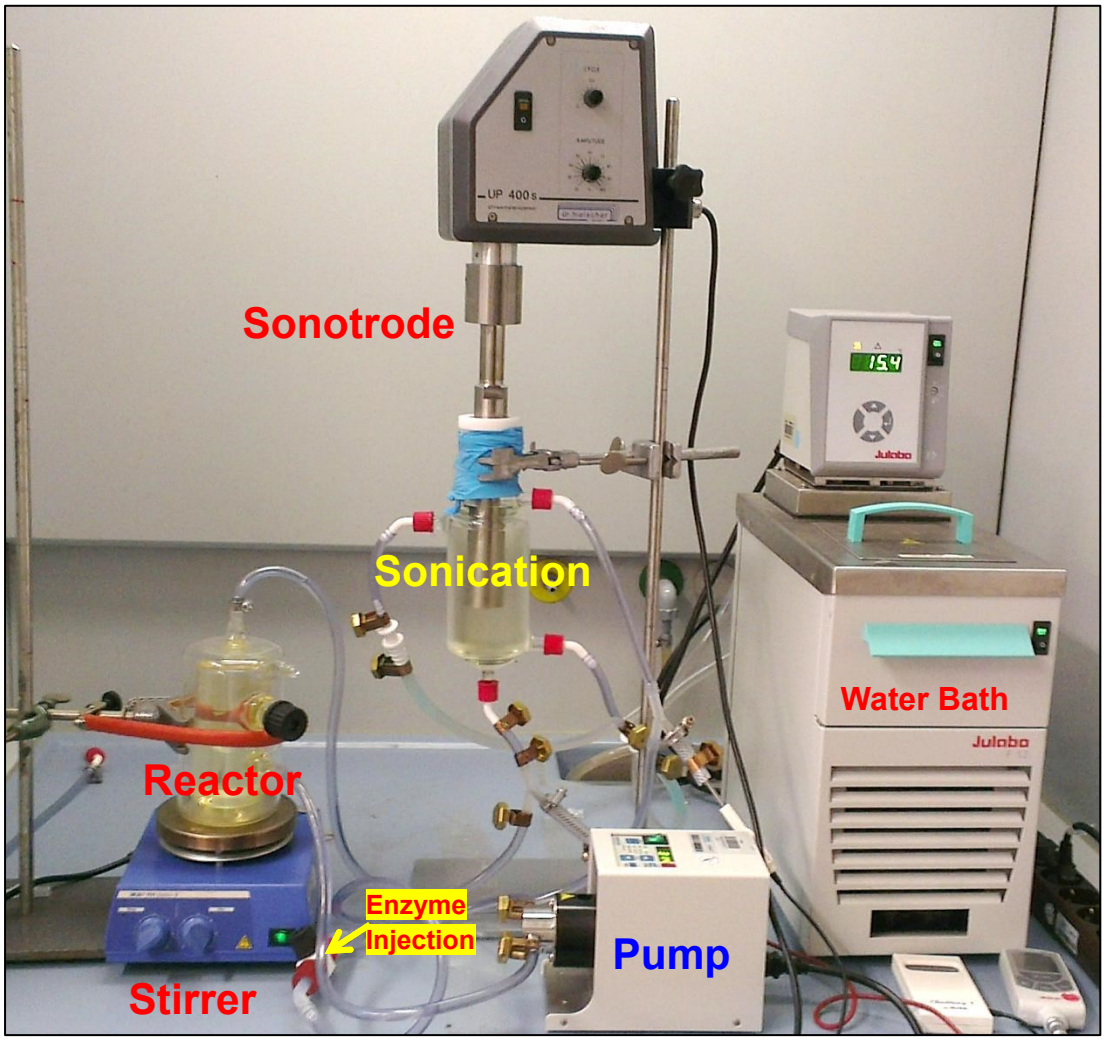


Figure 7.2. Flow through arrangement for an ultrasonic reactor

The reaction behavior obtained with flow through arrangement is shown in Figure 7.3. Flow through reactor arrangement gave a bit lower conversion in comparison to batch reactor. However, when compared with stirring, the reactor performance is certainly much better. The possible reason for lower conversion can be the improper mixing of enzyme as it was fed at pump discharge which flowed into reactor along feed. Enzyme mixing can be improved by injecting enzyme directly inside the sonication zone in a drop wise manner. This explanation is supported by the fact that in batch reactor (reactor B) lower conversion is obtained if enzyme is added at once. However, under identical conditions drop wise addition of enzyme in batch reactor gives improved conversion.

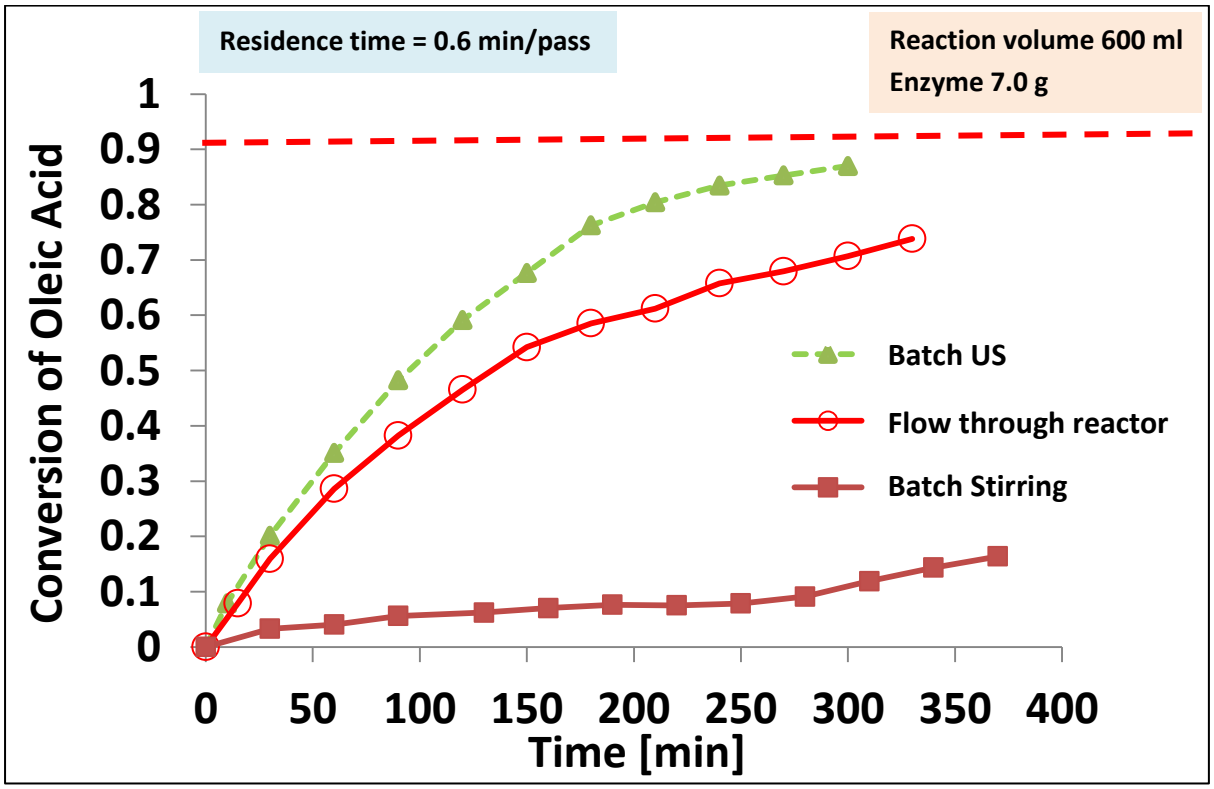


Figure 7.3. Comparison of the results from flow through and batch ultrasonic reactors

The second factor responsible for lower reaction rate can be attributed to inefficient mixing inside sonication chamber as shown in **Fehler! Verweisquelle konnte nicht gefunden werden.**. Mixing from sonotrode alone is not sufficient to ensure proper mixing of reactants in sonication chamber. As a result the stream of the reactant feed entering from bottom of the reactor moves vertically upward with minimal mixing in the radial direction. Mixing inside

sonication chamber can be improved by inserting a magnetic stirrer. However, with the used chamber it was not possible to use magnetic stirring as the feed inlet is at the bottom.

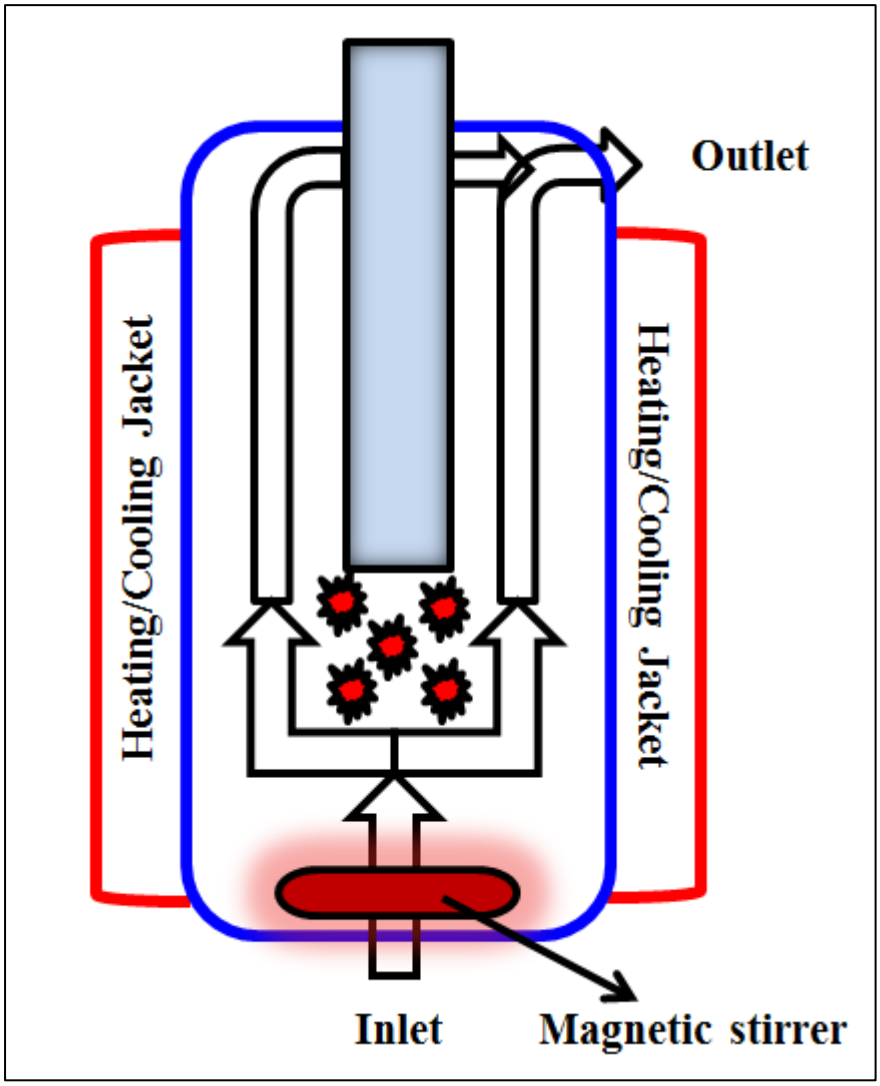


Figure 7.4 Problem of mixing in sonication chamber

Reproducibility of the results for flow through arrangements is demonstrated in Figure 7.5. As with previous experiments results obtained here are also highly reproducible.

Another important factor that covers the effect of circulation rate on reactor performance is compared in Figure 7.6 for 200 and 500 ml/min. This circulation rate effects the residence time of reactants inside the sonication chamber. Increasing circulation rate will decrease the residence time. Residence time at 200 ml/min is approximately 1.5 minute and 0.6 minute at 500 ml/min. Curves for both circulation rates are identical. Testing at higher flow rate was

not possible due to pump limitation. According to manufacturer UP400S can handle flow rates from 165-800 ml/min.

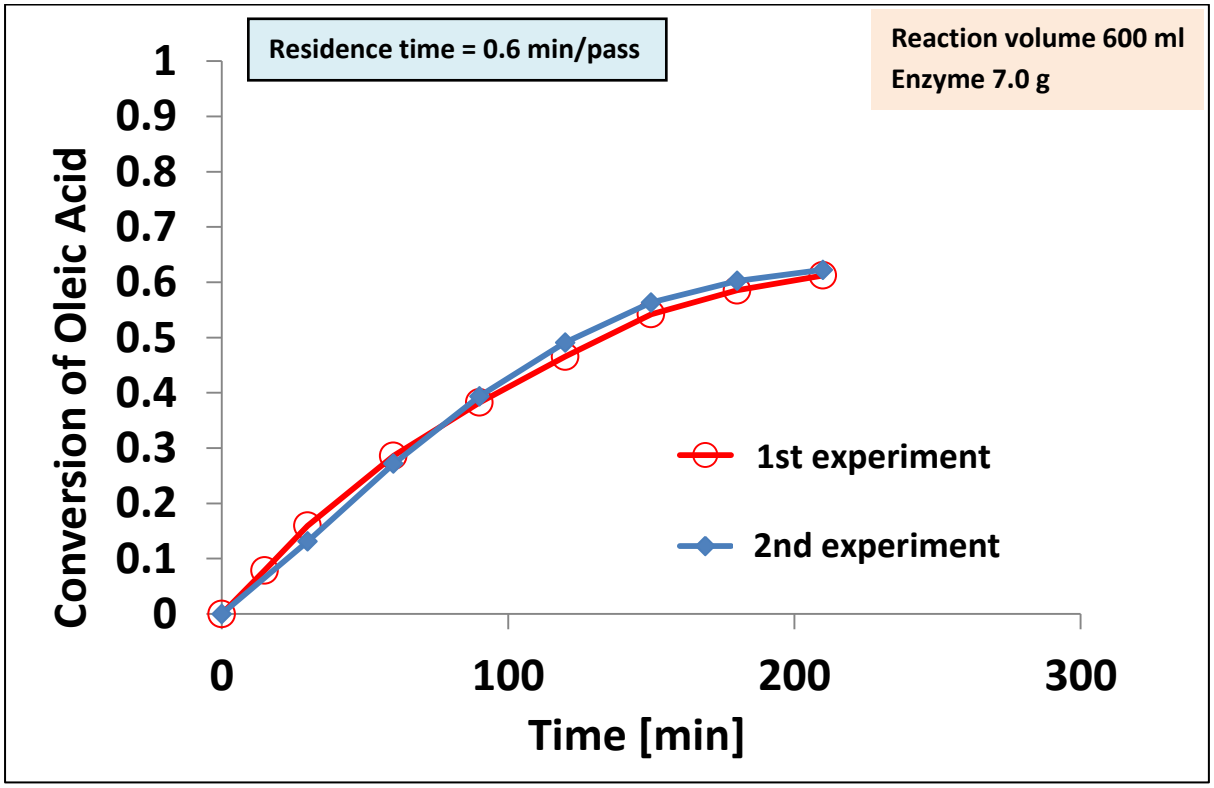


Figure 7.5. Reproducibility of results with the flow through reactor

For industrial scale production higher circulation rates are involved for which larger sonotrodes would be required. Sonotrodes capable of handling flow rates in range 2-10 m^3/day are also available from different manufacturers. This means using the larger sonotrode the demonstrated concept from Figure 7.2 can be easily adapted for large scale productions.

In conclusion, it can be said that the flow through ultrasonic reactor gives much better results when compared with stirring.

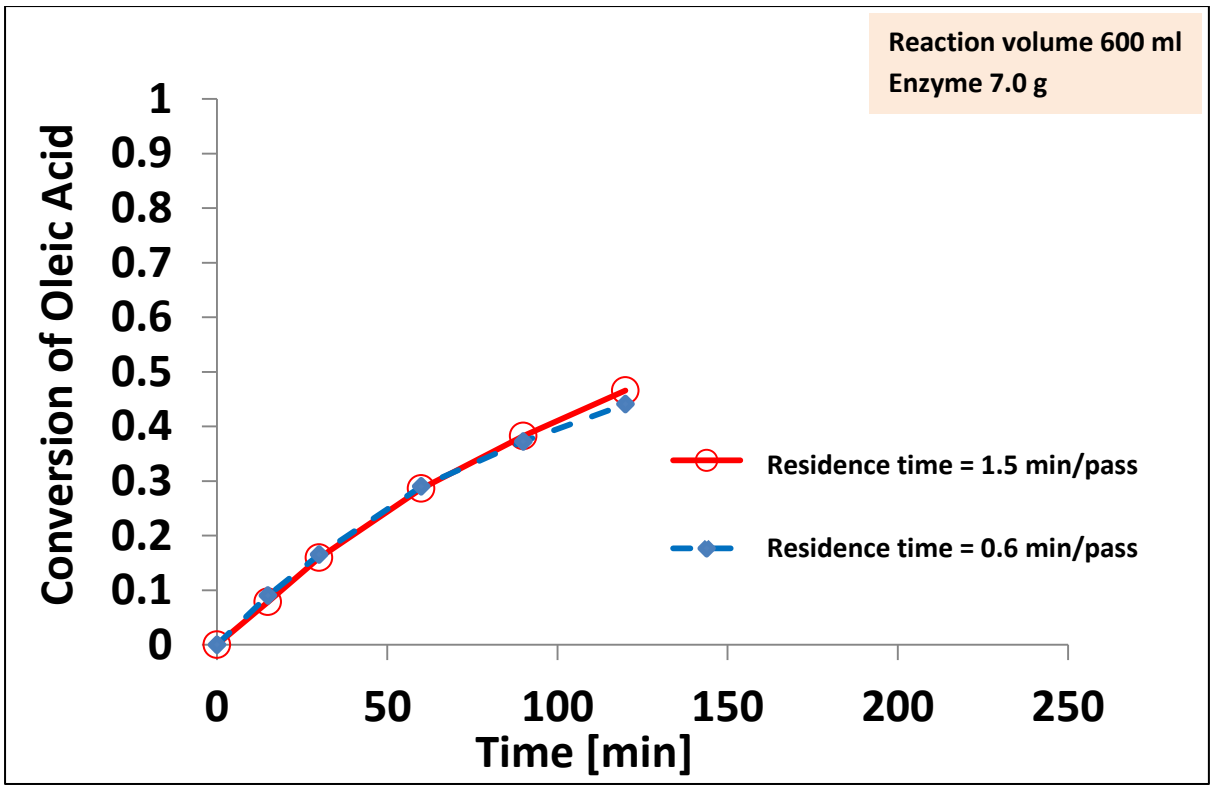


Figure 7.6. Effect of flow rate on performance of flow through reactor

8 Conclusions and Future Work

Effect of US on the intensification of lipase catalyzed esterification of oleic acid with n-hexanol was investigated in detail with an objective for industrial scale realization of the phenomenon. A comprehensive literature survey was carried out in order to determine the state of the art. Based on the literature survey following research objectives were defined which included following questions

- What type of US can bring intensification of enzyme catalyzed reactions i.e. non-cavitating and cavitating (as this information was necessary to determine the conditions and ultrasonic device necessary for obtaining intensification)?

- Whether the intensification observed using US is a result of improved enzyme activity or only the enhancement of mass transfer/mixing or both?

- Testing of the post sonication stability of enzymes

- Development of a concept for large scale realization of the phenomenon. To efficiently utilize the ultrasonic energy in reactor, studying the effect of relevant influencing parameters was also important

These objectives were achieved by using the developed research methodology shown in Figure 1.10. According to the proposed methodology research was divided into two main parts. One part focused on fundamental understanding of the phenomenon while in second part concept for large scale application of the phenomenon was developed and tested. In afore mentioned parts research activities were further divided into different steps. These steps were ordered in a manner that output of one step forms the basis for the next step. This systematic approach helped in gaining a comprehensive understanding of the phenomenon from process engineering perspective i.e. influence of US on hydrodynamics and enzyme molecule were studied separately. The developed concept was executed using an approach which employed a combination of experimental investigations and modeling/simulation.

Esterification of oleic acid with n-hexanol was selected as example reaction. For catalyzing the selected esterification reaction commercially available enzymes Lipozyme CALB L and Lipozyme-435 were used. In Lipozyme-435 CALB L is present in immobilized form. Due to same origin of enzyme in both cases it was possible to directly compare the results of all intensification tests. Processes based on these enzymes are well established. Therefore, gained knowledge will be beneficial both to existing as well as new processes. The intensification tests were done under influence of non-cavitating/cavitating US while tests under stirring conditions were taken as base case for comparison.

Developed mathematical model is capable of predicting acoustic pressure field inside the ultrasonic reactor as a function of influencing parameters such as frequency and power of applied US, nature of reaction medium, temperature etc. Enzyme particle trajectories resulting from combined effect of acoustic pressure field and stirring can also be simulated. This hydrodynamic information can be combined with intrinsic kinetics of the reaction to predict the performance of an ultrasonic reactor. For solving the model equations COMSOL multiphysics was used.

Following conclusions can be made from the obtained results:

- It is possible to sonicate a reaction medium with and without cavitation by using transducers of different frequencies and power. At higher frequencies (206 and 616 kHz) and lower power input, amplitude was lower and therefore, cavitation was not produced. But at lower frequency (24 kHz) and higher power the amplitude was also higher which lead to strong cavitation inside reaction medium. Effects of non-cavitating and cavitating US on studied reaction were not identical. Therefore, in discussing the role of US in intensification it is important to mention the presence or absence of cavitation.

- With cavitating US CALB L produced remarkably higher reaction rate compared to stirring. This higher rate with US was observed only upon addition of water. While testing the functioning of CALB L with different water concentrations it was learned that minimum ratio of water to enzyme is necessary to be maintained in order to achieve full activity of enzyme. This minimum ratio for the studied system was determined to be 0.43 gram water per gram of enzyme. Adding water in excess than minimum required had a slightly negative effect on reaction as water is also the

product of equilibrium reaction. It was also learned that CALB L shows higher activity as long as cavitation was present in reactor. Therefore, cavitation is a necessary phenomenon for obtaining intensification effect.

- Activation of Lipozyme CALB L with water can be explained on the basis of active and inactive conformation of lipase. It favors the hypothesis that in CALB L active site is covered by lid and as soon as water is added in presence of cavitation, active site is opened and catalytic activity is observed. In Lipozyme-435 lipase is immobilized in lid open conformation therefore, addition of water is not necessary to activate the enzyme. Due to this reason higher reaction rate is obtained with Lipozyme-435 under stirring.

- With Lipozyme CALB L remarkably higher reaction rate was obtained with cavitating US only upon addition of water. However, with cavitating US and Lipozyme-435 reaction rate did not increase even upon water addition. This points to the fact that US itself can't activate CALB L by interacting with it at structural level. It only helped to provide an environment for activating enzyme through better distribution/dispersion of water. Therefore, if such limitation is not present in enzyme or if it is already in active conformation then positive effect from US shall not be observable. This finding is an important step towards understanding the mechanism of enzyme activity improvement by US and has not been reported in the literature before.

- To test the reusability of enzyme after sonication enzyme stability tests were made. Stability tests showed that cavitation at 40 °C did not damage the enzyme up to 15 hours (Figure 6.13). However, with cavitation at 60 °C enzyme was deactivated after sonication of 3 hours. Based on this it can be concluded that suitable operating temperature for CALB L with cavitating US in studied reaction is 40 °C.

- While studying the effect of varying amplitude on reactor performance it was observed that it is important to determine the minimum required amplitude in order to operate ultrasonic reactor in energy efficient manner. Experimental data shows that

operating at higher amplitudes than required does not produce any positive effects on intensification of enzyme and is merely wastage of energy.

- Using pulse US it is possible to save up to 70% of energy. Running ultrasonic reactor in pulse mode it was necessary to use stirring. In continuous sonication use of stirring was not required. This also shows that in pulse mode contribution of US in bulk mixing of the reactor is diminished which needs to be compensated by stirring.

- Concept for large scale application of US in the form of the flow through reactor has been demonstrated successfully. The flow through reactor arrangement showed much better performance with Lipozyme CALB L when compared with stirring.

- With cavitating US reaction rate was almost double than with stirring. It was noted that positioning of the ultrasonic source in middle of the reactor (lab scale) gives as expected best performance. It was also observed that increase in amplitude beyond 21 μm did not further improve the reaction rate.

- Performance of Lipozyme-435 under stirring and non-cavitating US was identical. Therefore, it can be said that for studied reaction and enzyme non-cavitating US does not produce reaction intensification. Investigation of mass transfer resistances revealed that the studied reaction is not limited by external or internal mass transfer resistance. This absence of mass transfer resistances is probably the reason for ineffectiveness of non cavitating US.

- When developed mathematical model was used to predict trajectories of Lipozyme-435 particles in reactor, formation of standing waves was observed at 206.3 kHz. These standing waves caused formation of particle clumps. This phenomenon of clump formation was also observed in lab physically. It was assumed that this agglomeration of particles might cause a decrease in catalytic functioning. However, tests at 206.3 kHz showed that enzyme functioning was not affected due to clumping of enzyme particles. This can be attributed to high slip velocity caused by US.

- Cavitating US damaged the carrier particles completely and it was not possible to reuse them. Therefore, use of cavitating US for immobilized enzyme is not attractive

from economical viewpoint. Using stirring at 60 °C immobilized enzyme showed same performance as with cavitating US at 40 °C. The advantage with stirring was that enzyme particles were not damaged and could be reused for subsequent reaction cycles. Another advantage of stirring is far lower energy consumption than US.

8.1 Future work

A systematic and detailed work in context of present research project has helped in understanding a number of aspects related to sonication of enzyme catalysis. Gained knowledge not only provides a platform for further research but also points to the direction of future research activities.

- Cavitation is the phenomenon that is responsible for reaction intensification with Lipozyme CALB L. However, it is not yet clear that how cavitation helps in achieving this remarkable intensification effect. The literature survey has shown that cavitation can cause three kinds of effects i.e. mixing, localized temperature rise and generation of radical. Therefore, focus should be put on determining that which of the aforementioned effects are responsible for the observed effect. In other words what special effect cavitation produces to achieve this intensification? This understanding will help in determining that for which enzyme systems the use of US can give positive effects.

- Using available reactor it was possible to produce cavitation only at a single frequency i.e. 24 kHz. It would be interesting to study the effect of cavitation produced at higher frequencies. Cavitation bubbles produced at higher frequencies shall be smaller in size and will cause less damaging effect to enzyme. However, the number of bubbles produced shall increase at higher frequency. Larger number of bubbles with smaller size should help in further improving the intensification effects with minimal loss to enzymes.

List of Appendix

Appendix A: Calculation of effective power input for ultrasonic reactor

According to user manual transducer can provide power between 0-400 W. However, this power specified by manufacturer (P_t) is not actually transferred to the liquid medium in the form of ultrasound as there are losses in transducer. Therefore, actual ultrasonic energy going into liquid is less and can be calculated according to calorimetric method [87].

A thermometer was positioned inside reactor. Sonication was started and rise in temperature resulting from ultrasound was monitored against time. After measuring heating rate, transducer was turned off and as a result the temperature of the medium starting falling (due to heat losses from reactor wall). This rate of cooling was also recorded. This rise and fall in temperature was then plotted against time. From the slope of the heating/cooling lines rate of energy input was then calculated according to following correlation

$$P_{US} = mc_p \left[\left(\frac{\Delta T}{\Delta t} \right)_{Heating} - \left(\frac{\Delta T}{\Delta t} \right)_{Cooling} \right] \text{[W]} \qquad \text{[A1]}$$

From measured power it was possible to calculate an "effectiveness factor" for the used device,

$$\eta = \frac{P_{US}}{P_t} \qquad \text{[A2]}$$

Therefore,

$$P_{US} = \eta P_t \text{ [W]} \qquad \text{[A3]}$$

Intensity can be calculated by dividing P_{US} through area of the ultrasound emitting surface as

$$I_{US} = \frac{P_{US}}{A_t} = \frac{\eta P_t}{A_t} \text{ [W/m}^2\text{]} \qquad \text{[A4]}$$

Intensity and ultrasonic pressure are correlated according to the equation

$$I_{US} = \frac{(p)^2}{2\rho c} \text{ [W/m}^2\text{]} \qquad \text{[A5]}$$

Once we know the intensity we can calculate ultrasonic pressure as

$$p = \sqrt{2\rho c I_{US}} = \sqrt{2\rho c \frac{\eta P_t}{A_t}} \text{ [kPa]} \qquad \text{[A6]}$$

Calculation of effectiveness factor (0.20 for reactor A and 0.4 for reactor B) in simulation facilitates user. User need to specify rated power only and effective source pressure will be calculated automatically once simulation is started.

Appendix B: Stability Analysis for Acoustic Pressure

Reactor A, 2D model, Frequency = 206.3 kHz

max element size	DOF	max P (MPa)	min P (MPa)	Average P (MPa)	ΔP (Pa/DOF)		
0.200	6198	0.407	-0.411	-0.005544	7.7377	-9.7419	0.0535
0.100	24213	0.546	-0.587	-0.004579	12.1654	-13.7308	-0.0048
0.067	56129	0.934	-1.025	-0.004733	4.8873	-5.4139	-0.0024
0.050	93629	1.118	-1.228	-0.004822	0.9086	-1.0016	-0.0005
0.040	210158	1.224	-1.345	-0.004874	0.6830	-0.7485	-0.0004
0.033	227850	1.236	-1.358	-0.004880	0.1598	-0.1780	-0.0001
0.029	321937	1.251	-1.374	-0.004888	0.1007	-0.1089	-0.0001
0.025	386025	1.257	-1.381	-0.004891	0.0352	-0.0394	0.0000
0.022	540450	1.263	-1.388	-0.004894	0.0225	-0.0237	0.0000
0.020	654666	1.265	-1.390	-0.004896			

Reactor A, 2D model, Frequency = 616 kHz

max element size	DOF	max P (MPa)	min P (MPa)	Average P (MPa)	ΔP (Pa/DOF)		
0.200	6198	8.043	-6.574	-0.001914	-361.37	278.13	-0.0340
0.100	24213	1.533	-1.563	-0.002528	102.31	-109.65	0.0843
0.067	56129	4.798	-5.063	0.000163	-95.01	102.88	0.0098
0.050	93629	1.235	-1.207	0.000529	10.98	-10.73	0.0038
0.040	210158	2.515	-2.458	0.000973	-34.67	34.70	-0.0029
0.033	227850	1.901	-1.844	0.000921	-4.52	4.70	-0.0003
0.029	321937	1.475	-1.401	0.000893	-1.68	1.80	-0.0001
0.025	386025	1.367	-1.285	0.000888	-0.39	0.44	0.0000
0.022	540450	1.306	-1.216	0.000886	-0.16	0.20	0.0000
0.020	654666	1.288	-1.193	0.000886			

Appendix C: Particle velocity magnitude

From COMSOL

	P(W)	v (m/s)				
		Q1	Q2	Q3	max	mean
Reactor A, 2D						
f=206.3kHz	50	0.045	0.0929	0.154	0.789	0.111
	150	0.05	0.102	0.182	1.140	0.133
f=616kHz	50	0.1992	0.331	0.511	2.705	0.373
	150	0.2338	0.396	0.593	2.457	0.437
Reactor A, 3D	-	0.935	0.165	0.199	0.375	0.148

From Experiment

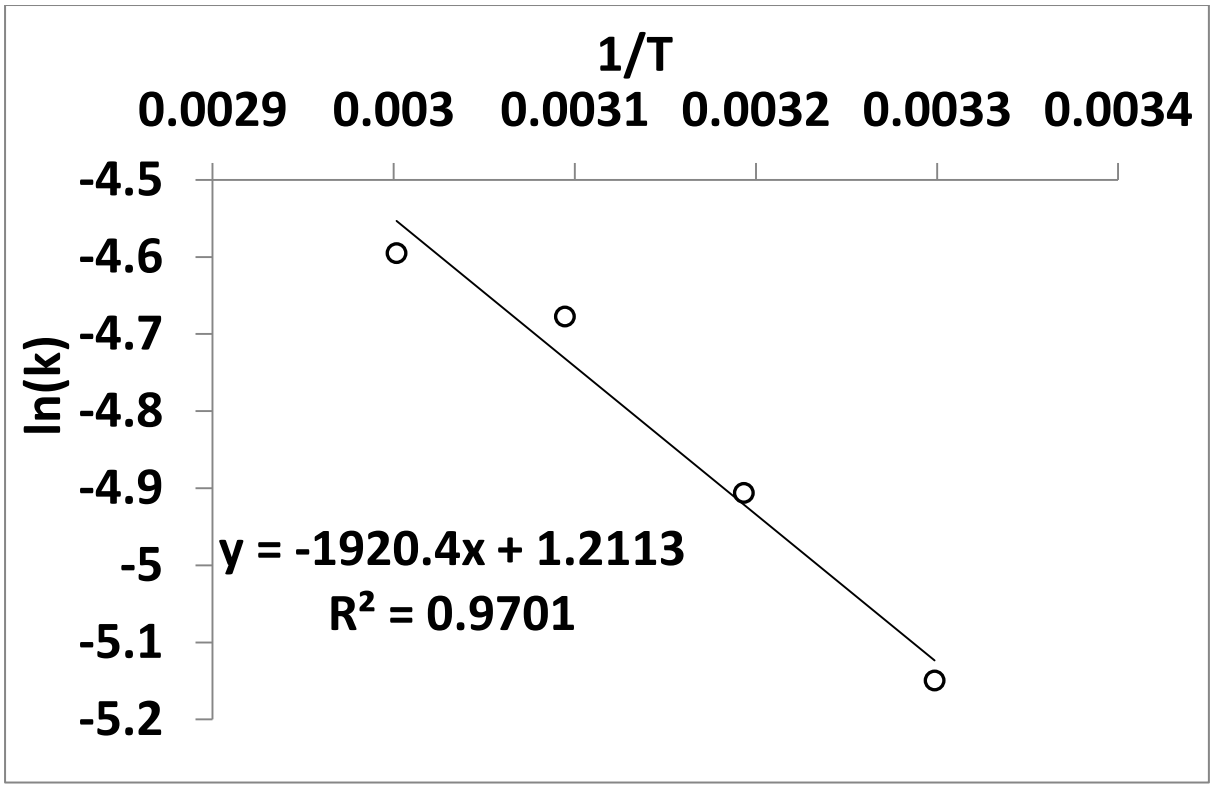
T(^{o}C)	f(kHz)	P(W)	v (m/s)				
			Q1	Q2	Q3	max	mean
Reactor A							
30	206.3	0	0.04	0.062	0.077	0.122	0.061
		56	0.056	0.083	0.095	0.142	0.073
		81	0.061	0.083	0.099	0.15	0.079
40		49	0.038	0.077	0.112	0.167	0.076
30	616	41	0.039	0.062	0.095	0.154	0.067
		82	0.043	0.066	0.097	0.159	0.069
		118	0.039	0.067	0.102	0.160	0.071
40		38	0.045	0.073	0.111	0.178	0.079
		134	0.054	0.073	0.113	0.181	0.083

Appendix D: Temperature dependence of rate constant

According to Arrhenius equation

$$k = A \cdot e^{-\frac{E_a}{RT}} \text{ [1/min]}$$

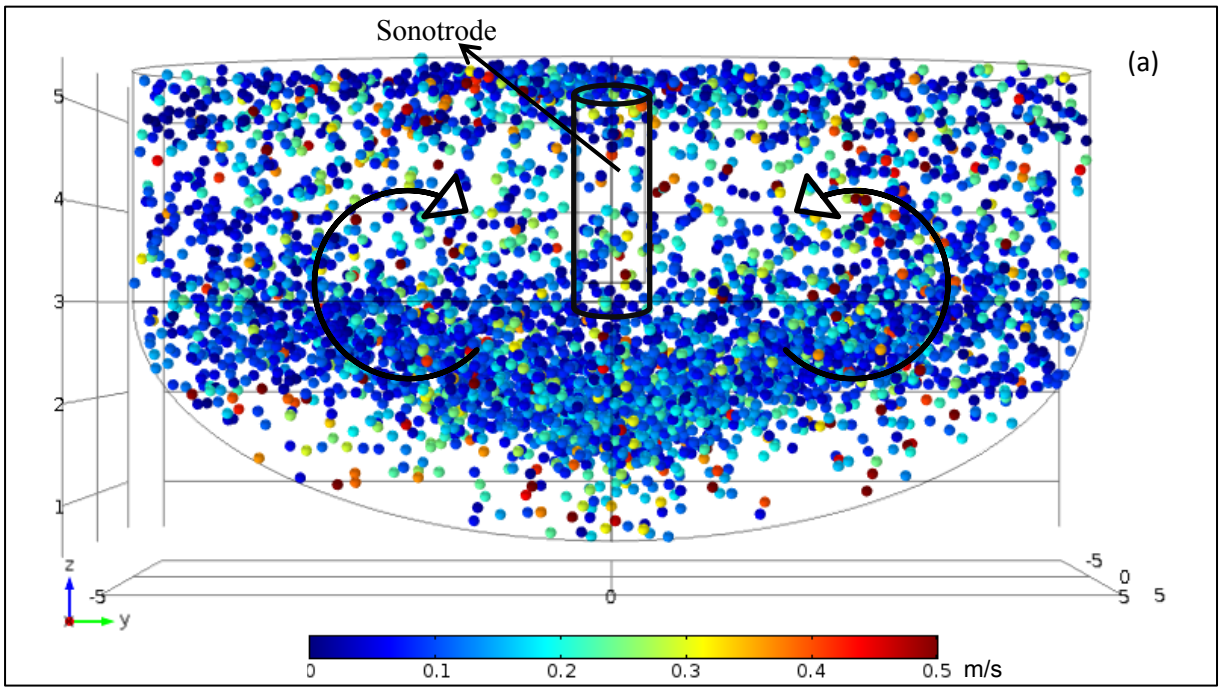
$$\ln(k) = \ln(A) + \frac{-E_a}{R} \cdot \frac{1}{T}$$



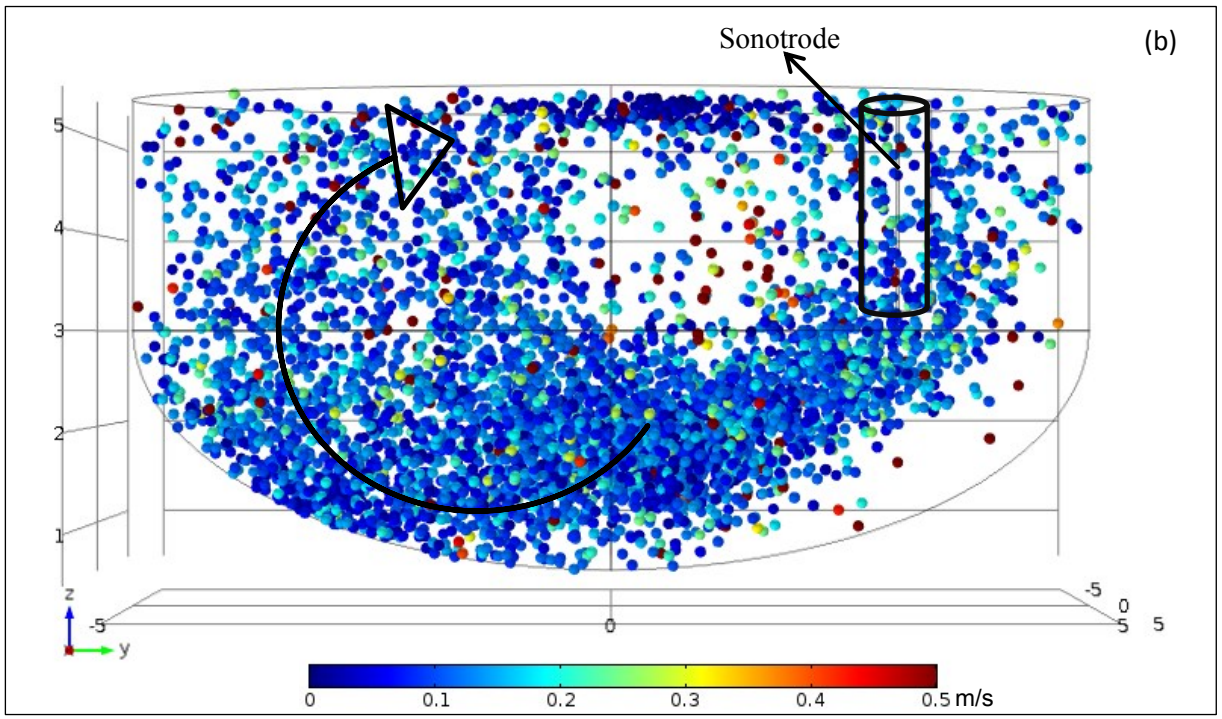
Activation Energy, E_a = 15967 [J/mol]

Arrhenius Rate Constant, A = 3.36 [1/min]

Appendix E: Particle trajectories for reactor B



Particle trajectories in reactor B with sonotrode positioned at middle of reactor



Particle trajectory in reactor B with sonotrode positioned offcenter

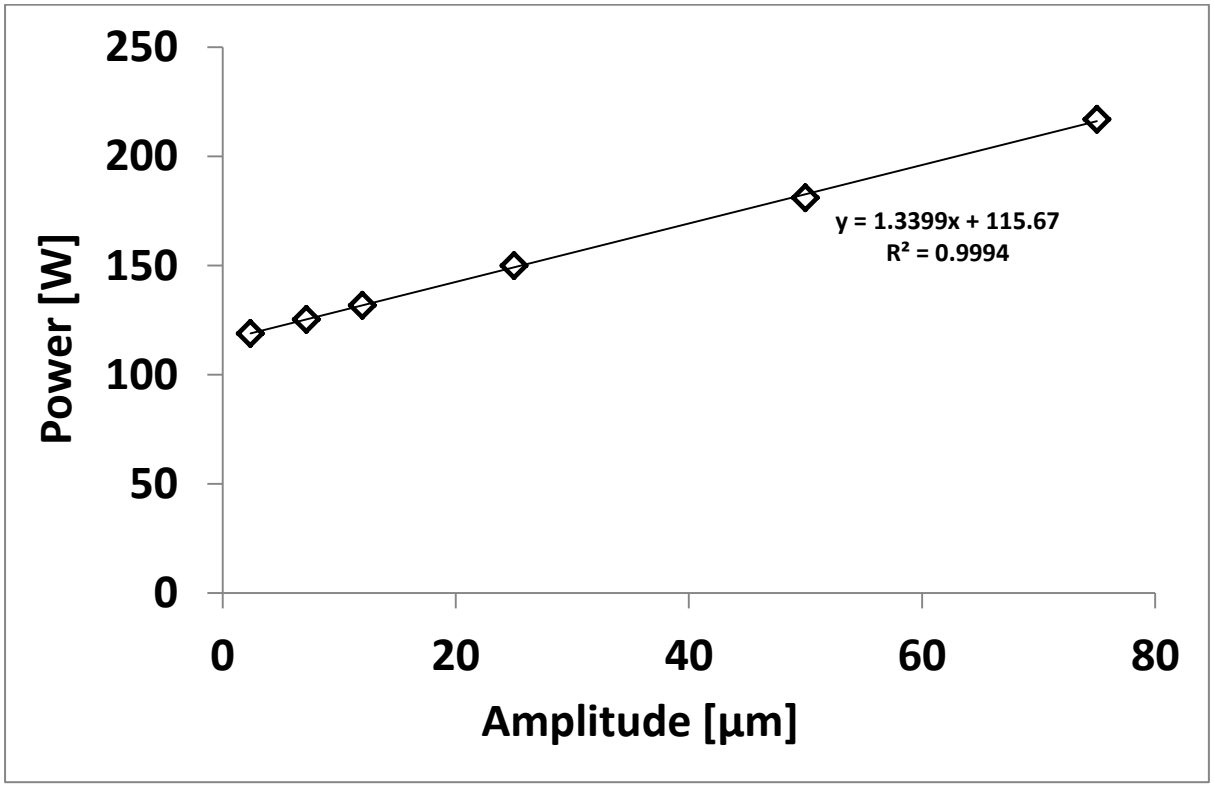
Appendix F: Transport properties for reaction system at 60 °C

Property	Value
Viscosity [Pa.s]	6.00E-03
d_P [m]	0.0006
R_P [m]	0.0003
ρ_P [kg/m^3]	890
ρ [kg/m^3]	847.92
D [m^2/s]	4.80E-10
ε [-]	0.5
τ [-]	6
$D_{eff} = \varepsilon D/\tau$ [m^2/s]	4E-11
v_t [m/s]	0.04
A_s [m^2]	0.029

Appendix G: Comparison of rate constants for different sonication times at 40°C

Sonication Time [h]	Rate Constant [l/(mole min)]	Rate Constant [% of fresh enzyme]
0	0.01	100
12	0.01	100
18	0.0064	64
28	0.0026	0.26

Appendix H: Effect of Amplitude on power consumption



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List of Bachelor and Master Thesis Supervised

Contribution of the bachelor and master thesis (supervised by author) in completion of this work is highly acknowledged. The list of the thesis is given below

1. Jan Kühl, Intensivierung enzymatisch katalysierter Reaktionen mittels Ultraschall, Bachelor Thesis, 2011
2. Muhammad Ahmad, Modeling ultrasonic energy distribution inside an enzyme catalyzed reactor, Master Thesis, 2011
3. Christian-Ole Möller, Entwicklung eines Anlagenkonzeptes zur Intensivierung (bio-) chemischer Reaktionen durch Ultraschall im Mikromaßstab, Studienarbeit, 2011
4. Sherly Octavia Rusli, Simulation of Sonicated Batch Reactor using COMSOL Multiphysics© version 4.3, Master Thesis, 2013
5. Till Ollroge, Auslegung und Optimierung eines Anlagenkonzeptes zur Intensivierung chemischer Reaktionen durch Ultraschall im Mikromaßstab, Bachelor Thesis, 2013
6. Karsten Gescher, Bestimmung des Geschwindigkeitsfeldes einer enzymkatalysierten Reaktion unter Ultraschalleinfluss, Bachelor Thesis, 2013
7. Yu Zhang, Process Intensification in Enzyme Catalyzed Reactions, Master Thesis, 2013
8. Lara Meyer, Prozessintensivierung in enzymkatalysierten ein- und zweiphasigen Reaktionssystemen mittels Ultraschall, Bachelor Thesis, 2014
9. Björn Wind, Untersuchung einer enzym-katalysierten Reaktion im einphasigen System unter dem Einfluss von Ultraschall, Bachelor Thesis, 2014
10. Vasudevan Tamilselvi, Madhu Sudhanan, Development of a Measurement System for Ultrasonic Reactors, Master Thesis, 2014
11. Vasudevan Tamilselvi, Madhu Sudhanan, CFD simulation of an agitated thin film evaporator using COMSOL, Master Thesis, 2014
12. Kugarajah Arulrajah, Einfluss der Betriebsparameter auf die Aktivität der immobilisierten Enzyme in einem Ultraschall-Reaktor, Bachelor Thesis, 2014
13. Eva Romero Montenegro, Investigating the potential of cavitating ultrasound in intensifying the kinetics of chemical reactions, Master Thesis, 2015
14. Charles Wiedenhöfer, Intensivierung der enzymatischen Transesterifikation von Triglyceriden zu Fettsäureethylestern, Project work, 2015
15. Paul Bene, Effect of ultrasound on trans esterification of butyl acetate with n-hexanol, Project work, 2015
16. Sukanya Arulrajah, Effect of ultrasound on enzyme catalyzed esterification of oleic acid with decanol, Bachelor Thesis, 2016
17. Thomas Marx, Auslegung und Kostenabschätzung einer Produktionsanlage für Biodiesel und ganzheitliche Optimierung, Project work, 2016

Curriculum Vitae

Personal data

Name:	Muhammad Ajmal
Date of Birth:	07.05.1976
Birthplace:	Multan, Pakistan

Education

06.1990 - 06.1992	Secondary School
09.1992 - 10.1994	Higher Secondary
11.1994 - 07.1997	Bachelor of Science
03.1998 - 07.2002	Bachelor of Science (Chemical Engineering) BZ University Multan, Pakistan
09.2002 - 07.2005	Master of Science (Process Engineering) Hamburg University of Technology
01.2010 – 03.2016	Research Scholar (PhD) Institute of Process and Plant Engineering Hamburg University of Technology