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Degree Project in Biotechnology KBTM01

Biocatalysis in Pickering emulsions

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Popular summary

The left foot is the mirror image of the right foot. Similar to that the left shoe does not fit on the right foot, the correct mirror image of a pharmaceutical molecule needs to be administered in order to reach the desired effects.
In classical chemical reactions, both mirror images of a molecule are obtained in a 50:50 ratio. Separating these is both costly and causes high amounts of solvent waste.
Enzymes facilitate chemical reactions in biological systems. They are very selective towards reactants, but also in the precision in terms of which mirror image of the product is formed. On top of this, these reactions are carried out at mild conditions. In industrial production, all these properties are very attractive. The successful implementation of enzymes would enable a big economization of steps and solvent use, and thereby a reduction in environmental pollution, usually caused by the energy use that is necessary to reach the high temperatures and pressures used, but also solvents. Economizing energy and solvents is reducing overall process costs. The downside of some enzymes, but especially concerning the ones used in this project, is the susceptibility towards surface contacts which is a problem as the enzymes perform the reaction in an aqueous phase, but the substrate is in the organic, non-polar phase. The presence of this surface will cause enzymatic deactivation.
Here, the employment of an emulsion was investigated. An emulsion is a mixture of two liquids that are immiscible, for example a well-mixed salad dressing made of oil and vinegar. An oil-vinegar dressing is splitting up into an oil and a vinegar phase again if it is not mixed any longer. This can be avoided by adding an emulsion stabilizing agent as it is done in mayonnaise with egg-yolk. Here, instead of egg-yolk, starch particles were used. The particles cover the surface of the non-polar liquid's droplets.
The hypothesis here was that the presence of the emulsion comes with a higher surface and thereby higher rates of substrate leakage into the water phase, but with longer lifetime of the enzymes as the starch particles might form a barrier, meaning surface contact is avoided, and thereby the source of deactivation might be reduced.
In the experiments, it turned out that the form of the vial that was used has a very big impact on the enzyme stability. It could be shown that employing Pickering emulsions might be beneficial, but more research is needed to understand the system better which is crucial for successful application.

Preface

This Master degree project was carried out from 2022-09-01 to 2023-04-12 at Lunds Tekniska Högskola, Lund Universitet, Faculty of Engineering, Department of Chemistry at the division of Biotechnology within the group of Bioorganic Chemistry.

Hypothesis of the project: The hypothesis of the project is that using a Pickering emulsion will result in less enzyme deactivation compared to a biphasic system.

Main objective: The main objective was designing an assay to investigate and compare the enzymatic activities three different experimental settings, namely a Pickering emulsion, a biphasic system, and in a monophase. As biocatalyst were used inactivated *E. coli* cells in which the enzyme, amine transaminase, was overexpressed.

Acknowledgements

A big thanks goes to Carl and Eimantas who gave me an awesome supervision.
Thank you, Carl, for giving always helpful input and inspiration, but also for the trust in my experimental skills (suspending 100 mg-cells/mL-buffer is not easy) and always an open ear for questions.
Thanks to Eimantas for trying to answer any (any!) question and helping whenever it was needed and possible.
Thanks to Patrick for examining!
Thanks to Regina for helping me with the drawing of the structure formulas and for proofreading. That was a huge help. Thanks to Carola for technical support.

Tusind tack till Malin och Helga.

Thanks to the fire brigade which would have come in case of necessity.

Abstract

Employing enzymes as catalysts in the production of chiral amines that are both used as building blocks and active pharmaceutical ingredients might be promising due to milder reaction conditions compared to non-biological catalysts, exclusion of heavy metals, high selectivity towards substrates and precision in chirality of the products. Transaminases are attractive for the use in this process. In an industrial setting, to obtain feasible yields, the amount of substrate supplied must be high. Most of the substrates are impolar organic solvents causing formation of two phases and the respective interphase which most of the transaminases are sensitive towards. This has as a consequence the deactivation of the enzymes in a usual biphasic system.
In this project, a possible solution was investigated, namely the use of Pickering emulsions. The hypothesis was that the Pickering emulsion will protect the enzyme from deactivation.
As biocatalyst, an ω-transaminase was employed, using the deactivated *E. coli* cells the enzymes were expressed in. Both the cells and the Pickering emulsion gave a colloidal system.
The aim was the development of an assay using spectrophotometry and to investigate the hypothesis with the help of it.
In each assay, a Pickering emulsion, a monophasic, and a biphasic system were compared. This was done by two reactions. The first reaction had the purpose to simulate a longterm exposure of the enzyme to the reactants, and the second reaction was used to determine the activity at the time point the sample was taken. Acetophenone was the reaction product of this second reaction. It has a characteristic absorbance maximum at 245 nm, so this wavelength was used in the spectrophotometric measurements.
Transferring a sample from the first reaction into a 40X diluted solution containing the reactants for the second reaction inside the quartz cuvette without any processing of the suspension and subsequent measurement of the absorbance every 5 seconds lead to unreliable results. The particles in the cuvette disturbed the beam in a manner that the curves resulting from this measurement became very craggy. This lead to the conclusion that removing particles before absorbance measurement is necessary.
To remove all particles, centrifugation was applied on samples taken from the second reaction at every second minute. After dilution, the absorbance was measured. The results were more reliable, so stop assay was the method of choice in all the following experiments.
The assay was performed in two different scales, namely in 4.5 mL glass vials and in Eppendorf tubes. Here, it could be shown that when using Eppendorf tubes, employing the ones with a volume of 2 mL is necessary.
An outcome of the project is that the geometry of the vials is crucial, so when applying 1000 rpm on 4.5 mL vials, the Pickering emulsion did not stay intact in contrast to applying this shaking speed on 2 mL Eppendorf tubes where the results seem to confirm the hypothesis, but this should be double-checked in future investigations.
As well in future research, a method for sampling at t_0 has to be developed, and the shaking speed that has to be applied for good mixing and an intact Pickering emulsion has to be found with respect to the vial used.

List of Abbreviations

ACE	acetone
ACP	acetophenone
ALA	L-alanine
API	Active Pharmaceutical Ingredient
TA	amine transaminase
av	average
BA	4-phenyl-2-butanone
EA	enzymatic activity
HPLC	High-Performance Liquid Chromatography
IPA	isopropylamine
LT	longterm
MPPA	1-methyl-3-phenylpropylamine
PEA	*S*-1-phenylethylamine
PLP	pyroxidal 5'-phosphate
PYR	pyruvate
OSA	octenylsuccinic anhydride
rcf	relative centrifugal force
rpm	rounds per minute
std	standard deviation
TRIS	tris(hydroxymethyl)aminomethane
UV/Vis	Ultraviolet-Visible Spectroscopy

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1 Introduction

1.1 Chirality

Biological systems distinguish very well and selectively between different enantiomers, both in macroscopic and molecular terms[1]. So do the human body's receptors, meaning that the two enantiomers of molecules have different effects which gets relevant in the administration of drugs. One example for this is methadone which is depicted in Figure 1.1.

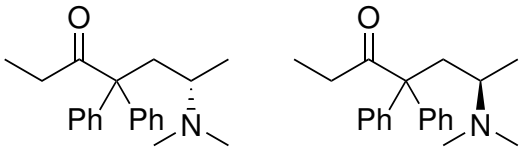

Figure 1.1: Molecular structure of *S*-methadone (left) and *R*-methadone (right).

The *R*-enantiomer, also called levomethadone, has analgesic properties[2]. In detail, this has as consequence that when comparing the effect of a racemic mixture of methadone and the enantiopure levomethadone, the levomethadone's effect is double as strong[3].
Methadone is a so-called chiral amine. Generally, chiral amines are important molecules in the pharmaceutical industry as they do not only serve as building blocks for amongst others active pharmaceutical ingredients (APIs), but also as APIs themselves. In the USA in 2018, over 30% of the 200 most sold pharmaceuticals using small molecules as API contained chiral amines[4].

1.2 Production of Chiral Amines

To produce chiral amines, different approaches are established. One of them is stereoselective synthesis, meaning that one enantiomer is produced in excess[5].
Sitagliptin, depicted in figure 1.2, is a chiral amine as well and used in the treatment of diabetes[6].

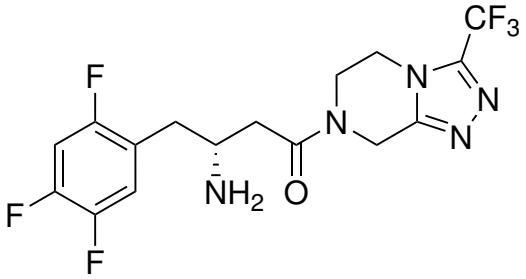

Figure 1.2: Sitagliptin, used in the treatment of diabetes. The enantiomer used as API is the *R*-form.

As Desai summarized in 2011[7] the implementation of an enzyme into the Sitaglipin synthesis process as well as the implementation of a one-pot synthesis is a good example of waste reduction. A severe problem in the pharmaceutical industry are the waste production ratios, also referred to as E-factor, of around 25 to 100 kg of waste per produced kg of product[8].

The first generation of the Sitagliptin synthesis was an extreme example with a waste production ratio of 250 kg-waste/kg product. The evolution of the Sitagliptin synthesis is depicted in Figure 1.3.

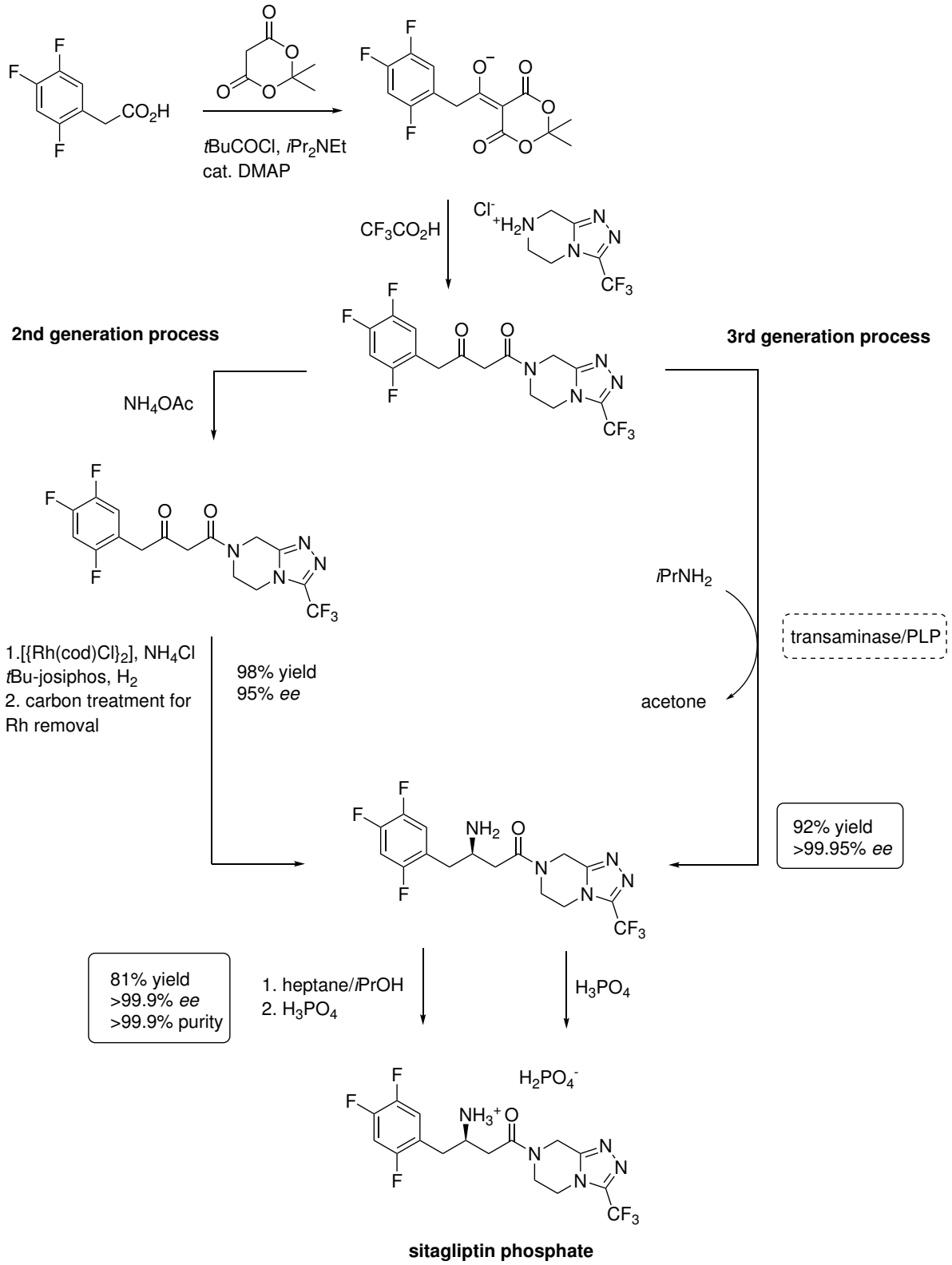

Figure 1.3: The Sitagliptin process development.

The switch to a one-pot synthesis of the pro-Sitagliptin which is a β-ketoamide and subsequent reaction to the β-amino amide resulted in a waste reduction to 50 kg-waste/kg-product.[9]
In the second and third generation of the process, transition metal catalysts and molecular hydrogen under high pressure are employed for the reaction of the β-ketoamide to the β-amino amide. The fourth generation of the process replaces this setting with a genetically engineered ω-amine transaminase (ω-ATA).[7] This has the advantage that 10 to 13% higher total yield is obtained, the enantiomeric excess of the product is raised to 99.99%, an increase by 53% in productivity per day measured in kg/L, and a 19% reduction in waste generation. On top of this, the use of a biocatalyst caused the process to become free from heavy metals[10]. Taking this from the perspective of the 12 Priciples of Green Chemistry, several of the principles are met[11]:

- **1** Prevention of waste, as the use of the ATA means 19% less waste
- **2** Atom economy, as the Mitsunobu reaction could be replaced
- **3** Less hazardous chemical synthesis, as the process does not require H_2 under high pressure any longer
- **4** Designing safer chemicals *via* the unemployment of heavy metals
- **6** Design for energy efficiency as the ATA needs lower temperatures and the high-pressure step for the reduction with H_2 could be replaced
- **12** Inherently safer chemistry for accident prevention which is a logical consequence from the effects described above

1.3 Amine Transaminases

Amine transaminases (TA), also referred to as aminotransferases ATA are enzymes that carry out reactions in which an amine group of one molecule is exchanged with the keto group of another molecule[12]. In this work, a thermostable ω-TA was used. ω-TA differ from α-TA in the way that α-TA are only carrying out the described reaction on substrates that have their above-mentioned functional group at the α-carbon atom, while ω-TA carry out the reaction independently from the position of it [13].
Without the cofactor pyridoxal 5'-phosphate (PLP), TAs do not have catalytic activity[14, 15]. PLP is under hydrolysis covalently bound to the enzyme, forming E:PLP. The transamination reaction happens *via* a transfer of the amine donor's amine moiety to the PLP which then forms the non-covalently bound pyridoxamine 5'-phosphate (PMP). Through the transfer of the amine group to the amine acceptor, a ketone, the cofactor is regenerated.[16, 17]

1.3.1 Challenges

Employment of ATA in an industrial process is accompanied by several challenges. Besides other, one of them is the stability. This work focuses on this aspect.
ATA are susceptible to contact with solvents[18], but also experience inactivation in contact with substrate[19].
As enzymes mostly are watersoluble, there will be a two-phase system when non-polar substrates are used, so the interaction between the enzyme and the substrate has to happen at and through

the interphase. This can be problematic as the interphase is a relatively small area which can be overcome by generating an emulsion which is kept existent *via* stirring. Stirring needs a lot of energy as well and creates shear stress on the enzymes which also can damage them.
Pickering emulsion may offer a solution for the mentioned downsides. Pickering emulsions are multiphase systems whose droplets in the dispersed phase are stabilized with particles[20].
If an emulsion is not stabilized with an emulgating substance or continuously stirred, the two phases will separate. Surfactants are due to their amphiphilic properties quiet often used. The individual surfactant molecules are hereby not anchored to a specific drop, but rather fluidly switch from one drop to the next. Instead of small molecules, in Pickering emulsions relatively big solid particles as starch or silica gel are employed. Here, the particles are wetted by both the impolar and the polar phase. As the detachment from the droplets needs a high energy, the particles can be described as nearly trapped on the droplets. In the case that will be investigated in the Master thesis, the enzyme will be in the watery phase and the substrate will be in the impolar phase, more precisely, the impolar phase will contain nothing else besides the substrate. Having a Pickering emulsion might be beneficial for working with ATAs and impolar substances as the surface in an emulsion is much larger than if just an unstirred two-phase system is present. A larger surface means more potential space for interaction between the enzyme and the substrate.
A further benefit is in the local separation of the enzyme and the substrate. Through this separation, the enzyme is not permanently in touch with an impolar substance, so the inactivation from this is supposed to be weakened a lot. The second advantage is that the applied substrate load can be much higher than in conventional systems as substrate inhibition gets less probable due to the different phases.

2 Aim of the study

The overall goal was to investigate whether the use of a Pickering emulsion as reaction system has a positive influence of the enzyme stability.
To reach this goal, an assay had to be developed that could be used to determine the enzymatic activity of enzymes that are employed inside the cells they were expressed in. This assay needs to be giving reliable results, but easy to carry out with standard laboratory equipment despite that the Pickering emulsion is a system of high complexity.

The reactions that are investigated are interfacial reactions in biphasic and Pickering emulsion systems in relation to monophase reactions which are assumed to be free of an organic-aqueous interphase.
In earlier research in the group, an automated HPLC system for measuring the enzymatic activity of the ω-TA was developed and applied[21]. This system had an output of well reproducible values when applied with dissolved purified enzymes.
Due to the particles present in the reaction system that was investigated here, HPLC cannot be used as monitoring method here because the column would clog as the systems investigated here are suspensions and Pickering emulsions, so particles are present. Spectrophotometry was the method of choice as it is reliable, cheap, easy to apply and widely spread.

3 Assay development

3.1 Experimental principles

Spray dried *E. coli* cells containing ω-amine transaminase (ω-ATA) are used as catalyst in the reactions. The applied ω-ATA can catalyse both the reaction from benzylacetone (BA) to (*S*)-(+)-1-methyl-3-phenylpropylamine (MPPA), further referred to as "reaction 1" as well as the conversion from *S*-1-phenylethylamine (PEA) to acetophenone (ACP), further referred to as "reaction 2", see Figures 3.1 and 3.2. The use of the enzymes in form of deactivated bacteria has the advantage that the separation and purification steps can be skipped.

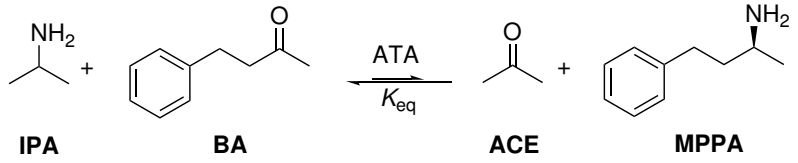

Figure 3.1: Reaction 1, BA to MPPA, which is used in the longterm experiments.

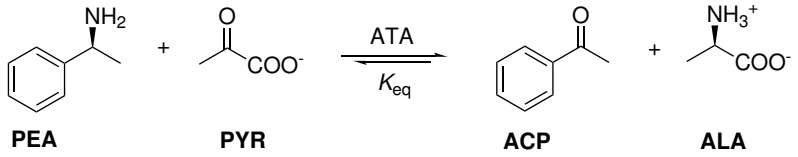

Figure 3.2: Reaction 2, PEA to ACP, which is used to determine r at n h.

The enzyme is considered as stable if its activity does not decrease when being exposed to the reaction environment over several hours.
As indicator of activity, the rate of product formation r is used which means that a decrease in r can be interpreted as a decrease in activity. Therefore, the terms "rate" and "enzyme activity" are used synonymously.
The methods described below were applied to determine r which can be expressed by the change of concentration c per change of time t, see equation 3.1.

$$r = \frac{\mathrm{d}c}{\mathrm{d}t} \tag{3.1}$$

To determine the activity, a sample from the longterm reaction vial (LT vial) was transferred in a second vial (EA vial) which contained the substrates for reaction 2, see Figure 3.3.

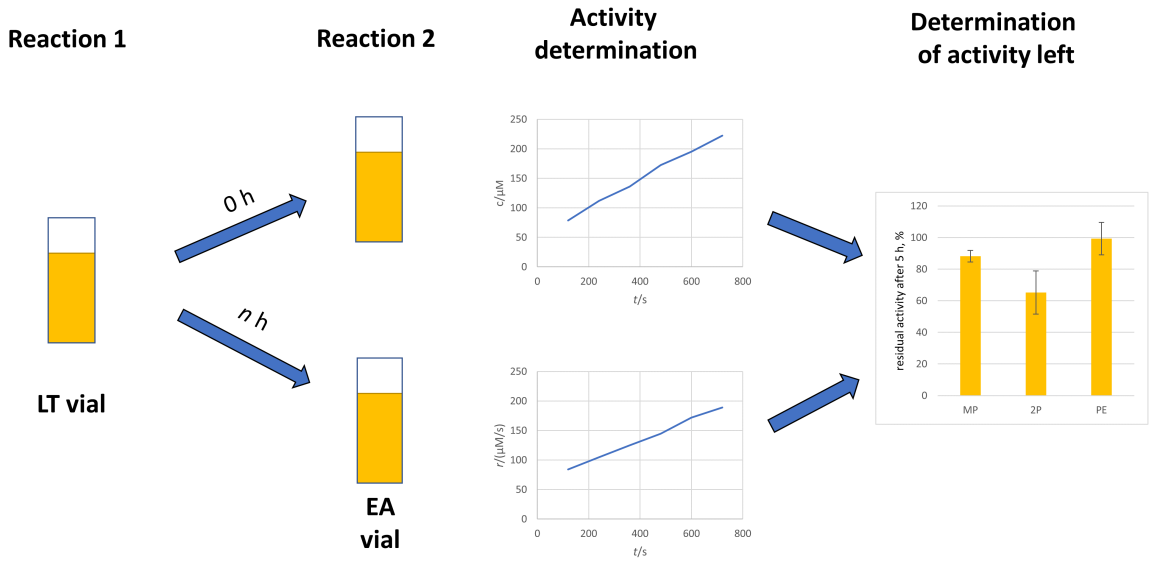

Figure 3.3: The sampling process scheme: In the vial labelled with "LT vial", the longterm (LT) reaction is run. At 0 and n h, a part of the LT vial's suspension is transferred to the vial in which the enzymatic activity (EA) is determined, here labelled with "EA vial". The residual activity was obtained by applying equation 3.2.

The enzyme activity was obtained by measuring the absorbance of the liquid in the EA vial at 245 nm as ACP has an absorption maximum at this wavelength[22]. With the help of a standard curve, see 3.2, these absorbances were transferred into the corresponding concentrations, which then were plotted against the time. Applying linear regression through these points gives r as it is described in equation 3.1.
Define that the following part is from the LT vial This enzyme activity determination was performed after n h at several time points t_n. To gain insights in the residual activity l, a_n which is the activity quantified at t_n, was set in relation to a_0 which is the activity measured at t_0, see equation 3.2.

$$l = \frac{a_n}{a_0} \tag{3.2}$$

3.2 Standard curve

To translate the absorbance into concentration, a standard curve was measured as described in 4.2.1 with both the quartz cuvette and the 96 well plates that were used in the experiments. To have as most precision as possible, the reaction was simulated, meaning that PEA, PYR, ACP, and ALA were added in the concentrations according to the status of the reaction. .
When using the quartz cuvette, the corresponding standard curve that is depicted in Figure 3.4 and for 96 well-plates, the curve that is depicted in Figure 3.5 has to be applied.
Linear regression of the absorbances resulted for cuvette measurements in equation 3.3 and for 96 well plates in equation 3.4.

$$A = 4.0819\ \mathrm{mM}^{-1} \cdot c + 0.0335 \tag{3.3}$$

$$A = 4.6668\ \mathrm{mM}^{-1} \cdot c + 0.0490 \tag{3.4}$$

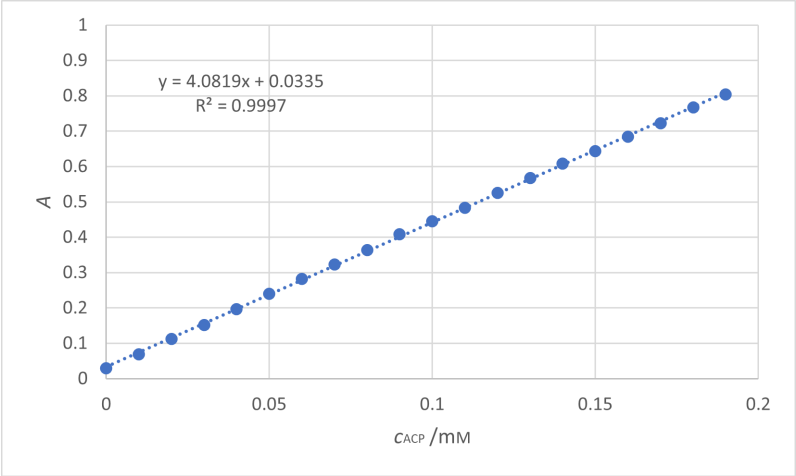

Figure 3.4: The standard curve that is applied for converting the absorbances obtained in measurements with the cuvette into the respective concentration.

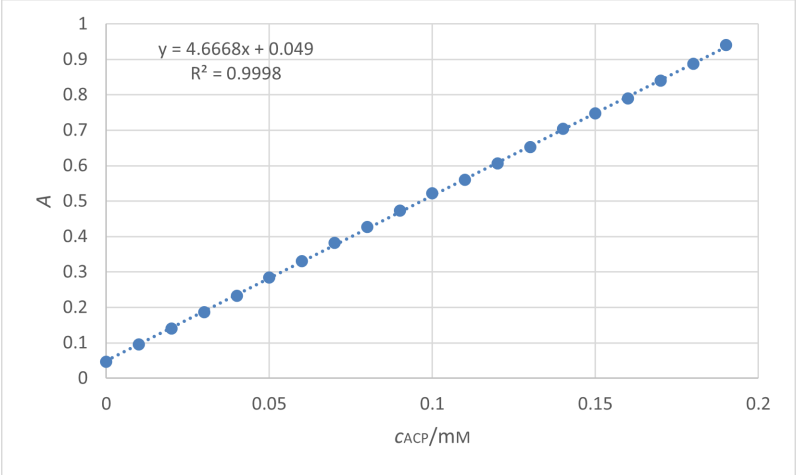

Figure 3.5: The standard curve for measurements that apply 96 well plates.

3.3 Continuous Assay

To run continuous assays, into the quartz cuvette 980 µL MilliQ water were added to 20 µL of sample from the LT vial of interest. This suspension was used to blank the spectrophotometer. The absorbance of this liquid was measured every 5 s over a time interval of 5 min, see description in detail in 4.2.2.

In the systems in which 15 µL of BA were added to 3 mL of IPA-buffer, the measurements gave graphs that had an oscillating absorbance curve. As an example, the curve in which the absorbance has been converted into the corresponding concentration is depicted in Figure 3.6. The rate r that is obtained if linear regression is applied is $r = 0.3034$ µM/s. It is visible that the determined concentration is negative.This means that the absorbance in the blank was higher than the measured absorbance. The spectrophotometer was blanked with the analysed suspension. As apparently the cell settled at the bottom of the cuvette after blanking, the liquid was more clear in the beginning of the measurement than at the blanking. Raw data to this graph can be found in table A.3.

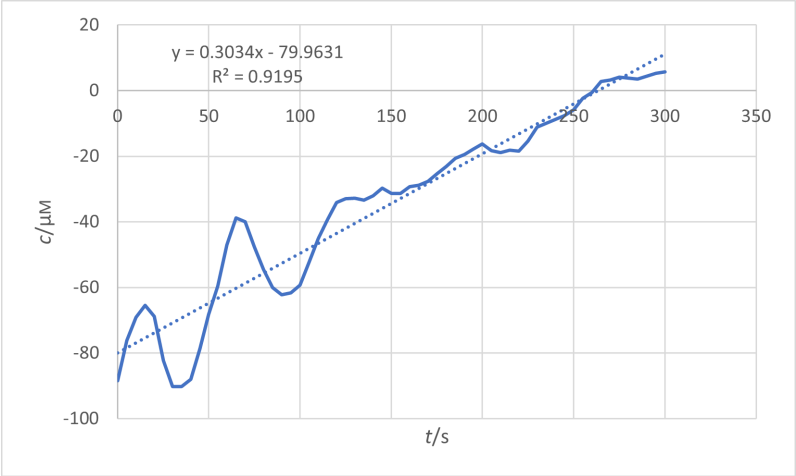

Figure 3.6: The curve that was obtained in the kinetic assay when adding 15 µL of BA to IPA-buffer at 0 h of measurement time including linear regression over the entire measurement interval.

Now an estimation is done of where the curve is less oscillating and looks more linear. One possibility is the time interval between t=115 s and t=300 s as it can be seen in Figure 3.7. When linear regression is applied, $r = 0.2492$ µM/s is obtained which is only 82% of the r that was obtained before.

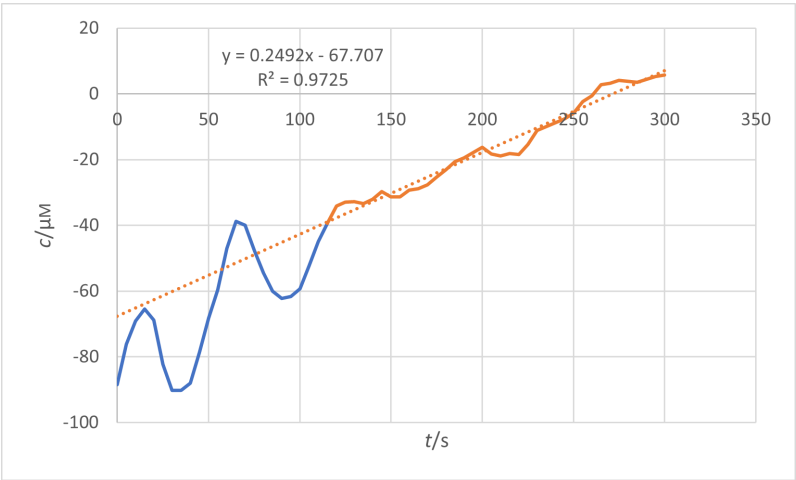

Figure 3.7: The curve including the same values as the curve in Figure 3.6, but linear regression is only applied over the time interval of t=115 s to t=300 s, here depicted in orange.

When cutting even more of the curve and only taking the time interval from t=155 s to t=300 s into account, $r = 0.2700$ µM/s is obtained. This scenario is depicted in Figure 3.8.

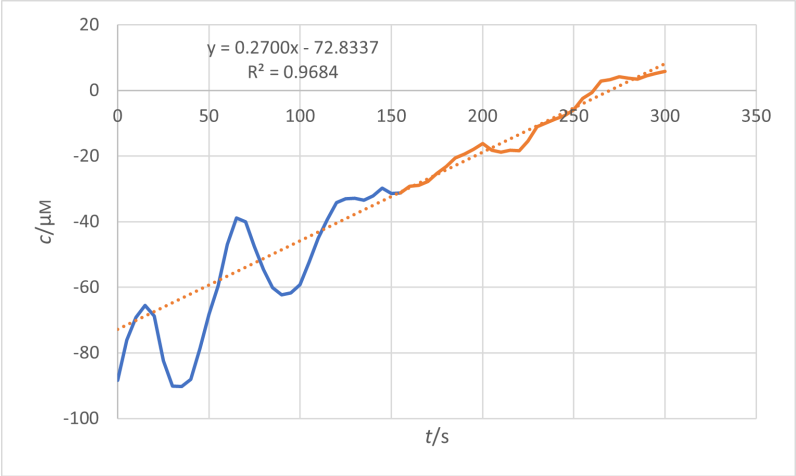

Figure 3.8: The concentration of ACP *vs* the respective time point applying the same values as in Figure 3.6. For the linear regression, the time interval from t=155 s to t=300 s is taken into account which is the orange curve.

In Figure 3.6, it can be seen that the amplitude of oscillation decreases over time which was observed as well at the other time points and in the other vial containing the system where 15 µL BA were added to 3 mL of IPA. An explanation for this might be that the cells settled down and the beam could go through the cuvette relatively undisturbed. It can be discussed whether the obtained activity is the true activity as the settled cells most probably did not transfer as much substrate into product as well dispersed cells would have done.
Both the phenomenons of an oscillating curve and that this oscillation decreased over time were observed in the systems in which 100 µL were added to 3 mL IPA-buffer as well. The raw data for all measurements can be found in A.2, starting from table A.4.
As the measurement of the first duplicate of the Pickering emulsion gave the graph that is depicted in Figure 3.9, it was not considered to be meaningful to measure the second duplicate nor to perform a longterm measurement.

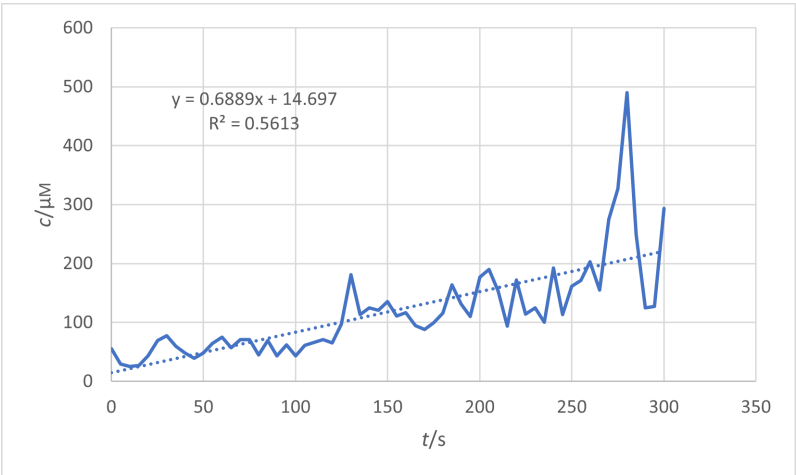

Figure 3.9: The curve from the continuous assay for the Pickering emulsion, c is plotted against t. For the linear regression, all values are taken into account.

Here, it is visible that the absorbance curve is very craggy. Applying linear regression on the time interval t=0 s to t=260 s gives also here a slope that significantly differs from the slope obtained when the linear regression is applied over the entire measurement interval. This is depicted in Figure 3.10.

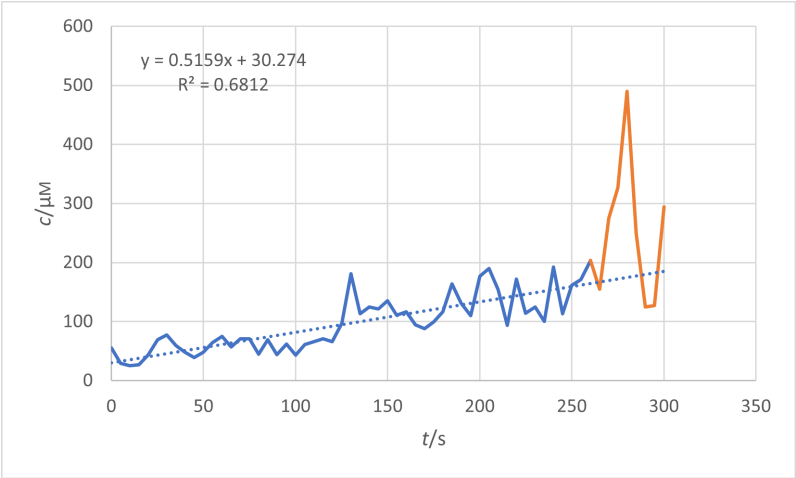

Figure 3.10: The curve obtained for the Pickering emulsion in the continuous assay, here, the orange part has been excluded from linear regression.

As the value of r depends on the regarded interval and setting this interval is very subjective, this method is not precise. Due to the light scattering, a decision has to be taken in terms of over which interval linear regression will be applied. As light scattering disturbs every measurement, this method cannot be applied for investigation of activity decrease as each measurement becomes too imprecise. As the oscillation of the curves most probably origins the light scattering from the particles in the system, a different method had to be applied.
Filtering or centrifuging the suspension before transfer into the cuvette for the kinetic measurement was not considered as helpful as it is assumed that the enzyme is in the cells. When centrifuging before adding the reaction liquid into the cuvette, only the enzyme-free IPA-buffer-substrate mixture is transferred to the EA vial which contradicts the purpose of enzymatic activity measurement as no enzyme is present that can perform the reaction.

3.4 Stop Assay

As r could not be measured directly inside the cuvette, an alternative method in which the measurement is performed on a system free from cells and particles must be found. Here, a stop assay was chosen.
The principle behind the stop assay is identical with the one applied in the continuous assay: the concentration of ACP in the EA vial is measured at specific time points. The difference is that all particles are removed *via* centrifugation before the spectrophotometric measurement.
Afterwards, an aliquot from the supernatant is taken and diluted, and the absorbance is measured in the spectrophotometer.
Sampling from the vial is repeated after specified time, so several concentrations for the corresponding time points were obtained.
Through these points, linear regression was applied. The slope of the obtained line is the rate.

The stop assay was performed on a cell suspension that was based on IPA buffer at 0 h, 1 h, and at least 4 h by transferring a sample from the LT vial to the EA vial. For obtaining the percentage of residual activity l, the average of measured activities at t=n h, $\bar{r_n}$ was set in relation to the average of activities determined at t=0 h $\bar{r_0}$, see equation 3.5.

$$l = \frac{\bar{r_n} \cdot 100}{\bar{r_0}} \tag{3.5}$$

Both values $\bar{r_n}$ and $\bar{r_0}$ exhibit a standard deviation so a Gauß propagation was applied to estimate the uncertainty of l, see equation 3.6.
Performing the partial derivations gives equation 3.7. As $\Delta\bar{r_0}$ and $\Delta\bar{r_n}$, the standard deviations of $\bar{r_0}$ and $\bar{r_n}$ were applied.

$$\Delta l = \sqrt{\left(\frac{\partial l}{\partial \bar{r_n}} \cdot \Delta\bar{r_n}\right)^2 + \left(\frac{\partial l}{\partial \bar{r_0}} \cdot \Delta\bar{r_0}\right)^2} \tag{3.6}$$

$$= \sqrt{\left(\frac{1}{\bar{r_0}} \cdot \Delta\bar{r_n}\right)^2 + \left(-\frac{\bar{r_n}}{\bar{r_0^2}} \cdot \Delta\bar{r_0}\right)^2} \tag{3.7}$$

3.4.1 4.5 mL glass vials at 1000 rpm with centrifugation followed by dilution

In this experiment, the change in activity of a monophase, biphase, Pickering emulsion, and pure cells was compared as it is described in 4.2.3. In table 3.1, the concentrations of substrates and cells that were applied here can be found.

Table 3.1: The concentrations of substrate and cells that were used in this experiment.

c_{IPA}/mM	$c_{\mathrm{cells,\ LT}}$/mg·mL^{-1}	c_{PEA}=c_{PYR}/mM	$c_{\mathrm{cells,\ EA}}$/mg·mL^{-1}
150	5	30	1.25

For sampling, the liquid taken from the EA vial was first centrifuged with the little table centrifuge for 30 s and then, it was diluted. The raw data from these absorbance measurements can be found in A.3. The consecutive step was transferring the absorbances into concentrations. These were plotted against the time points at which the samples were taken from the EA vial. Here, it has to be noticed that the measured absorbance cannot only origin from the ACP. If this was the case, the concentration obtained at t=0 min would expected to be 0 µM, but this is not the case, so it can be assumed that background contamination is present. Given that this contamination level stays constant over time, the slope of the line obtained from linear regression gives the respective activity. An example for how these graphs could look is depicted in Figure 3.11.

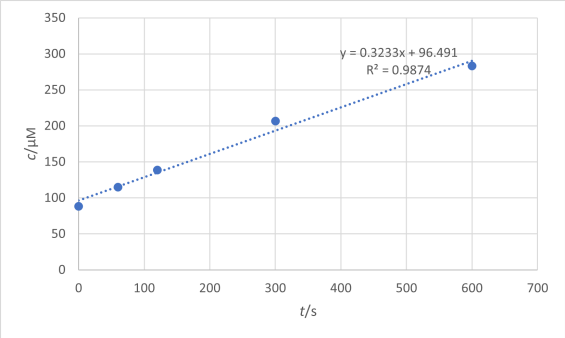

Figure 3.11: The graphs that are obtained after transferring the absorbances into concentrations and plotting them against the time at 0 h (left) and 5 h (right) for sample 1 of the monophase system. It has to be noticed that the concentration

An example how the graphs could look as well, meaning higher spread of the values, is depicted in Figure 3.12.

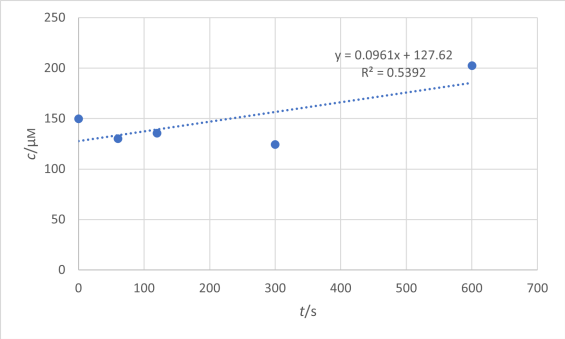

Figure 3.12: The plots of concentration against the time at 0 h (left) and 5 h (right) for sample 1 of the biphasic system's system to obtain the activity at the respective hour *via* linear regression.

A graphical comparison of the residual activities l is depicted in Figure 3.13.

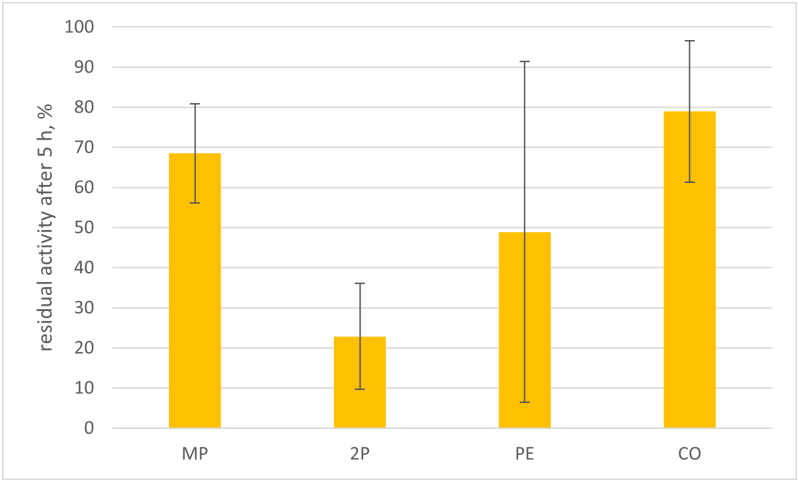

Figure 3.13: l of the investigated systems with using the table centrifuge and dilution of the supernatant: Monophase (MP): (68±13)%, two-phase (2P): (23±13)%, Pickering emulsion (PE): (49±43)%, cell suspension without substrate (CO): (79±18)%.

There can be drawn several conclusions from Figure 3.13. One, the shaking and/or the reaction environment impacts the enzymatic activity as it decreased by 21% in the sample without substrate. Two, employing the Pickering emulsion seems to have a protective effect, but on the other side, taking a look at the error bar of Δl, this conclusion seems not very sound. Three, l of the biphasic system is $(23 \pm 13)\%$ which means a high loss of activity.

3.4.2 4.5 mL glass vials at 1000 rpm with dilution followed by centrifugation

Looking into the results obtained in 3.4.1, depicted in Figure 3.13 it is visible that the standard deviation obtained in the Pickering emulsion measurement is so high that the result is unreliable. An approach to get values with a smaller standard deviation was swapping the order of dilution and centrifugation, using the big centrifuge, and doubling the cell concentration. Doubling the cell concentration was done to obtain an activity that ideally would be double as high than when using 5 mg·mL^{-1}, so the error also gets smaller.

Table 3.2: The concentrations of substrate and cells that were used in this experiment.

c_{IPA}/mM	$c_{\mathrm{cells,\ LT}}$ /mg·mL^{-1}	c_{PEA}=c_{PYR}/mM	$c_{\mathrm{cells,\ EA}}$/mg·mL^{-1}
150	10	30	2.5

Applying the method described in 4.2.4 resulted in the residual activities that are depicted in Figure 3.14. Raw data can be fond in A.4.

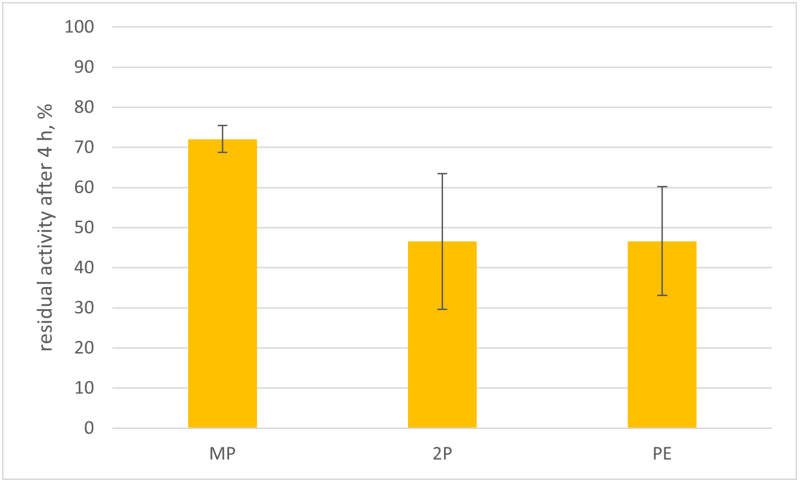

Figure 3.14: l of the different systems if the sample from the EA is first diluted and then centrifuged giving the following activities: MP: $(72 \pm 3)\%$, 2P: $(47 \pm 17)\%$, and PE: $(47 \pm 14)\%$.

Here, it can be seen that the standard deviations are smaller than before which means that the aforementioned measures taken were beneficial for the precision of the assay.
In contrast to 3.4.1, in this experiment, l was similar in the bi-phasic system and the Pickering emulsion.

The reason for this could be either that the Pickering emulsion did not stay intact throughout the process which would cause that the system was not applied as emulsion, but as biphasic system, or in case that the emulsion does stay intact, its application does not protect the enzyme from being deactivated.
In Figure 3.15, it can be seen that after the reaction, without any stirring, all the starch particles are at the bottom of the vial in contrast to the pre-experimental emulsion: Even after several days of storage, the major part of the emulsion is floating. When tilting both vials, white solid in the post-experimental vial settles again on the bottom while the aggregates of the emulsion stay where they are after stopping the tilting.
This suggests that the first mentioned option, namely that the Pickering emulsion did not stay intact during the experiment, is a valid explanation for the observed effect on l, meaning that a biphasic system might have been compared with a biphasic system.

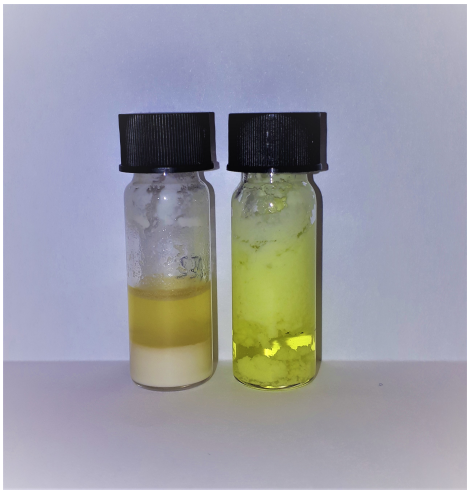

Figure 3.15: PE after running the experiment (left) *vs* before (right).

3.4.3 4.5 mL glass vials at 600 rpm with dilution followed by centrifugation

As mentioned in 3.4.2, 1000 rpm as stirring speed is too high for having an intact Pickering emulsion throughout the entire experimental time frame, so the shaking speed was lowered to 600 rpm. This was done after stirring a mixture of 3 mL water and 100 µL BA at 500 and 600 rpm for around 2 min. As 600 rpm gave a better mixing, this speed was chosen.

Table 3.3: The concentrations of substrate and cells that were used in this experiment.

c_{IPA}/mM	$c_{\mathrm{cells,\ LT}}$ /mg·mL^{-1}	c_{PEA}=c_{PYR}/mM	$c_{\mathrm{cells,\ EA}}$/mg·mL^{-1}
150	10	30	2.5

The experimental setup used in 3.4.2 was repeated at a lower shaking speed of 600 rpm. Instead of measuring the absorbance in a quartz cuvette, 96 well plates and a plate reader were used as measuring with the cuvette is very labour and time intense, see 4.2.5. The resulting activities which are computed from the absorbances, see A.5, are graphically expressed in Figure 3.16.

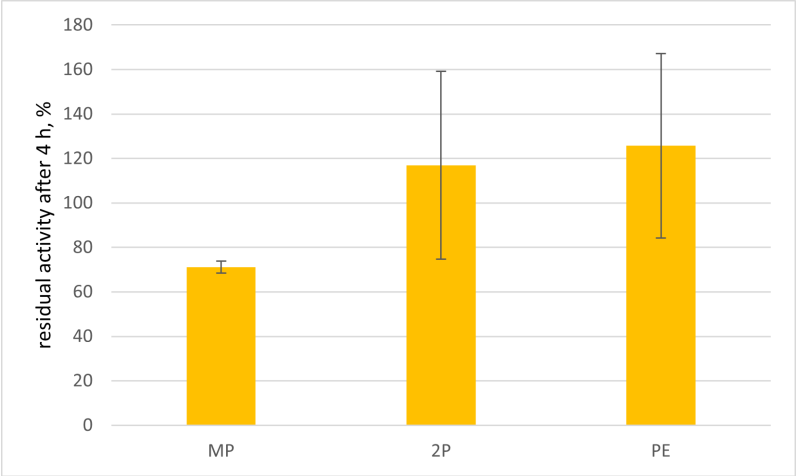

Figure 3.16: l of the different systems at 600 rpm: MP: $(71 \pm 3)\%$, 2P: $(117 \pm 42)\%$, and PE: $(126 \pm 41)\%$.

The outcome of this experiment is in regards to l_{2P} and l_{PE} unexpected as the activity seems to have increased over time.
The reason why this has happened can be that the suspension in the LT vial was not homogenous when being transferred to the EA vial which woud have resulted in different cell concentrations in the EA vial and thereby different activities. This hypothesis is supported by the activities that were obtained in the measurements, see table 3.4.

Table 3.4: r at the respective hours.

t/h	$r_{MP1}/(\mu M/s)$	$r_{MP2}/(\mu M/s)$	$r_{2P1}/(\mu M/s)$	$r_{2P2}/(\mu M/s)$	$r_{PE1}/(\mu M/s)$	$r_{PE2}/(\mu M/s)$
0	0.2166	0.2163	0.0548	0.1167	0.0539	0.1019
4	0.1479	0.1598	0.0985	0.102	0.1095	0.0864

The measured activities in the monophase samples are very similar, but in both the sample of the biphasic system and the Pickering emulsion, it can be seen that the activity in the first sample is about half of the one that is present in the second sample. When looking into these activities in a graphical way, see Figure 3.17, it is visible that the activities in the samples investigated at 0 h have a higher spread than the ones measured after 4 h.

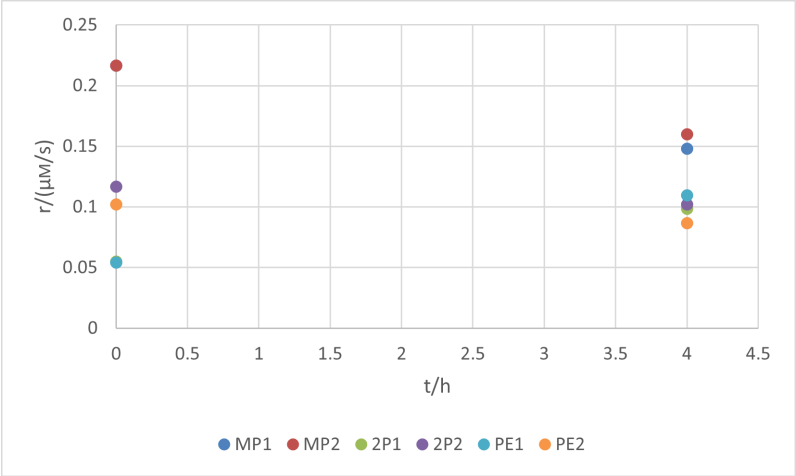

Figure 3.17: The activities at the different time points. At 0 h, only for points are visible as the activities of the MP duplicate very close and the activity of one of the PE sample is similar to the one in a 2P sample.

The outcome of this experiment is that before sampling from the LT vial, a mixing step has to be involved that ensures an equal spread of cells over the sample, but in a way influencing the enzyme activity as least as possible. As mixing means especially in the biphasic system contact between the organic phase and the cells, this is challenging. As time was not an eternal resource in this project, this has to be done in future investigations.

3.4.4 Eppendorf tubes at 500 rpm

The experiment was also performed in smaller scale to economize chemicals and catalyst. Coming to the timeline of the project, this experiment was done before taking into consideration that doubling the cell concentration might have a positive influence on the size of error bars, therefore, 5 mg/mL were used in the LT vial. 500 rpm were taken as a starting point for the stirring speed since the geometry of the Eppendorf tubes differs a lot from the glass vials.
For the assay, as it is described in 4.2.6, 1.5 mL tubes were used in the mono- and biphase samples, while 2 mL tubes were applied in the samples for the Pickering emulsion. There were not used 1.5 mL tubes for the Pickering emulsion samples because the blade of the UltraTurrax did not fit into the vial. The concentrations of substrates and cells in the suspension are displayed in 3.5.

Table 3.5: The concentrations of substrate and cells that were used in this experiment.

c_{IPA}/mM	$c_{\mathrm{cells,\ LT}}$ /mg·mL^{-1}	c_{PEA}=c_{PYR}/mM	$c_{\mathrm{cells,\ EA}}$/mg·mL^{-1}
150	5	30	1.25

The comparison between the activity at t=0 h and 5 h can be found in figure 3.18, for the raw data on which this computation is based on, see A.6.

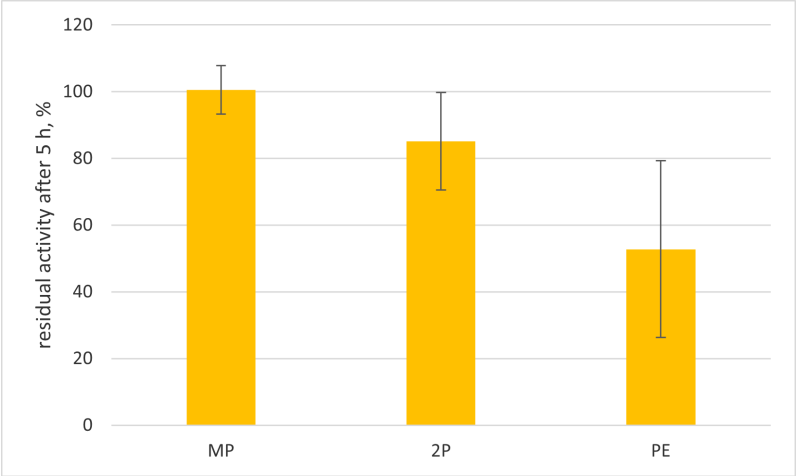

Figure 3.18: l of the different systems at 500 rpm: MP: $(101 \pm 7)\%$, 2P: $(85 \pm 15)\%$, and PE: $(53 \pm 26)\%$.

This outcome was not expected at all as the hypothesis is that the emulsion protects the enzyme from deactivation. The opposite seems to be the case here.
There was observed that in the 1.5 mL vials, the organic phase was shaken down underneath the aquaeous phase in the two-phase system. This effect could have caused less contact between the BA and the enzyme in the biphasic system than in the Pickering emulsion and thereby less deactivation happened. Another explanation can be that the starting hypothesis of the project is wrong, meaning that the Pickering emulsion does not protect the enzyme at all.

3.4.5 2 mL Eppendorf tubes at 1000 rpm

The fact that the organic phase was stirred under the aqueous phase most probably led to reaction conditions in the biphasic system that had a similarity to a monophase system as the phases were kept seperate from each others and not mixed. This was not the purpose of the experiment. It was tested with 900 µL water and 100 µL BA in a 2 mL Eppendorf tube whether the same effect would appear. This was not the case, so for this experiment, 2 mL Eppendorf tubes were used.
To compare the results that were obtained here with 3.4.2 where 4.5 mL glass vials were used, as stirring speed, 1000 rpm were chosen.

Table 3.6: The concentrations of substrate and cells that were used in this experiment.

$c_{\mathrm{IPA}}/\mathrm{mM}$	$c_{\mathrm{cells,\ LT}}/\mathrm{mg{\cdot}mL^{-1}}$	$c_{\mathrm{PEA}}{=}c_{\mathrm{PYR}}/\mathrm{mM}$	$c_{\mathrm{cells,\ EA}}/\mathrm{mg{\cdot}mL^{-1}}$
150	10	30	2.5

The experiment described in 3.4.4 was repeated with using 2 mL Eppendorf tubes as vials for both reaction 1 and 2, see 4.2.7, and gave the raw data that can be found in A.7. Transferring the data into concentrations and activities resulted in the deactivation that is visualized in Figure 3.19.

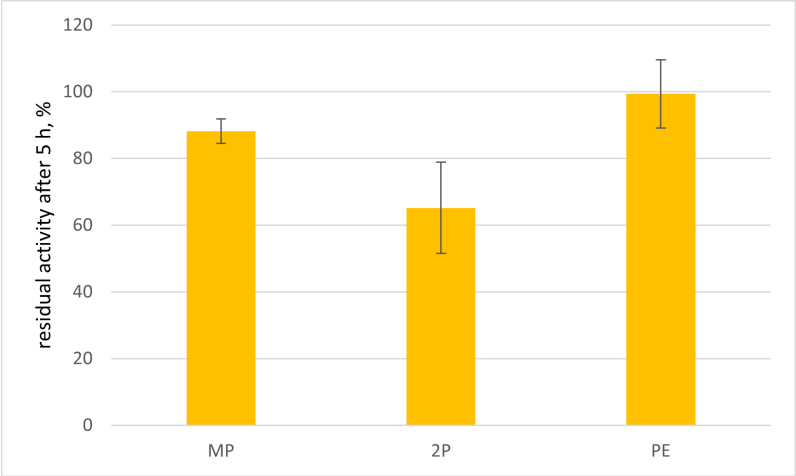

Figure 3.19: l of the different systems at 1000 rpm: MP: $(88 \pm 4)\%$, 2P: $(65 \pm 14)\%$, and PE: $(99 \pm 10)\%$.

Here, l of the Pickering emulsion is higher than l of the biphasic system which supports the hypothesis.

3.5 Conclusion

The main goal, a reliable investigation of whether the use of Pickering emulsion protects ω-TA from deactivation, could not be reached as the results were not consistent which could have had several reasons.
An assay using spectrophotometry in which the light scattering was eliminated was developed.
The assay development gave insight in other parameters that apparently influence the enzyme and emulsion stability.
An interesting observation is the loss in activity in the monophase: In the 4.5 mL vials at 1000 rpm and 600 rpm, see 3.4.2 and 3.4.3, l lied at $(72 \pm 3)\%$ and $(71 \pm 3)\%$, respectively, in 3.4.5 where 2 mL Eppendorf tubes were used, it is at (88 ± 4). Supported by the small Δl at all experiments, it can be said that the form of the vial has a bigger influence on the enzyme deactivation than the shaking speed as the difference of $l_{4.5\text{ mL, }1000\text{ rpm}}$ and $l_{4.5\text{ mL, }600\text{ rpm}}$ is very small, but when applying 1000 rpm on a 2 mL Eppendorf tube, the residual activity is significantly higher.
The data obtained in 3.4.2 compared to 3.4.5 regarding the Pickering emulsion point in the same direction: When applying the 2 mL Eppendorf tubes, no inactivation was observed in contrast to the 4.5 mL vials in which the combination of shaking at a high speed and the form of the vial seems to have caused conditions that were similar to the biphasic system.
Using the big centrifuge after having diluted the sample leads to more reliable results than using the small centrifuge with subsequent dilution.
Sampling at t_0 has to be improved. The method applied here gave widely spread a_0 values which most probably was caused by a heterogeneous distribution of cells over the vial. The sampling at t_0 has to happen when the cells are well mixed, but at the same time the enzymatic deactivation resulting from the mixing has to be as little as possible. This optimization has to happen in future projects.

To compare the enzymatic activity in these three different systems *via* applying spectrophotometry, performing a stop assay is recommended. At t_0 and t_n with $n \geq 4$ h, a sample from the LT vial has to be transferred into the EA vial. Concerning the optimum value of n, deactivation kinetics investigations should be run. The recommended sampling procedure from the EA vial is transferring every second minute 20 µL into a 1.5 mL Eppendorf tube and adding 980 µL MilliQ water with subsequent centrifugation with the big centrifuge at 15000 rcf for 1 min. For taking a sample from the Pickering emulsion from both the LT and EA vial, a cut-off pipette tip should be used to avoid clogging. It is recommended to use the plate reader for measuring absorbances as it is way less laborious than when the quartz cuvette is used. Measuring the absorbance with the plate reader should be done in triplicates to ensure a high enough precision. For determining the activity, the average of these absorbances converted into concentrations are plotted and linear regression is applied. The slope of this line gives the enzymatic activity.

Still, there are parameters which have to be optimized and/or investigated closer. The first one is the shaking speed. Here, it has to be be distinguished between the glass vials and the Eppendorf tubes. For the glass vials, the stirring speed on the thermoshaker should be lower than 600 rpm, but it has to investigated further which stirring speed enables a proper mixing of the biphasic system while the Pickering emulsion stays intact. For the Eppendorf tubes, a stirring speed of 1000 rpm seems not to be damaging, but it can be recommended to investigate this in further experiments. In both systems, the thermoshaker shaking speed optimum that enables a good mixing of the two-phase system, but also keeping the Pickering emulsion intact has to be found. It is not sure whether this optimum exists, meaning that it might be possible that a shaking speed that keeps the Pickering emulsion intact might be too slowly to enable enough mixing for the two-phase system, so this has to be figured out as well. As the geometry of the glass vials and the Eppendorf tubes differs so much, the shaking speed optimum most probably will lie at different points for each type of vial. On top of this, it could also be that the optimum exists only for one type of the vials.
In terms of whether the employment of the Pickering emulsion as reaction system using cell suspension is beneficial, one can take a look into the recently published study by Sun et al. [23]. Here, viable *E. coli*-cells were coated with dopamine and *N*-oleoyl dopamine to protect the cells from environmental stress. The coating is here describes as an "artificial sporulation" as the coating of the cells mimics the naturally occurring process of sporulation. In contrast to the natural sporulation which comes along with a highly reduced cell metabolism, the cells used here are fully active. First, the cells were used to overexpress different enzymes to perform example reactions. Secondly, cell coating was done and thirdly, a Pickering emulsion was generated using the coated cells as particles that were located in the interphase between the organic and aqueous phase. Then, the reaction was performed in this system. As comparison system, uncoated cells were used. The remaining enzymatic activity measured in the system using coated cells was after 2 h at around 50%, in the system using uncoated cells, it was around 30%. Comparing these results to the results obtained here, it can be concluded that using particles to generate the Pickering emulsion is beneficial for maintaining the enzymatic activity.

4 Materials and Methods

4.1 Materials

For the longterm experiments, a buffer (IPA-buffer) was used containing 150 mM IPA, 2 mM PLP, 100 mM TRIS, and 10 vol% glycerol.
For the activity measurements at t_n, a buffer (PEA-buffer) was used containing 40 mM PEA, 40 mM pyruvate, 2 mM PLP, and 100 mM TRIS. For the spectrophotometric measurements, this buffer was diluted 40X.
All chemicals were purchased at Sigma Aldrich.
The cells used in all experiments were inactivated *E. coli* cells containing CNB05-01 ω-transaminase from *CAMBREX*, Karlskoga AB, Sweden. The particles, further referred to as "starch", used as stabilisation agents for the Pickering emulsions were OSA modified quinoa starch particles under the brand name EQMULSE produced by Spexímo.
Pipettes from Finnpipette were used.
The reactions were carried out in 4.5 mL glass vials and 2 mL Eppendorf tubes. Centrifugation was done using 1.5 mL Eppendorf tubes. For the measurements in the spectrophotometer, a 1 mL quartz SUPRASIL cuvette type no. 1004.002-QS from HellmaAnalytics was used, for the plate reader, UV flat bottom Microtiter plates with 96 wells from Thermo were used.
For adjusting both of the buffers' pH values, the pH was determined with a pH meter (*pHenomenal*, VWR) and 4 mM HCl was added until pH=8 was reached. In both buffers, this was around 15 mL.
The thermoshakers used were Hettich MHR 13.
There were used two different centrifuges, the centrifuge that is described as "the little table centrifuge" is a mini centrifuge, the centrifuge that is referred to as "big centrifuge" (even though you do not need Andrius to transport it) is an Eppendorf 5424 centrifuge. All centrifugation was done with an Eppendorf 5424 centrifuge. The spectrophotometer used in all trials was an Evolution 300 UV-Vis and the plate reader used was a Multiskan Go, both by ThermoScientific.
For generating the Pickering emulsion, an UltraTurrax T25 from IKA was used.
The microscope used was produced by Olympus.

4.2 Methods

All spectrophotometric measurements were performed at λ=245 nm. For spectrophotometric measurements with the quartz cuvette, the spectrophotometer was blanked with MilliQ water prior to measurement.
All reactions were run at 37 ℃. Cells were always suspended in IPA-buffer, meaning a cell concentration of n mg/mL is mg of cells per mL of IPA-buffer.

4.2.1 Standard curve

To measure the standard curve, first a 2.5 mM TRIS-buffer had to be mixed. For this, 0.0485 g of TRIS were dissolved in 4 mL of water, giving a 100 mM TRIS-buffer. By adding 2.5 mL of this solution to 97.5 mL MilliQ-water, the 2.5-mM -TRIS-buffer was obtained, further entitled as "TRIS-buffer".
To 10 mL of TRIS-buffer, 2.54 µL of PEA were added, giving a 2 mM solution. In another 10 mL of TRIS-buffer, 0.0220 g of NaPYR were dissolved, giving a 20 mM solution. This solution was diluted by factor 10 with TRIS-buffer, giving a 2 mM solution.
5 mL of the 2 mM NaPYR-solution were mixed with 5 mL of the 2 mM PEA-solution, giving

10 mL of a 1 mM reactant solution.
The solution of the products was done similarly: To 10 mL of TRIS-buffer, 2.34 µL of ACP were added, giving a 2 mM solution. In another 10 mL of TRIS-buffer, 0.0178 g of ALA were dissolved, giving a 20 mM solution. The ALA-solution was diluted 10X with TRIS-buffer, so a 2 mM solution was obtained.
5 mL of the 2 mM the ALA solution are mixed with the 2 mM ACP-solution, so 10 mL of a 1 mM product solution were obtained.
The reactants and product solutions were mixed in a manner that simulated the reaction that is depicted in figure 3.2. The concentration range measured was from 0.00 to 0.19 mM of ACP with steps of 0.01 mM, including the corresponding amount left of a starting solution with 0.20 mM PEA and PYR. The precise values can be found in table A.1.
1 mL of each solution was pipetted into the cuvette and the absorbance was measured.
For the standard curve of the 96 well plates, for each concentration the absorbance of 200 µL of the respective solution was measured in 3 wells. For blank, water was added into three additional wells. Before plotting, the average blank value was subtracted from each measured sample absorbance. To obtain the standard curve, the absorbance was plotted against the concentration of ACP. This was done for both the values obtained from the cuvette and the 96 well plate.

4.2.2 Continuous Assay

To 3 mL of a suspension of 50 mg/mL, in one duplicate 15 µL, and in a second duplicate, 100 µL of BA were added. A third duplicate with a Pickering emulsion was mixed, using 2430 µL of IPA-buffer, 150 mg of starch, and 300 µL of BA. After 30 s of mixing with the UltraTurrax at $1.5 \cdot 10^4$ rpm, 270 µL of a cell suspension of 50 mg/mL was added.
The LT vials were put into a thermoshaker where they were shaken at500 rpm.
To measure the residual enzymatic activity, 50 µL from the LT vial were transferred into the cuvette which was filled with 950 µL of 40X diluted PEA-buffer. The spectrophotometer was blanked with this suspension. The absorbance was measured every 5 s over a total time interval of 300 s. As the result from the Pickering emulsion was very bad, it was decided to not measure the absorbance of the second sample.

4.2.3 4.5 mL glass vials at 1000 rpm with centrifugation followed by dilution

Four different systems with a cell concentration of 5 mg/mL were compared: monophase in a triplicate in which 4.44 µL of BA were dissolved in 3 mL of cell suspension, a two-phase system in a quadruplicate with 3 mL cell suspension to which 100 µL BA were added, a Pickering emulsion in one duplicate that was run for 4, and another duplicate that was run for 5 h, and 3 mL of the cell suspension (CO) in a quadruplicate without substrate. To obtain the Pickering emulsion, 2430 µL of IPA-buffer, 150 mg starch, and 300 µL BA were mixed with the UltraTurrax for 30 s at 15000 rpm. After this, 270 µL of a cell suspension with 50 mg/mL was added. The reactions were run in 4.5 mL vials at 1000 rpm. Sampling was done *via* transferring 500 µL into another 4.5 mL vial containing 1.5 mL PEA-buffer (EA vial) which as well was shaken at 1000 rpm. Sampling from the EA vial was done through transfer of 200 µL of the liquid into a 1.5 mL-Eppendorf tube, and 30 s of centrifugation with the little table centrifuge at the times that are denoted in the respective table in the appendix, see A.3. From the supernatant, 20 µL were transferred into

the quartz cuvette, then, 980 µL of MilliQ water were added. Absorbance measurements were performed with the spectrophotometer.

4.2.4 4.5 mL glass vials at 1000 rpm with dilution followed by centrifugation

This experiment was performed in duplicates on each system in 4.5 mL glass vials at 100 rpm. The systems investigated here were monophase, biphase, and Pickering emulsion. Preparation of the monophase and Pickering emulsion samples was done similarly to 4.2.3, for the biphase sample, to 2.7 mL of IPA-buffer, 300 µL of BA were added. Here, the working concentration of cells was 10 mg/mL.
Over the first 12 min of the process, 6 samples were taken from this vial, meaning that 20 µL of the mixture were transferred into a 1.5-mL-Eppendorf tube. After adding 980 µL of water, the sample was centrifuged with the big centrifuge for one minute at 15000 rcf. 600 µL of the supernatant were pipetted into the quartz cuvette and absorbance was measured.

4.2.5 4.5 mL glass vials at 600 rpm with dilution followed by centrifugation

The samples were prepared in duplicates similar to 4.2.4, besides that the shaking speed on the thermoshaker was lowered to 600 rpm.
Absorbance measurement was done with 96 well plates. For this, from the supernatant of the centrifuged EA vial sample, 3X200 µL were transferred into wells of a microtiter plate. The absorbance was measured in the plate reader.

4.2.6 Eppendorf tubes at 500 rpm

The monophase and biphase systems were held in 1.5 mL Eppendorf tubes, for the Pickering emulsion, 2 mL tubes were used. The cell suspension that was added to each sample had a concentration of 50 mg/mL. To obtain a monophase system, 3 mL IPA-buffer were mixed with 4.44 µL BA. To 900 µL of this mixture, 100 µL of cell suspension were added. To obtain the biphasic system, 800 µL of IPA-buffer were mixed with 100 mL of BA and 100 µL of cell suspension were added.For obtaining the samples for Pickering emulsion, 50 mg starch, 810 µL IPA-buffer, and 100 µL BA were mixed for 30 s with the UltraTurrax. To this resulting emusion, 90 µL of cell suspension were added. The reaction was run at 500 rpm. Sampling into the EA vial happened through transfer of 100 µL from the LT vial to 300 µL of PEA-buffer in a 1.5 mL Eppendorf tube. Sampling from the EA vial and subsequent measurement of absorbance with the plate reader was done every second minute in the first 12 min of reaction similarly to 4.2.5, see precise times in A.6.

4.2.7 2 mL Eppendorf tubes at 1000 rpm

The same methodology as in 4.2.6 was used besides the changes in the following parameters. For all LT and EA vials, 2 mL Eppendorf tubes were used. The stirring speed of the thermoshaker was at 1000 rpm, and the concentration of cell suspension that was added to each sample was 100 mg/mL. Sampling from the LT vial happened at 0 and 4 h of reaction time.

5 Future perspectives

As already mentioned in 3.4.3, the sampling from the LT vial has to be developed so that the sample transferred contains the same cell concentration as the remaining liquid in the vial. This means that a mixing method has to found, but in regards to the susceptibility of the enzyme towards contact with an organic compound, this method has to be performed that in each sample, the same deactivation takes place.
One development can be to perform the sampling for r_0 determination before adding the substrate. A concentration correction has to be done representatively relate the activities. As sampling is as challenging as described, applying headspace gas chromatographic measurements might be interesting.

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A Appendix

A.1 Standard curve

The reactant solution containing 1 mM PEA and 1 mM PYR is referred to as R, the product solution containing 1 mM ACP and 1 mM ALA is referred to as P.

Table A.1: The concentrations of PEA, PYR, ACP, and ALA, including the volumes and absorbances A obtained from the measurement both in the quartz cuvette (cuv) and the 96 well plate (96w) of the pipetted solutions. To all solutions, 1.6 mL of 2.5 mM TRIS-buffer were added. The absorbances in the 96 well plate were measured in triplicates.

c_{ACP}/mM	c_{ALA}/mM	c_{PEA}/mM	c_{PYR}/mM	V_{P} /µL	V_{S} /µL	A_{cuv}	$A_{\mathrm{96w,1}}$	$A_{\mathrm{96w,2}}$	$A_{\mathrm{96w,3}}$
0.00	0.00	0.20	0.20	0	400	0.02973	0.1210	0.1214	0.1215
0.01	0.01	0.19	0.19	20	380	0.06903	0.1698	0.1713	0.1700
0.02	0.02	0.18	0.18	40	360	0.11160	0.2151	0.2157	0.2156
0.03	0.03	0.17	0.17	60	340	0.15147	0.2625	0.2613	0.2635
0.04	0.04	0.16	0.16	80	320	0.19699	0.3120	0.3057	0.3067
0.05	0.05	0.15	0.15	100	300	0.23952	0.3594	0.3594	0.3588
0.06	0.06	0.14	0.14	120	280	0.28274	0.4047	0.4085	0.4052
0.07	0.07	0.13	0.13	140	260	0.32249	0.4544	0.4575	0.4589
0.08	0.08	0.12	0.12	160	240	0.36294	0.5034	0.5023	0.5002
0.09	0.09	0.11	0.11	180	220	0.40904	0.5456	0.5498	0.5501
0.10	0.10	0.10	0.10	200	200	0.44540	0.5927	0.5983	0.6003
0.11	0.11	0.09	0.09	220	180	0.48367	0.6338	0.6341	0.6382
0.12	0.12	0.08	0.08	240	160	0.52487	0.6873	0.6769	0.6818
0.13	0.13	0.07	0.07	260	140	0.56704	0.7272	0.7269	0.7300
0.14	0.14	0.06	0.06	280	120	0.60822	0.7767	0.7800	0.7821
0.15	0.15	0.05	0.05	300	100	0.64304	0.8219	0.8230	0.8246
0.16	0.16	0.04	0.04	320	80	0.68371	0.8631	0.8630	0.8681
0.17	0.17	0.03	0.03	340	60	0.72269	0.9067	0.9234	0.9173
0.18	0.18	0.02	0.02	360	40	0.76690	0.9691	0.9584	0.9597
0.19	0.19	0.01	0.01	380	20	0.80421	1.0171	1.0118	1.1018

Table A.2: The values obtained for blank in the 96-well-plate. The measurement was done with 200 µL of MilliQ water in each well.

$A_{\mathrm{H_2O,\,1}}$	$A_{\mathrm{H_2O,\,2}}$	$A_{\mathrm{H_2O,\,3}}$
0.0768	0.0742	0.00752

A.2 Continuous Assay

The raw data that were obtained in the continuous assay are displayed here. All absorbances were measured in the quartz cuvette.

Table A.3: The absorbances at the respective hours measured on sample 1 of a system of 3 mL IPA-buffer plus 15 µL BA. The spectrophotometer was blanked with the sample.

t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$	t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$
0	-0.32739	0.01662	0.05340	155	-0.09427	0.07375	0.12045
5	-0.27721	-0.01913	0.05238	160	-0.08595	0.10554	0.11988
10	-0.24869	-0.04208	0.04782	165	-0.08424	0.12845	0.11675
15	-0.23379	-0.05733	0.04634	170	-0.07950	0.14086	0.11432
20	-0.24721	-0.05587	0.05122	175	-0.06965	0.14661	0.11398
25	-0.30258	-0.02069	0.04263	180	-0.06106	0.14180	0.11632
30	-0.33464	0.04758	0.04350	185	-0.05061	0.13197	0.12314
35	-0.33484	0.07664	0.03999	190	-0.04577	0.12160	0.13049
40	-0.32587	0.12019	0.03838	195	-0.03956	0.11226	0.13573
45	-0.28773	0.12320	0.04618	200	-0.03275	0.11019	0.14166
50	-0.24485	0.08106	0.07253	205	-0.04095	0.11084	0.14835
55	-0.20950	0.03812	0.08215	210	-0.04354	0.10195	0.15186
60	-0.15818	0.01234	0.07524	215	-0.04065	0.09059	0.15593
65	-0.12501	0.00471	0.06786	220	-0.04166	0.08792	0.15958
70	-0.12973	0.00066	0.07412	225	-0.02945	0.10072	0.16048
75	-0.16063	0.00209	0.08260	230	-0.01172	0.12005	0.15973
80	-0.18903	0.01292	0.08563	235	-0.00697	0.12602	0.16083
85	-0.21161	0.03867	0.08327	240	-0.00230	0.12918	0.16480
90	-0.22060	0.06645	0.08139	245	0.00218	0.13163	0.16891
95	-0.21834	0.07568	0.08298	250	0.00946	0.13373	0.17059
100	-0.20833	0.08046	0.08614	255	0.02381	0.13737	0.16970
105	-0.17995	0.08146	0.08745	260	0.03113	0.14410	0.17098
110	-0.15031	0.07915	0.09412	265	0.04512	0.14654	0.17391
115	-0.12711	0.07280	0.09757	270	0.04683	0.14444	0.17933
120	-0.10594	0.07477	0.10107	275	0.05040	0.14156	0.18444
125	-0.10107	0.06944	0.10396	280	0.04882	0.14092	0.18749
130	-0.10048	0.05455	0.10691	285	0.04785	0.14180	0.19140
135	-0.10276	0.04318	0.11171	290	0.05161	0.14314	0.19625
140	-0.09749	0.03942	0.11243	295	0.05495	0.14513	0.19990
145	-0.08772	0.04347	0.11258	300	0.05708	0.15022	0.20337
150	-0.09453	0.04993	0.11479				

Table A.4: The absorbances at the respective hours measured on sample 2 of a system of 3 mL IPA-buffer plus 15 µL BA. The spectrophotometer was blanked with the sample.

t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$	t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$
0	-0.22457	-0.49172	-0.12624	155	0.01039	-0.13057	-0.03886
5	-0.18199	-0.41108	-0.14646	160	0.02930	-0.12897	-0.03148
10	-0.14362	-0.33918	-0.12178	165	0.04320	-0.12096	-0.02459
15	-0.12607	-0.25941	-0.10495	170	0.03967	-0.11246	-0.02049
20	-0.09381	-0.17058	-0.10245	175	0.03837	-0.09277	-0.01560
25	-0.05435	-0.16409	-0.10446	180	0.04530	-0.08978	-0.01060
30	-0.06720	-0.18063	-0.10351	185	0.04755	-0.09966	-0.00475
35	-0.07268	-0.19261	-0.09517	190	0.04327	-0.11165	-0.00426
40	-0.09002	-0.22097	-0.09828	195	0.04286	-0.12286	-0.00931
45	-0.12950	-0.24674	-0.10113	200	0.05118	-0.12465	-0.01599
50	-0.11867	-0.31247	-0.09935	205	0.06354	-0.12494	-0.01965
55	-0.10326	-0.38013	-0.09919	210	0.07655	-0.12021	-0.01923
60	-0.11217	-0.36742	-0.10907	215	0.08135	-0.10894	-0.01572
65	-0.11060	-0.30901	-0.12099	220	0.08110	-0.10039	-0.01029
70	-0.08540	-0.24223	-0.11391	225	0.08813	-0.08504	-0.00505
75	-0.05887	-0.16234	-0.10245	230	0.10518	-0.06675	-0.00306
80	-0.03738	-0.13458	-0.09614	235	0.12329	-0.04807	-0.00040
85	-0.02157	-0.13691	-0.08189	240	0.13074	-0.03228	0.00196
90	-0.01588	-0.13814	-0.06862	245	0.11546	-0.02196	0.00732
95	-0.02558	-0.15001	-0.05401	250	0.10102	-0.01893	0.01493
100	-0.01405	-0.16134	-0.04592	255	0.10050	-0.02399	0.02124
105	0.00409	-0.18440	-0.04854	260	0.10529	-0.02944	0.02488
110	0.00322	-0.21348	-0.05289	265	0.11090	-0.02742	0.02983
115	-0.00494	-0.23345	-0.05372	270	0.11212	-0.03125	0.03537
120	-0.02224	-0.24342	-0.05588	275	0.10990	-0.04345	0.03686
125	-0.02583	-0.25215	-0.05643	280	0.11172	-0.04640	0.03255
130	-0.01724	-0.24035	-0.05594	285	0.12357	-0.04149	0.03021
135	-0.01718	-0.19104	-0.05723	290	0.13545	-0.03351	0.03068
140	-0.02547	-0.15909	-0.05624	295	0.14891	-0.02590	0.03423
145	-0.02910	-0.14458	-0.05044	300	0.16285	-0.02400	0.04131
150	-0.01022	-0.13026	-0.04417				

Table A.5: The absorbances at the respective hours measured on sample 1 of a system of 3 mL IPA-buffer plus 100 µL BA. The absorbances are obtained after blanking the spectrophotometer with the sample.

t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$	t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$
0	-0.39779	-0.05256	0.19747	155	-0.01027	-0.02935	0.28899
5	-0.54313	-0.12824	0.20160	160	0.01546	-0.01986	0.28863
10	-0.62949	-0.12383	0.20323	165	0.02987	-0.01020	0.28966
15	-0.54620	-0.09670	0.21937	170	0.03591	-0.00202	0.29245
20	-0.42347	-0.07618	0.23369	175	0.04318	0.00386	0.29786
25	-0.30010	-0.06201	0.20281	180	0.03034	0.00782	0.30380
30	-0.22966	-0.05226	0.17274	185	0.01830	0.00767	0.30539
35	-0.20340	-0.04771	0.19318	190	0.01546	0.00295	0.31317
40	-0.18630	-0.04346	0.21866	195	0.02844	-0.00421	0.31990
45	-0.20926	-0.04451	0.24495	200	0.05125	-0.00926	0.32733
50	-0.27223	-0.04803	0.26332	205	0.07662	-0.00734	0.33434
55	-0.31315	-0.05931	0.27363	210	0.09548	-0.00022	0.33947
60	-0.32822	-0.08618	0.28022	215	0.10447	0.00907	0.34578
65	-0.34301	-0.09693	0.28201	220	0.11651	0.01652	0.35104
70	-0.25738	-0.08630	0.27975	225	0.12484	0.01915	0.35451
75	-0.23191	-0.06666	0.27607	230	0.12699	0.01977	0.35590
80	-0.20562	-0.05034	0.25952	235	0.12974	0.02292	0.35672
85	-0.11896	-0.03938	0.24402	240	0.12980	0.02598	0.35995
90	-0.05614	-0.03014	0.23327	245	0.13236	0.02956	0.36065
95	-0.03836	-0.02469	0.24286	250	0.14106	0.03292	0.36055
100	-0.04369	-0.02317	0.27164	255	0.14129	0.03617	0.36299
105	-0.07706	-0.02017	0.27910	260	0.13120	0.03855	0.36914
110	-0.11080	-0.01941	0.28258	265	0.12001	0.03939	0.37414
115	-0.14244	-0.02601	0.29120	270	0.11707	0.03888	0.37592
120	-0.16150	-0.03555	0.29562	275	0.12204	0.03645	0.37956
125	-0.13885	-0.04276	0.29940	280	0.13139	0.03530	0.38278
130	-0.11900	-0.04607	0.30610	285	0.14924	0.03702	0.38314
135	-0.11789	-0.04556	0.30934	290	0.16487	0.04008	0.38363
140	-0.11595	-0.04319	0.30547	295	0.17885	0.04283	0.38506
145	-0.08398	-0.03918	0.29820	300	0.19039	0.04408	0.38879
150	-0.04003	-0.03540	0.29337				

Table A.6: The absorbances at the respective hours measured on sample 2 of a system of 3 mL IPA-buffer plus 100 µL BA. The absorbances are obtained after blanking the spectrophotometer with the sample.

t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$	t/s	$A_{0\ \mathrm{h}}$	$A_{2.5\ \mathrm{h}}$	$A_{5.5\ \mathrm{h}}$
0	-0.04940	0.10927	0.00421	155	0.40149	0.21522	0.04373
5	-0.12967	0.09074	-0.00537	160	0.43684	0.21610	0.04694
10	-0.09563	-0.04386	-0.01090	165	0.43651	0.21557	0.05249
15	-0.02226	-0.04263	-0.01725	170	0.43320	0.22226	0.05504
20	0.07905	-0.02202	-0.03037	175	0.43667	0.23107	0.05900
25	0.16166	0.06015	-0.03943	180	0.43818	0.23503	0.06578
30	0.20132	0.09555	-0.02028	185	0.43815	0.23982	0.07241
35	0.15763	0.12741	0.00622	190	0.44301	0.24251	0.07776
40	0.13477	0.13528	0.00317	195	0.44372	0.24299	0.07997
45	0.14813	0.10999	0.00475	200	0.45516	0.23763	0.08070
50	0.17306	0.05553	0.00902	205	0.47347	0.23322	0.07931
55	0.12425	0.04072	0.02159	210	0.48557	0.24065	0.07744
60	0.07748	0.08720	0.02939	215	0.50464	0.25142	0.07555
65	0.10131	0.11500	0.02168	220	0.52147	0.26033	0.07702
70	0.14228	0.13958	0.01340	225	0.53304	0.26658	0.07895
75	0.16950	0.15360	0.01082	230	0.54688	0.27110	0.07624
80	0.28722	0.13356	0.00669	235	0.55470	0.27565	0.07699
85	0.37409	0.12594	-0.00124	240	0.55410	0.27908	0.08397
90	0.40547	0.14205	0.00136	245	0.55214	0.28478	0.09018
95	0.37542	0.17070	0.01055	250	0.55682	0.29452	0.09496
100	0.33999	0.19256	0.02242	255	0.56343	0.30211	0.09875
105	0.32112	0.19732	0.03326	260	0.56715	0.30452	0.10514
110	0.32547	0.18368	0.04530	265	0.57433	0.30460	0.10844
115	0.29402	0.15821	0.05313	270	0.58897	0.30664	0.11124
120	0.26541	0.14688	0.05835	275	0.60287	0.30773	0.11188
125	0.26066	0.16291	0.05764	280	0.61240	0.31333	0.11166
130	0.26187	0.16878	0.05512	285	0.61786	0.32190	0.11005
135	0.26017	0.16464	0.05271	290	0.61809	0.32791	0.11074
140	0.27640	0.17700	0.05053	295	0.62181	0.33225	0.11104
145	0.31946	0.19978	0.04668	300	0.63273	0.33673	0.10988
150	0.35823	0.21019	0.04350				

Table A.7: The absorbances measured on the Pickering emulsion sample at t=0 h. Also here, the spectrophotometer was blanked with the sample.

t/s	A	t/s	A
0	0.25957	155	0.48596
5	0.15378	160	0.51058
10	0.13587	165	0.41945
15	0.14395	170	0.39201
20	0.21013	175	0.43877
25	0.31675	180	0.50766
30	0.34872	185	0.70339
35	0.27743	190	0.56961
40	0.22958	195	0.48392
45	0.19300	200	0.75675
50	0.22992	205	0.80878
55	0.29725	210	0.66046
60	0.34057	215	0.41589
65	0.26753	220	0.73530
70	0.32319	225	0.49933
75	0.32240	230	0.54301
80	0.21525	235	0.44393
85	0.31651	240	0.81876
90	0.21182	245	0.49590
95	0.28646	250	0.69316
100	0.20926	255	0.73342
105	0.28280	260	0.86347
110	0.30411	265	0.66462
115	0.32329	270	1.15475
120	0.30138	275	1.36714
125	0.43050	280	2.03296
130	0.77109	285	1.05022
135	0.49620	290	0.54257
140	0.54197	295	0.55374
145	0.52728	300	1.23267
150	0.58599		

A.3 4.5 mL glass vials at 1000 rpm with centrifugation followed by dilution

A.3.1 Monophase

Table A.8: The absorbances measured on sample 1.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.39294	0.43819	0.41936
60	0.50296	0.33075	0.46452
120	0.59869	0.42702	0.51862
300	0.87782	0.65347	0.66129
600	1.18964	0.82304	0.85714

Table A.9: The absorbances measured on sample 2.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.51044	0.4015	0.22667
60	0.62183	0.39659	0.21832
120	0.64960	0.42779	0.23989
300	0.76972	0.59068	0.24925
600	1.06190	0.79361	0.26819

Table A.10: The absorbances measured on sample 3.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.51802	0.37515	0.48266
60	0.44402	0.52573	0.48261
120	0.67910	0.46547	0.55254
300	0.76669	0.59125	0.86900
600	1.08577	0.70985	0.85686

A.3.2 Biphasic system

Table A.11: The absorbances measured on sample 1.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.34252	0.46131	0.45302
60	0.35166	0.45010	0.45658
120	0.60084	0.45229	0.51267
300	0.82104	0.64919	0.45689
600	0.83270	0.60660	0.59082

Table A.12: The absorbances measured on sample 2.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.64518	0.38112	0.40087
60	0.56382	0.39477	0.52453
120	0.58620	0.44296	0.43282
300	0.54068	0.50102	0.54344
600	0.86005	0.58007	0.55772

Table A.13: The absorbances measured on sample 3.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.35094	0.41026	0.57106
60	0.38152	0.47078	0.52224
120	0.41589	0.49640	0.50301
300	0.53580	0.48445	0.49238
600	0.69021	0.67216	0.59026

Table A.14: The absorbances measured on sample 4.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.36088	0.41421	0.50297
60	0.38065	0.45986	0.47694
120	0.40879	0.44368	0.46046
300	0.59262	0.54830	0.51426
600	0.67933	0.57041	0.52339

A.3.3 Pickering emulsion

Table A.15: The absorbances measured on sample 1.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.53679	0.49877	0.45638
120	0.50965	0.46740	0.43303
240	0.53971	0.48327	0.47640
360	0.55063	0.46019	0.41550
480	0.60715	0.53771	0.46702
600	0.61571	0.44231	0.49396

Table A.16: The absorbances measured on sample 2.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{5\ \mathrm{h}}$
0	0.42089	0.51347	0.41012
120	0.52826	0.38883	0.44996
240	0.73825	0.39304	0.47063
360	0.63192	0.48822	0.46324
480	0.69810	0.54012	0.51600
600	0.79233	0.49158	0.53352

Table A.17: The absorbances measured on sample 3.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{4\ \mathrm{h}}$
120	0.35013	0.41688	0.37866
240	0.42408	0.37336	0.42127
360	0.39564	0.38839	0.38816
480	0.44011	0.46566	0.44791
600	0.40864	0.40958	0.38931
720	0.43828	0.43115	0.45737

Table A.18: The absorbances measured on sample 4.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	$A_{4\ \mathrm{h}}$
120	0.33206	0.38886	0.41342
240	0.42016	0.38818	0.41130
360	0.38804	0.45618	0.50734
480	0.43681	0.41580	0.42914
600	0.41634	0.45354	0.54913
720	0.49375	0.53895	0.57882

A.3.4 Cell suspension without substrate

Table A.19: The absorbances measured on sample 1.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	t/s	$A_{5\ \mathrm{h}}$
0	0.38869	0.34549	0	0.36375
60	0.40112	0.50237	120	0.33196
120	0.55946	0.36387	240	0.43730
300	0.60989	0.57792	360	0.50127
600	0.81023	0.68439	480	0.56502
			600	0.57794

Table A.20: The absorbances measured on sample 2.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	t/s	$A_{5\ \mathrm{h}}$
0	0.36510	0.40011	0	0.30003
60	0.38354	0.37152	120	0.35253
120	0.36733	0.38643	240	0.42375
300	0.56971	0.57265	360	0.43377
600	0.67512	0.73460	480	0.47223
			600	0.54615

Table A.21: The absorbances measured on sample 3.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	t/s	$A_{5\ \mathrm{h}}$
0	0.40022	0.31981	0	0.29349
60	0.36087	0.41167	120	0.35992
120	0.36882	0.48457	240	0.36916
300	0.60246	0.56013	360	0.43176
600	0.60781	0.61651	480	0.49263
			600	0.58916

Table A.22: The absorbances measured on sample 4.

t/s	$A_{0\ \mathrm{h}}$	$A_{1\ \mathrm{h}}$	t/s	$A_{5\ \mathrm{h}}$
0	0.42041	0.39471	0	0.30855
60	0.34266	0.38225	120	0.32494
120	0.41130	0.40444	240	0.38323
300	0.42764	0.54604	360	0.45927
600	0.63311	0.81148	480	0.47655
			600	0.5302

A.4 4.5 mL glass vials at 1000 rpm with dilution followed by centrifugation

A.4.1 Monophase

Table A.23: The absorbances measured on sample 1.

t/s	$A_{0\ \mathrm{h}}$	$A_{4\ \mathrm{h}}$
120	0.35396	0.37703
240	0.49090	0.46164
360	0.59021	0.54442
480	0.73787	0.62402
600	0.83032	0.73534
720	0.94102	0.80525

Table A.24: The absorbances measured on sample 2.

t/s	$A_{0\ \mathrm{h}}$	$A_{4\ \mathrm{h}}$
120	0.35726	0.37123
240	0.47608	0.46394
360	0.62023	0.53904
480	0.70457	0.61409
600	0.85718	0.73220
720	1.00681	0.82272

A.4.2 Biphasic system

Table A.25: The absorbances measured on sample 1.

t/s	$A_{0\ \mathrm{h}}$	t/s	$A_{4\ \mathrm{h}}$
120	0.43397	120	0.99834
240	0.51729	240	1.04310
360	0.60642	360	1.08638
480	0.71917	480	1.11840
610	0.82924	600	1.09708
720	0.92174	720	1.35050

Table A.26: The absorbances measured on sample 2.

t/s	$A_{0\ \mathrm{h}}$	$A_{4\ \mathrm{h}}$
120	0.44106	1.05532
240	0.55914	1.07123
360	0.59913	1.21444
480	0.67708	1.26303
600	0.78179	1.17926
720	0.84688	1.17036

A.4.3 Pickering emulsion

Table A.27: The absorbances measured on sample 1.

t/s	$A_{0\ \mathrm{h}}$	$A_{4\ \mathrm{h}}$
120	0.47911	0.98620
240	0.58738	1.04116
360	0.70591	1.09583
480	0.77405	1.11239
600	0.88857	1.15691
720	1.08931	1.17224

Table A.28: The absorbances measured on sample 2.

t/s	$A_{0\ \mathrm{h}}$	$A_{4\ \mathrm{h}}$
120	0.72164	1.01317
240	0.72346	0.98029
360	0.75560	1.13868
480	0.92098	1.17175
600	0.95736	1.19988
720	1.02168	1.21199

A.5 4.5 mL glass vials at 600 rpm with dilution followed by centrifugation

A.5.1 Monophase

Table A.29: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.3220	0.3319	0.3405	0.3963	0.3910	0.3860
240	0.4680	0.4837	0.4571	0.4482	0.4559	0.4499
360	0.5846	0.5874	0.5739	0.5193	0.5120	0.5245
480	0.7216	0.7029	0.7026	0.6091	0.6352	0.6394
600	0.8777	0.8295	0.8101	0.6958	0.7080	0.7059
720	0.9387	0.9353	0.9264	0.7960	0.7908	0.8072

Table A.30: The absorbances measured on sample 2.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	t/s	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.3737	0.3760	0.3780	120	0.3931	0.3963	0.3959
300	0.4982	0.5159	0.5150	240	0.4791	0.4895	0.4850
420	0.6027	0.6072	0.6221	360	0.5513	0.5847	0.5914
540	0.7549	0.7591	0.7645	480	0.6351	0.6395	0.6875
660	0.9240	0.9340	0.9353	600	0.7555	0.7625	0.7611
780	0.9920	1.0047	1.0264	720	0.8344	0.8458	0.8425

A.5.2 Biphasic system

Table A.31: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.7166	0.7245	0.7268	0.5394	0.5324	0.5413
240	0.7518	0.7607	0.7580	0.6763	0.6844	0.6903
360				0.7758	0.7855	0.7872
480	0.9827	0.9841	0.9858	0.7319	0.7461	0.7509
600	0.7565	0.7657	0.7688	0.8090	0.8345	0.8306
720	0.8858	0.8999	0.9082	0.8336	0.8519	0.8556

Table A.32: The absorbances measured on sample 2.

t/s	$\mathrm{A}_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	t/s	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
150	0.5529	0.5633	0.5587	120	0.6017	0.6020	0.6070
270	0.6891	0.6984	0.7051	240	0.6708	0.6722	0.6668
390	0.7205	0.7299	0.7358	360	0.6988	0.7172	0.7151
510	0.7316	0.7389	0.7390	480	0.7416	0.7569	0.7631
630	0.8502	0.8652	0.8749	600	0.8344	0.8467	0.8422
750	0.9015	0.9244	0.9179	720	0.8831	0.9008	0.8924

A.5.3 Pickering emulsion

Table A.33: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	t/s	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.8036	0.8163	0.8246	120	0.9049	0.9287	0.9341
240	0.8887	0.8951	0.903	255	0.9588	0.9707	0.9683
360	0.9332	0.9468	0.9538	360	0.9884	1.0091	1.0021
480	0.9113	0.9213	0.9355	480	1.1126	1.1266	1.1141
600	0.9703	0.9862	0.9896	600	1.1798	1.1395	1.1451
720	0.9688	0.9769	0.9898	720	1.2057	1.2005	1.2267

Table A.34: The absorbances measured on sample 2.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	t/s	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.8590	0.8759	0.8843	120	0.9358	0.9452	0.9503
240	0.8678	0.8884	0.8915	250	0.9536	0.9617	0.9757
360	0.9920	1.0247	1.0243	375	0.9469	0.9547	0.9713
480	1.0051	1.0384	1.0358	485	1.0359	1.0504	1.0687
600	1.0432	1.0664	1.0724	600	1.1083	1.1253	1.1406
720	1.1509	1.1698	1.1685	720	1.1559	1.1650	1.1795

A.6 Eppendorf tubes at 500 rpm

A.6.1 Monophase

Table A.35: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
120	0.3786	0.3721	0.3709	0.3993	0.4020	0.4039	0.4049	0.4062	0.4109
240	0.4015	0.4046	0.4074	0.4272	0.4309	0.4491	0.4345	0.4451	0.4437
360	0.4407	0.4450	0.4620	0.4690	0.4741	0.4855	0.4785	0.4853	0.4882
480	0.4835	0.4878	0.4908	0.4457	0.4514	0.4502	0.5283	0.5340	0.5362
600	0.5402	0.5424	0.5583	0.5616	0.5700	0.5756	0.5779	0.5855	0.6007
720	0.5598	0.5681	0.5674	0.6262	0.6464	0.6553	0.6123	0.6179	0.6180

Table A.36: The absorbances measured on sample 2.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	t/s	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
135	0.3875	0.3934	0.4019	120	0.3954	0.3996	0.4061	0.4071	0.4145	0.4096
255	0.4233	0.4281	0.4252	240	0.4351	0.4443	0.4480	0.4419	0.4494	0.4507
375	0.4772	0.4806	0.4798	360	0.4844	0.4925	0.4965	0.4908	0.4955	0.4996
495	0.5108	0.5206	0.5170	480	0.5371	0.5523	0.5507	0.5366	0.5448	0.5512
615	0.5545	0.5610	0.5699	600	0.5878	0.5997	0.6031	0.5827	0.5897	0.5874
720	0.5968	0.5967	0.6001	720	0.6419	0.6579	0.6596	0.6355	0.6499	0.6508

Table A.37: The absorbances measured on sample 3.

t/s	$A_{1,0\ \mathrm{h}}$	$A_{2,0\ \mathrm{h}}$	$A_{3,0\ \mathrm{h}}$	$A_{1,1\ \mathrm{h}}$	$A_{2,1\ \mathrm{h}}$	$A_{3,1\ \mathrm{h}}$	$A_{1,5\ \mathrm{h}}$	$A_{2,5\ \mathrm{h}}$	$A_{3,5\ \mathrm{h}}$
120	0.3787	0.3834	0.3869	0.3852	0.3868	0.3915	0.4013	0.4035	0.4059
240	0.4210	0.4237	0.4325	0.4269	0.4328	0.4372	0.4356	0.4448	0.4446
360	0.4643	0.4742	0.4766	0.4787	0.4898	0.4884	0.4808	0.4852	0.4917
480	0.5088	0.5216	0.5215	0.5284	0.5473	0.5414	0.5236	0.5301	0.5295
600	0.5677	0.5843	0.5761	0.5864	0.5951	0.6000	0.5815	0.5874	0.5855
720	0.6107	0.6278	0.6435	0.6376	0.6470	0.6544	0.6199	0.6343	0.6361

A.6.2 Biphasic system

Table A.38: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
120	0.3140	0.3655	0.4050	0.3288	0.3453	0.3589	0.3745	0.3818	0.3909
240	0.3750	0.4171	0.4460	0.3574	0.3686	0.3760	0.3757	0.3782	0.3796
360	0.4073	0.4184	0.4236	0.4241	0.4347	0.4503	0.4151	0.4235	0.4259
480	0.4620	0.4671	0.5265	0.4586	0.4702	0.4794	0.4476	0.4589	0.4593
600				0.5075	0.5156	0.5267	0.4649	0.4791	0.4793
720	0.4954	0.5275	0.6047	0.5333	0.5432	0.5521	0.5319	0.5353	0.5393

Table A.39: The absorbances measured on sample 2.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	t/s	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	t/s	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
120	0.3258	0.3534	0.3836	120	0.4183	0.4347	0.4362	120	0.3514	0.3559	0.3584
240	0.3519	0.3691	0.4489	240	0.3882	0.4094	0.4092	240	0.3858	0.3911	0.3911
360	0.3992	0.4303	0.4518	450	0.4276	0.4405	0.4436	360	0.4211	0.4249	0.4281
480	0.4321	0.4396	0.4481	570	0.4355	0.4405	0.4545	480	0.4525	0.4600	0.4600
600	0.4775	0.5336	0.5191	690	0.4892	0.5012	0.5052	600	0.4954	0.5032	0.5045
720	0.5485	0.5609	0.5872	810	0.5221	0.5310	0.5331	720	0.5344	0.5459	0.5411

Table A.40: The absorbances measured on sample 3.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
120	0.4013	0.4366	0.4174	0.3512	0.3561	0.3674	0.3916	0.4016	0.4066
240	0.4211	0.4424	0.4717	0.3841	0.3943	0.3968	0.4193	0.4279	0.4284
360	0.4329	0.4469	0.4647	0.4183	0.4286	0.4359	0.4566	0.4619	0.4658
480	0.4890	0.4972	0.5260	0.4488	0.4532	0.4651	0.4661	0.4758	0.4749
600	0.5486	0.5430	0.5726	0.4562	0.4645	0.4778	0.4963	0.5058	0.5076
720				0.5234	0.5354	0.5372	0.5205	0.5340	0.5356

A.6.3 Pickering emulsion

Table A.41: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
120	0.4895	0.5029	0.5204	0.8792	0.8974	0.9041	0.8830	0.9000	0.9092
240	0.7599	0.7781	0.7853	0.8342	0.8587	0.8608	0.9433	0.9676	0.9716
360	1.0967	1.1074	1.1052	0.7764	0.7891	0.7993	1.0225	1.0254	1.0513
480	0.8712	0.9135	0.8894	0.8263	0.8304	0.8512	0.9293	0.9477	0.9613
600	0.9781	0.9888	0.9918	0.8668	0.8881	0.8983	0.9796	0.9990	1.0010
720	1.0405	1.0340	1.0656	0.8616	0.8781	0.9092	1.1934	1.2252	1.2271

Table A.42: The absorbances measured on sample 2.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
120	0.6749	0.6846	0.6906	0.7431	0.7659	0.7692	0.8844	0.9072	0.9069
240	0.7391	0.7384	0.7540	0.8135	0.8331	0.8400	0.8615	0.8954	0.8999
360	0.8106	0.8274	0.8273	0.8727	0.8982	0.9049	0.9324	0.9484	0.9654
480	0.8569	0.8811	0.8821	0.8557	0.8691	0.8752	0.9493	0.9564	0.9824
600	0.8973	0.9218	0.9223	0.9884	1.0154	1.0221	1.0152	1.0231	1.0511
720	1.0236	1.0266	1.0398	0.9884	0.9886	1.0124	1.0077	1.0138	1.0310

Table A.43: The absorbances measured on sample 3.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 1\ \mathrm{h}}$	$A_{2,\ 1\ \mathrm{h}}$	$A_{3,\ 1\ \mathrm{h}}$	$A_{1,\ 5\ \mathrm{h}}$	$A_{2,\ 5\ \mathrm{h}}$	$A_{3,\ 5\ \mathrm{h}}$
120	0.8118	0.8288	0.8408	0.8826	0.8805	0.8822	0.8252	0.8345	0.8425
240	0.8890	0.9066	0.9261	0.9001	0.9179	0.9187	0.8712	0.8824	0.8815
360	0.9049	0.9116	0.9283	0.9427	0.9648	0.9696	0.7894	0.8084	0.8087
480	0.8191	0.8414	0.8416	0.9751	0.9766	1.0106	0.9329	0.9509	0.9629
600	0.8891	0.9071	0.9180	1.0138	1.0193	1.0316	0.9519	0.9420	0.9865
720	1.0364	1.0519	1.0629	1.1351	1.1631	1.1683	0.9288	0.9454	0.9558

A.7 2 mL Eppendorf tubes at 1000 rpm

A.7.1 Monophase

Table A.44: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.2826	0.2932	0.2948	0.3638	0.3708	0.3586
240	0.3560	0.3657	0.3702	0.4283	0.4289	0.4094
360	0.4222	0.4542	0.4501	0.4977	0.5023	0.5071
480	0.5199	0.5314	0.5139	0.5666	0.5705	0.8242
600	0.5974	0.6071	0.5849	0.6310	0.6312	0.6338
720	0.6733	0.6796	0.6877	0.7120	0.7216	0.7095

Table A.45: The absorbances measured on sample 2.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.2991	0.2988	0.3002	0.3552	0.3620	0.3625
240	0.3663	0.3682	0.3691	0.4155	0.4308	0.4289
360	0.4620	0.4651	0.4581	0.4871	0.4873	0.4946
480	0.5362	0.5597	0.5388	0.5723	0.5686	0.5752
600	0.6212	0.6378	0.6233	0.6287	0.6380	0.6356
720	0.6898	0.7088	0.7226	0.7003	0.7147	0.7086

Table A.46: The absorbances measured on sample 3.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.3016	0.2980	0.3041	0.3669	0.3657	0.3727
240	0.3857	0.3893	0.3896	0.4273	0.4395	0.4162
360	0.4641	0.4470	0.4643	0.4980	0.5098	0.5005
480	0.5317	0.5469	0.5253	0.5658	0.5779	0.5784
600	0.6263	0.6354	0.6382	0.6286	0.6557	0.6575
720	0.7220	0.7289	0.7401	0.7242	0.7409	0.7595

A.7.2 Biphasic system

Table A.47: The absorbances measured on sample 1.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.6454	0.6570	0.6620	1.0407	1.0410	1.0612
240	0.7938	0.8070	0.8082	1.1060	1.1300	1.1396
360	0.8823	0.9031	0.8989	1.0719	1.0865	1.0964
480	0.9430	0.9499	0.9487	1.2076	1.2176	1.2363
600	1.0317	1.0535	1.0547	1.2827	1.2812	1.3025
720	1.0977	1.1253	1.1437	1.2434	1.2506	1.2840

Table A.48: The absorbances measured on sample 2.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.7846	0.7973	0.7973	1.0978	1.1012	1.1515
240	0.7349	0.7392	0.7379	1.1571	1.1700	1.1956
360	0.7571	0.7736	0.7687	1.1997	1.2427	1.2665
480	0.9926	1.0092	1.0239	1.3255	1.3667	1.4165
600	0.9967	1.0180	1.0263	1.2530	1.2834	1.3139
720	1.1872	1.2067	1.2084	1.3441	1.3518	1.3812

Table A.49: The absorbances measured on sample 3.

t/s	$A_{1,\ 0\ \mathrm{h}}$	$A_{2,\ 0\ \mathrm{h}}$	$A_{3,\ 0\ \mathrm{h}}$	$A_{1,\ 4\ \mathrm{h}}$	$A_{2,\ 4\ \mathrm{h}}$	$A_{3,\ 4\ \mathrm{h}}$
120	0.7981	0.8113	0.8130	1.0895	1.0927	1.1135
240	0.8754	0.8850	0.8937	1.1319	1.1397	1.1690
360	1.0780	1.0968	1.1168	1.1428	1.1502	1.1966
480	1.0031	1.0191	1.0207	1.2661	1.2906	1.3062
600	1.0093	1.0109	1.0242	1.3396	1.3426	1.3618
720	1.1204	1.1610	1.1633	1.2819	1.3232	1.3410

A.7.3 Pickering emulsion

Table A.50: The absorbances measured on sample 1.

t/s	$A_{1,\,0\,\mathrm{h}}$	$A_{2,\,0\,\mathrm{h}}$	$A_{3,\,0\,\mathrm{h}}$	t/s	$A_{1,\,1\,\mathrm{h}}$	$A_{2,\,1\,\mathrm{h}}$	$A_{3,\,1\,\mathrm{h}}$	$A_{1,\,4\,\mathrm{h}}$	$A_{2,\,4\,\mathrm{h}}$	$A_{3,\,4\,\mathrm{h}}$
150	0.8927	0.8985	0.9433	120	0.9459	0.9620	0.9689	0.9640	0.9736	0.9892
300	0.9244	0.9236	0.9808	240	1.0120	1.0268	1.0437	1.0683	1.0801	1.0844
415	0.9907	0.9869	1.0186	360	0.9961	1.0057	1.0274	1.1012	1.1203	1.1222
525	1.0977	1.0798	1.1091	480	1.1331	1.1683	1.1770	1.1638	1.1676	1.1949
630	1.0909	1.0983	1.1274	600	1.1662	1.2027	1.2065	1.2488	1.2554	1.2695
745	1.1710	1.2023	1.2058	720	1.2305	1.2649	1.2592	1.2513	1.2861	1.2981

Table A.51: The absorbances measured on sample 2.

t/s	$A_{1,\,0\,\mathrm{h}}$	$A_{2,\,0\,\mathrm{h}}$	$A_{3,\,0\,\mathrm{h}}$	$A_{1,\,1\,\mathrm{h}}$	$A_{2,\,1\,\mathrm{h}}$	$A_{3,\,1\,\mathrm{h}}$	$A_{1,\,4\,\mathrm{h}}$	$A_{2,\,4\,\mathrm{h}}$	$A_{3,\,4\,\mathrm{h}}$
120	0.8863	0.8979	0.9083	1.0143	1.0297	1.0354	1.0221	1.0219	1.0654
240	0.9353	0.9489	0.9497	1.1474	1.1510	1.1859	1.0626	1.0699	1.0599
360	1.0509	1.0834	1.0755	1.1231	1.1441	1.1555	1.1631	1.1836	1.1815
480	1.1027	1.1210	1.1380	1.2737	1.2832	1.2886	1.2443	1.2638	1.2545
600	1.1850	1.1883	1.2164	1.3361	1.3656	1.3667	1.2786	1.3053	1.3172
720	1.2118	1.2510	1.2575	1.3505	1.3755	1.3899	1.3107	1.3386	1.3453

Table A.52: The absorbances measured on sample 3.

t/s	$A_{1,\,0\,\mathrm{h}}$	$A_{2,\,0\,\mathrm{h}}$	$A_{3,\,0\,\mathrm{h}}$	t/s	$A_{1,\,1\,\mathrm{h}}$	$A_{2,\,1\,\mathrm{h}}$	$A_{3,\,1\,\mathrm{h}}$	$A_{1,\,4\,\mathrm{h}}$	$A_{2,\,4\,\mathrm{h}}$	$A_{3,\,4\,\mathrm{h}}$
120	0.8522	0.8593	0.8728	120	0.9174	0.9382	0.9369	0.9973	1.0098	1.0226
240	0.9083	0.9218	0.9346	240	1.0472	1.0654	1.0872	1.1054	1.1149	1.1324
360	0.9905	1.0397	1.0282	360	1.1002	1.1065	1.1263	1.1732	1.1976	1.2092
480	1.0755	1.0929	1.0910	480	1.1633	1.1928	1.1923	1.2280	1.2418	1.2700
600	1.1047	1.1178	1.1255	600	1.3849	1.4208	1.4265	1.3189	1.3251	1.3337
745	1.1432	1.1697	1.1663	720	1.3213	1.3392	1.3663	1.3003	1.3214	1.3245