EFFECT OF LIPASE ADDITION IN OILY WATER CLEAN UP

Anas bin Abdullah@Abd halim, Puan Azzah Nazihah

Faculty of Chemical Engineering, Universiti Teknologi Mara

***Abstract*— The marine environment is open to large sources of toxic organic waste in the form of accidental oil spills.Lipase as we know is class of enzymes that break down dietary fats into smaller molecules which is called fatty acids S.Thakur (2005).Thus In this experimental study, the main objective is to see the effects of lipase towards the oily water and to observe the time period during the effects of lipase and the degree of hydrolysis.The oil for this experiment that used was olive oil since olive oil easily to have high reaction compare with other oil that meet with required.The preparation of the lipase solution were done at different concentration which at 2.0, 2.5 and 3.0 mg/ml.After that, 0.05 M concentration of olive oil was prepared and mix with isooctane as solvent at 50 ml.The time period taken for the sample were at 2, 4 and 6 hours.Then using the titration method which to find the degree of hydrolysis by using 0.01 N of NaOH.The result show at the different concentration of lipase which were at 2, 2.5 and 3 mg/ml give a slightly difference value of the amount of NaOH used.This show the sample was more acidic.From the result at period of 2 hours from the concentration of 2.0 mg/ml, the amount of the NaOH used was 0.5 ml while for 2.5 and 3 mg/ml were 1.1ml and 1.3 ml respectively .The result for time period also prove to be the main criteria to maximize the activity of lipase where as at 2 mg/ml with the time period at 6 hours the value of hydrolysis higher which was 2.26% follow by at 3 and 2.5 mg/ml the value of hydrolysis were 2.11% and 1.78% respectively.From there, the increase of time period cause the degree of hydrolysis to increase. The overall results indicate that lipase could be a promising toward the method to overcome the oil contaminant in near future.**

Keywords : Aspergillus niger,degree of hydrolysis,Lipase solution

I. INTRODUCTION

Petroleum industry well known to generates large volumes of oily waste water.The environmental been taken for the disposal of oily waste water is a new concern and problem to the petroleum industry.In the moment,the focused is on what the best to overcome the treatment of oily water.Furthermore,oily water treatment has growing towards a big concern, and it must be observed and resolved on all oil field and petroleum industry.Lipase was known an enzyme that can degraded oil layer but the possible to use on the oil that contain high hydrocarbon still developing.It proven,many lipase producing organisms (bacteria,fungi) can further be use to help degrading oil in the oily water.This study will look into addition of lipase in a free flow system.

Lipase is a category of hydrolased that catalyzed the hydrolysis of triglycerides to glycerol and free fatty acids over an oily water interface **S. Gupta, K. Singh (2010)**.The capability of the lipase to transform into a specific chemical biotransformation has make them increasingly popular among research uses which further the focus in the food, detergent, cosmetic, and organic synthesis. Lipases have evolved as one of the leading biocatalysts with prove the potentially to overcome the oil containment that increasingly demand for the oil and gas industry to been use for the purpose of cure the oil spill.

This show the result of the huge achievements made in the cloned and expression of enzymes from microorganisms and also had increased the demand for these biocatalysts with novel and specific characteristic such as specificity, pH value, stability, including the temperature . Lipases create from the bio metric of the animals, plants, and microorganisms. Microbial lipases have gaining special industry attraction due to their ability that can give a selectively, stability, and broad substrate specificity.**Serri , kamaruddin (2008)** Thus,for this experiment we focus on the ability of lipase on handling the oily water that contain high hydrocarbon such as heavy oil and light oil.Furthermore , to observe the effect of it and does it can be used for the purpose of degradation in oil and gas industry.If the experiment and the method potentially well made, this could be another achievement for one of the methods that can been use for the treatment of the oily water.Step by step been taken and a lot of method and material been use to over come the problem

II. METHODOLOGY

A. Materials

Aspergillus niger lipase(EC 3.1.1.3)(Sigma - Aldrich,USA), Polyethersulfone (PES) membrane (courtesy of postgraduate student), Isooctane (R&M Chemicals), Phenolphthalein (R&M Chemicals), Buffer solution (System-ChemAR) and olive oil were procured.

B. Preparations of lipase

At the first step, the lipase solution was taked out but make sure not expose to long towards the atmosphere which can effect the condition of the lipase.After that, the lipase was mixed with phosphate buffer solution at pH 7.0 for 12 hour with a curtain weight of fungal which to get the desire concentration.The reason use pH 7 phosphate buffer because want to avoid damage to the lipase and avoid the value pH conditions that have strongly acidic or alkaline as lipase were unstable in that condition **Shweta Gupta (2016)**.Thus also there were many research use at pH value of 7 not more and less.The next step was prepare 3 parameter concentration of lipase which were at 2.0, 2.5 and 3.0 mg/ml.From here we can see that at which of the concentration have the most activity of lipase and also does the time period increase gave a good value toward the hydrolysis.

C. Preparation of Oil

From here 0.05 M concentration of olive oil was prepared.Every of oil have its own characteristic and chemical material.So from there we can see which have a high strain of oil.After that place the oil into 1 liter of isooctane solvent and mix together.Make sure it avoid any bubble produce in the oil so that the result before the degradation process can be maximize.The mixed just prepare for 30 minutes and cannot be longer then that which can cause the chemical and material in the oil to dead and damage the result.From that also possible for no reaction of the lipase and the oil was high and need to avoid of the failure happen.

D. The membrane channel reactor

The membrane channel reactor was a custom made glass in form of square with a measure of 8 x 8 x 8 cm2 .Both side of the reactor were filled with the sample of mixture oily water and phosphate buffer solution and in the middle was an identical flat of the channel for holding the membrane with the size are 9.1 cm2, circle round on the glass of compartments The lipase mix directly at the side of oily water (olive oil and isooctane) at 0.05 M. Soon the both side were filled with two solution phase which it call the biphasis one. It was a combination of a bio reactor and separation step of the membrane. Membrane bio reactor offers in-situ separation capability that is lacking in other types of reactors showed the experimental set-up of the biphasis enzyme membrane reactor.The reaction of the sample and simultaneously the membrane surface took place for the separation, where the membrane was used both as catalyst support that give a better selective contact with the reactant and for selective removal of products **Shweta Gupta (2016)**.To start of an enzymatic reaction measurement, the lipase mix directly at the side of oily water (olive oil and isooctane) at 0.05 M was filled on the right side of the membrane, while on the left side 50 ml of phosphate buffer solution at pH 7.0 was filled on the right side. The experiments were carried out at room temperature which at 24 C. We only study olive oil of 0.05 M concentration.

E. Degree of Hydrolysis

In this step,we focus on the activity lipase based on the hydrolysis of olive oil by lipase.The determined it we use the titration method that involve with NaOH which prepare at 0.01 N (0.40g and 1 liter distilled water).First take the sample that goes through the channel reactor which is solution of isooctane and oil at 0.5 ml, put in the conical flask.Then put one drop of phenolphthalein in the sample as the indicator.As we know if the solution change to colourless it show acidic while the pink colour indicate base solution.Then record the volume of NaOH been used so that we can calculate the degree of hydrolysis.

F. Influence factor towards hydrolysis

The factor that influence the value of hydrolysis is effect of time period was to observe and determined the performance of lipase in the membrane reactor. The reactor was operated at different time period which is 2, 4, 6 hours. The hydrolytic experiments were carried out for olive oil. Second is the concentration of the lipase The reaction was done at room temperature for 6 hours using of phosphate buffer of pH 7.0 in aqueous phase and 0.05 M oils in organic phase.Then,the different type of concentration lipase which is 2, 2.5, 3 mg/ml that contribute towards the hydrolysis calculation.

G. Phosphate buffer solution

The important role of the phosphate buffer solution was to maintain the active site of the lipase activity and in this experiment we use the pH 7. It was avoid to proceed the experiment in unrelated value pH conditions that have strongly acidic or alkaline as lipase were unstable in that condition **Shweta Gupta (2016)**. Previous discussed that mention was pH will affects the stability, structure, and function of globular proteins due to their ability to influence the electrostatic interactions, the extent of hydrolysis varies at different pH. It was observed that at pH 7.0 the hydrolysis was maximum for oils studied. However the extent of hydrolysis was different for the oils.In this experiment, organic solvents, as isooctane were used as the reaction media for the enzymatic hydrolysis.Every enzyme has pH value on what it the best effectiveness sis of the optimum pH, this is because the exact arrangement of the active site of an enzyme is partly fixed by hydrogen and ionic bonds between –NH2 and –COOH groups of the polypeptides that make up the enzyme.Although a small change of the pH,the bonding maybe change shape of the active site **Lucas, Stephen (1894)**.The relatively higher polar organic solvents of the isooctane compare to other solvent such as hexane and heptane that strip more water off an enzyme and thereby dehydrate the catalyst thus the activity is destroyed.

III. RESULTS AND DISCUSSION

A. Concentration of lipase

Lipase solution that were prepare with different concentration which at 2.0, 2.5 and 3.0 mg/ml and use on the oily water which is the mixture of the olive oil and isooctane was showed a different amount degradation of the sample.The increase of the concentration lipase enhanced the chances for the produce more of fatty acid.From the result show that at concentration of lipase at 3 mg/ml give a slightly high value of the amount used of NaOH while for the 2 mg/ml and 2.5 mg/ml show slightly low which can refer on the Table 1, 2 and 3 for further understanding the trend of the activity.This show the increase in breakdown the sample into smaller molecule and turn into fatty acid with increase of concentration value.Other than that the experiment were also performed at different time period.

B. Time Period

The different of time period towards the lipase activity was shown in table 1, 2 and 3 which in period of 2, 4 and 6 hours that been taken on each of the sample of lipase concentration on the side that contain mixture of isooctane and oily water in the custom made enzyme membrane channel reactor.The purpose of the various time period is that we want to determined at which actual time that produce maximum yield of lipase activity.The calculation of hydrolysis of oil was obtained within a reaction period of 2, 4 and 6 hours. From there the result show, increase of time period cause the value of degree hydrolysis increase. This may be due to the activity of lipase produce in the oily water increase.

Concentration of Aspergillus at 2.0 mg/ml			
Time taken (h)	**NaOH Used(ml)**	**NaOH (N)**	**Weight Sample(g)**

2	**0.5**	**0.01**	**0.39**
4	**1.5**	**0.01**	**0.38**
6	**2.9**	**0.01**	**0.38**

Table 1 : Time period and the amount of NaOH used at 2.0 mg/ml

Concentration of Aspergillus at 2.5 mg/ml			
Time taken (h)	**NaOH Used(ml)**	**NaOH (N)**	**Weight Sample(g)**
2	**1.1**	**0.01**	**0.387**
4	**2.1**	**0.01**	**0.389**
6	**2.3**	**0.01**	**0.381**

Table 2 : Time period and the amount of NaOH used at 2.5 mg/ml

Concentration of Aspergillus at 3.0 mg/ml			
Time taken (h)	**NaOH Used(ml)**	**NaOH (N)**	**Weight Sample(g)**
2	**1.3**	**0.01**	**0.361**
4	**2.1**	**0.01**	**0.371**
6	**2.7**	**0.01**	**0.379**

Table 3 :Time period and the amount of NaOH used at 3.0 mg/ml

C. *Degree of hydrolysis*

In this result,the determination of the hydrolysis was to observe the activity of the lipase.The method for the hydrolysis was done by the titration process with the NaOH as our base solution.The process done with different time period that have been taken from the different value of concentration which at 2, 2.5 and 3 mg/ml. Phenolphthalein was placed into the the sample for the indicator by apply only a drop of it.Table 1, 2 and 3 show the value of the NaOH used until the sample change to pink colour which indicate the base solution.As we can see at the concentration of lipase at 2.0 mg/ml the volume of NaOH used at 2 hours was 0.5 ml while at the 2.5 and 3 mg/ml the volume of NaOH used were 1.1 and 1.3 ml respectively.This show that the increase of concentration cause the used of the NaOH to increase due to fatty acid produce.Then we calculated the degree of the hydrolysis,X% with the formula below;

$$\mathbf{X\%} = \frac{(mlNaOH)(MolarityNaOH)(MWfattyacid)}{10(Weightsample)}$$

The table 3,4 and 5 show the value of the hydrolysis from different time period and concentration of lipase.From the result shows the hydrolysis was at higher peak at 3 mg/ml with the average value is 0.38300% and for the 2 and 2.5 mg/ml are 0.1255% and 0.2807% respectively. As the lipase substrates of oil and fat which have limited solubility in water, lipase was known to act on oily water interface.A continuous micro environment for the reaction was assumed to exist where the two reactants come in contact with the lipase activity and reacted with each other during pass through the membrane surface. The hydrolysis took place in the organic phase that contain of the sample solution.Besides that the contribution of the hydrolysis in this experiment were the nature of organic phase known as the phosphate buffer solution, reaction time of the lipase, lipase concentration, and pH controls.Thus that give the different value of the hydrolysis.Furthermore, the fatty acid produce also part of the contribution effect of value hydrolysis.The experiment only focus on one type of oil which was olive oil and focus more on the activity by changing with different type of lipase concentration.The trend of the lipase concentration was as follows 3.0 mg/ml > 2.5 mg/ml > 2.0 mg/ml**.** Time variation hydrolysis also show the same trend irrespective of concentration

Aspergillus concentration of 2.0 mg/ml	
Time Taken(h)	**Degree of hydrolysis(%)**
2	**0.379**
4	**1.166**
6	**2.261**

Table 4 : Degree hydrolysis at 2.0 mg/ml

Aspergillus concentration of 2.5 mg/ml	
Time Taken (h)	**Degree of hydrolysis(%)**
2	**0.842**
4	**1.599**
6	**1.788**

Table 5 : Degree hydrolysis at 2.5 mg/ml

Aspergillus concentration of 3.0 mg/ml	
Time Taken (h)	**Degree of hydrolysis(%)**
2	**1.149**
4	**1.677**
6	**2.110**

Table 6: Degree hydrolysis at 3.0 mg/ml

The different value of the hydrolysis with different time period which were at (2, 4,6 h) was shown at table 4, 5 and 6. From there we can see the trend of the value of hydrolysis at the beginning of the time period at 2 hours, it were increase until the end of time period.This show the activity of lipase increase including the increasing of the amount of the fatty acid produce.The highest percentage conversion value happened after the hydrolysis reaction was carried out for 6 h. Thus this show a non growth substrate with high enzyme affinity will significantly decrease the rate at which the growth substrate is degraded, while a non growth substrate with low enzyme affinity will have much less effects, thus explaining the great parity in the abilities of these organisms to degrade crude oil hydrocarbons. **K. Chuks, Agha (2008).**This indicate that the potential activity of lipase increase for time been until it reach at optimize state which the lipase activity begin to decrease.Thus for further the experiment on different type of oil and different concentration of oil would have been discover more various on which part would best and suitable condition of maximize the activity of lipase

IV. CONCLUSION

In this study,lipase proves a significant ability of producing several enzymes for their survival within a wide range of substrates.From here the lipase show predominantly that can be used in several method which also can be apply on the degradation of oily water.The result also show at the different concentration of lipase solution which were at 2, 2.5 and 3 mg/ml give a slightly difference value of hydrolysis which play a important role on the activity of lipase.This due to the break down of the dietary acid towards the mixture of olive oil and isooctane into smaller component in every each of the others concentration.The result for time period also prove to be the main criteria to maximize get the optimum activity of lipase where as at 2 mg/ml with the time period at 6 hours, the value of hydrolysis was the higher which was 2.26% follow by at 3 and 2.5 mg/ml the value of hydrolysis were 2.11% and 1.78% respectively.This show the increase of time period cause the degree of hydrolysis to increase.Lipases contain their unique characteristics such as from over a wide temperature and pH range, substrate specificity, diverse substrate range that are the biocatalysts of choice for the present and future. The popularity demand for using the lipases has shifted the trend towards prospecting for study purposes, improving the properties of existing lipases for established technical applications and producing new enzymes for new areas of application. It also prove as one of the most versatile enzymes available in the market right now. They are unique in various aspects starting from their ability to act at the interface, to molecular imprinting and retention of activity in organic solvents. The tremendous potential of lipases in the industries shows the focus to develop for future use increase with effective technologies for increased production, scaling up and purification of this versatile enzyme. Furthermore, the lipase prove that this could be another achievement for one of the methods that can been use for the treatment of the oily water.For further study I believe there will be more significant chances that can be apply in the treatment of oil spill problem in the petroleum industry.

ACKNOWLEDGMENT

First of all, I would like to thank Allah S.W.T for give me the strength and the opportunity to embark on my degree and helping me through the journey accomplishing this successfully.I would personally want to thank my parent,whose got my back since day 1 until the end of my project with a love and guidance in everything I pursue.It give me good motivation to push myself and do the best in completing this Final Year Project II.They have become my inspiration for all ups and down time. Besides that, I would like to express my sincere gratitude to my supervisor, Puan Azzah Nazihah, Oil and Gas Engineering lecturer of “Universiti Teknologi MARA (UiTM)” for her support, motivation, enthusiasm, patience and immense knowledge. Without her kindly support, patience and ideas in assisting me with the project, I would not probably complete this Final Year Project successfully. Her guidance helped me throughout the research and writing of this thesis.Although with her tight schedule, she always spare her time in giving me the help and guidance to finish this task. Lastly, my sincere gratitude was to my colleagues and friends for their assistance throughout the whole semester. My sincerely thanks also goes to all people that helping me during this period of complete the Final Year Projects. My deepest appreciation is to all of you. Thank you.

REFERENCES

[1] 1. L.E.S. Brink, J. Tramper, Optimazation of organic solvent in multiphase biocatalysis, Biotechnol. Bioeng. 27 (1985) 1258.
[2] K.Chuks Ugochukwu, N.C Agha and J.N.Ogbulie ,Lipase activities of microbial isolates from soil contaminated with crude oil after bio remediation .
[3] An investigation into how the volume of lipase affects the rate of the hydrolysis of lipids by miss johnson, stephen lucas 1894
[4] Comparative study on hydrolysis of oils by lipase immobilized biocatalytic PS membranes using biphasic enzyme membrane reactor by Shweta Gupta 2016.
[5] Lipases, Its sources, Properties and Applications: A Review by S.Thakur 2012
[6] S. Gupta, K. Singh, A. Bhattacharya, Lipase immobilization on polysulfone globules and their performances in olive oil hydrolysis, Int. J. Biol. Macromol. 46 (2010) 445.
[7] N.A. Serri, A.H. Kamarudin, S.N.A. Rahaman, Preliminary studies for production of fatty acids from hydrolysis of cooking palm oil using C. rugosa lipase, J. Phys. Sci. 19 (1) (2008) 79.