Biodegradability of Ionic Liquid Waste

By

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Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

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CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

MUHAMMAD NURADI B MOHD ZAHARRI

CERTIFICATION OF APPROVAL

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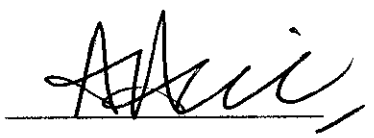
Approved by,



(Prof IR Abd Aziz Omar)

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TRONOH, PERAK
MAY 2012

ABSTRACT

Ionic liquids (ILs) are receiving increasing attention as reaction media and alternative solvents within the context of sustainable and green chemistry. Even though its growing use does not lead to pollution, we still need to figure out how to biodegrade it in case of accident mass release into the soil or water-courses. Biodegradation of ionic liquids in soil will be monitored in this project. For Part A of the project, tests will be carried out by analyzing the two soil samples from two different places which are soils from hill area and soils from coastal area. Those two samples will undergo a series of processes and will be monitored all the time. For part B, soil samples from coastal area and hill area will be used to degrade ionic liquid at different temperatures for 60 hours at three different temperatures. The quest for the biodegradation agent will take around 6 months.

ACKNOWLEDGEMENT

I want to take this opportunity to express my utmost gratitude to the individual that have taken the time and effort to assist the author in completing the project. Alhamdulillah praises to The Almighty Allah for blessing me with the strength and health towards completing this project. It is a pleasure to thank the many people who made this thesis possible. First and foremost, I would like to say a million of thankfulness to my supervisor, Prof IR Abdul Aziz Omar who has been very supportive from the beginning to the end of the project. His patience and support rendered to me is greatly appreciated and is contributory to the success of the research. Much appreciation is also accorded to PETRONAS Ionic Liquids Centre for guidance and advice on my project.

I would also like to thank the technicians in Unit Operation Lab, Block 3, Chemical Engineering Department, Universiti Teknologi PETRONAS especially Mr Shahafizan who provide helps towards experimental work of this research. My utmost appreciation goes to my family and friends who inspired, encouraged with never ending prayers and fully supported me for every trial that come in my way. Their advices will always be remembered and become the motivation in continuing the journey of my life. The love, support and precious time together has made this journey more meaningful.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Abbreviation

Ionic Liquids	ILs
Carbon Dioxide	CO_2
Potassium Hydroxide	KOH
Hydrochloric Acid	HCl
1-Butyl-3-Methylimidazolium Tetrafluoroborate	[bmim][BF_4]
1-Methoxyethyl-3-Methylimidazolium Tetrafluoroborate	[moemim][BF_4]
Room-temperature ionic liquids	RTIL's
Polyaromatic Hydrocarbons	PAHs
Polychlorinated Biphenyls	PCBs

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF STUDY

Chemical decomposition, analysis or breakdown is the separation of a chemical compound into elements or simpler compounds. It is sometimes defined as the exact opposite of a chemical synthesis. Chemical decomposition is often an undesired chemical reaction. The stability that a chemical compound ordinarily has is eventually limited when exposed to extreme environmental conditions like heat, radiation, humidity or the acidity of a solvent. The details of decomposition processes are generally not well defined, as a molecule may break up into a host of smaller fragments. Chemical decomposition is exploited in several analytical techniques, notably mass spectrometry, traditional gravimetric analysis, and thermo gravimetric analysis (Nic, Jirat, & Kosata, 2006).

1.2 PROBLEM STATEMENT

Ionic liquids are receiving increasing attention as reaction media and alternative solvents, within the context of sustainable and green chemistry, due to their thermal stability, negligible vapour pressure and non-flammability. Although their growing use does not lead to air pollution, possible release into the soil or water-courses could become persistent pollutants and pose environmental risks (Ranke, Stolte, Stormann, Arning, & Jastorff, 2007)

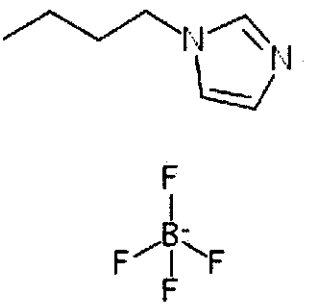

Figure 1: [bmim][BF4] or 1-Butyl-3-methylimidazolium tetrafluoroborate

1.3 OBJECTIVE

The main objective in this project is to study the biodegradation of ionic liquids and to identify the possible biodegradation agents. I chose imidazolium as the ionic liquid because of its high availability and it is widely used for laboratory works and in industries. It has also been shown that some imidazolium ionic liquids are resistant to photo degradation, their stability being enhanced with increasing length of the alkyl chain (Stepnowski, 2005)

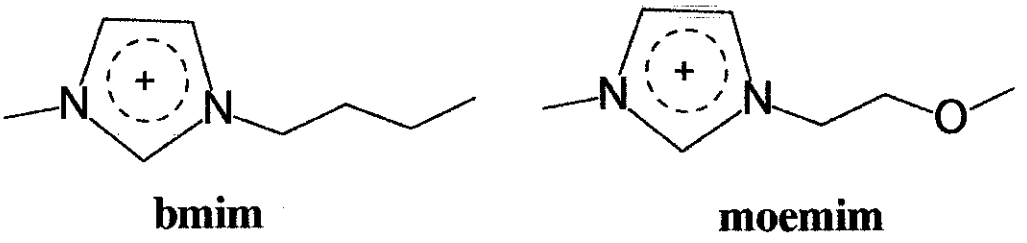

Figure 2: 1-R-3-methylimidazolium cations, with R = $CH_3(CH_2)_3$ and $CH_3O(CH_2)_2$

1.4 FEASIBILITY OF THE PROJECT

The time period for the project is 2 semesters, which is approximately 9 month to complete the project. There are also budget given to the student to purchase chemicals and each student is given RM 500 each. The apparatus for the experiment are also available in block 3, 4 and 5 but there are certain equipments and apparatus that are not available for a long period of time for example for three months use. But overall, based on the time period, budget and experimental apparatus, it is still feasible to do the project.

CHAPTER 2: LITERATURE REVIEW

2.1 IONIC LIQUIDS (ILs)

An ionic liquid is a salt in which the ions are poorly coordinated, which results in these solvents being liquid below 100°C, or even at room temperature (room temperature ionic liquids, RTIL's). At least one ion has a delocalized charge and one component is organic, which prevents the formation of a stable crystal lattice. The methylimidazolium and pyridinium ions have proven to be good starting points for the development of ionic liquids. Properties, such as melting point, viscosity, and solubility of starting materials and other solvents, are determined by the substituent's on the organic component and by the counter ion. Many ionic liquids have even been developed for specific synthetic problems. For this reason, ionic liquids have been termed "designer solvents".

One of the first RTILs was a mixture of [emim]Cl with $AlCl_3$ forming a series of equilibria between [emim][$AlCl_4$], [emim][Al_2Cl_7], and [emim][Al_3Cl_{10}]. This RTIL is not water stable. The discovery of water-insoluble RTILs such as [bmim][PF_6] allowed the development of new work-up methods, including the separation of water-soluble byproducts by simple extraction. Some transition metal catalysts that are soluble in ionic liquids may be recycled together with the ionic liquid, after extraction with water and the non-polar organic solvent used for product separation. The catalyst and ionic liquid may be recycled several times.

The absence of volatility is one of the most important benefits of ionic liquids, offering a much lower toxicity as compared to low-boiling-point solvents. Ionic liquids can also make for safer microwave synthesis methods, because sudden pressure surges are not possible. The dipole characteristics of ionic liquids translate into rapid excitation by microwaves, and consequently faster reactions (P. & W., 2000).

2.1.1 IMIDAZOLE

Imidazole is an organic compound with the formula $C_3H_4N_2$. This aromatic heterocyclic is a diazole and is classified as an alkaloid. Imidazole refers to the parent compound, whereas imidazoles are a class of heterocycles with similar ring structure, but varying substituents. This ring system is present in important biological building-blocks, such as histidine, and the related hormone histamine. Imidazole can serve as a base and as a weak acid. Many drugs contain an imidazole ring, such as antifungal drugs and nitroimidazole.

2.2 BIODEGRADATION BY SOIL MICROORGANISMS

Biodegradation is nature's way of recycling wastes, or breaking down organic matter into nutrients that can be used by other organisms. "Degradation" means decay, and the "bio-" prefix means that the decay is carried out by a huge assortment of bacteria, fungi, insects, worms, and other organisms that eat dead material and recycle it into new forms.In nature, there is no waste because everything gets recycled. The waste products from one organism become the food for others, providing nutrients and energy while breaking down the waste organic matter. Some organic materials will break down much faster than others, but all will eventually decay. By harnessing these natural forces of biodegradation, people can reduce wastes and clean up some types of environmental contaminants (Doyle & Krasny, 2003).

Organic material can be degraded aerobically with oxygen, or anaerobically, without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process. Biodegradable matter is generally organic material such as plant and animal matter and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by microorganisms.

Some microorganisms have a naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in microbial biodegradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions (Bernot, E.E, & Lamberti, 2005).

2.3 TYPES OF MICROORGANISMS IN SOIL

There are several types of microorganisms in soil that benefit plants. Together they make up an immense population of living organisms. One teaspoon of soil may contain millions of various types. Below is a list of common soil microorganisms found throughout the world.

Bacteria – Small, single cell organisms that make up the single most abundant type of microbe. They have a very wide range of conditions that they live in from the artic wastelands to the steaming waters of volcanic hot springs. In soils, they multiply rapidly under the proper conditions. When conditions are wrong for one species, it is right for another. This is not always a good thing since a balance is what is required.

Fungi – The largest microbe group in terms of mass. Some fungi are beneficial, called mycorrhiza, that form a symbiotic relationship with plant roots, either externally or internally. Within the fungi group are pathogen fungi. These are disease causing fungi, some of which can be quite devastating to plants.

Protozoas – Small single cell microbes that feed on bacteria.

Actinomycetes – Necessary for the breakdown of certain components in organic matter.

Algae – Beneficial groups such as blue-green algae, yellow-green algae and diatoms. Some of these can produce their own energy through photosynthesis.

Soil microorganisms are living, breathing organisms and, therefore, need to eat. They compete with plants for nutrients including Nitrogen, Phosphorus, Potassium and micronutrients as well. They also consume amino acids, vitamins, and other soil compounds. Their nutrients are primarily derived from the organic matter they feed upon. The benefit is that they also give back or perform other functions that benefit higher plant life.

CHAPTER 3: METHODOLOGY

3.1 RESEARCH METHODOLOGY FOR PART A

In each test, 2.0 g of ionic liquid was mixed with about 300 g of soil, sieved to 2-mm particle size and will be put in a desiccators. The soil will be collected only from the surface (maximum depth 10 cm). The pH of the soil will be measured. Distilled water will be added to bring the moisture content of the soil to about 90% of the moisture-holding capacity (29% of dry soil) (Standard Methods for the Examination of Water et al., 1989). The amount of CO2 produced will be determined by titrating 0.4 M KOH solutions with 0.25 M HCl to a phenolphthalein end-point.

The frequency of data recording will be done from daily to weekly, depending on the degradation rate. The temperature was 30 ± 2 °C over the three-month period (Cho, Pham, You-Chul Jeon, & Yeoung-Sang Yun, 2008).

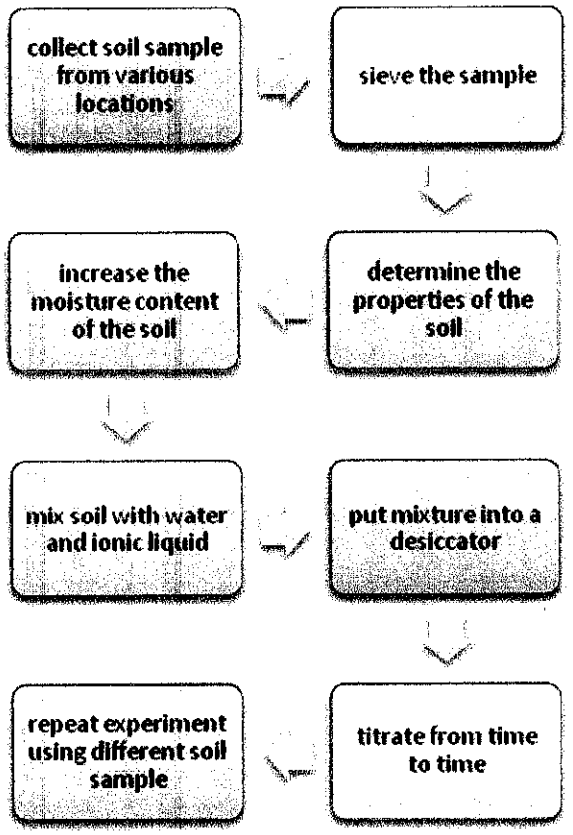

Figure 3: Flowchart of Project A's procedures

3.1.1 MEASUREMENT OF THE PH OF THE SOILS

For measuring the pH of the soil, first we have to dig a small hole in the soil. Then break up the soil within the hole and remove any foreign debris.

After that we fill the hole with distilled water until you have a muddy pool at the bottom. Next we will insert the test probe into the mud but make sure the tester is clean and calibrated. Wipe the probe with tissue or clean cloth to clean it. Hold there for 60 seconds and take a reading (Craze, 1990).

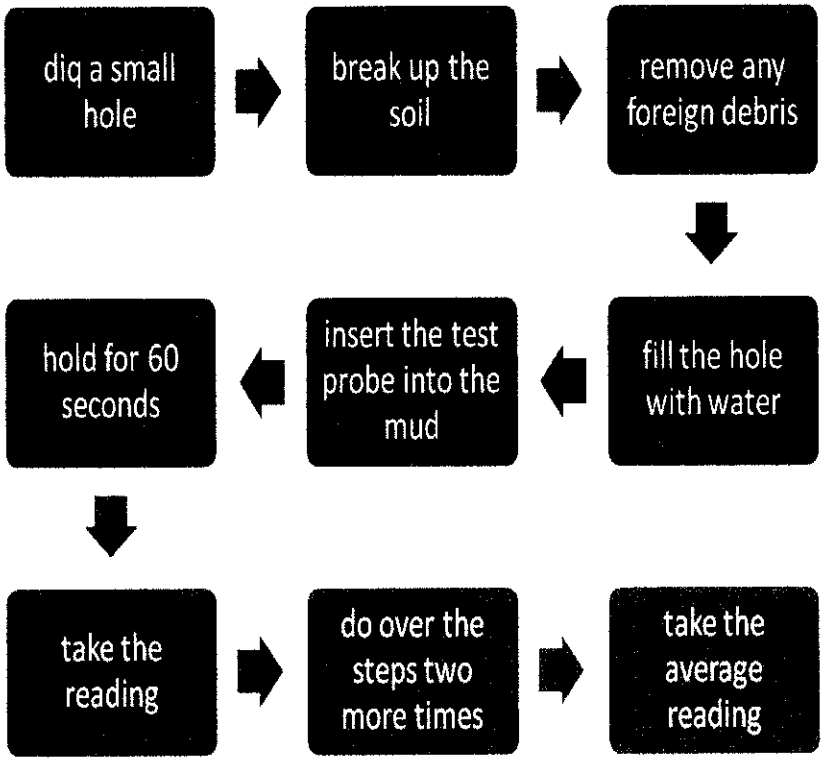

Figure 4: Measurement of the pH of the soils

3.1.2 MOISTURE CONTENT

For measuring the moisture content of the soil, first we have to weigh moisture sample immediately and record as "wet weight of sample". Then dry the wet sample to a constant weight at a temperature not exceeding 115° C. Next using suitable drying equipment to allow the sample to cool and then weigh the cooled sample again and record the weight as the "dry weight of sample". Finally put the data into the formula for the calculation of the moisture content (Procter & Meullenet, 1998).

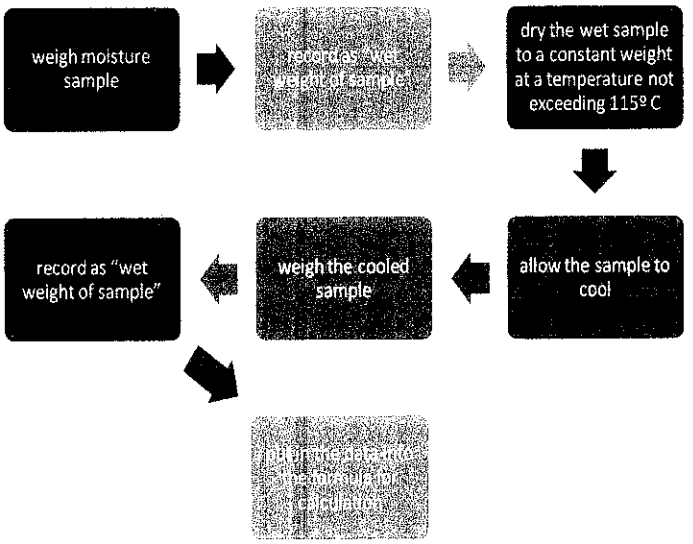

Figure 5: Moisture Content Measurement

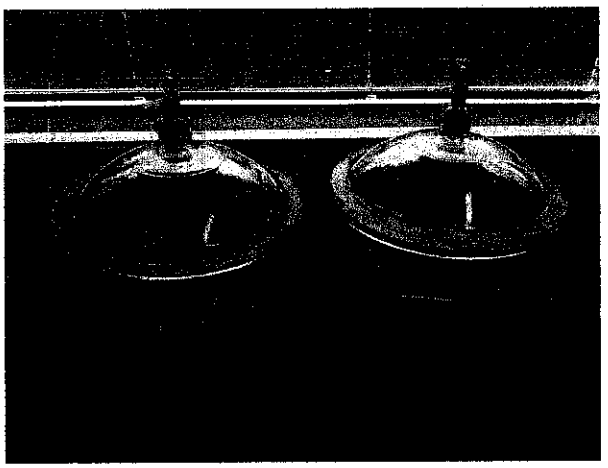

Figure 6: Soil samples in desiccators

3.2 RESEARCH METHODOLOGY FOR PART B

In each test, 10 g of [bmim][BF_4] was mixed with about 300 g of soil, sieved to 2-mm particle size and will be mixed with 800 ml of water and then be put in a 1 liter glass reactor with jacketed stirrer at a temperature of 20°C and 30°C. The soil will be collected only from the surface (maximum depth 10 cm). The amount of CO_2 produced will be determined by measuring the pH of the mixture.

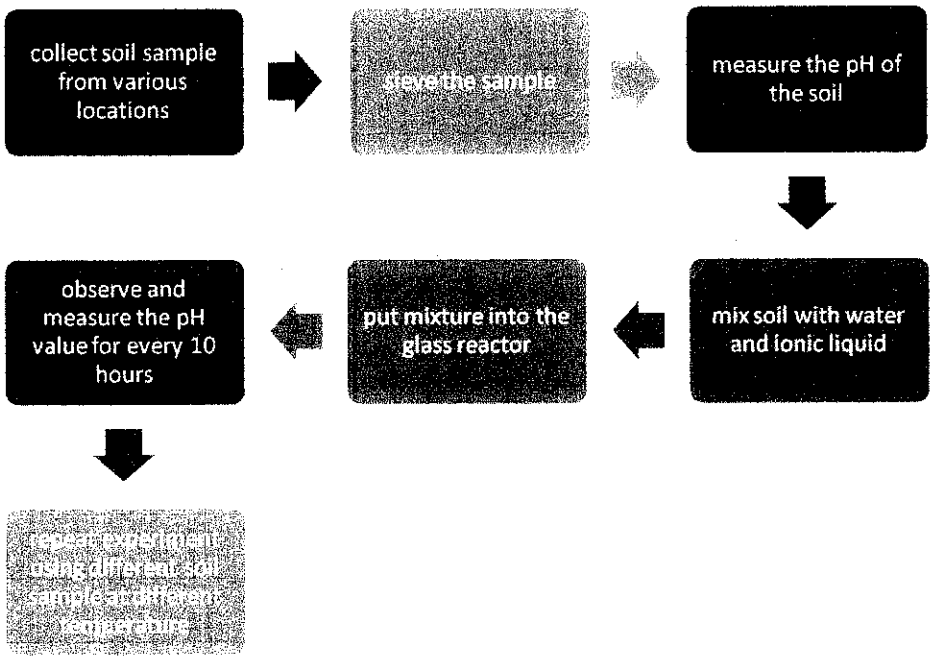

Figure 7: Flowchart of Project B's procedures

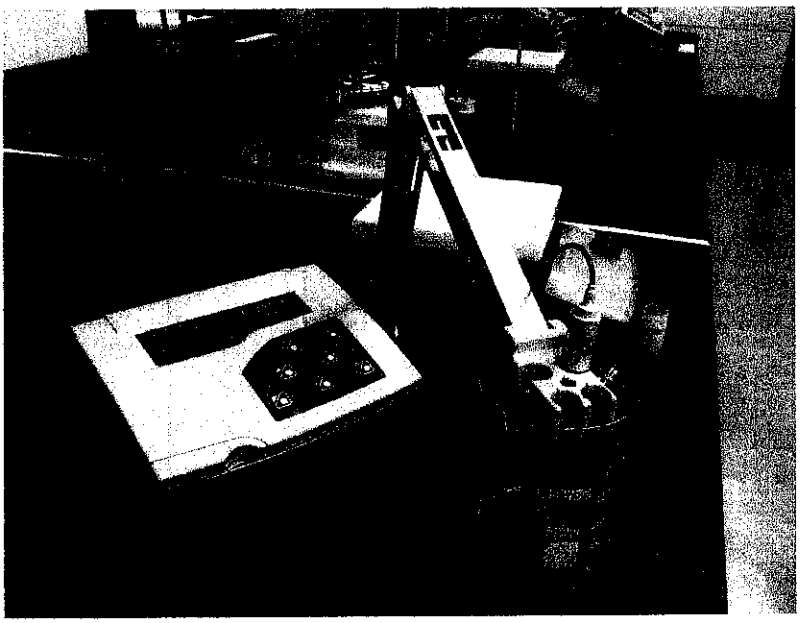

Figure 8: pH measurement

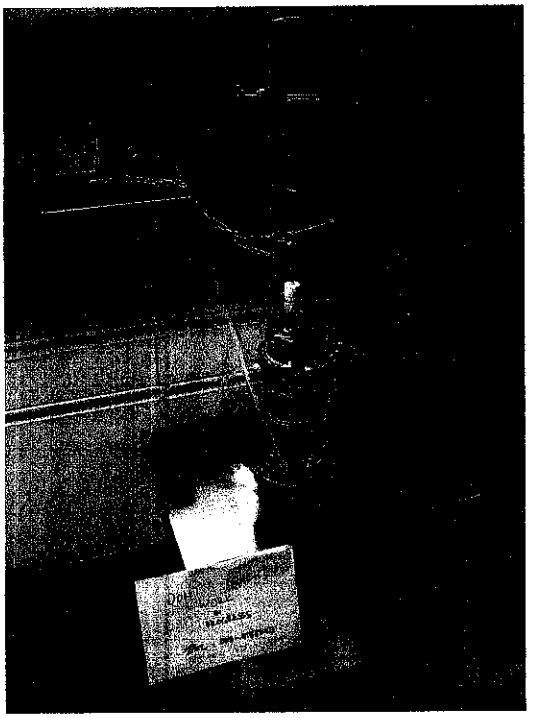

Figure 9: 1 litre glass reactor with jacketed stirrer

3.3 ACTIVITIES

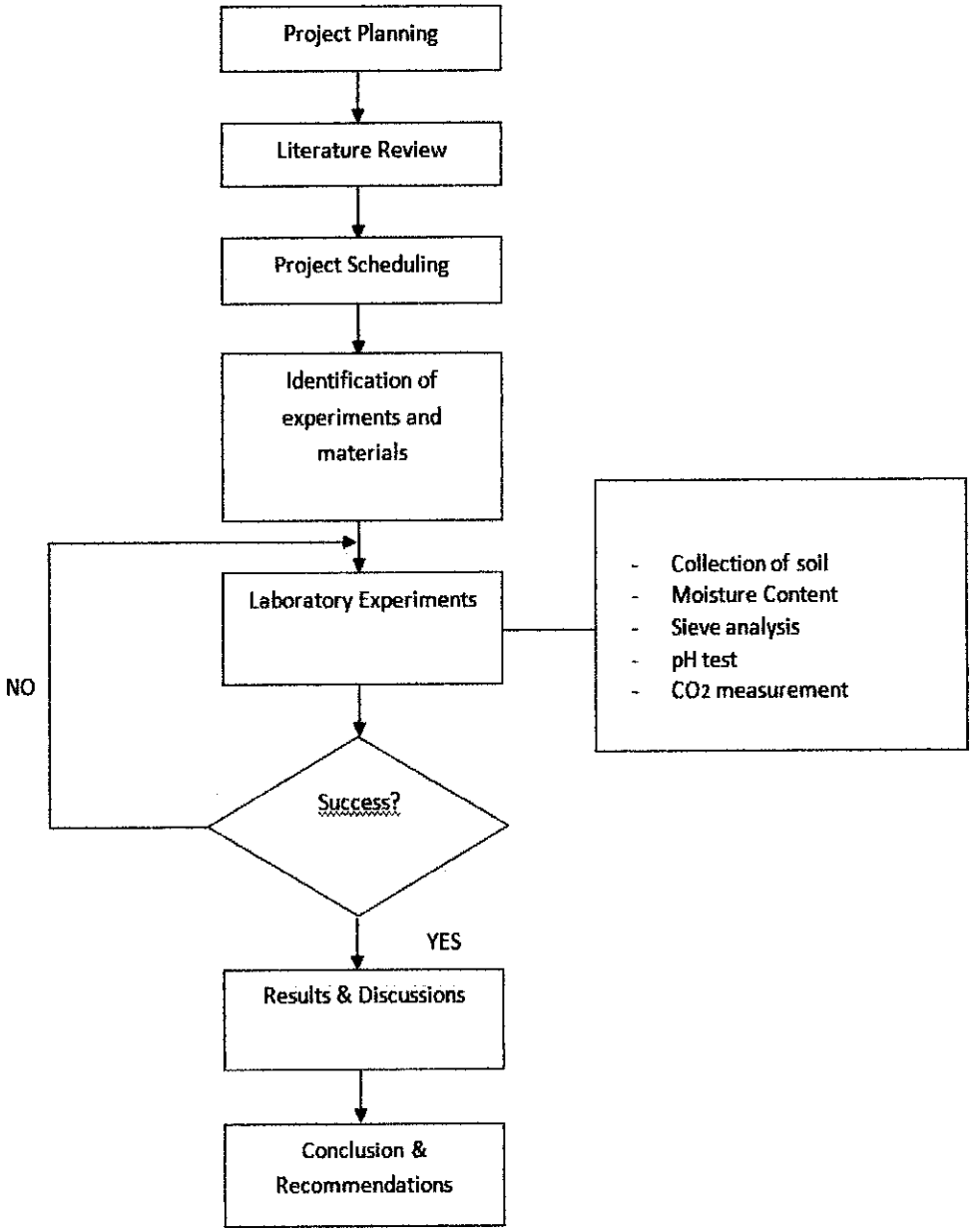

Figure 10: Flowchart of activities

3.4 PROJECT TIMELINE

Detail/ Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Selection of Project Topic														
2.Preliminary Research Work														
3.Submission of Extended Proposal														
4.Proposal Defense														
5.Project Work Continues														
6.Submission of Interim Draft Report														
7.Submission of Interim Report														

Table 1: FYP II Project Timeline

The important key milestones of the first part of this project are the submission of extended proposal defence, proposal defence and Interim Report.

Activities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Project Work Continues															
Submission of Progress Report															
Data collection and interpretation															
Pre-EDX															
Submission of Draft Report															
Submission of Dissertation (soft bound)															
Submission of Technical Paper															
Oral Presentation															
Submission of Project Dissertation															

Table 2: FYP II Project Timeline

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 RESULTS FOR PROJECT A

	Hill area	Coastal area
pH	6.0	7.4
	Moderate acid	Slightly alkaline
Moisture Content	Increased from 21% to 90%	Increased from 25% to 90%

Table 3: Properties of Soils

The data obtained after conducting the measurement of the pH and moisture content of the soils. The soil from hill area shows that it has an acidic condition while the soil from the coastal area shows that it has a slightly alkaline condition. So we can see in which condition the biodegradation will occur faster. Both of the soils' moisture content are increased to 90% from 21% (hill area) and 25% (coastal area) so that the soils will be fresh and healthy and provide a good environment to the microorganisms in it during the project period of three months.

4.1.1 AMOUNT OF CO_2 PRODUCED

The carbon content of [bmim][BF_4] is 42.5% by weight. A complete degradation of the 2.0 g of [bmim][BF_4] (850 mg of carbon) is expected to give rise to 68.4 mmol of CO_2.

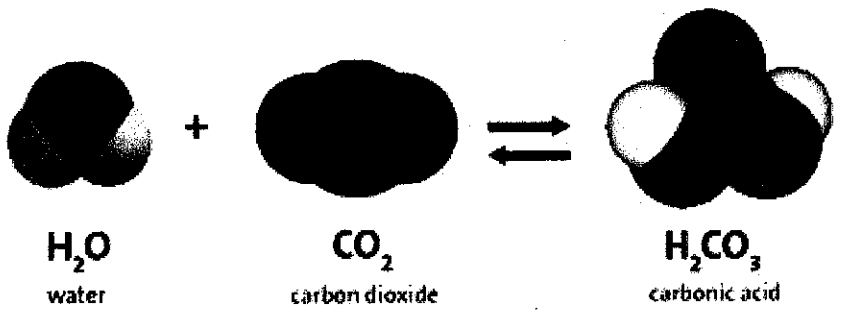

Figure 11: Reaction between carbon dioxide and distilled water

The CO_2 produced will be dissolved into distilled water to produce carbonic acid. The carbonic acid will be titrated by titrating 0.4 M KOH solutions with 0.25 M HCl to a phenolphthalein end-point.

$$0.4\frac{mol}{L}\ KOH \times 1\frac{L}{1000\ ml} \times X\ ml\ KOH = Y\ mol$$

$$0.25\frac{mol}{L}\ HCL \times \frac{1\ L}{1000\ ml} \times Z\ ml\ HCL = A\ mol$$

So mol of carbonic acid produced is $Y\ mol - A\ mol = B\ mol\ of\ H_2CO_3$ and since the stoichiometric coefficient of H_2CO_3 and CO_2 is 1:1, the mol of carbonic acid produced is equal to the mol of carbon dioxide produced.

The percentage of the biodegradation rate is measured by the percentage of the carbon consumed by the microorganisms which can be calculated with the following equation

$$\text{Degradation Rate (\%)} = \frac{\text{B mol of } H_2CO_3}{\text{68.4 mmol of } CO_2} \times 100\%$$

Time (week)	Degradation of IL in Hill Soil (%)	Degradation of IL in Coastal Soil (%)
Week 1	-	-
Week 2	-	-
Week 3	5	7
Week 4	5	8
Week 5	8	10
Week 6	13	15
Week 7	16	20
Week 8	19	24
Week 9	22	28
Week 10	26	33
Week 11	30	40

Table 4: Amount of CO_2 produced

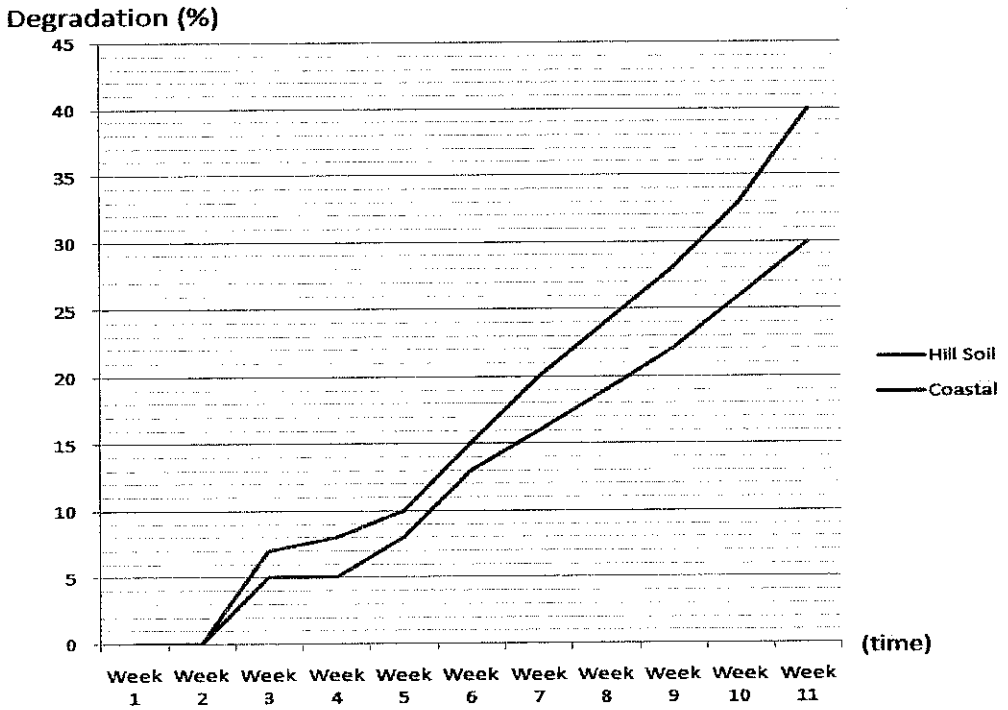

Figure 12: Biodegradation percentage

We can observe that the degradation percentage of ionic liquid in coastal area soil is higher which is 40% compared to hill area soil which is 30%. The effect of acidity has shown to greatly affect the biodegradation rate. According to the behaviour of the graphs, I believe that the biodegradation rate can be up to 60% if we add another four weeks into the observation period but unluckily due to time constraint I cannot add another month into the observation period.

As we see how a small different condition in the soils can contribute to a very huge biodegradation rate. I am eager to see the biodegradation rate at various temperatures such as 20 °C, 30 °C and 40 °C. By doing this, we can surely narrow down the biodegradation agents.

4.2 RESULTS FOR PROJECT B

Time (hour)	pH value for hill area soil at 25°C	pH value for coastal area soil at 25°C
0	6.13	7.40
10	5.43	5.30
20	4.10	3.69
30	3.93	3.60
40	4.80	4.50
50	5.11	4.90
60	4.90	4.80

Table 5: pH value for samples at 25 °C

Time (hour)	pH value for hill area soil at 30°C	pH value for coastal area soil at 30°C
0	6.13	7.38
10	5.03	3.95
20	3.75	3.33
30	3.99	4.56
40	4.90	4.91
50	5.30	4.78
60	5.28	4.91

Table 6: pH value for samples at 30 °C

Time (hour)	pH value for hill area soil at 35°C	pH value for coastal area soil at 35°C
0	6.08	7.39
10	4.12	5.25
20	3.50	3.96
30	4.50	4.07
40	4.60	4.93
50	4.70	5.06
60	4.75	6.07

Table 7: pH value for samples at 35 °C

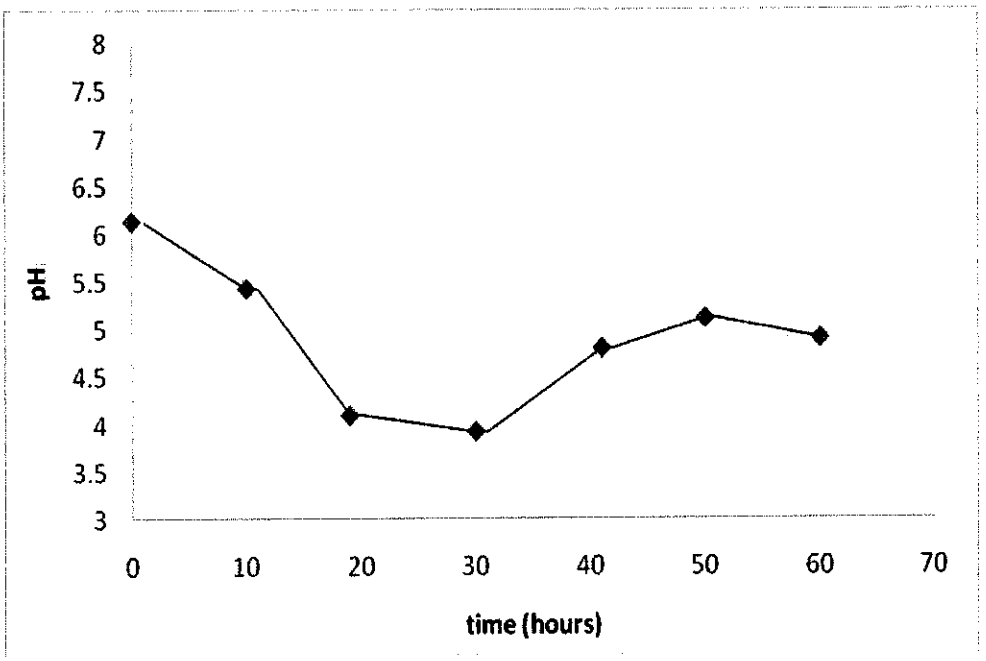

Figure 13: Hill area soil at 25 °C

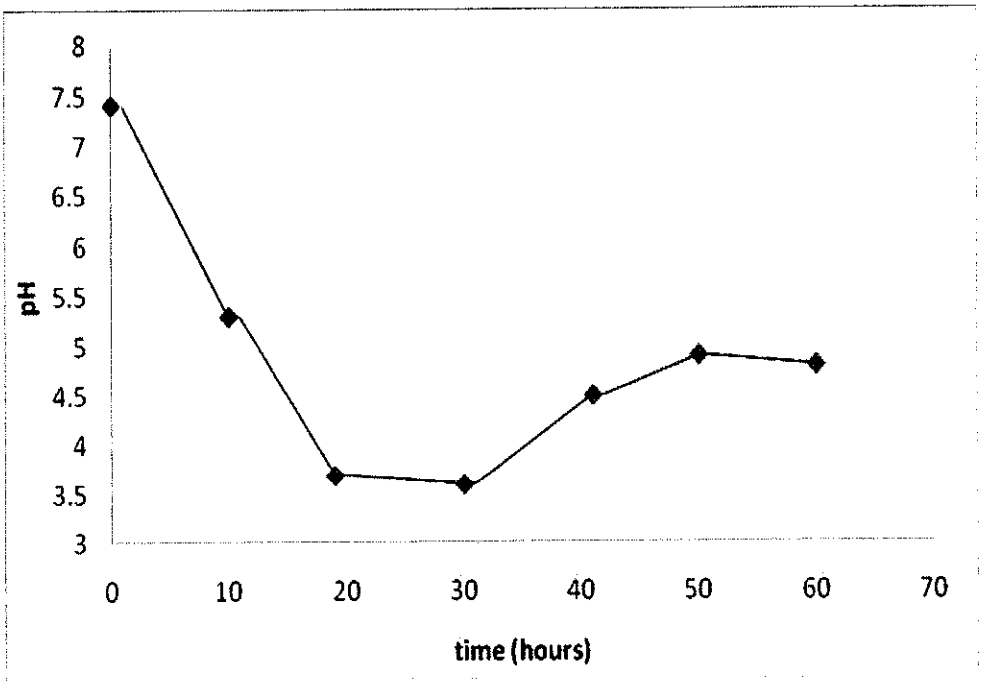

Figure 14: Coastal area soil at 25 °C

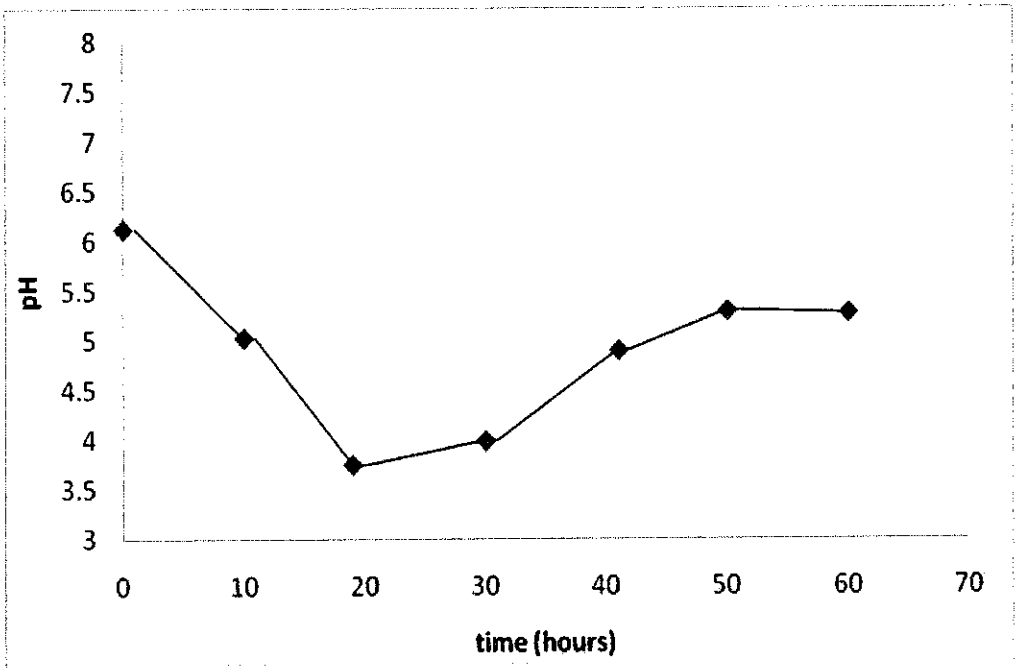

Figure 15: Hill area soil at 30 °C

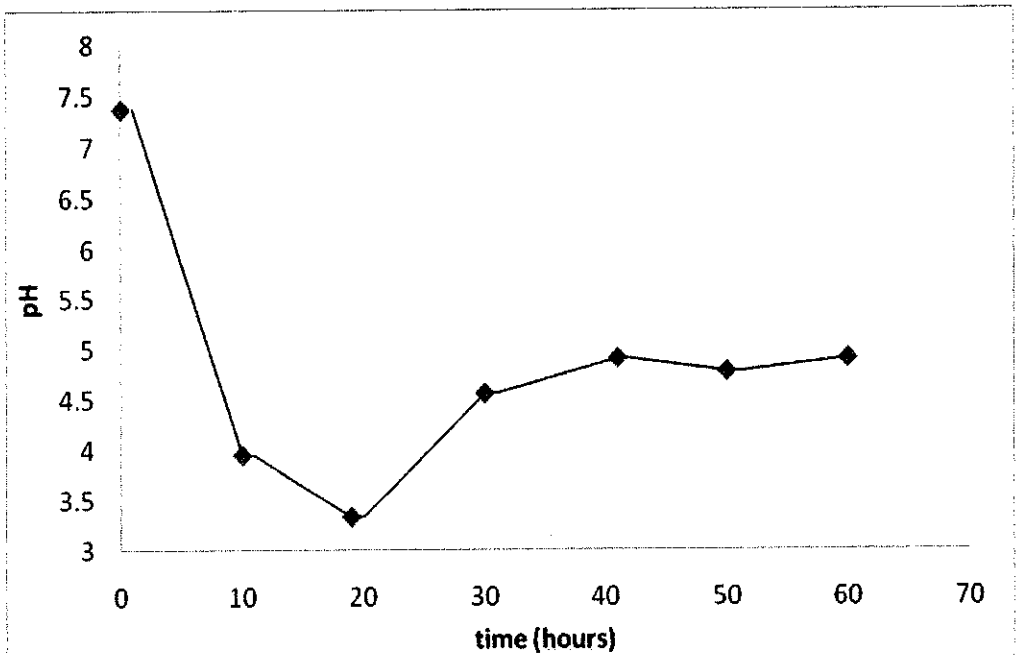

Figure 16: Coastal area soil at 30 °C

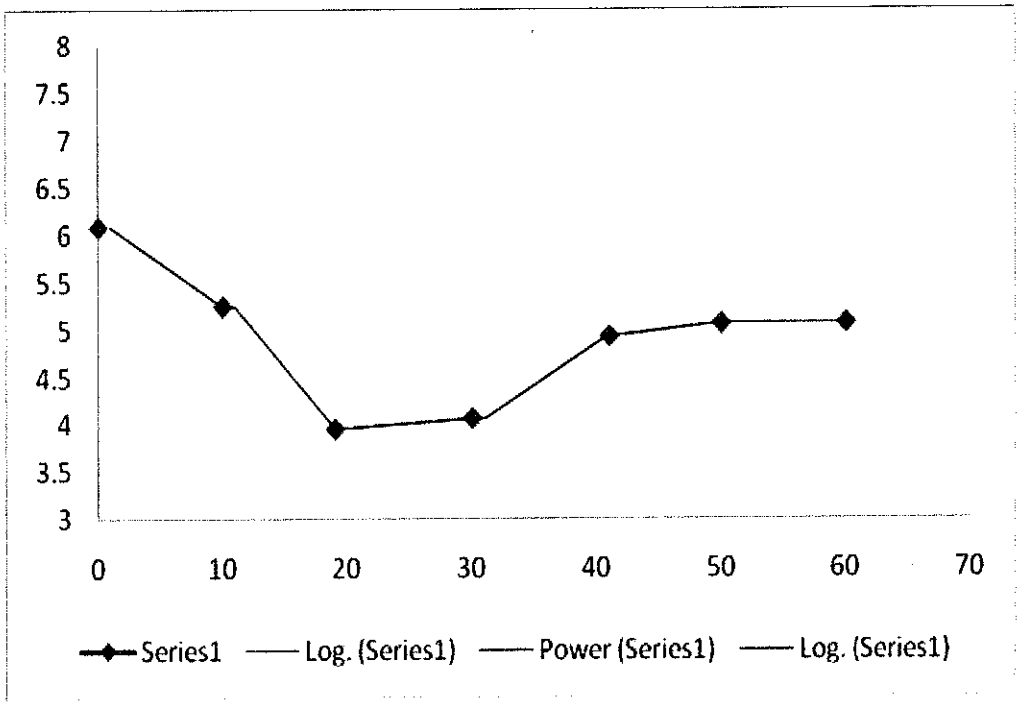

Figure 17: Hill area soil at 35 °C

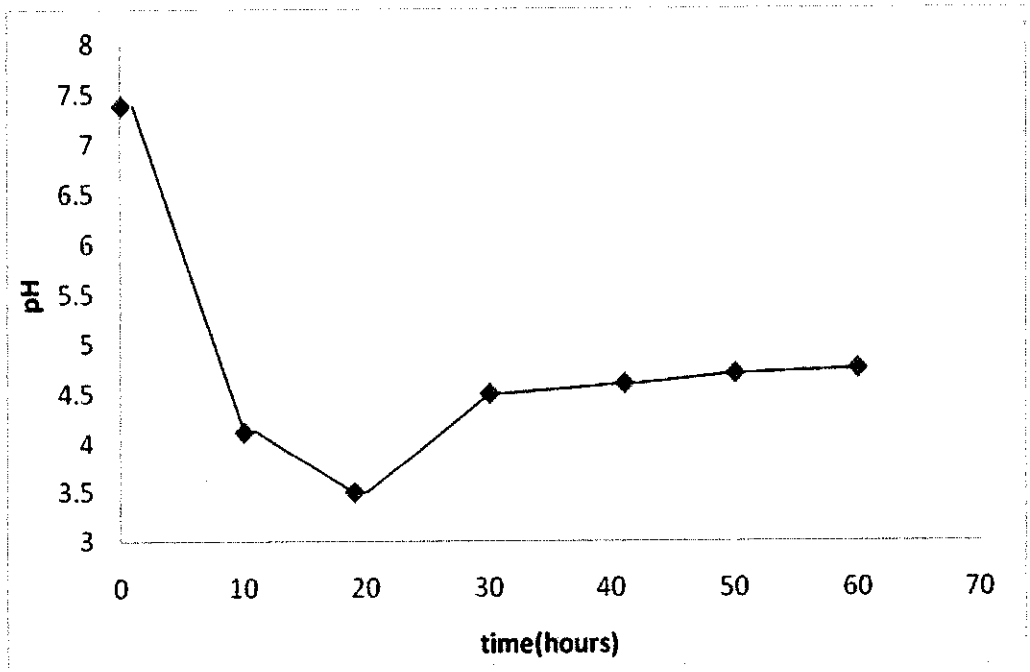

Figure 18: Coastal area soil at 35 °C

4.3 DISCUSSION

4.3.1 DISCUSSION FOR PART A

Based on the results from the experiment, there are several points that can be discussed to increase the understanding on the experiment result and to meet the objective that had been set.

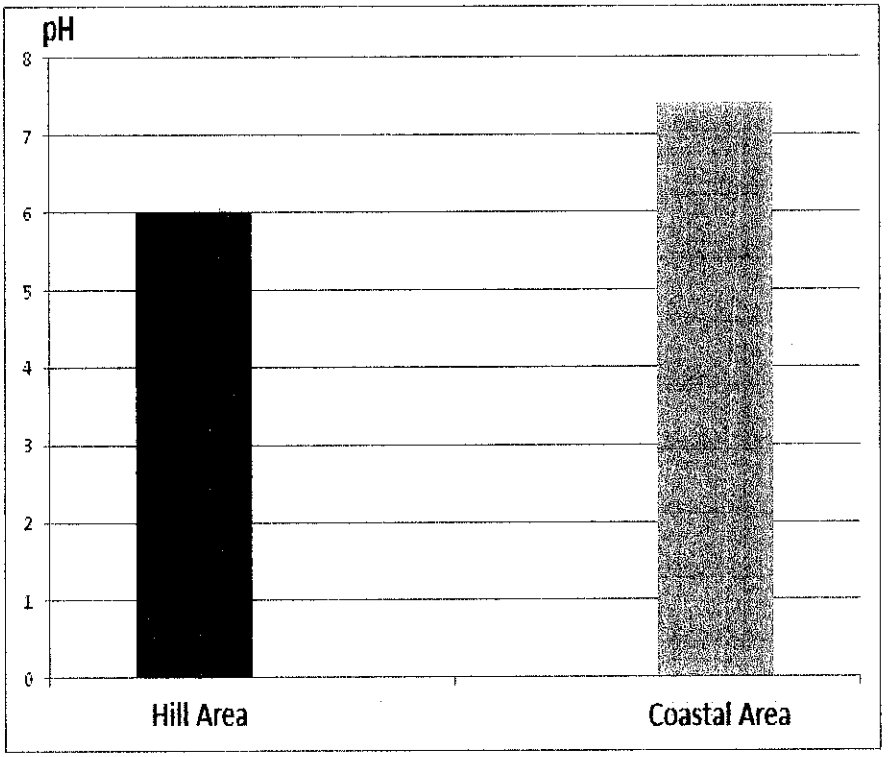

Figure 19: pH of soils

We can see the main different of those two soil samples is the pH of the soil. The hill area soil has the pH value of 6.0 and while the coastal area soil has the pH value of 7.4. Even though the pH difference is small, the effect of it is very big. Just 1.4 pH difference can result in almost 10% biodegradation percentage difference as the biodegradation percentage for hill area soil is 30% while the biodegradation percentage for coastal area soil is 40% in just three months period.

The reason behind this is because of the relationship between the pH of the soil and the activity of the microorganisms in the soil. This clearly shows us that the microorganisms in soil are active in the alkaline condition. That is what makes the biodegradation rate of [bmim][BF_4] in coastal area soil higher than the biodegradation rate in hill area soil. Even though microorganism can adapt so that they can survive in any conditions even in the most acidic condition of pH 0 up to 11.5 (Madigan, Martinko, & Perker, 2003), they still have an optimum condition that makes them to be most active and productive.

Soil reaction has a definite influence / effect on quantitative and qualitative composite on of soil microbes. Most of the soil bacteria, blue-green algae, diatoms and protozoa prefer a neutral or slightly alkaline reaction between PH 4.5 and 8.0 and fungi grow in acidic reaction between PH 4.5 and 6.5 while actinomycetes prefer slightly alkaline soil reactions.

Soil reactions also influence the type of the bacteria present in soil. For example nitrifying bacteria *(Nitrosomonas & Nitrobacter)* and diazotrophs like *Azotobacter* are absent totally or inactive in acid soils, while diazotrophs like *Beijerinckia, Derxia,* and sulphur oxidizing bacteria like *Thiobacillus thiooxidans* are active in acidic soils (Josephine, 2011).

We can say that the difference of biodegradation rate that we got as the result of this project is mainly caused by the difference of pH in the soil samples. But there are other factors that may affect the results obtained in this experiment. For example soil fertility, nature of the soil, root exudates and soil organic matter.

Soil fertility: Fertility level of the soil has a great influence on the microbial population and their activity in soil. The availability of N, P and K required for plants as well as microbes in soil determines the fertility level of soil. On the other hand soil micro flora has greater influence on the soil fertility level.

Nature of Soil: The physical, chemical and physico-chemical nature of soil and its nutrient status influence the microbial population both quantitatively and qualitatively. The chemical nature of soil has considerable effect on microbial population in soil. The soils in good physical condition have better aeration and moisture content which is essential for optimum microbial activity. Similarly nutrients (macro and micro) and organic constituents of humus are responsible for absence or presence of certain type of microorganisms and their activity. For example activity and presence of nitrogen fixing bacteria is greatly influenced by the availability of molybdenum and absence of available phosphate restricts the growth of *Azotobacter.*

Root Exudates: In the soil where plants are growing the root exudates also affects the distribution, density and activity of soil microorganism. Root exudates and sloughed off material of root surfaces provide an abundant source of energy and nutrients and thus directly or indirectly influence the quality as well as quantity of microorganisms in the rhizosphere region. Root exudates contain sugars, organic acids, amino acids, sterols, vitamins and other growth factors which have the profound effect on soil microbes.

Soil Organic Matter: The organic matter in soil being the chief source of energy and food for most of the soil organisms, it has great influence on the microbial population. Organic matter influence directly or indirectly on the population and activity of soil microorganisms. It influences the structure and texture of soil and thereby activity of the microorganisms.

4.3.1.1 AVERAGE DEGRADATION RATE

The biodegradation percentage can be observed in Figure 6 where the rate of degradation (percent of carbon per day) is given by the slope of the tangent to each curve.

Overall, the biodegradation rate is almost proportional to the period of observation and according to the behaviour of the graph, we predict that the biodegradation percentage can easily increase up to 60-80% if the project is being continued for another two months.

$$Biodegradation\ Rate = \frac{\Delta Degradation\ Percentage}{\Delta Time}$$

From week 3-7

$$Biodegradation\ Rate = \frac{(16 \quad 5)}{5(4)} = \frac{0.55\ \%}{day}$$

$$Biodegradation\ Rate = \frac{0.55\ \%}{day} = \frac{0.3762\ mmol\ CO_2}{day}$$

From week 8-11

$$Biodegradation\ Rate = \frac{(30 - 16)}{4(4)} = \frac{0.875\ \%}{day}$$

$$Biodegradation\ Rate = \frac{0.875\ \%}{day} = \frac{0.5985\ mmol\ CO_2}{day}$$

We can see that the difference of the biodegradation rate for week3-7 and for week 8-11. It shows that the biodegradation rate has increased from 0.3762 mmol CO2 per day to 0.5985 mmol CO_2 per day. So it is a proof that the microorganisms in the soil has become more active and become hungrier as the conversion of the ionic liquid to CO_2 is increasing.

4.3.1.2 CALCULATION OF MOISTURE CONTENT ADDITION

$$MC\% = \frac{W_2 - W_3}{W_3 - W_1} x\,100$$

Where:

W_1 = Weight of tin (g)

W_2 = Weight of moist soil + tin (g)

W_3 = Weight of dried soil + tin (g)

Before starting the experiment, the moisture content of both of the soil samples were calculated and then increased to 90%. Below are the calculations to increase moisture content of hill area soil to 90%

$$MC\% = \frac{W_2 - W_3}{W_3 - W_1} \times 100$$

$$MC\% = \frac{(300 + 352) - (248 + 352)}{(248 + 352) - 352} \times 100$$

$$MC\% = \frac{652 - 600}{600 - 352} \times 100$$

$$MC\% = 21\%$$

$$90 = \frac{(300 + 352 + x) - (248 + 352)}{(248 + 352) - 352} \times 100$$

$$223 = (652 + x) - 600$$

$x = 171\ g$ Of distilled water needed to be added to the hill area soil to increase the moisture content to 90%

Calculation to increase moisture content of coastal area soil to 90%

$$MC\% = \frac{W_2 - W_3}{W_3 - W_1} \times 100$$

$$MC\% = \frac{(300 + 355) - (240 + 355)}{(240 + 355) - 355} \times 100$$

$$MC\% = \frac{655 - 595}{595 - 355} \times 100$$

$$MC\% = 25\%$$

$$90 = \frac{(300 + 355 + x) - (240 + 355)}{(240 + 355) - 355} \times 100$$

$$216 = (655 + x) - 595$$

$x - 156\ g$ Of distilled water needed to be added to the coastal area soil to increase the moisture content to 90%

Moisture content is the quantity of water contained in a material, such as soil(called soil moisture), rock, ceramics, fruit, or wood. Moisture may be present as adsorbed moisture at internal surfaces and as capillary condensed water in small pores. At low relative humidity, moisture consists mainly of adsorbed water. At higher relative humidity, liquid water becomes more and more important, depending on the pore size.

In soil science, hydrology and agricultural sciences, water content has an important role for groundwater recharge, agriculture, and soil chemistry. Many recent scientific research efforts have aimed toward a predictive-understanding of water content over space and time. Observations have revealed generally that spatial variance in water content tends to increase as overall wetness increases in semiarid

regions, to decrease as overall wetness increases in humid regions, and to peak under intermediate wetness conditions in temperate regions (Lawrence & M., 2007).

Soil microorganisms (Flora & Fauna), just like higher plants depends entirely on soil for their nutrition, growth and activity. The major soil factors which influence the microbial population, distribution and their activity in the soil are soil fertility, cultural practices, soil moisture, soil temperature, soil aeration, light, the pH of the soil (H-ion Concentration), organic matter, food & energy supply, nature of soil and last but not least is microbial associations.

It is one of the important factors influencing the microbial population & their activity in soil. Water (soil moisture) is useful to the microorganisms in two ways. First it serves as source of nutrients and supplies hydrogen / oxygen to the organisms and it serves as solvent and carrier of other food nutrients to the microorganisms. Microbial activity & population proliferate best in the moisture range of 20% to 60%. Under excess moisture conditions / water logged conditions due to lack of soil aeration (Oxygen) anaerobic micro flora become active and the aerobes get suppressed. While in the absence of adequate moisture in soil, some of microbes die out due to tissue dehydration and some of them change their forms into resting stages spores or cysts and tide over adverse conditions. Therefore optimum soil moisture (range 20 to 60 %) must be there for better population and activity of microbes in soil.

4.3.2 DISCUSSION FOR PART B

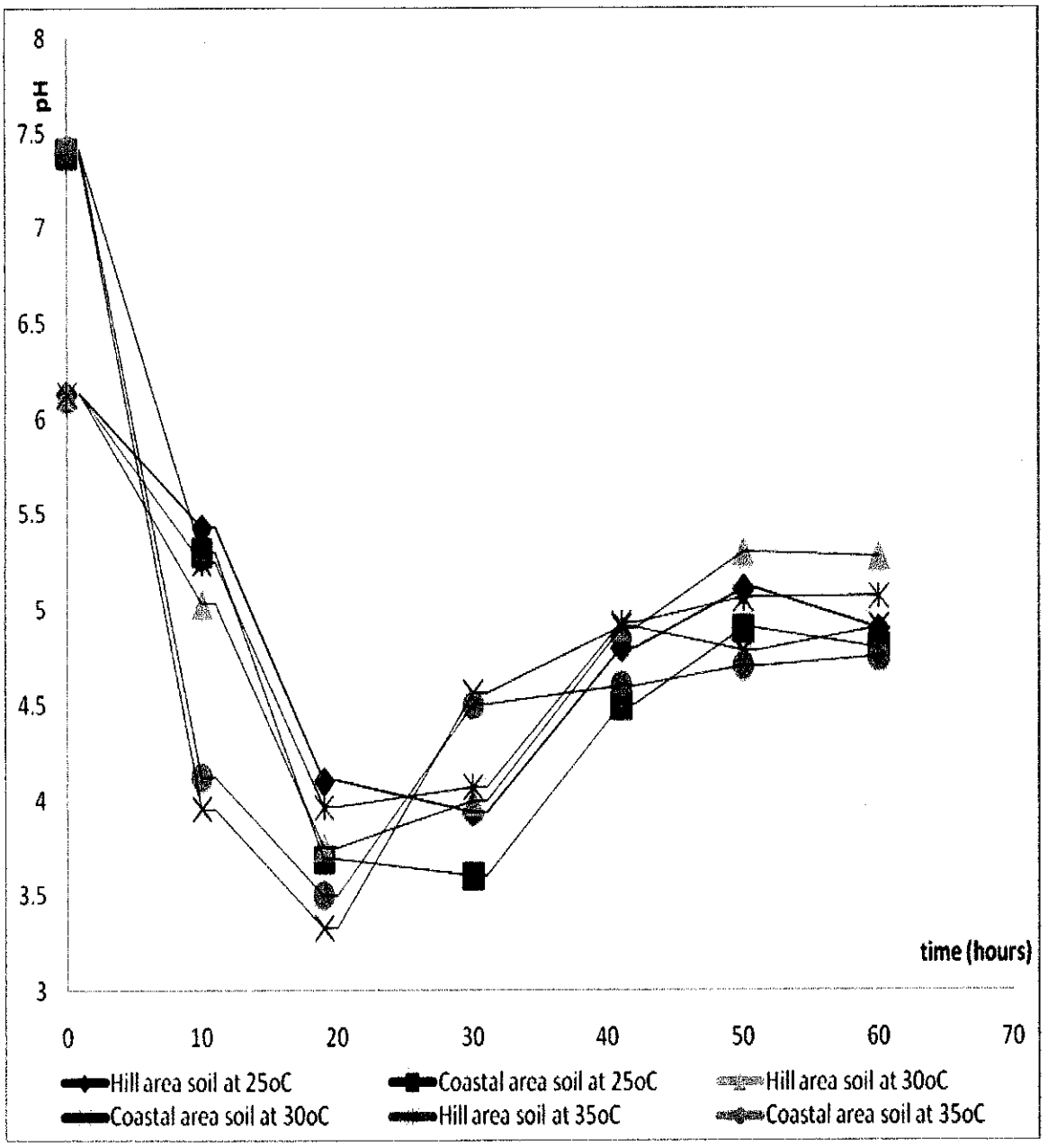

Figure 20: Comparisons on biodegradation between samples

4.3.2.1 PATTERNS OF THE GRAPHS

In each of the graph in *Figure 13 -18*, we can see three patterns in the graph where the value of the pH will first drop, then increase and after that fluctuate. The first drop of the pH value is because of the formation of carbonic acid through the

degradation of the [bmim][BF_4] where the degradation of the ionic liquid produces CO_2 and the CO_2 produced will straight be dissolved in the distilled water and that is where the carbonic acid will be formed. The formation of the carbonic acid will definitely reduce the pH value of the mixture. That explains the first drop of the pH value.

After the decrease, we can see that the pH value of the mixture will increase a bit. I believe that this is caused by the release of the CO_2 by the mixture. The carbonic acid that that formed earlier must have undergo a reverse reaction that causes the carbonic acid to produce back the carbon dioxide. This will result in the decrease of the concentration of the carbonic acid in the mixture thus increasing the pH value of the mixture. This is why we can observe the increasing pattern in the graph after the first drop of the pH value.

And the last part of the graph is the fluctuating part where the value of the pH will be fluctuate as the mixture is getting more stable.

4.3.2.2 EFFECTS OF pH AND TEMPERATURE ON DEGRADATION RATE

Observing from the pattern and the slope of the graphs, we can see that the ionic liquid got degraded faster by soil from coastal area and at temperature 30 °C - 35 °C. The rate of degradation can be seen by the degree of pH drop within the first 10-20 hours.

Coastal area soil degrades the ionic liquid faster than hill area soil because of its alkaline properties. The alkaline surrounding makes the microorganisms in the mixture to become more active. So when the microorganisms become more active it will consume more ionic liquid and convert it into CO_2 that later will react to form carbonic acid.

The temperatures during this experiment are 25 °C, 30 °C and 35 °C. According to the graph, the temperature that are ideal for biodegradation are somewhere in between 30 °C - 35 °C.

4.3.2.3 pH DROP

Based on *Figure 20,* we can see that the highest pH drop is experienced by coastal area soil at temperature of 30 °C and followed closely by coastal area soil at temperature of 35 °C. So the highest biodegradation rate occurred when coastal soil is at 30 °C -35 °C. It means the biodegradation agents inside the soil are most active and optimum when the condition is alkaline with temperature between 30 °C- 35 °C.

4.4 LIMITATIONS

Few problems had occurred and has forced me to change the project a bit. First is the insufficient equipment. Actually I wanted to run 12 samples so that i need 12 desiccators. But suddenly there was not enough desiccators. I feel very disappointed as I had requested at the beginning of the last semester and I was told that the request can be granted. So instead of having 12 samples I had to run with 2 samples only.

Secondly, there are not enough apparatus and equipments. At first I wanted to start my part B of this project first at I can add more parameters in it but due to limited equipments, I had to wait for other student to finish their work first. This is why the period for each run in part B of this experiment is only 60 hours instead of longer period for better results.

There are also ''no clean rooms'' available as at first I wanted to use pure bacteria instead of just using soils. So with these limitations, my experiment has become a very simple project but I believe my project will lead to an important discoveries.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

After conducting this experiment and studying the effects that affect the biodegradation of ionic liquids. We can clearly see on how the effect of the pH and temperature can accelerate the biodegradation of [bmim][BF4] that has been put in the soil. Even a small different can result in major different of biodegradation rate. We can relate that the acidic condition makes the microorganism that biodegrades ionic liquid become less active thus making the biodegradation process become slower. And soil microorganisms in the soil sample are most active at temperature between 30 °C to 35°C.

For the future research, it would be great if we can study other factors that can accelerate the biodegradation process so that we can effectively degrade the ionic liquids when needed.

5.2 RECOMMENDATION

There are several recommendations that can be done to achieve better results for this experiment. The recommendations are as follows:

1. Repetition for each sample to produce accurate result

For the experiment, there is no repetition done for each sample because of the time constraint. The repetition for each sample will surely produce a better result.2

2. Use variety of ionic liquids

Instead of just using [bmim][BF4] alone. I really want to add another parameter which is the effect of the ionic liquids. There we can use ionic liquids that contain oxygen element for example [moemim][BF4] or [moemim][dca]. So we can see which ionic liquid degrades faster, the ones with the oxygen or the ones without.

3. Equipments and Apparatus.

I really wish UTP will buy/provide more equipments and apparatus so that the next projects can be done without students facing a limitations caused by the unavailability of equipments and apparatus.

4. Prolong the period of the experiments.

In UTP, we final year students will have three semesters in our final year since UTP has changed its academic structure, then we enrol final project (fyp) subject in the final two semesters. So instead of having two semesters project, I hope fyp can be enrolled from the first semester of the final year study so that we can have longer period of experiment.

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