DEGRADATION OF BIOPOLYMER BASED ON SLOW RELEASE FERTILIZER IN WET SOIL

BY

MOHAMMAD AMIRUL FITRA B. SAZALI

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE

BACHELOR OF ENGINEERING (HONS)

(CHEMICAL ENGINEERING)

SEPTEMBER 2012

Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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Approved by,

_____

(DR NURLIDIA MANSOR)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

Sept 2012

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.

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MOHAMMAD AMIRUL FITRA B SAZALI

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ABSTRACT

Slow Released Fertilizer (SRF) coating is used in agriculture to gradually control the amount of nutrients released from fertilizers. Modified biopolymer is a newly developed technology that widely used as coating material for fertilizers. Studies on degradation impact of the slow release have found to be least studied in literature. To understand this process, the current experiment is designed to study the physical and chemical characterization of biopolymer material degradation and resultant urea release in wet (40% WHC) sterilized and unsterilized soil under different incubation period. Determination of microbial C and N biomass changes in relation to material degradation at different times will also be conducted. Soil is incubated with CRF and samples are collected over 0, 1, 3, 5, 7 weeks. The film samples are analyzed for physical changes using FTIR and FESEM. Spectrophotometer is used to quantify the biopolymer degradation and also urea release in soil to achieve the study objectives. The study will comprehend the suitability of biopolymer based CRF for paddy field soil and under different conditions over a period of times. From the study, it is observed that degradation had occurred and cause the morphology of the coating changed. Besides that, the result also shows the relation between the enzyme activities that is proportional to urea release. This proves that there are correlation between activity of enzyme, degradation of biopolymer and release of urea.

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CHAPTER 1

INTRODUCTION

1.1 Background Study

Fertilizer is any organic or inorganic material that is added to a soil to supply one or more plant nutrients essential to the growth of plants. Nevertheless, not all the nutrients come from fertilizers are absorbed by the plants. According to L. Wu and M. Liu (2008) about 40–70% of nitrogen applied as fertilizers is lost to the environment and cannot be absorbed by plants. However, the invention of slow release fertilizers (SRF) provides the solution to avoid such losses. According to K. Lubkowski and B. Grzmil (2007) mention that an application of the CRFs which release their nutrients in a way better fitting plant's requirements ensures an improved effectiveness of fertilizing through minimizing the losses between application and absorption. Since plants can take up nutrients continuously, it may be beneficial to provide them with a somewhat steady supply throughout their most active periods of growth.

According to the market, most of the coating used in CRF consists of chemical based substance which will eventually produce side effects which is detrimental to the environment. One of the alternatives is to replace chemical based coating of SRF with biopolymer. Biopolymers are polymers produced by living organisms. Biopolymers like starch, cellulose, chitin, and lignin are abundantly available from a variety of plant resources. The characteristic of biopolymer itself that is biodegradable makes it highly consider as substitute for CRF coating. Few modified biopolymers are succesfully used as a coating material for different fertilizers successfully. This includes chitosan [L. Wu and M. Liu(2008)], ethylcellulose [S. Pérez-García et. al(2007)], lignin-starch-cellulose [R. Wuet. al(2009)], and lignin [W. Mulder et. al (2011).

1.2 Problem Statement

The usages of biopolymers as coating are considered highly potential to replace current coating which mainly comes from synthetic materials. However, studies on CRF which analyse the biopolymer coating degradation in a period of time are rare.By understanding the degradation of biopolymer with the presence of certain element in soils, the current technology of CRF could be enhance and optimized. This project is also focuses on the degradation of coating in wet soil which is mainly in use at paddy field. Paddy field applies urea as its major fertilizer and due to its wet and flooding cycles, major losses of urea into different gases creates economic, plant yield and environmental issues.

1.3 Objectives of Study

The objectives of this project:

a) To study material degradation of biopolymer in wet sterilized and unsterilized soil under different incubation period.
b) To determine microbial C and N biomass changes in relation to material degradation at different times
c) To quantify the release of urea in soil from CRF

1.4 Scope of Study

This study involves the physical and chemical analysis for film and soil samples under two different soil treatment (sterilized and unsterilized) over different periods of time. In the study, wet soil is measures through determination of Water Holding Capacity (WHC) at which the value about 30 – 40 % (WHC). The used of sterilized and unsterilized soils are to indicate the effect of presence of microbe in degradation of biopolymer.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbial activity in soil

Soil microorganisms exist in large numbers in the soil and they are one of the factor that leading to degradation of organic matter. According to Jenkinson& Ladd (1981) and Sparling (1992), soil microbial biomass is a living pool containing 1-5% of the soil organic matter excluding root, meso- and macro-fauna. While, another research paper from James J. Hoorman & Rafiq Islam (2010) stated that there are more microbes in a teaspoon of soil than there are people on the earth. Soils contain about 8 to 15 tons of bacteria, fungi, protozoa, nematodes, earthworms, and arthropods. Table 1 below shows the distribution of microbial species in soil.

Table 1 : Relative number and biomass of microbial species at 0–6 inches (0–15 cm) depth of soil [James J. Hoorman&Rafiq Islam (2010)]

Microorganisms	Number/g of soil	Biomass (g/m^2)
Bacteria	10^8–10^9	40–500
Actinomycetes	10^7–10^8	40–500
Fungi	10^5–10^6	100–1500
Algae	10^4–10^5	1–50
Protozoa	10^3–10^4	Varies
Nematodes	10^2–10^3	Varies

Microbes need regular supplies of food in the soil to survive in the soil. Organic matter decomposition serves two functions for the microorganisms, providing energy for growth and supplying carbon for the formation of new cells. According to A Ashwin Kumar et. al. (June 2011), they mention that the breakdown of polymer materials may occur by microbial action, photo degradation, or chemical degradation. All three

methods are classified under biodegradation, as the end products are stable and found in nature. Many biopolymers are designed to be discarded in landfills, composts, or soil. In the other hand, a research paper from Jenkinson& Ladd (1981) mentioned that soil microbial biomass, a living part of soil organic matter, is an agent of transformation for added and native organic matter and acts as a labile reservoir for plant- available nitrogen, phosphorus and sulphur.

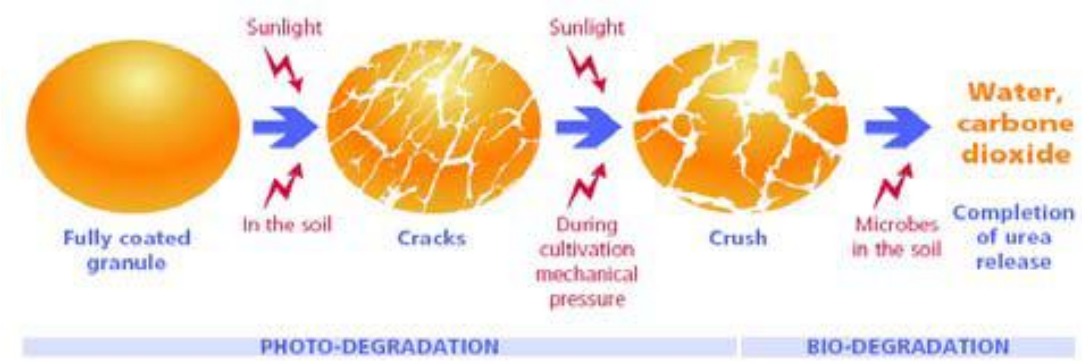


Figure 1 :Typical and critical steps in the process of degradation of polymer coated urea [James J. Hoorman&Rafiq Islam (2010)]

The steps in degradation process are shown above which consist of two parts that occur simultaneously that are photo degradation and biodegradation. Photo degradation is caused by the ray from the sunlight and also mechanical pressure from the soil. Biodegradation is a chemical degradation of polymers due to the action of naturally occurring microorganisms which the produce carbon dioxide and water. These two processes will eventually release the urea presence in the coating to the soil. Basically, microbes are used to transform chemicals, aid pesticide degradation, participate in soil formation and also contribute to soil aggregation.

The breakdown of organic residues by microbes is dependent upon the carbon to nitrogen (C:N) ratio. According to J. J.Hoorman & R. Islam (2010), a low nitrogen content or a wide (C:N) ratio is associated with slow organic matter decay. Immature or young plants have a higher nitrogen content, lower (C:N) ratios and faster organic matter decay. Microorganism populations change rapidly in the soil as organic matter products

are added, consumed, and recycled. The amount, the type, and availability of the organic matter will determine the microbial population and how it evolves.

The analysis for biopolymer coating is done in both non autoclaved and autoclaved soil. This is done to determine the effect of no microbes in the soil towards the degradation of the coating. Autoclaving uses moist heat and pressure to kill soil organisms and typically results in a decrease in microbial biomass and microbially mediated metabolism, such as enzyme activity [Tiwari et al. (1988)]. It is anticipated that there are no or less degradation occur in autoclaved soil.

2.2 Urea

Urea or carbamide is an organic compound with the chemical formula $CO(NH_2)_2$. The molecule has two NH_2 groups joined by a carbonyl (C=O) functional group. According Kesava Rao (1987), most of the applied fertilizer urea in wet agricultural soils is lost by various mechanisms such as surface runoff, leaching, ammonia volatilization and nitrification – denitrification. Urea hydrolyzes in wet soil under the action of urease enzyme into ammonia and carbon dioxide can be described by Equation 1.

$$NH2\text{-}CO\text{-}NH2 + H2O \Longleftrightarrow [\text{urea-urease complex}] \longrightarrow 2NH3 + CO2 \quad (1)$$

This statement is supported by Savant N K et. al. (1987) that concludes mobile urea present in soil solution diffuses and attaches to the immobilized urease enzyme, forming a complex and subsequently decomposing into ammonia and carbon dioxide irreversibly.

2.3Biopolymer Coating

Biopolymers like starch, cellulose, chitin and lignin are abundantly available from a variety of plant resources. These plant materials can be used as additives to enhance the desired properties of coating materials. The mechanism of the biopolymer coating degradation is caused by the enzyme produced by the microorganism. The table below shows the different enzymes for biopolymer degradation in soil.

Table 2 : Different Enzymes for Biopolymer degradation in soil [Z. Majeed et. al. (2012)]

Biopolymer	Hydrolytic Enzymes
Cellulose	Endo-cellulase
	B- Glucosidase
Hemicellulose	Xylanase
Chitin	Endo-chitinase
	N-acetylglucosaminidase
Starch	Amylase
Lignin	Phenoloxidase
	Peroxidase
	Mn-peroxidase

The analysis of biopolymer degradation is done by conducting the analysis of starch in the soil. It is being anticipated that the rate of degradation of starch has a direct relation with the rate of release of urea.

Starch is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by all green plants as an energy store. It is used by the plants themselves, by microbes and by higher organisms therefore there is a great diversity of enzymes that is able to catalyze its hydrolysis. Starch from all plant sources occurs in the form of granules which differ markedly in size and physical characteristics from species to species.

There are several reasons why starch is being considered as the component of coating. Firstly, the enzyme for the degradation of starch can be easily found in the soil. According to Haiyan Sun et. al. (2008), raw starch digesting enzymes (RSDE) refer to enzymes that can act directly on raw starch granules below the gelatinization temperature of starch. RSDE are ubiquitous and produced by plants, animals and microorganisms. Besides that, the cost of raw material of starch is also considerably cheap. According to Avérous L. (2004), Starch-based materials have been of particular interest because of its generally low cost of starch.

According to Rose (1980), starch occurs in the form of water-insoluble granules as the major reserve carbohydrate in all higher plants. Enzymes responsible for the breakdown of starch are widely distributed in nature. Among these are the amylases, which act on starch, glycogen and derived polysaccharides to hydrolyze α - 1, 4-glycosidic linkages. The amylase may thus be divided into three groups: the α -amylases (endoamylases), β-amylases(exo-amylases) and glucoamylases [Ajayi A and Fagade O. E. (2003)]**.**Figure 2 below shows the hydrolysis of starch by α –amylases enzymes.

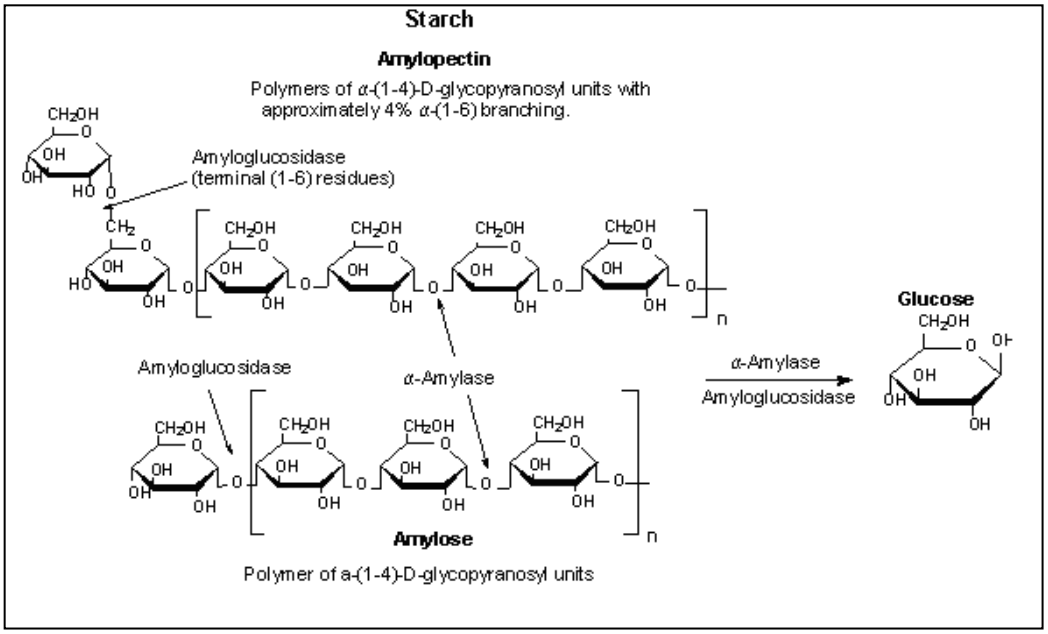


Figure 2 : The hydrolysis of starch to glucose catalyzed by α-amylase

CHAPTER 3

METHODOLOGY

For this project, the overall methodology can be referred as Figure 3 below.

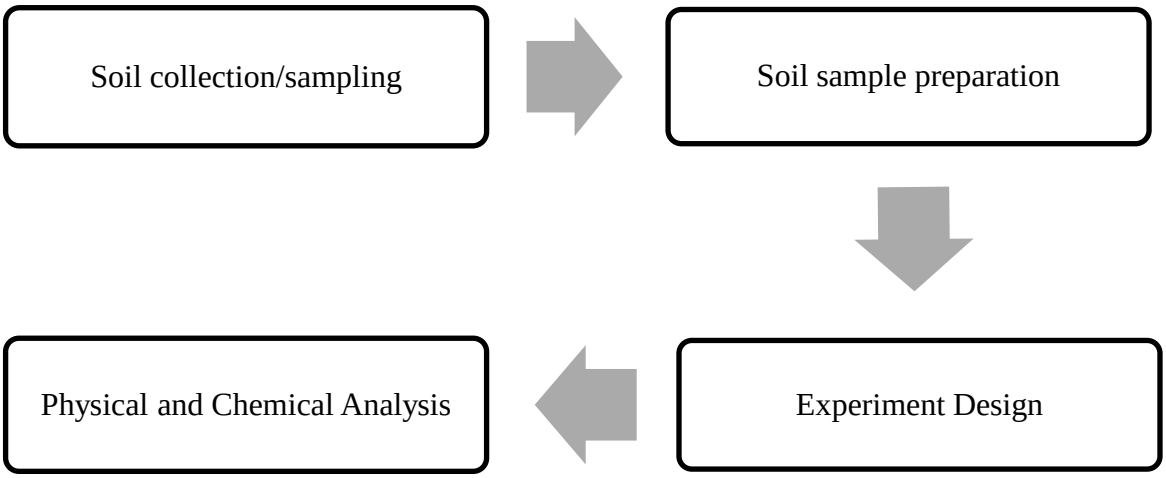


Figure 3 : Methodology for the author's project

3.1 Soil collection/sampling

The first step in doing this project is doing the collection of the soil for sample at paddy field located at Titi Gantung. The quantity of soil sample that needed to be collected is about 4-5 pots and each pots weighing 2-3 kg of soils. The soil for the sample must be taken within 20cm depth as the presences of microbes are at maximum within that depth.

3.2 Soil Sample Preparation

In soil sample preparation, all the samples are air dried until 30-40% moistures content. This is followed by meshing and crushing the soil sample and then seizing it through a 2mm-sized sieve. The samples are then store in a cold room at temperature of 4^{o}C.

3.3 Experiment Design

Before the experiment start, the conditions of the soil need to be maintained. The soil must be kept between30-40% WHC and its pH at 5.3. Any adjustment of the pH is either using 1N Sodium Hydroxide (NaOH) or 1N Sulphuric Acid.

The soils are then put in bottle at which each bottle contains 50g of the sample. Add 5g of biopolymer in each bottle and incubate soil at 25^{o}C for 0 week, 1 week, 3 week, 5 week and 7 week. Each sample runs in duplicate.

All the steps in the experiment are run parallel with autoclaved soil. Autoclaved soil is prepared by maintain the soil in oven at 121^{o}C and pressure at 0.1 kPa for 21 minutes **[**B. H. Anderson and F. R. Magdoff (2005)]. For autoclaved soil, each pot is adding few crystal of Sodium Nitrate.

After the soil sample are prepared and incubated according to the set up time, there are a total of 20 samples need to be analyzed (Table 3). The table shows NA which represents Non Autoclaved soil, A represents Autoclaved soil and R represents replicate.

Table 3 : Total Sample to be analyzed

No	Soil Sample	No	Soil Sample
1	NA/0	11	A/0
2	NA(R)/0	12	A(R)/0
3	NA/1	13	A/1
4	NA(R)/1	14	A(R)/1
5	NA/3	15	A/3
6	NA(R)/3	16	A(R)/3
7	NA/5	17	A/5
8	NA(R)/5	18	A(R)/5
9	NA/7	19	A/7
10	NA(R)/7	20	A(R)/7

3.4 Physical and Chemical Analysis

For each week that been set, the sample is taken for physical and chemical analysis. The details of the physical and chemical analysis conducted are listed below.

A. Physical Analysis

There are two experiments conducted for physical analysis of the sample which are Fourier Transformed Infrared-Attenuated Total Reflectance [RJ Cox PhDa (2000)] and also Field Emission Scanning Electron Microscopy (FESEM).

.

a) Fourier Transformed Infrared-Attenuated Total Reflectance (FTIR-ATR)

Analyses have been performed on the film samples after burial in soil for 7 weeks for both autoclaved and non-autoclaved. Each biopolymer coating was put in a sample holder and infrared spectrum was recorded at the wavelength of 4000 to 650 cm^{-1}.

b) Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) analyses have been performed on the coating film samples after burial in soil for 7 weeks for both autoclaved and non-autoclaved.

B. Chemical Analysis

There are several experiments conducted for chemical analysis of the sample which are Microbial Biomass Carbon and Nitrogen [Carol Grace et. al.(2006)], Urea Estimation [L. A. Douglas and J. M. Bremner (1970)], Starch Estimation [J. E. Hodge and B. T. Hofreiter (1962)] and also Total Amylase Activity Determination [H. Castillo-Michel et. al.(2007)].

a) Microbial Biomass Carbon and Nitrogen

In order to do the analysis of biomass carbon and nitrogen, the sample need to go fumigation and extraction method.

i. **Fumigation**

Place the jars into a desiccator, which should have some moistened tissue paper at the bottom, together with a 25 ml vial of soda lime and a 50 ml beaker containing at least 30 ml $CHCl_3$ and 2-3 anti-bumping granules. Evacuate the desiccator using a water pump or the air pump in room 310 until the $CHCl_3$ is boiling vigorously. Place the desiccator in the 25^{o}C constant temperature room and leave for 24 hours in the dark. After the fumigation, remove the jars and discard the tissue paper. Rinse the desiccator out thoroughly with distilled H_2O and dry. Replace the fumigated jars of soil in the desiccator and evacuate using a water pump for 2 minutes, 3 times, then using an electric vacuum pump, again 3 x 2 minutes. By this time the $CHCl_3$ should be undetectable by smell. Give 2 more 2-minute evacuations with the electric pump. Transfer the soil samples to 350 ml plastic screw-top bottles.

ii. **Extraction**

To the weighed triplicate soil samples in 350 ml plastic screw-top bottles, add 0.5 M K_2SO_4 in a ratio of 4:1 (i.e. 50 g soil is extracted with 200 ml K_2SO_4). Place the bottles upright on a reciprocal shaker set at "90" (approx. 200 strokes min^{-1}), and shake for 30 minutes. Remove the bottles from the shaker and stand them beside the labeled polythene bottles containing funnels and Whatman 42 filter papers. The bottles should be shaken thoroughly, and then allowed to stand so that the precipitate settles out again, before any extract is removed for analysis. Re-freeze the extracts for repeat analyses if required.

After the fumigation extraction had been done, the analysis for biomass carbon and also biomass nitrogen are done using Total Organic Carbon (TOC) Analyzer. All the sample is prepare and put into this analyzer.

b) Urea Determination

Place 10g of soil in a 250 ml bottle and add 100 ml of 2 M KCI-PMA solutions. Shake it on a mechanical shaker for 1 hour and filter the resulting suspension. To determine urea N, pipette an aliquot of the extract contain of urea N into a 50 ml volumetric flask, make the volume to 10 ml with 2M KCI-PMA solution and add 30 ml of color reagent. Swirl the flask and place it in an oven at 120 C. after 30 min, remove the flask, cool it with running water for 15 min, make the contents to 50 ml by adding water. Then transfer about 10 ml of this solution to a Klett-Summerson colorimeter tube and measure its red color intensity. Calculate the urea N content of the extract by reference to calibration graph plotted from the results obtained with standards. To prepare this graph, dilute 10 ml of standard urea N solution to 100 ml with 2 M KCI-PMA solutions in a volumetric flask and mix thoroughly. Then pipette 0, 1, 4 and 7 ml aliquots of this diluted standard solution into 50 ml volumetric flasks. Adjust the volumes to 10 ml by adding 2M KCI-PMA solution and proceed as described for urea N analysis of soil extract.

c) Starch Determination

Homogenize 0.1- 0.5 g of the sample in hot 80% ethanol to remove sugars. Centrifuge and retain the residue. Wash the residue repeatedly with hot 80%ethanol till the washings do not give colour with anthrone reagent. Dry the residue well over a water bath. To the residue add 5.0mL of water and 6.5 mL of 52% perchloric acid. Extract at 0^{o}C for 20 min. Centrifuge and save the supernatant. Repeat the extraction using fresh perchloric acid. Centrifuge and pool the supernatants and make up to 100 mL. Pipette out 0.1 mL of supernatant and make up the volume to 1 mL in each tube with water. Prepare the standards by taking 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard and make up the volume to 1 mL in each tube with water. Add 4 mL of anthrone reagent to each tube. Heat the tubes for eight minutes in a boiling water bath. Cool rapidly and read the intensity of green to dark green colour at 630 nm.

d) Total amylase activity determination

Before proceed to the experiment, the enzyme is extracted from the soil. To assaya-amylase activity, the enzyme extracts were heated for10 min at 70 ^{o}C to inactivate b-amylase and other heat sensitive enzymes. Extracts were centrifuged for 5 min at 4 ^{o}C at14,000 rpm in a refrigerated centrifuge. The reaction mixture was prepared in eppendorf tubes and set up at room temperature (25 ^{o}C). This contained 400 mL of enzyme extract and 700 mL of a 1%starch solution in 2 mM imidazole buffer (pH 7.0). In order to determine the linearity of the reaction with time, a 150 mL aliquot of reaction mixture was withdrawn at defined intervals as described below. The reaction was stopped by adding the 150 mL aliquot into 200 mL cold trichloroacetic acid(TCA) at periods of 0, 20, 40, 60, 80 and 90 min. After centrifuging for 5 min at 14,000 rpm in a refrigerated bench centrifuge,a 30 mL aliquot of the clear supernatant of the stopped reaction was mixed with 300 mL of iodine reagent (0.0075%iodine and 0.075% KI). The absorbance at 660 nm was measured in a spectrophotometer at room temperature approximately 20 min after the iodine-starch blue color was developed. Specific enzyme activity was calculated by making a standard calibration curve ranging from 0.33 to 0.03% starch.

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results of the study will be reported and discussed. There are total 6 analysis had been done to characterize the soil and the coating to determine the degradation of the biopolymer SRF.

4.1 Fourier Transformed Infrared-Attenuated Total Reflectance (FTIR-ATR)

FTIR is used to determine the information regarding the structure and the presence of functional groups in a molecule interest. The infrared (IR) spectrums of biopolymer coating are shown in figure 4 and figure 5.From the FTIR analysis for biopolymer coating, there are four major peaks identified in both non autoclaved soil and autoclaved soil. This indicates that both coatings have the same composition in terms of materials. For both non autoclaved soil and autoclaved soil, the first peak that could be observed are the absorption occurring at 3300.69 $cm^{-1}$and 3314.68 cm^{-1} which indicates the existence of free NH groups, that was probably attributed to urea that is present in the coating. The bands appearing at 2136.36 cm^{-1} and 2118.88 cm^{-1} are attributed to the formation of C=C stretching functional group which might be due to the presence of a long chained starch. Besides that, there are also absorption that occurring at 1634.99 cm^{-1}that represents a highly conjugated C=O stretching vibration and indicates the presence of urea which contains the C=O functional groups. The additional peaks at 1021.99 cm^{-1}indicate the presence of C-O-C vibrations in esters, which shows the characteristics of the starch. Overall, FTIR analysis of this spectra showed that the coating consist of two main structures which are urea and starch.

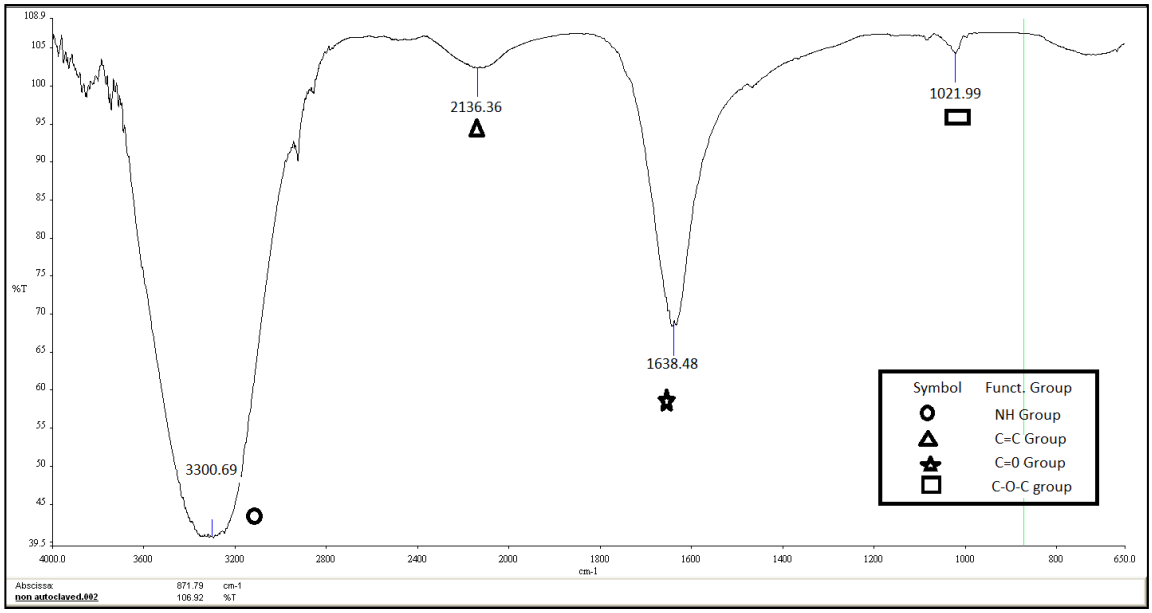


Figure 4 : FTIR Spectrum of Biopolymer Coating in Non Autoclaved Soil

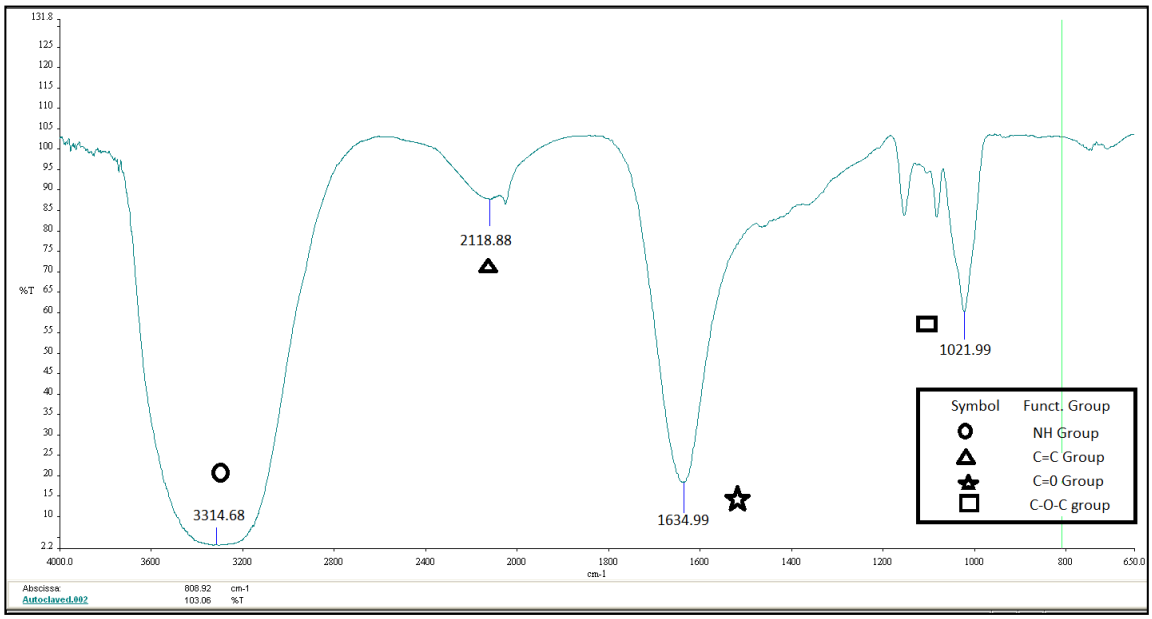
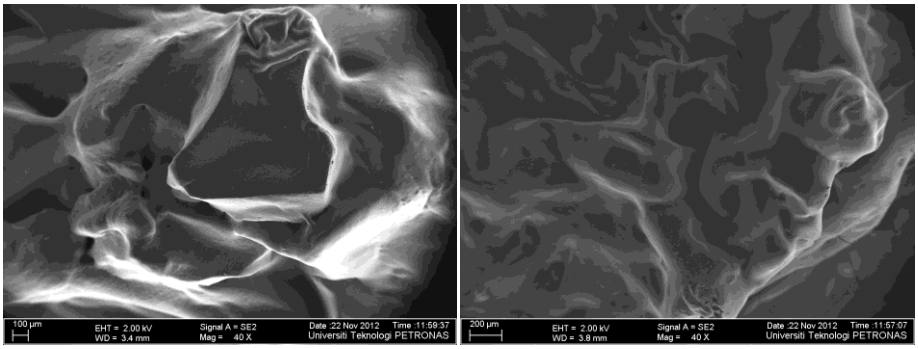


Figure 5 : FTIR Spectrum of Biopolymer Coating in Autoclaved Soil

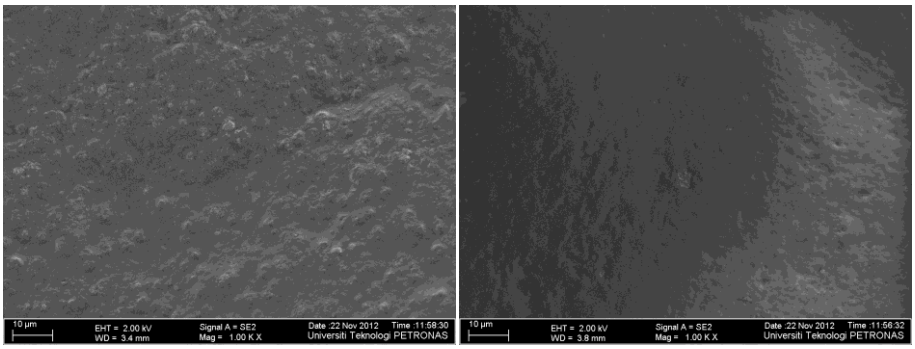
4.2 Field Emission Scanning Electron Microscopy (FESEM)

The morphological characteristic of biopolymer coating in both non autoclaved and autoclaved soil after 7 weeks burial was observed by using Field emission electron microscope (FESEM).



(a) (b)

Figure 6 : FESEM Photograph at 40x of (a) non autoclaved coating (b) autoclaved coating



(c) (d)

Figure 7 : FESEM Photograph at 1000x of (c) non autoclaved coating (d) autoclaved coating

The difference between both coatings could be easily detected at magnification 1000x as the surface of the biopolymer coating in non autoclaved soil is rougher and contain more pores compared to autoclaved soil. On the other hand, FESEM analysis of the coating in autoclaved soil exhibits much clearer surface with small amount of pores. The reason behind high porous and rough surface in non autoclaved soil is due to the structure of the coating that has been degraded and was destroyed by microorganisms.

4.3 Microbial Biomass Carbon and Nitrogen

The characterization for microbial biomass carbon and nitrogen is done by using fumigation extraction method. and then analyzed by Total Organic Carbon. (TOC). The results show the difference in TOC in the extracted solutions from the fumigated and the non fumigated samples which the content in fumigated soil is higher than non fumigated soil. The fumigation method is based on the assumption that the increased amount of organic C extracted from a sample relative to a control is due entirely from cell lyses caused by chloroform fumigation. The C from organisms that were killed and lysed during the fumigation process is readily mineralized to CO2, so that the difference in CO2 gas evolution between fumigated and non fumigated samples is a measure of the biomass C [Smith et al. (1995)].

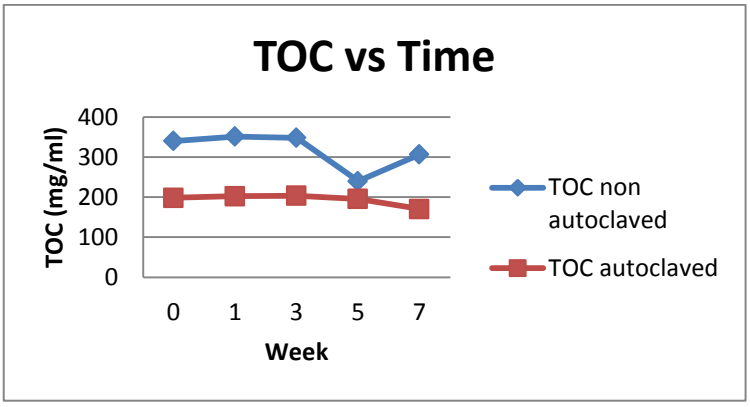


Figure 8 : Total Organic Carbon versus time

From figure 8, it could be observed that the content of microbial carbon in non autoclaved soil is slightly increases from week 0 until week 3 and then starts to decrease. The reason behind this increasing trend is due to microbial carbon growth throughout the week 0 to week 3. The growth of microbes is because of the degradation of the coating which gives the nutrient for the microbes' growth. The microbial carbon starts to reduce from week 5 and week 7. This is expected due to the content of starch which is reduced by that week due to degradation. In non autoclaved soil, it is found that there is not much difference for microbial carbon in autoclaved soil and it varies from 190 to 200 mg/ml. This trend shows that there are still left microbes in the soil even after autoclaving the soil.

4.4 Urea Estimation

For urea estimation, the analysis was done by extraction using KCI-PMA solutions and also color reagents. The extracts which were at observed to have red color intensity were then put through the spectrophotometer test to measure its red color intensity. The spectrophotometer result shows peak at about 525 nm which is supported by R. L Mulvaney (1979) that stated the presence of urea could be observed at wavelength around 500 nm – 550nm. In order to quantify of the amount of urea in the soils, the standards are prepared and performed the spectrophotometer test. The results of the test are as show in the table 4 and figure 9.The concentration shows the values in microgram of urea (μg).

Table 4: The absorbance and concentration of the standards for urea

Standard Table

	Sample ID	Type	Ex	Conc	WL527.0	Wgt.Factor	
1	std0	Standard		0.000	-0.021	1.000	
2	std40	Standard		40.000	0.173	1.000	
3	std70	Standard		70.000	0.285	1.000	
4	std100	Standard		100.000	0.377	1.000	
5							

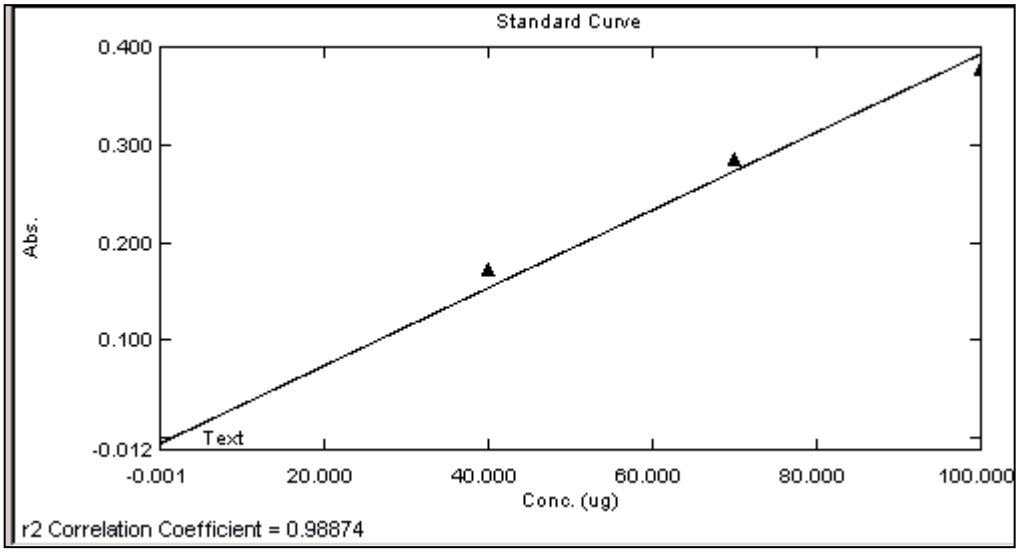


Figure 9 : Standard curves of urea for absorbance versus concentration

The quantification of urea in soil samples is then achieved by interpolates the absorbance of the soil samples with the standard that have been prepared. The interpolation is done by using the equation of a straight line. The formula for straight line equation is:

$y = mx + c$,

Where y is the value of absorbance, m is the value for gradient of standard curve, x is the concentration of urea and c is the value for y intercept of the standard curve. The data of the absorbance is used to compute the equation and obtain the concentration of urea in the soil. The graph of quantity of urea against the week incubated is then plotted.

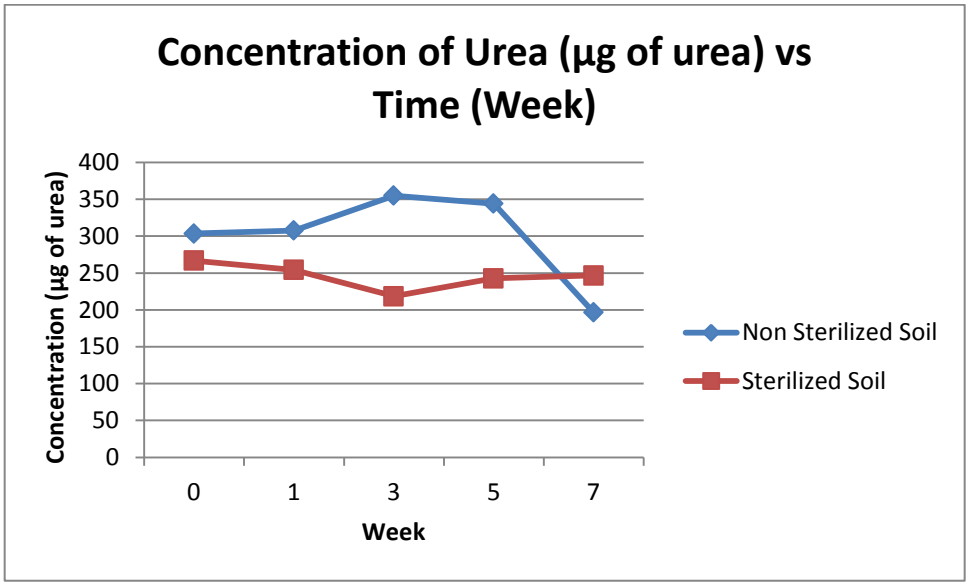


Figure 10 : Concentration of urea in both non autoclaved soil and autoclaved soil

Figure 10 shows the urea concentration in both non autoclaved soil and autoclaved soil. The concentration of urea for each week is calculated from the average urea contents of the original and replicate of soil sample that have been prepared. For the non autoclaved soil, it shows that there is an increase in concentration at week 3 which was maintained until week 5. The concentration then decrease sharply below its initial

concentration at 200 μg at week 7. The urea is observed to increase for the first three weeks due to growth of the microbial which lead to enzyme activity that degrade the coating and release the urea. This follows the literature review that stated one of the reasons for the coating to degrade is the microbial activity. However, the urea quantity in the soils starts to decrease after week 3 and it might occur due to two reasons. Firstly, the urea contents inside the coating itself is reduced over period of time. Secondly, the ureas in the soil samples have been broken down to become ammonia. This statement is supported by Savant N K et. al (1987) which stated that mobile urea present in wet soil diffuses and attaches to the immobilized urease enzyme, forming a complex and subsequently decomposing into ammonia and carbon dioxide.

In the autoclaved soil, the concentration of urea only varies slightly between 220 to 270 μg of urea per gram soil. In this sample, there should not be any presence of microbes which is one of the major factors of degradation. However, the degradation may still occur due to mechanical pressure from the soil and this explains why there is still urea present in the soil samples. Besides that, the urea also does not reduce as there should be no hydrolysis of urea due to the microbes being sterilized. This is supported by M. A. Tabatabai and J. M. Bremner (1972) which found that there is no hydrolysis of urea was detected in autoclaved soils. The difference between concentrations trends of autoclaved and non autoclaved soils show the influence of microbes towards urea concentration.

4.5 Starch Determination

The determination of starch is carried out by conducting the extraction of starch from the soil and measuring its concentration through spectrophotometer. All of the soil extracts are observed to be in dark green colour and thus prove presence of the starch. This is because the starch is hydrolyzed to glucose and dehydrated to hydroxymethyl furfural with exraction using hot ethanol. This compound reacts with anthrone reagent and forms a green coloured product. The soil extract for starch experiment is then analyzed by using spectrophotometer to measure its absorbance at wavelength 630nm. The concentration of starch is then measured by interpolate the absorbance value into the standard curve that have been prepared.

Table 5 : The absorbance and concentration of the standards for starch

Standard Table - (Active)

	Sample ID	Type	Ex	Conc	WL630.0	Wgt.Factor
1	0ppm	Standard		0.000	0.004	50.000
2	312.5ppm	Standard		312.500	0.021	50.000
3	625ppm	Standard		625.000	0.063	50.000
4	1250ppm	Standard		1250.000	0.102	50.000
5	2500ppm	Standard		2500.000	0.283	50.000
6	5000ppm	Standard		5000.000	0.570	50.000
7	10000ppm	Standard		10000.000	1.239	50.000

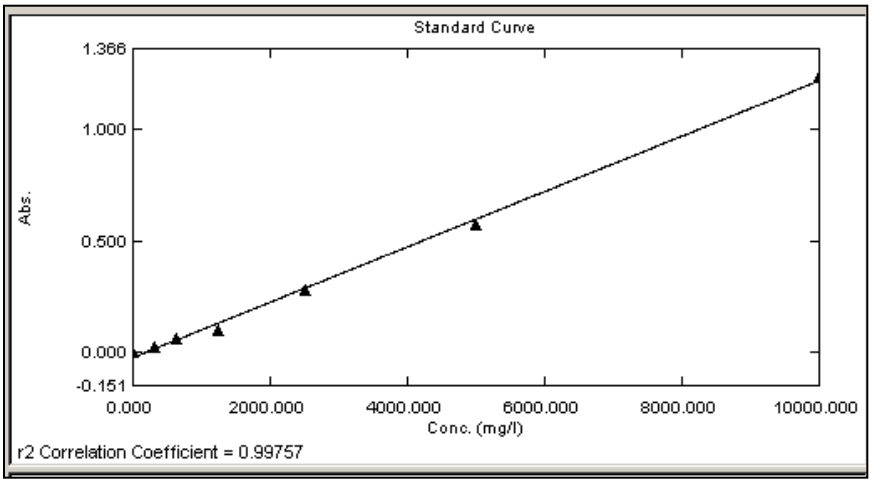


Figure 11 : Standard curves of starch for absorbance versus concentration

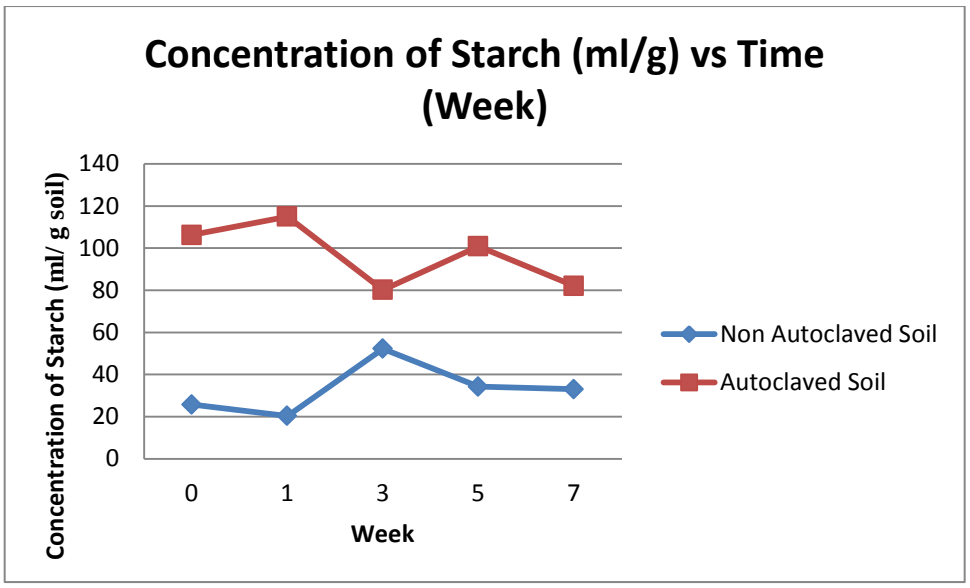


Figure 12 : Concentration of starch in both non autoclaved and autoclaved soil

Figure 12 shows the concentration of starch in both non autoclaved soil and autoclaved soil. From the graph, it could be observed that the starch contents in autoclaved soil are higher than in the non autoclaved soil. The reason behind this observation is due to the degradation process that occurs in non autoclaved and causes the starch to hydrolyze into smaller particles. The degradation is anticipated to occur less in autoclaved soil as the number of microbes in the soil is negligible once the soil has been sterilized. The microbes produce the enzymes which lead to the degradation of the coating and this is refer to work by A Ashwin Kumar et. al. (2011) who mentions that factor affecting the breakdown of polymer materials is microbial action.

From the graph, it can also could be observed that the trend for starch for non autoclaved increases until it reach concentration of 52 ml/ g soil on week 3 before reducing. The trends however show an unexpected results where the starch concentration increased throughout the time. The difference might due to the error in doing the characterization for week 0 and week 1. According to the method, the characterization of starch is done after repetitive dilution to the soil extract. The dilution will cause the extract to become more sensitive and even a slight error would affect the result. The error could also be observed in autoclaved soil on week 3. Therefore, extra handling needs to be done when performing the experiment. The recommendation that can be applied is by minimizing the loss of soil throughout the experiment.

4.6 Total Amylase Activity Determination

Total amylase activity was assayed based on Fuwa's colorimetric method of iodine-starch color reaction. All of the soil extract for this experiment is observed to be blue in colour and then analyzed by using spectrophotometer to measure its absorbance at wavelength 660nm. The extracted soil shows blue color characteristics due to the presence of starch which reacts with the iodine. The total amylase activity is then measured by interpolate the absorbance value into the standard curve that have been prepared.

Table 6 : The absorbance and concentration of the standards for enzyme activity

Standard Table

	Sample ID	Type	Ex	Conc	WL539.0	Wgt.Factor
1	0..06mg/ml	Standard		0.060	0.530	1.000
2	0.03mg/ml	Standard		0.030	0.173	1.000
3	0.015mg/ml	Standard		0.015	0.002	1.000
4	0.0075mg/ml	Standard		0.008	-0.077	1.000
5	0.12mg/ml	Standard		0.120	1.269	1.000
6	0.25mg/ml	Standard		0.250	2.883	1.000
7	0.00mg/ml	Standard		0.000	0.004	1.000
8	0.5mg/ml	Standard		0.500	5.000	1.000

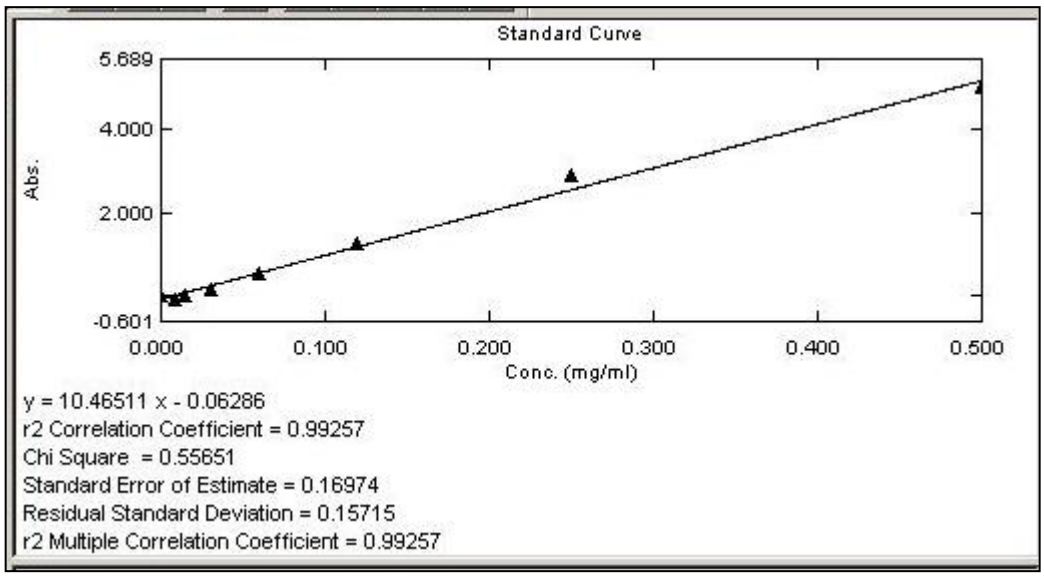


Figure 13 : Standard curves of enzyme activity for absorbance versus concentration

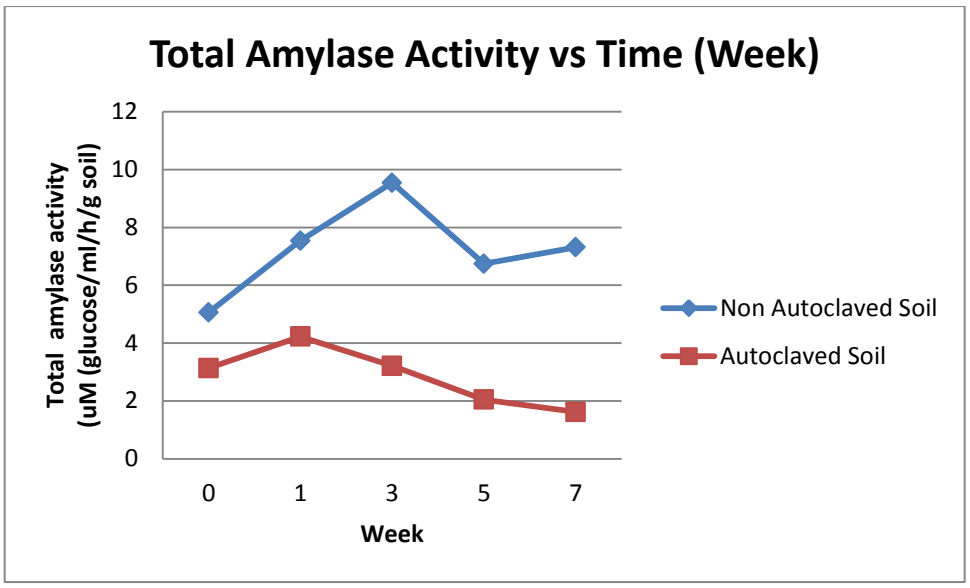


Figure 14 : Total Amylase Activity content in both non autoclaved and autoclaved soil

Figure 14 shows the graph for total amylase activity for both non autoclaved and autoclaved soil. From the calibration curves for non autoclaved soil, it can be observed that the enzyme activity increase until it reaches the peak at week 3 and then start to decrease. This trend can be roughly estimated to be similar as the curve for urea concentration in non autoclaved soil (Figure 10). This shows the relation between the enzyme activity and urea release which is proportional to each other. The enzyme activity increases for the first three weeks is expected due to the growth of the microbes which releases the enzyme for degradation. However from week 5 onwards, the enzyme activity started to decrease and it is due to the starch content in the coating had been reduced through time. This statement is supported by Skrabanja, V. and Tufvesson, F. (2000) that stated starch concentration has a direct effect on the enzymatic rate of amylase. As starch percentage was decreases enzymatic rate decreases.

For autoclaved soil, it could be observed that the total amylase activity varies from 2-4 µM glucose/ml/h/g soil. This trend shows small amounts of amylase activity as expected as there are no microbes in the non autoclaved soil.

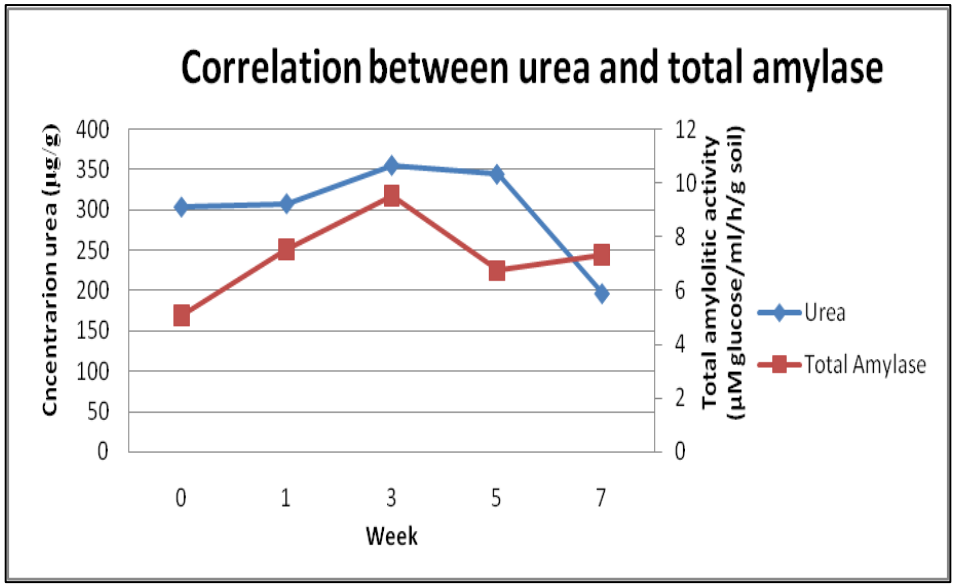


Figure 15 : Comparison for urea concentration and enzyme activity in non autoclaved soil

Figure 15 show the comparison between urea concentration and also enzyme activity and its exhibit correlation between activity of enzyme and release of urea. From the graph, it could be concluded that trend for enzyme activity can be roughly estimated to be similar as the curve for urea concentration in non-autoclaved soil. As the enzyme activity increase, more urea is release to the soil.

CHAPTER 5

CONCLUSION

The potential of biopolymer as coating in Slow Release Fertilizer is really needed for a better efficiency of nutrient release from fertilizer to the plant. The study of biopolymer degradation in wet soil through the physical and chemical analysis could lead to optimization of SRF in the future. The result that the author produces shows a positive physical degradation that occurs on the coating through morphology analysis by FESEM. Through FTIR characterization, the author had defined the component of the coating that is mainly from urea and also starch. Besides that, the results analyze from microbes, urea, starch and enzyme activity determination show correlation between activity of enzyme from microbes, degradation of biopolymer and release of urea. The project shows the relation between the enzyme activities that is proportional to urea release.

There are some recommendations that can be done to improve the effectiveness of the results. One of the recommendations for this experiment is that the entire sample must be done in one batch in order to avoid time delay effect in the result. Besides that, the soil samples need to be mixed thoroughly with soil samples before being analyzed in order to make sure soils are homogenized. The color reagent that is needed for characterization of urea, starch and also enzyme activity need to be prepared fresh before use as it will affect the results that need to be achieved.

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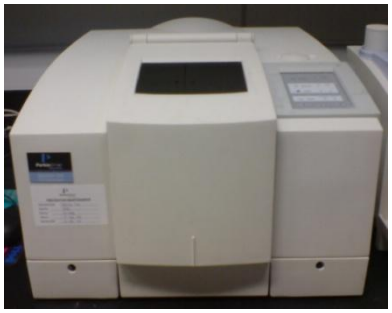
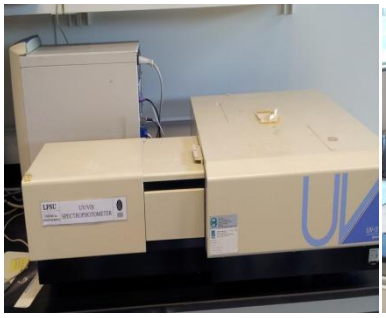
APPENDICES

Appendix 1 : Equipment used for characterization of the soil



FTIR

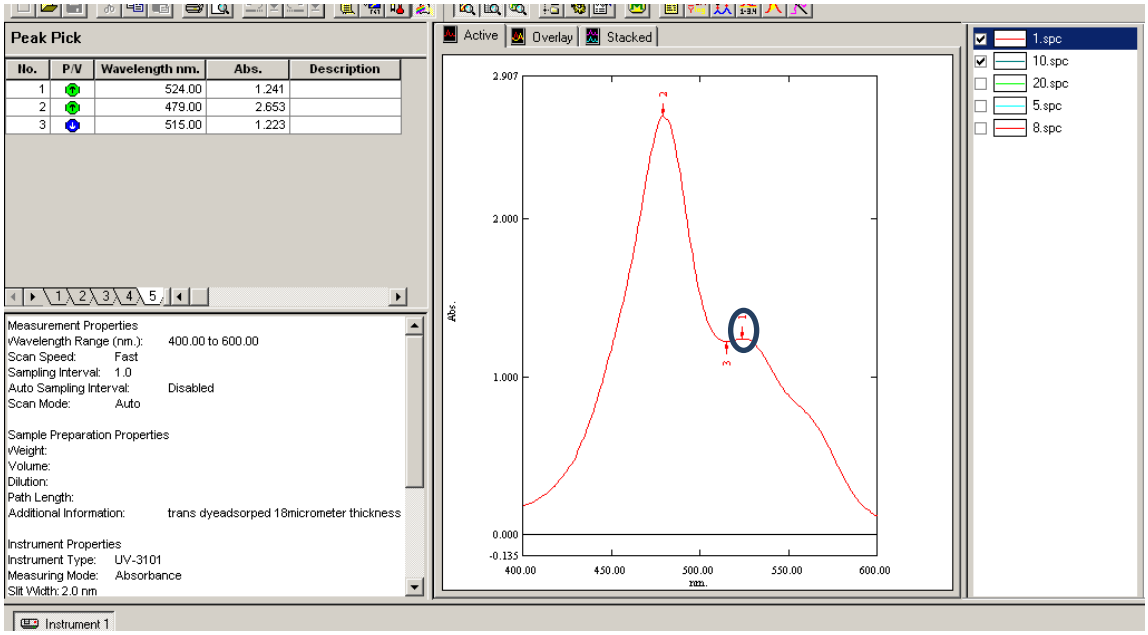
FESEM



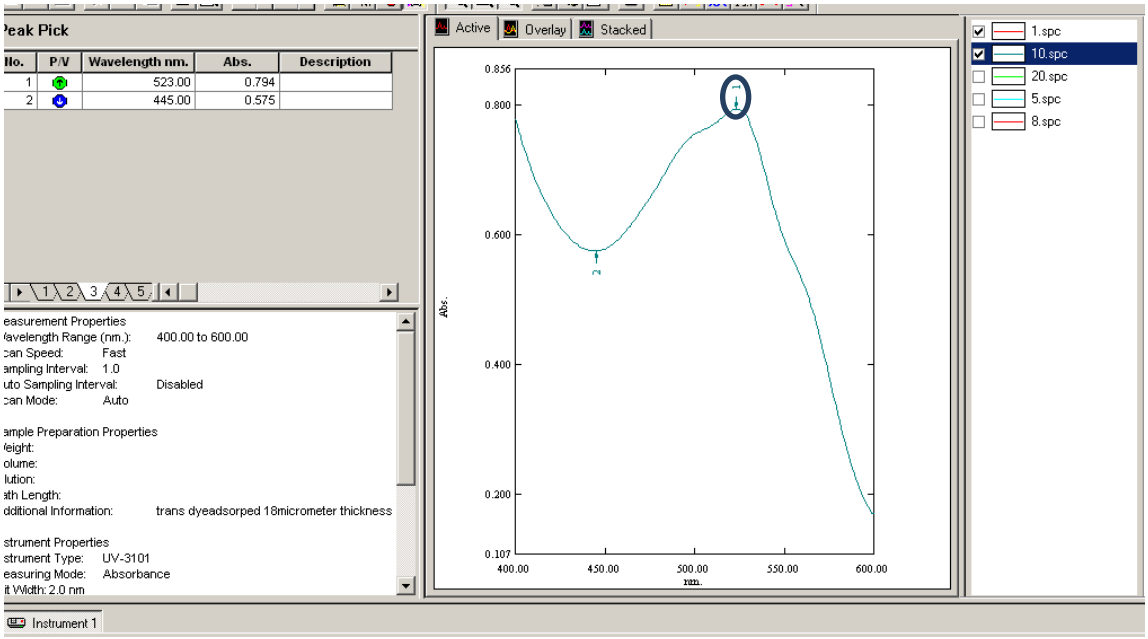
UV VIS Spectrophotometer

TOC

Appendix 2 : Sample result for urea determination using spectrophotometer



Spectrophotometer of urea determination result for week 0 non autoclaved soils



Spectrophotometer of urea determination result for week 7 non autoclaved soils