

University of Nottingham
UK | CHINA | MALAYSIA

DEVELOPMENT OF POLYCAPROLACTONE BASED CONTROLLED RELEASE FERTILIZER FOR MUNG BEAN CROP

HARSHINI PAKALAPATI, MSc

Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy

APRIL 2019

ABSTRACT

Pulses are a major source of protein, rich in vital elements supplying a substantial part of the calorie and nutrients. To ensure wholesome gram cultivation, nutrients are supplied to the soil for plant uptake. However, excessive release of nutrients leads to several environmental issues like depletion of soil quality, biological imbalance in water bodies and climate change. Thus, proper nutrient management is essential for a healthy crop in terms of both quality and quantity. This can be achieved through control release of nutrients by encapsulating the fertilizers within polymers. Therefore, this work focuses on the supply of essential nutrients to mung bean crop encapsulated within biodegradable polymers. Further, the effect of biochar dozed along with encapsulated system is investigated.

In this current research, initially the pot trials were carried out formulating with different levels of biochar and nutrients, results showed improved growth characteristics and crop yield when biochar of 20kg/ha and NPK of 30kg/ha was applied. Later, polycaprolactone (PCL) was synthesised using *Candida antarctica* lipase B (CALB) as catalyst, using design of experiments (DOE). Optimised parameters are found to be - time - 1hr, RPM - 500 rpm, temperature - 75°C and monomer/solvent ratio to 1:2, therefore successfully implementing D optimal design from DOE for optimisation of process parameters for PCL synthesis. Further, PCL was targeted to develop controlled release fertilizer (CRF) due to its biodegradability and non- toxic nature. Subsequently, two different techniques have been used to prepare control release fertilizers – solvent casting and blending techniques. When analysed with nutrient release properties and their effect on mung bean growth, blending technique is considered to be better. Since blending is easy to handle involving no solvent and potential scalable technique to prepare PCL based CRF blends. Out of the three-concentration used, 25% (i.e. blend with PCL/starch ratio is 5:1) was recorded to be less porous with significant controlled release of fertilizers. Moreover, in

final plant trials, PCL blended CRF had a notable effect on the growth and yield of the mung bean with 39% more yield (i.e. total seed weight) compared with non- PCL blended fertilizer (normal NPK fertilizer). Thus, PCL based controlled release fertilizers are developed which helps to maintain a sustainable cultivation system.

Key words: Polycaprolactone, Controlled release fertilizer, mung bean, biodegradable polymer, Design of experiment, D – optimal.

LIST OF PUBLICATIONS

Journals

- H Pakalapati, S K Arumugasamy, J Jewaratnam, Y.J Wong and M Khalid. Parametric optimization of polycaprolactone synthesis catalysed by Candida Antarctica lipase using response surface methodology. *Biopolymers, 2018.*
- H Pakalapati, M A Tariq and S K Arumugasamy. Optimization and modelling of enzymatic polymerization of ε- caprolactone to polycaprolactone using Candida Antarctica Lipase B with response surface methodology and artificial neural network. *Enzyme and Microbial Technology, 2018*
- H Pakalapati, Chih-Kai Chang, Pau Loke Show, S K Arumugasamy and John Chi-Wei La. Development of polyhydroxyalkanoates production from waste feedstocks and application. *Journal of Bioscience and Bioengineering, 2018*
- H Pakalapati, S K Arumugasamy and M Khalid. Comparison of response surface methodology and feedforward neural network modeling for polycaprolactone synthesis using enzymatic polymerization. *Biocatalysis and Agricultural Biotechnology, 2018.*
- H Pakalapati, A B Balaji, S K Arumugasamy and M Khalid. Optimisation of process parameters using D-optimal for enzymatic synthesis of polycaprolactone. *Polymer Bulletin, 2017.*

Book Chapter

- A B Balaji, H Pakalapati, M Khalid, R Walvekar and H Siddiqui. Natural and synthetic biocompatible and biodegradable polymers. *Biodegradable and Biocompatible Polymer Composites 2017*

Conference proceedings

- H Pakalapati, S K Arumugasamy and M Khalid. Development of Optimality Criterion for the Enzyme Catalysed Bio-polymerization" at the *International Conference on Chemical and Biochemical Engineering 2018, Germany.*
- H Pakalapati, S K Arumugasamy, M Khalid, J Jewaratnam and Y J Wong (2017*).* Polycaprolactone synthesis via enzymatic catalysis using response surface methodology, *30th Symposium of Malaysian Chemical Engineers 2017, Malaysia*
- H Pakalapati, F Fatihah, M Musa, A Singh, M Khalid and S K Arumugasamy. Influence of Biochar Type and Phosphorus on the yield of Green Gram (Vigna Radiata) in Tropical Sub- Humid Areas of Malaysia. *4th Postgraduate Colloquium for Environmental Research 2017, Malaysia*

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all my supervisor's Dr. Senthil Kumar Arumugasamy, Prof. Mohammad Khalid Siddiqui and Dr. Ajit Singh for all their insights and guidance throughout my PhD journey. I am very thankful for this opportunity given to me to pursue this project under their guidance. I would also like to thank Dr. Jega Lakshmi from the University of Malaya for giving me the opportunity to work under her guidance in established UM laboratory.

Many thanks to all the technical staff of faculty of engineering, especially Ms. Filzah, Ms. Khairani, Mr. Fareez, and Ms. Fatihah for all the help and continuous support.

I am extremely grateful to my dear parents P. Ramana Murthy Raju and P. Uma Devi and dear sister Sreedevi for their support and understanding throughout this journey. I thank them for believing me in all of my pursuits and inspiring me to follow my dream.

Finally, to all my friends and colleagues, thank you for listening, offering me advice, and supporting me through this entire process.

TABLE OF CONTENTS

ABSTRACT i

LIST OF PUBLICATIONS iii

ACKNOWLEDGEMENT iv

TABLE OF CONTENTS v

LIST OF FIGURES x

LIST OF TABLES xv

LIST OF EQUATIONS xvii

LIST OF ABBREVIATIONS xviii

CHAPTER 1 1

INTRODUCTION 1

1.1 Research background 1

1.2 Problem statement 3

1.3 Scope and outline of the research 4

1.4 Aim and objectives: 5

1.5 Thesis outline 6

1.6 Significance of research 8

CHAPTER 2 9

LITERATURE REVIEW 9

2.1 Introduction: 9

2.2 Biodegradable polymers 9

2.3 Synthesis of polycaprolactone 11

2.3.1 Different methods used for enzymatic polymerization of PCL 13

2.3.2 Different enzymes used to synthesize Polycaprolactone 15

2.3.3 Factors affecting enzymatic polymerisation of PCL 19

2.4 Fertilizers and importance of Control release 24
2.4.1 Influence of macro and micronutrients on mung bean 24
2.4.2 Influence of biochar on mung bean 26
2.5 Control release of fertilizers 28
2.5.1 Materials used for control release of fertilizers 29
2.6 Summary 35
CHAPTER 3 36
METHODOLOGY 36
3.1 Materials 36
3.1.1 Materials used for PCL synthesis 36
3.1.2 Materials used for pot trials 37
3.1.3 Materials used for preparation of PCL based CRF's 38
3.2 Preparation of PCL via enzyme catalysis 38
3.2.1 Design of experiments 38
3.2.2 Process for PCL synthesis using enzymes 41
3.2.3 Characterization for synthesised PCL 44
3.2.3.3 Fourier Transform Infrared Spectroscopy (FTIR) 44
3.2.3.4 Differential scanning calorimetry 45
3.2.3.5 Thermogravimetric Analysis 45
3.3 Pot trails 45
3.3.1 Pots preparation for optimum formulation of fertilizer and biochar 45
3.3.2 Pot trails for control release of PCL fertilizers 49
3.3.3 Data collection and analysis 50
3.4 Sample preparation for control release of fertilizer using PCL 52
3.4.1 Solvent casting technique 53
3.4.2 Melt mixing 54
3.4.3 Characterisations 55

CHAPTER 4 56

INFLUENCE OF NPK, FE AND BIOCHAR ON THE GROWTH CHARACTERISTICS OF MUNG BEAN 56

4.1 Introduction 56

4.2 Results and Discussion 57

4.2.1 Plant height, Number of leaves, Chlorophyll content, Anthocyanin content and leaf area 57

4.2.2 Number of pods plant^{-1}, pod length, pod weight, seeds pod^{-1}, seed weight, total dry weight and harvest index 63

4.2.3 Root length, root area, root volume, root diameter and nodules 66

4.3 Conclusion 74

CHAPTER 5 75

OPTIMISATION OF PROCESS PARAMETERS FOR PCL SYNTHESIS VIA ENZYMATIC POLYMERIZATION 75

5.1 Introduction 75

5.2 Design of experiments 76

5.2.1 Modelling using ANOVA 76

5.2.2 Response surface plots 86

5.2.3 Validation of the developed experimental model 90

5.3 Synthesis of PCL using *Thermomyces lanuginosus* lipase 94

5.3.1 Structural characterisation of the synthesised PCL 94

5.3.2 Effect of different reaction media on molecular weights: 98

5.3.3 Effect of enzyme concentration: 99

5.3.4 Thermal properties of synthesized PCL catalysed by CALB and TLL: 100

5.4 Summary 103

CHAPTER 6 105

PREPARATION OF PCL BASED CRF USING SOLVENT CASTING TECHNIQUE AND ITS INFLUENCE ON GROWTH AND YIELD OF MUNGBEAN 105

6.1 Introduction 105
6.2 Morphology and porosity 106
6.3 Biodegradation 112
6.4 Nutrient release 116
6.5 Influence of CRF on the growth and yield of mung bean 124
6.5.1 Plant height, Number of leaves, Chlorophyll content and Anthocyanin content 124
6.5.2 Leaf Area and dry weight 128
6.5.3 Number of pods plant^{-1}, pod length, pod weight, seeds pod^{-1} and seed weight 129
6.5.4 Root length, root area, root volume, root diameter and nodules 130
6.6 Summary: 134
CHAPTER 7 135
PREPARATION OF PCL BASED CRF USING BLENDING TECHNIQUE AND ITS INFLUENCE ON GROWTH AND YIELD OF MUNGBEAN 135
7.1 Introduction 135
7.2 Surface morphology and porosity 136
7.3 Biodegradation study 138
7.4 Nutrient release 141
7.5 Influence of Control release fertilizer on mung bean 145
7.5.1 Plant height, Number of leaves, Chlorophyll content and Anthocyanin content 146
7.5.2 Leaf Area and dry weight 150
7.5.3 Number of pods plant^{-1}, pod length, pod weight, seeds pod^{-1} and seed weight 152
7.5.4 Root length, root area, root volume, root diameter and nodules 154
7.6 Summary 156
CHAPTER 8 157
CONCLUSIONS AND 157
RECOMMENDATIONS FOR FUTURE STUDIES 157

8.1 Principal Findings 157
8.2 Limitation 159
8.3 Future studies 159
REFERENCES 161
APPENDIX 181

LIST OF FIGURES

Figure 1-1: Schematic representation of research outline.....4

Figure 2-1 : Different types of polymers (Jha et al., 2019).....10

Figure 2-2 Schematic representation of enzymatic ring opening polymerisation 15

Figure 2-3 : Mechanism of nutrient release from polymer coated fertilizer.....29

Figure 3-1: Schematic flow chart of PCL synthesis 38

Figure 3-2 : Schematic flow chart for methodology of PCL synthesis 43

Figure 3-3:The plot layout of the experiment placed based on randomization at 1st week after sowing in the Shed house of The University of Nottingham Malaysia Campus. 47

Figure 3-4: The plot layout of the experiment placed based on randomization at 3rd week after sowing in the Shed house of The University of Nottingham Malaysia Campus.....48

Figure 3-5:The plot layout of the experiment placed based on randomization at 6th week after sowing in the Shed house of The University of Nottingham Malaysia Campus.....48

Figure 3-6:The plot layout of the experiment placed based on randomization at 9th week after sowing in the Shed house of The University of Nottingham Malaysia Campus.....49

Figure 3-7: Schematic flow chart for methodology of application of PCL based CRF's 52

Figure 3-8 Procedure for preparing PCL based CRF's using solvent casting technique 54

Figure 4-1 Effect of Biochar and NPK on plant height and leaf number of mung bean. 58

Figure 4-2 Effect of biochar and NPK on chlorophyll and anthocyanin content 61

Figure 4-3 Effect of NPK and biochar on leaf area of mung bean. p stands for significance 63

Figure 4-4 : Effect of biochar and NPK on nodules of mung bean. Within a treatment group, bars with the same letters are not significantly different using Tukey's test at 5% level. SE bars are standard deviation values. p stands for significance; a& b are subsets indicating the significant difference between each level. 68

Figure 4-5 Effect of NPK and biochar on root volume and root diameter 69

Figure 4-6 : Effect of biochar and NPK on root parameters of mung bean. 70

Figure 4-7 : Plant roots with different treatments (B0- No Biochar, F0- No fertilizer, I0 – No iron, I15 – 15 kg/ha, F1- NPK at 15 kg/ha, F2 – NPK at 30 kg/ha) 71

Figure 4-8 : (B1- Biochar at 10 kg/ha, F0- No fertilizer, I0 – No iron, I15 – 15 kg/ha, F1- NPK at 15 kg/ha, F2 – NPK at 30 kg/ha) 72

Figure 4-9 : (B2 – Biochar at 15 kg/ha, F0- No fertilizer, I0 – No iron, I15 – 15 kg/ha, F1- NPK at 15 kg/ha, F2 – NPK at 30 kg/ha) 73

Figure 5-1: a) Normal plot for M_n b) Normal plot for M_w 83

Figure 5-2 : a) Actual vs predicted plot for M_n b) Actual vs predicted plot for M_w 84

Figure 5-3 : a) Studentized residual plot for Mn b) Studentized residual plot for Mw 85

Figure 5-4 : Response surface plots for number average molecular weight (M_n) 88

Figure 5-5 : Response surface plots for weight average molecular weights (M_w) 89

Figure 5-6 NMR spectra for PCL synthesised using (a) CALB (b) TLL 92

Figure 5-7 NMR spectra for PCL synthesised using (a) CALB (b) TLL 93

Figure 5-8 Ring opening polymerization of caprolactone catalysed by TLL for 96hrs. 97

Figure 5-9: Effect of solvent on the Molecular weight of the PCL catalysed by TLL 99

Figure 5-10: Effect of enzyme concentration on the molecular weight of the PCL catalysed by TLL 100

Figure 5-11 : DSC graph for synthesised PCL (using CALB and TLL) and Neat PCL 101

Figure 5-12 : TGA graph for synthesised PCL (using CALB and TLL) and Neat PCL 102

Figure 6-1 : (a) PCL-CRF prepared by Solvent casting technique (b)PCL - CRF packed in a mesh bag for degradation studies 106

Figure 6-2 : (a) PCL/starch-CRF prepared by Solvent casting technique (b)PCL/starch - CRF packed in a mesh bag for degradation studies 107

Figure 6-3 : a) NPK before coating b) NPK with PCL coating c) NPK with PCL/starch coating d) Surface morphology of PCL coating e) Surface morphology of PCL/starch coating 108

Figure 6-4 : a) Porosity of the PCL coated samples b) Porosity of PCL/starch coated samples 111

Figure 6-5 : Morphology of PCL based CRF at 3rd week a) 20% b) 30% c) 40% 112

Figure 6-6 : Morphology of PCL based CRF at 6th week a) 20% b) 30% c) 40% 113

Figure 6-7: Morphology of PCL based CRF at 9th week a) 20% b) 30% c) 40% 114

Figure 6-8 : Release of nutrients based on method a) release of nitrogen b) release of phosphorus c) release of potassium 118

Figure 6-9 : Release of nutrients based on molecular weight a) release of nitrogen b) release of phosphorus c) release of potassium 119

Figure 6-10: Release of nutrients based on concentration of PCL in coating structure. Within a treatment group, bars with the same letters are not significantly different using Tukeys test at 5% level. SE bars are standard deviation values. 121

Figure 6-11: Release of nutrients based on concentration of PCL in coating structure. Within a treatment group, bars with the same letters are not significantly different using Tukeys test at 5% level. SE bars are standard deviation values. 122

Figure 6-12 : The plot layout of the experiment placed to study the influence of CRF based on randomization in the Shed house of The University of Nottingham Malaysia Campus. .124

Figure 6-13: Difference between the Mungbean crops treated with CRF (encapsulated) and NPK.....125

Figure 6-14: Effect of CRF and NPK on plant height of mung bean. 126

Figure 6-15 : Effect of CRF and NPK on Leaf number of mung bean. 126

Figure 6-16 : Effect of CRF and NPK on Leaf area of mung bean. 128

Figure 6-17 : Effect of CRF and NPK on dry weight of mung bean. 129

Figure 6-18 : Influence of CRF and NPK on the pod weight, seed weight, pods & seeds per plant.....132

Figure 6-19: Effect of CRF and NPK on root parameters of mung bean. Within a treatment group, bars with the same letters are not significantly different using Tukey's test at 5% level. SE bars are standard deviation values. p stands for significance 133

Figure 7-1: PCL-CRF prepared by Blending technique 136

Figure 7-2 Surface morphology of the CRF (prepared via blending technique) 137

Figure7-3 : Porosity of CRF samples prepared via blending technique 137

Figure 7-4: Morphology of the PCL based CRF (prepared via blending technique) at 3rd week a) 5% b) 15) c) 25%.....139

Figure 7-5 : Morphology of the PCL based CRF (prepared via blending technique) at 6^{th} week 140

Figure 7-6: Morphology of the PCL based CRF (prepared via blending technique) at 9th week a) 5% b) 15) c) 25%.....141

Figure 7-7: Release of nutrients based on method a) release of nitrogen b) release of phosphorus c) release of potassium 142

Figure 7-8: Release of nutrients based on the concentration of PCL used a) release of nitrogen b) release of phosphorus c) release of potassium 143

Figure 7-9: the plot layout of the experiment placed to study the influence of CRF in early week based on randomization in the Shed house of The University of Nottingham Malaysia Campus. 145

Figure 7-10 the plot layout of the experiment placed to study the influence of CRF in later weeks based on randomization in the Shed house of The University of Nottingham Malaysia Campus. 146

Figure 7-11: Effect of CRF on plant height and leaf number of mung bean. 148

Figure 7-12 : Difference between the Mungbean crops treated with CRF (Blended) and NPK 149

Figure 7-13: Effect of CRF on the leaf area of Mung bean 151

Figure 7-14: Effect of CRF on Dry weight 151

Figure 7-15: Influence of CRF on the pod weight, seed weight, pods per plant and seeds per plant. p for significance; a& b are subsets indicating the significant difference between each level. 153

Figure 7-16: Effect of CRF on root parameters of mung bean. Within a treatment group, bars with the same letters are not significantly different using Tukey's test at 5% level. SE bars are standard deviation values. p stands for significance 155

LIST OF TABLES

Table 2-1: Advantages and disadvantages of different methods used in PCL synthesis.....14

Table 2-2 Different enzymes used in the synthesis of PCL.....18

Table 2-3 Brief summary of PCL synthesis and optimization.....23

Table 2-4 Different materials used for coating fertilizers.....33

Table 3-1: Properties of soil and biochar.....37

Table 3-2: Process parameters chosen for optimization by D optimal design.....40

Table 3-3: Treatment combinations with biochar, NPK and iron levels**.**.....46

Table 3-4: Treatment combinations included for studying the influence of CRF included with normal NPK and controls.....50

Table 4-1: Effect iron on chlorophyll and anthocyanin content.....60

Table 4-2 Influence of biochar, NPK and iron on the pod length, pod weight and seed weight, pods and seeds per plant, total dry weight of plant and harvest index.....65

Table 4-3 Influence of NPK, Biochar and Fe levels on the different parameters of the plant root.....67

Table 5-1: Experimental and predicted values of the polymer molecular weight.....78

Table 5-2 : ANOVA analysis for number average molecular weight (Mn).....79

Table 5-3 : ANOVA analysis for weight average molecular weight (Mw).....80

Table 5-4: Predicted optimised parameters by D-optimal.....90

Table 5-5: Molecular weights and conversion% of PCL catalysed by TLL for 96hrs.....97

Table 5-6 Degradation temperatures.....101

Table.6-1 Thickness of the coated samples (PCL & PCL/starch coatings) determined by SEM.....109

Table 6-2 : Effect of CRF and NPK application on chlorophyll and anthocyanin content..127

Table 6-3: Influence of CRF on the pod length 130

Table 7-1: Effect of CRF on chlorophyll and anthocyanin content 150

Table 7-2 : Influence of CRF on the pod length 154

LIST OF EQUATIONS

Equation 1:	Polynomial equation in DOE (methodology)	……37
Equation 2:	Porosity equation	……....51
Equation 3:	Polynomial equation for response 1(Mn)	……....53
Equation 4:	Polynomial equation for response 1(Mw)	……....53

LIST OF ABBREVIATIONS

ROP	Ring opening polymerisation
CALB	*Candida antarctica* lipase B
TLL	*Thermomyces lanuginosus* lipase
DP	Direct polycondensation
PCL	Polycaprolactone
PLA	Poly lactic acid
PGA	Poly glycolide acid
HIC	*Humicola insolens* cutinase
DOE	Design of experiments
RSM	Response surface methodology
PE	Polyethylene
PDI	Polydispersity index
THF	Tetrahydrofuran
PS	Poly styrene
PVC	Poly vinyl chloride
PVA	Poly vinyl alcohol
PP	Polypropylene
Mn	Number average molecular weight
Mw	Weight average molecular weight
FDS	Food and drug safety
MALDI TOF	Matrix assisted lazer dissociation ionisation time of flight
ANOVA	Analysis of variance
CRD	Completely randomized design
AAR	Applied agricultural resources
SPSS	Statistical package for social science
FTIR	Fourier transform infrared spectroscopy

FESEM	Field Emission scanning electron microscopy
TGA	Thermogravimetric Analysis
DSC	Differential scanning calorimetry
NMR	Nuclear Magnetic Resonance spectroscopy
$ScCO_2$	Super critical carbon dioxide
SPAD	Soil Plant Analysis Development
SEM	Standard error mean
CRF	Controlled release fertilizer (coated fertilizer)
NPK	Nitrogen phosphorus potassium (uncoated fertilizer)

CHAPTER 1

INTRODUCTION

1.1 Research background

Agriculture was the key in the rise of human civilisation, whereby farming of domesticated species created food surpluses that nurtured the development of civilisation. Although, agriculture comprises of cultivation and breeding of animals and plants (crops), which in turn used for food, fibre, biofuel, medicinal plants and other products; plant/crop is the one in high demand. However, agriculture in the 21st century is facing multiple challenges due to increased demand for food as the human population increases continuously. Thus, to increase the food supply, the agronomical industries have focused on developing techniques that can improve crop cultivation. One of the main ways to improve crop cultivation is through fertilizers; that is improving the manufacturing process as well as the implementation and release of fertilizers. This is because, fertilizers happen to be the principle compounds required to produce better crop growth and yield good products. Meanwhile, almost 50% of the fertilizers used are lost depending on the soil conditions and application method (Han et al., 2009). This improper utilization of fertilizer not only result in loss of crop productivity but also in environmental pollution and contamination of water. Subsequently, highlighting the challenges the agronomical industries face by the incomplete utilization of fertilizer and the need to address it.

One way to overcome the incomplete utilization of fertilizer is to develop a technique that can release fertiliser in a controlled manner. The main goal of this technique would be the gradual release of nutrients for a prolonged time along the growth of the plant with minimum wastage of fertilizers. In order to achieve the controlled release of fertilizer, the fertilizers are expected

to either coated or encapsulated with different type of materials. Previously, some polymers (e.g. polyvinylidene chloride (PVDC)-based, polyolefins, polyethylene, polyesters, alkyl resins etc.), fatty acid salts (e.g. Ca-stearate), rubber, guar gum, petroleum derived, wax were used for coating fertilizers (Trenkel, 1997, Al-Zahrani, 2000, Shaviv, 2001, Olad et al., 2018, Yang et al., 2018). Consequently, this helped to achieve the controlled release of fertilizers at the cost of leaving unwanted residues of the materials in the environment. This, in turn, caused negative effects on the living organisms.

Hence, in recent years, with the introduction of "green chemistry", the agronomical industries started to extend their scope into in-cooperating biodegradable polymers to coat or encapsulate fertilizers. This is because, as the name "biodegradable" refers to leaving no reminiscent of it. In other words, there is no residue remaining, thus overcoming the limitation of the conventional materials. In addition, biodegradable polymers have proven to be safe and suitable for numerous industrial applications such as food packaging, additives, thin films for wrapping and also in medical application like tissue engineering and drug delivery (Chilkoti et al., 2006) (Iqbal et al., 2019).

Initially, these biodegradable polymers were synthesised through conventional chemical polymerization, using chemicals and metals as catalyst (Labet and Thielemans, 2009). However, the residues left because of these catalysts has raised concern, especially in biomedical and agronomical applications due to their toxicity. Hence, enzyme catalysed bio-polymerization is used in place of these catalysts (which is eco-friendly). Moreover, enzymes have the advantages of mild reaction conditions, recyclability of enzymes and high enantio and regio-selectivity (Penczek et al., 2000). In the year 1993 Kobayashi and Uyama first reported the enzyme catalysed ring opening polymerization of lactones (Uyama and Kobayashi, 1993). Recently, there is a rapid increase in the number of publications on enzyme catalysed polymerization (Lassalle and Ferreira, 2008, Joseph et al., 2008, Levin et al., 2018, Subbiah et

al., 2018). Despite significant advances, a number of problems still exist in transferring the procedure of enzyme polymerization catalysed from the laboratory to an industrial level. Replacing enzymes with chemicals in the synthesis of biodegradable polymers is considered very important especially for their application in medical and agronomical sectors where the safety is of utmost importance.

1.2 Problem statement

According to the Fertilizer Industry Association of Malaysia (FIAM), the consumption of fertilizers increased tremendously in past years, reaching currently 545 million tons, recording 3% rise yearly. Considering the drawbacks of incomplete and excessive utilization of fertilizers; including several environmental issues like depletion of soil quality, biological imbalance in water bodies and climate change, the need to overcome these limitations is paramount. Thus, in an effort to control the release of fertilizer, researchers suggested coating or encapsulating the fertilizer with materials such as polymers. Nevertheless, the conventional materials initially used were successful in controlled release of fertilizer; however, there were still residues of the polymers causing environmental pollution. This led to further investigation of using biodegradable polymers to coat or encapsulate the fertilizer which is completely safe to the environment.

1.3 Scope and outline of the research

Figure 1-1 presents the schematic outline of this research

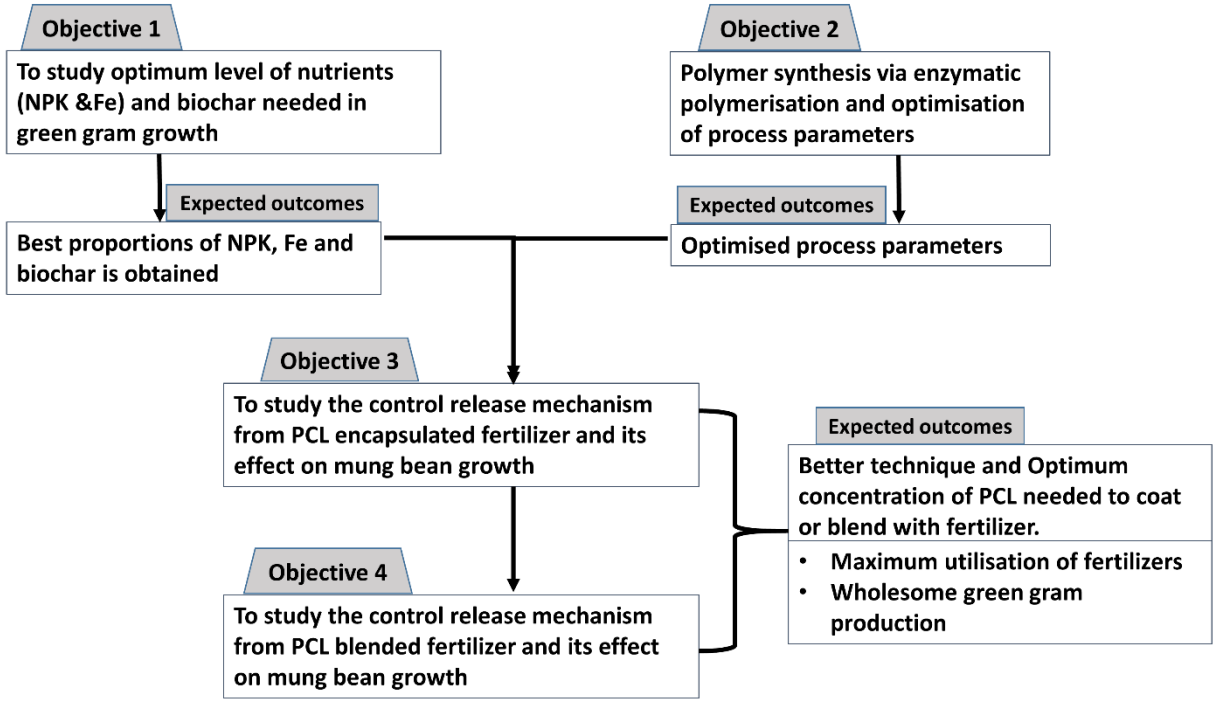


Figure 1-1: Schematic representation of research outline

Optimum formulation of nutrients: In order to obtain the optimum amounts required for improved growth and yield of mung bean**,** plants are treated with different amounts of fertilizers (NPK and Fe) and biochar. The treatments were laid out in a completely randomized design replicated three times to ensure no variability in experimental results. At harvest, data will be collected on yield components like plant height, number of leaves, pigment contents, number of pods per plant, pod weight, grain weight, total dry weight, harvest index and later subjected to analysis of variance (ANOVA) to verify the statistical significance of the data.

Synthesize of polycaprolactone: Synthesis of biodegradable polymer (polycaprolactone) is conducted using magnetic stirring in bench scale (250 ml) using enzyme *Candida antarctica*

lipase B**.** The optimisation of process parameters namely reaction time, reaction temperature, mixing effect, and monomer solvent ratio are established by running a set of experiments obtained using Design Expert 9^{TM} software. Polymer's characterisation is done using matrix assisted laser dissociation ionization time of flight (MALDI TOF) to determine the polymer molecular weight. Additionally, a novel enzyme catalyst - lipase from *Thermomyces lanuginosus* was tested to produce the polycaprolactone (PCL). Furthermore, the structural and thermal characteristics of the synthesised PCL were confirmed.

Preparation of PCL based CRF'S: Two different techniques were used to prepare the PCL based CRF's – Solvent casting and blending techniques. Effect of PCL based fertilizer is studied by polymer degradation mechanism and nutrient release, characterized by soil analysis for nitrogen, phosphorus and potassium (NPK), field emission scanning electron microscopy (FESEM) and porosity. Finally, pot trails are carried out with the optimum CRF to analyse its influence on growth and yield on mung bean.

1.4 Aim and objectives:

This study aims to synthesize and optimize the polycaprolactone from ε-caprolactone using enzyme catalyst i.e. lipase from *Candida Antarctica lipase B* and use it for application in control release of fertilizer.

Therefore, the aim of this study is met by considering the following objectives:

i. To investigate the optimum formulation of nutrients (Nitrogen, phosphorus and potassium & iron) and biochar on green gram growth.

ii. To synthesize, optimize and characterize polycaprolactone synthesis process via enzymatic polymerization.

iii. To study the fundamental degradation and nutrient release mechanism of PCL encapsulated fertilizer and their influence on growth and yield of green gram

iv. To study the fundamental degradation and nutrient release mechanism of PCL blended fertilizer and their influence on growth and yield of green gram.

1.5 Thesis outline

This thesis contains eight chapters. The outlines of the chapters in the thesis are as follows:

Chapter 1: Introduction: includes the background study, the problem statement, aims and objectives, scope and chapter outline of this study

Chapter 2: Literature Review

Initially presents a thorough review of the literature on PCL, different methods and enzymes used in PCL synthesis. Secondly, work done on studying the effect of NPK and biochar on the plants. Further control release of fertilizers and work done previously on related to control the release of fertilizers by different polymers were also mentioned

Chapter 3: Methodology - This chapter details the materials used and methods implemented in the experimental work of this project. Synthesis and optimization process for polycaprolactone synthesis have been presented. On the other hand, methods for monitoring the growth of mung bean. Later, methods implemented for the preparation of control release fertilizers are presented.

Chapter 4: Influence of different levels of NPK, iron and biochar on the growth characteristics of mung bean

Pot trails: To assess the influence of NPK, iron and biochar on the performance of green gram (*Vigna radiata*). Treatments consisted of a factorial combination of three biochar levels, three NPK and two levels of iron.

Chapter 5: Synthesis, optimization and characterization of PCL via enzyme catalysis

This chapter addresses the study on the effect of process parameters on the molecular weight of the PCL using CALB as catalyst. In addition, study on the lipase from *Thermomyces lanuginosus* as a catalyst for PCL synthesis was carried out. PCL synthesised was characterised by FTIR, NMR, TGA and DSC.

Chapter 6: Preparation of PCL based CRF via solvent casting technique and its effect on growth and yield of mung bean

In this chapter, solvent casting technique was studied for control release of fertilizers. Different characterizations were performed to determine the nutrient release and degradation of polymer. Finally, PCL based CRF was given to plants along with normal fertilizer and controls and study its effect on growth and yield of mung bean.

Chapter 7: Preparation of PCL based CRF by blending and its influence on the growth of mung bean

In this chapter, blending technique was used to prepare the PCL based CRF's. Further characterisations for CRF were performed to study the nutrient release and degradation mechanisms. Finally, the influence of PCL based CRF on the growth characteristics of mung bean was assessed along with normal NPK and controls.

Chapter 8: Conclusions and future works

This chapter summarizes the main findings of the research work based on the aims and objectives of the research. Also discusses the future work to be done.

1.6 Significance of research

This research looks into the potential application of biodegradable polymer PCL in controlled release of fertilizers. This sustainable system - control release of nutrients (NPK) with adding benefits of biochar, supports in improving crop cultivation with better yield qualitatively and quantitatively. Moreover, it helps to produce wholesome food to meet its surplus demand, subsequently for a healthy nation. This research also addresses one of the country's major challenges of declining soil fertility due to excessive chemical fertilizer usage. on other hand, In spite of excellent benefits in utilising PCL and its copolymers, enzyme-catalysed PCL without any traces of impurities is still not translated into the commercial arena. This research will aim to develop a polycaprolactone using enzymes as catalyst, meeting the need for biodegradable polymer with negligible chemical traces.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction:

This chapter presents a literature review on biodegradable polymer – PCL. The enzyme catalysts, preparation and optimization techniques related to synthesizing polycaprolactone are highlighted. In addition, work done on the formulation of fertilizers and biochar required for the improved cultivation of legumes are discussed; more focus was given to mung bean. Finally, importance of control release of fertilizers and the materials used previously are discussed.

2.2 Biodegradable polymers

One of the major research areas in recent years is biodegradable polymers. Due to their wide range of applications, excellent properties (biocompatibility, biodegradability) and the goal to become greener, all in turn has contributed to the increased demand of these biodegradable polymers.

There are a variety of polymers available, natural polymers from biological resources and synthetic polymers from petroleum resources (Vroman and Tighzert, 2009). **Figure 2-1** depicts different types of polymers with examples.

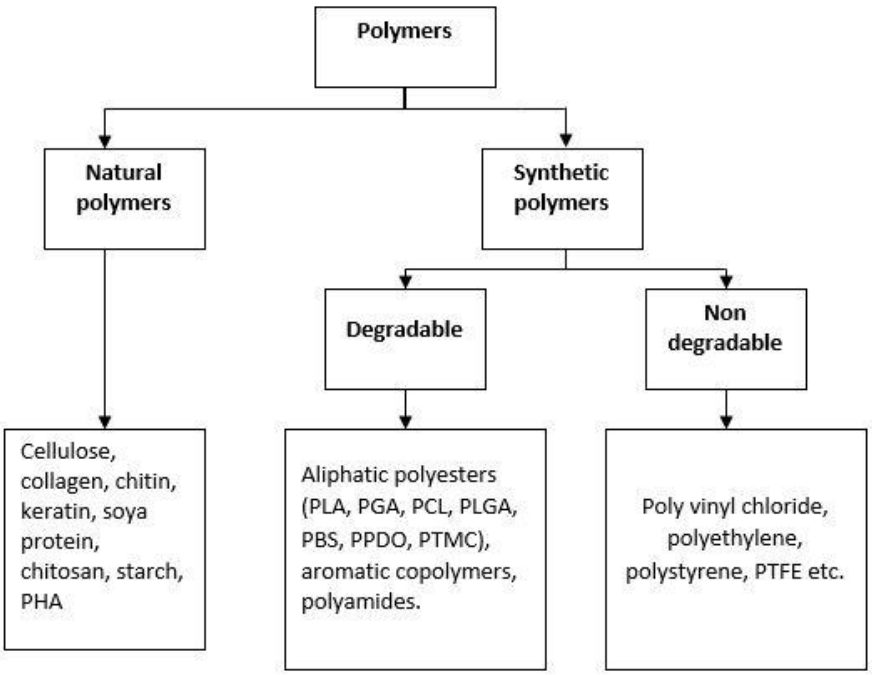


Figure 2-1 : Different types of polymers (Jha et al., 2019)

Synthetic polymers are further divided into degradable and non- degradable polymers. Non-degradable polymers (polyethylene, polyvinyl chloride, polytetrafluoroethylene, polystyrene) have served many uses like in automobiles (tires), water bottles, toys, carry bags, pipes, clothing industry and medical applications. In past decades, polymers/plastics have become a part of everyday life, but they have also contributed to the major problem of accumulation of waste material in the environment. Due to waste disposal problem and accumulation in the environment, they are replaced by bioplastics/ degradable polymers.

On the other hand, biodegradable polymers have gained a lot of interest due to their degradability, as the name suggests. Approximately 200 million tonnes of biodegradable polymers are consumed worldwide with 5% rise every year (Siracusa et al., 2008). According to a recent report, the market value of the biodegradable polymer exceeds $1.1 million and reaches $1.7 billion by 2023 (*George*, 2018). This value is likely to rise along with the increasing population. Recently, through ‘green chemistry’, the market penetration of

biodegradable polymers is expected to increase due to its safety and suitability for numerous applications particularly in medicine and agricultural applications where safety issues are given most priority.

Among these biodegradable polymers, polycaprolactone captured attention due to its properties like biodegradability, non-toxicity and biocompatibility with a large range of other polymers improving their properties (Woodruff and Hutmacher, 2010). This leads to more focus and research on polycaprolactone, which made it available commercially. The recent global market study reports that the PCL market value is expected to increase tremendously with CAGR of 10.5% (2018). The medical applications of PCL demand its synthesis process to be greener and safer with negligible traces of impurities.

2.3 Synthesis of polycaprolactone

PCL can be prepared either by polycondensation of 6-hydroxycarboxylic acid or ring opening polymerisation (ROP) of e-caprolactone (Labet and Thielemans, 2009, Albertsson and Varma, 2002). Among, ROP was suggested to be a preferred route (Wang et al., 2014).

Phenyl lithium gave high molecular weight polymer at 170^0C (Arbaoui and Redshaw, 2010), calcium alkoxide initiating system used for ROP of CL, resulted in wide range of molecular distribution (PDI up to 4) (Zhong et al., 2001). Stannous octoate is most commonly used catalyst for polymerisation of ε-caprolactone (Kowalski et al., 1998), the drawback of this catalyst is it requires high temperatures, resulting in inter and intramolecular esterification giving high PDI (Labet and Thielemans, 2009). Apart from these disadvantages, the usage of chemical catalysts to synthesise PCL is not recommended due to their biomedical applications. Any metal impurities remaining after the polymer degradation in the body may cause harmful effects (Gross et al., 2001).

Lately, these chemical catalyst are replaced by enzymes, which are considered as eco-friendly. Enzymes provide a huge benefit - easy handling, eco-friendly, non-toxic, effective in small amounts, energy savings, not consumed by the reaction they catalyse nor do they alter the equilibrium of these reactions. Compared to chemical catalysts, enzymes offers higher reaction rates, milder reaction conditions high reaction specificity with no side products, this change can enable new and more sustainable routes for the production of biodegradable polymers. Shiro kobayashi reported a review on lipase catalysed polyester synthesis, in the review it was reported that lipase catalysed polymerization shows high regio and chemo selectivity compared with chemical catalysts. So, polymerisation using enzymes Enzyme catalysis has become an increasingly important tool for medicinal process and polymer chemistry allowing the development of efficient and highly attractive products (Matsumura, 2006). Different enzymes used to synthesise the polycaprolactone are discussed in later section.

2.3.1 Different methods used for enzymatic polymerization of PCL

Most commonly used method for synthesising PCL is by carrying the reaction using thermostatic stirrer, in which the reactants are stirred until the reaction is terminated. Earlier many authors reported conventional method to synthesise the PCL (Ma et al., 2009, Kumar and Gross, 2000), among which Kumar et al reported PCL produced with a molecular weight of 40,000g/mol. A recent report on the synthesis of PCL reported similar method using enzyme and organocatalysts together (Huang et al., 2019). Other than thermostatic stirring, microwave irradiation was reported to synthesise of PCL (Ref). Few researchers reported this method using solvents, concluding microwave irradiation had a significant influence on the ring opening polymerization of caprolactone, without the molecular weight around 3000 - 16000 g/mol less than 2hrs (Fang et al., 2002) (Kerep and Ritter, 2006) (Barbier-Baudry et al., 2003). Later, Matos et al conducted PCL synthesis without the use of solvents, yet reported high molecular weight polymers around 20,600 g/mol in 4hrs (Matos et al., 2011). Comparing conventional and microwave irradiation method, the conversion and molecular weight are better in conventional method despite the reaction time is very less in microwave irradiation completing the reaction within no time and no involvement of organic solvents which is quite beneficial.

Ultrasonic irradiation was used in lipase catalysed ROP of caprolactone (Gumel et al., 2012). It was reported that synthesised polymer has narrow molecular weight distribution, high degree of crystallinity and with improved monomer conversion. Due to the frequency of the sonicator bath, the diffusion rates can be maximum resulting in more collisions between substrate and enzyme causing maximum conversion.

Supercritical CO_2 technology was reported using metal catalyst, tin alkoxides (Stassin et al., 2001), tin octoate (Bratton et al., 2005) and enzyme catalyst CALB (Loeker et al., 2004). Tin

alkoxides are reported to be efficient for ROP in this supercritical CO_2 technology, the polymerisation process is 14 times faster in toluene and 33 times faster in bulk compared to $ScCO_2$. Matsumura *et al* reported the transformation of PCL into repolymerisable oligomers in the presence of lipase from CALB, further repolymerised converts it into high molecular weight PCL establishing a novel methodology for recycling a polymer. (Matsumura et al., 2001).

The advantages and disadvantages of the above synthesis methods are mentioned in **Table 2.1.**

Table 2-1: Advantages and disadvantages of different methods used in PCL synthesis

Method	Advantages	Disadvantages	Reference
Conventional enzymatic method (normal set up, magnetic stirrer for mixing)	Simple to set up	Temperature fluctuations	(Hunsen et al., 2008), (Ma et al., 2009)
Microwave Irradiation	Reaction rate enhancement, Energy savings, Direct heating, High temperature homogeneity	Radiations may denature the enzymes	(Kerep and Ritter, 2006)
Ultra-sonication	Diffusion rate is high	Chances affecting immobilisation of enzymes	(Gumel et al., 2012)
NMR	Creates in vivo environment for the reaction to happen	Very costly	(Kumar and Gross, 2000)
Supercritical Co2	Green method, no involvement of organic solvent	Low solubility of polymers	(Jérôme and Lecomte, 2008)

2.3.2 Different enzymes used to synthesize Polycaprolactone

Polycaprolactone was initially synthesised using chemical catalysts, which are later replaced by enzymes, i.e. enzymatic ring opening polymerisation represented in **Figure 2-2**.

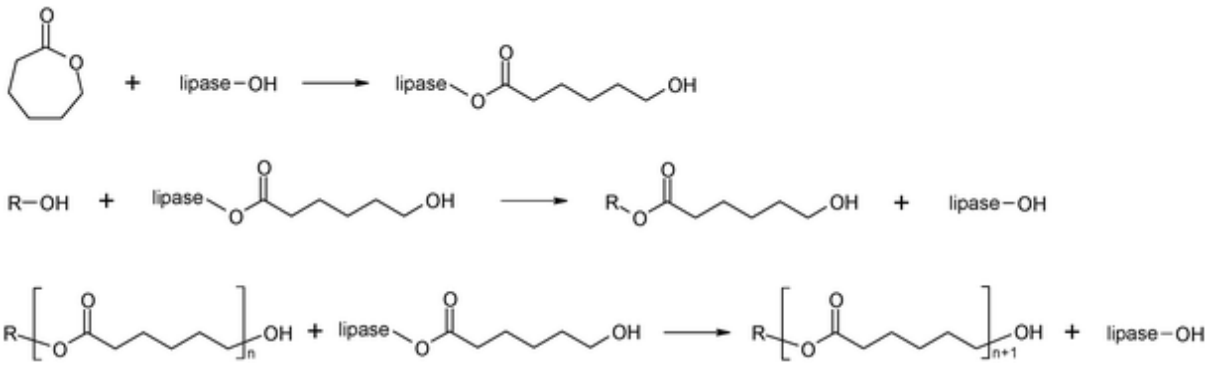


Figure 2-2 Schematic representation of enzymatic ring opening polymerisation

Initially, the enzyme/lipase reacts with the monomer to form lipase-activated CL complex, later the –OH group reacts with the enzyme monomer complex (Sivalingam and Madras, 2004) (Kobayashi, 2010). Water content in the reaction medium is important to achieve high molecular weight PCL with narrow low dispersity and also adding molecular sieves into medium will help maintaining the required water level. Apart from water content, pH plays a important role in ROP, it affects the ionisation of the amino acid residues at the active site of the lipase, which influences its ability to bind to substrates (Dong et al., 1998). Almost the lipase-mediated polymerization will takes place in solvents and in ionic liquids (Jérôme and Lecomte, 2008). In enzymatic ROP, the formation of lactone – lipase complex is considered to be the rate determining step, also enzymatic ROP is more favourable for large lactones (Duda et al., 2002). Unlike chemical ROP, in enzymatic ROP the large lactones react faster than the smaller ones (van der Mee et al., 2006).

The reason for the choice of enzymes over the chemicals was reviewed by Kobayashi et al (Kobayashi et al., 2001). There are many enzymes used until now to produce the polycaprolactone, mostly among them are lipases. All the enzymes used in synthesis of PCL are listed in a **Table2-*2***. Among all the enzymes, *Candida antarctic*a lipase B was reported to be more efficient than others (Hunsen et al., 2007). Kumar *et al.* carried out the polymerisation using CALB, to synthesise a polycaprolactone using caprolactone and studied the effect of temperature, time and different solvents. (Kumar and Gross, 2000).

Lipase from *Pseudomonas fluorescens* was initial lipase tested for ring opening polymerization of caprolactone (Namekawa et al., 1999). As presented in the **Table 2-2** *Pseudomonas fluorescens* lipase takes 10 days for the polymerisation reaction to complete reported by Hiroshi Uyama (Uyama et al., 1993). Lipase from *Fervidobacterium nodosum* also catalyses the ring opening polymerisation of caprolactone. Conversion of caprolactone to PCL took 72 hrs. It was reported that it has a higher affinity than CALB (Li et al., 2011b). Rivera et al. worked on a lipase isolated from a yeast *Yarrowia lipolytica* used in the ring opening polymerisation of lactones, polymerisation is carried under different temperature in presence of heptane for 15 days resulted in low molecular weight polymer with unique multiphase morphology (Barrera-Rivera et al., 2008). Lipases from other species like *Pseudomonas cepacia*, Pocrine pancreatic lipase, *Rhizopus muhei* are able to synthesise PCL with low molecular weights with a time period of about 240hrs. Cutinase from *Humicola insolens* was reported to catalyse the polymerisation of ε- caprolactone, synthesising a polycaprolactone. Hunsen et al compared the cutinase with CALB to catalyse the ring opening polymerization of caprolactone, reported high molecular weight PCL with CALB as catalyst (Hunsen et al., 2008). Considering the time as one of the factors except lipase B from *Candida Antarctica*, all the other enzymes take 3 days to 15 days which can be mentioned as a drawback to consider these enzymes as an effective catalyst.

Furthermore, polycaprolactone produced in solvent medium has more molecular weight than produced in bulk (without solvent). All the enzymes available are commercially immobilised. Compared to free enzymes the immobilised enzyme are proven to give better product, more stable for long hours when used in the presence of strong organic solvents. The main reason for immobilisation is to reuse the enzyme. Candida Antarctica which is immobilised can be reused up to 10 cycles without losing its activity producing results in every use (Poojari et al., 2013) which is very advantageous as it reduces the amount of enzyme required.

Table 2-2 *Different enzymes used in the synthesis of PCL*

Enzyme	Process Parameters			Product Properties			References
	Time (Hrs)	**Temp**	**Solvent**	**Mol wt**	**Monomer conversion**	**PDI**	
Lipases from							
1. Candida Antarctica lipase B	4	70	toluene	44800	>90	1.7	(Kumar and Gross, 2000)
2. Pseudomonas fluorescencs	10 days	60	-	3700			(Uyama et al., 1993)
3. Fervidobacterium nodosum	72	90	toluene	2350	100	1.3	(Li et al., 2011b)
4. Yarrowia lipolytica	15 days	60	N heptane	975	100	-	(Barrera-Rivera et al., 2008)
5. Candida rugose- a) free	5	65	Diisopropyl ether	-	75	-	(Foresti and Ferreira, 2004)
b)CR- immobilised	5	65	Diisopropyl ether	-	82-95	-	(Foresti and Ferreira, 2004)
6. Pseudomonas cepacia	240	60	Cyclohexane	6100	84	2.7	(Namekawa et al., 1999)
7. Candida cylindreacea	5	60	-	1900	92	2.0	(Namekawa et al., 1999)
8. Rhizopus muhei	5	65	Diisopropyl ether	-	45	-	(Foresti and Ferreira, 2004)
9. Rhizopus japonicas	240	60	-	840	83	1.7	(Namekawa et al., 1999)
10. Pocrine pancreatic lipase	240	60	-	1300	95	1.7	(Namekawa et al., 1999)
Cutinase from Humicola insulens	24	70	toluene	24900	90	1.7	(Hunsen et al., 2008)
Esterase from Archeaoglobus fulgidus	72	80	toluene	1400	100	1.21	(Ma et al., 2009)
A Recombinant Escherichia coli whole-cell biocatalyst	72	70	Cyclohexane	2000	100	1.47	(Li et al., 2011a)

2.3.3 Factors affecting enzymatic polymerisation of PCL

Different parameters are considered which can affect the bio-polymerisation process. Reaction temperature, reaction time, enzyme concentration, monomer or substrate concentration, monomer/solvent ratio, mixing speed are the parameters to be maintained during the bio-polymerisation process. Only a few have reported the effects of every parameter.

Effect of temperature on polymerisation is previously reported (Kumar and Gross, 2000, Hunsen et al., 2008). It is considered as the important factor in the polymerisation which may affect the stability of the biocatalyst (Fukuda et al., 2008) in turn effecting resultant polymer. At a high temperature in ring opening polymerisation, water molecules will access the chain initiation reaction, which would improve the conversion rate (Dong et al., 1999). But for thermal stability of the catalyst, one must also be considered before going to higher temperatures. Using *Humicola insolens* Cutinase (HIC) as enzyme, the range of temperature from 50 to 100^{o}C was studied on polymerisation process. Monomer conversion of 100% was obtained at 70^{o}C, later on, as the temperature increases there is a decrease in monomer conversion up to 20%, this may be because of occurrence of protein denaturation and deactivation at these elevated temperatures (Hunsen et al., 2008). Similarly, study on enzymatic polymerization of polycaprolactone using CALB as catalyst reported a similar trend to that of HIC, Where the rate of polymerization increased along with temperature (Kumar and Gross, 2000). Also, in this study increase of monomer conversion was observed starting from the temperature 60°C until 90°C, later with a further increase in temperature i.e. for 100°C and 110°C decrease in the monomer conversion was observed.

Solvent plays an important role in regulating the partitioning of substrates and products between the solvent and the enzyme. A large number of different solvents were tested (1,4-dioxane, acetonitrile, acetone, 2-butanone, *tert*-butyl alcohol, *tert*-butyl methyl ether, isopropyl

ether, benzene, carbon tetrachloride, heptane, cyclooctane, and isooctane) and it appeared that the efficiency of the polymerisation was linked to the hydrophobicity of the solvent used: when eROP was conducted in hydrophilic solvents, the conversion was very low after 10 days and the resulting polymers had a low molecular weight. Ma et al studied effect of different type of solvents for ring opening polymerisation of e-caprolactone. They reported, out of different type of solvents used – toluene, n hexane and cyclohexane gave a high molecular weight. Whereas, in toluene PCL obtained was almost with 100% monomer conversion with 1400 g/mol molecular weight (Ma et al., 2009). Li et al studied the ring opening polymerisation of e caprolactone using lipase from *Fervidobacterium nodosum*. They performed the reaction using different solvents, higher monomer conversions and Mn values was achieved using cyclohexane and n-hexane. PCL solubility was very low in these solvents and therefore it's hard to separate from reaction media (Li et al., 2011b) (Li et al., 2011a).Kumar *et al* reported different types of solvents used for the polymerisation of the polycaprolactone out of which toluene was reported to cause maximum monomer conversion than other organic solvents. Also, they studied the effect of monomer/solvent ratio taking the range from 1:1 to 1:10 (Kumar and Gross, 2000). The maximum molecular weight was obtained when the ratio is 2:1, it decreased with further increase in the ratio. Meanwhile, enzyme concentration has an intense effect on monomer conversion and product molecular weight (Dong et al., 1999). Ma et al reported enzyme concentration is inversely proportional to monomer conversion, but the molecular weight did not show much change with the increase of enzyme concentration (Ma et al., 2009).

Time and mixing also play a crucial role in the bio-polymerisation process. The mixing should be maintained such that allowing for more diffusion between the substrates. Low diffusion will not allow enough interaction between the monomer, substrate and enzyme. Therefore, fewer interactions leading to lower monomer conversions, low molecular weight polymers. So the

mixing plays an important role in the polymerisation process. This factor, mixing effect on the enzyme catalysed ring opening polymerisation has been less focused.

Optimization of process parameters is important to achieve desirable results. Previously, this optimization process was carried by single factor analysis/one factor at a time. This process is not only time consuming but also takes a lot of chemicals and other materials. As a result, the optimisation cost increases, making it difficult for the product in its commercial way. Lately, researchers made their effort to find alternatives via statistical approaches. Previously, enzymatic polymerization of PCL and its optimisation was done by single variate approach as presented in Table 2- 3. Except the few articles from our research team studied the optimisation using neural networks. Neural networks could predict the process parameters to achieve the desired molecular weight. Nevertheless, failed to explain the interaction between the parameters.

Recently, response surface methodology (RSM) in Design of experiments (DOE) lately gained a lot of interest for optimization of various chemical processes. RSM was used in many optimization processes such as fermentation process (Naveena et al., 2005), wastewater treatment (Körbahti et al., 2007), chitosan ester synthesis (Chaibakhsh et al., 2013). Nahit Aktas reported optimization of enzymatic bio polymerization using by central composite design (CCD) in response surface methodology. Successful optimization of catechol conversion to polycatchol using laccase enzyme was successfully done, by carrying out minimum runs (Aktaş, 2005). Optimization ofenzyme catalysed wax esters synthesis using RSM methodology was also reported (Gunawan et al., 2005). Aldemir et al reported optimization of aniline synthesis using enzyme as a catalyst, using CCD for optimisation showing good agreement of response with observed values (Aldemir et al., 2014).

Besides CCD, box Benken is another method in RSM which was significantly in use for many processes. This method was used to optimize the synthesis of lead implant polymers (Khajeh et al., 2011). Apart from polymer synthesis, it has been reported using in biodiesel synthesis (Kamath et al., 2011) and nanoparticle synthesis (Ba-Abbad et al., 2015). Out all other designs in response surface methodology, D optimal is the latest method in RSM which is least explored. Recently it was reported, to be used in the preparation of fermentation medium and some decolouration process (Yin et al., 2009).

Table 2.3 presents the brief summary of PCL synthesis with different chemical catalysts and its optimisation process.

Table 2-3 Brief summary of PCL synthesis and optimization

S.no	Polymerisation process	Catalyst used	Optimisation technique	References
1.	Ring opening polymerisation	Lithium metal	-	(Deng et al., 1999)
2.	Ring opening polymerisation	CALB	-	(Gokalp et al., 2016b)
3.	Polycondensation	-	-	(Braud et al., 1998)
4.	Ring opening polymerisation	Scandium	-	(Nomura et al., 2000)
5.	Ring opening polymerisation	Magnesium metal	-	(Zhong et al., 2001)
6.	Polycondensation of 6-hydroxycaproic acid	CALB	-	(Mahapatro et al., 2004)
7.	Ring opening polymerisation	stannous octoate	Single variate approach	(Fang et al., 2002)
8.	Ring opening polymerisation	lithium diisopropylamide	-	(Bhaw-Luximon et al., 2005)
9.	Ring opening polymerisation	Alumimium trifate	-	(Wang and Kunioka, 2005)
10.	Polycondensation	Tin metal	Single variate approach	(Labet and Thielemans, 2009)
11.	Ring opening polymerisation	Stannous 2 - ethylhexanoate	Single variate approach	(Lipik and Abadie, 2010)
12.	Ring opening polymerisation	CALB enzyme	Single variate approach	(Arumugasamy and Ahmad, 2011)
13.	Ring opening polymerisation	CALB enzyme	Neural network	(Arumugasamy et al., 2013)
14.	Ring opening polymerisation	CALB enzyme	Neural network	(Arumugasamy et al., 2012)

2.4 Fertilizers and importance of Control release

The steady agricultural sector ensures nations food security. Food security is the primary requirement of any nation, which prevents improper nourishment that has traditionally been one of the serious problems faced by the developing countries. Many of the countries rely on agronomical products as well as associated industries for their prime source of income. Global population is increasing exponentially and is expected to reach 9.5 billion by 2050 (Azeem et al., 2014). Sustainable agriculture is the farmer's ability to produce food without affecting the environment as well as the surrounding ecosystem. There are a few issues which are connected to agriculture and one of them is the biophysical issue. It is linked with activities like fertilizer usage, use of artificial nutrients and crop rotation along with the availability of resources like sunlight, water and wind.

While all these factors are equally important for plant growth, fertilizers are given extra importance, as they are the ones that help plants in the initial stages of growth. Fertilizers are materials of synthetic or natural origin, which are applied to the plant tissues or soil for supplying plant nutrients crucial to plant growth.

2.4.1 Influence of macro and micronutrients on mung bean

Fertilizers are meant for enhancing plant growth. The objective is met in two different ways: additives for providing nutrients and fertilizers for enhancing soil's effectiveness by modifying aeration and water rate. Plants get their minerals from the soil. Apart from their lack in the soil, their availability is also complicated. The process of micronutrient uptake, accumulation and their regulation is a dynamic process that should avoid deficiency in the plant. Hence, the importance of using fertilizers rises.

When it comes to fertilizers, Nitrogen, Potassium and Phosphorus are the three main elements or macronutrients. Nitrogen helps in leaf growth, whereas potassium aids sturdy stem growth, water movement and promotion of fruiting and flowering. The nutrients needed for healthy growth of plants are classified based on the elements. Rice crop yields are reported to be increased with the addition of NPK fertilizers compared to controls (Ayoola and Adeniyan, 2006). NPK fertilizers are responsible to increase the microbial mobility, which is a great sink for nutrients (Jonasson et al., 1996). Shaver et al reported that the growth and flowering responses were increased with NPK addition and are also sustained for few years (Shaver and Chapin, 1995). Not only growth and yields but also the nitrogen fixation and nodule formation was also reported to be improved (Becker et al., 1991). NPK fertilizers were also proved to be beneficial in cow pea, maize and also some intercropping system, by increasing both yield and other growth parameters (Jaliya et al., 2008, Abayomi et al., 2008).

However, NPK are the macronutrients very essential for physiology and growth of the plant. Apart from macronutrients (NPK), iron also plays a vital role in plant growth. Iron is necessary for normal functioning of plants. Iron deficiency affects photosynthesis and leads to reduced yield and dry matter production. Continuous deficiency of iron will cease the growth of root and shoot finally causing chlorosis (Mori, 1999). Higher contents of iron in rice will help in fighting iron deficiency anaemia (Lucca et al., 2001). Iron plays a major role in photosynthesis, nitrogen metabolism, for mediating redox reactions and preventing physiological disorders.

Mung bean (*Vigna radiata*) is a pulse crop belonging to legume family. It is consumed by millions of populations all over the world. It is a rich source of protein (20 – 24%) and a good cereal based diet with essential amino acids (Tang et al., 2014). Mung bean is of low cost and high nutritional values. By the same token, it is considered as poor man's meat. Besides serving as a high protein diet it also helps in improving soil fertility by fixing atmospheric nitrogen with the help of Rhizobium bacteria in its nodules (Hussain et al., 2010). Even though mung

bean is drought resistant crop, in order to achieve high yields and quality grains, addition of proportional amounts of nutrients is necessary. Little amount of nitrogen (N) as a starter dose, phosphorus (P) and potassium (K) are the major nutrients required by the crop to perform its functions. Apart from these, some micronutrients like iron, zinc, calcium, sulphur, molybdenum etc also play a critical role in plant growth and functions. Consequently, proper nutrition provided to crop will produce wholesome grains benefiting human health. Generally, soil serves as a nutrient source but due to many reasons like environmental factors, leaching etc. plant may not be able to receive complete nutrition.

Earlier studies on the effect of fertilizer application reported increased growth and yield of mung bean. Although legumes have an ability to fix nitrogen, the application of nitrogen in earlier stages was proved to be beneficial (Achakzai et al., 2012). Malik et al reported an increased yield of mung bean with the application of nitrogen and phosphorus (Malik et al., 2003). Researchers (Hussain et al., 2011, Malik et al., 2003) explored the effects of potassium application, consequently reporting it to beneficial, resulting in higher yields of mung bean. Evidently, addition of nitrogen, phosphorus and potassium proved to enhance the growth and yield of mung bean. Despite this, there were limited studies carried on the addition of NPK fertilizer as a whole. Hence in this study, the formulation of biochar together with the macro and micronutrients (NPK and Fe) on the growth and yield of mung bean was reported.

2.4.2 Influence of biochar on mung bean

These days along with fertilizers, biochar is also added to the soil. Biochar is a carbon-rich material produced via pyrolysis of bio-waste (Lehmann et al., 2011) and the use of it has been known as an ecological engineering tool to mitigate N_2O emission from croplands while to maintain soil productively (Ding et al., 2010) (Yuan et al., 2011). Today it is considered as a potential alternative to reduce non-renewable energy resources while enhancing agricultural

productivity. The large surface area with functional groups and generally high pH of biochar could be reactive to immobilize heavy metal cations in soils (Beesley et al., 2011). There had been increasing studies on biochar effect on metal mobility in soil (Hossain et al., 2011). Moreover, biochar may alter the physical properties of the soil, including increasing aeration and water holding capacity of certain soils. Biochar can increase pH by 0.5–1.0 unit in most cases for application rates of 30 Mg/ha of biochar, nutrients are directly available through the solubilization of ash in the solid biochar residue and other nutrients may become available through microbial utilization of a small labile carbon component of biochar. Apart from nutrients recently the benefits of biochar in agriculture are captivating. Biochar is a carbon-rich material produced via pyrolysis of biowaste (Lehmann, 2007). The use of it has been known as an ecological engineering tool to mitigate N_2O emission from croplands while to maintain soil productively (Liu et al., 2016, Hossain et al., 2011). Biochar's high pH and its large surface area with functional groups could be reactive to immobilize heavy metal cations in soils (Beesley et al., 2011). Subsequently, there had been increasing studies on biochar effect on metal mobility in soil (Méndez et al., 2012, Fellet et al., 2014, Ahmad et al., 2018). Furthermore, biochar may alter the physical properties of the soil, including increasing aeration and water holding capacity of certain soils. Besides, biochar can also increase the nutrient holding capacity and cation exchange capacity which in turn results in preventing nutrient leaching (Achakzai et al., 2012). Nutrients are directly available through the solubilization of ash in the solid biochar residue and other nutrients may become available through microbial utilization of a small labile carbon component of biochar. Therefore, considering the biochar is added benefit in improving the crop production.

As mentioned already, more than half of the total yield production is out of synthetic or inorganic fertilizers, which contains components like nitrogen, potassium, sulphur, calcium, magnesium, and so on. These chemicals and minerals, although help in boosting the growth of

plants, they also have their drastic side effects in the long run. The problem is that humans tend to use too much of fertilizers in the soil because they have to cater to the global demand for food. The fact is that using too much of fertilizers in the soil can alter the fertility of the soil by increasing the acid levels. Too much fertilizer in the soil also leads to eutrophication.

2.5 Control release of fertilizers

The most reliable and effective way to make the availability of nutrients coincide with plant requirements is by controlling their release into the soil solution, using control release fertilizers (CRF's). This is usually accomplished by coating the fertilizer with synthetic materials. Implementation of these type of fertilizers is believed to achieve nutrient supply along the plant growth, also reducing the number of application times. Besides CRF's will reduce the loss of fertilizer which is caused by leaching preventing soil pollution. Importantly they preserve environmental safety from excess fertilizers usage. **Figure 2.2** depicts the mechanism for control release of fertilizers. Many factors like polymer degradation, water, microbes, enzymes from microbes, sunlight will be playing a role in nutrient release from polymer coated fertilizer. The rate of utilization of the fertilizers is not 100% when applied in the soil. Its only 50% - 70% of nitrogen, 10% - 25% phosphorus and 50% - 60% of potassium will be utilized. Remaining all is considered to be wasted (Trenkel, 1997). This loss not only causes wastage of fertilizers but also the serious environmental damage. Utilization of the fertilizers depends on the method of application, mainly for nitrogen, phosphorus and potassium (Jarosiewicz and Tomaszewska, 2003).

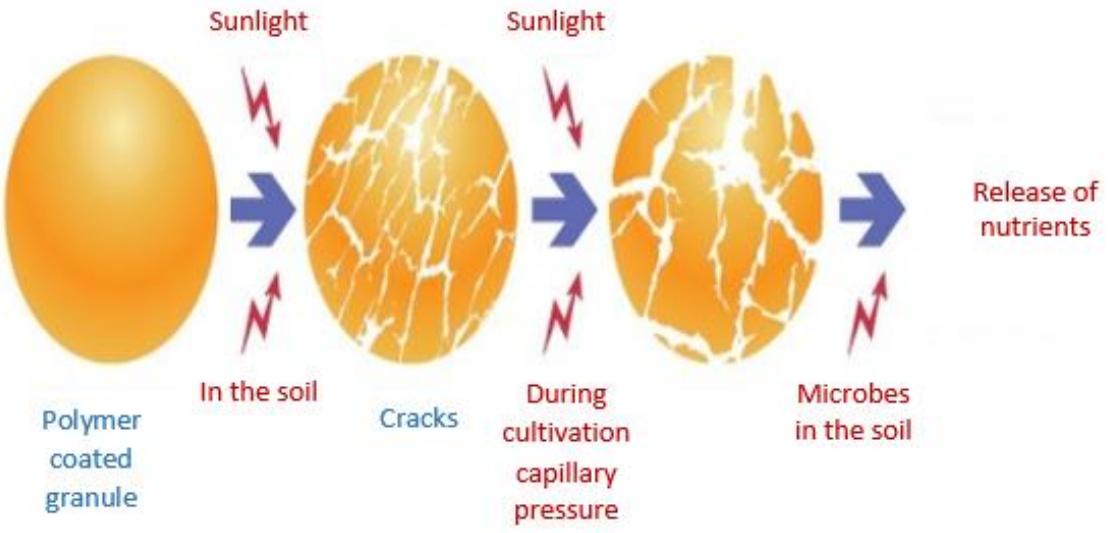


Figure 2-3 : Mechanism of nutrient release from polymer coated fertilizer

Dissolution rate of fertilizers can be reduced by coating them with organic materials and polymers. Previously, organic and synthetic materials were reported to be used in control release (González et al., 2015). However, the chemical nature and their existence in soil is so long which may affect the soil fertility. Recently, polymers are being widely used in control release of fertilizers. Polymer coated fertilizers provide good control release compared with organic based fertilizers (Shaviv et al., 2003). Coating material must be non-toxic concerning the plant growth and for environmental protection (Wu and Liu, 2008) for which biodegradable polymers perfectly fit.

2.5.1 Materials used for control release of fertilizers

Control release of fertilizers became a priority, aiming for agricultural benefits like improving nutrient uptake and reducing loss of fertilizer preventing environmental damage. Control release will help to meet the demand of nutrients along the growth of plant. It became a trend for the economic usage of fertilizers. **Table 2-3** presents few materials used previously for control release of fertilizers.

Chitosan and polyacrylic- co- acrylamide are used for the controlled release of urea (Wu and Liu, 2008). This polyacrylic- co- acrylamide is a superabsorbent polymer facilitating control release of fertilizer. It can absorb water more than its weight, facilitating water-absorbing capacity. The release rate is 75% in 30 days, despite with good release mechanism. Even though it can be monitored according to the coating layer. Polymer coated fertilizers are commercially available, which are usually coated with parafilm or polyurethane or sulphur coated. Polyurethane coated fertilizers are tested to know the degradation capacity and release rate of fertilizers. Du et al reported that 70% of polyurethane coated fertilizer is released in 70 days (Du et al., 2006). Fertilizers coated with polyethylene and parafilm are commercially available, tested for porosity and release rate. 45 to 52 days are needed to release 50% of the fertilizer (Al-Zahrani, 2000). Parafilm neither polyethylene are not degradable, chances of leaving more reminiscent in the environment. Anna et al reported the release rate and porosity of commercial granules/ fertilizer coated with polysulfone/cellulose acetate.

Polyacrylic polymer is used for urea fertilizer blended along with starch to increase its degradability. A double layer coating made up of chitosan and acrylic acid is used for NPK and urea fertilizer binding using epoxy. As a result, 75% of fertilizer was released in 30 days (Wu and Liu, 2008). Combination of polysulfone, cellulose acetate and polyacrynitrile were together used to achieve the coating for fertilizer. The casting is done using solvent casting technique (Tomaszewska and Jarosiewicz, 2002). Because of this solvent casting, the physical properties may be affected. Higher polymer concentration may result in low porosity. Addition of salt can be done to increase the porosity. Polysulfone, cellulose acetate and polyacrylonitrile are used for coating NPK fertilizer (Jarosiewicz and Tomaszewska, 2003). 100% nutritive release is reported with 60% porosity far less than coating layer with 48% porosity, the release rate is not more than 38%. This clearly refers that more the porosity more is the nutrient release. Study on control release of urea encapsulated by starch and polylactic acid prepared by solution

casting (Chen et al., 2008). They reported that usage of hydrophobic PLLA decreased the urea release because of its swelling. The release rate of urea can be adjusted by making changes in its graft efficiency. Starch when used as coating material the fertilizer is dispersed as ting particles and released through diffusion mechanism. Therefore, starch alone cannot be used for control release and can be considered only after any modifications.

Liang et al reported NPK fertilizer coated with polyacrylonitrile and kaolin has a good water retention capacity (Liang et al., 2007). When the coated layer has a high water retention capacity, it is good for slow release, moreover providing proper utilization of fertilizer along with water resource. Polyvinyl and starch blended together are used for encapsulation of urea fertilizer. Han et al reported that the released NH_4 ions, water absorbency and water permeability are increased along with the polyvinyl concentration (Han et al., 2009). Here it is important to note that the decrease in the starch content may affect the compatibility of the films blended. Crosslinkers used for enhancing the blending may also play a role in the absorbency and permeability. Formaldehyde, used as enhancer during coating purposes should be maintained at required levels. Due to its interference between the intermolecular and intramolecular linkage between the blends.

Polyvinyl alcohol and modified water borne starch blends were studied for the controlled release of fertilizers (Azeem et al., 2016). It has been reported that the release time is directly proportional to the coating thickness. Nevertheless, it is important to note that, porosity plays a role in the release rate of the nutrients. However, even with a thick layer of coating, the release rate can be considered good with high porosity. Consequently, high porosity coating can also increase the diffusion coefficient. A combination of good coating thickness along with least heterogeneity and film integrity is also important.

Xiao et al proposed a one-step preparation of starch based superabsorbent polymer for the slow release of fertilizers (Xiao et al., 2017). The melt mixing process is employed using the twin rotor mixer. Recently, a novel composite was reported in control release of fertilizer. Urea, paraformaldehyde and montmorillonite along with water were used to prepare novel nanocomposite for slow release of fertilizers. Reported efficient release of nitrogen fertilizers (Yamamoto et al., 2016). Xiaodo et al reported the use of wheat straw cellulose hydrogel in the slow release of fertilizers (Li et al., 2016)

Table 2-4 *Different materials used for coating fertilizers*

Composite	**Method for encapsulation**	**Degradation**	**Enhancers**	**References**
Chitosan /poly (acrylamic-co acrylamide) acid	Dissolving in epoxy	75% on the 30th day	super absorbent polymer for water retention	(Wu and Liu, 2008)
Polyurethane coated	Commercial	70% in 70 days	-	(Du et al., 2006)
Polyacrylic/urea/chitosan	Solution casting method	Not above 75% in 30 days	epoxy	(Wu et al., 2008)
Polysulfone/cellulose acetate/ polyacrylonitrile	Phase inversion technique	-	-	(Tomaszewska et al., 2002)
Polysulfone/cellulose acetate/ polyacrylonitrile	Phase inversion technique	50% - 60% in 5hrs	-	(Jarosiewicz and Tomaszewska, 2003)
Urea/ starch/ PLA	Solvent casting	100% in 26hrs	-	(Chen et al., 2008)
Poly(acrylic-co acrylamide) and kaolin	crosslinked using sodium alginate	50% in 7days	Sodium alginate	(Liang et al., 2007)
Starch and polyvinyl alcohol (PVA) films	No encapsulation	50% in 50 days	formaldehyde	(Han et al., 2009)
PCL/clay/starch	Hot press	65% in 60 days	cetyl pyridium chloride	(Wu et al., 2007)

Starch/polyvinyl alcohol	-	50% in 22hrs	Citric acid	(Azeem et al., 2016)
Starch based super absorbant polymers	Rheomix mixer	85% in 45 days	Methylene bisacrylamide	(Xiao et al., 2017)
Polyethylene/parafilm	commercial	40 to 52 days	-	(Al-Zahrani, 2000)

Wu et al reported the polycaprolactone in the encapsulating the phosphate solubilizing bacteria. Along with polycaprolactone, different blends PCL/ starch, PCL/ clay and PCL/starch clay are tested for this application. They reported that 65% PCL only was degraded in 60 days (Wu et al., 2007). Molecular weight of 80,000 was used in this study, which may also be considered playing a role in degradation time. Molecular weight is directly proportional to the degradation time. So the higher the molecular weight, higher is the degradation time. They reported that degradability is more in PCL/starch compared to other blends i.e. PCL/clay/and PCL alone.

In order to achieve good coating material for the fertilizers lot of factors are to be considered such as thickness of coating, porosity, enhancers used, method used, molecular weight of the materials preferred.

2.6 Summary

From the literature surveyed in this chapter, researchers have devoted more attention towards enzymatic ring opening polymerisation considering many advantages of enzyme catalyst. Also, single variate approach is the most common optimization process carried till date, except a very few studied on neural networks from MATLAB. With recent benefits of DOE, the process optimisation for enzymatic polymerisation of PCL using DOE is yet to be reported. It is also worth noting that fertilizers consumption has increased tremendously, out of which 50% is wasted due to improper fertilization techniques. Previously, controlled release fertilizers have been developed using polymers. However, there are still drawbacks especially due to the residues left by the polymers. Hence, in this study biodegradable polymer – polycaproaclactone was targeted to prepare controlled release fertilizer hoping to develop a sustainable fertilizer release system.

CHAPTER 3

METHODOLOGY

List of materials and methods used for sample preparation used in this study are listed out clearly in this chapter. Methods for polycaprolactone synthesis using enzyme catalysts, (CALB and TLL) and also optimization process using DOE are mentioned. Also, the pot trails procedure for optimization of the nutrients required for sample preparation for controlled release fertilizers using two different methods are briefly presented.

3.1 Materials

3.1.1 Materials used for PCL synthesis

Lipase acrylic resin from *Candida antarctica* with enzyme activity ≥5,000 U/g (Novozyme 435) and lipase from *Thermomyces lanuginosus* with ≥3000 U/g enzyme activity is immobilized on immobead 150, both were obtained from Sigma Aldrich. Both lipases were used as catalyst in the bio-polymerisation process. From the literature review, it observed that out of all lipases study for PCL synthesis, enzyme CALB produced the PCL with high m molecular weight. On the other hand, TLL was considered to study for its ROP function, observing that it has a similar structure to that of CALB. Further, ε-caprolactone and toluene are employed as monomer and solvent respectively. These are purchased from Merck Sdn Bhd, Malaysia. Further chloroform, used to terminate the polymerization reaction and methanol which have been used for precipitating the product after polymerisation both analytical grade were also obtained from Merck's. Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) as a matrix (purchased from Sigma Aldrich, Germany), tetrahydrofuran (THF) and Potassium hydroxide (KOH) are used for characterisation of synthesised polymer.

3.1.2 Materials used for pot trials

Red soil is used to conduct this experiment since it contains less nutrients and will be suitable for nutrient study. Soil sample and biochar was analysed for physico-chemical properties at Applied Agricultural Resources (AAR) Laboratories Sdn Bhd, Malaysia. NPK (15:15:15) and FeS (iron sulphate) were bought commercially in Input shop, Semenyih. The biochar used was sourced from Ladybird Organic Farm, Semenyih. Good quality mung beans are sowed in each pot containing 1.8 kg of soil. Soil and biochar properties are presented in **Table 3-1.**

Table 3-1: Properties of soil and biochar

Soil properties		**Biochar properties**	
Properties	**Values**	**Properties**	**Values**
p^H	4.7	pH (H20)	6.73
Carbon %	0.36	Total P2O5 (%)	0.14
N %	0.05	Carbon (%)	18.40
C/N	7.2	Total N (%)	0.27
K	0.09	S (%)	0.07
Ca	0.54	K2 O (%)	0.48
Mg	0.07	CaO (%)	0.18
Na	3.4	MgO (%)	0.13
Al	0.56	Na (%)	0.01
Texture	Sandy clay loam	Al (ppm)	82.0
		C/N ratio	68.4
		Total ash (%)	62.3
		Conductivity(µs/cm)	1728.5

3.1.3 Materials used for preparation of PCL based CRF's

Polycaproalctone, grade CAPA 6250 and CAPA 6400 with molecular weights of 25000g/mol and 34000g/mol were supplied by Perstorp Company, Sweden. NPK (15:15:15) was bought commercially in Input shop, Semenyih. Starch was purchased from Sigma Aldrich, Germany.

3.2 Preparation of PCL via enzyme catalysis

Figure 3.1 depicts the **s**chematic flow chart for PCL synthesis.

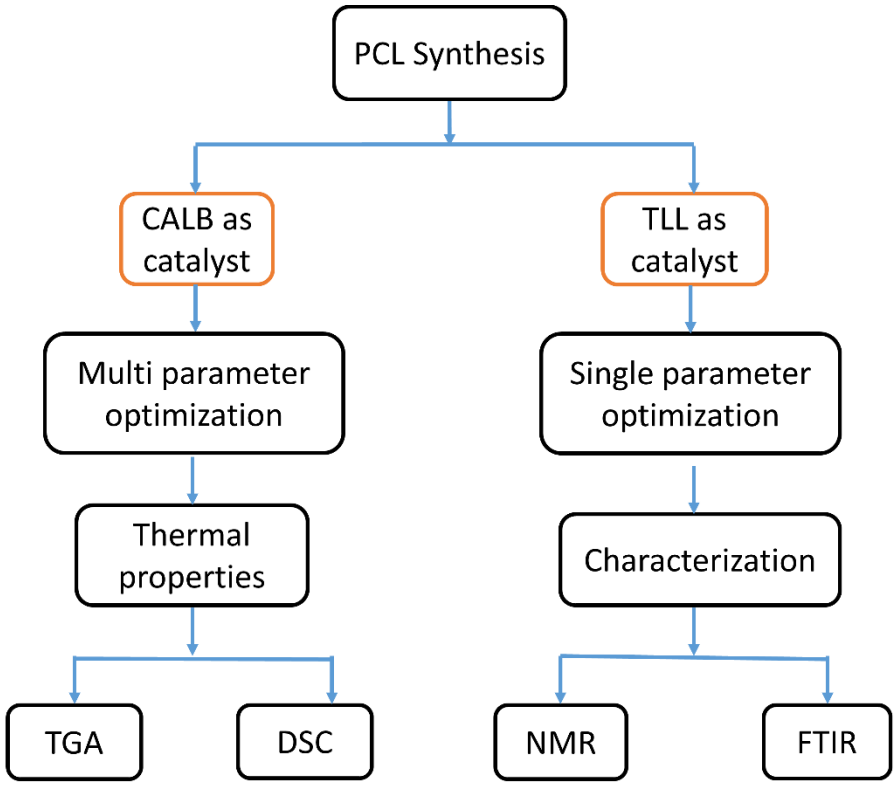


Figure 3-1: Schematic flow chart of PCL synthesis

3.2.1 Design of experiments

Response surface methodology in DOE software was used to develop a model for predicting the optimised conditions for polycaprolactone synthesis. Since optimised parameters can be

obtained by performing fewer runs compared to single variate approach (Jagadish et al., 2017). It has been carried out based on the response chosen i.e. number average molecular weight (Mn) and weight average molecular weight (Mw). Where Mn is the total weight of all the polymer molecules divided by the total number of polymer molecules in the samples whereas the Mw is calculated based on the fact that bigger molecules contain more total mass of polymer. Molecular weights (Mn and Mw) was chosen as a response because the physical, thermal, mechanical properties and degradation rate of PCL are greatly influenced by molecular weight, Also, the ratio between Mn and Mw, will gives the poly dispersity index which explains to understand the molecular weight distribution of the polymer. (Lam et al., 2007). Temperature, mixing speed, time and monomer/solvent ratio are the process parameters, which have been considered for optimisation. Stat ease software version 9 was used in this study. In response surface methodology (RSM), D-optimal design package was selected, in order to optimise the process parameters to obtain desired molecular weight. It also has an adaptable structure allowing the numeric factors in either way i.e. discrete or continuous manners, unlike other RSM designs such as Box–Behnken and CCD where the parameters are varied on fixed levels. D optimal is also the latest method in RSM which is least explored. D optimal will interpret the effect of these parameters on the polymer molecular weight based on the 25 set of experiments. The specific ranges of the process parameters chosen for this experiment are taken from the previous literature (Kumar and Gross, 2000). Till date most of the studies (Ma et al., 2009, Hunsen et al., 2008) reported the effect of time, temperature and monomer/solvent ratio on enzyme catalysed polycaprolactone synthesis process, but no reports on the effect of mixing speed. In this study effect of mixing speed from 50 – 500 rpm has been included, since mixing speed plays an important role in the polymerization process (Fathi Roudsari et al., 2015) causing more interactions between substrates resulting in long chain polymer. All the parameters have been considered as continuous factors, i.e. considering a

range from low to high values. All the experimental range of these parameters are presented in **Table 3-2.** The model developed by D optimal design tool will illustrate the effect of process parameters and their interaction statistically.

Table 3-2: Process parameters chosen for optimization by D optimal design

Parameters	Coded name	Range	Units	Type
Temperature	A	50 - 110	°C	Continuous
Time	B	1-7	hr	Continuous
Mixing speed	C	50 - 500	RPM	Continuous
Monomer/solvent ratio	D	1:1 to 1:6	-	Continuous

In this experimental design process, the chosen parameters and responses are fitted to the polynomial equation (shown in Equation 1),

$$Y = \beta_0 + \sum \beta i \, Xi + \sum \beta ij \, Xi \, Xj \qquad \text{(Eq 1)}$$

Where Y stands for the response, β0 is independent constant coefficient, βi is the individual coefficient, Xi stands for independent parameters (i = A, B, C, D), βij is for interaction coefficient effects (i = A, B, C, D and j = A, B, C, D)

Data were analysed through analysis of variance i.e. ANOVA, to investigate process parameters significance along with their interactions. Statistical significance and the quality of

the model fit was expressed by *F*-test and the p-value along with few more terms explained in later sections.

3.2.2 Process for PCL synthesis using enzymes

3.2. Using lipase from *Candida Antarctica*

Synthesis of polycaprolactone using *Candida antarctica* lipase B as catalyst and toluene as solvent, was performed using a magnetic hot plate with constant stirring. CALB and toluene were selected, since from previous literature, out of all the enzyme catalysts used CALB proved to be efficient enzyme in producing PCL. Similarly, toluene as solvent gave high molecular weight PCL and monomer conversion compared to other solvents (Kumar and Gross, 2000) (Ma et al., 2009) . Process parameters: temperature, time, monomer/solvent ratio and mixing speed were maintained as per the experimental design obtained from D optimal design. This procedure was taken from previous literature (Kumar and Gross, 2000). Firstly, the ε-caprolactone (monomer), toluene (solvent) and CALB (enzyme) were added to the 250 ml conical flask. Amount of enzyme (1g) is kept constant throughout the procedure. Other parameters - temperature, time, mixing speed and monomer/solvent ratio were set to the required values obtained from DOE (25 set of experiments). After the specified time interval (as per in DOE) for every experimental run, the polymerisation was terminated by adding cold chloroform in excess amounts. Later the enzyme is filtered and the excess chloroform is eliminated by rotatory evaporation. Methanol was used to precipitate the polymer obtained in concentrated solution. Finally, the precipitate obtained was removed and dried in the oven (50^{o}C, overnight). The dried polymer is used for further characterisations.

Using lipase from *Thermomyces lanuginosus*

Bio polymerization process was carried out in 250 ml conical flask. In the initial trial, the amount of monomer and enzyme used were followed similar to CALB catalysed procedure. Since it's a new enzyme catalyst, previous procedure used for CALB was followed. 10 ml of ε-caprolactone, 20 ml of solvents- (hexane, THF and toluene) and 1gm of the enzyme was taken in a conical flask and allowed to polymerize with constant stirring aided by magnetic stirrer. Solvents and parameters (temperature and mixing speed) were chosen based on the previous work on *Thermomyces lanuginosus* lipase catalysed reactions (Hernández-Martín and Otero, 2008) (Yang et al., 2005, Torres et al., 2002). Polymerization was continued until the solution became viscous (From the observations during the PCL synthesis using CALB). In-between aliquots were withdrawn intermittently at specified time intervals, to check for polymer formation. Chloroform was added to terminate the polymerization reaction. Excess chloroform was removed by rotatory evaporation. Further, methanol was added to precipitate the polymer and the remaining concentrated solution is kept in an oven at 50°C until it's completely dried. The dried polymer is removed from the oven and further characterizations – NMR, FTIR, TGA and DSC were performed. A similar procedure is carried out to study the effect of solvents and effect of enzyme concentrations.

Figure 3-2 depicts the procedure for the PCL synthesis in general for the enzymes.

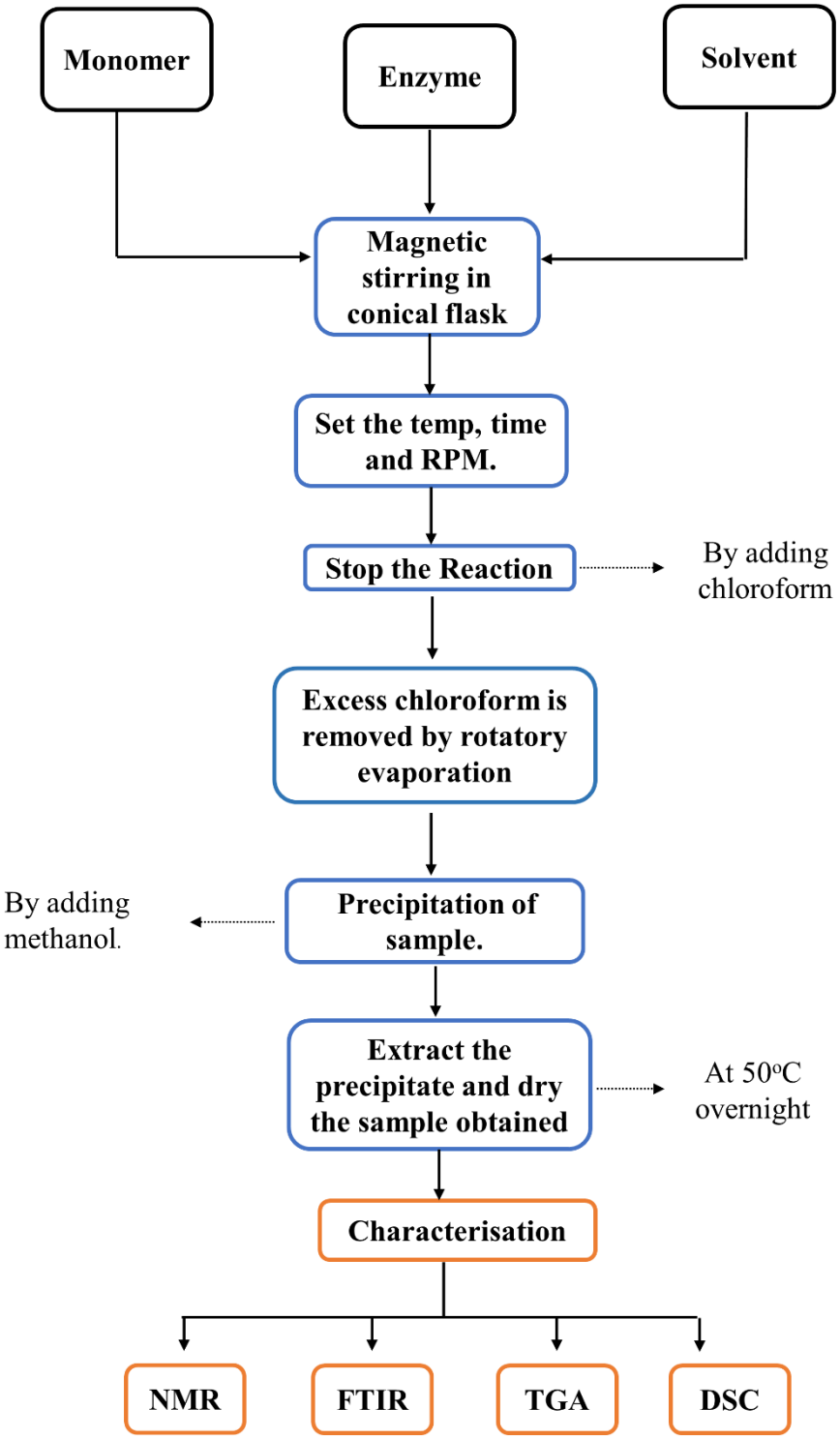


Figure 3-2 : Schematic flow chart for methodology of PCL synthesis

3.2.3 Characterization for synthesised PCL

3.2.3.1 Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectroscopy was used to characterise the number of protons to determine the basic chemical structure of the synthesised polymer. H NMR spectra was measured on Bruker NMR spectrophotometer at 300MHz in Sunway University, Petaling jaya, Selangor. Deuterated chloroform (CDCl3) was used as a solvent. Spectra for the CDCl3 was referenced at 7.27 ppm respectively.

3.2.3.2 Matrix-assisted laser desorption/ionisation time of flight (MALDI TOF)

Molecular weights (Mn and Mw) of the synthesised polymer were determined using microflex MALDI TOF mass spectroscopy system (Bruker). MALDI analyses were carried out in University of Malaya, Jalan Universiti, Kuala Lumpur. Synthesised polymer sample (5mg/ml), DCTB (Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] (10mg/ml) and sodium trifluoroacetate (0.1mol/L) are dissolved in tetrahydrofuran. The sample, matrix (DCTB) and doping salt (KOH) are mixed in 5:25:1 ratio. 0.5ul of this mixture is spotted on the target plate and irradiated by UV light. Usually, few laser shots are taken and used to generate a peak. Later the peaks are analysed by polymerix software^{TM} to obtain the molecular weight of the sample.

3.2.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

Polymer functional groups were characterised using Perkin Elmer FT-IR Spectrometer Frontier model. The spectrometer scanning was regulated at 4 cm^{-1} resolution performing 50 scans for every sample. The transmittance spectra were observed in the region ranging between 4000-400 cm^{-1} since the polymer functional groups can be identified between this ranges. Later, the absorbance unit was recorded for all the FTIR spectra.

3.2.3.4 Differential scanning calorimetry

The melting temperature of the synthesised PCL was measured using a Perkin Elmer STA 6000 thermal analyser. The melting point of PCL was determined by heating a polymer sample from 25°C to 160°C. Heating rate was maintained at 10°C/min. Sample scanning was performed under the nitrogen flow of 10ml/min.

3.2.3.5 Thermogravimetric Analysis

The thermogravimetric analysis (Mettler Toledo TGA with STARe system) was used to determine the degradation temperature of the synthesised polymer. Approximately 10-15 mg of polymer sample was heated from 25°C to 500°C at the heating rate of 10°C/min. The range was selected because the polymer degradation temperature will be around 400°C to 450°C. To avoid undesirable oxidation, the analysis was carried out under the nitrogen atmosphere maintained with a constant flow rate of 20ml/min.

3.3 Pot trails

3.3.1 Pots preparation for optimum formulation of fertilizer and biochar

Pot trails were carried out in greenhouse, University of Nottingham, Malaysia Campus. Treatments included factorial combination of 3 levels of biochar (0, 10 & 20 t/ha), 3 levels of NPK fertilizer (0, 15:15:15, 30:30:30) and 2 levels of iron (0 & 15kg/ha) with total of 18 treatment combinations including controls presented in **Table 6.**

Table 3-3: Treatment combinations with biochar, NPK and iron levels**.**

1. Biochar (kg/ha soil)	***2. NPK (kg/ha)***	***3. Fe levels (kg/ha soil)***
0	0	0
10	15:15:15 (1)	15
20	30:30:30 (2)	

No	***Treatment***	***Abbreviation***
1	Soil (no biochar, no NPK, no iron)	B0F0I0
2	Soil+ iron@15kg/h	B0F0I5
3	Soil+ NPK (1)	B0F1I0
4	Soil+ NPK (1)+ Iron@15kg/ha	B0F1I5
5	Soil+ + NPK (2)	B0F2I0
6	Soil+ + NPK (2)+ Iron@15kg/ha	B0F2I5
7	Soil+ biochar@10t/ha	B10F0I0
8	Soil+ biochar@10t/ha + Iron@15kg/ha	B10F0I5
9	Soil+ biochar@10t/ha + NPK (1)	B10F1I0
10	Soil+ biochar@10t/ha + NPK (1)+ Iron@15kg/ha	B10F1I5
11	Soil+ biochar@10t/ha + NPK (2)	B10F2I0
12	Soil+ biochar@10t/ha + NPK (2)+ Iron@15kg/ha	B10F2I5
13	Soil+ biochar@20t/ha	B20F0I0
14	Soil+ biochar@20t/ha + Iron@15kg/ha	B20F0I5
15	Soil+ biochar@20t/ha + NPK (1)	B20F1I0
16	Soil+ biochar@20t/ha + NPK (1)+ Iron@15kg/ha	B20F1I5
17	Soil+ biochar@20t/ha + NPK (2)	B20F2I0
18	Soil+ biochar@20t/ha + NPK (2)+ Iron@15kg/ha	B20F2I5

Each treatment has 3 replications and each replication was further repeated additional time to reserve for the destructive samplings at periodic interval. Total of 108 pots are laid out in a completely randomized design (CRD). NPK, biochar and Fe requirements for each pot was computed based on 2,000,000 kg soil mass per hectare.

Initially, the soil was collected, air-dried and thoroughly mixed, 1.8 kg of the air-dried soil was used for each pot. Later mixed with the biochar accordingly and was thoroughly incorporated in the soil prior to sowing. Consequently, after sowing fresh water will be manually irrigated daily to ensure sufficient moisture for germination. Regular monitoring on samplings will be taken in order to detect and to prevent phytopathogenic and insect damage. Destructing sampling was done at 7^{th} week for root analysis. Finally, after physiological maturity harvesting will be carried out by cutting at the base of the plant. The plot layout during the growth period of the plants is depicted in **Figures 4 to 7.**



Figure 3-3:The plot layout of the experiment placed based on randomization at 1st week after sowing in the Shed house of The University of Nottingham Malaysia Campus.



Figure 3-4: The plot layout of the experiment placed based on randomization at 3rd week after sowing in the Shed house of The University of Nottingham Malaysia Campus.



Figure 3-5:The plot layout of the experiment placed based on randomization at 6th week after sowing in the Shed house of The University of Nottingham Malaysia Campus.



Figure 3-6:The plot layout of the experiment placed based on randomization at 9th week after sowing in the Shed house of The University of Nottingham Malaysia Campus

3.3.2 Pot trails for control release of PCL fertilizers

Pot trails with PCL coated/blended fertilizers were carried out in greenhouse, University of Nottingham, Malaysia Campus. Treatments include optimised concentrations from the blending and solvent casting technique, with neat PCL and synthesised PCL via enzyme catalysis. Total of four treatment combinations including normal NPK (non PCL coated/ non PCL blended fertilizer) and controls presented in **Table 3.4**

Table 3-4: Treatment combinations included for studying the influence of CRF included with normal NPK and controls

S no	Treatment	Abbreviation
1	Soil + biochar 20t/ha	C
2	Soil + biochar 20t/ha + NPK	NPK
3	Soil + biochar 20t/ha + PCL blend 25	B25*
5	Soil + biochar 20t/ha + PCL Coated 30	C30*

*B25 - PCL blend 25, SC30 – PCL coated 30

Each treatment has 3 replications and each replication was further repeated additional time to reserve for the destructive samplings at 7th week of the study. Total of 36 pots are laid out in a completely randomized design (CRD).

Initially, the soil was collected and air-dried. Each pot was filled with 1.8 kg of the air-dried soil along with biochar at 20t/ha (this amount was taken from the previous trail since biochar at 20t/ha got the significant effect). Soil and biochar are properly mixed before sowing the seeds. Freshwater will be manually irrigated daily to ensure sufficient moisture for germination. After the plant saplings are visible and blended and coated fertilizer are added to the plants. Regular monitoring will be done on samplings to prevent any damage from insects and pathogens. Readings for growth parameters will be taken at 3^{rd}, 6^{th} and 9^{th} week of the trail. Destructing sampling was carried out at 7^{th} week for root analysis. Finally, after physiological maturity harvesting will be carried out by cutting at the base of the plant.

3.3.3 Data collection and analysis

The data for growth parameters were taken for every two weeks i.e. 3^{rd}, 6^{th} and 9^{th} week. Data collected are plant height, number of leaves, leaf area index, chlorophyll content (SPAD value), anthocyanin content, leaf area, shoot- fresh weight & dry weight, root- fresh weight & dry weight, plant dry weight, root length, root volume, root diameter, root surface area. Number

of pods per plant, height of the pod, pod weight, number of grains per pod and grain weight are collected.

The procedure for the data collection in all the experiments is as described below.

1) *Plant height* - Plant height was measured at 3, 6, and 9 weeks after sowing using meter rule and recorded against each treatment.

2) Number of leaves - Number of leaves per plant are counted at 3, 6 and 9 weeks after sowing and recorded

3) Anthocyanin content- Plant leaf anthocyanin content was measured using anthocyanin meter.

4) Plant chlorophyll content- Plant leaf chlorophyll content was measured using SPAD Chlorophyll Meter, SPAD 502 plus, KONICA MINOLTA, INC. Made in Japan.

5) Leaf area - Leaf area was measured using LI-3100C Area Meter. The leaflets from each plant were collected to measure leaf area.

6) Shoot and root weights – Soon after the destructive sampling, the fresh weight of root and shoot are recorded. Moreover, to obtain dry weight, harvested plants were dried in an oven until constant weight and later dry weight is recorded.

7) Number of pods per plant - Pods are harvested from plants at the mature stage. Number of pods per plant was counted and the average number of pods per plant was recorded. The height and weight of pods were also recorded.

8) *Number of seeds per pods*- Pods were shelled manually and seeds were separated. Number of seed per pods were counted and weighed. The average and total number of seeds per pod were recorded.

9) *Root studies*

Root studies were carried out at 7th week after sowing. At destructive sampling period, the tops were severed from the roots using a knife and immediately taken for analysis. The roots were carefully washed, floated on water and scanned using Flatbed Scanner, Epson Expression 11000XL, Seiko, Japan with WinRhizo Pro version 2013 (Regent Instruments, Canada) software. Thereafter, the roots were dried in the oven with the temperatures maintained at 70°C for 72 hrs. Root and shoot dry weight were also determined.

The data collected will be subjected to two-way analysis of variance procedure (ANOVA) for completely randomized design (CRD) using SPSS software.

3.4 Sample preparation for control release of fertilizer using PCL

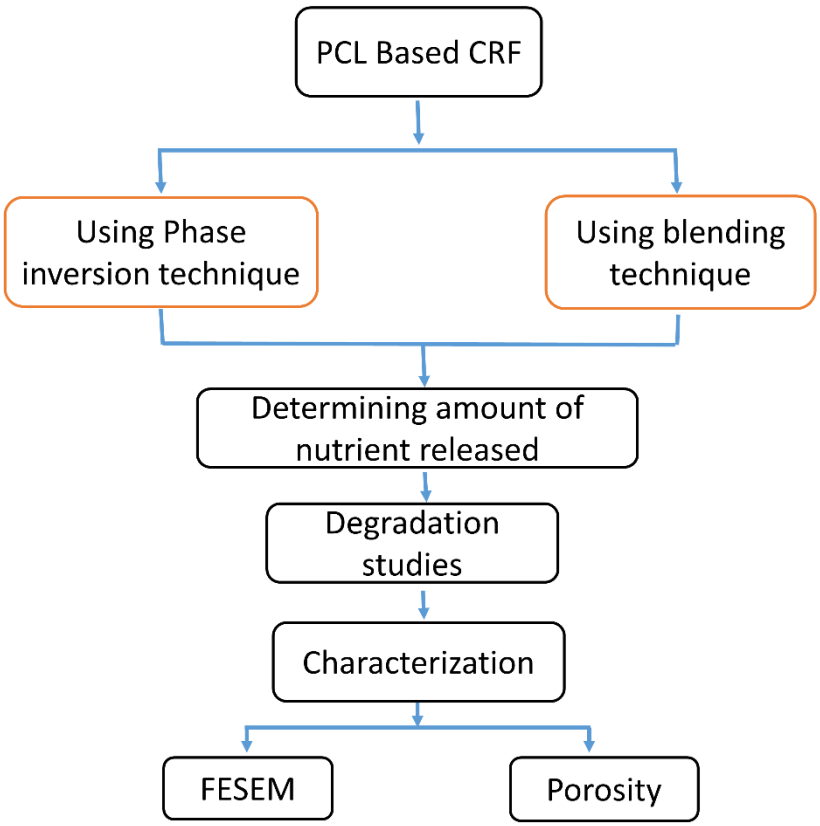


Figure 3-7: Schematic flow chart for methodology of application of PCL based CRF's

3.4.1 Solvent casting technique

Polymer coated fertilizer was prepared by solvent casting technique at laboratory scale. The fertilizer used was NPK 15:15:15, it's granular and water soluble. Commercially bought PCL and PCL synthesised via enzyme catalysed polymerization was used to prepare the polymer coating NPK. THF was used for dissolving the polymer. Three different concentrations i.e. 20, 30 and 40% were prepared in order to compare them later with polymer blends. Polymer solution was prepared by dissolving the PCL pellets in THF. Later, coating was achieved by solvent casting technique using water precipitation bath.

Prior to coating, screening of the granules was done with diameter 3 to 5 mm, since this size facilitates the easy encapsulation and transferring of the granule. Initially, the PCL was dissolved in THF to form the polymer solution by constant stirring. Later, NPK pellets are added into the polymer solution. Once the NPK granules are covered by the layer of polymer film, they are dropped into precipitation bath maintained at 19°C from a height of 10cm approximately using forceps. This helps for the gelation process, ensuring the formation of polymer coating. Later the granules are immediately removed from precipitation bath and are dried in the oven at 377K (104°C) up to constant mass (Tomaszewska and Jarosiewicz, 2002). Procedure is outlined in the figure 3-8. On the other hand, PCL/starch coating was prepared by adding starch to the polymer solution under constant stirring. Further, NPK granules were added to the solution and immediately transferred to petri dish and allowed to dry until constant weight.

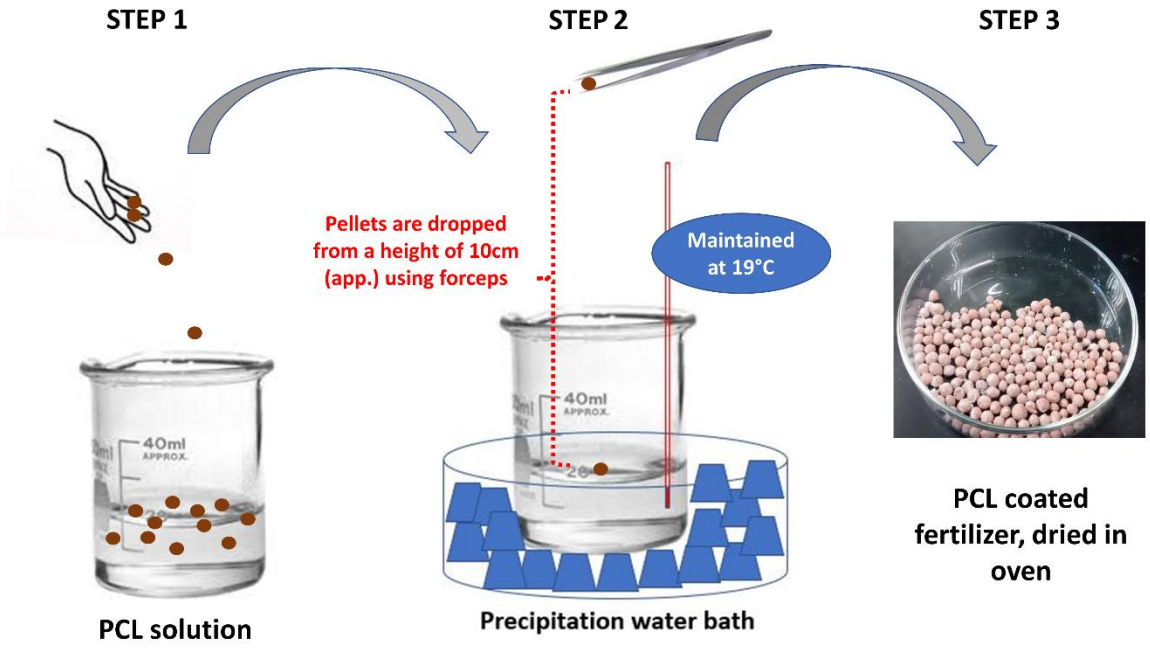


Figure 3-8 *Procedure for preparing PCL based CRF's using solvent casting technique*

3.4.2 Melt mixing

PCL, NPK and starch are melt mixed using an internal mixer (Haake Remix Polydrive R600/610 equipped with twin screw and volumetric capacity of 69 cm^3). Melt mixing was performed for 10 mins at temperature - 80°C with a rotor speed of 50 rpm. PCL was first allowed to melt for 5 mins in the mixing chamber, later followed by the addition of starch and fine powder of NPK. PCL, starch and NPK were allowed to mix for another 5 mins before collecting the blend. The collected blend was later made into sheets using compression moulding, enabling to get uniform thickness. The different concentrations of the blends prepared are presented in table. Blends were collected from the internal mixer and made into sheets using compression molder, later cut into small spherical pieces.

3.4.3 Characterisations

3.4.3.1 Field Emission scanning electron microscopy

Surface morphology of the polymer and polymer blends were examined using field emission scanning electron microscopy (FESEM, FEI Quanta 400). The polymer blends were subjected to low vacuum at an accelerating voltage of 10kV, current of 60-90mA.

3.4.3.2 Porosity using Pycnometer

The porosity of the blends was measured using the pycnometer (Lenz Laborglas, Germany), which works on the principle of Archimedes principle. Porosity was determined by the equation

$$Porosity = \left[1 - \left(\frac{Vs}{Vt}\right)\right] \times 100\% \qquad \text{(Eq 2)}$$

Where Vs is volume of the solids and Vt is the volume of the tablets

CHAPTER 4

INFLUENCE OF NPK, FE AND BIOCHAR ON THE GROWTH CHARACTERISTICS OF MUNG BEAN

4.1 Introduction

In the present chapter, second objective of this work is addressed, whereby the influence of nutrients (NPK and Fe) along with biochar on the growth and yield of mung bean was discussed. Mung bean serves as a protein rich food for the population all around the globe (*Tang et al.*, 2014). It also plays an important role in crop rotation maintaining the soil fertility. As Malaysian soils are quite leached and not fertile, bringing the need for fertilizer application. Generally, soil serves as a nutrient source but due to many reasons like environmental factors, leaching etc. plant may not be able to receive complete nutrition leading to improper growth and yields of the crop. Earlier studies (*Achakzai et al.*, 2012) (*Malik et al.*, 2003) (*Liu et al.*, 2016) (*Hossain et al.*, 2011) (*Ahmad et al.*, 2018) on the effect of individual nutrients on the growth of mung bean reported improved plant growth and along with the yield. Apart from nutrients, recently benefits of biochar in agriculture are captivating. Biochar is a carbon-rich material produced via pyrolysis of biowaste (Lehmann, 2007). The use of it has been known as an ecological engineering tool to mitigate N_2O emission from croplands while to maintain soil productively (Liu et al., 2016, Hossain et al., 2011). Hence, in this study, pot trials were carried out with the formulation of different levels of biochar (0, 10, 15 t/ha) and nutrients (NPK- 0, 15:15:15, 30:30:30 & Iron 0, 15kg/ha). Finally, the data was collected at regular intervals and later was subjected to a two-way analysis of variance (ANOVA) procedure for completely randomized design (CRD) using SPSS software.

4.2 Results and Discussion

4.2.1 Plant height, Number of leaves, Chlorophyll content, Anthocyanin content and leaf area

Influence of different levels of NPK, biochar and iron on the plant height, number of leaves, anthocyanin and chlorophyll content along with control are shown in **Figure 4-1 &Table 4-1.**

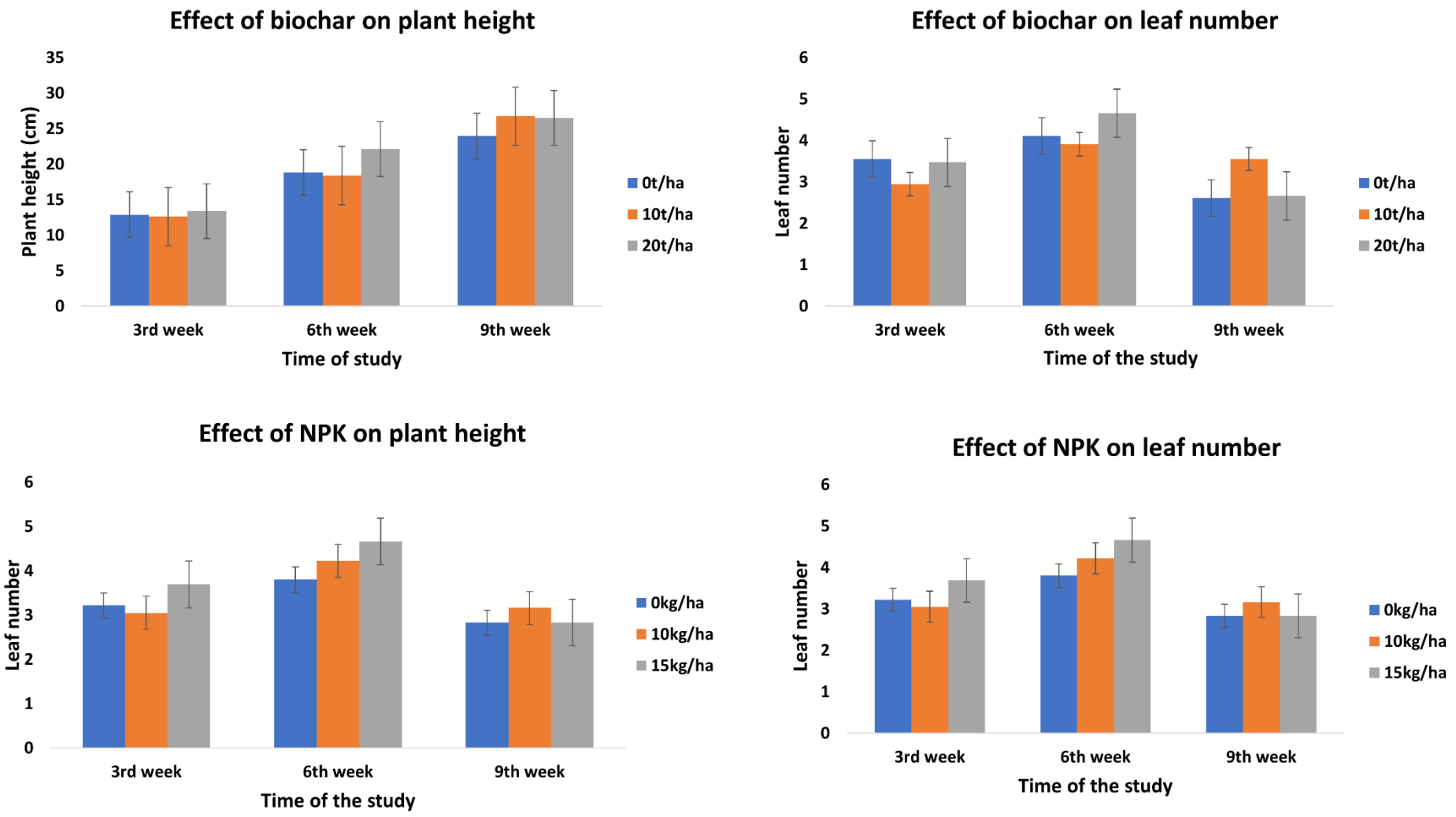


Figure 4-1 *Effect of Biochar and NPK on plant height and leaf number of mung bean.*

Figure 4-1 depicts a significant effect ($P<0.05$) on the plant height and leaf number during the application of biochar and NPK. Application of biochar at 15t/ha had recorded highest plant height at 6th week (22.10 cm) compared to the application at 10t/ha and the control (18.40 &18.82 cm, respectively). However, during the 9th week, 15 & 10 t/ha showed similar results (26.51 & 26.73 cm) and were comparatively higher compared to the control (23.95 cm). Biochar application showed a significant effect on plant height after the 3rd week. The significance of applying biochar may be attributed by the fact that it prevents fertilizer leaching, which will the improves the plant growth. These results were coherent with Graber et al. (2010) where the report says that plant height increases with the increase in biochar application and better growth when compared to control. Apparently, biochar application (at 10 & 15 t/ha) had no significant effect on the number of leaves. On the other hand, NPK application of 15kg/ha and 30kg/ha showed a significant ($P<0.05$) effect on plant height and leaf number during the trail. Higher values were recorded with 30 kg/ha at all the intervals 3rd (13.66 cm), 6th (23.13 cm) and 9th (27.59 cm) week when compared to 15 kg/ha (12.25, 18.30 & 24.39 cm) and the control (12.93, 17.89 & 24.39 cm). Applied NPK fertilizer helps to nourish the plant with essential nutrients and also improving the plant height. Besides potassium being known as quality element plays a critical role in improving the plant size. Furthermore, plant height is vital characteristics coordinating with plant productivity (Shittu, 2007). Investigations carried on the effect of nitrogen and phosphorus on mung bean reported a similar trend showing an increase of plant height with the increase in nitrogen and phosphorus content (Malik et al., 2003). Similarly, leaf number also showed a significant effect ($P<0.05$) for NPK at 3rd and 6th week WAS (weeks after sowing) recording higher values than controls.

Figure 4-2 presents the effect of different levels of NPK and biochar on chlorophyll and anthocyanin content during different growth periods of the plant. It was observed that chlorophyll content had increased with biochar application in 6th week compared to 3rd week

and later decreased during the 9th week. Furthermore, anthocyanin content had a significant effect with biochar application at 3rd and 6th week with higher values recorded than in 9th week. Besides, there is no significant effect observed with chlorophyll and anthocyanin content during the NPK application. Chlorophyll and anthocyanin content showed a similar trend, which has increased from 3rd week to 6th week, this increase is may be due to increase in leaf thickness which increases along the growth of the plant (Prsa et al., 2007). Consequently, a decrease in later weeks may be due to leaf senescence. As Table 4-1 depict no significant effect of iron on chlorophyll and anthocyanin content.

Table 4-1: Effect iron on chlorophyll and anthocyanin content

Treatment	**Chlorophyll content**			**Anthocyanin content**		
	3rd week	6th week *	9th week	3rd week*	6th week*	9th week
Iron						
0	37.35	43.77	30.99	6.99	8.54	5.53
15	37.54	45.46	37.98	6.70	8.88	7.03
SEM	1.00	1.33	2.38	0.31	0.43	0.75
P	0.892	0.37	0.04	0.51	0.58	0.16

*Within a treatment group, means in a column followed by same letter(s) are not significantly different using Tukeys test. p & SEM stands for significance and standard error mean;

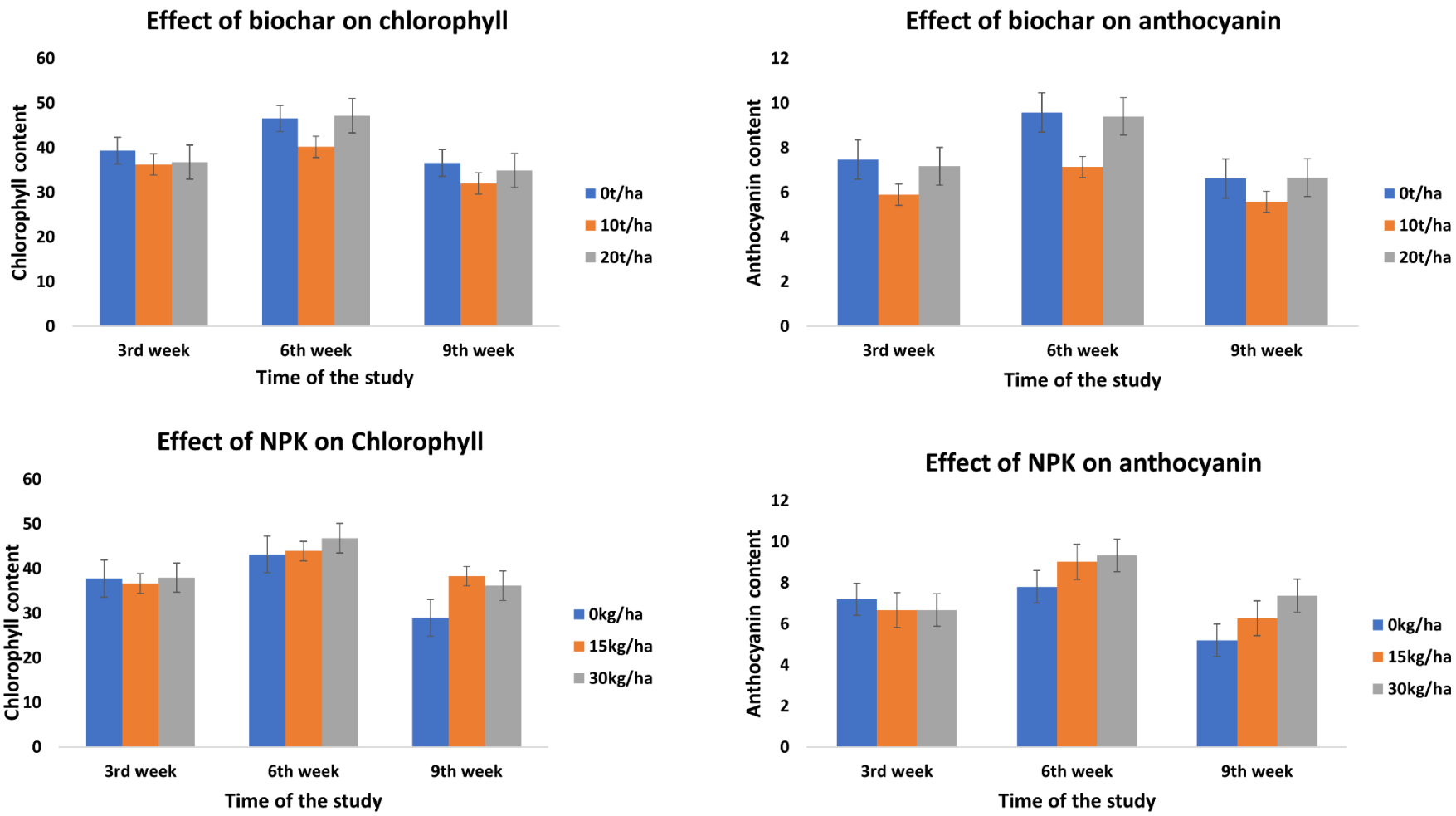


Figure 4-2 Effect of biochar and NPK on chlorophyll and anthocyanin content

Figure 4-2 depicts the influence of biochar and NPK levels on the leaf area. Application of biochar at 10 and 15t/ha had a better result than control, even though the effect is not statistically significant (P>0.05). On the other hand, application of 10 kg/ha NPK was significant showing recorded higher values (116cm^2) than the application of 30 kg/ha NPK (104 cm^2) and control (64 cm^2). The increase of leaf area with an increase in biochar and NPK can be considered rational. Biochar will improve a wide range of plant parameters: leaf area, number of nodes causing improved plant growth on a whole (Graber et al., 2010). Secondly, the availability of more potassium is known to amplify cell division and cell expansion causing the increase in leaf area (Gairola et al., 2009). Later the subsequent decrease of leaf area in the final week is may be due to change in climate conditions. Hussain et al. (2011) reported similar observations regarding the leaf area, where application of NPK (15:15:15) shows a better result than control (Hussain et al., 2011).

As for iron treatment, there is no significant (p>0.05) effect observed in plant height, leaf number, chlorophyll and anthocyanin content and leaf area of the crop. This was possibly due to the limited mobility of iron in phloem (Fang et al., 2008).

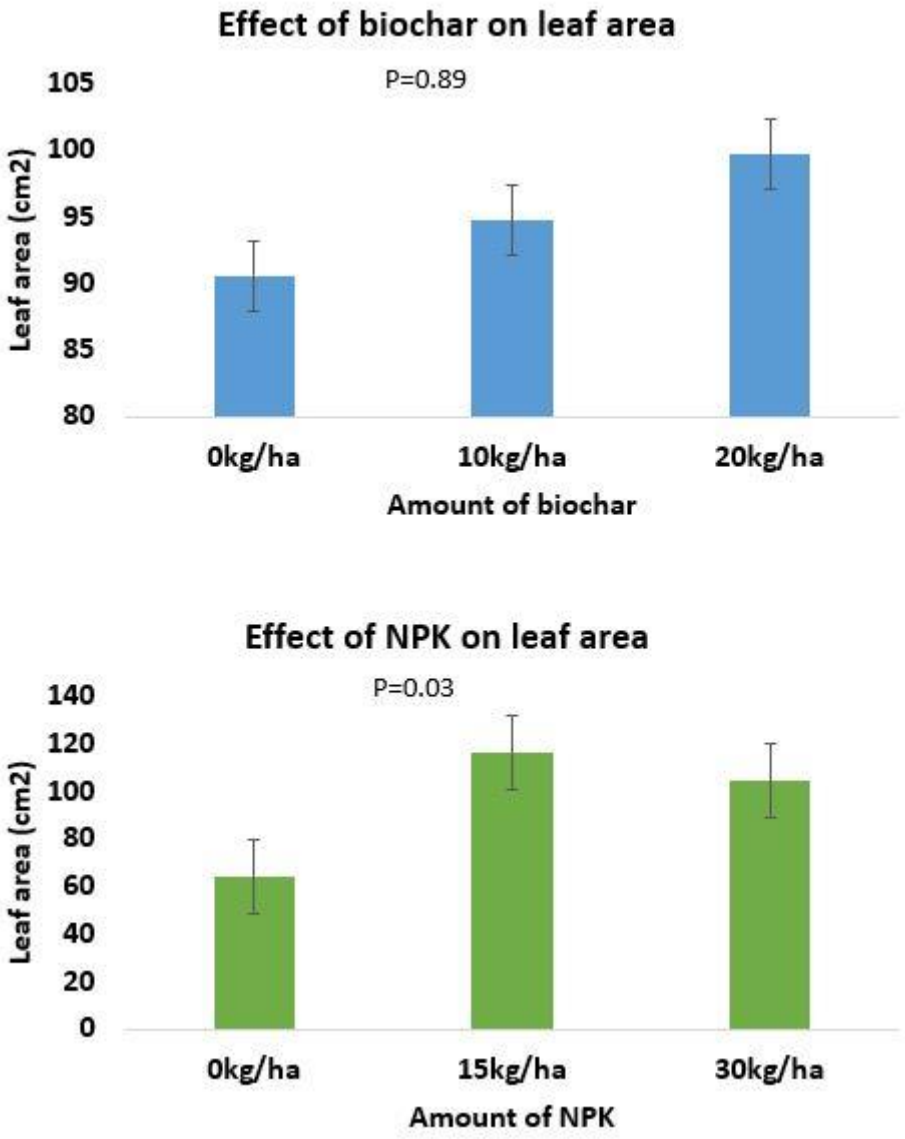


Figure 4-3 Effect of NPK and biochar on leaf area of mung bean. p stands for significance

4.2.2 Number of pods plant^{-1}, pod length, pod weight, seeds pod^{-1}, seed weight, total dry weight and harvest index

Influence of biochar, NPK and iron levels on the number of pods per plant, pod length, pod weight, seeds per pod and seed weight of green gram is presented in **Table 4-2.**

Significant ($p<0.05$) effect of NPK application on the number of pods per plant, total pod weight and grain weight of green gram was observed during the trial. Application of 30 kg

NPK/ ha recorded a higher number of pods per plant (3.83) than 15 kg NPK /ha per plant. Ahmad et al reported similar type of results, presenting 11% increase in yield when the fertilizer used to that of control (Ahmad et al., 2003). Also, application of 30 kg NPK/ha recorded the highest total pod weight and total seed weight (2.30 and 1.62 g per plant) followed by the application of 15 kg NPK/ha (1.64 and 1.17 g per plant) and the least (1.24 and 0.87 g per plant) was recorded in the treatments receiving no NPK application. Out of nitrogen, phosphorus and potassium, potassium plays a vital role in quality and quantity of the fruits/grains of the plant (Kafkafi et al., 2001). Potassium is responsible for the activation of many enzymes, like enzymes involved in photosynthesis, ATP formation and starch formation. It's also causes opening and closing of stomata. Proper regulation of stomata is essential for photosynthesis, water and nutrient uptake and preventing water loss due to transpiration. Therefore, increase in its quantity in soil may constitute better yield from the plant. Application of NPK helps in vegetative growth finally resulting in more yield from the plant. (Nadeem et al., 2004) and (Ahmad et al., 2003) reported a similar significant effect of fertilizer level on the yield of the plant. Besides, pod length, seed per pod was not influenced by NPK application.

Pod length, pod yield, seed yield and total dry weight has a significant effect on the application of biochar. Application of biochar at 15 t/ha has recorded higher values of pod weight (2.11g) and seed weight (1.50g) compared with at 10 t/ha (1.55 & 1.09g) and control values (1.57 &1.08g). Similarly, application of biochar has a significant effect ($p<0.05$) on number of pods where the higher level of biochar (15 t/ ha) produced more number of pods (4.02) compared to 10t/ ha (2.58) and control (3.07). Presence of biochar at higher levels will increase the soil field capacity, which in turn has a positive impact on plant productivity (Alburquerque et al., 2014). Shamim et al reported similar type of results, were yield increased with the increase in biochar (Shamim et al., 2015).

No significant (p>0.05) effect of iron on the number of pods per plant, pod length, seeds per pod and seed weight, pod weight, grain weight of the crop was observed during the trial.

Table 4-2 Influence of biochar, NPK and iron on the pod length, pod weight and seed weight, pods and seeds per plant, total dry weight of plant and harvest index

Treatment	Pod length (cm)	Pods per plant*	Pod weight (g) *	Seed per plant^{-1}	Seed weight(g)*	Total dry weight (g)*	Harvest index
Biochar							
0	6.79	3.08b	1.57b	5.49	1.08b	1.03b	52.36
10	6.69	2.58b	1.55b	5.67	1.09b	1.31b	48.49
20	6.76	4.02a	2.11a	5.88	1.50a	1.41a	48.47
SEM	0.248	0.263	0.158	0.382	0.115	0.08	4.62
P	0.954	0.002	0.027	0.766	0.02	0.012	0.79
NPK							
0	6.53	2.55b	1.29b	5.40	0.87b	0.90b	47.3
15	6.72	3.30b	1.64b	5.24	1.17b	1.33b	46.6
30	6.48	3.83a	2.30a	6.39	1.62a	1.53a	55.4
SEM	0.418	0.263	0.158	0.382	0.115	0.08	4.62
P	0.70	0.006	0.00	0.084	0.00	0.001	0.33
Iron							
0	6.74	3.18	1.74	5.7	1.22	1.193	49.49
15	6.75	3.27	1.75	5.64	1.22	1.32	50.06
SEM	0.202	0.214	0.129	0.312	0.094	0.071	3.7
P	0.967	0.762	0.929	0.879	0.969	0.20	0.91

*Within a treatment group, means in a column followed by same letter(s) are not significantly different using Tukey's test. p & SEM stands for significance and standard error mean; a& b are subsets indicating the significant difference between each level.

According to **Table 4-2**, Significant (p<0.05) effect on total dry weight of the plant was observed in the NPK and biochar application, Application of 15 t biochar /ha recorded 1.41gms of total dry weight which was comparatively higher than the value observed at 10t biochar/ha (1.31g) & control (1.03). On the other hand, 30 kg NPK/ha has a higher value of dry weight (1.53g), followed by the application of 15 kg NPK/ha (1.33g) and the least (0.90) was recorded

in the treatments receiving no NPK application. Improved dried weight can be associated with an increase in leaf area, which enhances photosynthesis leading to the growth of plant. Subsequently, results in improved dry matter (Gairola et al., 2009). NPK and biochar application had no significant effect on harvest index

4.2.3 Root length, root area, root volume, root diameter and nodules

Influence of biochar, NPK and iron levels on the root length, root volume, root diameter, root surface area and nodules are depicted in **Figure 4-3 & 4-4. Figure 4-6 to 4-8** depicts the difference between the growths of roots for all the treatment combinations.

Out of different parameters of root, significant ($p<0.05$) effect of NPK application on root length, root volume, root surface area and nodules were observed during the trial. Application of 30 kg NPK/ha on the crop resulted in the highest root length (2236.3cm), root volume (4.4cm^3), root surface area (353 $cm^{2)}$ and nodules (67) followed by the application of 15 kg NPK/ha (1947 cm, 3.6 cm^3, 296 cm^2 & 30), and the least (1621 cm, 2.9 cm^3, 242 cm^2 & 13) was recorded in the treatments receiving no NPK application. Phosphorus and potassium are the two major nutrients instigate the growth of roots in plants. Specifically, they encourage plants to grow more number of roots strengthen existing root system (Fageria and Moreira, 2011). This means that high levels of phosphorus and potassium especially help to develop the different root parameters during the active growing season. Previous studies on the effect of nitrogen and phosphorus on root development reported improved root length with high levels of nutrient supply (Fageria et al., 2014, Fageria, 2016). In **Figure 4-6, 4-7 and 4-8**, image (f) depicts that the roots treated with a higher amount of fertilizer showing denser roots than other treatment and control. Similarly, number of nodules are higher when treated with NPK compared with controls where it's almost zero. However, in **Figure 5-3,** long error bars have been noticed, indicating there is large spread out between the concentrations of the values. As

depicted in figure 5-3, the difference between each concentration is more than usual, which could have led to these long error bars. In **Figure 4-6, 4-7 and 4-8**, image (f) depicts that the roots treated with a higher amount of fertilizer showing denser roots with more number of nodules than other treatment and control depicted in the image (a). However, the root diameter is not statistically significant ($P<0.05$), but it still shows the highest value for high level of NPK (30 kg/ha) compared to NPK at 15 kg/ha and control.

Similarly, application of 15 t/ha of biochar has a significant effect on root length (2247 cm), root volume (4.5 cm^3), root diameter (0.50 mm) and root surface area (355 cm^2) compared to application at 10 t/ha and control, whose values are almost similar (root length (1867cm), root volume (3.29 cm^3), root diameter (0.45 mm) & root surface area (259 cm^3). Improved root parameters along with biochar level is possibly due to its nutrient and water retention property along with increase in availability of ions in the soil (Howard, 2011). Xiang et al studied the effects of biochar on different root parameters and suggests that application of biochar will benefit the development of root morphology (Xiang et al., 2017). Further studies on the effect of biochar on root systems of soybean and crabgrass suggest the beneficial and positive effect of biochar on roots (Wang et al., 2018, Mitchell et al., 2018). Roots in **figure 4-6** have no application of biochar, whereas the **figure 4-7 & 4-8** depicts roots treated at 0 t/ha and 15 t/ha. It is clearly evident that the roots in **Figure 4-8** are thicker and condense than **Figure 4-7 & 4-6**, out of all the biochar treatments the growth in control is much lesser. Number of nodules does not show much effect with biochar application.

No significant ($p>0.05$) effect of iron application on root length, root volume, root diameter, root surface area and nodules were observed during the trial.

Table 4-3 *Influence of NPK, Biochar and Fe levels on the different parameters of the plant root*

Treatment	Nodules	Root length (cm)	Root volume (cm^3)	Root diameter (mm)	Root surface area (cm^2)
Iron					
0	37.96	2011.26	3.67	0.473	303.80
15	35.96	1858.97	3.68	0.48	291.15
SEM	5.611	119.04	0.261	0.010	19.113
P	0.802	0.372	0.983	0.320	0.643

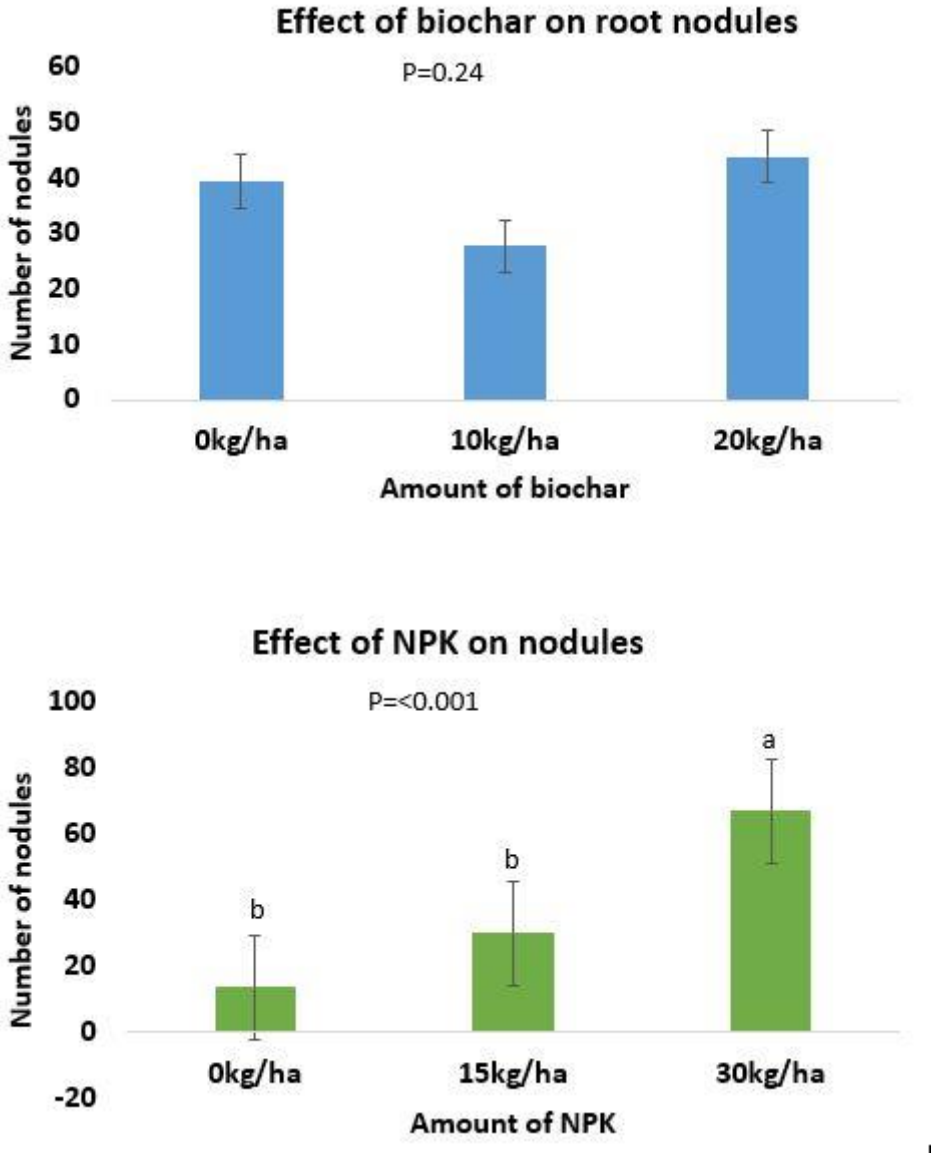


Figure 4-4 : Effect of biochar and NPK on nodules of mung bean. Within a treatment group, bars with the same letters are not significantly different using Tukey's test at 5% level. SE bars are standard deviation values. *p stands for significance; a& b are subsets indicating the significant difference between each level.*

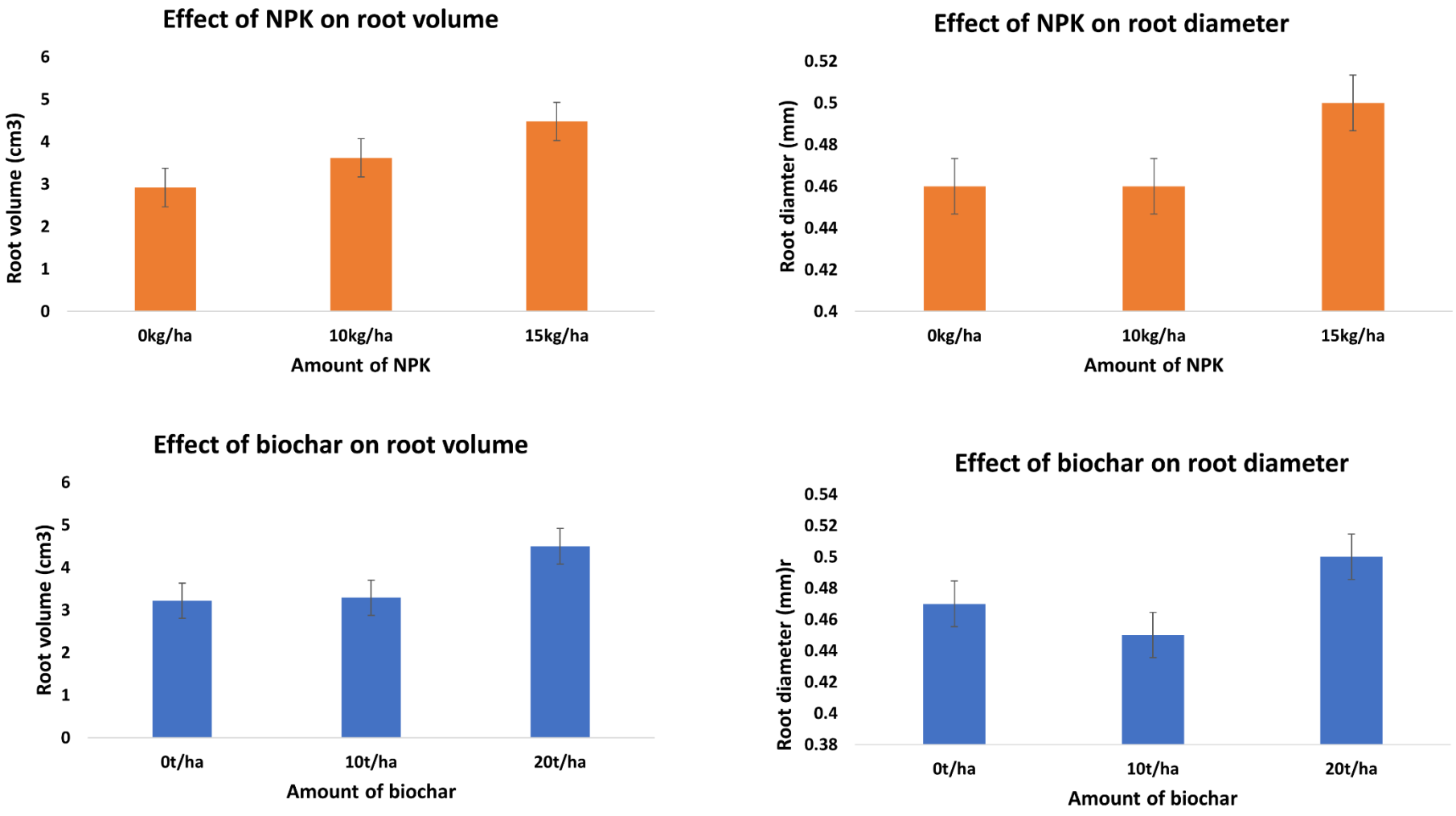


Figure 4-5 *Effect of NPK and biochar on root volume and root diameter*

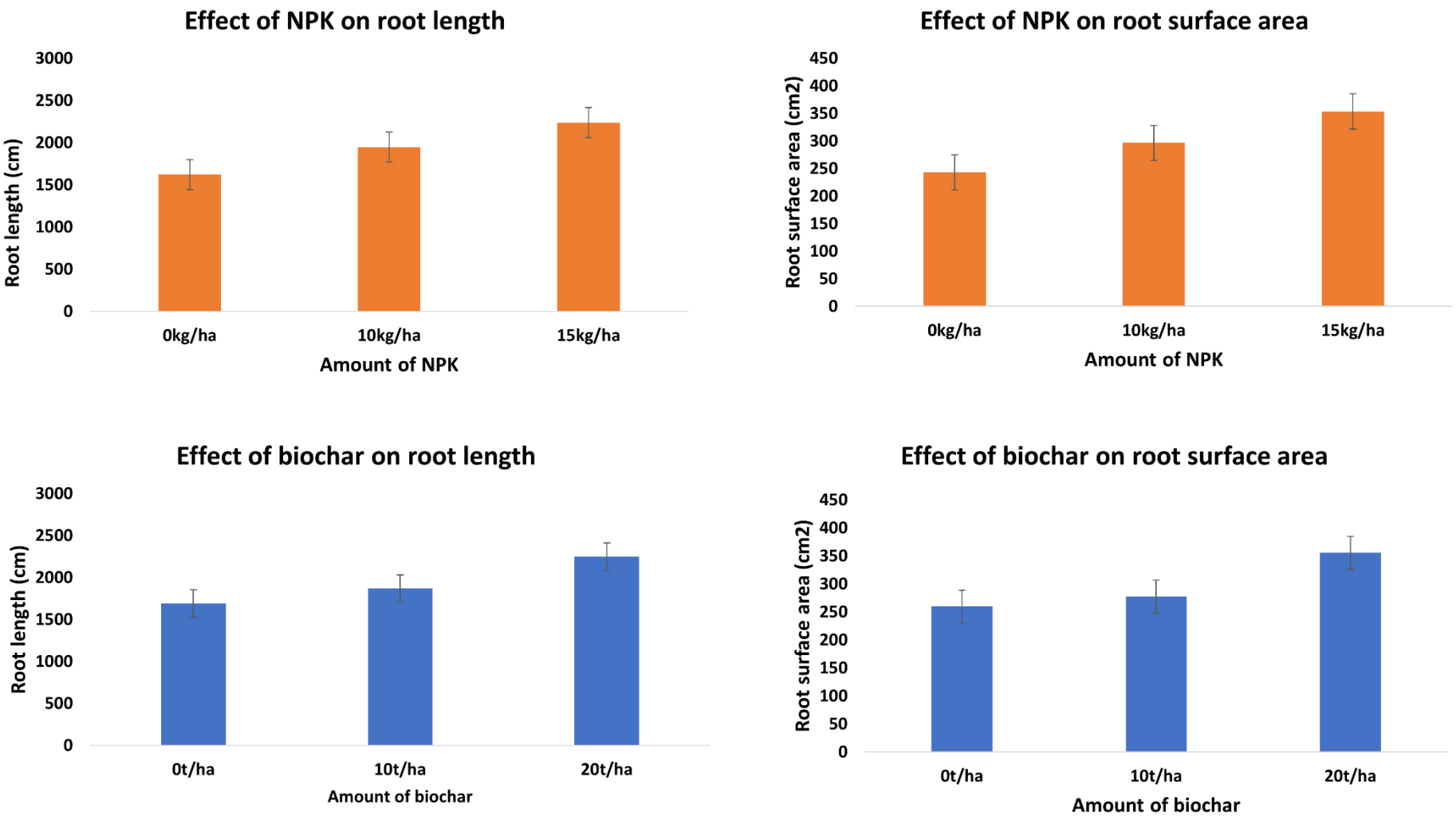


Figure 4-6 : Effect of biochar and NPK on root parameters of mung bean.

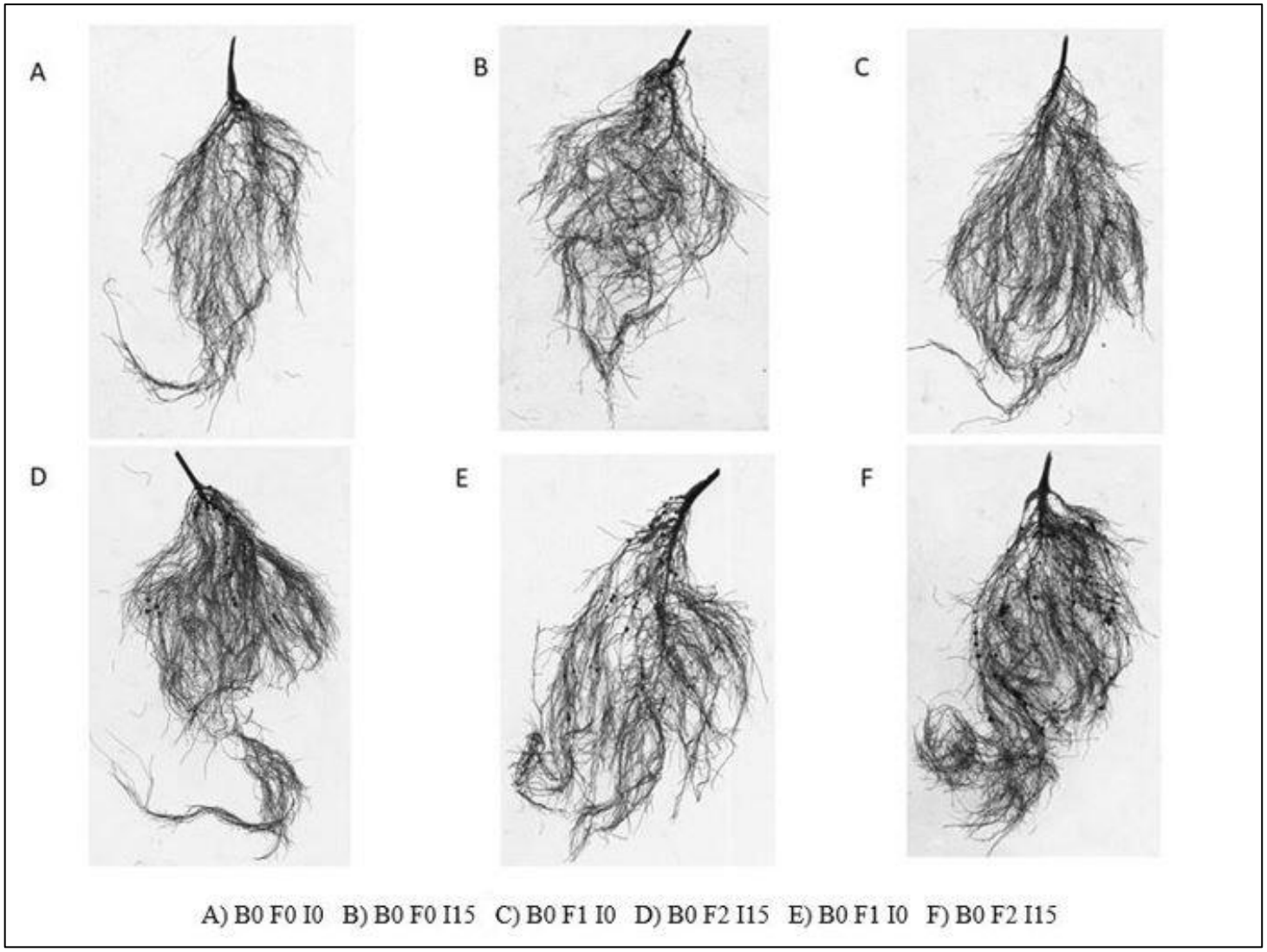


Figure 4-7 : Plant roots with different treatments (B0- No Biochar, F0- No fertilizer, I0 – No iron, I15 – 15 kg/ha, F1- NPK at 15 kg/ha, F2 – NPK at 30 kg/ha)

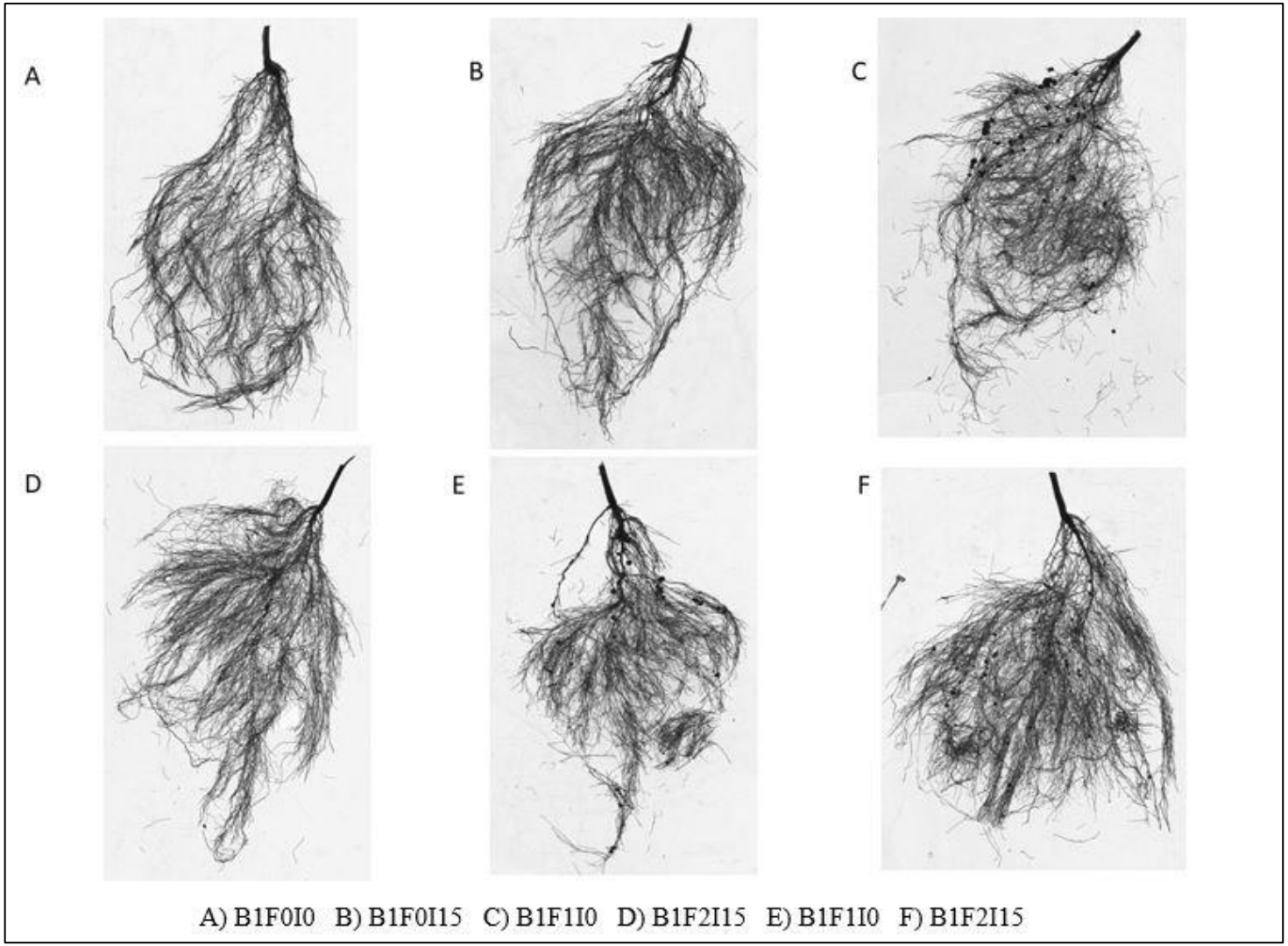


Figure 4-8 : (B1- Biochar at 10 kg/ha, F0- No fertilizer, I0 – No iron, I15 – 15 kg/ha, F1- NPK at 15 kg/ha, F2 – NPK at 30 kg/ha*)*

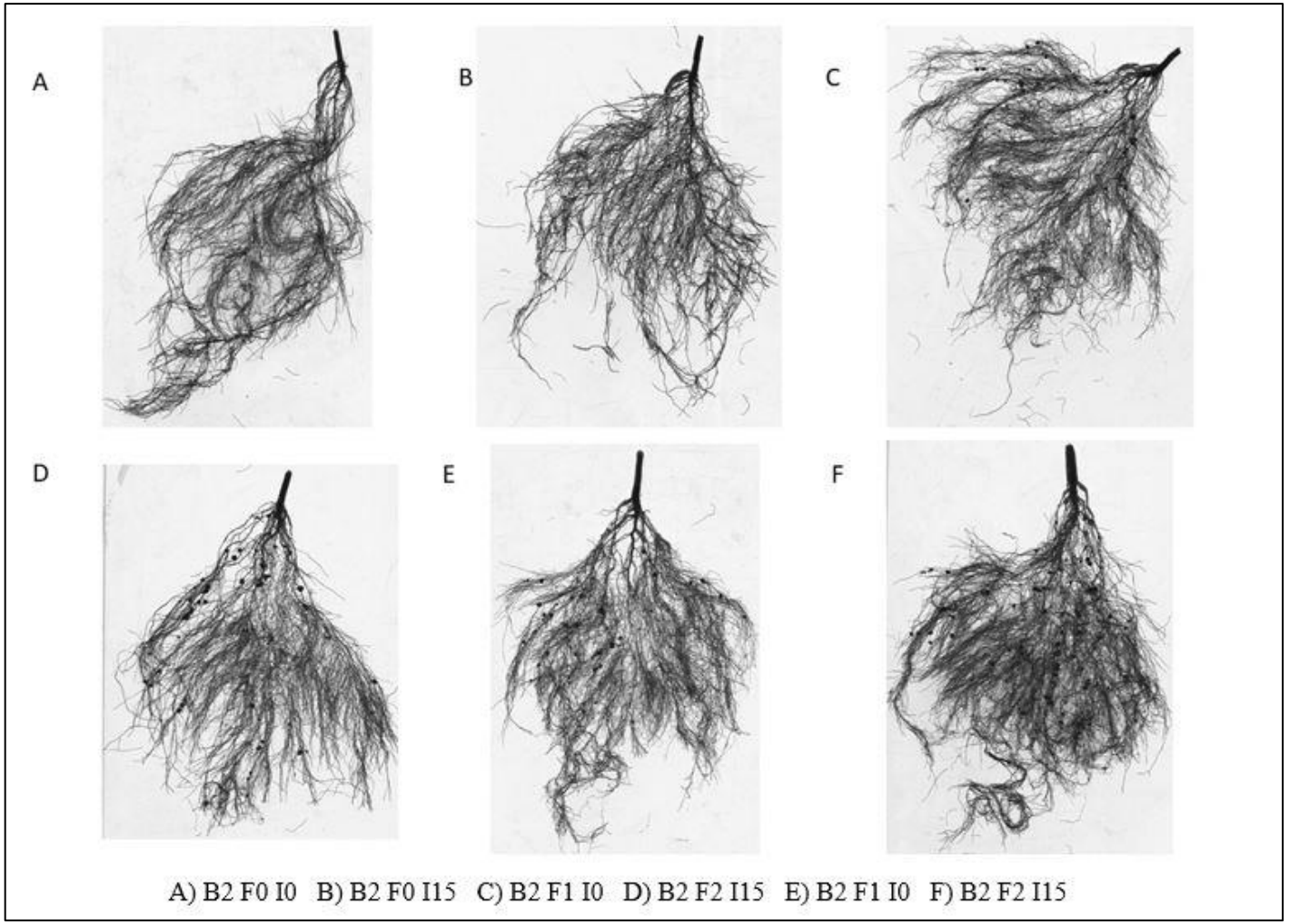


Figure 4-9 : (B2 – Biochar at 15 kg/ha, F0- No fertilizer, I0 – No iron, I15 – 15 kg/ha, F1- NPK at 15 kg/ha, F2 – NPK at 30 kg/ha)

4.3 Conclusion

Results obtained revealed a significant effect of NPK application at 30kg/ha and biochar application at 15 t/ha on the growth characteristics of mung bean. The yield has been increased by 86% and 38% with application of NPK and biochar compared with control. Whereas the application of iron did not influence the performance of the crop at any stage. But it cannot be suggested as the optimised amount since the next level of application may or may not show a significant effect. Therefore, further studies should be carried out for optimization. From this study, the application of 30 kg NPK /ha can be adopted for increased production of the crop in the study area. Besides, the application of biochar at 15 t/ha also can have a huge influence on the performance of the crop. Higher concentration of Iron (> 15kg /ha) can be considered for further studies.

CHAPTER 5

OPTIMISATION OF PROCESS PARAMETERS FOR PCL SYNTHESIS VIA ENZYMATIC POLYMERIZATION

5.1 Introduction

This chapter addresses the first objective of this study i.e. synthesis of PCL using enzyme catalyst CALB and optimisation of process parameters using design of experiments. The PCL synthesis was carried out using a thermostatic magnetic stirring process. Initially, the optimisation of different process parameters such as the temperature (50-110°C), reaction time (1-7 hrs), mixing speed (50-500 rpm) and monomer/solvent ratio (1:6) and their effect on the polymer molecular weight was investigated using D optimal in Design Expert 9^{TM}. In general, the quality of the synthesised PCL is assessed by its molecular weights (M_n and M_w). This is because the physical, thermal, mechanical properties and degradation rate of PCL are greatly influenced by molecular weights (number average molecular weight and weight average molecular weights) Meanwhile, this molecular weight is dependent on process parameters such as temperature, time, and speed of the. Previously, many researchers have studied the effect of these parameters through single factor analysis using various. Among them, Kumar et al. synthesised PCL using *Candida antarctica lipase B* enzyme at flask level, optimising process parameters—temperature, monomer/solvent ratio, and time in a single variate approach. Therefore, the novelty of this objective lies in the optimization of process parameters multi variate approach, using response surface methodology (RSM) based on D-optimal design. Moreover, it will also offer interaction between the individual parameters. Apart from optimisation process, synthesis of PCL using TLL as catalyst and the characterisation of the synthesised PCL was also carried out. Later, PCL was synthesised using the TLL as a catalyst and further effect of enzyme concentration and effect of different solvents

were studied. Finally, the thermal properties of the synthesised PCL from CALB and TLL with respect to neat PCL were also discussed.

5.2 Design of experiments

A statistical approach with D-optimal design was used to optimize the process parameters for polycaprolactone (PCL) synthesis. The variables selected were temperature (50-110°C), time (1-7 hrs), mixing speed (50-500 rpm) and monomer/solvent ratio (1:1-1:6). Molecular weights i.e. Mn and Mw were chosen as responses and were determined using matrix-assisted laser desorption/ionization time of flight (MALDI TOF). Mn is the total weight of all the polymer molecules divided by the total number of polymer molecules in the samples whereas the Mw is calculated based on the fact that bigger molecules contain more total mass of polymer. In general, the quality of the synthesised PCL is assessed by its molecular weight. This is because the physical, thermal, mechanical properties and degradation rate of PCL are greatly influenced by molecular weight (Lam et al., 2007). Meanwhile, this molecular weight is dependent on process parameters such as temperature, time, and speed of the stirrer (Kumar and Gross, 2000). Using the D-optimal method in design of experiments, the interactions between parameters and responses were analysed and validated. DOE will emphasize the interactions of parameters in the process with the minimal number of experimental runs. This complete knowledge can lead to breakthroughs in understanding the process, therefore minimising the production cost with improved quality and profits. It also has an adaptable structure allowing the numeric factors in either way i.e. discrete or continuous manners, unlike other RSM designs such as Box–Behnken and CCD where the parameters are varied on fixed levels. This study primarily aims to determine the combination of experimental parameters that will yield polymer with desired molecular weight.

5.2.1 Modelling using ANOVA

25 sets of experiments were developed by D-optimal factorial design with different combinations of experimental parameters along with predicted and experimental values are presented in **Table 5.1**. The outcome of experimental design was used to fit the observed data into polynomial equation (Equation 3 & 4) which represents the effect of parameters and their possible interactions.

$$Y1 = +2066.17 - 402.69\,A - 249.36B + 632.12C - 363.18D + 749.33AC + 285.58AD - 510.50BC - 226.18BD \quad \textbf{(Eq 3)}$$

$$Y2 = +2807.72 - 527.93A - 472.72B + 994.31C - 488.10D + 1006.59AC + 352.96AD - 768.67BC - 327.46BD \quad \textbf{(Eq 4)}$$

Where Y1 & Y2 represents number average molecular weight (M_n) and weight average molecular weight (M_w). A, B, C & D are temperature, time, mixing speed and monomer/solvent ratio. **Table 5-2 & 5-3** present the ANOVA results of responses i.e. number average molecular weight (M_n) and weight average molecular weight (M_w)

Table 5-1: Experimental and predicted values of the polymer molecular weight

Run	Factor A	Factor B	Factor C	Factor D	Response 1 (M_n)			Response 2 (M_w)		
					Experimental	Predicted	% error	Experimental	Predicted	% error
1	110	7	50	10	1127.19	1163.68	3.2	1321.65	1360.37	2.8
2	85	4	50	30	1024.77	1048.39	2.3	1339.35	1333.85	0.4
3	101	1	500	60	1950.09	2134.14	8.5	2878.90	2895.28	0.5
4	79	1	219	10	2222.66	2312.51	3.8	2988.62	3162.81	5.4
5	50	1	500	20	1845.60	1988.71	7.1	2690.50	2736.97	1.6
6	50	7	500	10	2415.70	2349.03	2.7	3138.45	3222.98	2.6
7	110	7	405	10	2073.90	2199.21	5.6	2970.90	3003.60	1.0
8	50	6	500	60	982.67	1036.49	5.1	1226.52	1296.51	5.4
9	110	7	500	60	2228.15	2280.82	2.3	2972.98	2918.81	1.9
10	110	1	500	10	2395.00	2386.43	0.3	3188.27	3237.10	1.5
11	50	4	50	10	3143.41	2980.75	5.1	3986.71	3868.87	2.9
12	50	1	151	30	2752.80	2693.05	2.1	3769.29	3569.59	5.3
13	80	7	50	60	1124.10	1165.46	3.5	1323.51	1317.25	0.4
14	110	4	208	60	1025.66	1116.72	8.1	1314.81	1447.24	9.1
15	50	7	500	10	2525.27	2349.03	6.9	3441.78	3322.98	3.4
16	101	1	500	60	3591.17	3387.14	5.6	5201.45	4995.28	3.9
17	110	4	208	60	3762.88	3826.72	1.6	5754.22	5697.24	0.9
18	87	5	500	30	2614.02	2482.33	5.0	3422.34	3486.35	1.8
19	50	7	223	40	1634.90	1620.72	0.8	2912.30	2789.69	4.2
20	50	1	50	60	1824.04	1872.66	2.5	2548.47	2672.09	4.6
21	110	1	50	30	1072.89	1125.70	4.6	1405.22	1516.98	7.3
22	110	1	500	10	4390.20	3986.43	9.2	5925.86	5839.10	1.5
23	99	7	259	40	1643.84	1701.62	3.5	2965.51	2888.17	2.6
24	50	1	500	20	3379.52	3288.71	2.6	5116.38	5136.97	0.4
25	70	3	311	60	2590.79	2498.78	3.5	3517.86	3401.74	3.3

The model adequacy is determined by F and p values. F value is the ratio of means (means of squared deviations and means of squared error). The F value greater than 4 indicates a notable

effect of parameters on the chosen response (Yang and Tarng, 1998). From the ANOVA tables of the responses shows that the F value for responses (M_n & M_w) are 15.98 and 17.02 respectively indicating that all the four parameters chosen have a significant effect on determining the molecular weight of the polymer. Finally, p value lesser than 0.05 indicates a significant model. As the p values for Mn & Mw are 0.004 & 0.003, the model developed is significant.

Table 5-2 : ANOVA analysis for number average molecular weight (Mn)

Source	Sum of squares	Degree of freedom	Mean of squares	F	p-value Prob > F
Model	1.139	8	1.423	15.98	0.0004 (significant)
A-Temperature	1.111	1	1.111	12.47	0.0077
B-Time	4.305	1	4.305	4.83	0.0591
C- RPM	3.615	1	3.615	40.59	0.0002
D- Volume of solvent	1.426	1	1.426	16.01	0.0039
AC	2.940	1	2.940	33.01	0.0004
AD	6.691	1	6.691	7.51	0.0254
BC	1.353	1	1.353	15.19	0.0046
BD	4.280	1	4.280	4.81	0.0597
Residual	7.125	8	89061.34		
Lack of fit	7.065	7	1.009	16.81	0.18 (non-significant)
Pure error	6003.19	1	6003.19		
Core total	1.210	16			
SD	424.79	R^2	0.9445		
Mean	2917.71	Adj R^2	0.8890		
CV	14.56	Pred R^2	0.7075		
PRESS	7.609	Adeq Precision	14.325		

Table 5-3 : ANOVA analysis for weight average molecular weight (Mw)

Source	Sum of squares	Degree of freedom	Mean of squares	F	p-value Prob > F
Model	2.45	8	3.071	17.2	0.0003 (significant)
A-Temperature	1.91	1	1.910	10.58	0.0116
B-Time	1.54	1	1.547	8.57	0.0191
C- RPM	8.94	1	8.945	49.57	0.0001
D- Volume of solvent	2.57	1	2.576	14.27	0.0054
AC	5.30	1	5.305	29.40	0.0006
AD	1.02	1	1.022	5.66	0.0446
BC	3.06	1	3.068	17.00	0.0033
BD	8.97	1	8.972	4.97	0.0563
Residual	1.44	8	1.804		
Lack of fit	1.39	7	1.997	4.34	0.354 (non-significant)
Pure error	46004.54	1	46004.54		
Core total	2.601	16			
SD	293.43	R^2	0.9411		
Mean	2155.64	Adj R^2	0.8822		
CV	13.84	Pred R^2	0.7283		
PRESS	3.287	Adeq Precision	13.44		

Others terms like R^2 (multiple correlation coefficient) and its value near to 1 signifies the model is fit. The value of R^2 for M_n and M_w is 0.941 and 0.944 implying the model is well fitted. On

the other hand, the difference between Adjusted R^2 and predicted R^2 must be less than 0.2. From **Table 5-2** we can notice that the difference between Adjusted R^2 and predicted R^2 for M_n and M_w is 0.15 and 0.16 indicating the significance of the developed model. Both R2 and the adjusted R2 give the idea of how many data points fall within the line of the regression equation. However, the main difference between R2 and the adjusted R2 is that R2 assumes that every single variable explains the variation in the dependent variable whereas the adjusted R2 tells the percentage of variation explained by only the independent variables that actually affect the dependent variable. Where, the independent variables (e.g. parameters chosen - temperature, time, mixing speed and monomer/solvent ratio) are the one which can be controlled and will affect the dependent variable (e.g. response chosen - polymer molecular weight, which changes in response to the independent variables). One more statistic here is the one-labelled Adeq Precision. It measures the ratio of the range of variation in the predicted response to an estimate of the standard error of the predictions. Its value more than 4 indicates that the variation we are observing is large in relation to the underlying uncertainty of the fitted model (Maran et al., 2013). It can be noted from **Table 5-2 & 5-3** that the value of Adeq precision for M_n and M_w are 14 & 13 respectively,. clearly suggesting that the model is fit.

Apart from these terms, evaluation of the model can be done by the normality plot. Ideally, the plot should be a straight line indicating no abnormalities. As shown in **Figure 5-1**, the normality plot was satisfied as residual plots follow approximately a straight line following normal distribution of data without any deviation. **Figure 5-2** depicts actual vs predicted plot for M_n and M_w. These plots show how the model predicts over the range of data. The random scatter of the data falling on both the sides of regression line indicates good agreement between that the predicted and actual values. **Figure 5-3** shows the plot of residual vs run number, it is used to check the regression model. Generally, an ideal plot has all the points between within the line ± 4 without any trend. As it is evident from the **figure 5-3**, data points did fall between

the lines implying the adequacy of the proposed model. On the other hand, data does fall between the ± 4 residual without any outliers inferring the model significance.

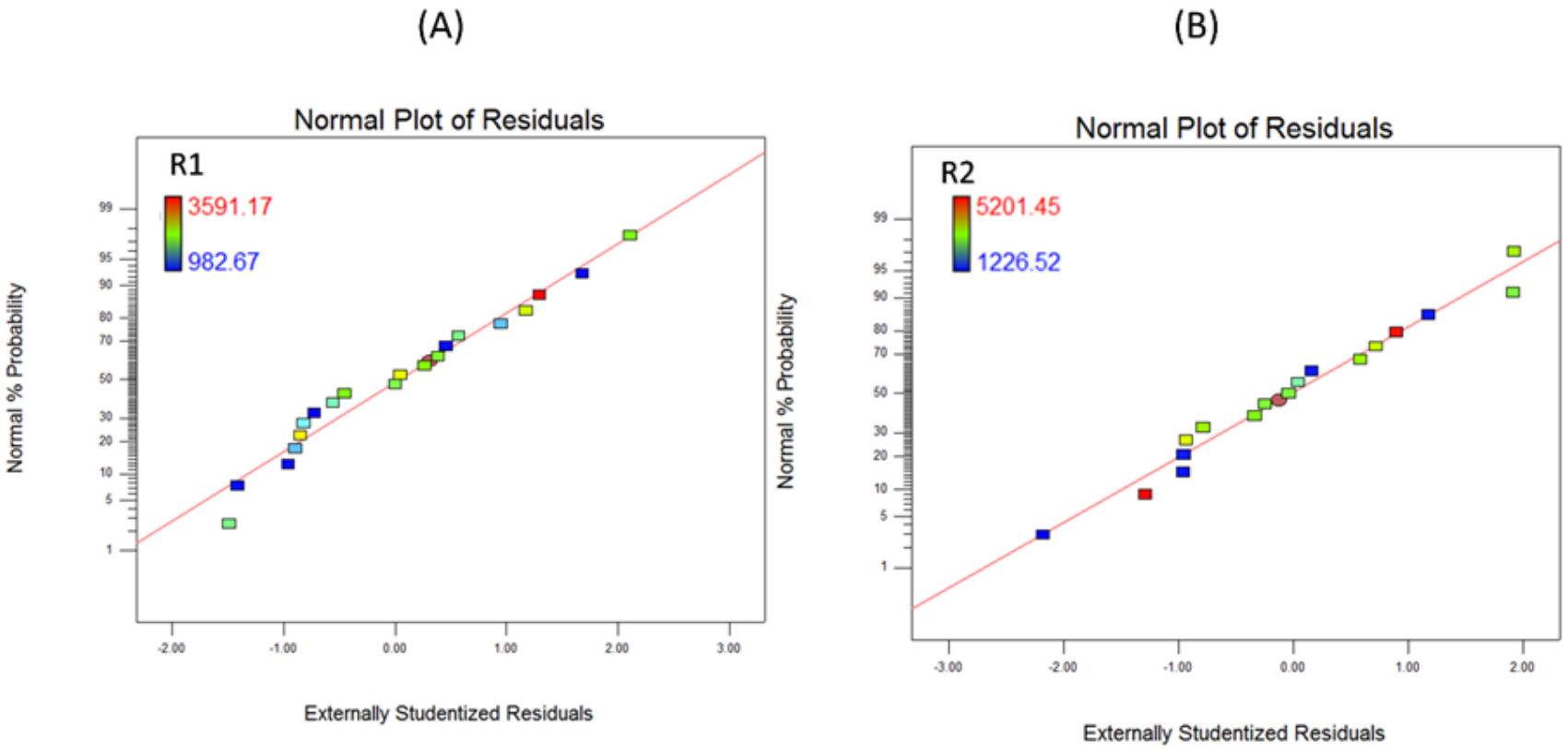


Figure 5-1: a) Normal plot for M_n b) Normal plot for M_w

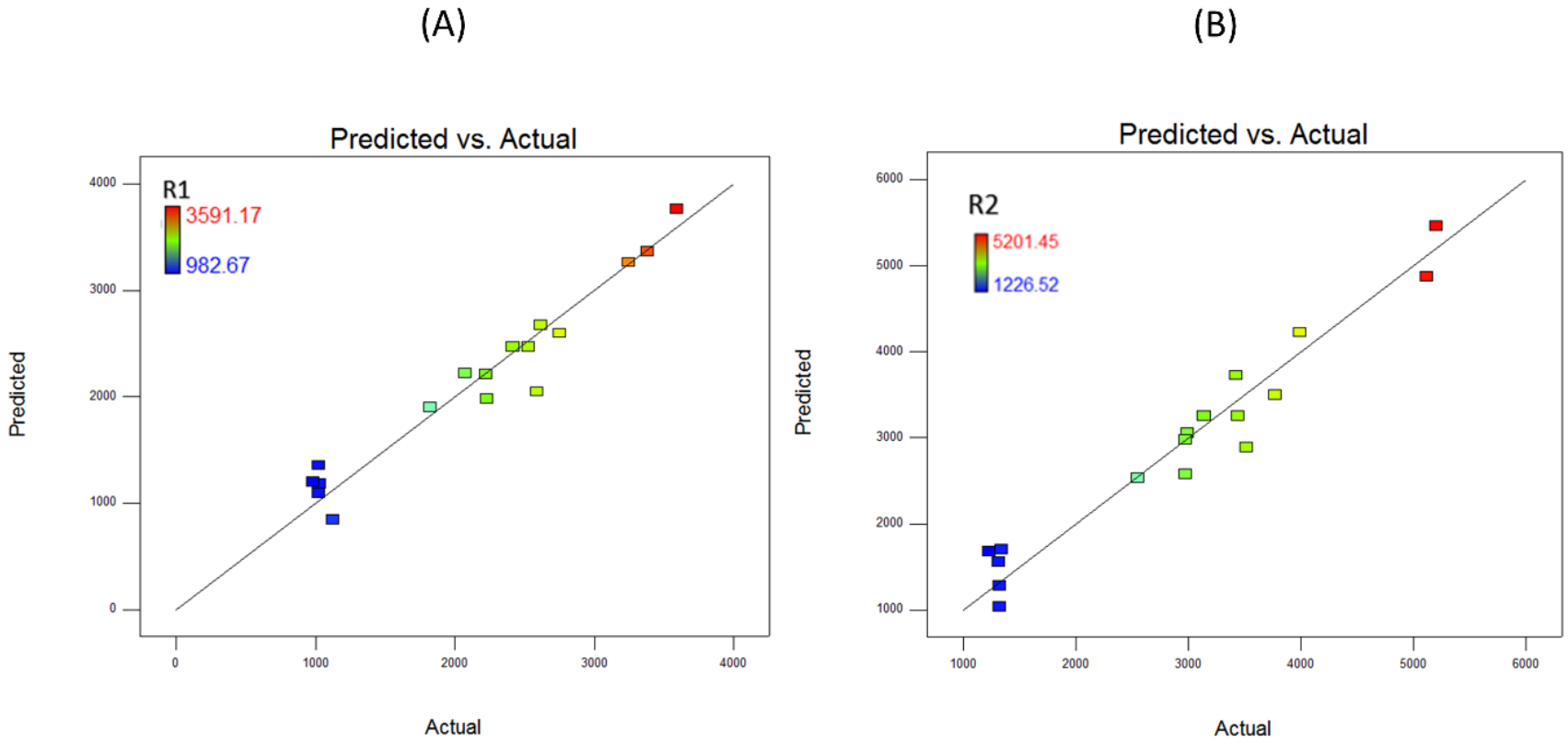


Figure 5-2 : a) Actual vs predicted plot for M_n b) Actual vs predicted plot for M_w

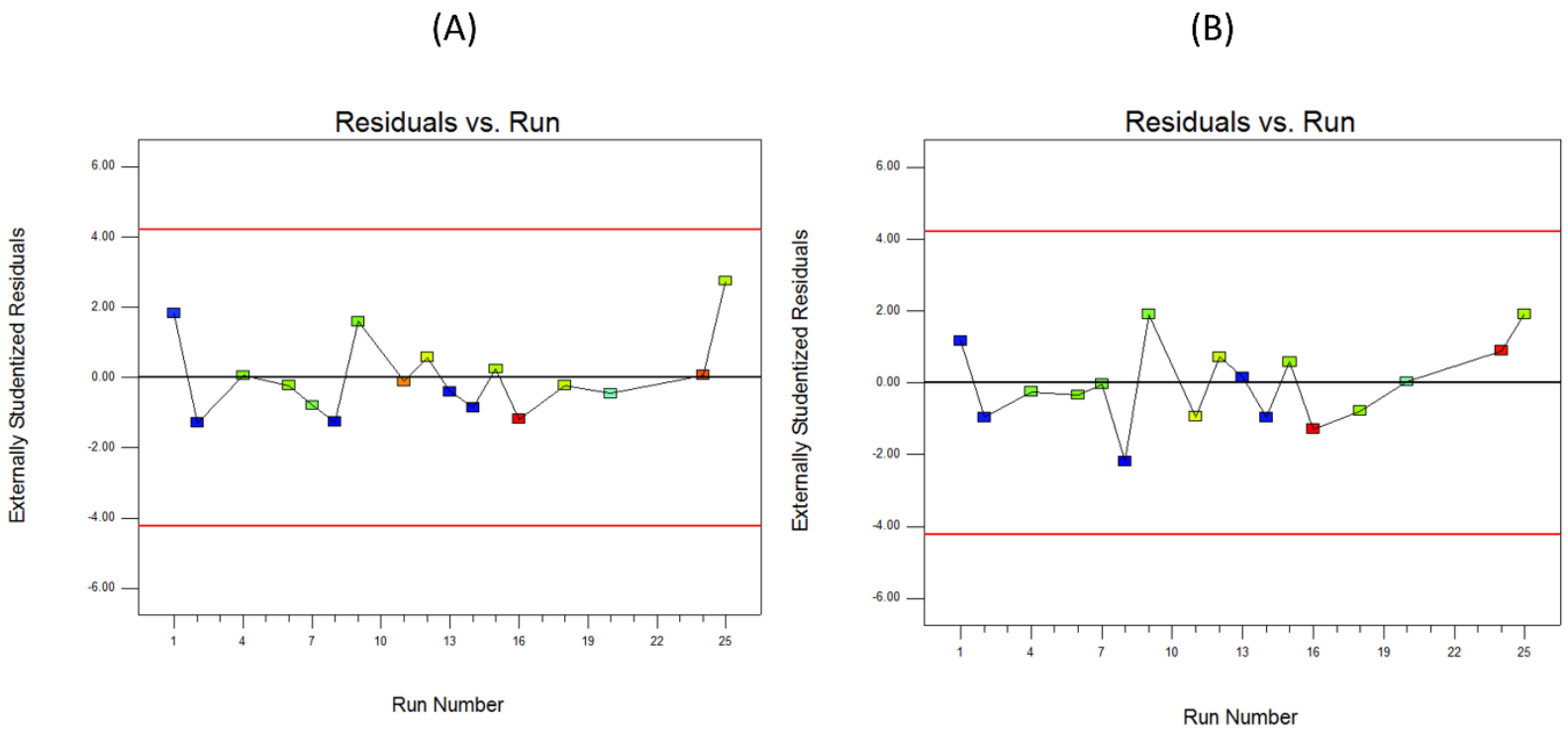


Figure 5-3 : a) Studentized residual plot for Mn b) Studentized residual plot for Mw

5.2.2 Response surface plots

3D response surface plots analyse the effect of variables on the response. They allow getting a deeper understanding of a process and identifying the process conditions to satisfy the goal. **Figure 5-4 & 5-5** depict the interactions between the process parameters and the chosen responses i.e. M_n and M_w. Figure 5-4a & 5-5a show that at low temperature with a constant increase in mixing speed has led to an increase in molecular weights. This may be due to the fact that increase in rpm may cause more diffusion, allowing more interactions (Fathi Roudsari et al., 2015). Further, more chances for molecules to react allowing more linkage of the monomers forming polymer chains. Moreover, from Figures 5-4a & 5-5a, it is also observed that the effect of temperature is inversely proportional to polymer molecular weight. Findings from Hunsen *et al*. and Kumar *et al*. stated that increase in temperature may cause protein denaturation which in turn may induce the reduction of enzyme activity (Kumar and Gross, 2000, Hunsen et al., 2008).

Figure 5-4b & 5-5b explain the combined response of process temperature and volume of solvent on the molecular weight distribution of the polymer. It also indicates that low temperature and low volume of solvent favours the synthesis of high molecular weight polymer. Solvents in enzymatic polymerization act a lubricant and will help to make solution less viscous and absorb the heat during the reaction (Klibanov, 2001b). This heat absorption will reduce the rate of reaction. Therefore, in order to avoid maximum reduction of reaction rate, solvent should be in minimum amounts. Evidently, previous studies also reported higher molecular weight of the polymer when less amount of solvent is used (Kumar and Gross, 2000).

The combined effect of mixing speed and reaction time on the molecular weight distribution of the synthesised polymer is depicted in Figure 5-4c & 5-5c. Higher molecular weights were

observed at the highest mixing speed (500 rpm) and less reaction time (1 hr). Higher molecular weights were achieved during the first hour of polymerisation, this may be due to the maximum mixing speed causing more interactions. Similarly, Figure 5-4d & 5-5d depicts the combined effect of time and volume of solvent. It shows that the maximum result has occurred when using less volume of solvent (10 ml) at less reaction time (1 hr). It can be seen from the above analysis that reaction temperature and mixing speed has more impact on the bio-polymerization process compared to time and volume of solvent. Therefore, this report presents the importance of mixing speed in polymer synthesis. Moreover, it is important to note the interaction of all the four process parameters and their effect on the molecular weight of the polymer is quite notable. Besides, from previous studies, it is noted that the range of process parameters used does not affect the enzyme activity. Yadav *et al* studied on reusability and effect of mixing speed (up to 800 rpm) on transesterification catalysed by novozyme 435. They reported an increase in the rate of conversion with increasing mixing speed, and the activity of the enzyme remains as fresh enzyme, unaffected when reused after washing and later drying under vacuum (Yadav and Devi, 2004). Mahapatro *et al* studied the novozyme 435 activity at different reaction times, reported that enzyme retains its 75% activity even after 48 hr at 90°C (Mahapatro et al., 2004). This implies no decrease in enzyme activity even at a high rate of mixing speed, high temperature and long reaction time additionally good for reusing as well. In this work, the enzyme beads are collected after the process without any disruption, which can be reused for the next reactions.

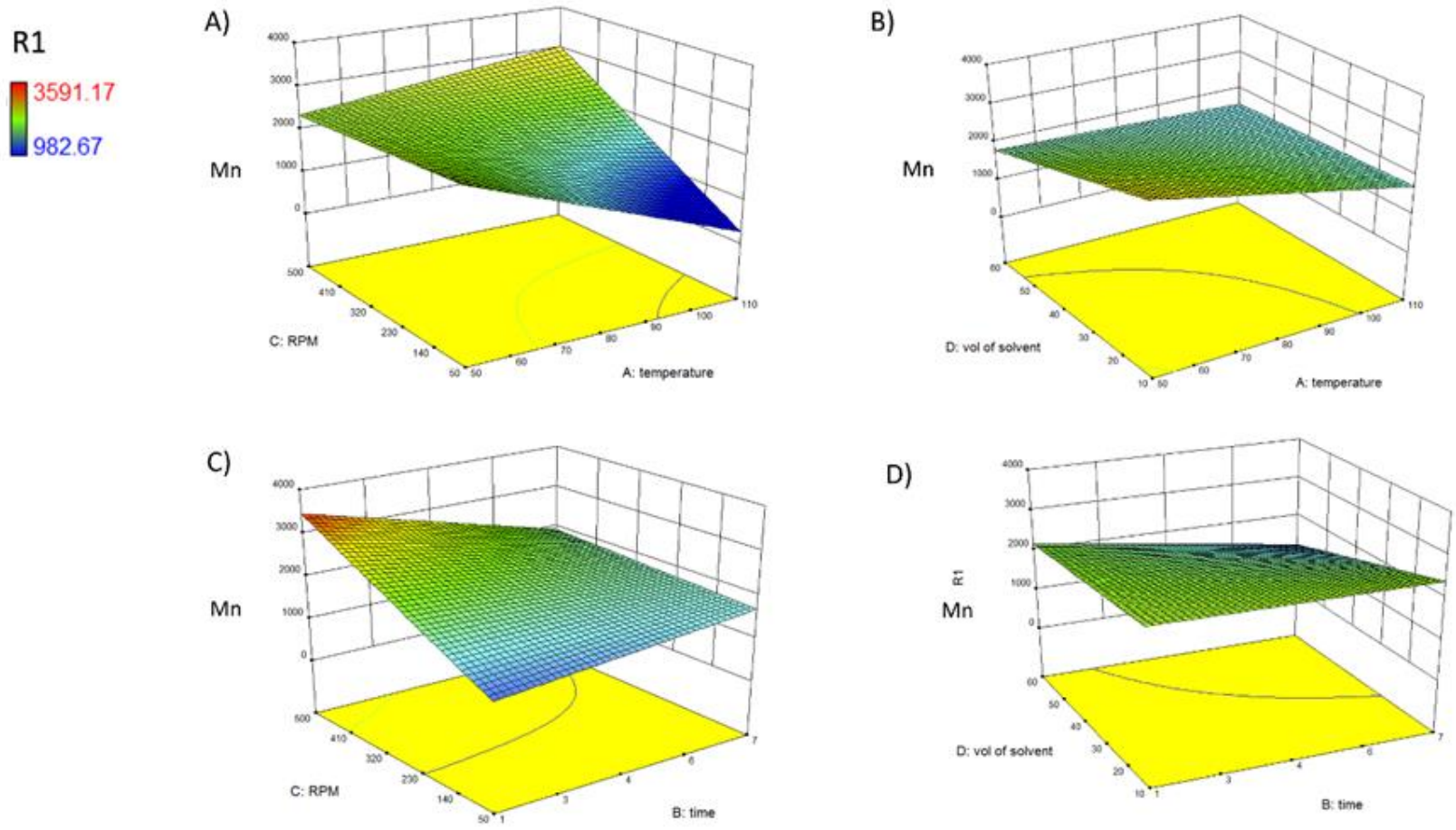


Figure 5-4 : Response surface plots for number average molecular weight (M_n)

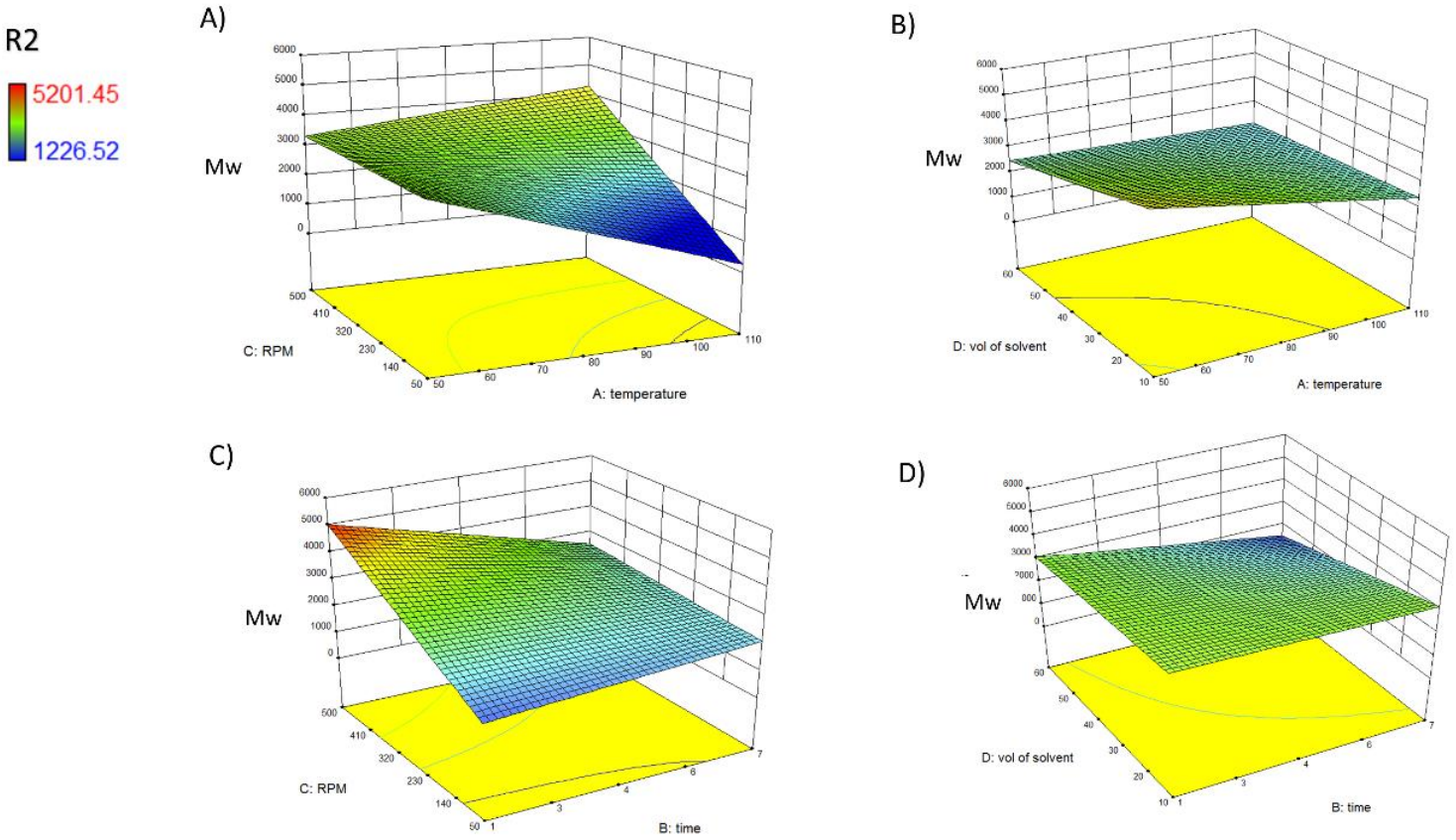


Figure 5-5 : Response surface plots for weight average molecular weights (M_w)

5.2.3 Validation of the developed experimental model

The model developed was validated by performing the experimental run under the optimised conditions predicted by design of experiment. **Table 5-4** presents the predicted optimized conditions of the process parameters and the observed experimental values carried based on these predicted conditions. Conventionally, till now most of the research work pertaining to polycaprolactone synthesis via enzymatic ring opening polymerization has been investigated through univariate approach. As in this approach except for the chosen parameter under examine, remaining all the process parameters are kept constant. For this reason, the univariate approach may not be able to present the actual time process which could oversight the interactions between the process parameters. Thus, this study attempts to achieve desired molecular weight, examining the possible combinations of all the process parameters, which was not investigated during univariate approach. Besides, we could examine different ranges of parameters by performing only 25 experimental trails, making this process viable for industrial purposes. Molecular weights determined are proximal to predicted values with acceptable error percentage of less than 10% with a monomer conversion percentage of 76%.

Table 5-4: Predicted optimised parameters by D-optimal

	Temperature (°C)	Time (hr)	Monomer/solvent ratio	Mixing speed (rpm)	M_n	M_w
Predicted values	75.29	1.001	11.6	500	3564.61	5168.23
Experimental values	75	1	11	500	3359.47	4920.85
Percentage error (%)	-	-	-	-	6%	8%

Therefore, the validation results obtained based on the predicted values by DOE were almost similar to the observed experimental values, this clearly portrays the robustness of design of experiment.

Synthesised PCL was further characterized by NMR, FTIR. **Figure 5-6** illustrates the H NMR spectra of the synthesized PCL a) using CALB as catalyst b) using TLL as catalyst, the signals at 4.15 ppm (a) representing methyl group attached to hydroxyl group (-CH_2OH), signals at 1.65 ppm and 1.38 ppm (c) represents other repeating methyl groups (-$CH_2CH_2CH_2$-). Finally, the signal at 2.31ppm (b) represents methylene end with carboxyl group (-CH_2COOH) which are the characterized chemical shifts for the polymer chain (Darbandy et al., 2011). The peaks at 3.65ppm represent the overlapping of hydroxyl methylene (-CH_2OH) and the carboxyl group adjacent to the methylene (-CH_2CO_2H) in the polycaprolactone chain.

Figure 4-7 depicts the FTIR spectrum of synthesised PCL by using and commercial PCL. From the structure of PCL [-(CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CO – O-) n -] it is evident that the functional groups present in the polycaprolactone are methyl group (CH_2), carbonyl group (C=O), and ester groups (O= C-O) and others C-C & C-O. The characteristics bands for PCL can be observed at 2800 to 3000, 1720 to 1750 and from 600 to 1400 representing functional groups of the PCL (Elzein et al., 2004). The two peaks around 2800 cm^{-1} and 2900 cm^{-1} represents CH_2 group in amorphous and crystalline state. The sharp peak at 1750 cm^{-1} is because of the presence of characteristic carbonyl group. Bands located between 600 to 1600 cm^{-1}, as we can see are all overlapped and difficult to explain individually. They usually belong to the ester group during trans-isomerization and due to the stretching of methyl groups of PCL (Phillipson et al., 2014). Synthesised PCL peaks are almost same as that of neat PCL having all the characteristics peaks of PCL. Except the intensity of peaks from the 600 to 1600 cm^{-1} which is low. This can be due to the difference in the intensity of crystallization (Phillipson et al., 2014).

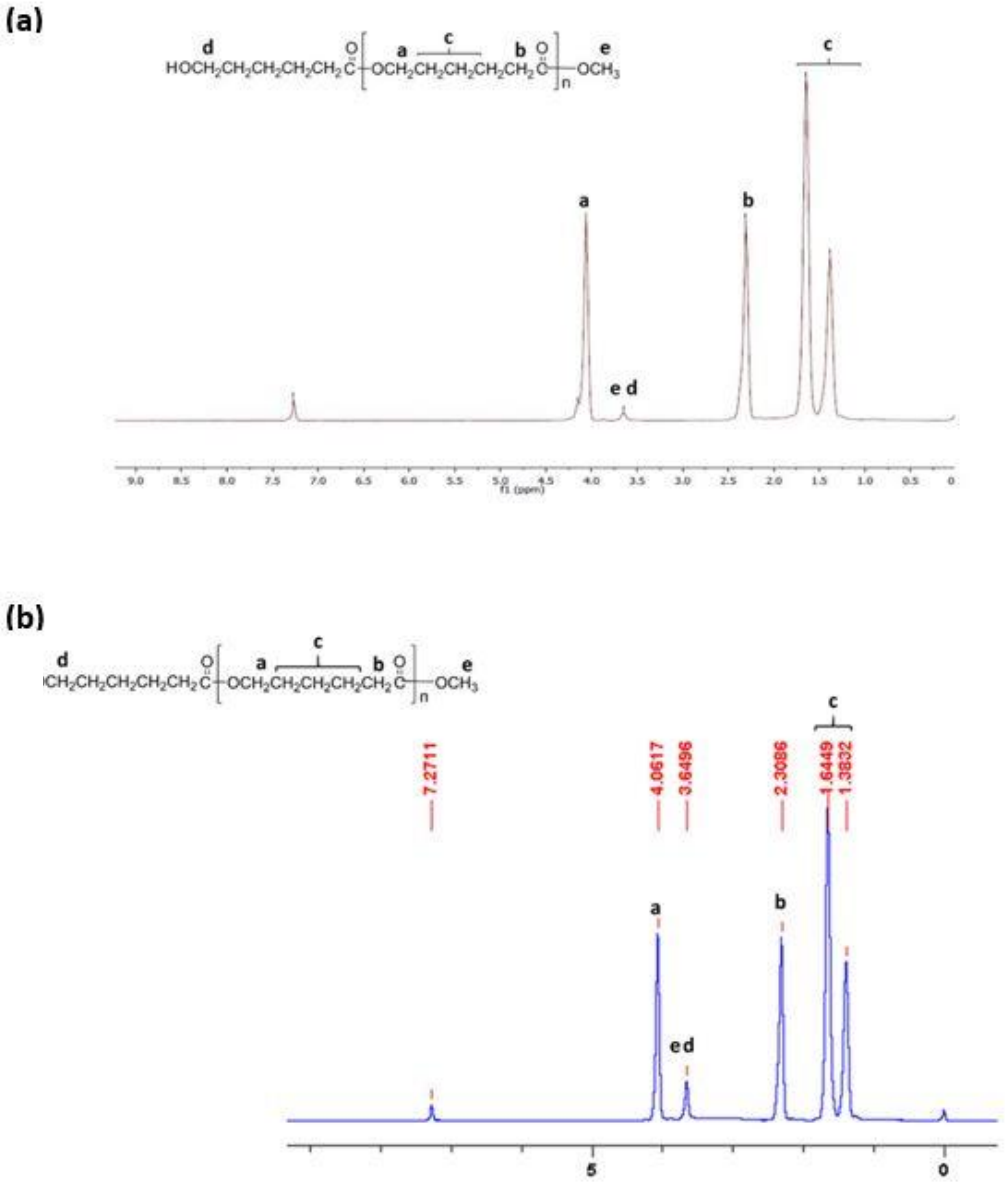
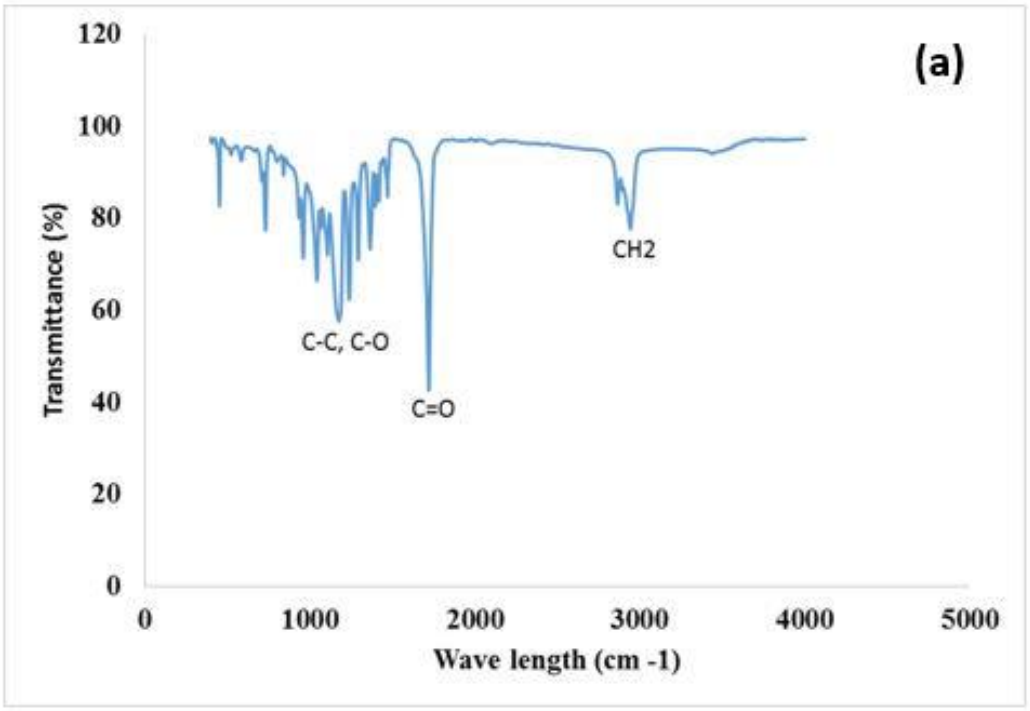


Figure 5-6 *NMR spectra for PCL synthesised using (a) CALB (b) TLL*



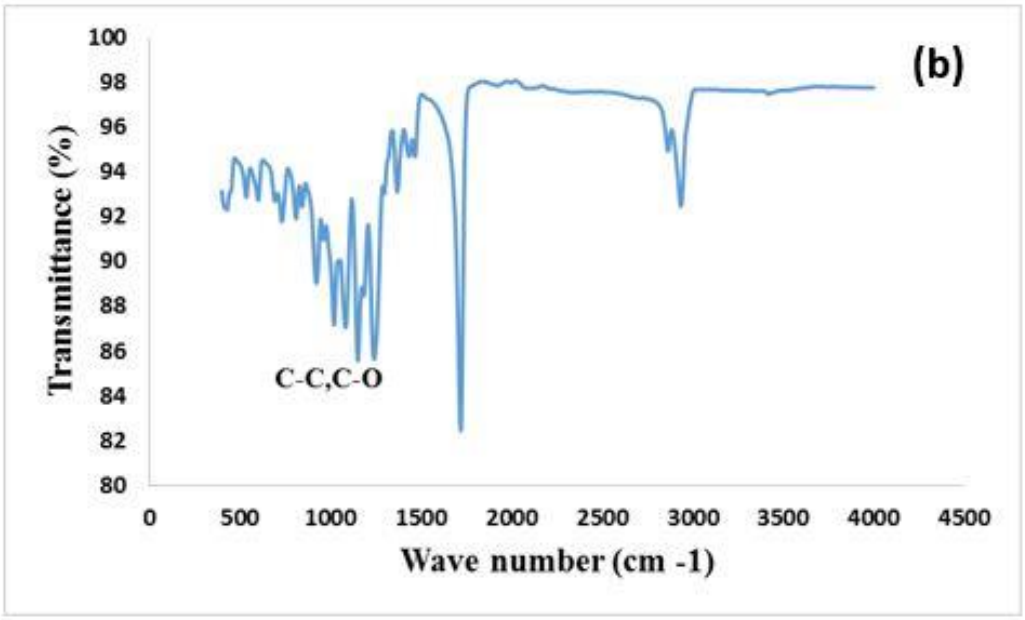


Figure 5-7 *NMR spectra for PCL synthesised using (a) CALB (b) TLL*

5.3 Synthesis of PCL using *Thermomyces lanuginosus* lipase

Lipase from *Thermomyces lanuginosus* (TLL) is one of the potential biocatalyst used in many biosynthesis and bio transformation process (Rodrigues et al., 2009). Its thermophilic nature and high stability have allowed its application in various types of reaction media from water to strong organic solvents (de Maria et al., 2009). Recently, its aid in esterification reaction is reported. Gumel *et al* reported the synthesis of natural flavor esters, methyl butanoate and methyl benzoate catalysed by *Thermomyces lanuginosus* lipase (Gumel and Annuar, 2016). A comparison study of ring opening polymerization of oxiranes catalyzed by three lipases, including TLL was reported. Comparing the activity of TLL with other enzymes, it stated that TLL showed the highest activity than other enzymes including lipase from Candida Antarctica lipase (Rajkhowa et al., 2005). Furthermore, this lipase has catalysed bio reactions similar to lipase from *Candida Antarctica* (which is reported to be the best biocatalyst for the polycarolactone synthesis) (Hunsen et al., 2008). That being said until there are few enzymes reported for the synthesis of polycaprolactone which includes lipases, cutinases and esterases. Even though TLL has proved to be the potential biocatalyst, its activity in polymer synthesis is never explored till date. Inspired from these facts that TLL is active for transesterification and polymerization reaction, studies were performed to determine the TLL activity for lactone ring-opening polymerisation of caprolactone. Therefore, in this section, the ring opening polymerisation of the ε- caprolactone catalysed by TLL has been reported. Different reaction medium and different enzyme concentration have been investigated. TLL catalysed ROP was performed in bulk and solvents.

5.3.1 Structural characterisation of the synthesised PCL

To begin with, identifying the synthesised polymer H NMR, FTIR and MALDI were performed. The H NMR spectra obtained was demonstrated in **Figure 5-6 (b).** And it contains

peaks that definitely identifies the product as polycaprolactone, which is similar to **Figure 5-6 (a)** that presents the H NMR peaks obtained during PCL synthesis using CALB as catalyst. The structural groups of PCL are represented by the peaks at 4.06 ppm (main chain, - CH_2O), 3.64 ppm (chain end -OCH_3 and – CH_2OH), 2.30 ppm (CH_2CO), 1.64 and 1.38 ppm (repeating methyl group (CH_2-CH_2-CH_2) confirming the PCL.

Figure 5.7 (b) depicts the FTIR spectra of the synthesised PCL catalysed by TLL. The characteristics peaks of PCL are methyl group (CH_2), carbonyl group (C=O), and ester groups (O= C-O) are represented by the IR spectra at 2947 & 2867, 1720 and other bands at 457 to 1472 cm-1, respectively. Similarly, **Figure 5.7 (a)** i.e. FTIR spectra for PCL catalysed by CALB shows similar peaks as well. Also, other studies related to synthesis of PCL (Gokalp et al., 2016a) (Ma et al., 2009), suggests the similar kind of peaks at 1752 and 1720 cm-1 representing the characteristic carbonyl group and other peaks obtained at 2945, 2865 and 1192.5 cm -1 representing methyl and ester groups. Based on these reports, it can be stated that the FTIR spectrum obtained has all the characteristics IR bands of PCL and that the PCL was synthesised successfully using TLL as catalyst.

Further to determine the effect of time and determine the optimum time for PCL synthesis, ring opening polymerization of Ɛ- caprolactone catalysed by TLL was carried out for 96 hr at 50°C with 220 rpm. Molecular weights (Mn and Mw) and polydispersity index of the synthesised PCL was analysed by GPC. **Table 5-5** presents the results obtained. From Table 4-5, the highest molecular weight i.e. Mn - 2971 g/mol, Mw – 4456 g/mol with PDI 1.5 were obtained at 5^{th} hr of the bio polymerization reaction with a conversion of 56% calculated using Equation 1. Even though the polymer obtained at the first hour of the reaction, the molecular weights Mn and Mw obtained is low and conversion is reported to be 21% which is very low. Furthermore, molecular weight increased initially up to 5^{th} hr later there is a decrease in the molecular weight along the time period, this was depicted in **Figure 5-8**. PDI is ranging from 1.2 to 1.8 throughout the run.

While the highest monomer conversion rate 69% was obtained at 48hr after which the values have been decreased to 57% at 96 hr. This can be explained by the fact that ring opening polymerization of ε-CL is a reversible process, where the degradation can also take place at the same time (Kobayashi et al., 2001). This could be the reason for the decrease of molecular

weights after a certain time. Compared to CALB this enzyme provides low molecular weight PCL, which are applicable for drug delivery and also as a soft segment for polyurethane production.

Table 5-5: Molecular weights and conversion% of PCL catalysed by TLL for 96hrs

Hour	Mn (g/mol)	Mw (g/mol)	PDI	Conversion (%)
1	601	1352	2.2	21
3	1476	2131	1.4	33
5	2971	4456	1.5	56
7	740	933	1.2	58
12	808	1083	1.3	62
24	1080	1416	1.3	67
48	652	1188	1.8	69
72	735	1072	1.4	55
96	968	1340	1.3	57

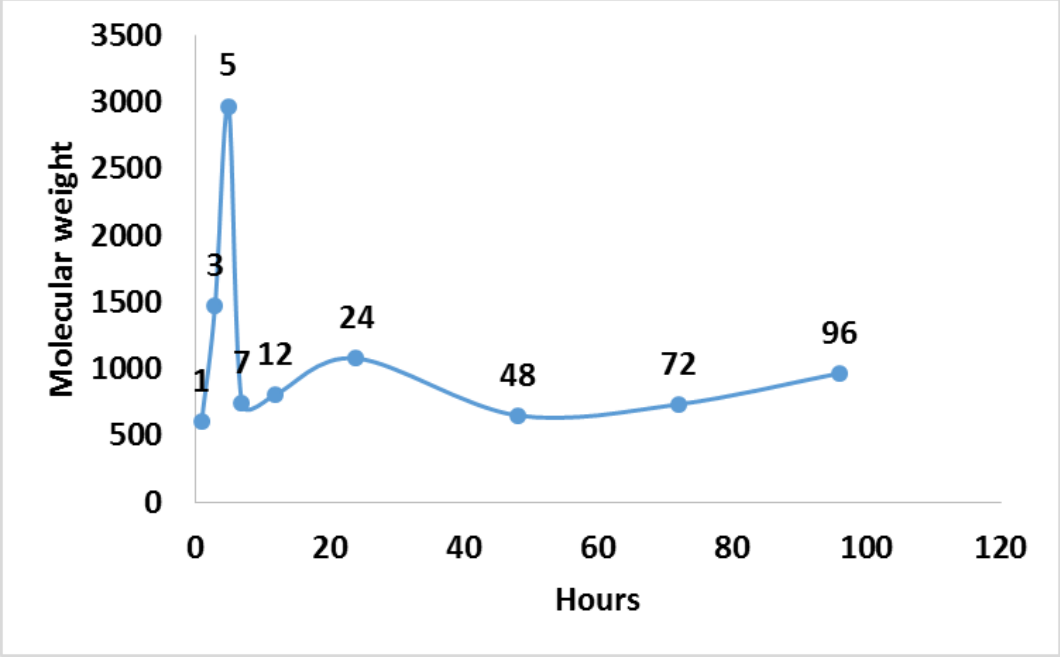


Figure 5-8 *Ring opening polymerization of caprolactone catalysed by TLL for 96hrs.*

5.3.2 Effect of different reaction media on molecular weights:

Type of solvents employed during the bio polymerization reaction plays an important role in determining the quality of the polymer. Also, the solvents help in segregating the substrate, enzyme catalyst and the polymer synthesised (Gross et al., 2001, Klibanov, 2001a). Three most common solvents for polymerization reaction i.e. toluene, tetrahydrofuran and hexane are used to study the effect of different solvents on the synthesised polymer's molecular weights and monomer conversion. Enzymatic polymerisations carried out in hydrophobic solvents usually afford high monomer conversion and Mn values (Li et al., 2011a). These experiments were conducted at 50°C for 7 hrs since during the initial run highest molecular weight was obtained at 5^{th} hr and later decreased. **Figure 5-9** depicts the effect of different solvents on the molecular weight of PCL obtained by the ring opening polymerization of caprolactone catalysed by TLL. The highest molecular weight (6317 g/mol) was obtained when toluene was used as a solvent with a conversion of 69% while comparing with other solvents the MW and conversion are low. Similar reports have been presented for solvent effect in enzymatic polymerization (Gross et al., 2001) were higher Mn and monomer conversion was obtained where toluene was used as a solvent. It can be explained by the fact that hydrophobic solvents will retain the water molecules on the surface of the enzymes, which helps in maintaining the catalytic activity with necessary confirmations (Li et al., 2011a). However, there was fluctuation in toluene during the 3^{rd} hour of the reaction, this could be due to the alterations in dipole moment and solvent geometry, which could have affected the enzymatic polymerization process, interfering with the monomer conversion and molecular weight (Ma et al., 2009).

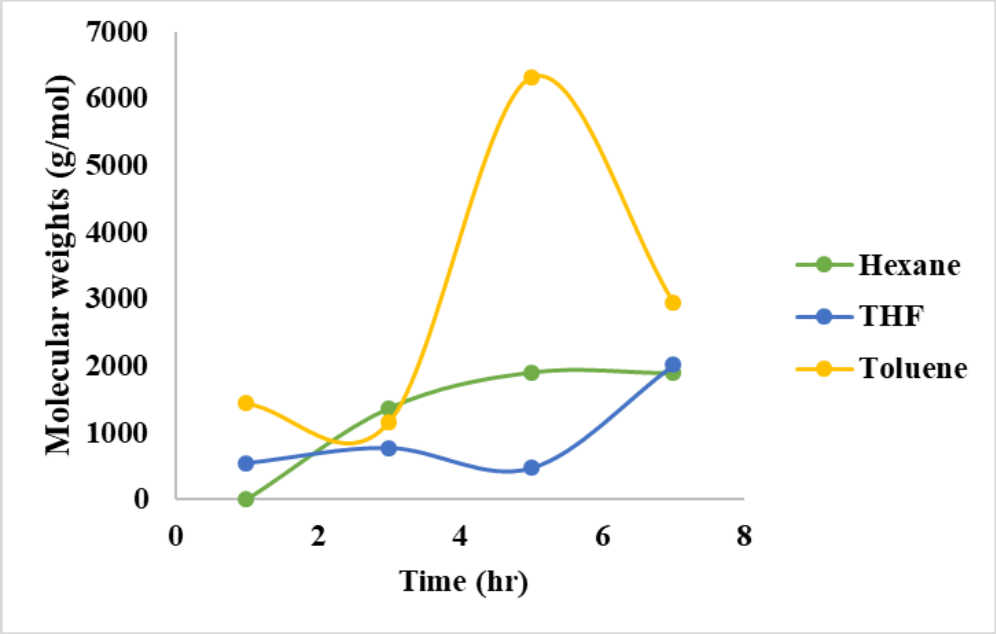


Figure 5-9: Effect of solvent on the Molecular weight of the PCL catalysed by TLL

5.3.3 Effect of enzyme concentration:

The effect of enzyme concentration on PCL synthesis was determined to find out the optimum amount of enzyme catalyst required to achieve high Mn values. The variation in the enzyme concentration showed a profound effect on the product molecular weight and PDI. Experiments are performed using a different concentration of enzyme while other parameters kept constant. The reaction was carried out at 50°C for 7hrs. **Figure 5-10** depicts the effect of enzyme concentration on the molecular weight of the polymer. From the analysis, we can determine that the enzyme concentration is directly proportional to molecular weight. As the enzyme concentration increases, the molecular weight also increases. Goklap et al reported a similar result where the molecular weights increased with the increase in enzyme concentration (Gokalp et al., 2016a). PDI range obtained was 1.1 to 1.5, which is considered a good range. Since the natural polymers have a PDI of 1. Generally, in enzymatic polymerisation, process starts with initiation of lactone ring and the formation of the acyl enzyme intermediate, further followed by chain propagation. However, during the 1st and 2nd hr of polymerisation, polymer

molecular weight for 1g and 2g enzyme concentration shows similar trend. This could be due to the 1g difference, which may not affect initial step in enzymatic polymerization where initiation of polymer chain takes place, forming the intermediates at initial reaction hours, resulting in similar results.

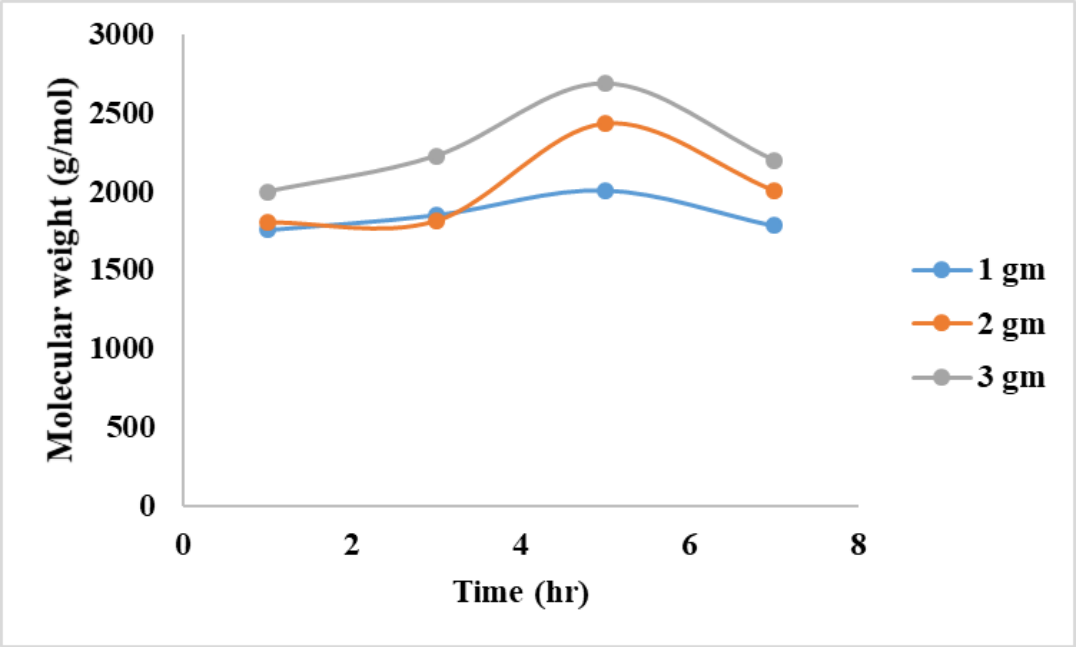


Figure 5-10: Effect of enzyme concentration on the molecular weight of the PCL catalysed by TLL

5.3.4 Thermal properties of synthesized PCL catalysed by CALB and TLL:

Thermal properties of the synthesised PCL catalysed by CALB and TLL were compared to neat PCL. **Table 5-6** presents the degradation temperatures and the melting points of CALB PCL, TLL PCL and neat PCL.

Table 5-6 Degradation temperatures

	PCL - CALB	PCL - TLL	Neat PCL
Melting point	62	60.1	66
Onset Temperature at 5% mass loss	310	304	372

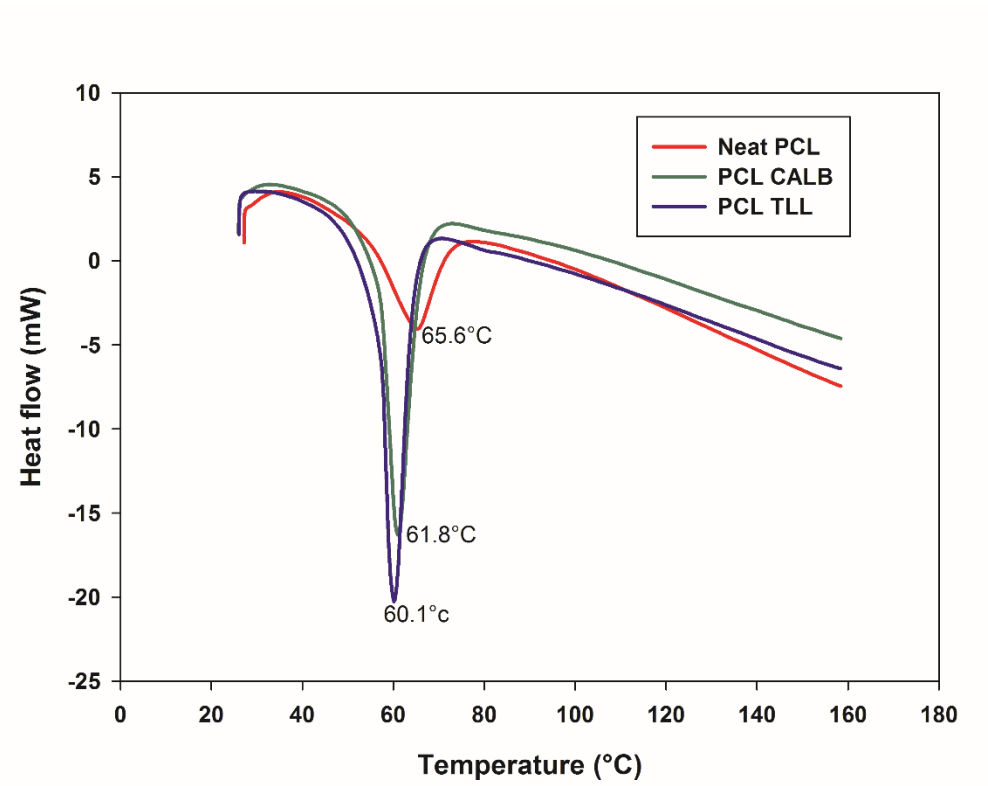


Figure 5-11 : DSC graph for synthesised PCL (using CALB and TLL) and Neat PCL

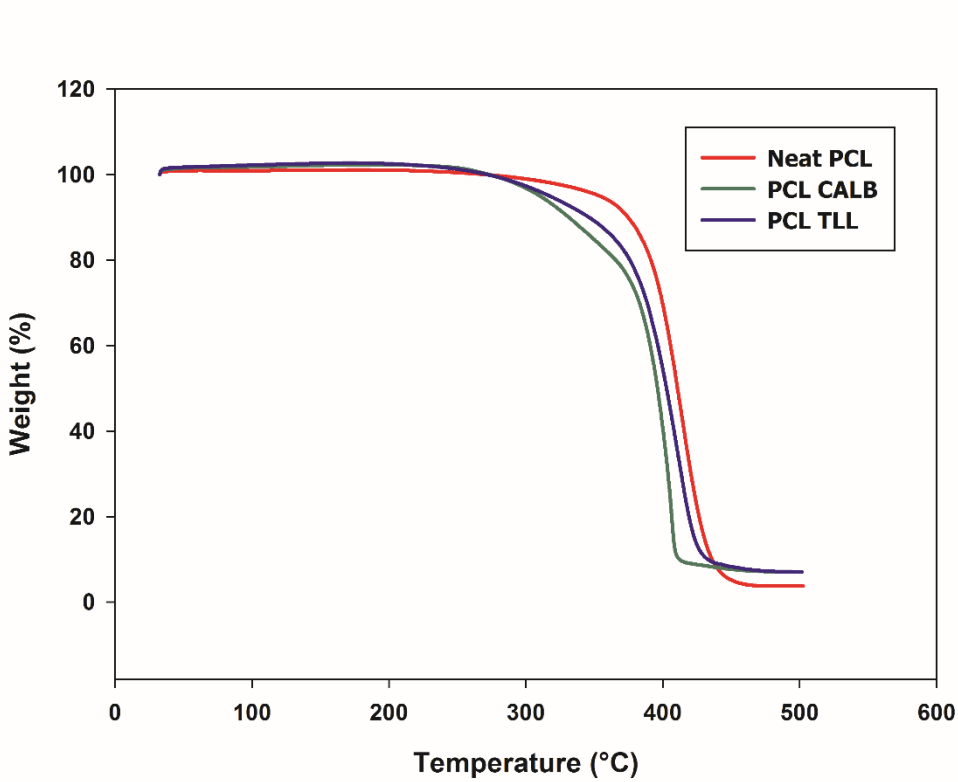


Figure 5-12 : TGA graph for synthesised PCL (using CALB and TLL) and Neat PCL

Figure 5-11 and 5-12 depict the DSC and TGA analysis of the two synthesised PCL along with neat PCL. The melting point of synthesised PCL by CALB (66°C) and by TLL (60°C) is lesser compared to the neat PCL (66°C). This may be due to the difference in molecular weights of the polymer. However, it is important to note that the melting point of PCL is 60°C, considering this fact synthesised PCL still holds a good melting point. Figure 13 shows the degradation temperature representing the thermal stability of the synthesised PCL. Although there is a similar trend in degradation pattern for both the synthesised PCL and neat PCL, the temperature onset of synthesised PCL and neat PCL at 5% mass loss is 310°C, 304°C and 372°C. This indicates the degradation is faster in synthesised PCL compared to neat PCL owing to their difference in molecular weights. Degradation rate will vary depending on the molecular weight of the polymer (Park et al., 1992). Since the synthesized PCL has molecular weight of

4920g/mol, whereas the neat PCL has molecular weight of 25000 g/mol, this could be reason for earlier degradation in synthesised PCL.

5.4 Summary

Optimization of enzyme catalysed bio-polymerization was achieved by conducting a minimal number of runs. The optimal conditions from the developed model were obtained at temperature: 75°C, time: 1 hr, monomer/solvent ratio: 1:1 and mixing speed: 500 rpm resulted a polymer with molecular weight of Mn – 3357.47g/mol and Mw – 4920.85g/mol. The predicted and experimental values were in good agreement with a percentage error ± 8%. It is concluded that mixing speed has a huge impact on enzyme catalysed polymerization process. Among chosen parameters, mixing speed and temperature has a more profound effect on the molecular weight distribution compared to solvent volume and time. Overall, all four selected parameters had an impact on polymer molecular weight. On the other hand, TLL was proved to be a novel potential catalyst for PCL synthesis. Among the solvents used to synthesise PCL with TLL, toluene reported the high molecular weight PCL.

Although we could achieve the successful implementation of D optimal design from DOE to optimise the process parameters of enzyme catalysed PCL synthesis, we could not achieve high molecular weight polymer. The reason to obtain a low molecular weight polymer during enzymatic polymerisation could be due to strong binding nature of the monomers to the active site. Due to this, the transfer of acyl group to hydroxyl/ amino groups of other monomers could have been restricted, causing termination in polymer chain formation (Divakar, 2012). However, the low molecular weight PCL obtained cannot be used for further application in preparing the PCL based controlled release fertilizers. Because the low molecular weight polymers tend to degrade faster (Chrissafis et al., 2006). Besides, for controlled release fertilizer, other external factors like water, microorganisms, sunlight will also interfere

increasing the degradation rate (Naz and Sulaiman, 2016). Polycaprolactone degradation studies revealed that for low molecular weight PCL will degrade faster that PCL with high molecular weights (Mochizuki and Hirami, 1997) (Hakkarainen and Albertsson, 2002) (Lam et al., 2008). As, the synthesised PCL has low molecular weight of Mn – 3357.47g/mol and Mw – 4920.85g/mol, also Figure 5.12 depicts the degradation of synthesised PCL started much earlier compared to neat PCL, due to these reasons, we couldn't use synthesised PCL for application studies. Therefore, in next chapters to develop controlled release fertilizers commercial PCL (Grades CAPA 6250 and CAPA 34000 with molecular weight of 25000g/mol and 34000g/mol) was used considering it would be appropriate for the scope of this project.

CHAPTER 6

PREPARATION OF PCL BASED CRF USING SOLVENT CASTING TECHNIQUE AND ITS INFLUENCE ON GROWTH AND YIELD OF MUNGBEAN

6.1 Introduction

This chapter presents the third objective of the present study, which aims to employ PCL based control release fertilizers (CRF). Previously for preparing CRF's, solvent casting technique was mostly studied using different polymers like polysulfone, polyvinyl alcohol, and polylactic acid (Chen et al., 2008) (Azeem et al., 2016). Among all the biodegradable polymers available in the market, PCL had received utmost interest due to its extreme flexibility and biodegradability along with its hydrophobic nature (Wu et al., 2007). Therefore, this study aims to employ PCL based fertilizer granules, which could potentially encapsulate the fertilizer causing a slow release. Initially, the project was aimed to use PCL which is synthesised using enzyme catalyst, however as discussed in the previous chapter, synthesised PCL with very low molecular weights will not be suitable for preparing controlled release fertilizers. Therefore, the commercial PCL (two grades: CAPA 6420 and CAPA 6400 bought from perstop LTD, Sweden) was used to develop the controlled release fertilizer.

In this present objective, the development of the CRF was carried out with PCL and PCL/starch composites via solvent casting technique. Addition of starch to PCL alters its physical and mechanical properties, hydrophobicity (Averous et al., 2000) and importantly its biodegradation rate, which plays a key role in control release of fertilizer (Wu et al., 2007). Starch was opted among other natural biopolymers because it is fully degradable, renewable and also available abundantly for a lesser cost. In this study, PCL with two different molecular weights 25000g/mol and 34000g/mol, three different concentrations i.e. 20, 30, 40% were considered. Further, surface morphology, porosity, biodegradation of the coated fertilizers and

the nutrient release (nitrogen, potassium and phosphorus) is discussed. Finally, the influence of CRF on growth and yield of mung bean was studied.

6.2 Morphology and porosity

Figure 6-1 & 6-2 (a & b) depicts the PCL and PCL/starch based CRF prepared by solvent casting technique, packed in mesh bags in order to determine the amounts of nutrient released and morphology of degraded samples. Morphology of the NPK and coated fertilizer were characterised by performing Field Emission Scanning Electron Microscope (FESEM). **Figure 6.3** depicts the coated samples, i.e. the NPK before and after coating with PCL and PCL/starch. After the solvent casting, as presented in **Figure 6.3(b)** the polymer layer is formed around the NPK. **Figure 6.3c** depicts the coating formed by polymer and starch composites, this layer is



(a) (b)

Figure 6-1 : (a) PCL-CRF prepared by Solvent casting technique (b)PCL - CRF packed in a mesh bag for degradation studies



(a) (b)

Figure 6-2 : (a) PCL/starch-CRF prepared by Solvent casting technique (b)PCL/starch - CRF packed in a mesh bag for degradation studies

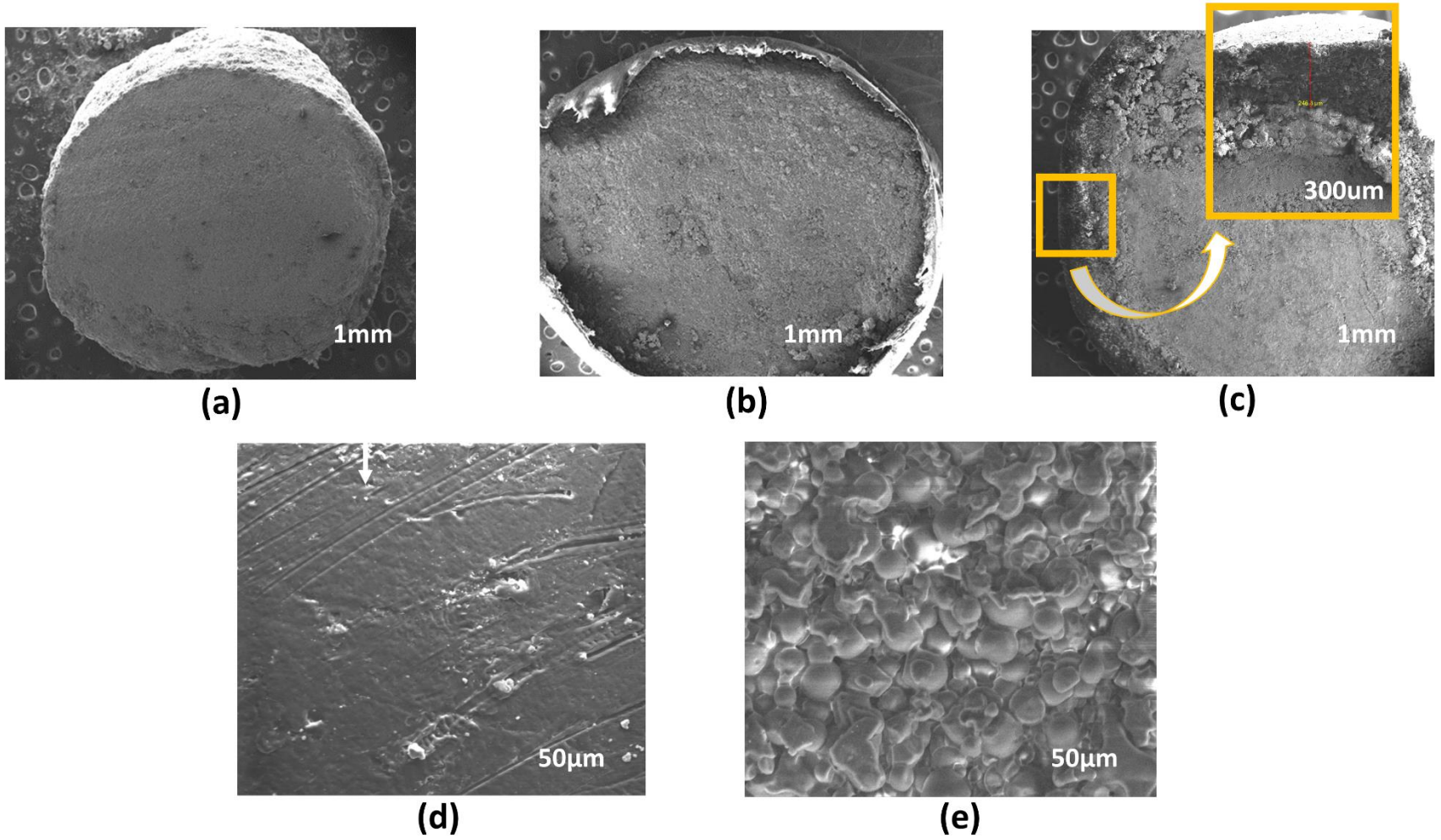


Figure 6-3 : a) NPK before coating b) NPK with PCL coating c) NPK with PCL/starch coating d) Surface morphology of PCL coating e) Surface morphology of PCL/starch coating

thicker compared to only PCL layer, due to the presence of starch. Whereas the surface morphology of the coated samples is depicted in Figure **6.3(d) and 6.3(e)** determining the absence and presence of starch. The incorporation of starch molecules tends to increase the surface area of the samples and at the same time increasing the water absorption capacity. Additionally, starch also plays a role in degradation and porosity of the sample, which will be explained in the later section.

Thickness of the coating layer plays an important role in better nutrient release properties (Azeem et al., 2016). Depending on the concentration of the polymer in the coat forming solution, the thickness of the layer will vary. The thickness of the samples was measured by SEM, the average of three measurements values was taken. **Table 6.1** presents the thickness of the different concentration coatings determined by SEM. From **Table 6.1,** it is evident that when only PCL was used the thickness increased along with the concentration. This may be due to the fact that an increase in polymer concentration in the coating solution results in increased viscosity forming a thicker layer (Kasten et al., 2016). Further, coatings formed by PCL/ starch are very much thicker than only polymer coatings, here the thickness increased with the increase in starch percentage, this could be due to the presence of more starch in the coat forming solution.

Table.6-1 Thickness of the coated samples (PCL & PCL/starch coatings) determined by SEM

S no	Composition of coatings	Concentrations (%)	Thickness (µm)
1.	Only PCL	20	26.27
		30	32.12
		40	56.99
2.	PCL/starch	20	1154.6
		30	627.1
		40	246.3

Correspondingly, porosity of the samples plays an important role in the control release of nutrients. It restrains the diffusion rates, i.e. the water to enter the fertilizer and the nutrient to come out from the granule. The porosity was determined using pycnometer. **Figure 6-4 (a) & (b)** depicts the porosity of the different concentration of the samples. The porosity obtained was ranging between 6.6 to 14.6 % in case of only PCL, and 13 to 18 % in PCL/starch coatings. The samples with PCL/starch are more porous compared to only PCL. This is due to the presence of starch molecules embedded all along making the sample more porous. The incorporation of starch tends to increase the surface area of the samples, same time increasing the water absorption capacity. While the only PCL coated fertilizer shows less porosity, could be due to the hydrophobic nature of the PCL. The coatings prepared from the higher concentration has lower porosity and vice versa. This can be explained by the fact that when a higher concentration of polymer is used for coating the precipitation rate will be more, forming a dense layer on the surface. This thick layer could obstruct the diffusion of water inwards the capsule and the fertilizer outwards the capsule, acting as a barrier. It is said that the exchange rate of solvent and water will be slower in case of the high polymer concentration solutions resulting in denser and less porous layer (Tomaszewska and Jarosiewicz, 2002). From these results, it can be drawn that the porosity is inversely proportional to the polymer concentration suggesting its huge influence on the porosity of the sample.

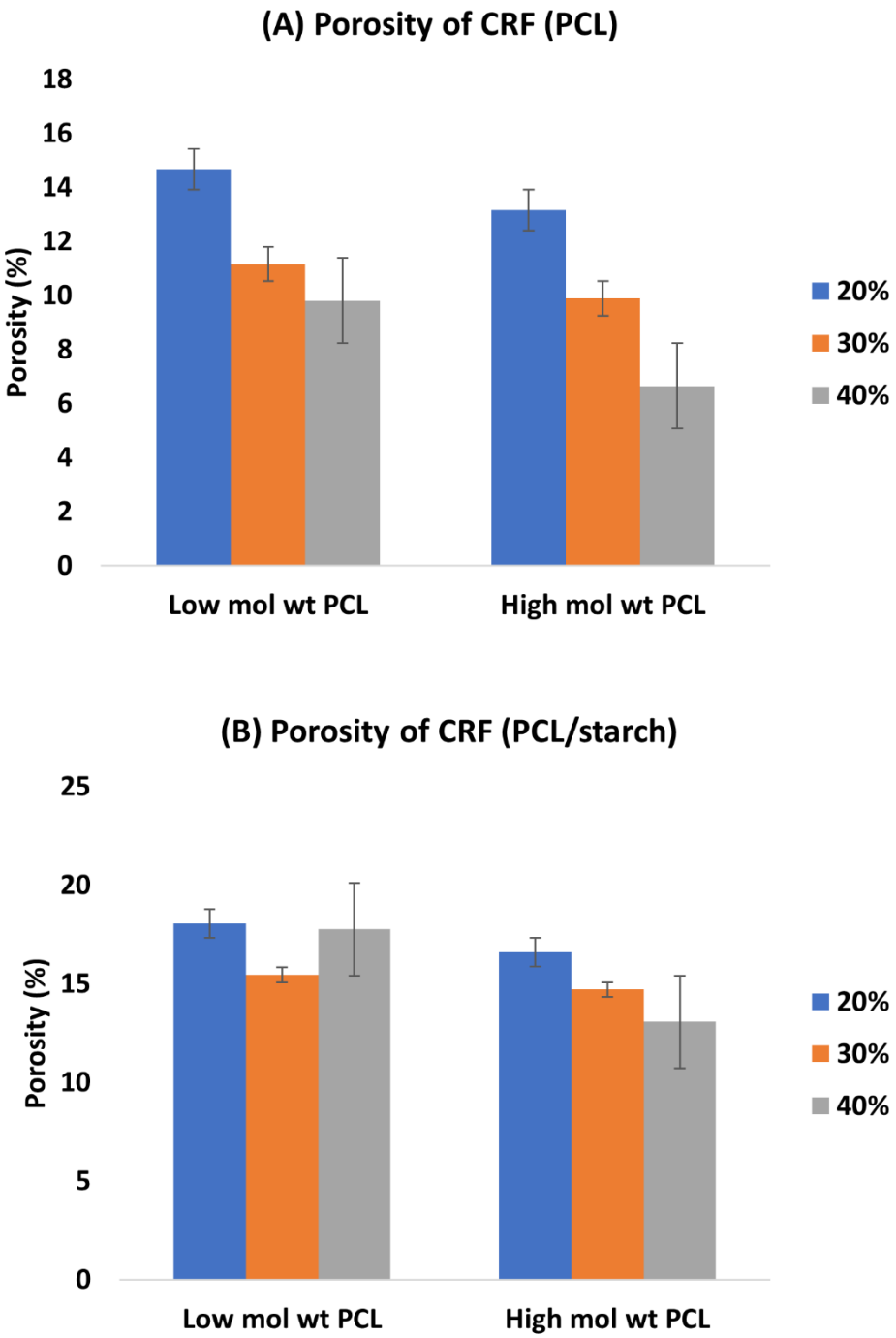


Figure 6-4 : a) Porosity of the PCL coated samples b) Porosity of PCL/starch coated samples

6.3 Biodegradation

To investigate the degradation of PCL, which eventually leads to the release of nitrogen, potassium and phosphorus, 9 weeks of the degradation studies were carried out using the litterbags, these are packed with the coated fertilizers and kept in the soil. CRF samples were collected for every 3rd, 6th and 9th week to study the morphology of the sample. As PCL is considered to be a biodegradable polymer, it is prone to biodegradation. Bacteria present in the soil will also be effectively working on the degradation of the coating eventually leading to the release of nutrients.

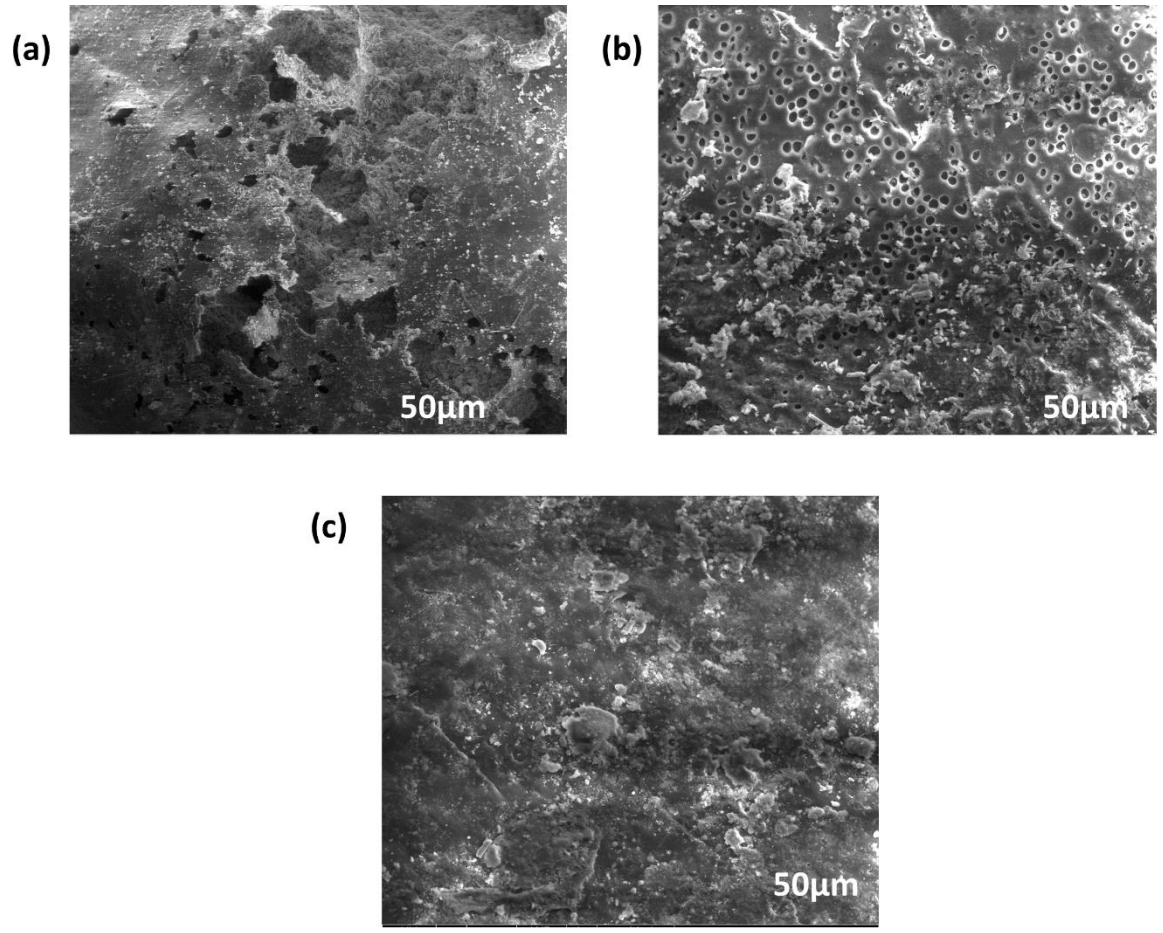


Figure 6-5 : *Morphology of PCL based CRF at 3rd week a) 20% b) 30% c) 40%*

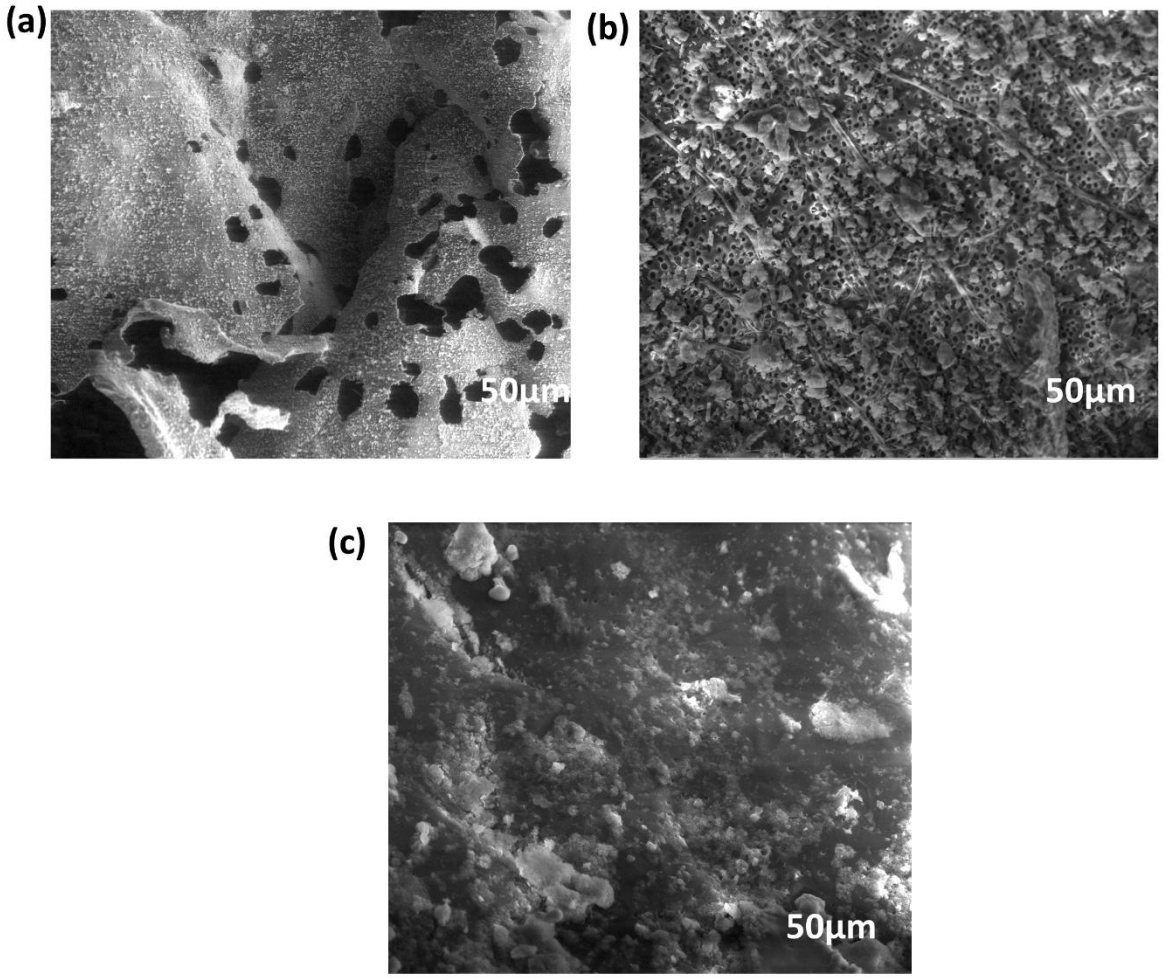


Figure 6-6 : Morphology of PCL based CRF at 6th week a) 20% b) 30% c) 40%

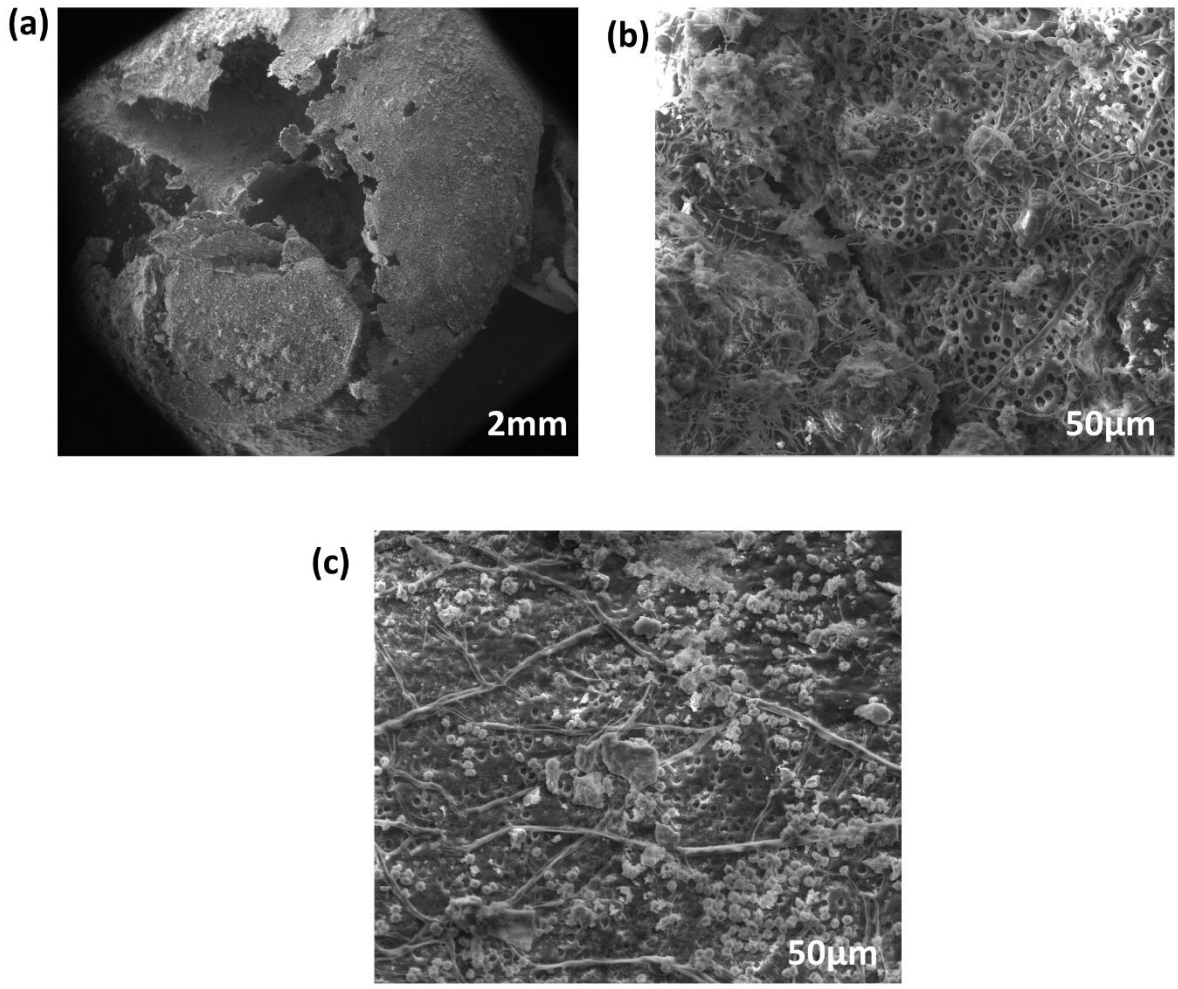


Figure 6-7: Morphology of PCL based CRF at 9th week a) 20% b) 30% c) 40%

The changes in the morphology of the PCL coated fertilizer along the course of 9 weeks' time for different concentrations used was depicted in **Figures 6.5, 6.6 and 6.7**. **Figure 6.5** depicts the changes observed after the 3rd week, from this figure, the degradation is evident for 20 and 30 % coating layer. For 20% there was a degradation of the polymer layer (Figure 6.5 a), and in 30% small pores are appeared on the surface (Figure 6.5 b). Whereas in 40% the surface appeared to be less affected (Figure 6.5 c). After the 6th week, for PCL 20% and 30%, the degradation became more obvious, showing more damage in 20% (Figure 6.6 a) and bigger pores in 30% (figure 6.6 b). On the other hand, in 40% there were very small pores started to appear (figure 6.6 a). After 9 weeks, the degradation was significant in 20%, where there was

as maximum damage done to polymer coating as depicted in figure 6.7(a), whereas the degradation was comparatively lesser than 30 (figure 6.7 b) and 40% (figure 6.7 c). In these two concentrations, the surface was damaged along with increased pores and pore size. Therefore, compared to 20 and 30%, the PCL 40 % shows lesser degradation.

As PCL is a biodegradable polyester, soil bacteria and fungi help in its degradation. Since the microorganisms are ubiquitous, they can easily associate and grow on any type of materials (Bano et al., 2017). Biodegradation is the surface erosion process caused by layer of microorganisms, growing on the surface of the polymer. Organic matter present in the soil will help to improve the microorganism's growth along with their enzyme production and its activity. These bacterial enzymes will be responsible for the degradation of the polyester by breaking the ester bonds (Nowak et al., 2016). At the same time, microorganisms will utilize the hydrocarbons in the polymer backbone as their carbon source for their survival which also leads to degradation (Bhatia et al., 2014). As we can see in the above SEM figures, the degradation is faster in coating with lower polymer concentration compared to the coating with higher polymer concentration. This could be due to the presence of more percentage of polymer taking more time for degradation. This is because, the solution with high polymer concentration will contain polymer chains overlap with each other, whereas in lower polymer concentration the polymer chains will be present more dispersed comparatively (Lyu and Untereker, 2009).

6.4 Nutrient release

The nutrients released at 3rd week, 6th week and 9th week were assessed by performing test methods (Distillation method for nitrogen, phosphor-vanadomolybdate complex method for phosphorus and Kjeldahl flask method for potassium) to analyse nutrients in the soil samples.

Figure 6.8 depicts the amount of nutrients released for a period of 9 weeks, differentiating the difference between the CRF (prepared via solvent casting), uncoated NPK and control (no fertilizer). Amount of nutrients (Nitrogen, phosphorus and potassium) released at 3rd week recorded the lowest values for CRF (0.07, 226, 1.39) samples compared with uncoated fertilizer (0.08, 272, 1.6) and control (0.03, 94, 0.56). It is evident from the analysis that there is a significant difference ($P < 0.05$) between control release fertilizers and uncoated fertilizer, where the amount of nutrients released are much lesser in CRF samples suggesting a slow release of nutrients. Later in the 6th week, the nutrients released from uncoated fertilizer (0.07, 245, 1.33) are lesser compared with CRF (0.06, 262, 1.62). Further, in the 9th week similar trend appeared i.e. the amount of nutrients released from uncoated fertilizer (0.07, 236, 1.3) are lesser compared with CRF (0.09, 323, 1.8).

From **Figure 6.8**, it is evident that the nutrient release from the uncoated fertilizer was lesser in 6th and 9th week compared to 3rd week. Maximum content was released in earlier weeks could be due to the dissolution of the fertilizer caused by regular addition of water. As a consequence, leaching may occur which results in wastage of fertilizer and also polluting the soil. On the other hand, CRF tends to release nutrients slowly due to the presence of polymer layer making them available to plant along its growth. Except in nitrogen, since the nitrogen is unstable and volatile element, due to which the amount of nitrogen released into soil can be

fluctuating. A similar trend was reported by (Tomaszewska and Jarosiewicz, 2002) where polysulfone was used for coating the fertilizer.

Figure 6.9 depicts the amount of nutrients released along a period of 9 weeks' time, based on the polymer molecular weight along with uncoated fertilizer (only NPK) and control. Amount of nutrients (Nitrogen, phosphorus and potassium) released at 3rd week recorded the lowest values for both the high molecular weight (0.06, 221, 1.35) and low molecular weight (0.06, 224, 1.36) samples compared with uncoated fertilizer (0.08, 272, 1.6) and control (0.03, 94, 0.56). Later in the 6th week, the nutrients released from uncoated fertilizer (0.07, 245, 1.35) are lesser compared with high molecular weight polymer (0.07, 257, 1.56) and low molecular weight polymer (0.06, 262, 1.6). Further, in the 9th week, similar trend appeared i.e. the amount of nutrients released from uncoated fertilizer (0.07, 236, 1.3) are lesser compared with HMwF (0.08, 303, 1.74) and solvent LMwF (0.09, 320, 1.81). Even though there is a significant difference between control release fertilizers (differed in the polymer molecular weight) and uncoated fertilizer, there is no significant difference ($p>0.05$) between the two molecular weights used for coating the fertilizer, where the higher molecular weight is better than low molecular polymer causing relatively slow release than low molecular polymer. Since the difference between the molecular weight is less, there will not have much difference in their chain structure, resulting in no significant difference in the nutrients release (Jintakanon et al., 2008).

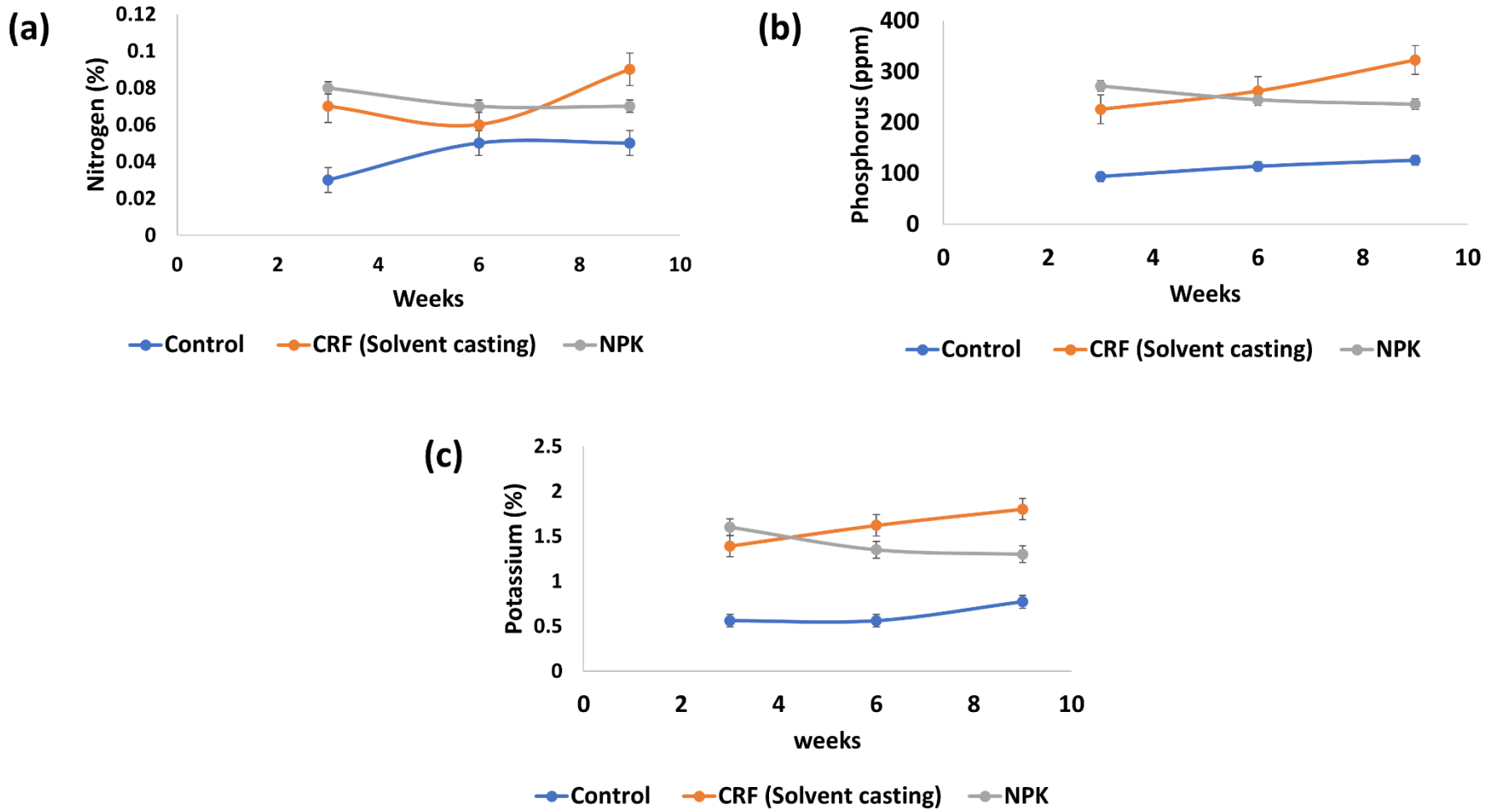


Figure 6-8 : Release of nutrients based on method a) release of nitrogen b) release of phosphorus c) release of potassium

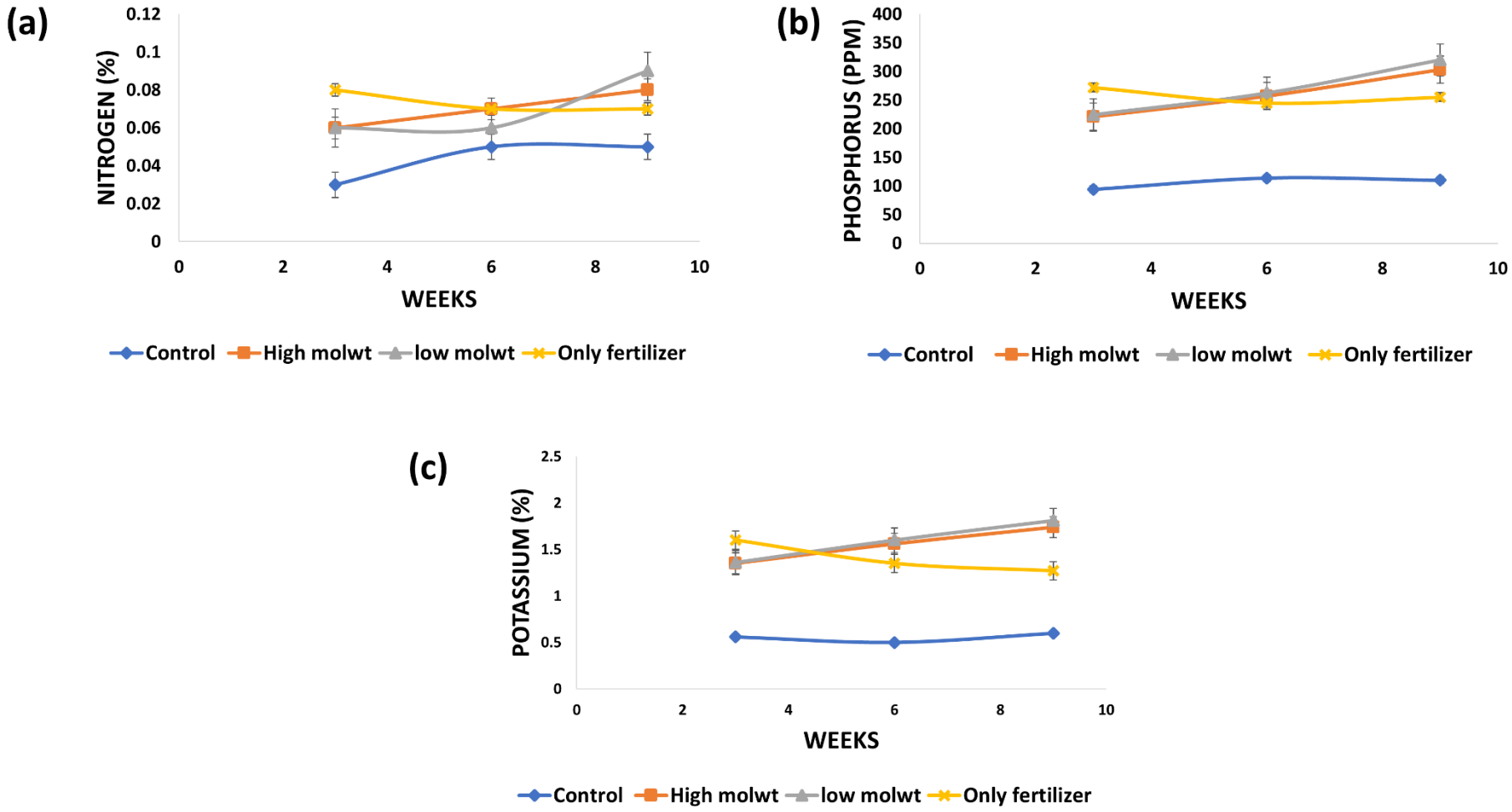


Figure 6-9 : Release of nutrients based on molecular weight a) release of nitrogen b) release of phosphorus c) release of potassium

Figure 6.10 displays the percentage of nutrients released by different concentration of the polymer coatings formed PCL at 3rd week, 6th week and 9th week. The nutrient release rate is the task of the coating membrane, which is also controlled by other parameters i.e thickness and porosity of the coating structure. As presented in the **Figure 6.10**, the nutrient release rate from the coated fertilizer decreases with the increase in the polymer concentration of the coating solution, with a significant difference ($p<0.05$) between uncoated NPK and CRF. At 3rd week, the nutrients (N, P & K) from the CF 20% were released rapidly (0.07, 260, 1.58) which are almost similar to that uncoated NPK (0.08, 272, 1.6). This was influenced by the thinner coating layer coupled with higher porosity (figure1) of the coating. The nutrients from the CF 30% (0.07, 213, 1.44) and CF 40% (0.06, 208, 1.43) was released comparatively much slower than CF 10%. Higher concentration clearly shows a slow release of nutrients. As shown in the Figure 6.10, after 6 weeks, the nutrient release was lesser CF 20% (0.07, 301, and 1.46) compared with CF 30% (0.07, 257, 1.68) and CF 40% (0.07, 243, and 1.84). After 9weeks similar trend appeared where nutrient release is more in higher concentration CF 40% (0.08, 299, 1.92) and CF 30% (0.08, 317, 2.1) compared to lower concentration CF 20% (0.06, 251, 1.54). The results obtained indicate the slow release pattern of CRF's in high concentration. This could be because CF 40% has a lower porosity compared with the other two concentrations as depicted in **Figure 6.4**. Less porosity with a thicker coating layer decreases the rate of nutrient release. A similar pattern was observed in (Tomaszewska and Jarosiewicz, 2002). Thus, the increase of polymer concentration in the coat-forming solution will result in the decrease of nutrient release rate.

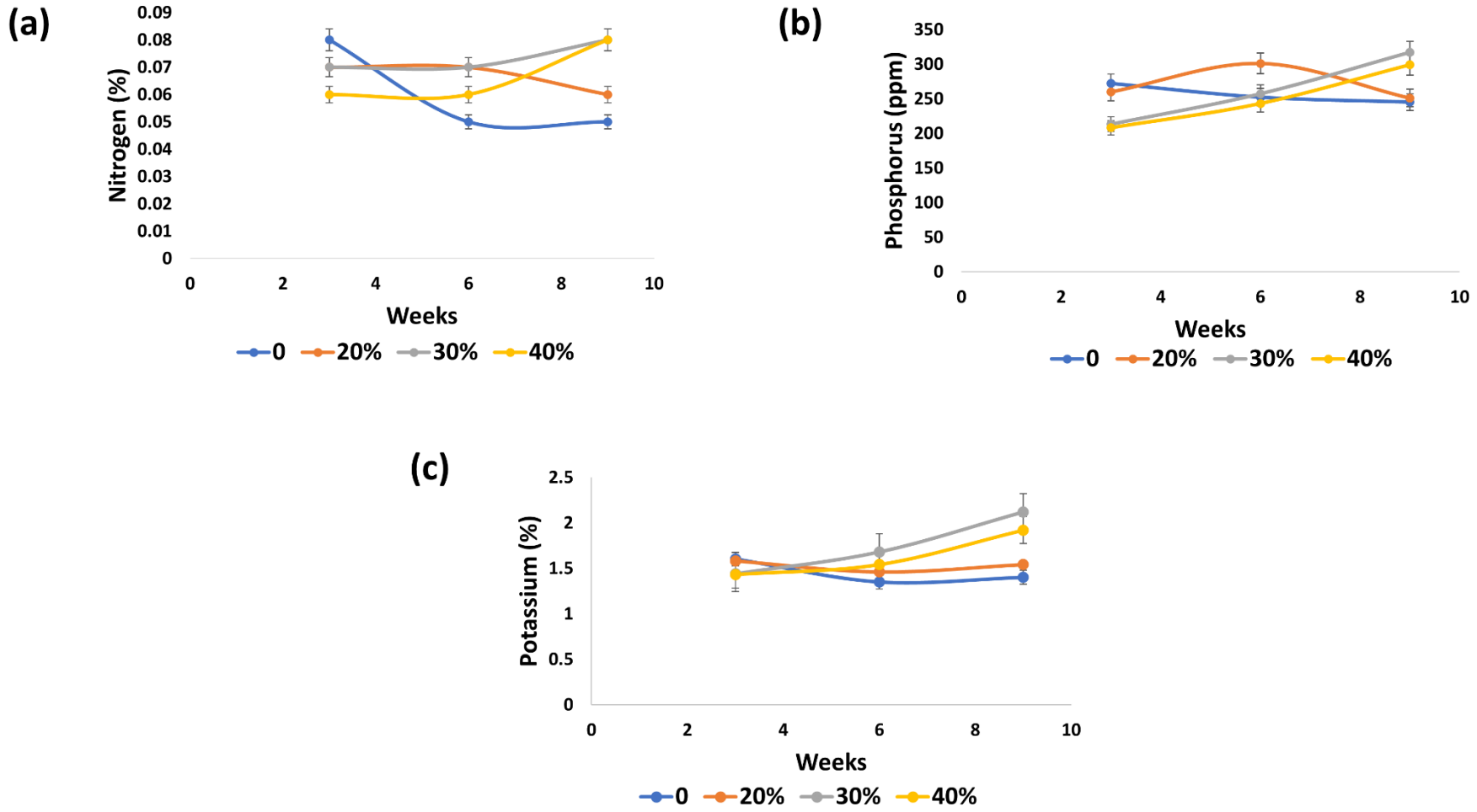


Figure 6-10: Release of nutrients based on concentration of PCL in coating structure. Within a treatment group, bars with the same letters are not significantly different using Tukeys test at 5% level. SE bars are standard deviation values.

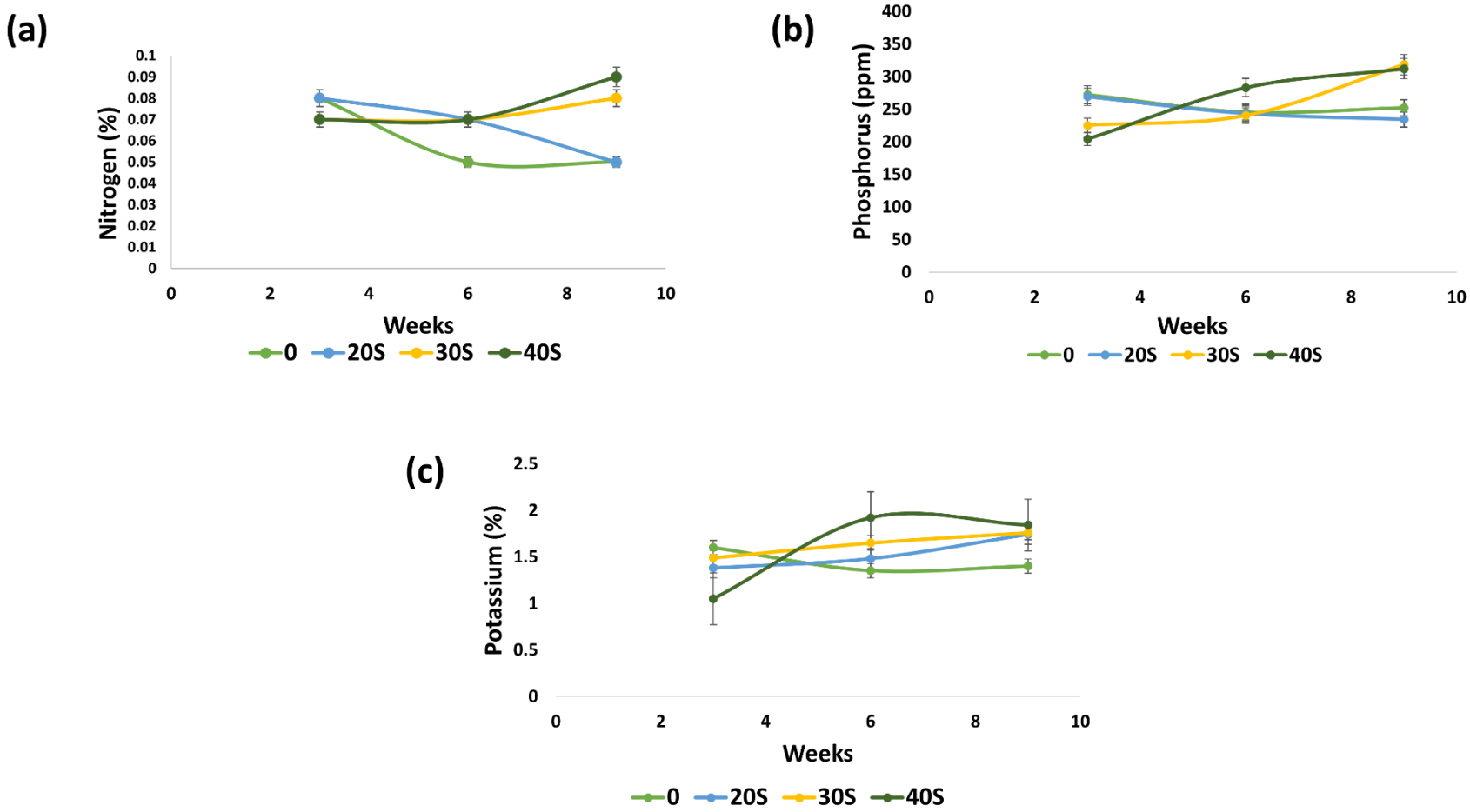


Figure 6-11: Release of nutrients based on concentration of PCL in coating structure. Within a treatment group, bars with the same letters are not significantly different using Tukeys test at 5% level. SE bars are standard deviation values.

Figure 6.11 displays the percentage of nutrients released by different concentration of the coatings prepared by PCL/starch composites at 3rd week, 6th week and 9th week. As presented in Figure 6.9, at 3rd week, the nutrients (N, P & K) from the CF 20S were released rapidly (0.08, 259, 1.38) which are almost similar to that uncoated NPK (0.08, 272, 1.6). The nutrients from the CF 30S (0.07, 225, 1.49) and CF 40S (0.06, 213, 1.44) was released comparatively much slower than CF 20S. Later in 6th week, the nutrient release was lesser in CF 20S (0.05, 243, and 1.48) compared with CF 30S (0.07, 257, 1.65) and CF 40S (0.07, 301, and 1.68), Similar trend is noticed after 9th week, where nutrient release is more in higher concentration i.e. CF 30S (0.08, 318, 1.76) and CF 40S (0.1, 337, 1.84) compared to lower concentration CF 20% (0.05, 343, 1.74), except the phosphorus content, the release is low in 30S compared to 40S.

From **Figures 6.8 and 6.9**, it is clearly evident that the PCL coated fertilizer shows a slow release compared to the fertilizer without coating. But, when coming to individual concentration, only PCL coating with a higher concentration (40%) showed a significant difference from normal NPK without coating. Whereas the low concentration most of the time, are under the same subset indicating the non-significant difference. Same trend is observed in PCL/starch coatings as well, no significant difference between the different concentrations. This could be due to the imperfect coatings formed during the solvent casting.

When compared to only PCL coating the release is faster in PCL/starch coatings, more porosity and presence of starch explains their faster release of nutrients. This could also be due to the biodegradation, which will be more in CRF samples with starch. Wu et al studied the biodegradability of PCL and PCL/starch composites also reported similar observations in their study that starch improves the biodegradability when blended with PCL (Wu et al., 2007).

6.5 Influence of CRF on the growth and yield of mung bean

Figure 6-12 depicts the arrangement of the plants in the plot layout to the study of effect of PCL CRF on the growth and yield of the mung bean. PCL coated 30 [Fertilizer coated with only PCL (30%)] was studied for its effect on growth of mung bean, since it showed maximum release of nutrients in 9 weeks.



Figure 6-12 : The plot layout of the experiment placed to study the influence of CRF based on randomization in the Shed house of The University of Nottingham Malaysia Campus.

6.5.1 Plant height, Number of leaves, Chlorophyll content and Anthocyanin content

Figure 6.14, 6.15 and Table 6.2 presents the effect of CRF and normal NPK granules on the plant height, number of leaves, anthocyanin and chlorophyll content along with control**.** Figure 6.14 depicts significant effect ($P<0.05$) on the plant height and leaf number during the application of CRF after the 3rd week i.e. during 6th and 9th week. At 3rd week, the plant height is more in NPK (13.6 cm) compared to CRF and control (12.2 & 9.9 cm). CRF recorded the highest plant height at 6th week (28.6 cm) compared to NPK and the control (25.9 & 20.7 cm,

respectively). Similar trend was observed during the 9th week, where CRF has recorded the highest value (33.3cm) than NPK and control (27.7 & 24.6 cm). Plant height shows a significant difference between the CRF, NPK and control, as depicted in **Figure 6.13**. This significant effect of CRF application could be attributed to the fact that it provides the nutrients throughout the growth of the plant preventing fertilizer leaching. Similar trend was observed in leaf number, where a significant effect was observed after 3rd week. During 3rd week, there is no prominent effect (P=0.3) between CRF (3), NPK (2) and control (2) falling under the same subset. Subsequently, higher values were recorded with CRF at 6th and 9th (11 & 13) week when compared to NPK (9 & 8) and the control (7 & 6).

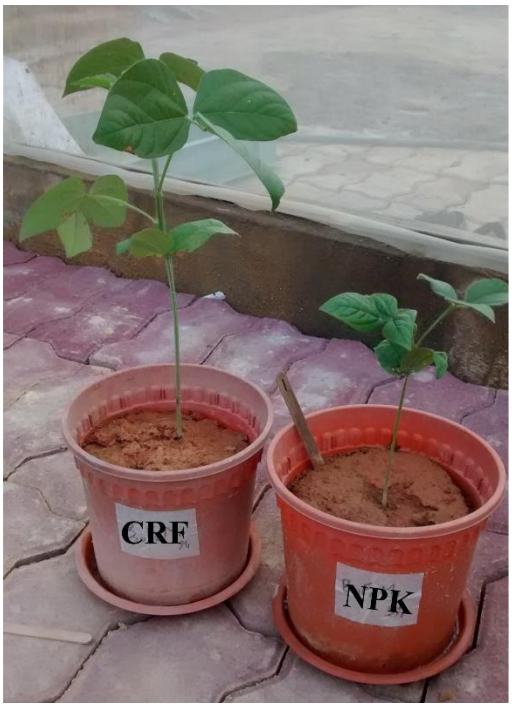


Figure 6-13: Difference between the Mungbean crops treated with CRF (encapsulated) and NPK

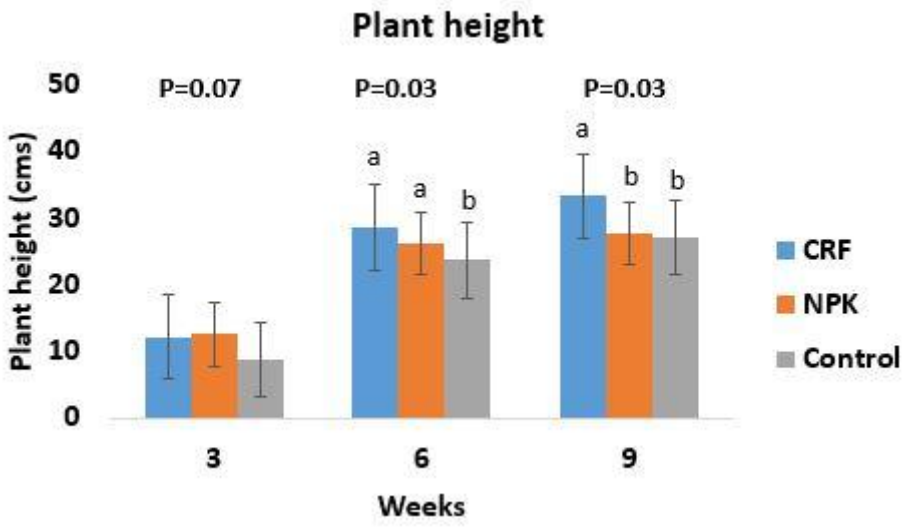
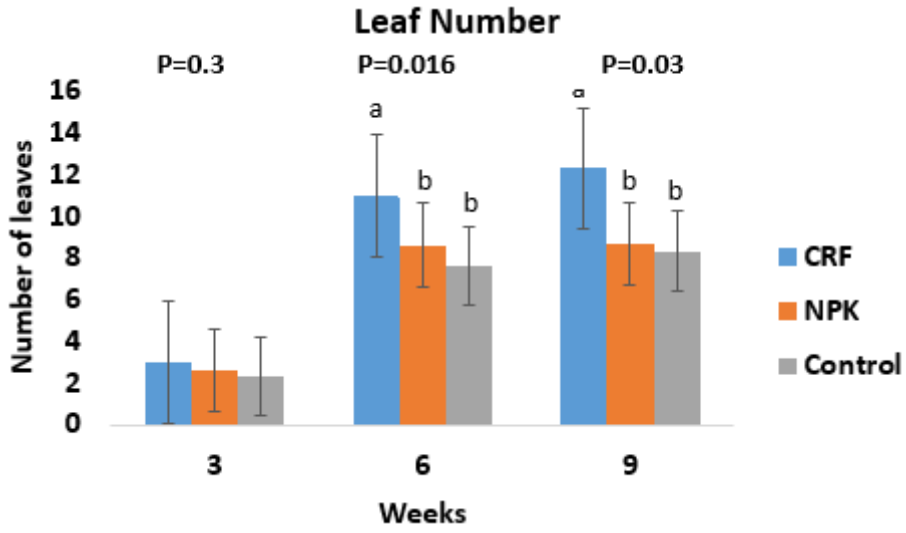


Figure 6-14: Effect of CRF and NPK on plant height of mung bean.



*p stands for significance; a& b are subsets indicating the significant difference between each level.

Figure 6-15 : Effect of CRF and NPK on Leaf number of mung bean.

Table 6-2 : Effect of CRF and NPK application on chlorophyll and anthocyanin content

	Chlorophyll content		
	3rd week	6th week	9th week*
CRF	48.2	39.1	42.5a
NPK	50.7	42.7	32.8a
Control	31.4	40	26b
SEM	6.4	6.3	3.04
P	0.180	0.400	0.006
	Anthocyanin content		
	3rd week	6th week	9th week
CRF	3.7	7.1	8
NPK	6.1	5.7	4.9
Control	4.2	4.5	6.2
SEM	0.8	1.05	1.17
P	0.200	0.2000	0.180

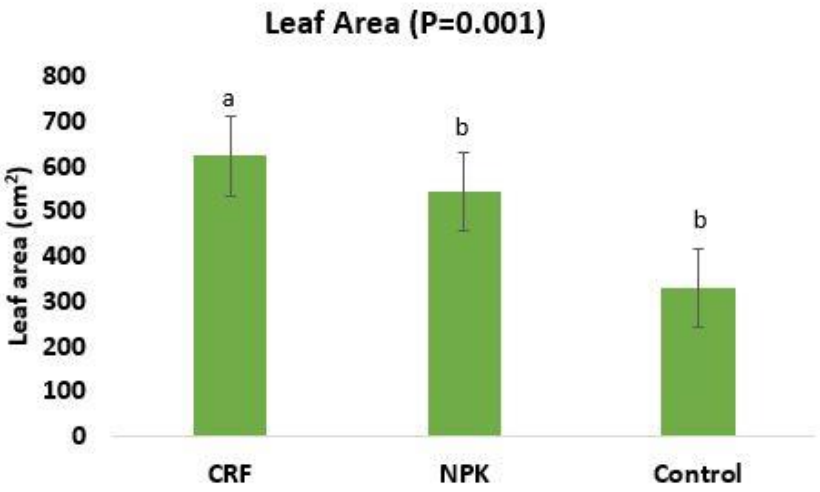
Table 6.2 presents the effect of CRF and NPK application on chlorophyll and anthocyanin content during the 9weeks of plant growth period. It was observed that chlorophyll content in CRF and NPK was not significantly different ($P>0.05$) in 3rd and 6th week. As shown in the table, during 3rd week, the plant given with NPK has more chlorophyll content compared with CRF and control. Later in 6th week after sowing, same pattern was observed. Finally, during 9th week, CRF has recorded higher values (42.5) compared to NPK (32.8) and control (26.0). Apparently, anthocyanin content had no significant effect observed with application of CRF to that of NPK.

Significant effect ($P<0.05$) was observed on plant height, leaf number and chlorophyll content for the plants treated with CRF after the 3rd weeks after sowing (WAS). Where during the 3rd

week the NPK had recorded the highest values. This could be due to the fact that, at early stages, plants treated with NPK will have more nutrients than plants treated with CRF. From earlier results obtained for nutrient release (Figures 6.8 and 6.10) exhibits that nutrients are released slowly from CRF, which increase gradually in 6th and 9th WAS. This explains the significant growth in plants treated with CRF after 6th and 9th week. Study on the effect of CRF on the growth of calla reported similar types of results, where plant height, leaf number and chlorophyll content showed significant results for application CRF. (HOU et al., 2008).

6.5.2 Leaf Area and dry weight

The influence of CRF, NPK and control on the leaf area and total dry weight was depicted in **Figure 6.16 and 6.17.**



*p stands for significance; a& b are subsets indicating the significant difference between each level.

Figure 6-16 : Effect of CRF and NPK on Leaf area of mung bean.

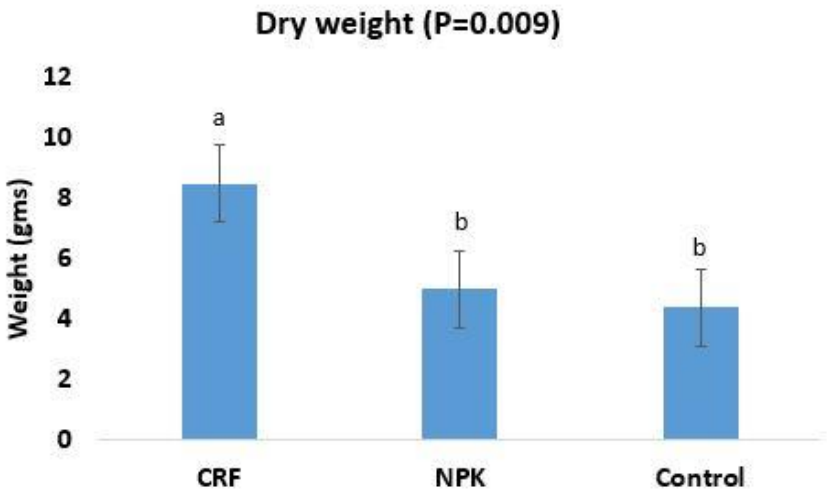


Figure 6-17 : Effect of CRF and NPK on dry weight of mung bean.

Significant effect (P<0.05) was observed on leaf area and total dry weight during the CRF application. The leaf area was recorded highest values for CRF (624.1) when compared to NPK (515.1) and control (330.5). **Figure 6.17** depicts the influence of CRF and NPK on total dry weight of the plant. According to the figure, significant (P<0.05) effect on total dry weight of the plant was noted. Application of CRF had recorded 8.5gms of total dry weight, which was comparatively higher than the value observed for NPK (5gms) & control (4.6gms). Improved dried weight can be associated with an increase in leaf area, which enhances photosynthesis leading to the growth of plant. Apparently, Tian et al reported the improved amount of dry matter of the plant when treated with CRF (Tian et al., 2016)

6.5.3 Number of pods plant^{-1}, pod length, pod weight, seeds pod^{-1} and seed weight

Figure 6.18 and Table 6.3 presents the impact of CRF and NPK on the number of pods per plant, pod length, pod weight, seeds per pod and seed weight of green gram.

Application of CRF recorded a higher number of pods and seeds per plant (8 & 51) than NPK (6 & 47) and control (4 & 31). Also, application of CRF recorded the highest total pod weight

(5.8 gms) and total seed weight (4.7gms) followed by NPK (4 and 3.9 g) and the least was recorded in control (3.2g and 2.8 g). Table 6.3 presents the CRF effect on pod length, demonstrating no notable difference between CRF and NPK. Although, the values recorded highest for all the parameters are higher for CRF, there is no significant difference (P >0.05) noted between CRF and NPK during this trial.

Table 6-3: Influence of CRF on the pod length

Treatment	Pod length (cms)
CRF	8.1
NPK	7.8
Control	6.9
SEM	0.64
P	0.2

*p & SEM stands for significance and standard error mean

6.5.4 Root length, root area, root volume, root diameter and nodules

Influence of CRF and NPK included with control on the root length, root volume, root diameter, root surface area and nodules are depicted in **Figure 6.19.**

Application of CRF on the crop resulted in the highest root length (2674.7cm), root volume (11.3cm^3), root diameter (0.79mm) and root surface area (622.8cm^2) followed by the application of NPK (2584.3 cm, 10.3 cm^3, 0.72mm & 606.1 cm^2), and the least (2021 cm, 8.08

cm^3, 0.61mm and 525.1 cm^2) was recorded in the treatments receiving no NPK application. Out of different parameters of the root, application of CRF demonstrated a significant ($p<0.05$) effect of on root diameter and root surface area during the trial.

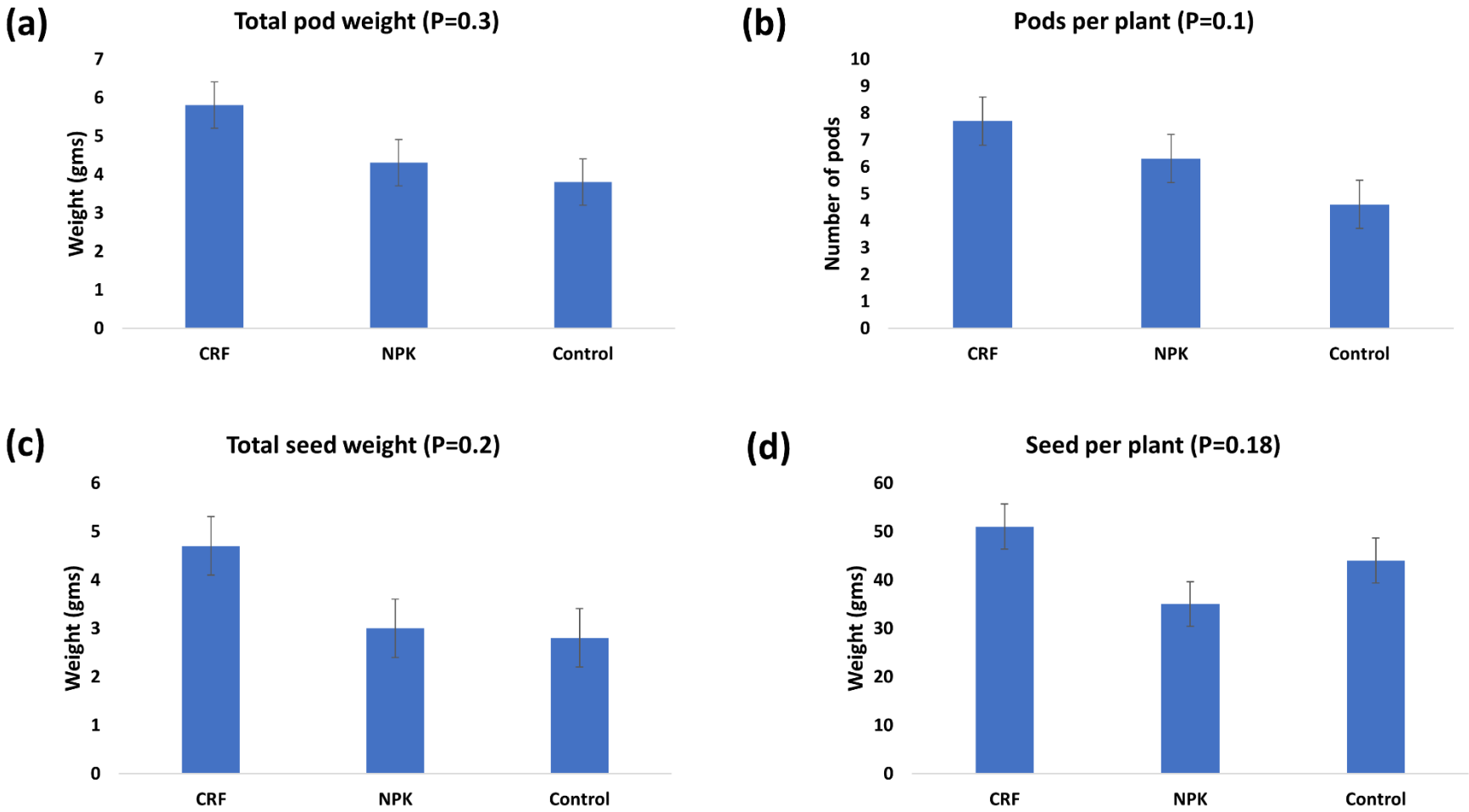


Figure 6-18 : Influence of CRF and NPK on the pod weight, seed weight, pods & seeds per plant

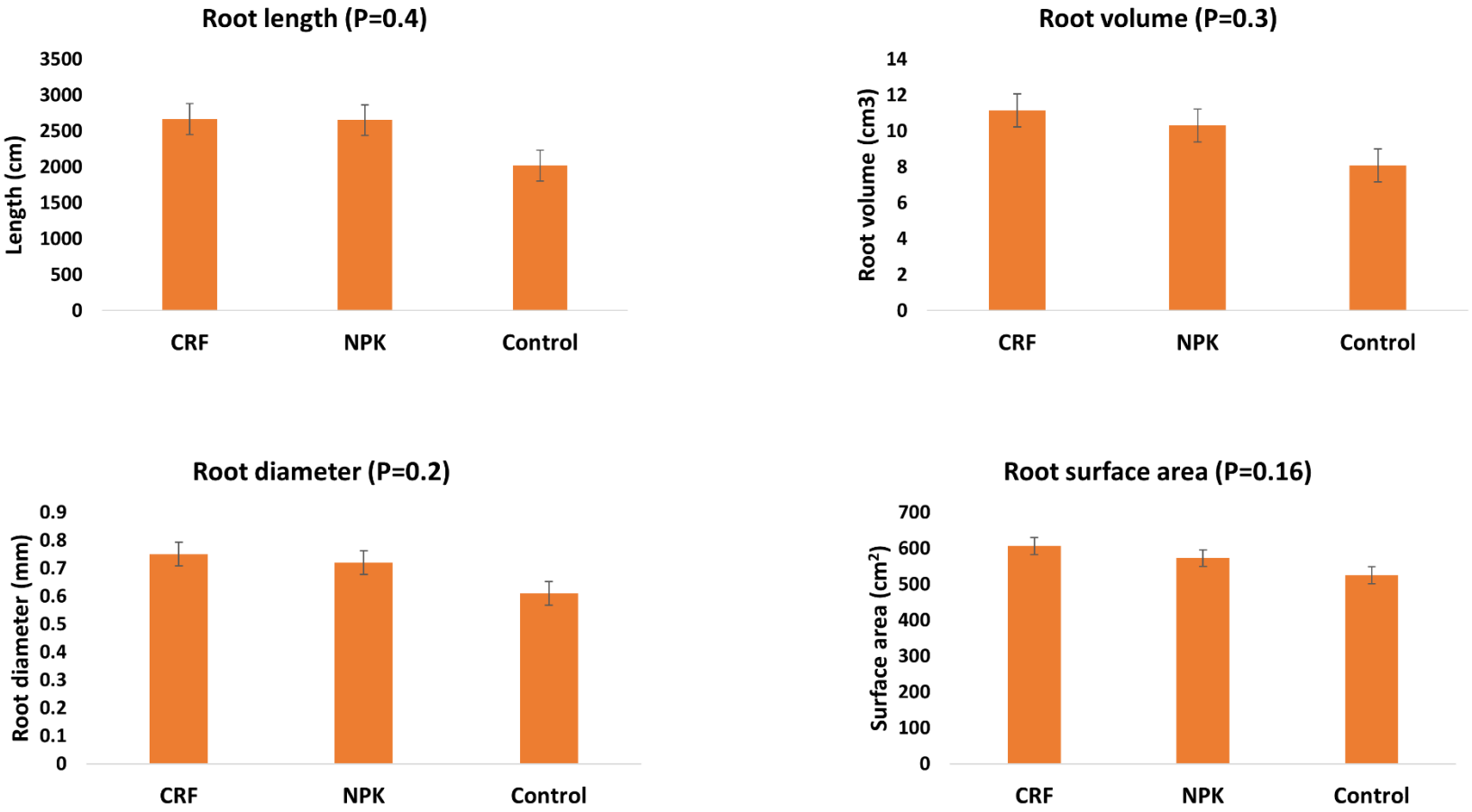


Figure 6-19: Effect of CRF and NPK on root parameters of mung bean. Within a treatment group, bars with the same letters are not significantly different using Tukey's test at 5% level. SE bars are standard deviation values. *p stands for significance* .

6.6 Summary:

The use of polycaprolactone for coating the NPK fertilizer via solvent casting was successfully achieved. Polymer concentration in the coat forming solution plays an important role in porosity and thickness of the coating. It is observed that as PCL concentration increased the thickness of the coating layer increased, while porosity decreased. Litter bag experiment revealed that more degradation has occurred in the case of lower concentrations 20% compared to higher concentration 40%. Subsequently, the release of nutrients is higher in lower concentration (PCL 20%) followed by 30% and slowest released by observed in high concentration 40%. On the other hand, PCL/starch coatings did not show a notable difference between the concentrations. Application of CRF for mung bean plants showed a significant difference in few growth characteristics such as plant height, leaf number, leaf area and dry biomass. No significant effect was observed in the yield of mung bean.

CHAPTER 7

PREPARATION OF PCL BASED CRF USING BLENDING TECHNIQUE AND ITS INFLUENCE ON GROWTH AND YIELD OF MUNGBEAN

7.1 Introduction

This chapter addresses the fourth objective of this work, which is the preparation of the control release fertilizers using blending technique. In the previous chapter, where CRF are prepared using solvent casting technique, significant result was observed only with PCL coating but not for PCL/starch. Therefore in this chapter, blending technique was used to prepare the CRF's by preparing PCL/starch/NPK blends. Blending/melt mixing technique is considered easy and simple to handle with a very minimum time required for sample preparation. In addition, the use of a solvent can be totally eliminated. Therefore, in this present chapter three combination of polymer and starch (5:1, 1:1 and 5:1) mixed along with NPK (10 grams) were blended together for preparing the CRF samples. PCL with only one molecular weight was opted in this chapter (molecular weight of 36000g/mol) since in the earlier chapter, no significant difference was observed between the two molecular weights used. Further, surface morphology, porosity, biodegradation studies and nutrient release (nitrogen, potassium and phosphorus) analysis of the PCL based blended fertilizers was discussed. To the best of author's knowledge, polycaprolactone and NPK blends are never prepared and examined for their nutrient release characteristics till date. Finally, the influence of PCL based CRF (prepared by blending technique) on the growth and yield of mung bean was studied in this chapter.

7.2 Surface morphology and porosity

Surface morphology of the blended and coated samples was characterised by performing FESEM. **Figure 7.2** displays the surface morphology of the blended samples (NPK, PCL and starch) confirming the presence of NPK and starch distributed all along the sample. The thickness of CRF samples prepared was 1 mm, maintained for all the samples.



Figure 7-1: PCL-CRF prepared by Blending technique

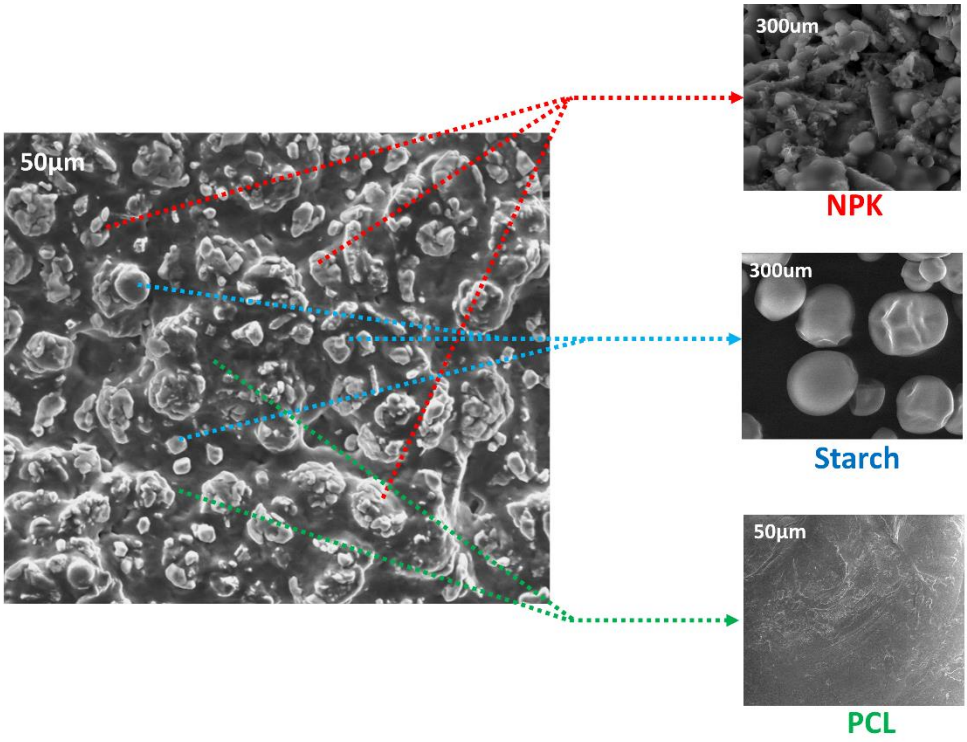


Figure 7-2 Surface morphology of the CRF (prepared via blending technique)

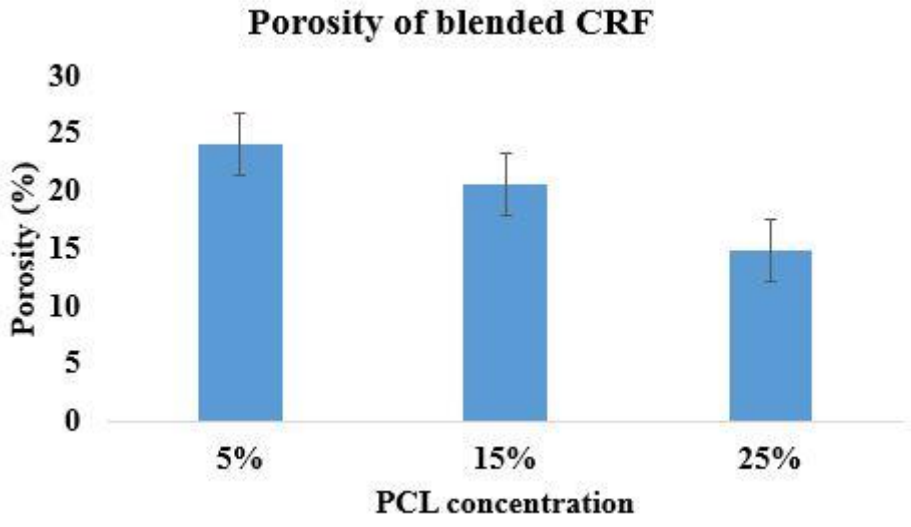


Figure7-3 : Porosity of CRF samples prepared via blending technique

Correspondingly, the porosity of the samples plays an important role in the control release of nutrients. By regulating the exchange of the water and the nutrients, in and out of the granules. **Figure 7.3** depicts the porosity of the different concentration of the samples. The porosity of the CRF samples was ranging from 14.86 % to 26.5 %. The blended CRF samples are more porous compared to CRF samples prepared by solvent casting technique. This could be due to the presence of starch and NPK molecules embedded all along making the sample porous. As said before, the incorporation of starch tends to increase the surface area of the samples, same time increasing the water absorption capacity (Jarosiewicz and Tomaszewska, 2003). Porosity of high concentration sample (25%) shows lesser porosity 14.8 % compared to other two concentrations (15% & 5%) having 24.9% and 20.5 % porosity. Also, almost same pattern was obtained when the solvent casting technique was used confirming that porosity decreases with the increase in the polymer concentration. A similar trend was observed in related studies to nutrient release from polymer coated fertilizer (Tomaszewska et al., 2002).

7.3 Biodegradation study

Time course morphology of the blended CRF samples at 3rd, 6th and 9th week are depicted in **Figures 7.4, 7.4 and 7.6.** After 3 weeks of incubation in the soil, the surface of 5% CRF shows more deterioration compared to 15% and 25 % **(Figure 7.4).** After 6weeks, the damage became obvious **(Figure 7.5**) which is due to the biodegradation of the samples. With further increase of time, the degradation increased, as depicted in **Figure 7.6** shows the samples after the incubation for 9weeks. If the polymer concentration is more it will slow down the degradation. Study on biodegradability of polycaprolactone and starch blends has reported a similar trend related to polymer concentration and degradation (Wu et al., 2007).

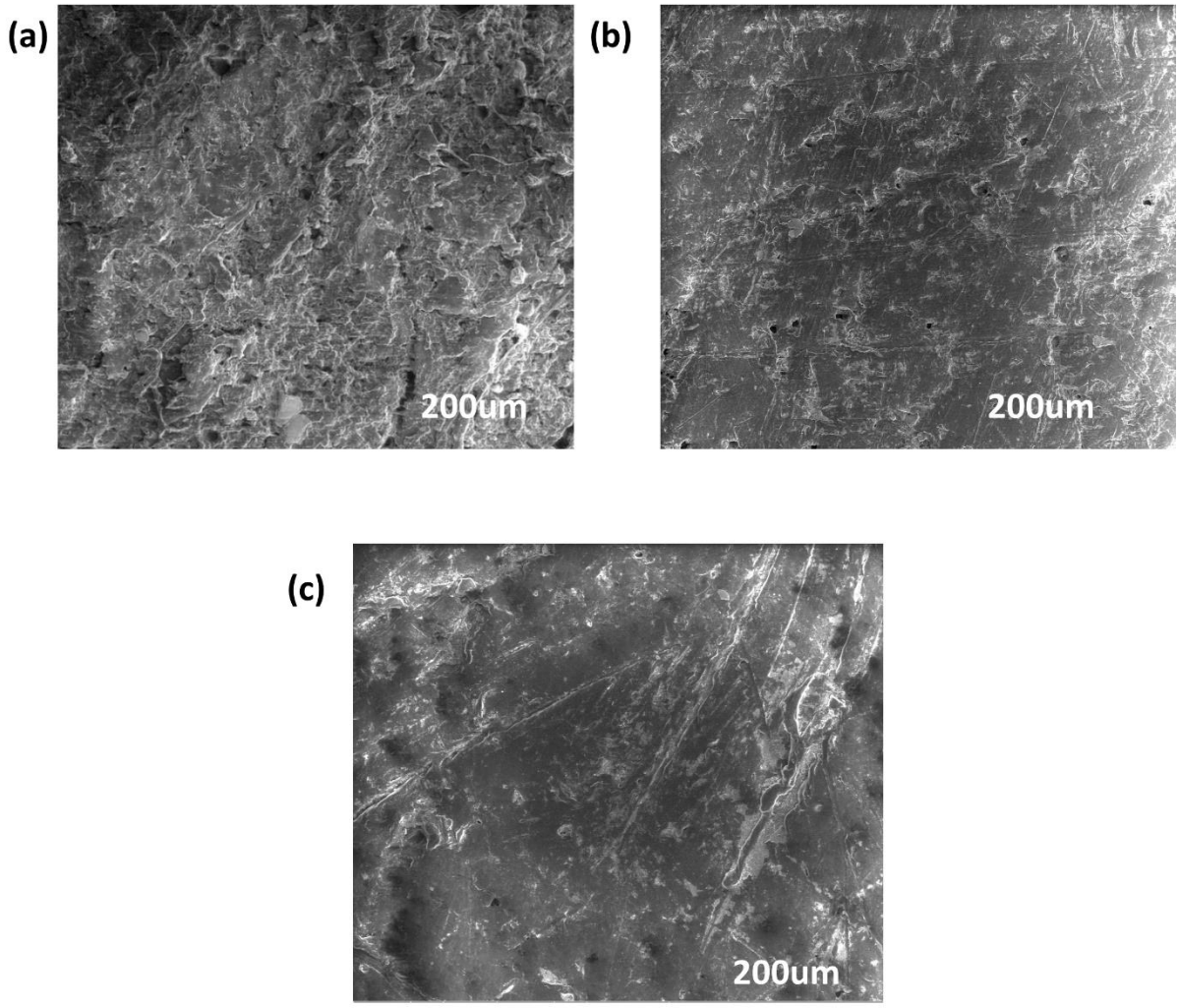


Figure 7-4: Morphology of the PCL based CRF (prepared via blending technique) at 3rd week a) 5% b) 15) c) 25%

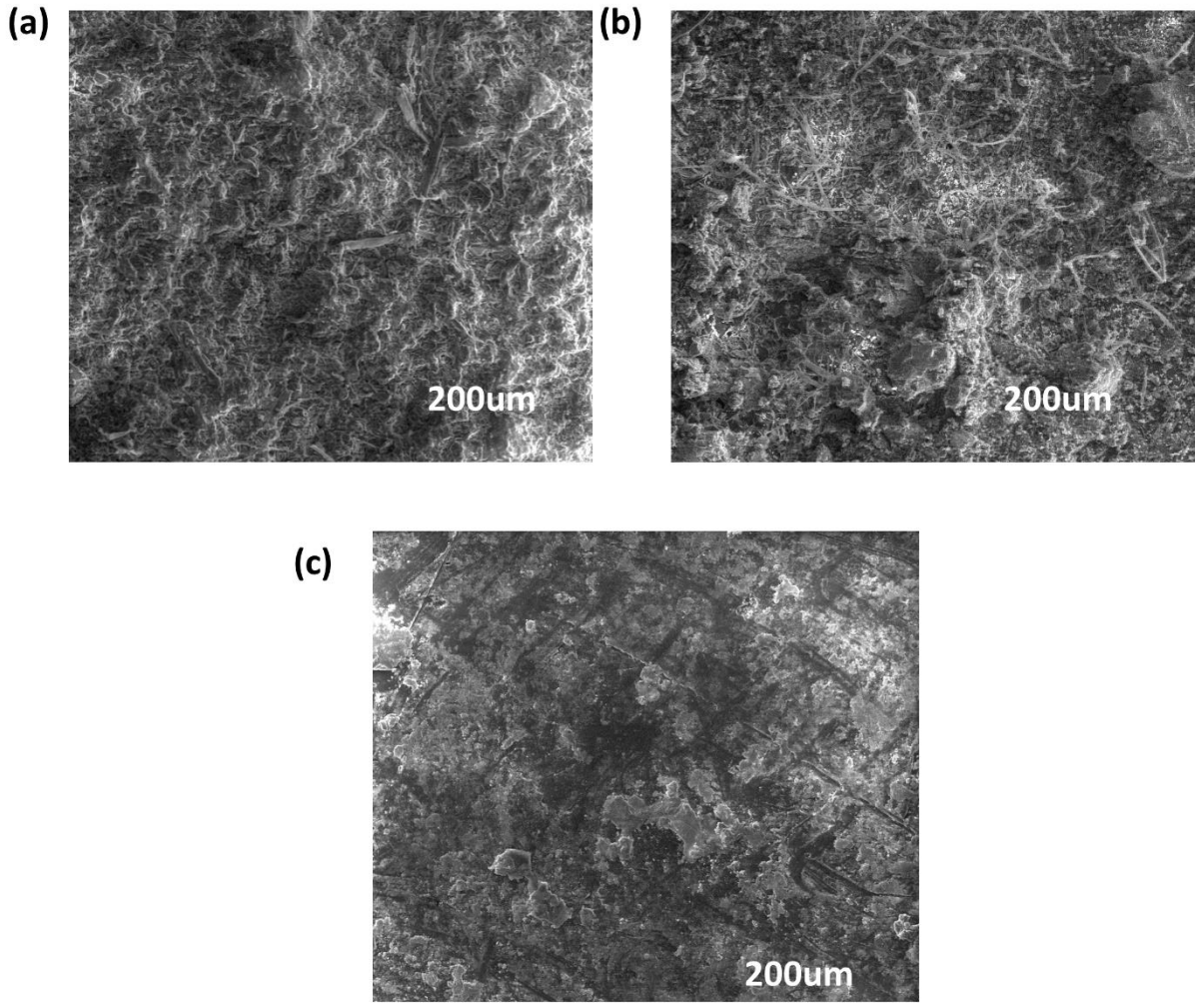


Figure 7-5 : Morphology of the PCL based CRF (prepared via blending technique) at 6th week

a) 5% b) 15) c) 25%

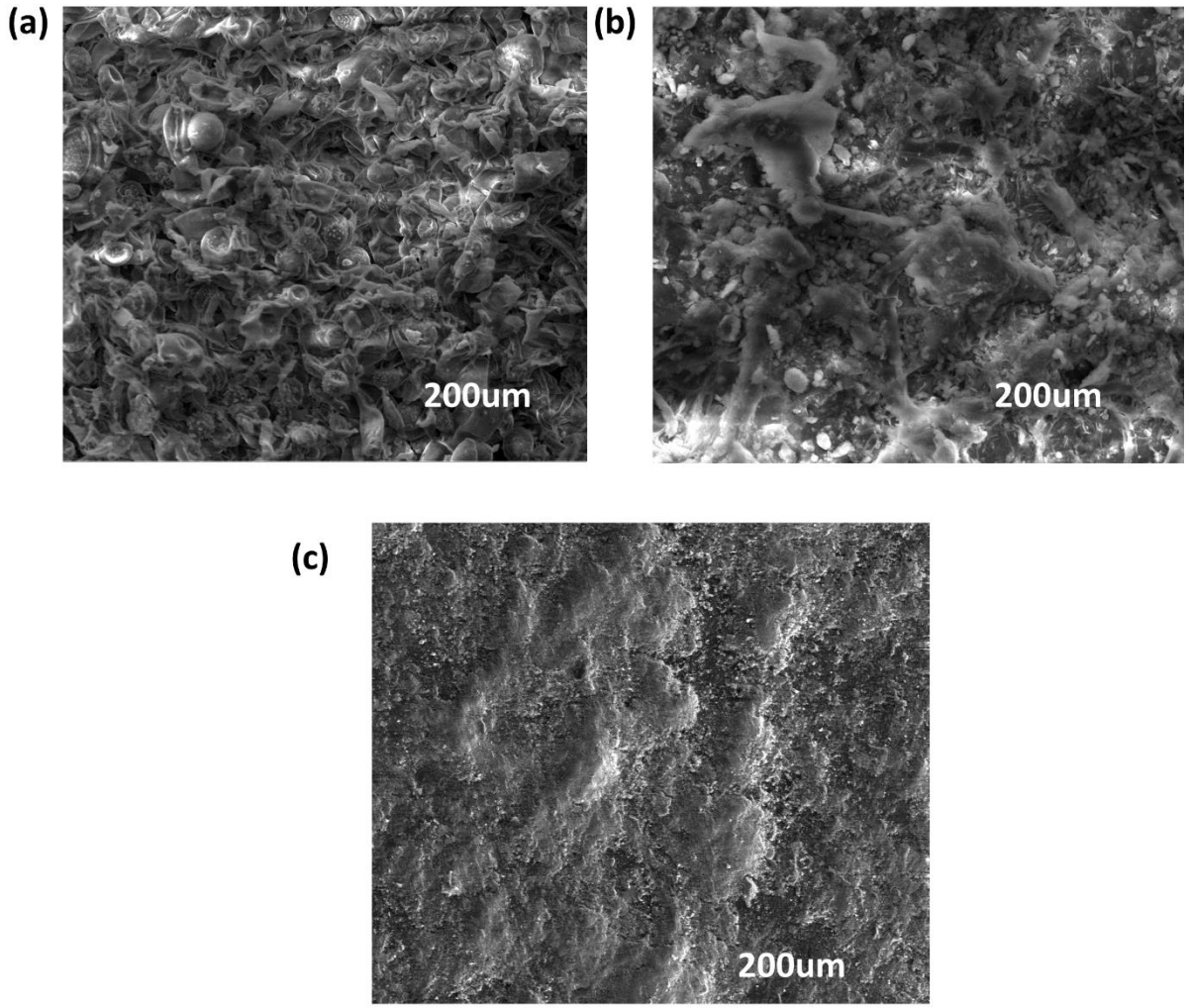


Figure 7-6: Morphology of the PCL based CRF (prepared via blending technique) at 9th week a) 5% b) 15) c) 25%

7.4 Nutrient release

The amount of nutrients released along the growth period i.e. at 3rd 6th and 9th week was assessed by performing test methods (Distillation method for nitrogen, phosphor-vanadomolybdate complex method for phosphorus and Kjeldahl flask method for potassium at applied agricultural research) to analyse nutrients in the soil samples

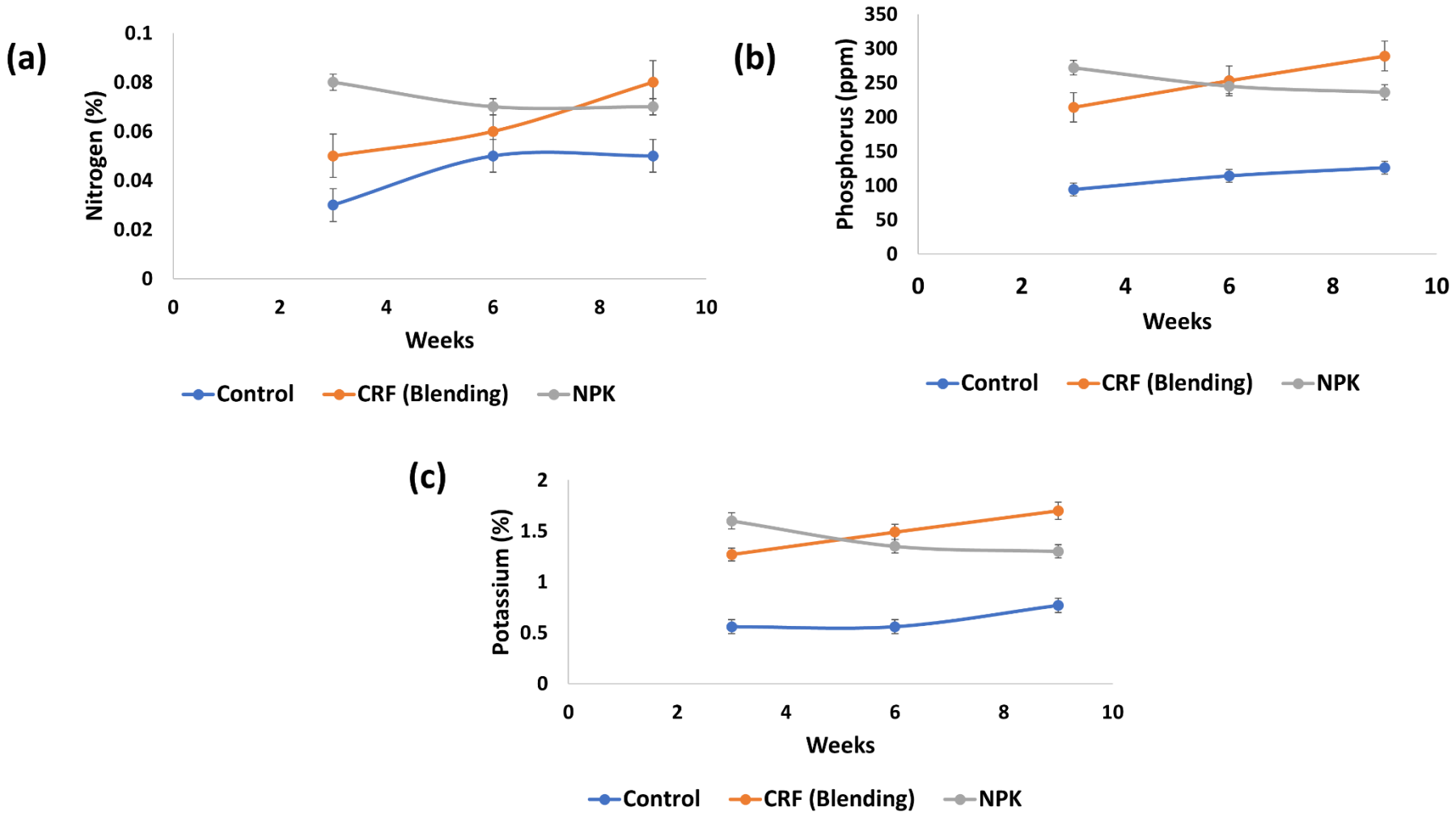


Figure 7-7: Release of nutrients based on method a) release of nitrogen b) release of phosphorus c) release of potassium

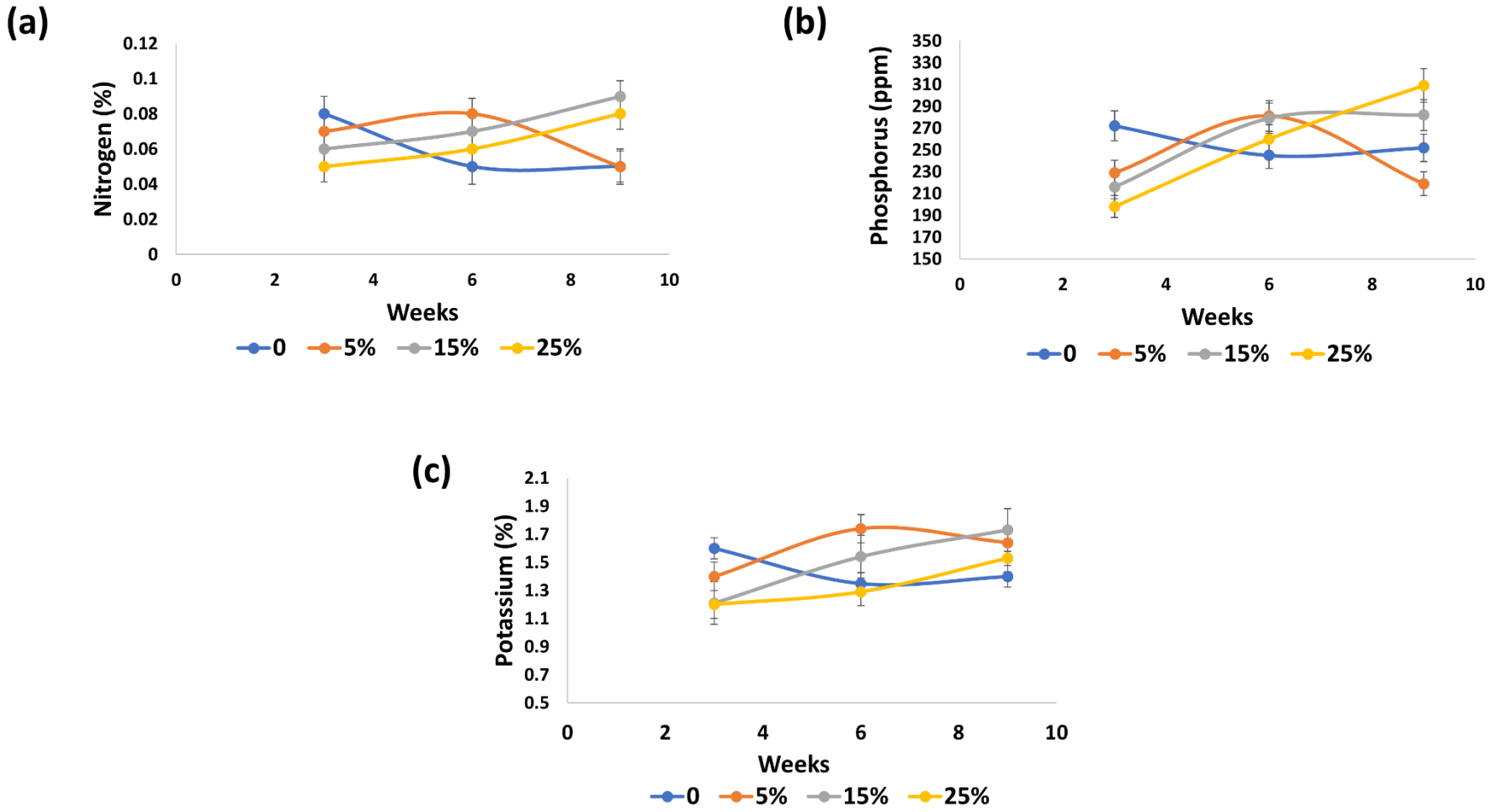


Figure 7-8: Release of nutrients based on the concentration of PCL used a) release of nitrogen b) release of phosphorus c) release of potassium

Amount of nutrients (N, P and K) released at 3rd week recorded the lowest values for CRF (0.05, 214, 1.27) samples compared with uncoated fertilizer (0.08, 272, 1.6) and control (0.03, 94, 0.56). It is evident from the analysis that there is a significant difference ($P< 0.05$) between control release fertilizers and uncoated fertilizer, where the amount of nutrients released are much lesser in CRF samples suggesting a slow release of nutrients. Later in the 6th week, the nutrients released from uncoated fertilizer (0.07, 245, 1.35) are lesser compared with CRF (0.06, 253, 1.49), except the nitrogen content. Further, in the 9th week similar trend appeared i.e. the amount of nutrients released from uncoated fertilizer (0.07, 236, 1.3) are lesser compared with CRF (0.08, 289, 1.7). From Figure 7.7, it is evident that the nutrient release from the uncoated fertilizer was lesser in 6th and 9th week compared to 3rd week. Similar trend was observed in 3rd week for CRF prepared via solvent casting.

Figure 7.8 displays the percentage of nutrients released by different concentration of the polymer coatings formed PCL at 3rd week, 6th week and 9th week. The nutrient release rate is controlled by the thickness and porosity of the coating structure. As presented in Figure 7.8, the nutrient release rate from the coated fertilizer decreases with the increase in the polymer concentration of the coating solution, with a significant difference ($p<0.05$) among the three concentration used. At 3rd week, the nutrients (N, P & K) from the CF 5% were released rapidly (0.07, 229, and 1.4) almost similar to normal NPK. The nutrients from the CF 15% (0.06, 216, 1.3) and CF 25% (0.05, 198, 1.2) was released comparatively much slower than CF 5%. Higher concentration clearly shows a slow release of nutrients. As shown in figure 7.7, after 6 weeks, the nutrient release showed a similar pattern between for the nutrients release. That is, amount of nutrient released from CF 5% (0.08, 281, and 1.74) are more compared with CF 15% (0.06, 279, 1.54) and CF 25% (0.07, 260, and 1.29). After 9weeks, nutrient release in CF 5% (0.05, 252, and 1.64) decreased compared with higher concentrations CF15% (0.08, 219, and 1.73) and CF 25% (0.09, 282, and 1.52). The results obtained indicate the slow release pattern of

CRF's in high concentration. This could be because CF 25% having lower porosity compared with the other two concentrations as depicted in figure 7.2. Thus, the increase of polymer concentration in the coat-forming solution will result in the decrease of nutrient release rate. Whereas in CRF 5% and normal NPK the nutrients release is more in the early weeks, later on, decreased along the time course. This probably due to the leaching of nutrients which are released early.

7.5 Influence of Control release fertilizer on mung bean

Figure 7-10 & 7-11 depicts the arrangement of the plants in the plot layout to the study of effect of PCL blended CRF on the growth and yield of the mung bean. Blend 25% of PCL i.e. (blend 5:1) was given to the plants.



Figure 7-9: the plot layout of the experiment placed to study the influence of CRF in early week based on randomization in the Shed house of The University of Nottingham Malaysia Campus.



Figure 7-10 the plot layout of the experiment placed to study the influence of CRF in later weeks based on randomization in the Shed house of The University of Nottingham Malaysia Campus.

7.5.1 Plant height, Number of leaves, Chlorophyll content and Anthocyanin content

Influence of CRF (prepared via blending technique) and normal NPK granules on the plant height, number of leaves, anthocyanin and chlorophyll content along with control are shown in **Figure 7.11** and **Table 7.1.** Figure 7.11 *depicts* a significant effect ($P<0.05$) on the plant height and leaf number during the application of CRF. At 3rd week, the plant height is more in NPK (12.6 cm) compared to CRF and control (11.3 & 8.7 cm). Application of CRF application showed a significant effect on plant height after 3rd week. CRF recorded the highest plant height at 6th week (30.6 cm) compared to NPK and the control (26.2 & 23.7 cm, respectively). Similar trend was observed during the 9th week, where CRF has recorded the highest value (35.8cm) than NPK and control (28.2 & 27.1cm). Plant height shows a significant difference between the CRF, NPK and control. This significant effect of CRF application could be attributed to the

fact that it provides the nutrients throughout the growth of the plant preventing fertilizer leaching.

Also generally in crops, good amounts of nutrients induce to improve the plant protein content (Silva and Uchida, 2000). So when plants given with enough amounts of nutrients during its growth period, it improves the protein content same time improving metabolic process helping to improve vegetative growth of the plant (Lawlor, 2002). Similar results were obtained for the study on the effect of polymer coated urea on the rice crop (Binti Said et al., 2014) where plants treated with CRF have better growth compared with normal fertilizer. An equally significant effect was observed in the case of leaf number. Initially, NPK recorded more number of leaves (3) compared with CRF (2) and control (2). Later, higher values were recorded with CRF at 6^{th} and 9^{th} (11 & 14) week when compared to NPK (9 & 8) and the control (8 & 7).

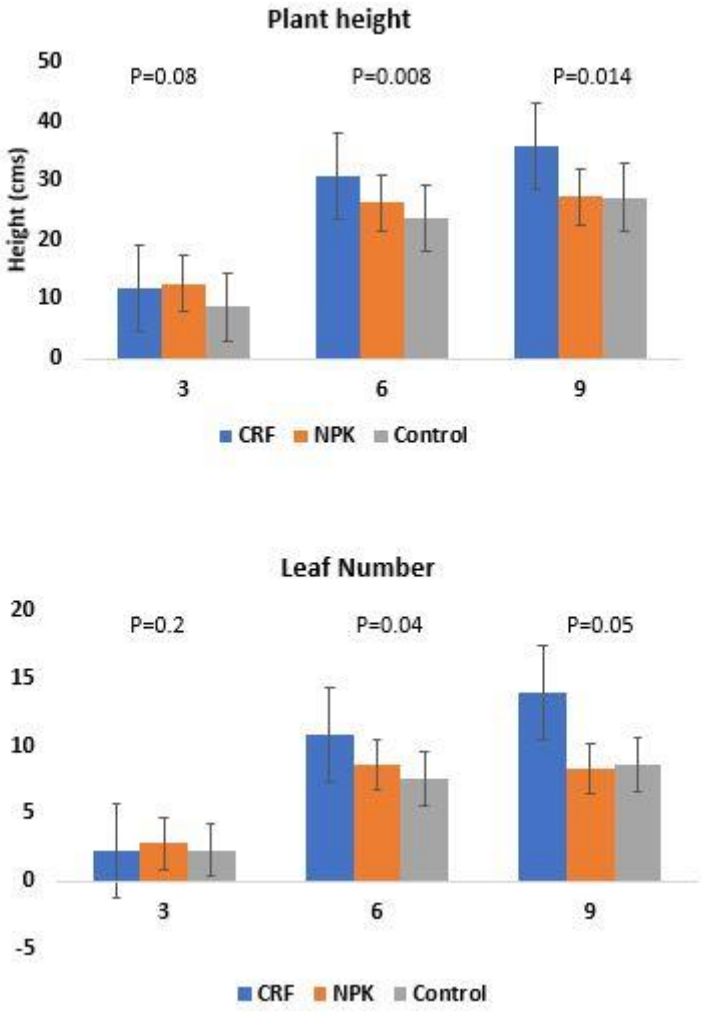


Figure 7-11: Effect of CRF on plant height and leaf number of mung bean.

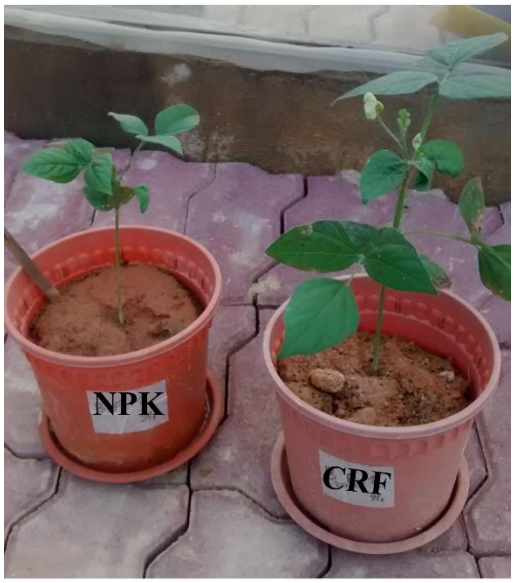


Figure 7-12 : Difference between the Mungbean crops treated with CRF (Blended) and NPK

Table 7.1 presents the effect of CRF and NPK along with control on chlorophyll and anthocyanin content during different growth periods of the plant. It was observed that chlorophyll content in CRF and NPK has no significant difference, but comparatively better than control. During 3rd week, the plant given with NPK has more chlorophyll content (51.2) compared with CRF (47.8) and control (34.9). Later in 6th week and 9th week, CRF has recorded higher values (43.3, 37.8) compared to NPK (40.6, 37.1) and control (40.3, 27.6). Chlorophyll content had increased during 6th week compared with the 3rd week and later decreased during the 9th week. Apparently, anthocyanin content had no significant effect observed with application of CRF to that of NPK. Also anthocyanin content showed a similar trend, which has increased from 3rd week to 6th week. Similar trend appeared during the initial plant trail. As explained earlier chapter, this increase could be due to an increase in leaf thickness which increases along the growth of the plant (Prsa et al., 2007). And later consequent decrease could be the result of leaf senescence, it's a plant aging process where the leaves turns into yellow and starts shedding.

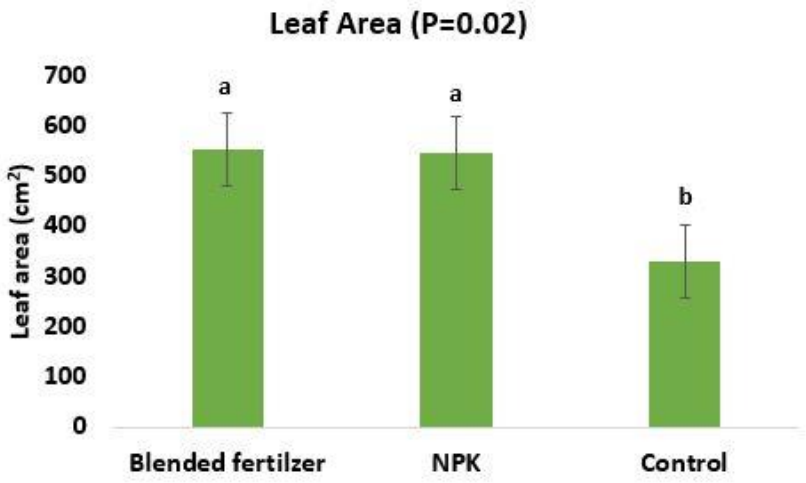
Table 7-1: Effect of CRF on chlorophyll and anthocyanin content

	Chlorophyll content		
	3rd week *	6th week	9th week*
CRF	47.8ab	43.3	37.8a
NPK	51.2a	40.6	37.1a
Control	34.9b	40.3	27.6b
SEM	4.9	3.8	3.5
P	0.03	0.7	0.04
	Anthocyanin content		
	3rd week	6th week	9th week
CRF	5.2	7.6	7.4
NPK	5.1	7.1	7.1
Control	3.7	6.2	5.5
SEM	0.8	1.16	1.08
P	0.2	0.5	0.2

*Within a treatment group, means in a column followed by same letter(s) are not significantly different using Tukeys test. p & SEM stands for significance and standard error mean; a& b are subsets indicating the significant difference between each level.

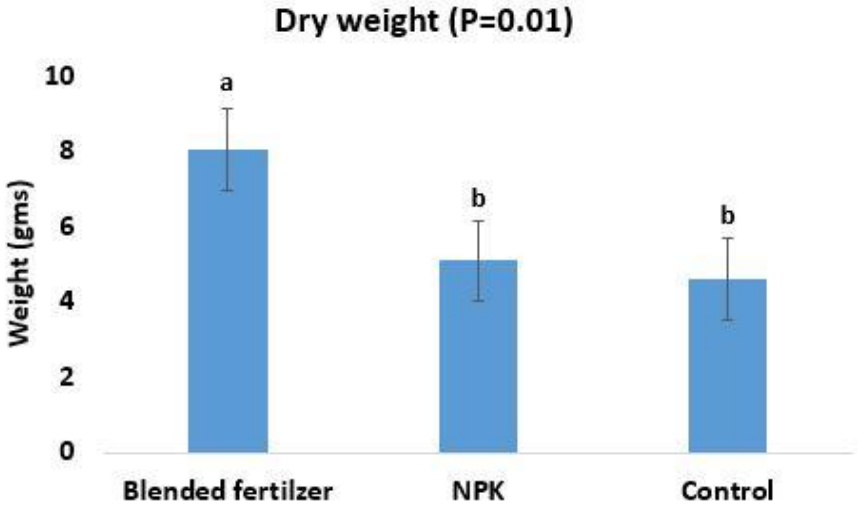
7.5.2 Leaf Area and dry weight

Figure 7.13 ***depicts*** the influence of CRF, NPK and control on the leaf area. The leaf area was recorded to be almost nearer values for CRF (553.4) and NPK (545.1), but when compared to control (330.5) they have a much higher value. Application of CRF had still a better result than NPK, even though the effect is not statistically significant falling in the same subset.



*p & SEM stands for significance and standard error mean; a& b are subsets indicating the significant difference between each level.

Figure 7-13: Effect of CRF on the leaf area of Mung bean



*p for significance; a& b are subsets indicating the significant difference between each level.

Figure 7-14: Effect of CRF on Dry weight

Influence of CRF and NPK on the total dry weight of the plant were depicted in **Figure 7.14**. According to figure, significant ($P<0.05$) effect on the total dry weight of the plant was observed in the CRF and NPK application, Application of CRF had recorded 8.04gms of total dry weight which was comparatively higher than the value observed for NPK (5.09gms) & control (4.6gms). As said earlier improved dried weight can be associated with increase in leaf area, which enhances photosynthesis leading to the growth of plant. Subsequently, results in improved dry matter (Gairola et al., 2009).

7.5.3 Number of pods plant^{-1}, pod length, pod weight, seeds pod^{-1} and seed weight

Figure 7.15 and Table 7.2 presents the impact of CRF and NPK on the number of pods per plant, pod length, pod weight, seeds per pod and seed weight of green gram.

Significant ($p<0.05$) effect of CRF application on the number of pods per plant, number of seeds per plant, total pod weight and seed weight of green gram were observed during the trial. Application of CRF recorded a higher number of pods and seeds per plant (9 & 78) than NPK (6 & 44) and control (4 & 35). Also, application of CRF recorded the highest total pod weight (6.7gms) and total seed weight (5.07gms) followed by NPK (4.3 and 3.07 g) and the least was recorded in control (3.8g and 2.5 g). Therefore, the increase in its quantity in soil may constitute better yield from the plant. Application of NPK helps in vegetative growth finally resulting in more yield from the plant. Besides, pod length, seed per pod was not influenced by NPK application. From the economic aspect, the yield is an important characteristic for considering the preparation of CRF's, with the main purpose of having increased yield. CRF prepared via blending technique shows a significant difference ($P<0.05$) with grain yield compared with uncoated NPK. Similar research studies on the effect of CRF on the plants reported improved grain yield (Tian et al., 2016) (Hashim et al., 2017).

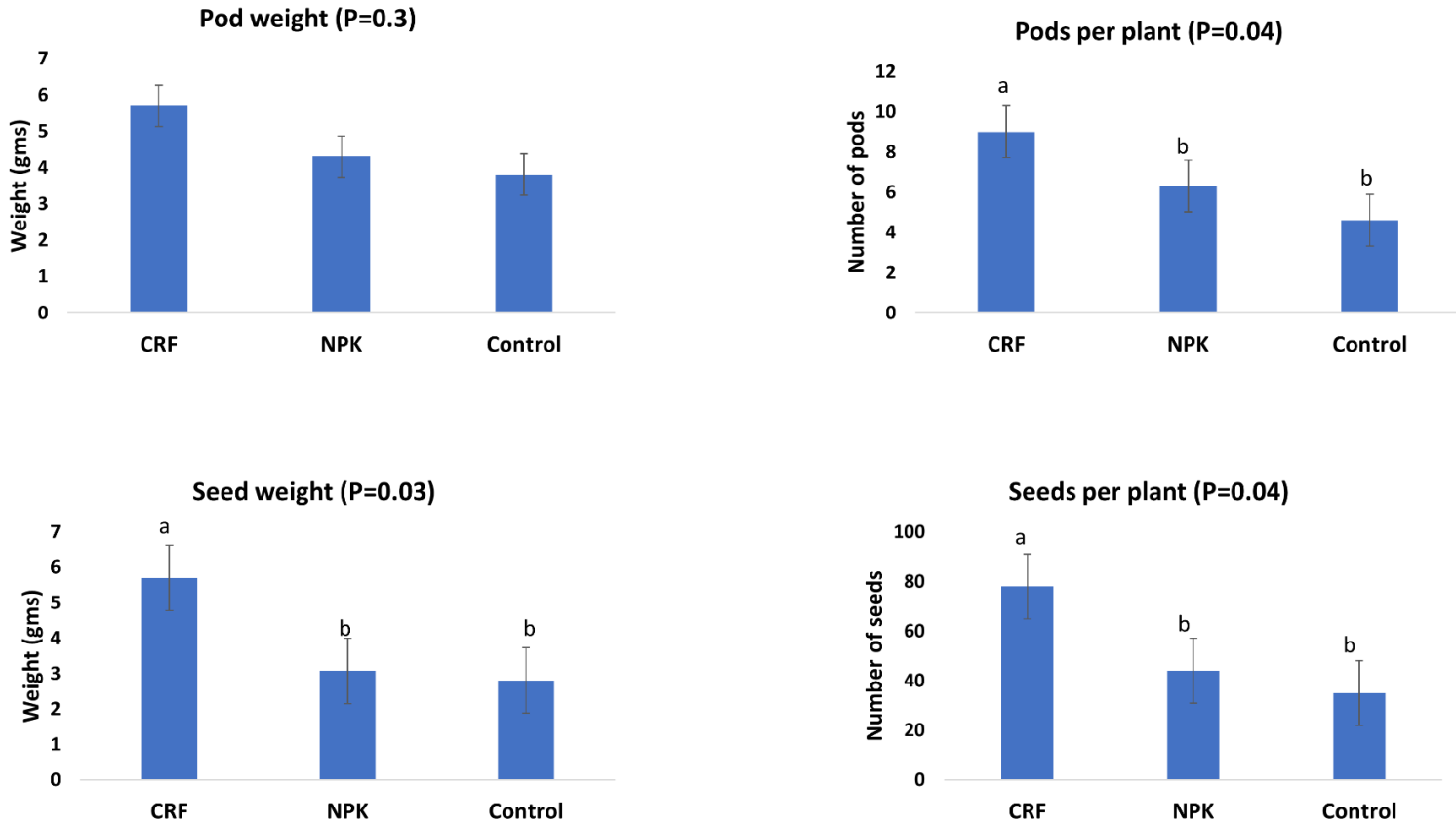


Figure 7-15: Influence of CRF on the pod weight, seed weight, pods per plant and seeds per plant. *p for significance; a& b are subsets indicating the significant difference between each level.*

Table 7-2 : Influence of CRF on the pod length

	Pod length (cms)
CRF	8.01
NPK	7.3
Control	7.1
SEM	0.67
P	0.4

p & SEM stands for significance and standard error mean

Table 7.2 presents the effect of CRF and NPK on the pod length. As seen the results, there is no significant effect observed between CRF and NPK during this trial.

7.5.4 Root length, root area, root volume, root diameter and nodules

Influence of CRF and NPK included with control on the root length, root volume, root diameter, root surface area and nodules are depicted in **Figure 7.16.**

Out of different parameters of root, significant ($p<0.05$) effect of CRF application on root length was observed during the trial. Application of CRF for the crop resulted in the highest root length (2664.4cm) followed by the application of NPK (237.12cm), and the least (2015 cm) was recorded in the treatments receiving no NPK application. Slow release of nutrients from CRF helps the plant to put down a dense collection of new roots throughout the growth, strengthening the existing root system (Fageria and Moreira, 2011). On the other hand, CRF does not show any significant effect ($p>0.05$) on the root volume, root diameter and root surface area. However, the values obtained for root volume, root diameter and root surface area is not statistically significant ($P<0.05$), but it still shows the highest value for CRF compared to NPK and control.

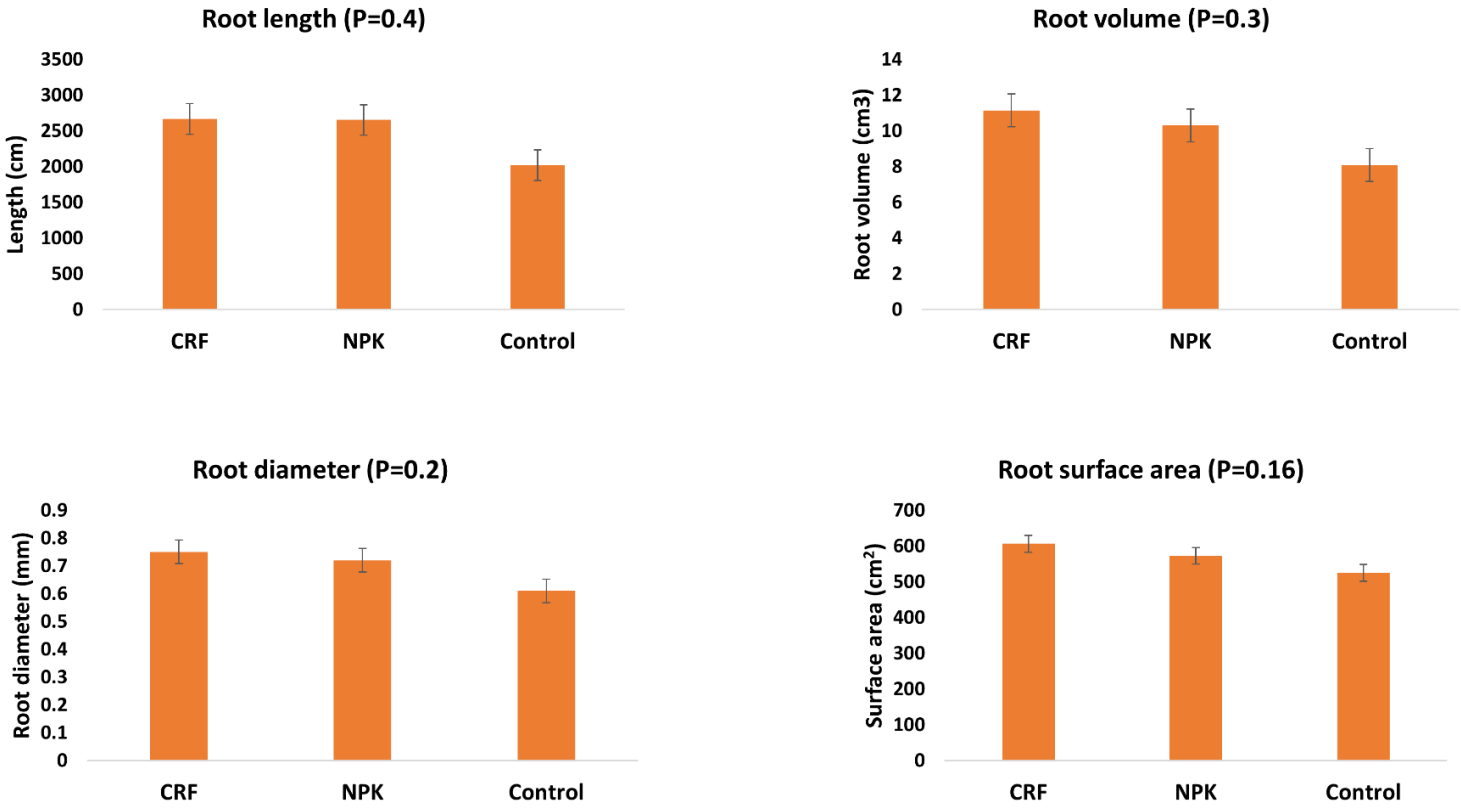


Figure 7-16: Effect of CRF on root parameters of mung bean. Within a treatment group, bars with the same letters are not significantly different using Tukey's test at 5% level. SE bars are standard deviation values. *p stands for significance* .

7.6 Summary

Blending technique was successfully implemented to prepare PCL based CRF. From this chapter, it is concluded that PCL/starch/NPK blends are suitable for developing a sustainable system for control release of fertilizers. The blends can be processed in the presence of fertilizer resulting in a very ordered porous structure. NPK are homogeneously dispersed in PCL domains, which are released into the environment slowly in a sustained manner. Three concentrations studied for control release of nutrients showed a significant difference, the one with high concentration PCL 25% showed slow release followed by 15% and least by 5%. Blended CRF shows better results in plant height, leaf number, dry weight and grain yield. There was an improvement of 39% of yield for the plants (i.e. total seed weight) treated with blended CRF compared with normal uncoated fertilizer.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

8.1 Principal Findings

This study was focused on utilising PCL for control release of fertilizers for the mung bean crop. Also, a comprehensive study on optimization of process parameters for enzymatic synthesis was carried out. Following are the significant findings from this study

Pot trials with mung bean were carried out with the formulation of different levels of biochar (0, 10, 15 t/ha) and the nutrients (NPK- 0, 15, 30 kg/ha & Iron 0, 15kg/ha). Improved crop performance was observed with the application of 30kg/ha of NPK and 15 t/ha of biochar. There was a significant improvement in the characteristics of the plant such as height, leaf number and root parameters. Final yield i.e. total seed weight obtained has been increased 86% and 38% with application of NPK and biochar compared with control. Most importantly dry biomass and seed yield have been improved greatly compared with control. On the other hand, the application of iron did not influence the crop performance. Therefore, the application of 30 kg NPK /ha 15 t/ha of biochar can be adopted for increased production of the mung bean. Besides, application of biochar at 15 t/ha also can have huge influence on the performance of the crop.

D optimal in DOE was implemented successfully for the optimization of process parameters for polycaprolactone synthesis using CALB as enzyme. Due to this multivariate approach, the relation and the interactions between the four process parameters chosen (temperature, time, mixing speed and monomer solvent ratio) with respect to the molecular weight of the polymer has been obtained. Polymer with molecular weight of Mn – 3359.47 and Mw 4920.85 has been achieved with process parameters maintained at - time 1hr, mixing speed 500 rpm, temperature

of 75°C and monomer/solvent ratio 1:2. Additionally, TLL has been recognized for its catalytic activity for synthesising PCL. Up to our knowledge, there are no reports till date using TLL enzyme for PCL synthesis. Based on NMR, FTIR, TGA and DSC results acquired, the synthesised PCL (using CALB and TLL) displays all the properties similar to PCL. However, the molecular weight achieved will not be suitable to prepare the controlled release fertilizers. Therefore, commercial PCL with molecular weight of 25000 and 34000 g/mol were bought for application studies.

Two different techniques have been used to prepare control release fertilizers – solvent casting and blending techniques. Previous works done on the preparation of CRF were all performed in water, referring actual studies should be conducted in soil. So, this project fills the gap by giving the information about CRF behaviour in soil. In the solvent casting technique, NPK was coated with a polymer layer prepared with three different concentrations (20wt%, 30wt% and 40wt% with and without starch). The porosity and the biodegradation showed a notable difference among the different concentration used. Besides, PCL/starch coatings are not very firm, causing disruption easily. Further for nutrient release, CRF had a remarkable slow release compared with the control. Among all the concentrations used, most slow and control release of nutrients was observed in polymer coating with 40wt% followed by 30wt %. However, there is no significant difference between them, except in the case of potassium release. Finally, the plants given with CRF (prepared by solvent casting) showed some improved physical characteristics such in plant height and leaf number, but did not show any considerable variance in the yield of the crop from that plants treated with normal NPK.

Further, blending technique is considered to be easy, solvent free and scalable technique to prepare CRF blends. In the previous technique used, CRF prepared with PCL/starch coatings were not firm with a non-significant slow release of nutrients. Therefore, the limitation of including starch in the developing CRF has been overcome by the blending technique. Since,

PCL/NPK/starch blends have been successfully prepared. Out of the three combinations of PCL/starch concentrations, blend with the ratio 5:1 i.e. blend with highest concentration 25% PCL was recorded to be less porous with minimum degradation observed. In addition, the nutrient release had a significant slow release for all the nutrients. Moreover, in final plant trials, CRF prepared by blending technique had a significant effect on the growth and yield of the mung bean. 39% more yield was obtained when using CRF compared with NPK.

8.2 Limitation

- Three different concentrations of PCL were only examined for preparing blended CRF's due to the limitation in the equipment and resources.
- PCL synthesised via enzymatic catalysis couldn't be used to prepare controlled release fertilizer because of the low molecular weight obtained.

8.3 Future studies

The following future works are recommended in order to fully develop PCL based CRF and to take the enzymatic synthesis to industrial levels

a) Scale up for PCL synthesis using CALB can be performed using the multivariate approach, DOE. On the other hand, process parameters - temperature, time, mixing speed and monomer solvent ratio can be optimized to produce PCL using TLL as catalyst
b) Influence of higher concentration of Iron (> 15kg /ha) can be considered for further studies.
c) PCL/NPK blends can be tested for control release, for the plants with longer growth period. Addition of starch can be optimal depending on the plant growth period.
d) Thickness of the blends can be optimised, which will vary the release time of nutrients.

e) Potential application of PCL synthesized via enzymatic polymerization should be considered for medical applications like tissue engineering and drug delivery

For commercialization of PCL based CRF, cost would be major challenge, but due to its advantages like complete degradability, not effecting the soil fertility and environment. It would be worth the cost.

REFERENCES

ABAYOMI, Y., AJIBADE, T., SAMMUEL, O. & SAADUDEEN, B. 2008. Growth and yield responses of cowpea (Vigna unguiculata (L.) Walp) genotypes to nitrogen fertilizer (NPK) application in the Southern Guinea Savanna zone of Nigeria. *Asian J. Plant Sci,* 7**,** 170-176.

ACHAKZAI, A. K. K., HABIBULLAH, B. H. S. & WAHID, M. A. 2012. Effect of nitrogen fertilizer on the growth of mungbean [Vigna radiata (L.) Wilczek] grown in Quetta. *Pak. J. Bot,* 44**,** 981-987.

AHMAD, M., USMAN, A. R., AL-FARAJ, A. S., AHMAD, M., SALLAM, A. & AL-WABEL, M. I. 2018. Phosphorus-loaded biochar changes soil heavy metals availability and uptake potential of maize (Zea mays L.) plants. *Chemosphere,* 194**,** 327-339.

AHMAD, R., IKRAAM, M., ULLAH, E. & MAHMOOD, A. 2003. Influence of different fertilizer levels on the growth and productivity of three mung bean {Vigna radiata) cultivars. *Int. J. Agric. Biol,* 5**,** 335-338.

AKTAŞ, N. 2005. Optimization of biopolymerization rate by response surface methodology (RSM). *Enzyme and microbial technology,* 37**,** 441-447.

AL-ZAHRANI, S. 2000. Utilization of polyethylene and paraffin waxes as controlled delivery systems for different fertilizers. *Industrial & engineering chemistry research,* 39**,** 367-371.

ALBERTSSON, A.-C. & VARMA, I. K. 2002. *Degradable aliphatic polyesters,* Springer Berlin.

ALBURQUERQUE, J. A., CALERO, J. M., BARRÓN, V., TORRENT, J., DEL CAMPILLO, M. C., GALLARDO, A. & VILLAR, R. 2014. Effects of biochars produced from different feedstocks on soil properties and sunflower growth. *Journal of Plant Nutrition and Soil Science,* 177**,** 16-25.

ALDEMIR, A., BIRHANLı, E., AKTAŞ, N. & YEŞILADA, Ö. 2014. Optimization of Aniline Biopolymerization Reaction Conditions By Response Surface Methodology (RSM). *International Journal of Bio-Science and Bio-Technology,* 6**,** 97-112.

ARBAOUI, A. & REDSHAW, C. 2010. Metal catalysts for ε-caprolactone polymerisation. *Polymer Chemistry,* 1**,** 801-826.

ARUMUGASAMY, S. K. & AHMAD, Z. 2011. Candida antarctica as catalyst for polycaprolactone synthesis: effect of temperature and solvents. *Asia-Pacific Journal of Chemical Engineering,* 6**,** 398-405.

ARUMUGASAMY, S. K., UZIR, M. & AHMAD, Z. 2013. Neural Network Based Modeling for Polycaprolactone Synthesis by Bio-Polymerization of [epsilon]-caprolactone. *International Journal of Bioscience, Biochemistry and Bioinformatics,* 3**,** 56.

ARUMUGASAMY, S. K., UZIR, M. H. & AHMAD, Z. Modeling of polycaprolactone production from ε-caprolactone using neural network. International Conference on Neural Information Processing, 2012. Springer, 444-451.

AVEROUS, L., MORO, L., DOLE, P. & FRINGANT, C. 2000. Properties of thermoplastic blends: starch–polycaprolactone. *Polymer,* 41**,** 4157-4167.

AYOOLA, O. & ADENIYAN, O. 2006. Influence of poultry manure and NPK fertilizer on yield and yield components of crops under different cropping systems in south west Nigeria. *African Journal of Biotechnology,* 5.

AZEEM, B., KUSHAARI, K. & MAN, Z. 2016. Effect of Coating Thickness on Release Characteristics of Controlled Release Urea Produced in Fluidized Bed Using Waterborne Starch Biopolymer as Coating Material. *Procedia Engineering,* 148**,** 282-289.

AZEEM, B., KUSHAARI, K., MAN, Z. B., BASIT, A. & THANH, T. H. 2014. Review on materials & methods to produce controlled release coated urea fertilizer. *Journal of Controlled Release,* 181**,** 11-21.

BA-ABBAD, M. M., CHAI, P. V., TAKRIFF, M. S., BENAMOR, A. & MOHAMMAD, A. W. 2015. Optimization of nickel oxide nanoparticle synthesis through the sol–gel method using Box–Behnken design. *Materials & Design,* 86**,** 948-956.

BANO, K., KUDDUS, M., R ZAHEER, M., ZIA, Q., F KHAN, M., GUPTA, A. & ALIEV, G. 2017. Microbial enzymatic degradation of biodegradable plastics. *Current pharmaceutical biotechnology,* 18**,** 429-440.

BARBIER-BAUDRY, D., BRACHAIS, L., CRETU, A., GATTIN, R., LOUPY, A. & STUERGA, D. 2003. Synthesis of polycaprolactone by microwave irradiation—An interesting route to synthesize this polymer via green chemistry. *Environmental Chemistry Letters,* 1**,** 19-23.

BARRERA-RIVERA, K. A., FLORES-CARREÓN, A. & MARTÍNEZ-RICHA, A. 2008. Enzymatic ring-opening polymerization of ε-caprolactone by a new lipase from Yarrowia lipolytica. *Journal of applied polymer science,* 109**,** 708-719.

BECKER, M., DIEKMANN, K., LADHA, J., DATTA, S. & OTTOW, J. 1991. Effect of NPK on growth and nitrogen fixation of Sesbania rostrata as a green manure for lowland rice (Oryza sativa L.). *Plant and soil,* 132**,** 149-158.

BEESLEY, L., MORENO-JIMÉNEZ, E., GOMEZ-EYLES, J. L., HARRIS, E., ROBINSON, B. & SIZMUR, T. 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environmental Pollution,* 159**,** 3269-3282.

BHATIA, M., GIRDHAR, A., TIWARI, A. & NAYARISSERI, A. 2014. Implications of a novel Pseudomonas species on low density polyethylene biodegradation: an in vitro to in silico approach. *SpringerPlus,* 3**,** 497.

BHAW-LUXIMON, A., JHURRY, D., MOTALA-TIMOL, S. & LOCHEE, Y. Polymerization of ε-Caprolactone and its Copolymerization with γ-Butyrolactone using Metal Complexes. Macromolecular symposia, 2005. Wiley Online Library, 60-68.

BINTI SAID, F. N., YUSOP, M. K. & OAD, F. C. 2014. Nutrient uptake, pH changes and yield of rice under slow release sulfur-coated urea fertilizers. *Australian Journal of Crop Science,* 8**,** 1359.

BRATTON, D., BROWN, M. & HOWDLE, S. M. 2005. Tin (II) ethyl hexanoate catalyzed precipitation polymerization of ε-caprolactone in supercritical carbon dioxide. *Macromolecules,* 38**,** 1190-1195.

BRAUD, C., DEVARIEUX, R., ATLAN, A., DUCOS, C. & VERT, M. 1998. Capillary zone electrophoresis in normal or reverse polarity separation modes for the analysis of hydroxy acid oligomers in neutral phosphate buffer. *Journal of Chromatography B: Biomedical Sciences and Applications,* 706**,** 73-82.

CHAIBAKHSH, N., RAHMAN, A., JESUNATHAN, K. & BASRI, M. 2013. Optimization of biocatalytic synthesis of Chitosan Ester using response surface methodology. *Chemistry of Solid Materials,* 1**,** 41-55.

CHEN, L., XIE, Z., ZHUANG, X., CHEN, X. & JING, X. 2008. Controlled release of urea encapsulated by starch-g-poly (L-lactide). *Carbohydrate polymers,* 72**,** 342-348.

CHILKOTI, A., CHRISTENSEN, T. & MACKAY, J. A. 2006. Stimulus responsive elastin biopolymers: applications in medicine and biotechnology. *Current opinion in chemical biology,* 10**,** 652-657.

CHRISSAFIS, K., PARASKEVOPOULOS, K. & BIKIARIS, D. 2006. Effect of molecular weight on thermal degradation mechanism of the biodegradable polyester poly (ethylene succinate). *Thermochimica Acta,* 440**,** 166-175.

DARBANDY, S. M., BAKHTIARY, A. & TAHERI, H. 2011. Novel and efficient catalyst for the synthesis of polycaprolactone. *IPCBEE,* 23**,** 125-129.

DE MARIA, P. D., FERNANDEZ-ALVARO, E., TEN KATE, A. & BARGEMAN, G. 2009. Role of apparent pKa of carboxylic acids in lipase-catalyzed esterifications in biphasic systems. *Journal of Molecular Catalysis B: Enzymatic,* 59**,** 220-224.

DENG, X., YUAN, M., XIONG, C. & LI, X. 1999. Polymerization of lactides and lactones. IV. Ring-opening polymerization of ε-caprolactone by rare earth phenyl compounds. *Journal of applied polymer science,* 73**,** 1401-1408.

DING, Y., LIU, Y.-X., WU, W.-X., SHI, D.-Z., YANG, M. & ZHONG, Z.-K. 2010. Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns. *Water, Air, & Soil Pollution,* 213**,** 47-55.

DIVAKAR, S. 2012. *Enzymatic transformation,* Springer Science & Business Media.

DONG, H., CAO, S. G., LI, Z. Q., HAN, S. P., YOU, D. L. & SHEN, J. C. 1999. Study on the enzymatic polymerization mechanism of lactone and the strategy for improving the degree of polymerization. *Journal of Polymer Science Part A: Polymer Chemistry,* 37**,** 1265-1275.

DONG, H., WANG, Z., LI, Z. Q., YOU, D. L., HAN, S. P., CAO, S. G. & SHEN, J. C. 1998. Enzyme-Catalyzed Synthesis of Polyester: Effect of the Microenvironment of Enzyme on Polyesterification. *Annals of the New York Academy of Sciences,* 864**,** 263-266.

DU, C.-W., ZHOU, J.-M. & SHAVIV, A. 2006. Release characteristics of nutrients from polymer-coated compound controlled release fertilizers. *Journal of Polymers and the Environment,* 14**,** 223-230.

DUDA, A., KOWALSKI, A., PENCZEK, S., UYAMA, H. & KOBAYASHI, S. 2002. Kinetics of the ring-opening polymerization of 6-, 7-, 9-, 12-, 13-, 16-, and 17-membered lactones. Comparison of chemical and enzymatic polymerizations. *Macromolecules,* 35**,** 4266-4270.

ELZEIN, T., NASSER-EDDINE, M., DELAITE, C., BISTAC, S. & DUMAS, P. 2004. FTIR study of polycaprolactone chain organization at interfaces. *Journal of colloid and interface science,* 273**,** 381-387.

FAGERIA, N., CARVALHO, M. & DOS SANTOS, F. 2014. Root growth of upland rice genotypes as influenced by nitrogen fertilization. *Journal of plant nutrition,* 37**,** 95-106.

FAGERIA, N. & MOREIRA, A. 2011. The role of mineral nutrition on root growth of crop plants. *Advances in agronomy.* Elsevier.

FAGERIA, N. K. 2016. *The use of nutrients in crop plants,* CRC press.

FANG, X., SIMONE, C. D., VACCARO, E., HUANG, S. J. & SCOLA, D. A. 2002. Ring-opening polymerization of ϵ-caprolactam and ϵ-caprolactone via microwave irradiation. *Journal of Polymer Science Part A: Polymer Chemistry,* 40**,** 2264-2275.

FANG, Y., WANG, L., XIN, Z., ZHAO, L., AN, X. & HU, Q. 2008. Effect of foliar application of zinc, selenium, and iron fertilizers on nutrients concentration and yield of rice grain in China. *Journal of Agricultural and Food Chemistry,* 56**,** 2079-2084.

FATHI ROUDSARI, S., DHIB, R. & EIN-MOZAFFARI, F. 2015. Mixing effect on emulsion polymerization in a batch reactor. *Polymer Engineering & Science,* 55**,** 945-956.

FELLET, G., MARMIROLI, M. & MARCHIOL, L. 2014. Elements uptake by metal accumulator species grown on mine tailings amended with three types of biochar. *Science of the Total Environment,* 468**,** 598-608.

FORESTI, M. L. & FERREIRA, M. L. 2004. Synthesis of Polycaprolactone Using Free/Supported Enzymatic and Non-Enzymatic Catalysts. *Macromolecular rapid communications,* 25**,** 2025-2028.

FUKUDA, H., HAMA, S., TAMALAMPUDI, S. & NODA, H. 2008. Whole-cell biocatalysts for biodiesel fuel production. *Trends in Biotechnology,* 26**,** 668-673.

GAIROLA, S., UMAR, S. & SURYAPANI, S. 2009. Nitrate accumulation, growth and leaf quality of spinach beet (Beta vulgaris Linn.) as affected by NPK fertilization with special reference to potassium. *Indian Journal of Science and Technology,* 2**,** 35-40.

GEORGE, C. 2018. *ISH MarkIt releases biodegradable polymers report.* [Online]. Recycling today. Available: http://www.recyclingtoday.com/article/ish-markit-biodegradable-polymers-report/ [Accessed July 27 2018].

GOKALP, N., ULKER, C. & GUVENILIR, Y. A. 2016a. Enzymatic ring opening polymerization of ε-caprolactone by using a novel immobilized biocatalyst. *Advanced Materials Letters,* 7**,** 144-149.

GOKALP, N., ULKER, C. & GUVENILIR, Y. A. 2016b. Synthesis of Polycaprolactone via Ring Opening Polymerization Catalyzed by Candida Antarctica Lipase B Immobilized onto an Amorphous Silica Support. *Journal of Polymer Materials,* 33**,** 87-100.

GRABER, E. R., HAREL, Y. M., KOLTON, M., CYTRYN, E., SILBER, A., DAVID, D. R., TSECHANSKY, L., BORENSHTEIN, M. & ELAD, Y. 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant and soil,* 337**,** 481-496.

GROSS, R. A., KUMAR, A. & KALRA, B. 2001. Polymer synthesis by in vitro enzyme catalysis. *Chemical Reviews,* 101**,** 2097-2124.

GUMEL, A., ANNUAR, M., CHISTI, Y. & HEIDELBERG, T. 2012. Ultrasound assisted lipase catalyzed synthesis of poly-6-hydroxyhexanoate. *Ultrasonics sonochemistry,* 19**,** 659-667.

GUMEL, A. M. & ANNUAR, M. 2016. Thermomyces lanuginosus lipase-catalyzed synthesis of natural flavor esters in a continuous flow microreactor. *3 Biotech,* 6**,** 24.

GUNAWAN, E. R., BASRI, M., RAHMAN, M. B. A., SALLEH, A. B. & RAHMAN, R. N. Z. A. 2005. Study on response surface methodology (RSM) of lipase-catalyzed synthesis of palm-based wax ester. *Enzyme and Microbial Technology,* 37**,** 739-744.

HAKKARAINEN, M. & ALBERTSSON, A. C. 2002. Heterogeneous biodegradation of polycaprolactone–low molecular weight products and surface changes. *Macromolecular Chemistry and Physics,* 203**,** 1357-1363.

HAN, X., CHEN, S. & HU, X. 2009. Controlled-release fertilizer encapsulated by starch/polyvinyl alcohol coating. *Desalination,* 240**,** 21-26.

HASHIM, M. M. A., YUSOP, M. K., OTHMAN, R. & WAHID, S. A. 2017. Field Evaluation of Newly-Developed Controlled Release Fertilizer on Rice Production and Nitrogen Uptake. *Sains Malaysiana,* 46**,** 925-932.

HERNÁNDEZ-MARTÍN, E. & OTERO, C. 2008. Different enzyme requirements for the synthesis of biodiesel: Novozym® 435 and Lipozyme® TL IM. *Bioresource technology,* 99**,** 277-286.

HOSSAIN, M. K., STREZOV, V., CHAN, K. Y., ZIOLKOWSKI, A. & NELSON, P. F. 2011. Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge biochar. *Journal of Environmental Management,* 92**,** 223-228.

HOU, X.-L., ZHANG, M., DUAN, L.-L., WANG, H. & SONG, R.-L. 2008. Effects of Controlled Release Compound Fertilizers on Leaching Loss of Nutrient and Growth of Calla [J]. *Journal of Soil and Water Conservation,* 4.

HOWARD, T. 2011. The Effect of Biochar on the Root Development of Corn and Soybeans in Minnesota Soil and Sand.

HUANG, W., ZHU, N., LIU, Y., WANG, J., ZHONG, J., SUN, Q., SUN, T., HU, X., FANG, Z. & GUO, K. 2019. A novel microfluidic enzyme-organocatalysis combination strategy for ring-opening copolymerizations of lactone, lactide and cyclic carbonate. *Chemical Engineering Journal,* 356**,** 592-597.

HUNSEN, M., ABUL, A., XIE, W. & GROSS, R. 2008. Humicola insolens cutinase-catalyzed lactone ring-opening polymerizations: kinetic and mechanistic studies. *Biomacromolecules,* 9**,** 518-522.

HUNSEN, M., AZIM, A., MANG, H., WALLNER, S. R., RONKVIST, A., XIE, W. & GROSS, R. A. 2007. A cutinase with polyester synthesis activity. *Macromolecules,* 40**,** 148-150.

HUSSAIN, A., ALI, A., AKHTAR, J. & YASIN, M. 2010. Effects of phosphorus in combination with rhizobium inoculation on growth and yield parameters of mungbean. *Crop Environ,* 1**,** 53-56.

HUSSAIN, F., MALIK, A., HAJI, M. & MALGHANI, A. 2011. Growth and yield response of two cultivars of mungbean (Vigna radiata L.) to different potassium levels. *J. Anim. Plant Sci,* 21**,** 622-625.

IQBAL, N., KHAN, A. S., ASIF, A., YAR, M., HAYCOCK, J. W. & REHMAN, I. U. 2019. Recent concepts in biodegradable polymers for tissue engineering paradigms: a critical review. *International Materials Reviews,* 64**,** 91-126.

JAGADISH, P. R., KHALID, M., AMIN, N., LI, L. P. & CHAN, A. 2017. Process optimisation for n-type Bi2Te3 films electrodeposited on flexible recycled carbon fibre using response surface methodology. *Journal of Materials Science,* 52**,** 11467-11481.

JALIYA, M., FALAKI, A., MAHMUD, M. & SANI, Y. 2008. Effects of sowing date and NPK fertilizer rate on yield and yield components of quality protein maize (Zea mays L.). *ARPN Journal of Agricultural and Biological Science,* 3**,** 23-29.

JAROSIEWICZ, A. & TOMASZEWSKA, M. 2003. Controlled-release NPK fertilizer encapsulated by polymeric membranes. *Journal of Agricultural and Food Chemistry,* 51**,** 413-417.

JÉRÔME, C. & LECOMTE, P. 2008. Recent advances in the synthesis of aliphatic polyesters by ring-opening polymerization. *Advanced drug delivery reviews,* 60**,** 1056-1076.

JHA, K., KATARIA, R., VERMA, J. & PRADHAN, S. 2019. Potential biodegradable matrices and fiber treatment for green composites: A review.

JINTAKANON, N., OPAPRAKASIT, P., PETCHSUK, A. & OPAPRAKASIT, M. Controlled-release materials for fertilizer based on lactic acid polymers. Advanced Materials Research, 2008. Trans Tech Publ, 905-908.

JONASSON, S., MICHELSEN, A., SCHMIDT, I. K., NIELSEN, E. V. & CALLAGHAN, T. V. 1996. Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. *Oecologia,* 106**,** 507-515.

JOSEPH, B., RAMTEKE, P. W. & THOMAS, G. 2008. Cold active microbial lipases: some hot issues and recent developments. *Biotechnology advances,* 26**,** 457-470.

KAFKAFI, U., XU, G., IMAS, P., MAGEN, H. & TARCHITZKY, J. 2001. *Potassium and chloride in crops and soils: The role of potassium chloride fertilizer in crop nutrition*, International Potash Institute.

KAMATH, H. V., REGUPATHI, I. & SAIDUTTA, M. 2011. Optimization of two step karanja biodiesel synthesis under microwave irradiation. *Fuel Processing Technology,* 92**,** 100-105.

KASTEN, G., SILVA, L. F. C. & LEMOS-SENNA, E. 2016. Development of low density azithromycin-loaded polycaprolactone microparticles for pulmonary delivery. *Drug development and industrial pharmacy,* 42**,** 776-787.

KEREP, P. & RITTER, H. 2006. Influence of Microwave Irradiation on the Lipase-Catalyzed Ring-Opening Polymerization of ε-Caprolactone. *Macromolecular rapid communications,* 27**,** 707-710.

KHAJEH, M., HEIDARI, Z. S. & SANCHOOLI, E. 2011. Synthesis, characterization and removal of lead from water samples using lead-ion imprinted polymer. *Chemical engineering journal,* 166**,** 1158-1163.

KLIBANOV, A. M. 2001a. Improving enzymes by using them in organic solvents. *nature,* 409**,** 241.

KLIBANOV, A. M. 2001b. Improving enzymes by using them in organic solvents. *nature,* 409**,** 241-246.

KOBAYASHI, S. 2010. Lipase-catalyzed polyester synthesis–a green polymer chemistry. *Proceedings of the Japan Academy, Series B,* 86**,** 338-365.

KOBAYASHI, S., UYAMA, H. & KIMURA, S. 2001. Enzymatic polymerization. *Chemical Reviews,* 101**,** 3793-3818.

KÖRBAHTI, B. K., AKTAŞ, N. & TANYOLAÇ, A. 2007. Optimization of electrochemical treatment of industrial paint wastewater with response surface methodology. *Journal of Hazardous Materials,* 148**,** 83-90.

KOWALSKI, A., DUDA, A. & PENCZEK, S. 1998. Kinetics and mechanism of cyclic esters polymerization initiated with tin (II) octoate, 1. Polymerization of ε-caprolactone. *Macromolecular rapid communications,* 19**,** 567-572.

KUMAR, A. & GROSS, R. A. 2000. Candida a ntartica Lipase B Catalyzed Polycaprolactone Synthesis: Effects of Organic Media and Temperature. *Biomacromolecules,* 1**,** 133-138.

LABET, M. & THIELEMANS, W. 2009. Synthesis of polycaprolactone: a review. *Chemical Society Reviews,* 38**,** 3484-3504.

LAM, C. X., SAVALANI, M. M., TEOH, S.-H. & HUTMACHER, D. W. 2008. Dynamics of in vitro polymer degradation of polycaprolactone-based scaffolds: accelerated versus simulated physiological conditions. *Biomedical materials,* 3**,** 034108.

LAM, C. X., TEOH, S. H. & HUTMACHER, D. W. 2007. Comparison of the degradation of polycaprolactone and polycaprolactone–(β-tricalcium phosphate) scaffolds in alkaline medium. *Polymer international,* 56**,** 718-728.

LASSALLE, V. & FERREIRA, M. L. 2008. Lipase-catalyzed synthesis of polylactic acid: an overview of the experimental aspects. *Journal of chemical technology and biotechnology,* 83**,** 1493-1502.

LAWLOR, D. W. 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of experimental Botany,* 53**,** 773-787.

LEHMANN, J. 2007. A handful of carbon. *Nature,* 447**,** 143-144.

LEHMANN, J., RILLIG, M. C., THIES, J., MASIELLO, C. A., HOCKADAY, W. C. & CROWLEY, D. 2011. Biochar effects on soil biota–a review. *Soil Biology and Biochemistry,* 43**,** 1812-1836.

LEVIN, G., GOMEZ, S., GLODOWSKY, A., CASCONE, O. & HERNÁIZ, M. 2018. Two-step enzymatic strategy for the synthesis of a smart phenolic polymer and further immobilization of a β-galactosidase able to catalyze transglycosydation reaction. *International journal of biological macromolecules*.

LI, Q., LI, G., MA, F., ZHANG, Z., ZHENG, B. & FENG, Y. 2011a. Highly efficient ring-opening polymerization of ε-caprolactone catalyzed by a recombinant Escherichia coli whole-cell biocatalyst. *Process Biochemistry,* 46**,** 477-481.

LI, Q., LI, G., YU, S., ZHANG, Z., MA, F. & FENG, Y. 2011b. Ring-opening polymerization of ε-caprolactone catalyzed by a novel thermophilic lipase from Fervidobacterium nodosum. *Process Biochemistry,* 46**,** 253-257.

LI, X., LI, Q., XU, X., SU, Y., YUE, Q. & GAO, B. 2016. Characterization, swelling and slow-release properties of a new controlled release fertilizer based on wheat straw cellulose hydrogel. *Journal of the Taiwan Institute of Chemical Engineers,* 60**,** 564-572.

LIANG, R., LIU, M. & WU, L. 2007. Controlled release NPK compound fertilizer with the function of water retention. *Reactive and Functional Polymers,* 67**,** 769-779.

LIPIK, V. T. & ABADIE, M. J. 2010. Process Optimization of Poly (ε-caprolactone) Synthesis by Ring Opening Polymerization.

LIU, Q., LIU, B., AMBUS, P., ZHANG, Y., HANSEN, V., LIN, Z., SHEN, D., LIU, G., BEI, Q. & ZHU, J. 2016. Carbon footprint of rice production under biochar amendment–a case study in a Chinese rice cropping system. *Gcb Bioenergy,* 8**,** 148-159.

LOEKER, F. C., DUXBURY, C. J., KUMAR, R., GAO, W., GROSS, R. A. & HOWDLE, S. M. 2004. Enzyme-catalyzed ring-opening polymerization of ε-caprolactone in supercritical carbon dioxide. *Macromolecules,* 37**,** 2450-2453.

LUCCA, P., HURRELL, R. & POTRYKUS, I. 2001. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *TAG Theoretical and Applied Genetics,* 102**,** 392-397.

LYU, S. & UNTEREKER, D. 2009. Degradability of polymers for implantable biomedical devices. *International journal of molecular sciences,* 10**,** 4033-4065.

MA, J., LI, Q., SONG, B., LIU, D., ZHENG, B., ZHANG, Z. & FENG, Y. 2009. Ring-opening polymerization of ε-caprolactone catalyzed by a novel thermophilic esterase from the archaeon Archaeoglobus fulgidus. *Journal of Molecular Catalysis B: Enzymatic,* 56**,** 151-157.

MAHAPATRO, A., KUMAR, A. & GROSS, R. A. 2004. Mild, Solvent-Free ω-Hydroxy Acid Polycondensations Catalyzed by Candida a ntarctica Lipase B. *Biomacromolecules,* 5**,** 62-68.

MALIK, M. A., SALEEM, M. F., ALI, A. & MAHMOOD, I. 2003. Effect of nitrogen and phosphorus application on growth yield and quality of mungbean (Vigna radiata L.). *Pakistan Journal of Agricultural Sciences,* 40**,** 133-136.

MARAN, J. P., MANIKANDAN, S., THIRUGNANASAMBANDHAM, K., NIVETHA, C. V. & DINESH, R. 2013. Box–Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. *Carbohydrate Polymers,* 92**,** 604-611.

MARKETREPORT. 2018. *Global Market Study on Polycaprolactone: Rising Awareness and Shift Towards the Adoption of Biodegradable Polymers Enabling Increasing Usage of Polycaprolactone* [Online]. Persistance market research. Available: https://www.persistencemarketresearch.com/market-research/polycaprolactone-market.asp.

MATOS, T. D., KING, N., SIMMONS, L., WALKER, C., MCCLAIN, A. R., MAHAPATRO, A., RISPOLI, F. J., MCDONNELL, K. T. & SHAH, V. 2011. Microwave assisted lipase catalyzed solvent-free poly-ε-caprolactone synthesis. *Green Chemistry Letters and Reviews,* 4**,** 73-79.

MATSUMURA, S. 2006. Enzymatic synthesis of polyesters via ring-opening polymerization. *Enzyme-Catalyzed Synthesis of Polymers.* Springer.

MATSUMURA, S., EBATA, H., KONDO, R. & TOSHIMA, K. 2001. Organic Solvent-Free Enzymatic Transformation of Poly (ϵ-caprolactone) into Repolymerizable Oligomers in Supercritical Carbon Dioxide. *Macromolecular Rapid Communications,* 22**,** 1325-1329.

MÉNDEZ, A., GÓMEZ, A., PAZ-FERREIRO, J. & GASCÓ, G. 2012. Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. *Chemosphere,* 89**,** 1354-1359.

MITCHELL, K., FRENCH, E., BECKERMAN, J., IYER-PASCUZZI, A., VOLENEC, J. & GIBSON, K. 2018. Biochar Alters the Root Systems of Large Crabgrass. *HortScience,* 53**,** 354-359.

MOCHIZUKI, M. & HIRAMI, M. 1997. Structural effects on the biodegradation of aliphatic polyesters. *Polymers for advanced technologies,* 8**,** 203-209.

MORI, S. 1999. Iron acquisition by plants. *Current opinion in plant biology,* 2**,** 250-253.

NAMEKAWA, S., SUDA, S., UYAMA, H. & KOBAYASHI, S. 1999. Lipase-catalyzed ring-opening polymerization of lactones to polyesters and its mechanistic aspects. *International journal of biological macromolecules,* 25**,** 145-151.

NAVEENA, B., ALTAF, M., BHADRAYYA, K., MADHAVENDRA, S. & REDDY, G. 2005. Direct fermentation of starch to L (+) lactic acid in SSF by Lactobacillus amylophilus GV6 using wheat bran as support and substrate: medium optimization using RSM. *Process Biochemistry,* 40**,** 681-690.

NAZ, M. Y. & SULAIMAN, S. A. 2016. Slow release coating remedy for nitrogen loss from conventional urea: a review. *Journal of Controlled Release,* 225**,** 109-120.

NOMURA, N., TAIRA, A., TOMIOKA, T. & OKADA, M. 2000. A catalytic approach for cationic living polymerization: Sc (OTf) 3-catalyzed ring-opening polymerization of lactones. *Macromolecules,* 33**,** 1497-1499.

NOWAK, B., RUSINOWSKI, S., CHMIELNICKI, B., KAMIŃSKA-BACH, G. & BORTEL, K. Degradation of PVC/rPLA Thick Films in Soil Burial Experiment. IOP Conference Series: Earth and Environmental Science, 2016. IOP Publishing, 052029.

OLAD, A., ZEBHI, H., SALARI, D., MIRMOHSENI, A. & TABAR, A. R. 2018. Slow-release NPK fertilizer encapsulated by carboxymethyl cellulose-based nanocomposite with the function of water retention in soil. *Materials Science and Engineering: C,* 90**,** 333-340.

PARK, T. G., COHEN, S. & LANGER, R. 1992. Poly (L-lactic acid)/pluronic blends: characterization of phase separation behavior, degradation, and morphology and use as protein-releasing matrixes. *Macromolecules,* 25**,** 116-122.

PENCZEK, S., DUDA, A., KOWALSKI, A., LIBISZOWSKI, J., MAJERSKA, K. & BIELA, T. On the mechanism of polymerization of cyclic esters induced by tin (II) octoate. Macromolecular Symposia, 2000. Wiley Online Library, 61-70.

PHILLIPSON, K., HAY, J. & JENKINS, M. 2014. Thermal analysis FTIR spectroscopy of poly (ε-caprolactone). *Thermochimica Acta,* 595**,** 74-82.

POOJARI, Y., BEEMAT, J. S. & CLARSON, S. J. 2013. Enzymatic synthesis of poly (ε-caprolactone): Thermal properties, recovery, and reuse of lipase B from Candida antarctica immobilized on macroporous acrylic resin particles. *Polymer bulletin,* 70**,** 1543-1552.

PRSA, I., STAMPAR, F., VODNIK, D. & VEBERIC, R. 2007. Influence of nitrogen on leaf chlorophyll content and photosynthesis of 'Golden Delicious' apple. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science,* 57**,** 283-289.

RAJKHOWA, R., VARMA, I., ALBERTSSON, A. C. & EDLUND, U. 2005. Enzyme-catalyzed copolymerization of oxiranes with dicarboxylic acid anhydrides. *Journal of applied polymer science,* 97**,** 697-704.

RODRIGUES, R. C., BOLIVAR, J. M., VOLPATO, G., FILICE, M., GODOY, C., FERNANDEZ-LAFUENTE, R. & GUISAN, J. M. 2009. Improved reactivation of immobilized-stabilized lipase from Thermomyces lanuginosus by its coating with highly hydrophilic polymers. *Journal of biotechnology,* 144**,** 113-119.

SHAMIM, M., DIJKSTRA, F. A., ABUYUSUF, M. & HOSSAIN, A. I. 2015. Synergistic effects of biochar and NPK fertilizer on soybean yield in an alkaline soil. *Pedosphere,* 25**,** 713-719.

SHAVER, G. & CHAPIN, F. S. 1995. Long–term responses to factorial, NPK fertilizer treatment by Alaskan wet and moist tundra sedge species. *Ecography,* 18**,** 259-275.

SHAVIV, A. 2001. Advances in controlled-release fertilizers. *Advances in agronomy,* 71**,** 1-49.

SHITTU, S. O. S. 2007. Effect of NPK fertilizer rates and method of application on growth and yield of okra (Abelmoschus esculentus (L.) Moench) at Ado-Ekiti Southwestern, Nigeria. *International Journal of Agricultural Research,* 2**,** 614-619.

SILVA, J. A. & UCHIDA, R. S. 2000. Plant nutrient management in Hawaii's soils: approaches for tropical and subtropical agriculture. University of Hawaii.

SIRACUSA, V., ROCCULI, P., ROMANI, S. & DALLA ROSA, M. 2008. Biodegradable polymers for food packaging: a review. *Trends in Food Science & Technology,* 19**,** 634-643.

SIVALINGAM, G. & MADRAS, G. 2004. Modeling of lipase catalyzed ring-opening polymerization of ε-caprolactone. *Biomacromolecules,* 5**,** 603-609.

STASSIN, F., HALLEUX, O. & JÉRÔME, R. 2001. Ring-opening polymerization of ε-caprolactone in supercritical carbon dioxide. *Macromolecules,* 34**,** 775-781.

SUBBIAH, N., KRISHNAMOORTHY, L., YADAVALI, S. P., AYYAPILLAI, T., VELLAISAMY, S. & C. UMA, M. 2018. Lipase Catalysed Synthesis of Furan Based Oligoesters and their Self-Assembly Assisted Polymerization. *ChemSusChem*.

TANG, D., DONG, Y., REN, H., LI, L. & HE, C. 2014. A review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (Vigna radiata). *Chemistry Central Journal,* 8**,** 4.

TIAN, C., ZHOU, X., LIU, Q., PENG, J.-W., WANG, W.-M., ZHANG, Z.-H., YANG, Y., SONG, H.-X. & GUAN, C.-Y. 2016. Effects of a controlled-release fertilizer on yield, nutrient uptake, and fertilizer usage efficiency in early ripening rapeseed (Brassica napus L.). *Journal of Zhejiang University-SCIENCE B,* 17**,** 775-786.

TOMASZEWSKA, M. & JAROSIEWICZ, A. 2002. Use of polysulfone in controlled-release NPK fertilizer formulations. *Journal of agricultural and food chemistry,* 50**,** 4634-4639.

TOMASZEWSKA, M., JAROSIEWICZ, A. & KARAKULSKI, K. 2002. Physical and chemical characteristics of polymer coatings in CRF formulation. *Desalination,* 146**,** 319-323.

TORRES, C. F., MUNIR, F., BLANCO, R. M., OTERO, C. & HILL JR, C. G. 2002. Catalytic transesterification of corn oil and tristearin using immobilized lipases from Thermomyces lanuginosa. *Journal of the American Oil Chemists' Society,* 79**,** 775-781.

TRENKEL, M. E. 1997. *Controlled-release and stabilized fertilizers in agriculture*, International fertilizer industry association Paris.

UYAMA, H. & KOBAYASHI, S. 1993. Enzymatic ring-opening polymerization of lactones catalyzed by lipase. *Chemistry Letters***,** 1149-1150.

UYAMA, H., TAKEYA, K. & KOBAYASHI, S. 1993. Synthesis of polyesters by enzymatic ring-opening copolymerization using lipase catalyst. *Proceedings of the Japan Academy. Ser. B: Physical and Biological Sciences,* 69**,** 203-207.

VAN DER MEE, L., HELMICH, F., DE BRUIJN, R., VEKEMANS, J. A., PALMANS, A. R. & MEIJER, E. 2006. Investigation of lipase-catalyzed ring-opening polymerizations of lactones with various ring sizes: kinetic evaluation. *Macromolecules,* 39**,** 5021-5027.

VROMAN, I. & TIGHZERT, L. 2009. Biodegradable polymers. *Materials,* 2**,** 307-344.

WANG, C., ALIDOUST, D., YANG, X. & ISODA, A. 2018. Effects of bamboo biochar on soybean root nodulation in multi-elements contaminated soils. *Ecotoxicology and environmental safety,* 150**,** 62-69.

WANG, Y. & KUNIOKA, M. Ring-Opening Polymerization of Cyclic Monomers with Aluminum Triflate. Macromolecular Symposia, 2005. Wiley Online Library, 193-206.

WANG, Y., LIU, B., WANG, X., ZHAO, W., LIU, D., LIU, X. & CUI, D. 2014. Immortal ring-opening polymerization of ε-caprolactone by a neat magnesium catalyst system: an approach to obtain block and amphiphilic star polymers in situ. *Polymer Chemistry,* 5**,** 4580-4588.

WOODRUFF, M. A. & HUTMACHER, D. W. 2010. The return of a forgotten polymer—polycaprolactone in the 21st century. *Progress in Polymer Science,* 35**,** 1217-1256.

WU, K.-J., WU, C.-S. & CHANG, J.-S. 2007. Biodegradability and mechanical properties of polycaprolactone composites encapsulating phosphate-solubilizing bacterium Bacillus sp. PG01. *Process Biochemistry,* 42**,** 669-675.

WU, L. & LIU, M. 2008. Preparation and properties of chitosan-coated NPK compound fertilizer with controlled-release and water-retention. *Carbohydrate Polymers,* 72**,** 240-247.

WU, L., LIU, M. & LIANG, R. 2008. Preparation and properties of a double-coated slow-release NPK compound fertilizer with superabsorbent and water-retention. *Bioresource technology,* 99**,** 547-554.

XIANG, Y., DENG, Q., DUAN, H. & GUO, Y. 2017. Effects of biochar application on root traits: a meta-analysis. *Gcb Bioenergy*.

XIAO, X., YU, L., XIE, F., BAO, X., LIU, H., JI, Z. & CHEN, L. 2017. One-step method to prepare starch-based superabsorbent polymer for slow release of fertilizer. *Chemical Engineering Journal,* 309**,** 607-616.

YADAV, G. D. & DEVI, K. M. 2004. Kinetics of hydrolysis of tetrahydrofurfuryl butyrate in a three phase system containing immobilized lipase from Candida antarctica. *Biochemical Engineering Journal,* 17**,** 57-63.

YAMAMOTO, C. F., PEREIRA, E. I., MATTOSO, L. H., MATSUNAKA, T. & RIBEIRO, C. 2016. Slow release fertilizers based on urea/urea–formaldehyde polymer nanocomposites. *Chemical Engineering Journal,* 287**,** 390-397.

YANG, P., LI, H., LIU, Q., DONG, H., DUAN, Y. & ZHANG, J. Plasticization of Poly (lactic) acid Film as a Potential Coating Material. IOP Conference Series: Earth and Environmental Science, 2018. IOP Publishing, 022062.

YANG, T., REBSDORF, M., ENGELRUD, U. & XU, X. 2005. Monoacylglycerol synthesis via enzymatic glycerolysis using a simple and efficient reaction system. *Journal of Food Lipids,* 12**,** 299-312.

YANG, W. P. & TARNG, Y. 1998. Design optimization of cutting parameters for turning operations based on the Taguchi method. *Journal of materials processing technology,* 84**,** 122-129.

YIN, H., CHEN, Z., GU, Z. & HAN, Y. 2009. Optimization of natural fermentative medium for selenium-enriched yeast by D-optimal mixture design. *LWT-Food Science and Technology,* 42**,** 327-331.

YUAN, J.-H., XU, R.-K. & ZHANG, H. 2011. The forms of alkalis in the biochar produced from crop residues at different temperatures. *Bioresource technology,* 102**,** 3488-3497.

ZHONG, Z., DIJKSTRA, P. J., BIRG, C., WESTERHAUSEN, M. & FEIJEN, J. 2001. A novel and versatile calcium-based initiator system for the ring-opening polymerization of cyclic esters. *Macromolecules,* 34**,** 3863-3868.

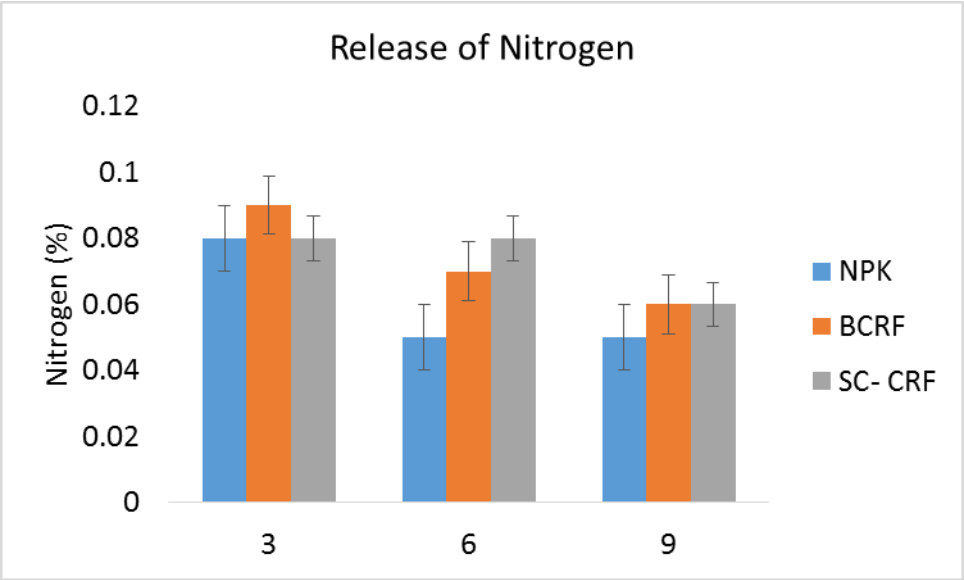


Figure A 1: Amount of nitrogen released from PCL (synthesised) based CRF

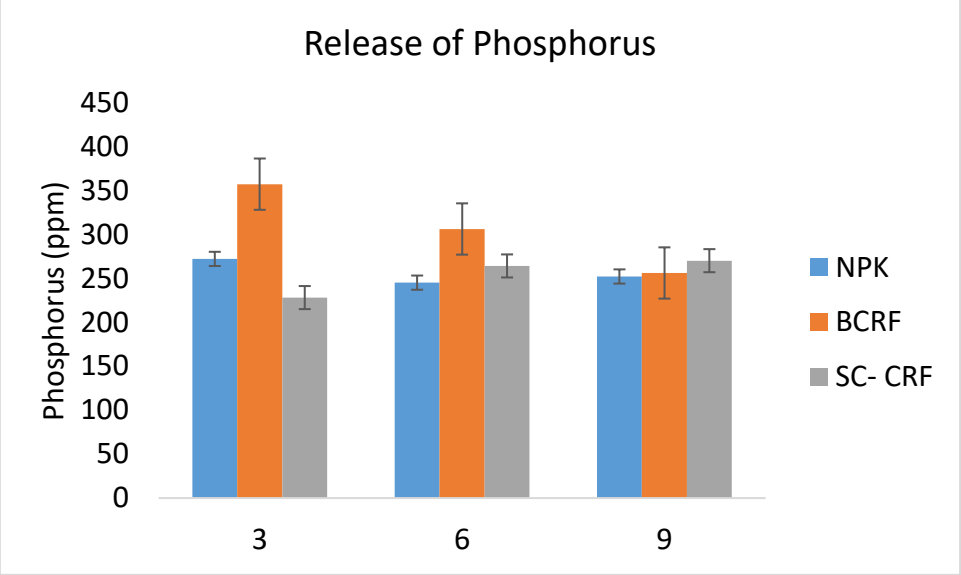


Figure A 2: Amount of Phosphorus released from PCL (synthesised) based CRF

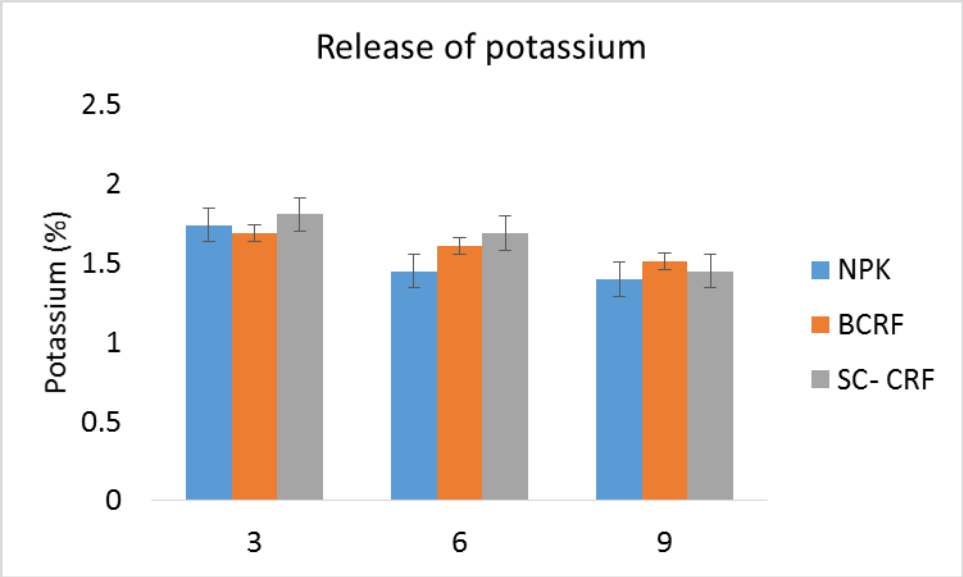


Figure A 3: Amount of Potassium released from PCL (synthesised) based CRF