PHYSICOCHEMICAL AND MICROBIAL PROFILE OF OIL PALM EMPTY FRUIT BUNCHES COMPOST TREATED WITH EFFECTIVE MICROORGNISMS

LIM LI YEE

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Dedicated to my parents, siblings and friends for their love and understanding.

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ABSTRACT

This study investigated the effects of Effective Microorganisms (EM·1™) on the composting process of ground oil palm empty fruit bunches (EFB). The maturity of the compost was monitored based on its important physical (temperature, pH and colour), chemical (organic and inorganic components) and microbiological properties. The compost with the addition of EM·1™ (ETC) matured 8-10 days earlier than the control sample (Ctl). The prolonged thermophilic phase was not observed for ETC because ETC could reach 50-55 ^{o}C within 2 to 3 hours on the first day of a 10-day experiment and maintained the temperature between 26-42 ^{o}C until the end of the process. Compared to control, ETC decreased the total organic carbon (TOC) and carbon to nitrogen (C/N) ratio by 10.78% and 17.26, respectively, but increased the microbial population (37.4% higher than Ctl) and the production of essential inorganic elements (Mg, K, Ca, Cu and B), as well as the key metabolite (5-aminolevulinic acid) for plant growth. The increment of nitrate nitrogen (NO_3^--N) was found to be higher in ETC, this may be due to a decrease of ammonium nitrogen (NH_4^+-N) in the process of nitrogen mineralization. Both Ctl and ETC reached a NH_4^+/NO_3^- ratio of 0.16 after maturation of the compost. Interestingly, the pH of the compost was found acidic (between pH 4.5 and 5.0) for the first week of composting, and then gradually increased to neutral and slightly alkaline pH (around pH 7.5-8.5) at the end of the process. The acidic condition was due to the accumulation of organic acids. The findings of this study concluded that the inoculation of EFB compost with EM·1™ could provide a better degradation of EFB compared to Ctl.

ABSTRAK

Kajian ini bertujuan untuk menyelidik kesan Mikroorganisma Efektif (EM·1™) dalam proses pengkomposan sisa tandan kosong buah kelapa sawit (EFB). Kematangan kompos dipantau dengan berasaskan ciri-ciri fizikal (suhu, pH dan warna), kimia (komponen organik dan inorganik) dan mikrobiologi yang penting. Kompos yang ditambahkan dengan EM·1™ (ETC) matang 8-10 hari lebih awal daripada kompos kawalan (Ctl). Fasa termofilik berpanjangan tidak dikesan untuk ETC kerana suhu setinggi 50-55 °C dikekalkan selama 2-3 jam dalam sehari sahaja bagi tempoh masa sepuluh hari yang pertama. Seterusnya, suhu kompos dikekalkan antara 26-42 °C hingga proses tamat. ETC menunjukkan penurunan dalam jumlah karbon organik (TOC) dan nisbah karbon kepada nitrogen (C/N), iaitu masing-masing 10.78% dan 17.26. Penambahan polulasi mikrob (37.4% lebih tinggi daripada Ctl), penghasilan unsur inorganik penting (Mg, K, Ca, Cu dan B) dan metabolit penting (asid 5-aminolevulinik) untuk pertumbuhan tumbuhan juga dapat diperhatikan. Penambahan nitrogen nitrat (NO_3^--N) adalah lebih tinggi bagi ETC akibat daripada penurunan kandungan nitrogen ammonium (NH_4^+-N) semasa proses peminerelan nitrogen. Nisbah NH_4^+/NO_3^- 0.16 tercapai apabila sampai waktu pematangan kompos. Kompos mempunyai pH berasid (pH 4.5-5.0) pada minggu pertama dan pHnya semakin meningkat ke neutral dan alkali lemah (pH 7.5-8.5) disebabkan pengumpulan asid organik. Kesimpulannya, penambahan EM·1™ untuk pengomposan EFB dapat menghasilkan degradasi EFB yang lebih baik daripada Ctl.

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LIST OF ABBREVIATIONS

EFB	-	Oil palm empty fruit bunches
EM·1™	-	Effective Microorganisms
TOC	-	Total organic carbon
ALA	-	5-Aminolevulinic acid
OM	-	Organic matters
MA	-	Microbiological additives
Cfu	-	Colony-forming unit
PGRs	-	Plant growth regulators
SB	-	Sugar beet
DOC		Dry olive cake
AM	-	Arbuscular Mycorrhizal
LAB	-	Lactic acid bacteria
BOD	-	Biological oxygen demand
COD	-	Chemical oxygen demand
PDT	-	Photodynamic therapy
PPIX	-	Protoporphyrin IX
PDD	-	Photodynamic diagnosis
TDPH	-	Tetrapyrroles-dependent herbicides
Phen	-	1,10-phenanthroline
Glu	-	glutamic acid

PS	-	photosystem
IAA	-	Indole-3-acetic acid
EMAS	-	Effective Microorganisms activated solution
ETC	-	EM·1™-treated compost
Ctl	-	Control sample
TOM	-	Total organic matter
LMWOAs	-	Low molecular weight organic acids
LCMS	-	Liquid chromatography mass spectrometry
ESI	-	Electrospray ionization
TON	-	Total organic nitrogen
ATP	-	Adenosine triphosphate
ADP	-	Adenosine diphosphate
NA	-	Nutrient agar
MRS	-	deMan-Rogosa-Sharps agar
AIA	-	Actinomycetes isolation agar
RBC	-	Rose bengal chloramphenicol agar
PBG	-	porphobilinogen
ALAD	-	5-Aminolevulinic acid dehydratase
ALAS	-	5-Aminolevulinic acid synthetase
POME	-	Palm oil mill effluents

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LIST OF SYMBOLS

C	-	Carbon
N	-	Nitrogen
CO_2	-	Carbon dioxide
P	-	Phosphorus
Mg	-	Magnesium
Zn	-	Zinc
Cu	-	Copper
Mn	-	Manganese
H_2S	-	Hydrogen sulfide
NO_2^-	-	Nitrite
Ca	-	Calcium
Fe	-	Iron
B	-	Boron
NH_3	-	Ammonia
NH_4^+	-	Ammonium
NO_3^-	-	Nitrate
H	-	Hydrogen
O	-	Oxygen
N_2	-	Nitrogen gas

CHAPTER 1

INTRODUCTION

1.1 Research Background

Malaysia is the major palm oil production country after Indonesia (MPOB, 2014). The solid wastes from oil plantation such as trunks, fronds and oil palm empty fruit bunches (EFB), have always been discussed in environmental conservation forums. Thus, composting is likely to become an effective alternative in managing these organic wastes. Technically, composting is a bioconversion process which allows the conversion of organic wastes into stable amorphous dark brown to black colloidal humus-like substances (de Bertoldi *et al.*, 1983). Therefore, composting recycles organic wastes and the mature compost can also be used as bio-fertilizer for a wide range of crops. This microbial added bio-fertilizer is believed to promote soil health for sustainability of crop production. This indirectly overcomes the high cost, environment pollution and soil organic matter depletion problems of excessive chemical fertilizer usage.

As a microbiological process, the addition of microbial additive which consisted of consortia of beneficiary microorganisms would increase the composting rate and the product quality. One of the commonly used microbial additives in Malaysia is Effective Microorganisms (EM·1™) from Japan. This microbial additive enhances microbial diversity of soil, detoxifies pesticides, suppresses plant disease and soil borne pathogens, enhances nutrient cycling and produces soil enhancing

metabolites to increase plant yield (Higa and Wididana, 1991; Monica *et al.*, 2011). It contains a variety of natural-occurring beneficial local microorganisms such as lactic acid bacteria, yeast, fungi, photosynthetic bacteria and actinomycetes. However, the scientific data of EM·1™ covering the exact strains and composition of various strains are often not revealed and generally remained as trade secrets. The performance of EM·1™ on EFB composting was investigated in this study before large scale application.

A complete composting process can be examined by various physical, biological and chemical parameters including temperature, microbial activities, total organic carbon content (TOC), total nitrogen content, carbon-to-nitrogen (C:N) ratio, formation of humic substances, CO_2 production, moisture content and pH level (de Bertoldi *et al.*, 1983; Michael and Kathleen, 2001). These parameters are of great importance to be an indicator for compost maturity and stability (Marta Benito *et al.*, 2003; M. Benito *et al.*, 2005; Forster *et al.*, 1993; Tiquia, 2005). The application of immature compost can be problematic to the ecosystem. This is because decomposition process is still continuing and hence inducing the anaerobic conditions in soil pores.

As a major plant growth regulation factor (PGRF), the production of 5-aminolevulinic acid (ALA)is not only detected in plants, but also in some microorganisms including anoxygenic and oxygenic photosynthetic bacteria, *Pseudomonas* sp., *Escherichia coli*, *Clostridium thermoaceticum*, *Methanosarcina* sp. and so on as a primary or secondary metabolite (Choorit *et al.*, 2011; Gilles *et al.*, 1983; Noparatnaraporn *et al.*, 2000; Sasaki *et al.*, 2002; Sonhom *et al.*, 2012). ALA can be used as herbicides or insecticides when it is applied at high concentration (>0.6mM), but become a plant growth promoter at low concentration (0.0006-2mM). The detection of ALA production during composting process can further confirm the agronomic value of the final product.

1.2 Problem Statement

The main objective of composting was to convert organic waste into value added bio-product such as bio-fertilizer. The application of compost as bio-fertilizer can only be carried out using mature compost. A non-matured compost is not suitable to be applied because of the presence of potential harmful substances and toxic, emergence of pathogens, as well as high loss of nutrients (Bertoldi *et al.*, 1983). As compost maturity is based on the degree of degradation of organic matters (OM), the transformation of C and N become one of the major functions for the study of compost maturity. Many researches on the OM degradation, the transformation of element C and N, the formation of humic substances together with some physical analysis such as appearance, temperature profile, pH and moisture content of compost are always performed for the determination of compost maturity and stability, but limited study has been carried out on the composting of EFB, which is the major agricultural waste in Malaysia, using Effective Microorganisms, EM·1™ as microbial inoculants.

1.3 Significance of the Study

Since composting is a microbial process, the addition of microbiological additives (MA) can be significantly importance in the acceleration of the decomposing rate, especially during the initiation of composting. Although many researchers have focused on the use of Effective Microorganisms, EM·1™ for municipal solid waste management, wastewater treatment and agricultural application, the roles of microbial biomass and their interactions during composting have not been deeply studied based on scientific approach. The application of EM·1™ on different plants at different locations in terms of efficacy and consistency remains a major challenge since the performance could vary due to the variance in soil fertilities, soil and microbial composition and constituents, plant types, and climates change. Therefore, the fundamental understanding of the microbial

populations throughout the composting process is essential for the determination of composting maturity and stability.

Furthermore, the degradation of the OM during composting also releases some plant growth promoting metabolites such as 5-aminolevulinic acid (ALA). The production of the metabolites is strongly depended on the raw material, its composition of OM, as well as the microorganisms present. Besides, the presence of the metabolites in the mature compost can further enhance the compost quality as a bio-fertilizer. Since the metabolite profile of compost is poorly studied so far, ALA production in the EM·1™-inoculated EFB compost is important to be carried out using the non EM·1™-inoculated sample as control.

1.4 Objective

The main objective of this study was to characterize the physical (temperature, pH and moisture content), chemical (total organic carbon, total nitrogen, carbon to nitrogen ratio, 5-aminolevulinic acid and low molecular weight organic acids contents) and microbial profile (total microbial, actinomycetes, lactobacillus and yeast count) of ground oil palm empty fruit bunches (EFB) compost using Effective Microorganisms (EM·1™) as inoculants.

1.5 Scopes of Study

The scopes of the study included,

1. To determine the compost maturity and stability by monitoring the physical (temperature, pH and moisture content) and chemical properties (C:N ratio, elemental analysis, 5-aminolevulinic acid concentration and low molecular weight organic acids content).

2. To identify the compost maturity and stability based on the microbial profile (total bacteria, lactic acid bacteria, actinomycetes and yeast).

3. To investigate the relationship between the physical, chemical and microbial profile of the EM-inoculated EFB compost experimentally.

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Appendix A

Grinding machine

Figure A1 Grinding machine.

Commercial EM·1™ microbial inoculants and molasses and the EM solutions before and after activation

Figure A2 Commercial EM·1™ microbial inoculants and molasses.

Figure A3 EM solutions before and after activation. Fully activated EM gave a lighter color and gas production can be observed along the one week activation process due to the microbial respiration.

Appendix C

pH meter used for the pH determination

Figure A4 pH meter used for the pH determination.

Spectroquant® test kits and Spectroquant® NOVA 60 analyzer used for macro- and micro-nutrients analysis

Figure A5 (a) Spectroquant® test kits and (b) Spectroquant® NOVA 60 analyzer used for macro- and micro-nutrients analysis. From left to right are the test kits for (first row) Potassium, Magnesium and Phosphorus; (second row) Calcium, Iron and Boron; (third row) Copper, ammonium, nitrate and nitrite.

Appendix E

Detailed macro-nutrient cell test

- **Potassium (K) Cell Test**

For K cell test, 2.0mL of the pretreated sample with the pH range of 3.0 – 12.0 was pipetted into the reaction cell (20 – 30°C). The cell was then closed and mixed thoroughly. After that, the pH of the sample was adjusted to a range of 10.0 – 11.5. Following that was the addition of 6 drops of Reagent K-1K. After mixing, a microspoon of Reagent K-2K was added into the cell and mixed thoroughly. The cell was left for an exactly 5 minutes of reaction time. Then, the sample was measured using a photometer and the amount of K was expressed as mg/L K (MERCK, Spectroquant® NOVA 60 analyzer).

- **Magnesium (Mg) Cell Test**

For Mg cell test, 1.0mL of pretreated sample (20 – 30°C) with the pH range of 3.0 – 9.0 was pipetted into the reaction cell. The cell was then closed and mixed thoroughly. 1.0mL of reagent Mg-1K was added and the mixture was left for anexactly 3 minutes of reaction time. 3 drops of reagent Mg-2K was added and mixed well. The absorbent was measured in a photometer and expressed as mg/L Mg (MERCK, Spectroquant® NOVA 60 analyzer).

- **Calcium (Ca) Cell Test**

For Ca cell test, 1.0mL of pretreated sample (20 – 30°C) with the pH range of 3.0 – 9.0 was pipetted into the reaction cell. The cell was then closed and mixed thoroughly. 1.0 mL of Reagent Ca-1K was then added and mixed well. The mixture was left for an exactly 3 minutes of reaction time. After that, 0.5 mL of Reagent Ca-2K was added and mixed well. The concentration of Ca was measured using a photometer and expressed as mg/L Ca (MERCK, Spectroquant® NOVA 60 analyzer).

Appendix E

Detailed macro-nutrient cell test

- **Phosphate (P) Cell Test**

For P cell test, 1.0mL of pretreated sample was pipetted into the reaction cell. The cell was then closed and mixed thoroughly. One dose of Reagent P-1K was added and mixed well. The cell was then heated at 120°C for half an hour. The cell was cooled down and adjusted the pH to 0 – 10.0. 5 drops of Reagent P-2K was added and mixed well. One dose of Reagent P-3K was then added and mixed vigorously until the reagent was completely dissolved. The mixture was left for an exactly of 5 minutes reaction time. The concentration of P was measured using a photometer and expressed as mg/L P (MERCK, Spectroquant® NOVA 60 analyzer).

Appendix F

Detailed micro-nutrient cell test

- **Iron (Fe) Cell Test**

For Fe cell test, the total Fe(II) and Fe(III) were determined by adding 1.0mL of pretreated sample (5 – 35°C) with the pH range within 3.0 – 8.0 into the reaction cell and mixed thoroughly. One dose of Reagent Fe-1K was then added and mixed vigorously until the reagent was completely dissolved. The mixture was then left for an exactly 5 minutes of reaction time. The concentration of total Fe(II) and Fe(III) was measured using a photometer and expressed as mg/L Fe (MERCK, Spectroquant® NOVA 60 analyzer).

- **Boron (B) Cell Test**

For B cell test, 1.0 mL of Reagent B-1K was pipetted into the reaction cell and mixed well in 4.0mL of pretreated sample (15 – 40°C) with the pH range within 2.0 – 12.0. The mixture was left for an exactly 60 minutes of reaction time. The concentration of B was measured using a photometer and expressed as mg/L B (MERCK, Spectroquant® NOVA 60 analyzer).

- **Copper (Cu) Cell Test**

For Cu cell test, 5.0mL of pretreated sample (10 – 30°C) with the pH range within 4.0 – 10.0 was pipetted into the reaction cell. The cell was then closed and mixed thoroughly. After that, the pH of the sample was adjusted to the pH of 7.0 – 9.5. Following that was the addition of 5 drops of Reagent Cu-1K. The cell was then mixed well and left for an exactly of 5 minutes reaction time. The concentration of Cu was measured using a photometer and expressed as mg/L Cu (MERCK, Spectroquant® NOVA 60 analyzer).

Appendix G

Detailed inorganic nitrogen cell test

- **Ammonium (NH_4^+) Cell Test**

A 0.5mL of pre-treated sample (20 – 30 °C) with pH range within 4.0 – 13.0 was pipetted into the reaction cell and mixed thoroughly. One dose of Reagent NH_4-1K was then added and mixed vigorously until the reagent was completely dissolved. The mixture was then left for an exactly 15 minutes of reaction time. The concentration of NH_4^+/N was measured using a photometer and expressed as mg/L NH_4^+/N (MERCK, Spectroquant® NOVA 60 analyzer).

- **Nitrate (NO_3^-) Cell Test**

A 0.5mL of pre-treated sample (5 – 25 °C) with the pH range within 1.0 – 3.0 was pipetted into the reaction cell and mixed thoroughly. 1.0mL of Reagent NO_3-1K was then added and mixed vigorously until the reagent was completely dissolved (the cell becomes hot and must be held only by the screw cap during mixing). The mixture was then left for an exactly of 10 minutes reaction time. The concentration of NO_3^-/N was measured using a photometer and expressed as mg/L NO_3^-/N (MERCK, Spectroquant® NOVA 60 analyzer).

- **Nitrite (NO_2^-) Cell Test**

Two level blue microspoons of Reagent NO_2-1K was placed into the reaction cell. A 8.0mL of pre-treated sample (15 – 25 °C) with the pH range within 1.0 – 12.0 was then pipetted into the cell and mixed vigorously until the reagent was completely dissolved. The mixture was then left for an exactly of 20 minutes reaction time. The concentration of NO_2^-/N was measured using a photometer and expressed as mg/L NO_2^-/N (MERCK, Spectroquant® NOVA 60 analyzer).

Appendix H

SPECTRONIC 200 UV-Vis spectrophotometer

Figure A6 SPECTRONIC 200 UV-Vis spectrophotometer.

Chemical composition of the agar plates used for the identification of each microorganism group

Table A1 Chemical composition of the agar plates used for the identification of each microorganism group.

Agar Plate	Chemical and Its Composition per Liter		Condition
Total Bacteria (Nutrient Agar)	• Agar • Peptic digest of animal tissue • Sodium chloride • Beef extract • Yeast extract	15.0g 5.0g 5.0g 1.5g 1.5g	With final pH of 7.4 ± 0.2 at 25°C
Actinomycetes (Actinomycete Isolation Agar)	• Agar • Glycerol • Sodium propionate • Sodium caseinate • K_2HPO_4 • Asparagine • $MgSO_4 \cdot 7H_2O$ • $FeSO_4 \cdot 7H_2O$	15.0g 5.0g 4.0g 2.0g 0.5g 0.1g 0.1g 1.0mg	With final pH of 8.1 ± 0.2 at 25°C
Lactobacillus (Lactobacillus deMan-Rogosa-Sharpe Agar)	• Agar • Glucose • Beef extract • Peptone • Sodium acetate • Yeast extract • Ammonium citrate • Na_2HPO_4 • Tween™ 80 • $MgSO_4 \cdot 7H_2O$ • $MnSO_4 \cdot 5H_2O$	15.0g 20.0g 10.0g 10.0g 5.0g 5.0g 2.0g 2.0g 1.0g 0.1g 0.05g	With final pH of 6.5 ± 0.2 at 25°C
Yeast (Rose Bengal Chloramphenicol Agar)	• Agar • Glucose • Papaic digest of soybean meal • KH_2PO_4 • $MgSO_4 \cdot 7H_2O$ • Rose Bengal • Chloramphenicol solution	15.0g 10.0g 5.0g 1.0g 0.5g 0.05g 10.0mL	With final pH of 7.0 ± 0.2 at 25°C

Correlationship between TOC degradation and carbon dioxide emission rate

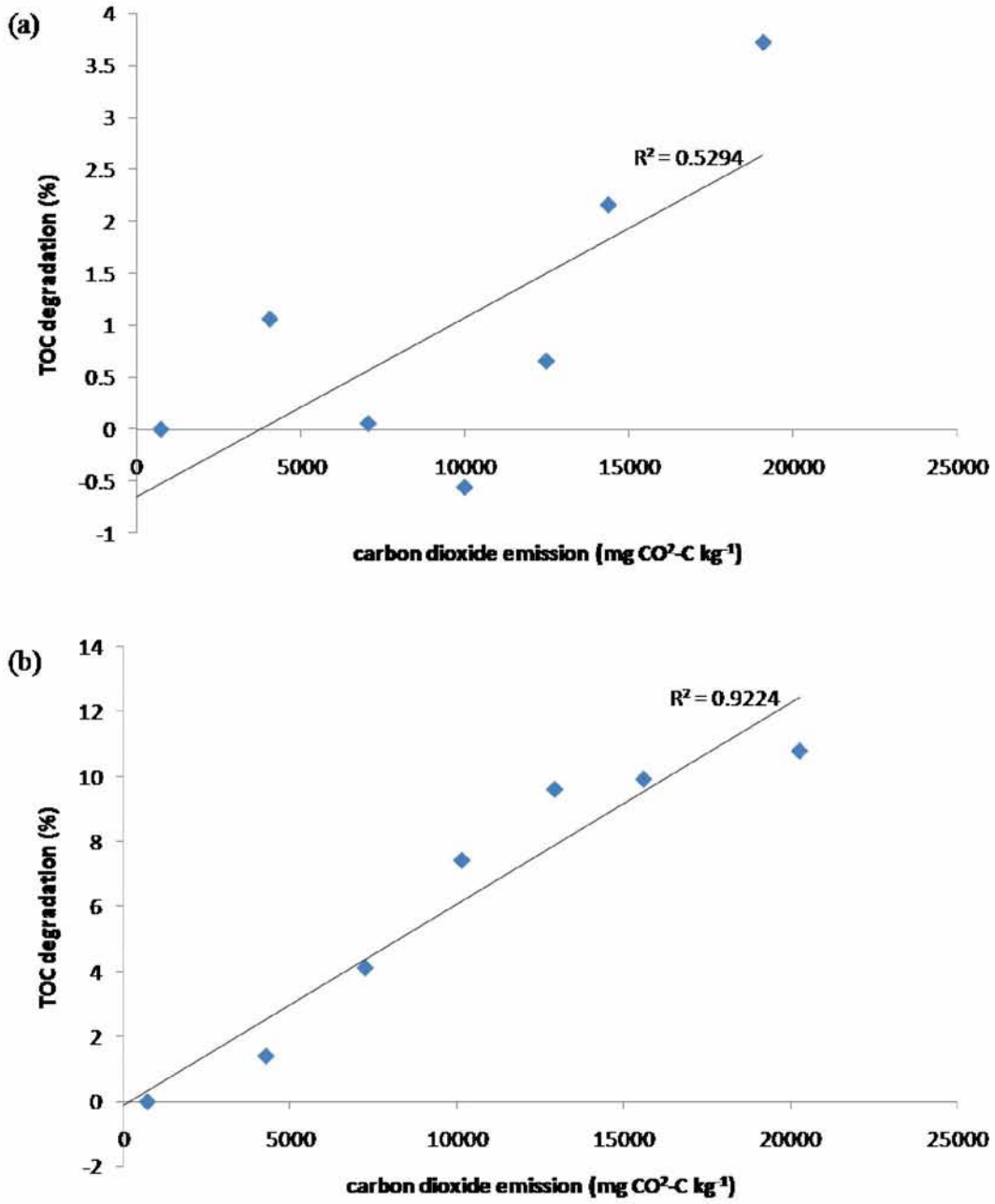

Figure A6 Relationship between the TOC degradation and the carbon dioxide emission for (a) Ctl and (b) ETC.

Appendix K

Correlationship of TOC and TON to C/N ratio

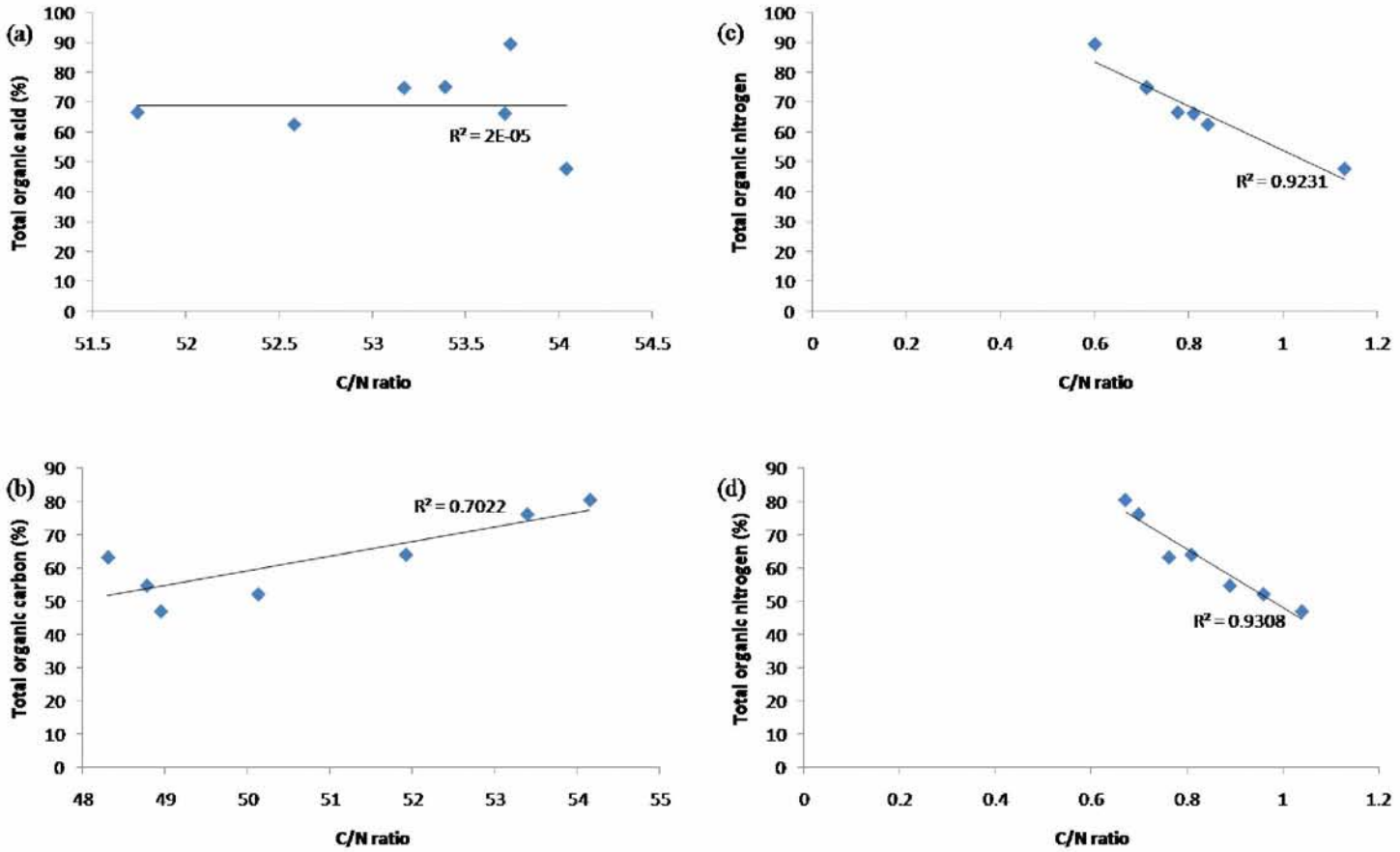

Figure A7 The relationship betwwen TOC and C/N ratio for (a) Ctl and (b) ETC and the relationship between TON and C/N ratio (c) Ctl and (d) ETC.