Titre: Title:	Co-composting as a means of remediating petroleum hydrocarbon-contaminated soil
Auteur: Author:	Nathalie Beaudin
Date:	1995
Type:	Mémoire ou thèse / Dissertation or Thesis
Référence: Citation:	Beaudin, N. (1995). Co-composting as a means of remediating petroleum hydrocarbon-contaminated soil [Master's thesis, École Polytechnique de Montréal]. PolyPublie. https://publications.polymtl.ca/32595/

UNIVERSITÉ DE MONTRÉAL

CO-COMPOSTING AS A MEANS OF REMEDIATING PETROLEUM HYDROCARBON-CONTAMINATED SOIL

NATHALIE BEAUDIN

DÉPARTEMENT DE GÉNIE CHIMIQUE

ÉCOLE POLYTECHNIQUE DE MONTRÉAL

MÉMOIRE PRÉSENTÉ EN VUE DE L'OBTENTION

DU DIPLÔME DE MAÎTRISE ÈS SCIENCES APPLIQUÉES

(GÉNIE CHIMIQUE)

DÉCEMBRE 1995

ISBN 0-612-11477-5

Nom Nathalie, BEAUDIN

Dissertation Abstracts International est organisé en catégories de sujets. Veuillez s.v.p. choisir le sujet qui décrit le mieux votre thèse et inscrivez le code numérique approprié dans l'espace réservé ci-dessous.

Génie Chimique — SUJET

0542 — CODE DE SUJET

U·M·I

Catégories par sujets

HUMANITÉS ET SCIENCES SOCIALES

COMMUNICATIONS ET LES ARTS
- Architecture 0729
- Beaux-arts 0357
- Bibliothéconomie 0399
- Cinéma 0900
- Communication verbale 0459
- Communications 0708
- Danse 0378
- Histoire de l'art 0377
- Journalisme 0391
- Musique 0413
- Sciences de l'information 0723
- Théâtre 0465

ÉDUCATION
- Généralités 515
- Administration 0514
- Art 0273
- Collèges communautaires 0275
- Commerce 0688
- Économie domestique 0278
- Éducation permanente 0516
- Éducation préscolaire 0518
- Éducation sanitaire 0680
- Enseignement agricole 0517
- Enseignement bilingue et multiculturel 0282
- Enseignement industriel 0521
- Enseignement primaire. 0524
- Enseignement professionnel 0747
- Enseignement religieux 0527
- Enseignement secondaire 0533
- Enseignement spécial 0529
- Enseignement supérieur 0745
- Évaluation 0288
- Finances 0277
- Formation des enseignants 0530
- Histoire de l'éducation 0520
- Langues et littérature 0279
- Lecture 0535
- Mathématiques 0280
- Musique 0522
- Orientation et consultation 0519
- Philosophie de l'éducation 0998
- Physique 0523
- Programmes d'études et enseignement 0727
- Psychologie 0525
- Sciences 0714
- Sciences sociales 0534
- Sociologie de l'éducation 0340
- Technologie 0710

LANGUE, LITTÉRATURE ET LINGUISTIQUE
- Langues
  - Généralités 0679
  - Anciennes 0289
  - Linguistique 0290
  - Modernes 0291
- Littérature
  - Généralités 0401
  - Anciennes 0294
  - Comparée 0295
  - Médiévale 0297
  - Moderne 0298
  - Africaine 0316
  - Américaine 0591
  - Anglaise 0593
  - Asiatique 0305
  - Canadienne (Anglaise) 0352
  - Canadienne (Française) 0355
  - Germanique 0311
  - Latino-américaine 0312
  - Moyen-orientale 0315
  - Romane 0313
  - Slave et est-européenne 0314

PHILOSOPHIE, RELIGION ET THÉOLOGIE
- Philosophie 0422
- Religion
  - Généralités 0318
  - Clergé 0319
  - Études bibliques 0321
  - Histoire des religions 0320
  - Philosophie de la religion 0322
- Théologie 0469

SCIENCES SOCIALES
- Anthropologie
  - Archéologie 0324
  - Culturelle 0326
  - Physique 0327
- Droit 0398
- Économie
  - Généralités 0501
  - Commerce-Affaires 0505
  - Économie agricole 0503
  - Économie du travail 0510
  - Finances 0508
  - Histoire 0509
  - Théorie 0511
- Études américaines 0323
- Études canadiennes 0385
- Études féministes 0453
- Folklore 0358
- Géographie 0366
- Gérontologie 0351
- Gestion des affaires
  - Généralités 0310
  - Administration 0454
  - Banques 0770
  - Comptabilité 0272
  - Marketing 0338
- Histoire
  - Histoire générale 0578
  - Ancienne 0579
  - Médiévale 0581
  - Moderne 0582
  - Histoire des noirs 0328
  - Africaine 0331
  - Canadienne 0334
  - États-Unis 0337
  - Européenne 0335
  - Moyen-orientale 0333
  - Latino-américaine 0336
  - Asie, Australie et Océanie 0332
- Histoire des sciences 0585
- Loisirs 0814
- Planification urbaine et régionale 0999
- Science politique
  - Généralités 0615
  - Administration publique 0617
  - Droit et relations internationales 0616
- Sociologie
  - Généralités 0626
  - Aide et bien-être social 0630
  - Criminologie et établissements pénitentiaires 0627
  - Démographie 0938
  - Études de l' individu et de la famille 0628
  - Études des relations interethniques et des relations raciales 0631
  - Structure et développement social 0700
  - Théorie et méthodes. 0344
  - Travail et relations industrielles 0629
- Transports 0709
- Travail social 0452

SCIENCES ET INGÉNIERIE

SCIENCES BIOLOGIQUES
- Agriculture
  - Généralités 0473
  - Agronomie. 0285
  - Alimentation et technologie alimentaire 0359
  - Culture 0479
  - Élevage et alimentation 0475
  - Exploitation des pâturages 0777
  - Pathologie animale 0476
  - Pathologie végétale 0480
  - Physiologie végétale 0817
  - Sylviculture et faune 0478
  - Technologie du bois 0746
- Biologie
  - Généralités 0306
  - Anatomie 0287
  - Biologie (Statistiques) 0308
  - Biologie moléculaire 0307
  - Botanique 0309
  - Cellule 0379
  - Écologie 0329
  - Entomologie 0353
  - Génétique 0369
  - Limnologie 0793
  - Microbiologie 0410
  - Neurologie 0317
  - Océanographie 0416
  - Physiologie 0433
  - Radiation 0821
  - Science vétérinaire 0778
  - Zoologie 0472
- Biophysique
  - Généralités 0786
  - Medicale 0760

SCIENCES DE LA TERRE
- Biogéochimie 0425
- Géochimie 0996
- Géodésie 0370
- Géographie physique 0368
- Géologie 0372
- Géophysique 0373
- Hydrologie 0388
- Minéralogie 0411
- Océanographie physique 0415
- Paléobotanique 0345
- Paléoécologie 0426
- Paléontologie 0418
- Paléozoologie 0985
- Palynologie 0427

SCIENCES DE LA SANTÉ ET DE L'ENVIRONNEMENT
- Économie domestique 0386
- Sciences de l'environnement 0768
- Sciences de la santé
  - Généralités 0566
  - Administration des hipitaux 0769
  - Alimentation et nutrition 0570
  - Audiologie 0300
  - Chimiothérapie 0992
  - Dentisterie 0567
  - Développement humain 0758
  - Enseignement 0350
  - Immunologie 0982
  - Loisirs 0575
  - Médecine du travail et thérapie 0354
  - Médecine et chirurgie 0564
  - Obstétrique et gynécologie 0380
  - Ophtalmologie 0381
  - Orthophonie 0460
  - Pathologie 0571
  - Pharmacie 0572
  - Pharmacologie 0419
  - Physiothérapie 0382
  - Radiologie 0574
  - Santé mentale 0347
  - Santé publique 0573
  - Soins infirmiers 0569
  - Toxicologie 0383

SCIENCES PHYSIQUES
Sciences Pures
- Chimie
  - Généralités 0485
  - Biochimie 487
  - Chimie agricole 0749
  - Chimie analytique 0486
  - Chimie minérale 0488
  - Chimie nucléaire 0738
  - Chimie organique 0490
  - Chimie pharmaceutique 0491
  - Physique 0494
  - PolymÇres 0495
  - Radiation 0754
- Mathématiques 0405
- Physique
  - Généralités 0605
  - Acoustique 0986
  - Astronomie et astrophysique 0606
  - Electronique et électricité 0607
  - Fluides et plasma 0759
  - Météorologie 0608
  - Optique 0752
  - Particules (Physique nucléaire) 0798
  - Physique atomique 0748
  - Physique de l'état solide 0611
  - Physique moléculaire 0609
  - Physique nucléaire 0610
  - Radiation 0756
- Statistiques 0463

Sciences Appliqués Et Technologie
- Informatique 0984
- Ingénierie
  - Généralités 0537
  - Agricole 0539
  - Automobile 0540
  - Biomédicale 0541
  - Chaleur et ther modynamique 0348
  - Conditionnement (Emballage) 0549
  - Génie aérospatial 0538
  - Génie chimique 0542
  - Génie civil 0543
  - Génie électronique et électrique 0544
  - Génie industriel 0546
  - Génie mécanique 0548
  - Génie nucléaire 0552
  - Ingénierie des systèmes 0790
  - Mécanique navale 0547
  - Métallurgie 0743
  - Science des matériaux 0794
  - Technique du pétrole 0765
  - Technique minière 0551
  - Techniques sanitaires et municipales 0554
  - Technologie hydraulique 0545
- Mécanique appliquée 0346
- Géotechnologie 0428
- Matières plastiques (Technologie) 0795
- Recherche opérationnelle 0796
- Textiles et tissus (Technologie) 0794

PSYCHOLOGIE
- Généralités 0621
- Personnalité 0625
- Psychobiologie 0349
- Psychologie clinique 0622
- Psychologie du comportement 0384
- Psychologie du développement 0620
- Psychologie expérimentale 0623
- Psychologie industrielle 0624
- Psychologie physiologique 0989
- Psychologie sociale 0451
- Psychométrie 0632

Name _____

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

_____ SUBJECT TERM

☐☐☐☐ SUBJECT CODE

U·M·I

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION

General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523

Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES

American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce-Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES

Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences

Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences

Applied Mechanics 0346
Computer Science 0984
Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY

General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

UNIVERSITÉ DE MONTRÉAL

ÉCOLE POLYTECHNIQUE DE MONTRÉAL

Ce mémoire intitulé:

CO-COMPOSTING AS A MEANS OF REMEDIATING

PETROLEUM HYDROCARBON-CONTAMINATED SOIL

présenté par: **BEAUDIN Nathalie**

en vue de l'obtention du diplôme de: Maîtrise ès sciences appliquées

a été dûment accepté par le jury d'examen constitué de:

M. SAMSON Réjean, Ph.D., président

M. RAMSAY Bruce, Ph.D., membre et directeur de recherche

M. LEGROS Robert, Ph.D., membre et co-directeur de recherche

M. RHO Denis, Ph.D., membre

... to

my parents

ACKNOWLEDGMENTS

I am indebted to *Bruce Ramsey* and *Robert Legros* who initiated and supervised this project. I would like to thank them for their support, both financial and academic.

Special thanks are due to *Richard F. Caron* who was responsible for the start-up and in great part the continuation of this project.

I would also like to thank *Juliana Ramsay* (Queen's University) and Larry Lawlor (Esso, Sarnia) for their advice on microbiology and hydrocarbon analysis, respectively.

I would also like to acknowledge *Michael Mitchell* who designed the min-reactors and *Claude Bélanger* for her work on the bioaugmentation and $CaCO_3$ and PO_4^{-3} experiments.

This study was financed by Esso ESTAC and FCAR-CQVB.

RÉSUMÉ

Dans cette étude, un sol sablonneux, contaminé avec 17,000 ppm d'huiles et graisses minérales (HGM), dont 40 % (massique) étaient aliphatiques, 28 % aromatiques, et 32 % polaires, a été compostées avec des feuilles d'érable et de la luzerne. Les buts de cette recherche étaient de déterminer le potentiel du co-compostage pour la rémédiation des sols contaminés aux hydrocarbures et l'effet de certains paramètres tels l'addition d'un co-substrat, la bioaugmentation, le ratio C/N, l'addition du $CaCO_3$ et du PO_4^{-3} et la température sur la biodégradation des hydrocarbures dans le co-compostage.

Pendant les essais de co-compostage, la température et la quantité de bioxyde de carbone dans le gaz de sortie étaient mesurées à intervalles réguliers Les échantillons de co-compost ont été analysés pour l'humidité, les solides non-volatiles (cendres), le pH et les comptes de bactéries et de moisissures. Ils ont aussi été analysés afin de déterminer leur contenu en HGM et dans certains cas les huiles et graisses ont été séparées sur colonne par chromatographie afin de déterminer leur contenu en huiles aliphatiques, aromatiques et polaires.

Plusieurs expériences de co-compostage ont été réalisées dans un réacteur de 8 L. Typiquement, le pH du co-compost, bien qu'acide initialement avait atteint une valeur de 8.5 après 19 jours. Une montée de température a été observée pendant cette période. La température du co-compost était ambiante au début de l'essai, mais avait augmentée jusqu'à

53^0C après 2 jours. Pendant les jours qui ont suivi, la température est graduellement redescendue à la température ambiante. Les taux de génération de CO_2 et de consommation d'O_2 étaient faibles pendant les premiers 15 heures du traitement. Au deuxième jour, les taux ont atteint un maximum de 135 mmol de CO_2 (hr-kg co-compost sec initial)$^{-1}$ et 180 mmol d'O_2 (hr-kg co-compost sec initial)$^{-1}$. Après 12 jours de traitements, les taux étaient inférieur à 10 mmol/(hr-kg co-compost sec initial). Le Quotient Respiratoire (taux de CO_2 molaire généré (taux d'O_2 molaire consommé)$^{-1}$) était à 0.9 initialement, mais a ensuite descendu à 0.7 (deuxième journée) et s'est subséquemment maintenu à une valeur entre 0.7 et 0.8. Le taux maximal de dégradation d'HGM (environ 600 mg (kg co-compost sec initial-jour)$^{-1}$) a été observé au début du traitement de co-compostage. Environ 50 % des HGM provenant du sol étaient dégradées dans les 105 premiers jours de co-compostage, ce qui était supérieur au 'landfarming' (étude avec le même sol) où seulement 30 % des HGM provenant du sol étaient dégradées après 180 jours de traitement. Soixante pourcent des aliphatiques, 54 % des aromatiques et 83 % des polaires ont été dégradées durant les 178 premiers jours de co-compostage. Après 287 jours, au moins 73 % des HGM provenant du sol étaient dégradées.

Les conditions nécessaires à l'optimisation du procédé de co-compostage ont été déterminées à partir d'une série d'essais d'une durée de 30 jours en mini-composteurs (pots Mason de 1 L modifiés). La dégradation des HGM provenant du sol (HGM moins HGM dans un contrôle sans sol) a augmenté de 6 à 45 % lorsque la quantité de co-substrat ajouté a augmenté de 0 à 73 %, même si ce co-substrat contenait aussi des HGM. L'addition de $CaCO_3$ ou de PO_4^{-3} (KH_2PO_4 et K_2HPO_4) individuellement a stimulé la dégradation des HGM, mais aucun effet positif n'a été observé lorsque les deux nutriments étaient ajoutés

simultanément. De meilleurs résultats ont été obtenus avec l'addition du $CaCO_3$ au début de l'essai suivi de PO_4^{-3} après deux semaines. Cette approche a permis de stimuler la dégradation en HGM de 23 à 48 %. La bioaugmentation n'avait soit aucun effet ou soit qu'elle inhibait la dégradation des HGM, dépendant de la souche ajoutée. La dégradation en HGM augmentait avec la réduction du ratio C/N du compost initial. A un ratio C/N de 17, 48 % des HGM ont été dégradées, tandis qu'à un ratio C/N de 40 il n'y avait point de dégradation des HGM. Cinquante-sept pourcent des HGM étaient dégradées si le co-compost était maintenu à température ambiante, tandis que 70 % des HGM étaient dégradées si la température était maintenue à 50°C pendant les 30 jours de l'essai. La réduction en HGM observée durant le co-compostage n'était pas causée par la volatilisation. L'addition du $CaCO_3$ et du PO_4^{-3}, le ratio C/N initial du co-compost et la température étaient les facteurs les plus importants pour l'obtention d'une dégradation d'HGM maximale lors du co-compostage.

ABSTRACT

In this study, a weathered hydrocarbon-contaminated sandy soil containing 17,000 ppm mineral oil and grease (MOG), of which 40 % (wt/wt) was aliphatic, 32 % (wt/wt) polar and 28 % (wt/wt) aromatic, was co-composted with maple leaves and alfalfa. The aim was to determine the feasibility of co-composting for the treatment of a sandy soil contaminated with weathered crude oil and to investigate the effect of various parameters (the addition of a co-substrate, bioaugmentation, C/N ratio, the addition of $CaCO_3$ and phosphate, and temperature) on hydrocarbon degradation during co-composting.

During the co-composting runs, temperature, and carbon dioxide levels in the off-gas were periodically measured. Tests for humidity, non-volatile solids (ash), pH, and heterotrophic bacterial and fungal counts were made throughout the co-composting runs. Co-compost samples were also analysed for MOG content. In some cases the MOG extract was further treated to determine the percentage of aliphatic, aromatic, and polar fractions present.

Several co-composting experiments were conducted in a laboratory-scale reactor. Typical co-compost experiments show that the co-compost pH though initially acidic rose to 8.5 by day 19. During the same period, the co-compost temperature rose from ambient to 53°C (day 2) and subsequently, gradually returned to room temperature. The rates of carbon dioxide generation and oxygen consumption were low during the first 15 hours. They

subsequently rose to 135 mmol CO_2 (hr-kg initial dry co-compost)$^{-1}$ and 180 mmol O_2 (hr-kg initial dry co-compost)$^{-1}$. By day 12, the rates had descended to less than 10 mmol (hr-kg initial dry co-compost)$^{-1}$. The Respiratory Quotient (RQ) (molar rate of CO_2 generation (molar rate of O_2 consumption)$^{-1}$) was initially about 0.9, but had decreased to 0.7 by day 2 and subsequently maintained a value between 0.7 and 0.8. The maximum rate of MOG degradation (about 600 mg (kg initial dry co-compost-day)$^{-1}$) occurred at the beginning of the experiment. About 50 % of the MOG of soil origin was degraded in the first 105 days of treatment comparing favourably with landfarming studies with the same soil where only 30 % of the MOG of soil origin had been degraded after 180 days. MOG fractionation demonstrated that 60 % of the aliphatics, 54 % of the aromatics and 83 % of the polars were degraded during the first 178 days of co-composting. After 287 days, at least 73 % of MOG of soil origin had been degraded.

The conditions necessary for optimal co-composting of the contaminated soil were determined in a series of 30 day tests in mini-composters (modified 1 L Mason jars). Degradation of MOG of soil origin (total MOG degradation minus MOG degradation in a control with no soil) increased from 6 % to 45 % as the quantity of co-substrate added increased from 0 % to 73 %, even though the co-substrate also contained MOG. Addition of calcium carbonate ($CaCO_3$) or PO_4^{-3} (KH_2PO_4 and K_2HPO_4) individually, stimulated degradation of MOG but when added together there was no beneficial effect. Better results were obtained when $CaCO_3$ was added at the beginning followed by addition of PO_4^{-3} two weeks later. This treatment increased MOG degradation from 23 % to 48 %. Bioaugmentation had either no effect or inhibited MOG degradation, depending on the

inoculum added. MOG degradation increased with decreasing initial C/N compost ratio. At a C/N ratio of 17, 48 % of the MOG was degraded (29 % of the MOG of soil origin), while at a C/N ratio of 40 there was no MOG degradation. Maintaining the co-compost at room temperature (23°C) resulted in 57 % MOG degradation whereas holding the temperature at 50°C for the entire 30 day period resulted in 70 % MOG degradation. The MOG reduction during co-composting was not due to volatilisation. Of the four parameters investigated, $CaCO_3$ and PO_4^{-3} addition, the C/N ratio, and the temperature profile were the most critical factors in obtaining maximum MOG degradation during co-composting experiments performed in mini-composters.

CONDENSÉ EN FRANÇAIS

Le co-compostage est un processus capable de convertir les déchets dangereux en produits inoffensifs. Ceux-ci inclus, entre autres, les explosifs tel le 2,4,6-trinitrotoluène, le chlorophénol, et les hydrocarbures aromatiques polycycliques. Au début du procédé de co-compostage, le matériel contaminé (sol) est mélangé avec des matériaux organiques (co-substrat). Le mélange est placé dans une pile ou dans un réacteur de co-compostage, où les micro-organismes consomment les contaminants.

Dans cette étude, un sol contaminé avec des hydrocarbures a été co-composté avec des feuilles d'érable et de la luzerne. Le sol était sablonneux, et était contaminé avec 17,000 ppm d'huiles et graisses minérales (HGM), dont 40 % (massique) étaient aliphatiques, 28 % aromatiques, et 32 % polaires. Le sol ne contenait aucun métaux lourds qui auraient pu être toxiques pour la flore microbienne. Le but était de déterminer le potentiel du co-compostage pour la rémédiation des sols contaminés aux hydrocarbures, et l'effet de certains paramètres (l'addition d'un co-substrat, la bioaugmentation, le ratio C/N, l'addition du $CaCO_3$ et du phosphate, et la température) sur la dégradation des hydrocarbures.

Les huiles et graisses minérales (HGM) ont été utilisées comme indicateur du degré de contamination en hydrocarbures parce qu'elles sont souvent mentionnées dans les guides du gouvernement. La technique des HGM a plusieurs variations. lorsque cette expérience a débuté. Une méthode en cuvée a été utilisée pour séparer les huiles et graisses minérales des

huiles et graisses totales. Il est possible qu'il soit restées une plus grande proportion de polaires dans les huiles et graisses minérales que si une méthode de séparation sur colonne avait été utilisée.

Pendant les essais de co-compostage, la température, et la quantité de bioxyde de carbone dans le gaz de sortie étaient mesurés continuellement. Des tests d'humidité, de solides non-volatiles (cendres), de pH, et des comptes de bactéries et de moisissures héterotrophiques ont aussi été faits. Les échantillons ont été analysés afin de déterminer leur contenu en HGM et dans certains cas les huiles et graisses ont été séparés sur colonne afin de déterminer leur contenu en huiles aliphatique, aromatique et polaire.

Plusieurs expériences de co-compostage ont été réalisées en réacteur de 8 L. Le réacteur était cylindrique (diamètre interne de 16 cm, et hauteur de 42 cm) et avait un faux fond perforé pour distribuer l'air plus uniformément. L'aération du compost avait comme but de maintenir les conditions aérobies, et de limiter la température du compost à environ 50°C. Le mur externe du réacteur était chauffé afin de minimiser la perte de chaleur. Pendant le compostage, une forte montée de température a été observée, de la température ambiante au début, jusqu'à 53^0C après 2 jours. Il a été nécessaire d'augmenter le taux d'aération afin de contrôler la température. Pendant les jours qui ont suivi, la température est graduellement redescendue à la température environnante. Un faible taux de génération de CO_2 et de consommation d'O_2 a été observé pendant les premiers 15 heures de traitement. Par la suite, les taux ont rapidement monté à 135 mmol de CO_2 (hr-kg co-compost sec initial)$^{-1}$ et 180 mmol d'O_2 (hr-kg co-compost sec initial)$^{-1}$. Après 12 jours de traitements, les taux étaient redescendus à moins de 10 mmol (hr-kg co-compost sec initial)$^{-1}$. Le Quotient Respiratoire

(taux de CO_2 généré (taux d'O_2 consommé)$^{-1}$) était à 0.9 initialement, mais a ensuite descendu à 0.7 (deuxième journée) et s'est subséquemment maintenu à une valeur entre 0.7 et 0.8. Le taux maximale de dégradation d'HGM (environ 600 mg (kg co-compost sec initial-jour)$^{-1}$) a été observé au début du traitement. Par contre, le ratio du taux d'HGM dégradées et du taux de matière organique dégradée a augmenté pendant les premiers 20 jours de co-compostage. Des extractions ont démontré que 50 % des HGM provenant du sol étaient dégradées dans les 105 premiers jours de co-compostage, ce qui était supérieur au 'landfarming' (étude avec le même sol) où seulement 30 % des HGM provenant du sol ont été dégradées en 180 jours de traitement. Soixante pourcent des aliphatiques, 54 % des aromatiques et 83 % des polaires ont été dégradées durant les 178 premiers jours de co-compostage. Après 287 jours, au moins 73 % des HGM provenant du sol étaient dégradées.

Les conditions nécessaires à optimisation du co-compostage ont été déterminées dans une série d'essais de 30 jours en mini-composteurs (pots Mason de 1 L modifiés). La dégradation en HGM provenant du sol a augmenté de 6 à 45 % lorsque la quantité de co-substrat ajouté a augmenté de 0 à 73 %, même si ce co-substrat contenait aussi des HGM. L'addition de $CaCO_3$ ou de PO_4^{-3} (KH_2PO_4 et K_2HPO_4) individuellement a stimulé la dégradation des HGM, mais aucun effet positif significatif n'a été observé lors de l'addition simultanée des deux nutriments. De meilleurs résultats ont été obtenus avec l'addition du $CaCO_3$ au début de l'essai suivi de PO_4^{-3} après deux semaines. Ce traitement a augmenté la dégradation en HGM de 23 à 48 %. La bioaugmentation n'avait soit aucun effet positif significatif ou soit qu'elle inhibait la dégradation des HGM, dépendant de la souche ajoutée. La dégradation en HGM augmentait avec la réduction du ratio C/N du compost initial. A un

ratio C/N de 17, 48 % des HGM ont été dégradées, tandis qu'à un ratio C/N de 40 il n'y avait point de dégradation des HGM. La dégradation en HGM était aussi sensible à la température. Des plages de températures de 23 à 60°C (plateau de 5 jours à cette température suivi de 24 jours à la température de la pièce) ont été étudiées. La plus importante quantité d'HGM (56 %) a été dégradée à 23°C même si une réponse bimodale avait été observée. Trente-huit, 33 et 47 % des HGM ont été dégradées lorsqu'il y avait un plateau de cinq jours à 30, 40 et 50°C respectivement. Seulement 23 % des HGM ont été dégradés lorsque le profil de température comprenait un plateau à 60°C. De plus, la dégradation en HGM augmentait à 70 % si la température du mini-composteur était maintenue à 50°C pour 29 jours au lieu de 5, et cette dégradation était supérieure à la quantité dégradée à 23°C. D'après les résultats obtenus, la réduction en HGM observée durant le co-compostage n'était pas causée par la volatilisation. Des paramètres étudiés, l'addition de $CaCO_3$ et de PO_4^{-3} , le ratio C/N, et la température étaient les facteurs les plus importants pour obtenir une dégradation maximale d'HGM durant le co-compostage.

Conclusions:

1) Le co-compostage est un traitement efficace pour des sols contaminés avec des contaminants récalcitrants. Quatre-vingt-neuf pourcent des HGM et au moins 73 % des HGM provenant du sol ont été dégradées en 287 jours de co-compostage, ce qui est supérieur aux résultats obtenus avec le "landfarming".

2) Le traitement d'une tonne de sol contaminé nécessite l'addition de 1.7 tonnes de co-substrat. Par contre, la masse finale du co-compost n'est pas aussi importante qu'on pourrait le croire parce qu'il y a dégradation de la matière organique. Par exemple, dans l'expérience mentionné çi-haut la masse finale du compost était 62 % de la masse originale après 287 jours.

3) Une expérience de co-compostage avec un sable qui n'était pas contaminé a démontré que 89 % des HGM provenant du co-substrat (feuilles et luzerne) ont été dégradées dans les premiers 3.5 mois de co-compostage. La conclusion est que les HGM provenant du co-substrat sont facilement dégradées et que presque toutes les HGM demeurant après 3.5 mois de co-compostage provenaient du sol.

4) Dans les expériences de co-compostage, une plus grande partie des substrats facilement assimilables ont été utilisés au début de l'essai. Ceci est indiqué par la haute valeur du Quotient Respiratoire et le bas ratio du taux d'HGM dégradées/taux de matériaux organiques dégradés.

5) Les mini-composteurs sont utiles parce qu'il est difficile de prédire les paramètres optimaux pour la dégradation d'un contaminant. Les conditions optimales dépendent non seulement du contaminant mais aussi du type de sol à traiter et de la souche indigène au sol. Par contre, moins d'HGM ont été dégradées lors d'essais en mini-réacteur qu'en réacteur de 8 L. Donc, les mini-réacteurs sont utiles pour faire des comparaisons, mais ne peuvent être utilisés pour déterminer la quantité d'HGM dégradables.

6) Les conclusions suivantes ont été faites durant les tests d'optimisation en mini-réacteurs:

i) Des nutriments, tel le phosphate, étaient limitant dans le sol. L'addition d'une solution de nutriments au sol a permis d'augmenter la quantité d'HGM dégradées.

ii) L'ajout de co-substrats a eu comme résultat d'accélérer la croissance microbienne, ce qui a permit plus de minéralisation et de co-oxydation.

iii) La bioaugmentation était inefficace. Les micro-organismes ajoutés n'ont pas compétitionnés efficacement avec la population indigène du sol. De plus, la bioaugmentation n'était pas nécessaire parce qu'une population microbienne capable de dégrader les hydrocarbures était déjà présente dans le sol.

iv) La dégradation d'HGM a augmentée avec la diminution du ratio C/N du co-compost. Le plus haut pourcentage d'HGM dégradées a été obtenu à un ratio C/N de 15. Il n'y avait pas de dégradation apparente à un ratio C/N initial de 40. Donc, il y a eu plus de dégradation des HGM lorsque le ratio C/N initial était bas (moins de 20) dû à la limitation en carbone et une dégradation préférentielle des co-substrats (plus facilement dégradables que les hydrocarbures) à un ratio C/N initial de 40.

v) La population microbienne capable de dégrader les hydrocarbures était plus active à 23°C et 50°C qu'à 30°C, 40°C et 60°C.

vi) La faible quantité de CO_2 générée à 60°C suggère qu'il y a eu une baisse dans le nombre de micro-organismes à cette température.

vii) Il y a eu plus de dégradation d'HGM à 50°C qu'à 23°C lorsque cette température était maintenue pendant 14 jours ou plus. C'est une indication qu'un système en réacteur serait préférable à un système en andain pour le co-compostage à grande échelle afin d'allonger et de contrôler la phase thermophilique.

Recommandations:

Cette étude a démontré que le co-compostage est un moyen efficace pour le traitement d'un sol sablonneux contaminé aux hydrocarbures. Il reste toutefois a trouver un substitut à la luzerne (Purina rabbit chow), parce que ce co-substrat est trop coûteux pour qu'il soit utilisable à grande échelle. Les caractéristiques du substitut idéal seraient: un ratio C/N inférieur à 15, un faible contenu de matières pouvant être extraites au fréon, et un coût très bas (peu dispendieux).

Un des désavantages du co-compostage est le coût additionnel des co-substrats. Le coût des co-substrats a été estimé à $104 par tonne de sol traité. Ce nombre n'inclut pas le coût additionnel du transport des co-substrats jusqu'au site de traitement. De la luzerne (directement de la ferme) plutôt que le "rabbit chow" de Purina a été utilisée dans cette analyse économique. Le coût de la luzerne était $90 tonne^{-1}, une portion considérable du coût total. Donc, le procédé deviendrait plus économique si un déchet était utilisé comme substitut pour la luzerne.

Une fois le substitut choisi, plusieurs expériences devraient être faites en réacteur de 8L pour déterminer si ce nouveau co-substrat donne des résultats acceptables. Des essais à l'échelle pilote devraient ensuite être effectués. Une nouvelle méthode pour analyser les hydrocarbures devrait être considérée à cause des problèmes rencontrés avec la méthode des HGM.

Bien que cette étude ait permis de conclure qu'il y avait plus de dégradation des HGM à un ratio C/N initial de 15. Il est possible que cette condition soit impossible à rencontrer à grande échelle. Une plus grande masse de co-compost pourrait nécessiter l'ajout d'une plus

grande proportion de feuilles afin d'améliorer la porosité et de maintenir les conditions aérobies (nécessaires pour les oxygénases, souvent impliquées dans les premières étapes de la dégradation des hydrocarbures). Une autre possibilité serait l'ajout d'un agent qui donne du volume comme des copeaux de bois.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

C/N	:	Mass ratio of carbon and nitrogen
C/N/P	:	Mass ratio of carbon, nitrogen and orthophosphate
comp.	:	Co-compost
cumm.	:	Cummulative
d. c.	:	Dry compost
org. deg.	:	Degradation
dp	:	Particle diameter
HGM	:	Huiles et graisses minérales
i.d.	:	Internal diameter
MOG	:	Mineral oil and grease
MSM	:	Mineral salts medium
PAH	:	Polycyclic aromatic hydrocarbons
pH	:	Hydrogen ion activity
ppm	:	Parts per million
RQ	:	Respiratory quotient
rpm	:	revolutions per minute

v	:	volume
wt	:	weight

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1. Development of Objectives

In the last few decades, pollution has been recognised as a major problem. Concern for the quality of our air and water forced a general restructuring in our waste management strategies. However, we have only recently realised the magnitude of our soil pollution problem to which petroleum products are a major contributor.

Petroleum products are complex mixtures of aliphatic, alicyclic and aromatic compounds. Some of these components such as polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and mutagenic (Cerniglia, 1981), and have the potential for bioaccumulation in the food chain (Means, 1980). Their negative impact on health and the environment necessitates the remediation of petroleum-contaminated soils.

Bacteria and fungi are the principal microbes involved in petroleum biodegradation in soils, although the relative contribution of each is not clear. A number of actinomycetes also have hydrocarbon-degrading abilities, but their role is less important since they do not compete successfully in contaminated soils (Jensen, 1975). Hydrocarbon-degrading microorganisms occur naturally in soil. Studies have demonstrated that their activity and

numbers increase (Dibble and Bartha, 1979), but that their species diversity decrease when a soil is contaminated with oil (Jensen, 1975).

Different factors affect microorganisms and therefore the hydrocarbon degradation rate. The primary factor affecting microbial growth in soil is the scarcity or absence of an energy source. Second; optimal soil pH for microorganisms is between 6 and 8, but can be lower for fungi. Third; soil temperature affects the degradation rate of hydrocarbons. Fourth; microorganisms are sensitive to the soil moisture level. And finally, all the nutrients necessary for the growth of microorganisms must be present (nitrogen, phosphorous and oxygen are the most important) in the proper amounts. One bioremeditaion method that would facilitate control of these factors is co-composting.

Co-composting is relatively simple, inexpensive, and environmentally sound. It involves the addition of a carbon rich soil amendment which typically increases soil permeability, improves oxygen transfer and soil texture, and provides an energy source to rapidly establish a large microbial population. The aim is for the microbes to consume the contaminants (hydrocarbons in this case) as well as the soil amendment. Contaminants that have been successfully treated by co-composting include explosives (Williams and Myler, 1990), chlorophenol (Valo et al., 1986) and PAHs (Hogan et al., 1988).

Mineral oil and grease (MOG) was chosen as an indicator for hydrocarbon content in this study because it is often cited in Government guidelines. There are several variations on the technique. In this study, a batch method with silica gel was used to seperate the non-mineral oil and grease from the total oil and grease. As a result, more polars may have

remained in the MOG than would have been the case if a column separation method had been used.

The main objective of this research is to assess the feasibility of co-composting to treat a soil contaminated with a weathered crude oil (see Appendix H for soil characterization). Feasibility studies were carried out in a laboratory-scale reactor and optimisation studies in a series of experiments in mini-composters.

1.2 Thesis Structure

This thesis is structured in five chapters: introduction, literature review, two articles and conclusions and recommendations. The first article (Chapter 3) was submitted to the journal *Compost Science & Utilization.* It investigated the feasibility of co-composting to treat a sandy soil contaminated with weathered crude oil. The second article (Chapter 4) was submitted to *Applied and Environmental Microbiology*. This paper evaluated the effect of various parameters (co-substrate addition, bioaugmentation, Ca and P addition, C/N ratio and temperature) on hydrocarbon degradation in a series of 30 day tests carried out in mini-composters (modified 1 L Mason jars).

CHAPTER 2

LITERATURE REVIEW

2.1 Composting

Composting is an aerobic microbial process for the degradation of organic solids usually employing a thermophilic phase. Traditionally, composting has been a product-oriented process in which an agricultural waste was converted into a stabilized humic-like organic soil amendment (compost) (Haug, 1993). Today composting is used more and more as a treatment technology for hazardous waste in which the objective is solely its conversion to innocuous end-products (Williams and Myler, 1990).

In soil co-composting, the contaminated soil is mixed with suitable organic materials and placed in a pile or enclosed vessel. The organic material serves to improve the soil texture for increased aeration and drainage and provides a nutrient and energy source to rapidly establish a large microbial population. The aim is for the microbes to consume the hazardous materials as well as the supplied organics. The highly active, heterotrophic environment increases the possibility that some contaminants will be degraded by co-oxidation (Williams and Keehan, 1992). Although the bacteria, fungi and actinomycetes are responsible for most of the degradation, algae, viruses and protozoa as well as the macrofauna: nematodes, mites, ants, springtails, mili/centipedes, spiders,

beetles and earthworms are also present (IWM Scientific and Technical Committee, 1994).

The changes in temperature observed during the composting process have been used to divide the process into four stages: 1) mesophilic (temperature, ambient to 45°C), 2) thermophilic (45 to 75°C), 3) second mesophilic and 4) final maturation. At the beginning of the mesophilic stage, the microorganisms are very active, oxygen, water and food are abundant. The population increases rapidly (in particular that of the bacteria) and results in the production of a large amount of heat. This heat is trapped due to the insulating effect of the compost heap and results in a rapid rise in temperature (Figure 2.1). As the temperature rises the conditions rapidly become thermophilic, resulting in the death of the mesophilic organisms and the succession of a thermophilic population. Above 45°C, growth is restricted to a few species of thermophilic bacteria. Thermophilic fungi and bacteria start to die at around 60°C. Only extremely thermophilic endospore forming bacteria and some actinomycetes are still active. Compost temperatures continue rising, and may reach 80°C. Most of the organisms are now dead or dying, resulting in a dramatic drop in activity and a gradual decline in the temperature. As the compost cools, bacteria and fungi repopulate the pile from the outer surfaces. The self-heating process begins once more and cellulose and lignin breakdown commences. Eventually, most of the nutrients are consumed and the pile cools. The decreasing temperature results in a second mesophilic stage. Predation and antagonism occur as food becomes scarce and biodiversity decreases to a few hundred species or less. The stage may last several months depending on the initial feed. Maturation is the final stage of composting and may last six months or more (IWM Scientific and Technical Committee, 1994).

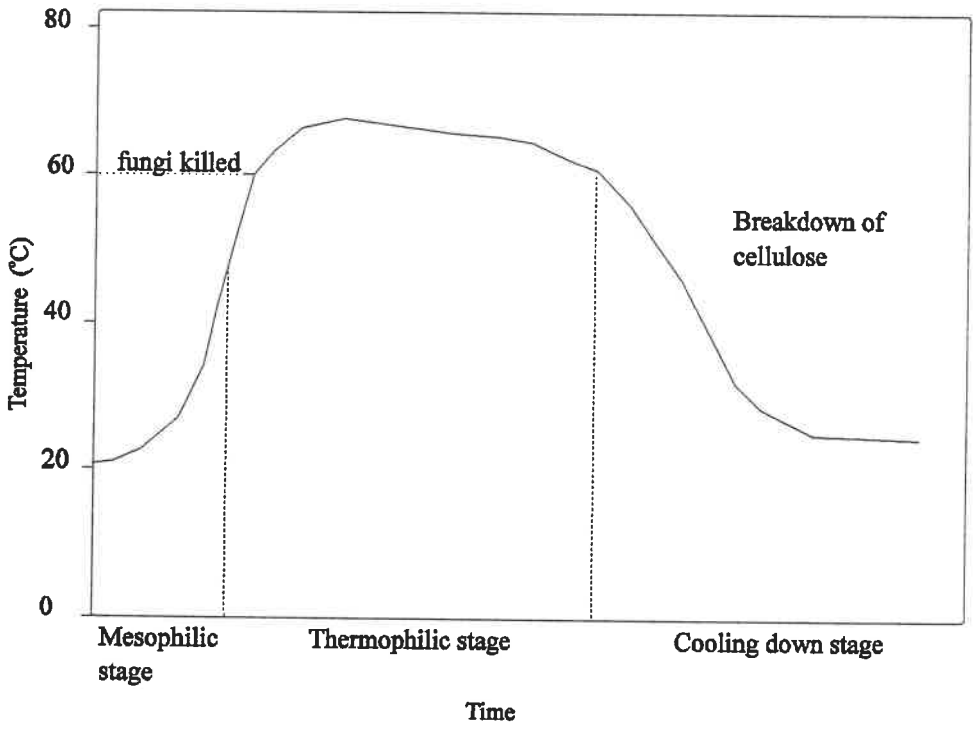


Figure 2.1. Typical temperature profile in a compost heap (IWM Scientific and Technical Committee, 1994).

The potential of this process for the bioremediation of contaminated soils is promising primarily because of the dynamics of the population within the composting environment. The population concentration has increased by several orders of magnitude due to the aerobic, nutrient-rich environment. Second, the metabolic activity of those microorganisms is increased. Finally, the changing microbial populations results in a large variety of diverse microorganisms to which the contaminant is exposed (Strom, 1985, Williams and Keehan, 1992).

Composting of botanical waste is not new, it has been practiced for thousands of years. The chinese are thought to have had aerated static piles using hollow bamboo poles 2000 years ago (IWM Scientific and Technical Committee, 1994). Co-composting

however is relatively new. It is only in the last few decades that co-composting of the biodegradable portion of municipal waste has been practiced (Briedenbach, 1971). The concept of co-composting of hazardous wastes came about when the National Canner's Association studied the biodegradation of pesticides in 1968 (Hart, 1991). Since then, attempts have been made to co-compost many hazardous wastes. These include explosives, chlorophenol and polynuclear aromatic hydrocarbons (Williams and Myler, 1990; Valo *et al.*, 1986; Hogan *et al.*, 1988). Only a few studies have been published on co-composting of hydrocarbon contaminated soil. Van den Munckhof and Veul (1991) co-composted an oil-contaminated soil with household garbage. They determined, in laboratory tests, that oil decomposition was possible within two weeks at mesophilic temperatures (30-35°C) with adequate moisture and nutrient levels. Pilot-scale studies were not as successful because optimal temperatures were not achieved. A study was also reported on the co-composting of oily production pit sludges (containing 10.8 % extractable hydrocarbons) with wood chips and manure. The concentration in extractable hydrocarbons was reduced by 92 % after 4 weeks of co-composting (McMillen *et al.*, 1992).

2.2 Operating Conditions

Co-composting allows for the adjustment of several factors in order to improve the hydrocarbon-degradation rate. These include the choice of matrix (carbon-rich nutrient source), the initial pH (usually between 6 and 8), moisture content, temperature and oxygen supply.

2.2.1 Moisture Content

Although high aeration levels may cause water stripping, there is also a considerable amount of water produced in a compost system. The humidity level is very important as it affects the physiology of the microflora during co-composting. An excessive amount will cause the interstices within the organic mass to become filled with water. This restricts aeration causing the development of anaerobic zones which results in odors and a significant decrease in the rate of decomposition. Excessively dry conditions will also hinder decomposition, resulting in process failure because the filamentous bacteria (which are primarily responsible for the most active phase of composting) are unable to physically colonize the substrate (Miller, 1989). Optimal moisture content changes for each material composted depending on the particle size and the materials' capacity for absorbing water. The maximum moisture content which allows for optimal breakdown rates also depends on the type of composting system which is employed, because mixing promotes microbial colonization. Consequently, composting processes which incorporate mixing can tolerate a lower level of moisture (Robinson and Stentiford, 1993).

Although 50 to 60 % water content usually gives the best results (Suler and Finstein, 1977) values ranging from 25 to 80 % have been reported.

2.2.2 Aeration

Compost is normally continuously aerated during its initial phases, fulfilling three process requirements. Aeration removes the water and heat produced during composting, as well as supplying oxygen. Aeration is especially important in

bioremediation as the first step in hydrocarbon degradation usually involves oxygenases (the incorporation of one or two oxygen molecules into the substrate) (Cookson, 1995). A study on crude oil degradation in a basal salts medium found that increased aeration, although not having an effect on either the rate of growth or the total amount of microbial cells produced, did increase the utilization of the saturate fraction (Jobson *et al.*, 1972). A study on jet fuel degradation in a humid, fertilized and tilled soil showed that oxygen limitation strongly attenuated microbial response to the contaminant (Song and Bartha, 1990).

2.2.3 Temperature

In composting, a basic process control objective is to maximize microbial activity at the expense of the waste being treated. This results in the temperature of the composting mass increasing from ambient to as high as 80 degrees Celsius (Figure 2.1). In a moist well-aerated system, temperatures will remain high until the readily degradable material is used up. The process is characterized by the expansion and subsequent collapse of an indigenous mesophilic population, followed by a repetition of similar events in a thermophilic population.

The effect of temperature in composting has been studied extensively. Composters operating at a maximum of 45°C are more active (in terms of CO_2 production) than at 55° or 65°C (McGregor *et al.*, 1981). Although reports do not agree on what is the optimum temperature, it is generally held that 60°C is the temperature at which a system becomes self-inhibited (McGregor *et al.*, 1981; McKinley *et al.*, 1984). This is caused by a decrease in the total number and species diversity of microorganisms at high temperatures (Strom, 1985).

2.2.4 pH

The optimal hydrogen ion activity (pH) of a composting system lies somewhere between 6 and 8. This can be explained by the fact that most bacteria found in a compost ecosystem have their optimum in this range. Fungi are more versatile and will tolerate a pH range of 5 to 8.5 but tend to be acidophilic.

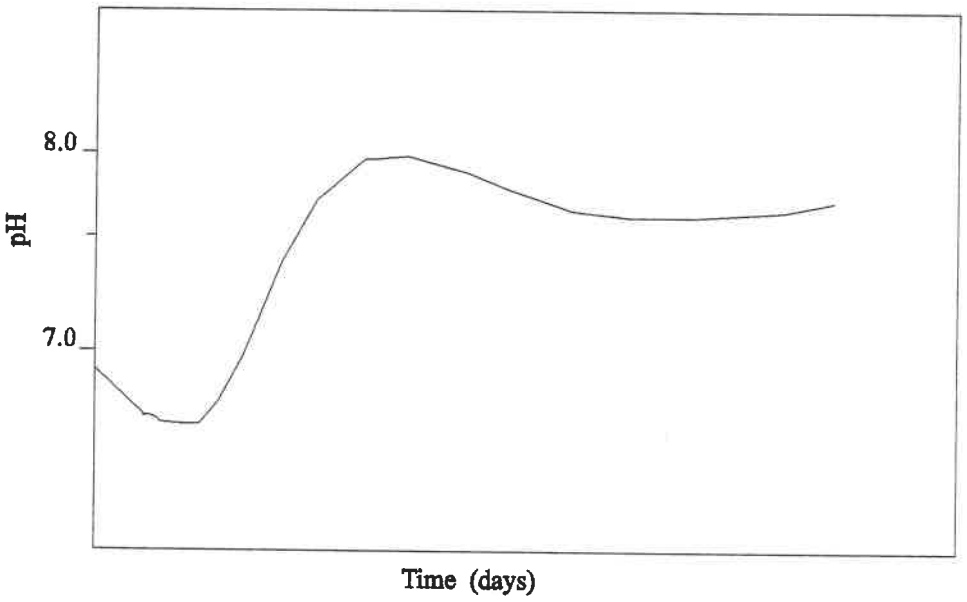


Figure 2.2. Typical pH profile for a batch composting system (Mustin, 1987).

The pH of a compost changes with time (Figure 2.2). It is affected by both acidifying and alkalinizing reactions as well as influenced by microorganisms within the system. A study on composting, found that a pH of 8.2 gave the highest composting rate (in terms of O_2 consumed; 6.7, 7.5 and 8.2 tested) (Jeris and Regan, 1973). A landfarming study (conducted with the objective of studying the effects of environmental

parameters on the biodegradation of an oily sludge) also recommended a slightly alkaline pH though for different reasons. The soil pH's were adjusted to 5, 6, 7, and 7.8 with $CaCO_3$, and 7.8 was found to give the most hydrocarbon degradation (Dibble & Bartha, 1979).

2.2.5 Carbon, Nitrogen, and Phosphorous

Microorganisms need carbon as well as at least 11 essential minerals (of which nitrogen and phosphorous are the most important), in the proper amounts and ratios. Microorganisms generally consume fifteen to thirty times more carbon than they do nitrogen. Historically, in municipal waste composting a C/N ratio of 30 to 40 has been recommended. However, C/N ratios around 22 give higher peak and cumulative carbon dioxide evolution rates (Nakasaki *et al.*; 1981; Michel *et al.*, 1993). Phosphorous is also important. Optimal values are thought to be given by a N/P ratio of 2 to 5.

2.3 Crude Oil

Crude oil is a complex mixture of aliphatic, alicyclic, and aromatic hydrocarbons. There are several hundred individual components in every crude oil and the composition of each crude oil varies with its origin. The most common aliphatic components are the n-alkanes, of which chain lengths of 5 to more than 35 carbon atoms can be found. The shorter chain molecules can cause membrane disruption in many microorganisms and are therefore relatively toxic (Atlas, 1984; Cookson, 1995). Alkenes are rare in crudes while branched alkanes are common. Cyclic aliphatics are common and may be monocyclic, bicyclic, substituted, or unsubstituted. As with alkanes, the lower molecular weights are

disruptive to membranes. A wide range of aromatics are biologically available in petroleum.

2.4 Degradation

The biodegradation of petroleum products is the modification or decomposition of the product by microbes. If taken to completion the process theoretically produces only microbial cells, carbon dioxide and water. The biodegradation of a petroleum product is generally described by the following equation.

$$\text{Petroleum Product} + O_2 + \text{Nutrients} \xrightarrow{\text{Biomass}} CO_2 + H_2O + \text{Byproducts}$$

The susceptibility of petroleum products to biodegradation varies with the types and sizes of the components. For example, the n-alkanes , n-alkyl aromatics, and simple aromatic molecules in the C_{10} to C_{22} range are the least toxic and the most readily degradable. Smaller molecules, although biodegradable, tend to be more toxic and in most systems are removed by volatilization, rather than biodegradation. Long-chain n-alkanes are more recalcitrant due to their hydrophobicity and the fact that they are viscous or solid at ambient temperatures. Branched alkanes and cycloalkanes (C_{10} to C_{22} range) are less biodegradable than their n-alkane and aromatic analogs. The most recalcitrant molecules are the polynuclear aromatics and the asphaltene fraction. It becomes evident that the individual components of an oil will be degraded at different rates (Bossert and Bartha, 1984). The interaction between substrates further complicates

the situation. Microorganisms may ignore all other substrates until they have consumed those more readily biodegradable.

2.4.1 Degradation Pathways

Different hydrocarbon compounds have different biodegradation pathways. In the case of alkanes, the initial attack occurs by monooxygenases (Figure 2.3) or dioxygenases. In the first case, one atom of oxygen is incorporated into the alkane yielding a primary alcohol. Although the most common degradation pathway is oxidation at the terminal methyl group, subterminal attack does occasionally occur. In the second

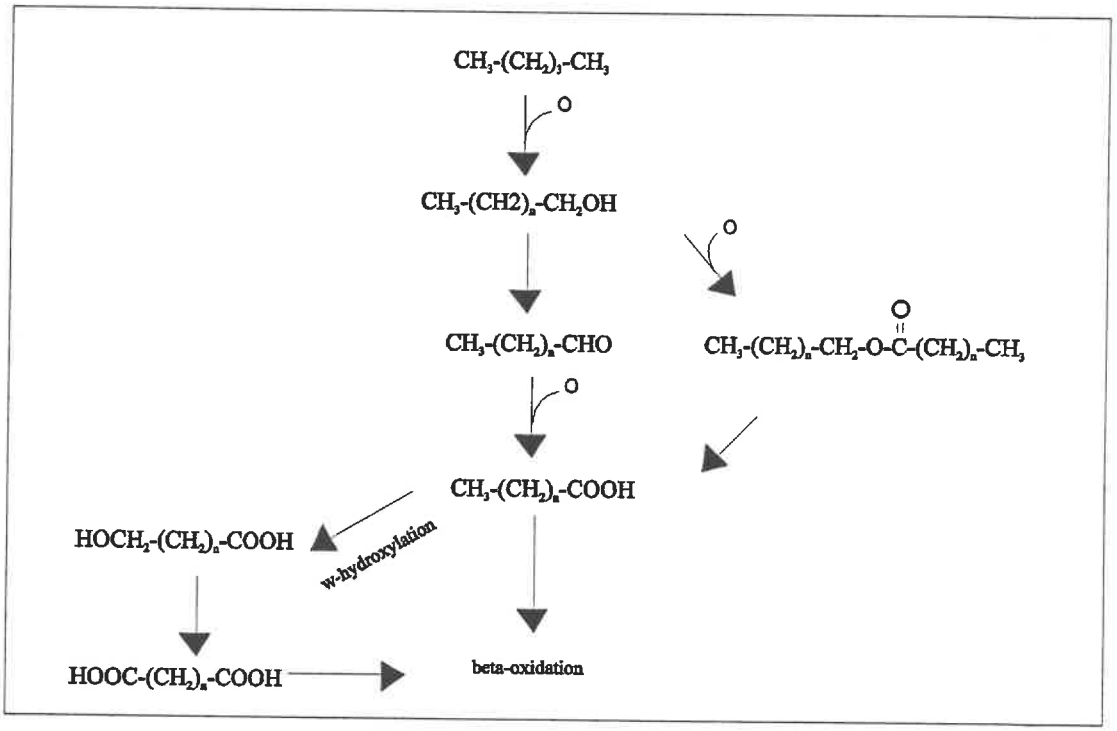


Figure 2.3. Oxidation of n-alkanes by attack on the terminal methyl group (Britton, 1984).

case, both atoms of oxygen are transferred to the alkane, yielding a labile hydroperoxide intermediate that is subsequently reduced by $NADPH_2$ to an alcohol and H_2O. The resulting alcohol (in both cases) is then oxidized to an aldehyde and a fatty acid. Occasionally both terminal methyl groups are oxidized in this manner yielding a dicarboxylic acid (ω-oxidation). Further catabolism occurs by the β-oxidation sequence (the successive removal of two carbon units) (Atlas and Bartha, 1980; Cookson, 1995).

The degradation of cycloalkanes usually occurs by the oxidation of the terminal methyl unit yielding a primary alcohol (Cookson, 1995). In the case of alicyclic hydrocarbons having no terminal groups, hydroxylation by a mixed function oxidation leads to an alicyclic alcohol (Figure 2.4). Dehydrogenation produces a ketone and oxidation a lactone, which is subsequently hydrolyzed. The hydroxyl group is oxidized, in sequence to an aldehyde and carboxyl group and further metabolized by β-oxidation (Atlas and Bartha, 1980).

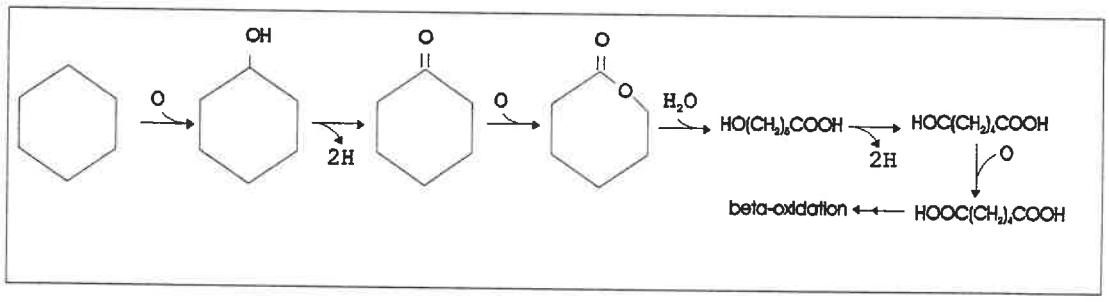


Figure 2.4. Oxidation of Cyclohexane as an example for metabolism of alicyclic hydrocarbons (Atlas and Bartha, 1987).

Microorganisms capable of degrading alicyclic hydrocarbons are not as predominant as those for the degradation of aliphatic hydrocarbons (Cookson, 1995). Furthermore, organisms capable of growing on these compounds often cannot

completely metabolize the molecule (Atlas and Bartha, 1980). Consequently, commensalism and co-metabolism play an important role in the biodegradation of alicyclic hydrocarbons (Perry, 1984; Trudgill, 1984).

PAHs are a large group of xenobiotic pollutants that consist of benzene rings fused into various arrangements (Figure 2.5). Their degradation pathways, by bacteria, have been studied extensively (Gibson, 1977; Cerniglia, 1981). Two atoms of molecular oxygen are initially incorporated into the substrate to form a dihydriol with *cis* configuration (Park *et al.*, 1990). Further oxidation of *cis* dihydriols leads to the formation of catechols that are substrates for another dioxygenase that brings about enzymatic fission of the aromatic ring (Dagley, 1971; Gibson, 1977).

Co-oxidation has been proposed as a possible mechanism for the loss of recalcitrant PAHs from soils (Alexander, 1965; Perry, 1979; Sims and Overcash, 1983). This could explain why 5 and 6-ring PAHs are slowly biodegraded in soils even though they are apparently not usable as an energy source by bacteria (Sims and Overcash, 1983). In co-oxidation (cometabolic transformation) non-growth hydrocarbons are oxidized when a primary substrate is furnished for growth. The transformation is limited, because the next enzyme of the organism that should attack in sequence, has a higher specificity and does not recognize the product of oxidation as a substrate. In the case of a pure culture, oxidation would be a dead-end transformation without benefit to the organism. In a mixed culture, however, such an initial cometabolic transformation may pave the way for subsequent attack by another organism (Atlas and Bartha, 1980).

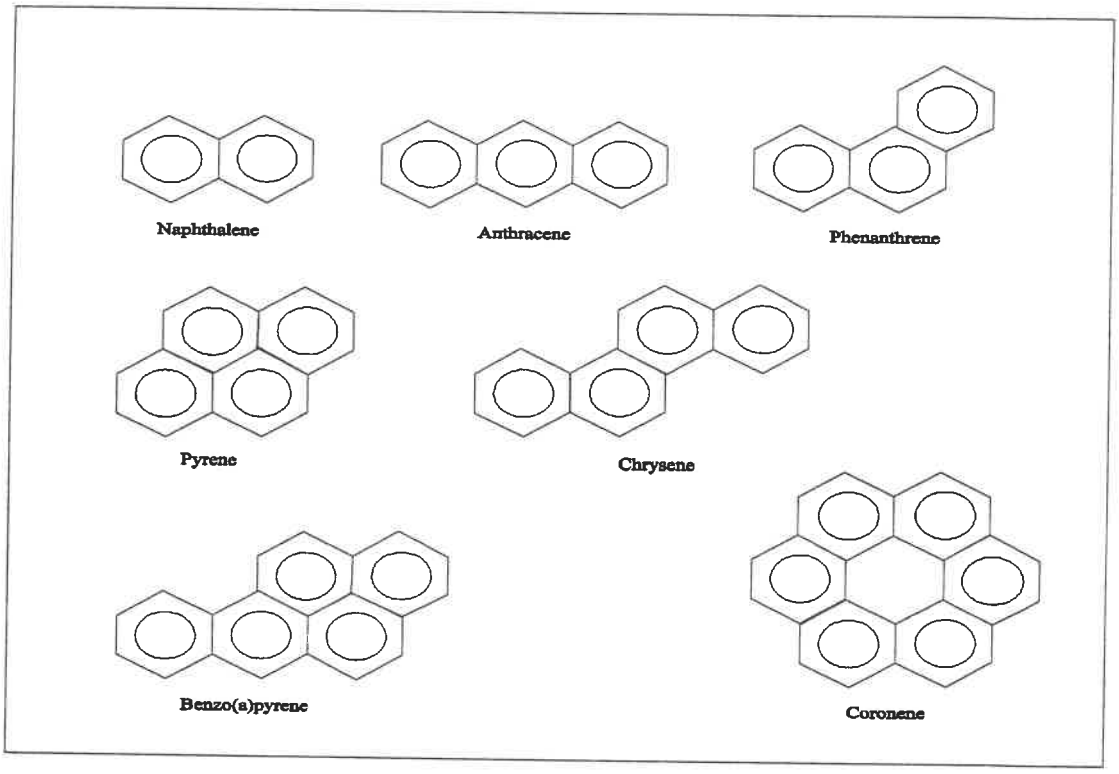


Figure 2.5. Some Polynuclear Aromatic Hydrocarbons (PAHs).

2.4.2 Degradation Pathways of Fungi

The PAH metabolites produced by fungi include phenols, trans-dihydrodiols, quinones and tetralones (Cerniglia and Crow, 1981; Cerniglia and Gibson, 1977; Cerniglia *et al.*, 1978). All of these fungal metabolites are produced by reactions similar to those known in pharmacology as primary metabolism (Davis, 1988). Most of the metabolites produced from PAHs by fungi are less toxic than the parent compound, resulting in detoxification (Cerniglia *et al.*, 1985; McMillan *et al.*, 1988).

The first step in the fungal metabolism of an unsubstituted PAH involves ring epoxidation by a monooxygenase enzyme complex (Figure 2.6) (Ferris *et al.*, 1973;

Ferris *et al.*, 1976). The product of epoxidation is an unstable arene oxide (Cerniglia *et al.*, 1983), which is immediately either hydrated by epoxide hydrolase to trans-dehydrodiols or rearranged non-enzymatically to a phenol (Cerniglia *et al.*, 1989; Cerniglia *et al.*, 1992). The monooxygenase enzyme complex that catalyzes the formation of arene oxides generally contains an inducible, membrane bound enzyme, cytochrome P-450 (Cerniglia *et al.*, 1992; Ferris *et al.*, 1976; King and Wiseman, 1987).

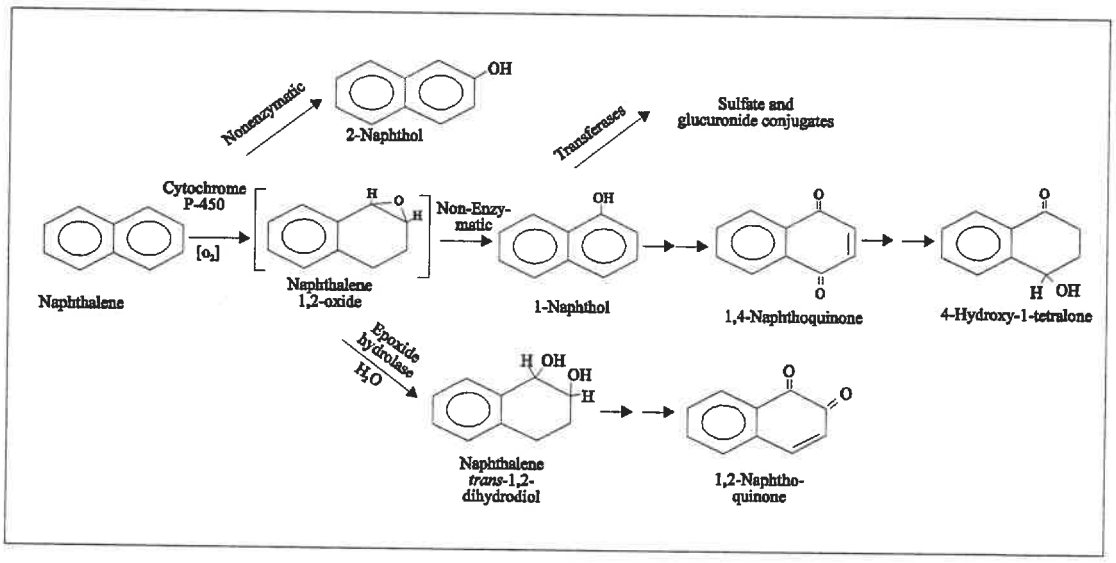


Figure 2.6. Metabolism of naphthalene by fungi (Sutherland, 1992; Cerniglia and Gibson, 1977).

Several species of fungi will produce a quinone from the previous step (Cerniglia *et al.*, 1978). Others will produce tetralones (Cerniglia and Crow, 1981; Cerniglia and Gibson, 1978; Cerniglia *et al.*, 1978). When a monooxygenase catalyzes the further oxidation of a PAH trans-dihydrodiol (previous step), the product of the reaction is a dihydrodiol epoxide (Cerniglia and Gibson, 1980; Gibson, 1982) which can be

metabolized further by epoxide hydrolase to tetrahydrotetraols (Cerniglia and Gibson, 1980).

Secondary metabolism, may lead to the detoxification of phenols and trans-dihydrodiols by conjugation with another molecule (Cerniglia *et al.*, 1982; McMillan *et al.*, 1988). The conjugates produced include sulfates (Cerniglia *et al.*, 1982), glucosides (Cerniglia *et al*, 1989), glucoronides (Cerniglia *et al.*, 1982), and xylosides (Sutherland, 1992).

White rot fungi such as *Phanerochaete chrysosporium* belong to a class of fungi capable of degrading cellulose in wood During growth on carbohydrates such as glucose, they produce nonspecific extracellular enzymes that can oxidize xenobiotics. These enzymes, known as lignin peroxidases and manganese-dependent peroxidases are produced by the fungi in response to low levels of key sources of carbon, nitrogen or sulfur nutrients (Tien 1984). Unlike many other organisms, white rot fungi do not require preconditioning to a particular pollutant. The degrading system of white rot fungi is induced by nutrient deprivation. White rot fungi are not the only organisms that have demonstrated lignin-degrading peroxidases. Lignin peroxidase is present in brown rot fungi as well (Dey *et al*, 1991), and peroxidase activity and genes that control this activity have been found in actinomycetes (Zimmerman, 1990).

One way to understand the nonspecific ability of white rot fungi to degrade pollutants is to consider the complexity of lignin. Lignin is a three-dimensional polymer consisting of non-repeating phenyl propanoid units linked by various carbon-carbon and ether bonds. The stereoirregularity of lignin and the fact that chiral carbons exist in both the L and D configurations render lignin very resistant to biodegradation because most microorganisms do not possess enzyme systems capable of degrading molecules which lack structural and stereoregularity (Bumpus and Aust, 1994). The same unique non-

specific mechanism that give these fungi the ability to degrade lignin also allow them to degrade a wide range of pollutants to CO_2. Some organic compounds which have been degraded by white rot fungi are 3 and 4 ring PAHs, benzene, toluene, ethylbenzene, xylene, pentachlorophenol, polychlorinated biphenyls, phenanthrene and 2,4,6-trinitrotoluene (Davis *et al*, 1993; Yadav and Reddy, 1993; Lamar and Deitrich, 1990; Sassek *et al.*, 1993; Bumpus, 1989; Fernando *et al.*, 1990).

2.5 Composting Processes

Various processes have been used for the high-rate and curing phases of composting (Figure 2.7). Composting processes are divided between reactor and non-reactor systems. Systems that use reactors are termed 'mechanical', 'enclosed', or 'in-vessel' whereas those that do not are termed 'open' systems.

Non-reactor processes are divided into two classes: those that maintain an agitated solids bed (i.e. compost is mixed at some point during the process) and those that employ a static bed (static pile). The most popular example of a non-reactor agitated solids bed system is the windrow process. Mixed feedstocks are placed in rows and turned periodically. While the size of he windrow varies depending on the substrates and equipment used, it generally will not surpass 3 feet in height. Oxygen is either supplied by natural ventilation and gas exchange during turning or by forced or induced aeration from blowers. Static pile processes usually have a distribution system to enable forced or induced aeration. This system is similar to the windrow with the exception that there is no bed agitation.

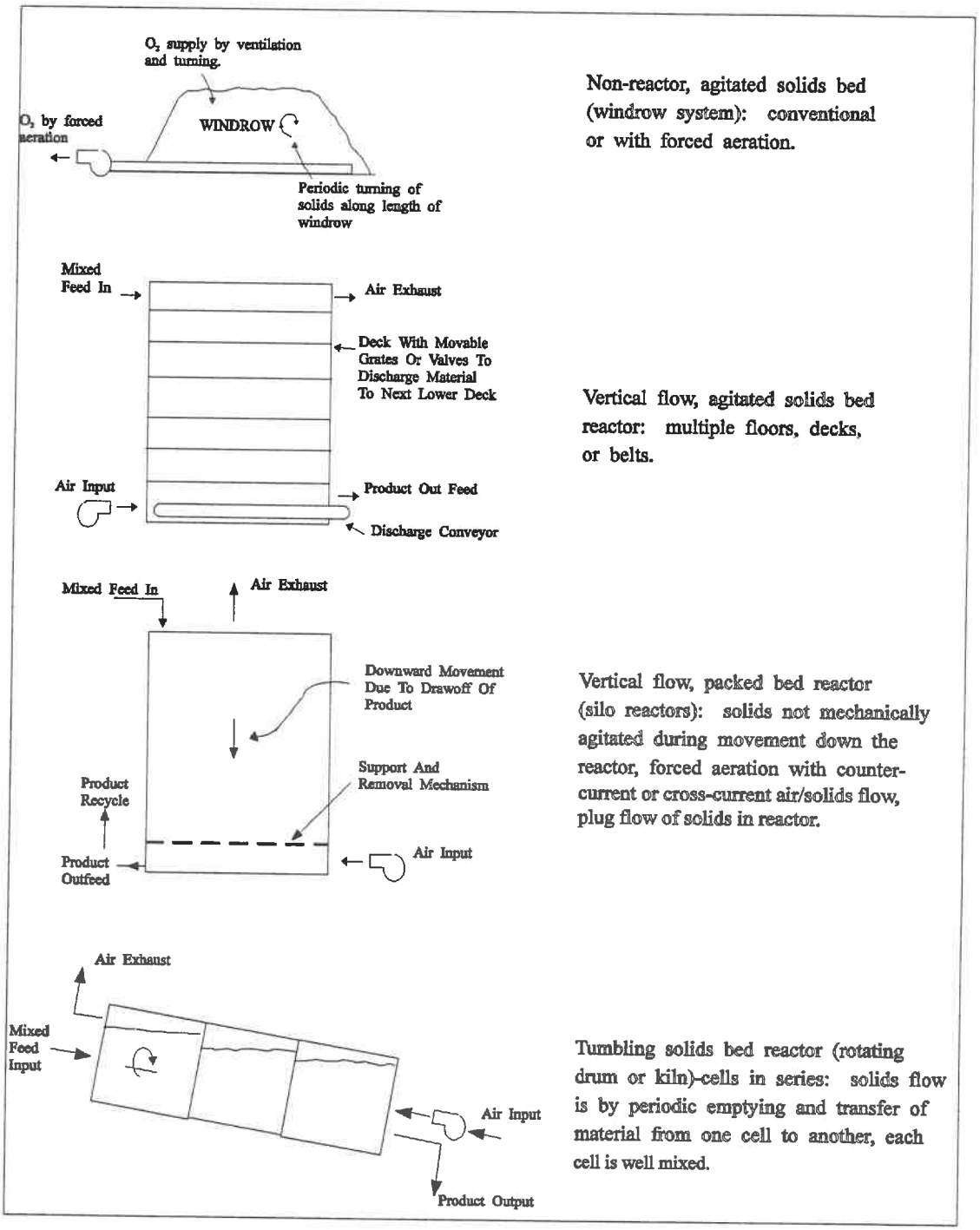


Figure 2.7. Some composting processes (adapted from Haug, 1993).

Reactor processes are classified as either vertical flow reactors or horizontal flow reactors according to the manner of solids flow. Vertical flow reactors are further defined as either agitated bed reactors or packed bed reactors. Horizontal flow reactors are divided into those that employ a rotating or rotary drum (tumbling solids bed reactors), and that use a bin structure of varying geometry and method of agitation (agitated solids bed reactors), and those that use a bin type structure but with a static solids bed (static solids bed reactors).

2.5.1 Laboratory-Scale Reactors

Compost experimentation in the field is costly and difficult to control indicating a need for a laboratory apparatus in which fieldlike behavior can be simulated. Factors of concern in laboratory-scale systems are the generation and transfer of heat and the outer surface to volume ratio. In the first case the process is controlled by the deliberate removal of heat by ventilation. This also ensures well-oxygenated conditions. In the second case, the much larger surface to volume ratio of any laboratory-scale reactor (compared to field scale processes) results in a disproportionately large conductive heat loss making simulation difficult. For faithful simulation, the conductive heat flow must be minimized. Two methods have generally been used to solve this problem. The first is to place the reactor in a temperature-controlled environment and force the compost to follow a set temperature profile by heating the surroundings. The second, which is to allow self-heating to set the temperature profile but to minimize the conductive heat flow

in some manner. The exterior wall temperature is usually adjusted to that of the compost center as insulation is generally ineffective (Hogan et al., 1989).

Over the years, various laboratory-scale systems have been used to simulate composting conditions. For example, a reactor (30 cm i.d. by 40 cm) was constructed using polyvinyl chloride and placed in a polystyrene foam box in order to maintain thermal insulation (Chayansk et al.). The matrix was periodically turned and air was supplied at 23 L/h. Bach et al. (1984) used a 26 cm i.d. by 30 cm continuously mixed isothermal reactor. The reactor was operated isothermally, aerated at 48 L/h and heated by an external heater. A similar reactor was later developed (Bach et al., 1985) in which the compost temperature was maintained by the heat of metabolic reaction and regulated by air flow to the reactor. Strom (1985) used a temperature preset bench scale 4.4 L reaction vessel. The vessel was continually aerated (1.4 - 4.2 L/h), with O_2 concentration in the exhaust maintained between 10-18 % (v/v) to optimize biological activity and subsequent heat generation. Hogan et al. (1989) proposed a prototype laboratory system (21 cm i.d. by 45 cm) using ventilative air control to simulate heat loss processes observed in the field. Heat was ventilatively removed to maintain a microbially favorable temperature and maximize aerobic activity. A conductive flux apparatus was designed to minimize conductive heat flux thereby retaining the compost-generated heat. Magalhães et al., (1993) proposed a small vessel system (400 cm^3) consisting of a glass canning jar with a modified lid. The lid was equipped with an air inlet and outlet and openings for inserting up to 3 thermocouples. Air was injected through a false floor at 0.8 or 1.2 L/h. Walls were insulated with 2.54 cm fiberglass insulation and a conductive heat flux control

system was used to minimize conductive heat loss. With time, laboratory-scale systems have become increasingly sophisticated in order to better simulate windrow and other field-scale composting systems.

CHAPTER 3

CO-COMPOSTING OF WEATHERED HYDROCARBON-CONTAMINATED SOIL

This chapter contains an article entitled **"Co-composting of weathered hydrocarbon-contaminated soil"** submitted to ***Compost Science & Utilization***. The paper demonstrated that co-composting is an effective treatment for soils with recalcitrant contaminants such as weathered hydrocarbons. Data from three co-composting experiments are presented, including the MOG breakdown into its component fractions, aliphatic, aromatic and polar, and the % degradation of each.

CO-COMPOSTING OF WEATHERED HYDROCARBON-CONTAMINATED SOIL

N. Beaudin, R. F. Caron, R. Legros, J. Ramsay[1], L. Lawlor[2] and B. Ramsay[*]

Department of Chemical Engineering, École Polytechnique, C. P. 6079, Succursale Centreville, Montréal, Québec, Canada, H3C 3A7

[1] Current address: Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada, K7L 3N6

[2] Imperial Oil Ltd., Research Department, P.O. Box 3022, Sarnia, Ontario, Canada, N7T 7M1

[*] Corresponding Author (Phone: 514-340-4525, Fax: 514-340-5913. E-mail: Bruce.Ramsay@Mailsrv.Polymtl.ca)

ABSTRACT

Weathered, hydrocarbon-contaminated soil containing 17,000 ppm mineral oil and grease (MOG) of which 40 % (wt/wt) was aliphatic, 32 % (wt/wt) polar and 28 % (wt/wt) aromatic was co-composted with maple leaves and alfalfa in a laboratory-scale reactor. The co-compost pH was acidic initially (6.4) but subsequently rose to 8.5 by day 19. During this period the co-compost temperature had risen from ambient to 53°C (day 2) and subsequently, gradually returned to room temperature. The rates of carbon dioxide generation and oxygen consumption were both low during the first 15 hours after which they rapidly rose to 135 mmol CO_2/(hr-kg initial dry co-compost) and 180 mmol O_2/(hr-kg initial dry co-compost). By day 12 the rates had descended to less than 10 mmol/(hr-kg initial dry co-compost). The respiratory quotient (RQ) (molar rate of CO_2 generation/molar rate of O_2 consumption) was initially about 0.9, but it had decreased to 0.7 by day 2. The maximum rate of MOG degradation (about 600 mg/kg initial dry co-compost-day) occurred at the beginning of the experiment. Soxhlet extraction indicated that 50 % of the MOG of soil origin was degraded in the first 105 days comparing favourably with landfarming studies with the same soil where only 30 % of the MOG of soil origin had been degraded after 180 days. MOG fractionation demonstrated that 60 % of the aliphatics, 54 % of the aromatics and 83 % of the polars were degraded during the first 180 days of co-composting. After 287 days, at least 73 % of MOG of soil origin had been degraded.

INTRODUCTION

Petroleum products are complex mixtures of aliphatic, alicyclic, and aromatic compounds. Some of these components such as polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and mutagenic (Cerniglia, 1981), and have the potential for bioaccumulation in the food chain (Means, 1980). Their negative impact on health and the environment necessitates the remediation of petroleum-contaminated soils.

Studies have shown that many hazardous wastes may be converted to innocuous end-products via co-composting. Field demonstrations at the Louisiana Army Ammunition Plant have shown that the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-trizine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine could be degraded by co-composting (Williams and Myler, 1990). Chlorophenol, though not the polychlorinated phenoxyphenol impurities present in technical chlorophenol, has also been successfully co-composted (Valo *et al.*, 1986). Pilot co-composting studies have demonstrated effective degradation of PAHs (1-octadecene; 2,6,10,15,19,23-hexamethyl-tetracosane, phenanthrene, fluoranthene and, pyrene) and Aroclor 1232 under mesophilic and thermophilic conditions (Hogan *et al.*, 1988).

Bioremediation by co-composting is both environmentally sound and economical. The cost is comparable to the biopile process and only slightly more expensive than landfarming (Englert *et al.*, 1993). Co-composting and biopile processes bear some similarities. In the biopile process, bulking agents and nutrients may be required but it is not standard practice to add exogeneous carbon to increase microbial activity. Consequently biopiles unlike co-composting do not have a thermophilic phase and have less microbial diversity.

In the weathering process, certain petroleum fractions may be lost quickly by volatilization, dissolution/leaching, or biodegradation upon the release of a petroleum product to the environment. The residual 'weathered' product is much more persistant or recalcitrant than the original contaminant and may require a more aggressive bioremediation treatment than landfarming or biopile.

The purpose of this study was to determine if co-composting could be used for the remediation of sandy soils that could not be adequately treated by a landfarming or biopile process due to their weathered crude oil content.

MATERIALS AND METHODS

Soil.

Two soils were used. One was hydrocarbon-contaminated while the other was not. The hydrocarbon-contaminated soil (provided by Imperial Oil, Sarnia, Ontario) consisted of 83.5 % (wt/wt) sand (0.075mm<dp<2mm), 14.7 % stones (dp>2mm) and 1.8 % (wt/wt) particles of several mm in diameter (probably oil-saturated coal particles). It contained 10.5 % (wt/wt) volatile matter (determined by ash tests) of which 7 % (wt/wt) was total organic carbon, and 0.18 % (wt/wt) was total nitrogen (C/N/P=39/1/0, analyzed by Novamann (Québec) Inc., Lachine, Québec, Canada). The soil contained 17,000 ppm MOG (on average) of which 40 % (wt/wt) was aliphatic, 32 % (wt/wt) polar and 28 % (wt/wt) aromatic. The clean soil (Bomix Golden Sand, Daubois Inc., Montreal, Que.) consisted of 97.1 % (wt/wt) sand

(0.075mm<dp<2mm), 0.3 % (wt/wt) stones (dp>2mm) and 2.6 % (wt/wt) fines (dp<0.075mm) and contained only 0.5 % (wt/wt) volatile matter. MOG content was negligeable (less than 100 ppm).

Co-substrate.

The co-substrate consisted of leaves (mostly maple) and alfalfa (5315 Purina Rabbit Chow, Ralston Purina Canada Inc., Woodstock, Ont.). The leaves contained 47 % (wt/wt) total organic carbon, 1.1 % (wt/wt) total nitrogen, and 0.049 % (wt/wt) orthophosphate. The alfalfa contained 38 % (wt/wt) total organic carbon, 2.8 % (wt/wt) total nitrogen, and 0.055 % (wt/wt) orthophosphate.

MOG Analysis.

A 15 g sample of humid co-compost was placed in a mortar with 5 g Na_2SO_4 and 10 g $MgSO_4$. Once all the moisture had been absorbed by the $MgSO_4$, the mixture was pulverized and placed in a paper extraction thimble. The top of the thimble was filled with glass wool. The oil and grease were extracted in a Soxhlet apparatus using 150 ml 1,1,2-trichloro-1,1,2-trifluoroethane (Freon 113, Anachemia Science, Montreal, Quebec), at a rate of 20 cycles/h for 4.5 hours. Fifteen grams of silica gel (Anachemia Science, Grade 12, 28-200 mesh, activated at 105°C for 24 hours) were added to the extraction solvent. The container was stoppered and mixed with a magnetic bar for 10 minutes. The solvent was then filtered

through glass wool, placed in a clean pre-weighed flask and evaporated. The flask was dried at room temperature for 24 hours and weighed to obtain the amount of MOG extracted.

MOG Fractionization.

MOG components were separated on a silica gel column. About 50 g of activated silica gel (same as above) was packed in a 2 cm diameter column and saturated with hexane. The dried MOG sample was dissolved in hexane and placed at the top of the silica column. The fraction of MOG not soluble in hexane was dissolved in toluene in a later step and placed at the top of the silica column. The class fractionations of the MOG were accomplished by successive elution in a discontinuous solvent gradient of increasing polarity. The aliphatic, aromatic and polar classes were eluted with 70 ml of hexane, then toluene, and then chloroform/methanol (1:1 v/v) respectively.

Dry Mass, Ash Content and pH Determination.

A 10 gram sample of humid co-compost was mixed with 100 ml of distilled water. The sample was mixed for five minutes before determining the pH with an electrode.

Approximately 10 grams of the humid co-compost was placed in a preweighed aluminum weigh dish and dried at 105°C for 24 hours. The dish with sample was weighed before and after drying to obtain the percent dry mass. The dry sample was placed in a preweighed porcelain combustion dish, weighed and heated at 550°C for 1.5 hours. The dish was then

placed in a desiccator to cool for 30 minutes and then weighed to obtain the percent ash. The ash content was used to calculate the mass of MOG per mass of initial dry co-compost from the measured MOG concentration.

Carbon Dioxide Generation and Oxygen Consumption.

A quadrupole mass spectrometer (VG Quadrupoles, Middlewich, England) was used for on-line analysis of the gas at the outlet of the composter. The flow rate of the gas entering the spectrometer was maintained between 100 and 150 ml/min, and the settling time for analysis was 60 s. The air at the inlet of the composter was used as a reference to calculate the carbon dioxide generation and oxygen consumption.

Microbial Counts.

A 1 g sample of co-compost was diluted in a sterile saline solution and used as inoculum. Nutrient agar was used for the bacterial counts. Sabouraud agar, with 10 mg chlortetracycline/ml added after sterilisation was used for the fungal counts. Twenty ml of the appropriate molten agar-containing medium (15 minutes at 121°C) was poured over the inoculum (1 ml) in a petri dish and mixed by hand rotating each dish on the table surface. The cooled plates were stacked upside down in the incubator. Total heterotrophic bacteria and fungi were enumerated after 2, 5 and 7 days incubation at 30°C.

RESULTS

Laboratory-scale Composter.

An 8-liter in-vessel system (Figure 3.1) was built to simulate an interior portion of a large compost heap. The laboratory-scale composter was cylindrical in shape (internal diameter 16 cm and height 42 cm) and had a perforated plate at the bottom for more uniform air distribution. The much larger surface to volume ratio of any laboratory-scale composter results in a large conductive heat loss making simulation difficult (Hogan *et al.*, 1989; Magalhães *et al.*, 1993). To avoid this problem, the reactor shell was surrounded by a water-jacket through which heated water was circulated. The reactor temperature was adjusted to that of the centre of the co-compost to limit heat loss. The co-compost temperature was limited to about 50°C by increasing the aeration rate once that temperature had been achieved.

Co-composting.

Several co-composting runs were performed of which the following was typical. Contaminated soil (35.4 % (wt/wt)), alfalfa (35.4 %), leaves (20.2 %), innoculum (5.0 %) (90 day old co-compost originating from a previous run), KH_2PO_4 (1.4 %), and K_2HPO_4 (2.6 %) were loaded into the reactor and co-composted. The initial total organic carbon to total nitrogen to orthophosphate mass ratio (C/N/P) was 20/3/1 (analyzed by Novamann (Québec) Inc., Lachine, Que, Canada). The temperature in the composter remained at ambient for the

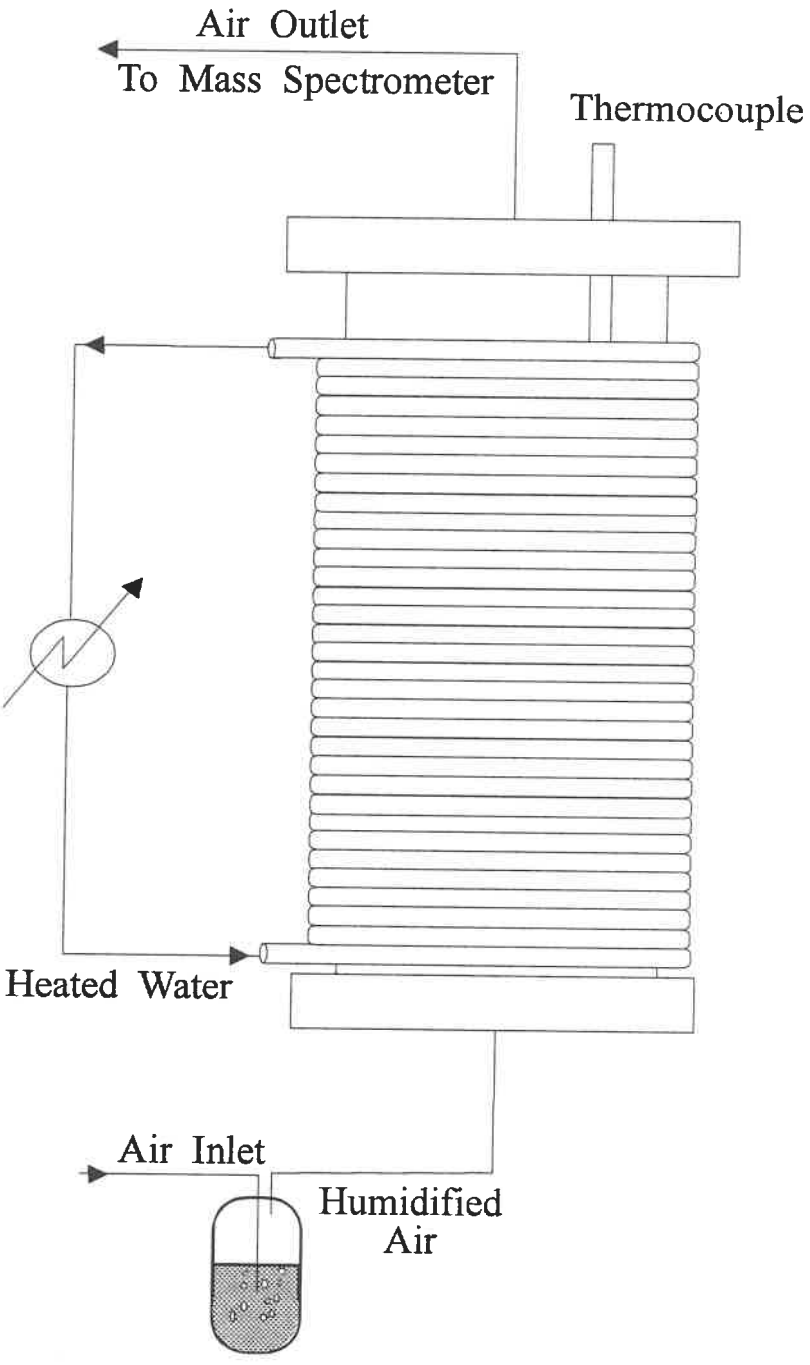


Figure 3.1. Schematic diagram of the 8-L laboratory-scale composter.

first six hours then quickly rose to 40°C (Figure 3.2a) eventually reaching 53°C on the second day. The aeration rate was increased at this time to control the temperature (Figure 3.2a). Over the next three days, the temperature decreased to 35°C and then gradually descended to room temperature.

Carbon dioxide generation (Figure 3.2b) and oxygen consumption rates varied with the rate of heat generation. Both the rates of carbon dioxide generation and oxygen consumption were very low during the first 15 hours after which they rapidly rose to 135 mmol CO_2/(hr-kg initial dry co-compost) and 180 mmol O_2/(hr-kg initial dry co-compost). By day 5, the rates had decreased to 25 mmol CO_2/(hr-kg initial dry co-compost) and 20 mmol O_2/(hr-kg initial dry co-compost), and by day 12, they were less than 10 mmol/(hr-kg initial dry co-compost). The respiratory quotient (RQ = molar CO_2 generation/molar O_2 consumption) was initially about 0.9, but it had decreased to 0.7 by day 2 (Figure 3.2c). The RQ remained between 0.7 and 0.8 for the next 17 days. The pH was initially slightly acidic at 6.4, but by day 19 it had risen to 8.5 (Figure 3.2c) which is potentially an inhibitory value. It subsequently decreased and levelled off at 7.8 (day 30). The humidity of the co-compost was kept between 50 and 60 %, except for days 5 to 9 when the humidity was slightly higher (61-65 %).

The nutrient-rich environment resulted in a rapid increase in the heterotrophic bacteria and fungi populations. By day 5, they had increased from 10^7 to 10^{11} bacteria per g of dry co-compost, and 10^5 to 10^9 fungi per g of dry co-compost.

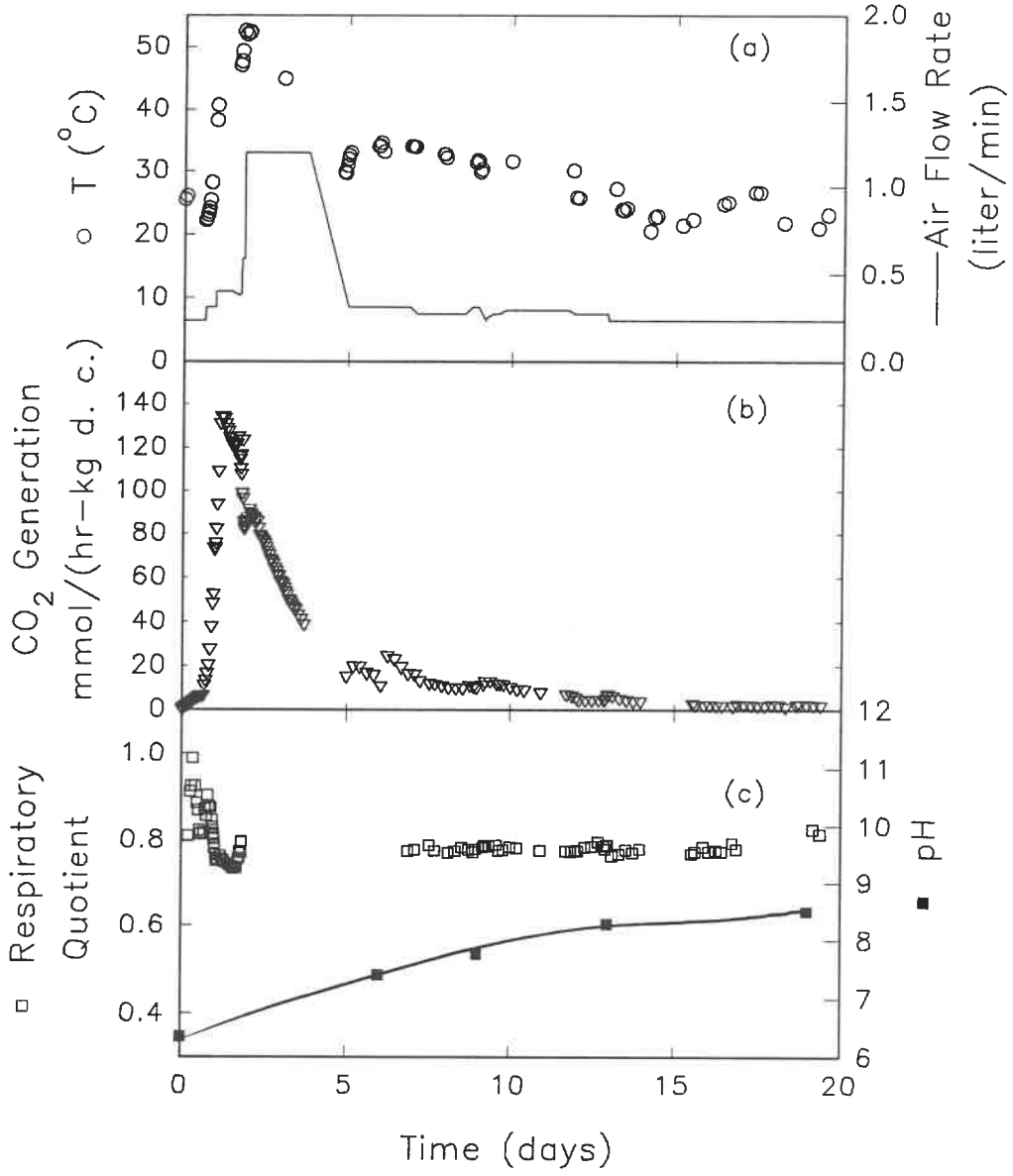


Figure 3.2 Initial 19 days of co-composting (36.4 % contaminated soil, 35.4 % alfalfa, 20.2 % leaves, 5.0 % inoculum, 1.4 % KH_2PO_4, and 2.6 % K_2HPO_4), a) The compost temperature (○) and the air flow rate (—). b) The CO_2 evolution rate (mmol/hr-kg initial dry co-compost). c) The RQ (molar ratio of CO_2 generation to O_2 consumption) (□) and the pH (■).

MOG Degradation.

The MOG concentration of the co-compost decreased from 17,800 to 7,500 mg/(kg dry co-compost) over a 178 day period (Figure 3.3a). All MOG fractions (aliphatic, aromatic, and polar) were degraded although not to equal extents (Figure 3.3b).

A second experiment, comprising two co-composting runs was conducted to determine the quantity of MOG of co-substrate origin remaining after several months of co-composting. The laboratory-scale reactor was loaded with alfalfa (39.3 %), leaves (15.1 %), inoculum (5.8 %) and $CaCO_3$ (4.9 %) (C/N/P=878/48/1, i.e. P limited). Contaminated soil (34.9 %) was added to the mixture in one run and the same amount of uncontaminated soil was added to the mixture in the other run. Phosphate was added 60 days into the runs (0.9 % KH_2PO_4, 1.8 % K_2HPO_4 of initial dry co-compost). After 105 days of co-composting, 89 % of the MOG of co-substrate origin had been degraded. Assuming that the same quantity of MOG of co-substrate origin was degraded in the co-compost with contaminated soil as in the one with clean soil, then 50 % of the MOG of soil origin (9200 ppm to 4600 ppm MOG of soil origin) had been degraded after 105 days.

In a third experiment conducted under the same conditions as the run with the contaminated soil in the second experiment, the MOG content decreased from 13,000 ppm (the initial MOG concentration was different from the first experiment due to the heterogeneity of the soil) to 3,500 ppm over a 287 day period. Overall, MOG degradation was 83 %. If it is assumed that all the MOG remaining was of soil origin, (i.e. MOG of co-

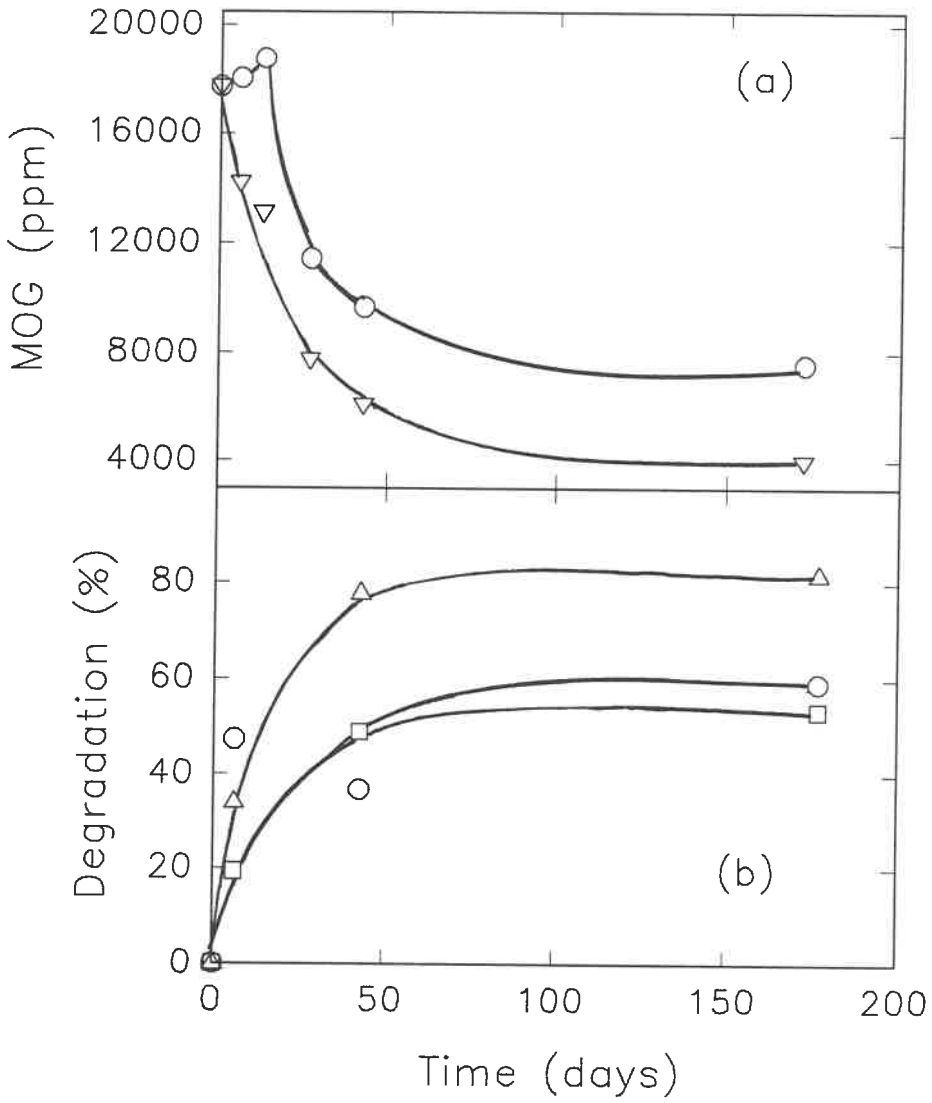


Figure 3.3. Degradation of MOG and MOG fractions over a 170 day period a) O mg MOG/(kg dry compost), ∇ mg MOG/(kg initial dry compost). b) Percent degradation of O aliphatics, □ aromatics and Δ polars

substrate origin was assumed to have been degraded first) then at least 73 % of the MOG originally in the soil fraction must have been degraded.

DISCUSSION

Although more specific methods are now being adopted, MOG content is still often used in government guidelines pertaining to total petroleum hydrocarbons in soils. Substances such as chlorophyl are also extracted by 1,1,2-trichloro-1,1,2-trifluoroethane during MOG determination (APHA, 1985). This could lead to problems in the practical application of co-composting to petroleum contaminated soils as leaves and alfalfa are chlorophyl-rich. However, MOG extraction after 105 days of co-composting with leaves, alfalfa and clean soil demonstrated that most of the MOG of co-substrate origin (89 %) was degraded.

After 178 days of co-composting, considerably more polars had been degraded (83 %) than aliphatics (60 %) or aromatics (54 %) (Figure 3.3b). In typical processes for the bioremediation of soil containing petroleum hydrocarbons, degradation rates increase in the following sequence: aliphatics > aromatics > polars. The observed opposite degradation behaviour in our study may be due to the unique biology of the co-composting environment or to changes in the sorption of the different hydrocarbons present during the co-composting experiments. Co-composting has been shown to enhance the stabilization of pollutants since they bind to the humic acids formed during co-composting (Benoit and Barriuso, 1995) The proportion immobilized (unextractable) will be different for different hydrocarbon fractions. If

a larger proportion of a MOG fraction had been bound (in this case the polar fraction), the apparent degradation would be greater.

The ratio of CO_2 generated to O_2 consumed varies with the substrate degraded as it is dependant on the stoichiometry of the molecule. For example, aerobic glucose degradation has a theoretical RQ of 1.0, lignin of 0.75 and oil and grease of 0.69 based on complete mineralization to H_2O and CO_2. The high RQ ratios obtained during the first day of co-composting suggest that sugars from plant material made up a high proportion of the substrate metabolized. The lower ratio over the rest of the run indicates that the less assimilable substrates such as lignin and MOG were being degraded.

Co-substrate addition may cause utilization of the more easily degradable carbon amendments in place of the targeted contaminants (Swindoll *et al.*, 1988). This does not appear to be a problem in this study. The highest rate of MOG degradation occured at the beginning of treatment (Figure 3.4a) when co-compost activity was at its maximum. However, although the MOG degradation rate decreased with co-composting activity, the ratio of MOG to organic degradation rate increased during the first 20 days meaning that the proportion of MOG degraded to other substrates was increasing (Figure 3.4b).

Simulated biopile studies in a mini-composter resulted in only 6% of the MOG being degraded in 30 days, whereas co-composting under similar conditions resulted in 25 % degradation during the same period (Table E.2). Landfarming studies with the same soil resulted in only 30 % degradation of the MOG after 180 days (Imperial Oil, unpublished data) whereas the present study demonstrated that co-composting resulted in 50

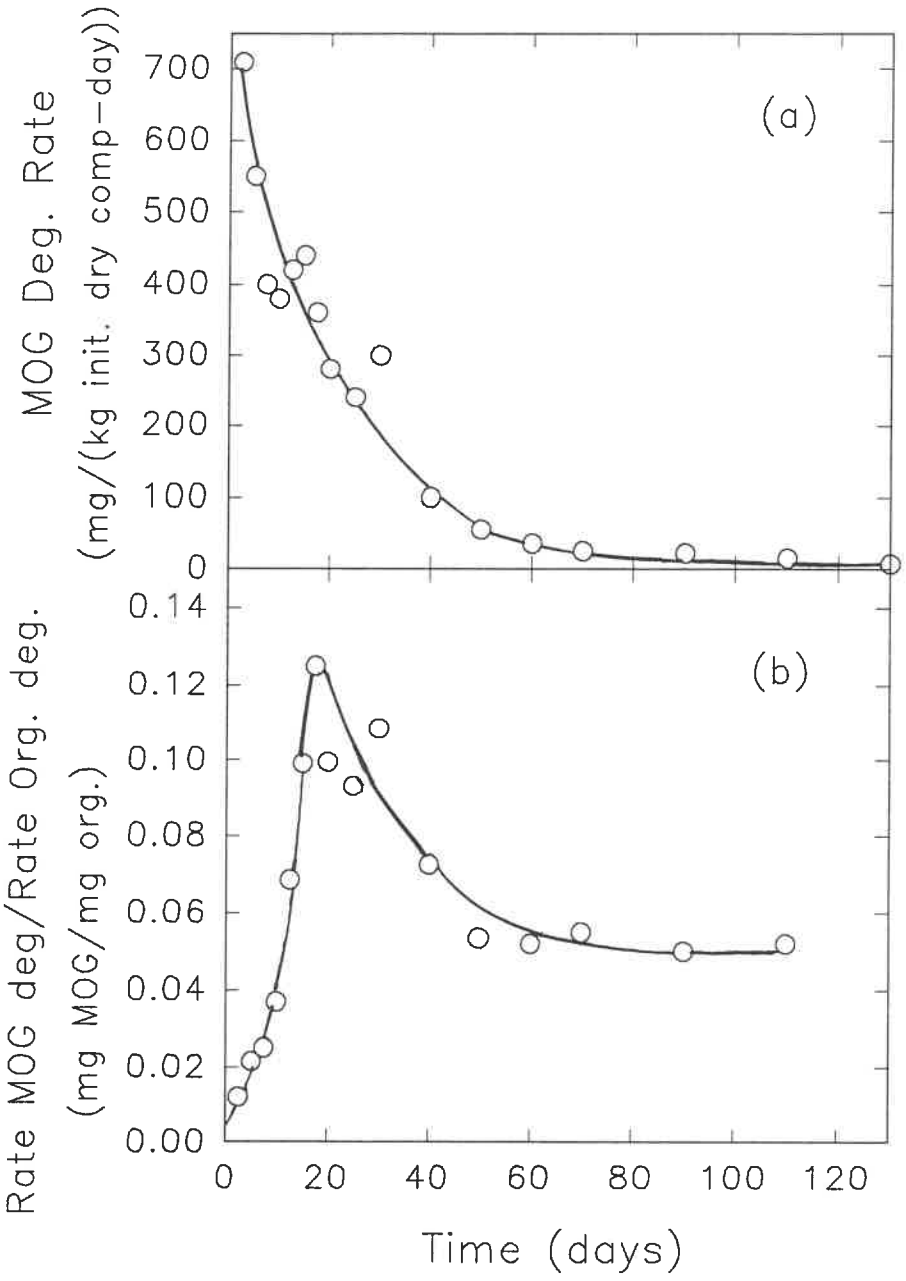


Figure 3.4. MOG degradation rate and ratio of MOG degraded to organics degraded. a) mg MOG degraded/(kg initial dry compost-day). b) The MOG degradation rate to organic degradation rate ratio (mg MOG/mg organics).

% degradation of the MOG of soil origin in 105 days and 73 % degradation of the MOG of soil origin after 287 days.

Biodegradation is dependant on microbial activity, but hydrocarbons are often present in too low a concentration to support an active microbial population. Furthermore, some hydrocarbons, such as 5-ring and 6-ring PAHs cannot be used as an energy source by microorganisms and must be degraded by co-oxidation (Sims and Overcash, 1983). The nutrient-rich co-composting environment results in an increase in the microbial population by several orders of magnitude. The changing conditions in the composting matrix also cause the development of a large variety of microorganisms. Increased microbial diversity favours complete contaminant degradation (Texas Research Institute, 1982). The changing temperatures typical of co-composting may also favour more complete degradation as studies indicate that different hydrocarbon components may be degraded at different temperatures (Atlas, 1975; Westlake *et al.*, 1974; Jobson *et al.*, 1972).

Co-composting appears to be uniquely suited for the bioremediation of recalcitrant contaminants and should be considered when other relatively inexpensive approaches such as biopile prove ineffective.

ACKNOWLEDGEMENTS

This research was supported by Imperial Oil ESTAC, and FCAR-CQVB.

REFERENCES

APHA, AWWA and WPCF 1985. Standard methods for the examination of water and wastewater. Sixteenth Edition. APHA, Washington, DC.

Atlas, R. M. 1975. Effects of temperature and crude oil composition on petroleum biodegradation. *Appl. Microbiol.* 30:396-403.

Benoit, P., and E. Barriuso. 1995. Effect of straw composting on the degradation and stabilization of chlorophenols in soil. *Comp. Sci. Util.* 3(3):31-37.

Cerniglia, C. E. 1981. Aromatic hydrocarbons: metabolism by bacteria, fungi and algae. *Rev. Biochem. Toxic.* 3: 321-361.

Englert, C. J., E. J. Kenzie and J. Dragun. 1993. Bioremediation of petroleum products in soil. *In* Principles and Practices for Petroleum Contaminated Soils. E. J. Calabrese and P. T. Kostecki (Eds.). Lewis Publishers, Chelsea, MI, p. 111-130.

Hogan, J. A., G. R. Toffoli, R.C. Miller, J. V. Hunter and M. S. Finstein. 1988. Composting physical model demonstration: mass balance of hydrocarbons and PCB's. *In* The International Conference on Physicochemical and Biological Detoxification of Hazardous Wastes, Vol. 2. X. C. Wu (Ed.). Technomic Publication, Lancaster, Pa., p. 742-758.

Hogan, J. A., F. C. Miller and M. S. Finstein. 1989. Physical modeling of the composting ecosystem. *Appl. Environ. Microbiol.* 55:1082-1092.

Jobson, A., F. D. Cook and D. W. S. Westlake. 1972. Microbial utilization of crude oil. *Appl. Microbiol.* 23:1082-1089.

Magalhães, A. M. T., P. J. Shea, M. D. Jawson, E. A. Wicklund and D. W. Nelson. 1993. Practical simulation of composting in the laboratory. *Waste Manage. Res.* 11:143-154.

Means, J. C., S. G. Ward, J. J. Hasset and W. L. Banwart. 1980. Sorption of polynuclear aromatic hydrocarbons by sediments and soils. *Environ. Sci. Technol.* 14:1524-1528.

Sims R. C. and M. R. Overcash. 1983. Fate of polynuclear aromatic compounds (PNAS) in soil-plant systems. *Residue Rev.* 88:1-68.

Swindoll, C. M., C. M. Aelion and F. K. Pfaender. 1988. Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation of subsurface microbial communities. *Appl. Environ. Microbiol.* 54:212-217.

Texas Research Institute. 1982. Enhancing the microbial degradation of underground gasoline by increasing the available oxygen. Report to the American Petroleum Institute, Washington, D.C.

Valo, R. and M. Salkinoja-Salonen. 1986. Bioreclamation of chlorophenol-contaminated soil by composting. *Appl. Microbiol. Biotechnol.* 25:68-75.

Westlake, D. W. S., A. Jobson, R. Phillippe and F. D. Cook. 1974. Biodegradability and crude oil composition. *Can. J. Microbiol.* 20: 915-928.

Williams, R. T. and C. A. Myler. 1990. Bioremediation using composting. *Biocycle* 31(11):78-82.

CHAPTER 4

EFFECTS OF CO-COMPOSTING PARAMETERS ON THE REMEDIATION OF HYDROCARBON-CONTAMINATED SOIL

This chapter contains the article entitled **"Effect of co-composting parameters on the remediation of hydrocarbon-contaminated soil"** submitted to the journal ***Applied and Environmental Microbiology***.

The co-composting process is complicated and not enough is known to predict the effect of different parameters on hydrocarbon-degradation. This paper demonstrates that mini-composters are useful in the determination of optimal parameters such as co-substrate addition, bioaugmentation, $CaCO_3$ and phosphate addition, initial C/N ratio and temperature. It also contributes to the general knowledge on the effect of these parameters on the co-composting of hydrocarbon-contaminated soil.

EFFECTS OF CO-COMPOSTING PARAMETERS ON THE REMEDIATION OF HYDROCARBON-CONTAMINATED SOIL

N. Beaudin, R. F. Caron, R. Legros, J. Ramsay[1] and B. Ramsay*

Department of Chemical Engineering, École Polytechnique, C.P. 6079, Succursale Centreville, Montréal, Québec, Canada, H3C 3A7

* Corresponding author (Phone: 514-340-4525, Fax: 514-340-5913. E-mail: Bruce.Ramsay@Mailsrv.Polymtl.ca)

[1] Current address: Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada, K7L 3N6

ABSTRACT

The conditions necessary for optimal co-composting of a hydrocarbon-contaminated soil were evaluated in a series of 30-day tests in mini-composters. Soil containing 17,000 ppm mineral oil and grease (MOG) of which 40 % (wt/wt) was aliphatic, 32 % (wt/wt) polar and 28 % (wt/wt) aromatic was co-composted with maple leaves and alfalfa. Although the co-substrate (leaves and alfalfa) also contained MOG, degradation of MOG of soil origin (total MOG degradation minus MOG degradation in a control with no soil) increased from 6 to 45 % as the quantity of co-substrate added increased from 0 to 73 %. Addition of $CaCO_3$ to batches containing 59 % co-substrate followed by addition of phosphate two weeks later, increased MOG degradation from 23 % to 48 %. Bioaugmentation had no positive effect. MOG degradation increased with decreasing C/N ratio. At a C/N ratio of 17, 48 % of the MOG was degraded in 30 days, while at a C/N ratio of 40 there was no MOG degradation. When temperatures ranging from 23 to 60°C were investigated, 50°C maintained for 29 days resulted in the most MOG degradation (68 %). The MOG reduction during co-composting was not due to volatilization. Of the parameters investigated, $CaCO_3$ and PO_4^{-3} addition, the C/N ratio, and the temperature profile were the most critical factors in obtaining maximum MOG degradation during co-composting.

INTRODUCTION

Many hazardous wastes may be converted to innocuous end-products via co-composting. These include explosives such as 2,4,6-trinitrotoluene, as well as chlorophenol, and polynuclear aromatic hydrocarbons (PAHs) (Williams and Myler, 1990; Valo *et al.*, 1986; Hogan *et al.*, 1988). During co-composting, the contaminated material (eg. soil) is initially mixed with suitable organic materials (co-substrate). The mixture is placed in a pile or enclosed vessel where microorganisms consume the supplied organics as well as the contaminating materials, possibly by co-oxidation. Microbial activity is responsible for the high temperatures (50 to 60°C) observed during composting runs.

The amount of contaminant degraded depends on the co-composting conditions especially the composition of the co-substrate. Although few studies have been published regarding the effects of co-composting conditions, optimal composting conditions have been the subject of many studies. Moisture contents between 50 and 60 % give the best composting results (Suler *et al.*, 1977) although values ranging from 25 to 80 % have been reported. Composters operating at 45°C have been shown to be more active (in terms of CO_2 production) than at 55 or 65°C and became self-inhibiting around 60°C (McGregor *et al.*, 1981; McKinley *et al.*, 1984). Maximum organic degradation rates (based on cumulative and peak CO_2 generation) occur at C/N ratios of 22 (Nakasaki *et al.*, 1992; Michel *et al.*, 1993). N/P ratios of 2 to 5 are considered optimal in municipal

composting. However, it is not known how these parameters affect hydrocarbon degradation in co-composting.

As with composting, experimentation with co-composting conditions is lengthy and affected by climatic conditions if conducted outdoors. Thus there is a need for laboratory apparatus in which fieldlike behavior can be simulated. Such a system must be able to run a number of duplicate experiments simultaneously to determine the effect of operating conditions on hydrocarbon degradation in a reasonable length of time. Small laboratory composters (mini-composters) have been used to achieve these objectives (Magalhães *et al.*, 1993; Kaplan and Kaplan, 1982; McFarland *et al.*, 1992). The objective of the work presented in this paper was to develop such a system and apply it in the determination of the effects of C/N ratio, co-composting temperature, length of the thermophilic phase as well as the addition of micro-elements and inoculum on degradation of mineral oil and grease (MOG) in a hydrocarbon contaminated soil during co-composting.

MATERIALS AND METHODS

Soil.

Two soils were used. One was contaminated with hydrocarbons. The hydrocarbon contaminated soil (provided by Imperial Oil, Sarnia, Ontario) consisted of 83.5 % (wt/wt) sand (0.075 mm<dp<2mm), 14.7 % (wt/wt) stones (dp>2mm), and 1.8 %

(wt/wt) particles of several mm (probably oil-saturated coal particles). It contained 10.5 % (wt/wt) volatile matter of which 7 % (wt/wt) was total organic carbon and 0.18 % (wt/wt) was total nitrogen (C/N/P=39/1/0, analyzed by Novamann (Québec) Inc., Lachine, Quebec, Canada). The soil contained 17,000 ppm MOG of which 40 % (wt/wt) were aliphatic, 32 % (wt/wt) polar and 28 % (wt/wt) aromatic. The clean soil (Bomix Golden Sand, Daubois Inc., Montreal, Que.) consisted of 97.1 % (wt/wt) sand ($0.075<dp<2mm$), 0.3 % (wt/wt) stones ($dp>2mm$) and 2.6 % (wt/wt) fines ($dp<0.075mm$) and contained only 0.5 % (wt/wt) volatile matter. MOG content was undetectable (less than 100 ppm).

Co-Substrate.

The co-substrate consisted of leaves (mostly maple) and alfalfa (5315 Purina Rabbit Chow, Ralston Purina Canada Inc., Woodstock, Ont.). The leaves contained 47 % (wt/wt) total organic carbon, 1.1 % (wt/wt) total nitrogen, and 0.049 % (wt/wt) orthophosphate. The alfalfa contained 38 % (wt/wt) total organic carbon, 2.8 % (wt/wt) total nitrogen, and 0.055 % (wt/wt) orthophosphate. The leaf/alfalfa ratio was changed to obtain the desired C/N ratio. The mineral salts medium (MSM) used in the co-substrate experiment was composed of (per liter) 2.45 g KH_2PO_4 , 6.23 g K_2HPO_4 , 18 g $(NH_4)_2SO_4$, 0.36 g $MgSO_4$, 0.108 g $CaCl_2$, 0.108 g $FeSO_4$ and 3.6 ml TES (trace element solution). The TES contained (per liter) 0.1 g $ZnSO_4 \cdot 7H_2O$, 0.03 g $MnCl_2 \cdot 4H_2O$,

0.30 g H_3BO_3 , 0.20 g $CoCl_2 \cdot 6H_2O$, 0.01 g $CuSO_4 \cdot 5H_2O$, 0.02 g $NiCl_2 \cdot 6H_2O$ and 0.03 g Na_2MoO_4.

MOG Analysis.

A 15 g sample of humid co-compost was placed in a mortar with 5 g Na_2SO_4 and 10 g $MgSO_4$. Once all the moisture had been absorbed by the $MgSO_4$, the mixture was pulverized and placed in a paper extraction thimble. The top of the thimble was filled with glass wool. The oil and grease were extracted in a Soxhlet apparatus using 1,1,2-trichloro-1,1,2-trifluorethane (Freon 113, Anachemia Science, Montreal, Que), at a rate of 20 cycles h^{-1} for 4.5 hours. Fifteen grams of silica gel (Anachemia Science, Grade 12, 28-200 mesh, activated at 105^0C for 24 hours) were added to the extraction solvent. The container was stoppered and mixed with a magnetic bar for 10 minutes. The solvent was then filtered through glass wool placed in a clean pre-weighed flask and evaporated. The flask was dried at room temperature for 24 hours and weighed to obtain the amount of MOG extracted.

MOG Fractionation.

MOG components were separated on a silica gel column. About 50 g of activated silica gel (same as above) was packed in a 2 cm diameter column and covered in hexane. The dried MOG sample was dissolved in hexane and placed at the top of the silica

column. The fraction of MOG not soluble in hexane was dissolved in toluene in a later step and placed at the top of the silica column. The class fractionations of the MOG were accomplished by successive elution in a discontinuous solvent gradient of increasing polarity. The aliphatic, aromatic and polar classes were eluted with 70 ml of hexane, then toluene, and then chloroform/methanol (1:1; v/v), respectively.

Dry Weight, Ash Content and pH Determination.

A 10 g sample of humid co-compost was mixed with 100 ml of distilled water. The sample was mixed for five minutes before determining the pH with an electrode.

Approximately 10 g of the humid co-compost was placed in a preweighed aluminum weigh dish and dried at 105°C for 24 hours. The dish with sample was weighed before and after drying to obtain the % dry mass. The dry sample was placed in a preweighed porcelain combustion dish, weighed and combusted at 550°C for 1.5 hours. The dish was placed in a desiccator to cool for 30 minutes and then weighed to obtain the % ash. The ash content was used to calculate the mass of MOG per mass of initial dry compost from the measured MOG concentration.

Carbon Dioxide Generation.

A quadrupole mass spectrometer (VG Quadrupoles, Middlewich, England) was used for on-line analysis of the gas at the inlet and outlet of the composter. The flow rate

of the gas entering the mass spectrometer was maintained between 100 and 150 ml min^{-1}, and the settling time for analysis was 60 s. The air flow rate and the difference in CO_2 content between the inlet and outlet were used to calculate the total CO_2 generation.

Inocula.

Three inocula were used in this study: microorganisms native to the contaminated soil, microorganisms from a 90 day old co-compost, and a bacterial consortium known to degrade anthracene (supplied by Prof. M. A. Pickard, University of Alberta, Edmonton, Alberta). In the first case, gravel and the contaminated soil were placed in a column. Mineral salts medium (MSM) was refluxed through the column with a peristaltic pump (0.2 liter min^{-1}) for one week at room temperature. Soil from the column (containing microorganisms) was used as an inoculum. A sample of co-compost that had begun 90 days previously, was also used. Microorganisms known to degrade anthracene were cultured in 200 ml MSM and crude oil (K_2HPO_4 0.5 g $liter^{-1}$, Na_2SO_4 2.0 g $liter^{-1}$, $MgSO_4$ 0.2 g $liter^{-1}$, KCl 1.45 g $liter^{-1}$, NH_4NO_4 1.55 g $liter^{-1}$, $FeSO_4$ (2 grains), whole crude oil (11.5 % polar, 48.1 % aliphatic, 40.4 % aromatic) 1.0 g $liter^{-1}$) in a 500 ml Erlenmeyer flask at 25°C on a rotary shaker at 120 rpm for 2 weeks and used as the third inoculum.

RESULTS

Mini-composter design.

Eighteen mini-composters were used to evaluate the effect of co-composting parameters on the remediation of hydrocarbon-contaminated soil. The mini-composters were 1-liter Mason jars, 18 cm in height with an internal diameter of 8 cm. Two openings were made in the cover into which 1/4 inch diameter stainless steel tubes were inserted (Figure 4.1) to serve as an air inlet and outlet. A perforated plate was placed 2 cm from the bottom to permit uniform air distribution. Humidified air was injected under the plate at a rate of 0.2 liter min^{-1} .

Co-composting conditions.

A co-compost experiment had previously been conducted in an 8-liter, laboratory-scale reactor designed to simulate a portion of a windrow (Figure C.1, run 1). That data was used to select an imposed temperature profile (Figure 4.2) typical of uncontrolled windrow conditions (Fogarty and Tuovinen, 1991).

Soil was mixed with leaves and alfalfa, and loaded into the composting units. The mini-composters were placed in a temperature controlled oven and were continuously aerated and heated to the temperature profile mentioned above. All experiments lasted

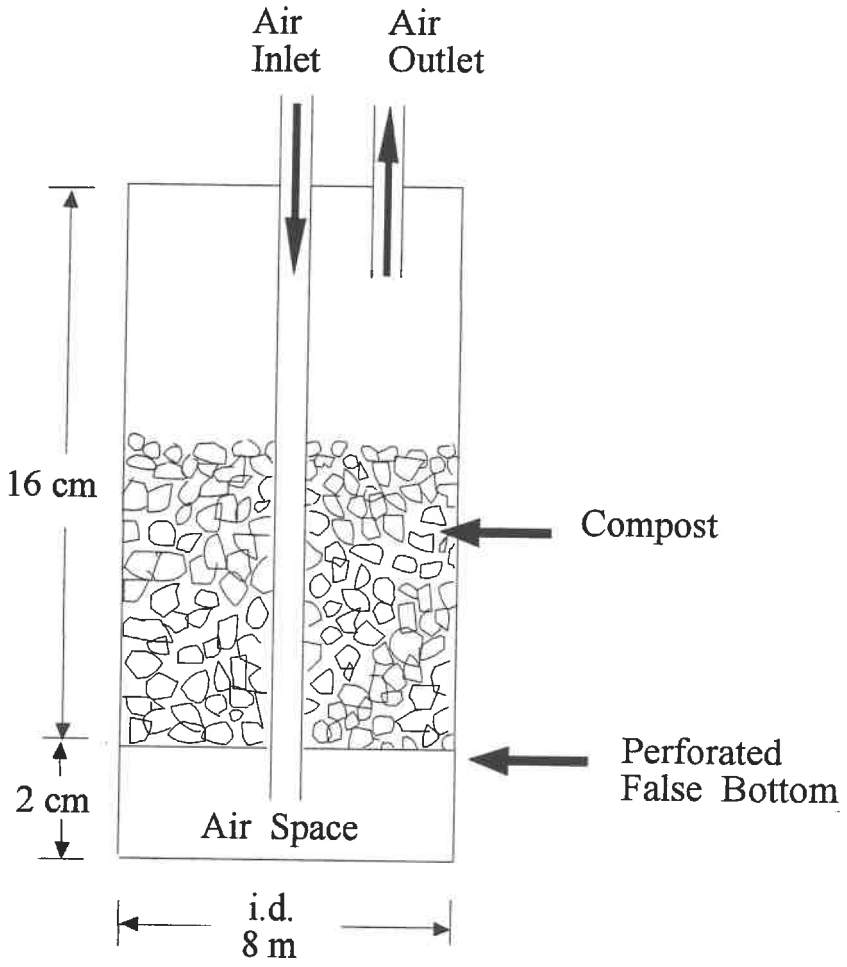


Figure 4.1. Mini-composter design.

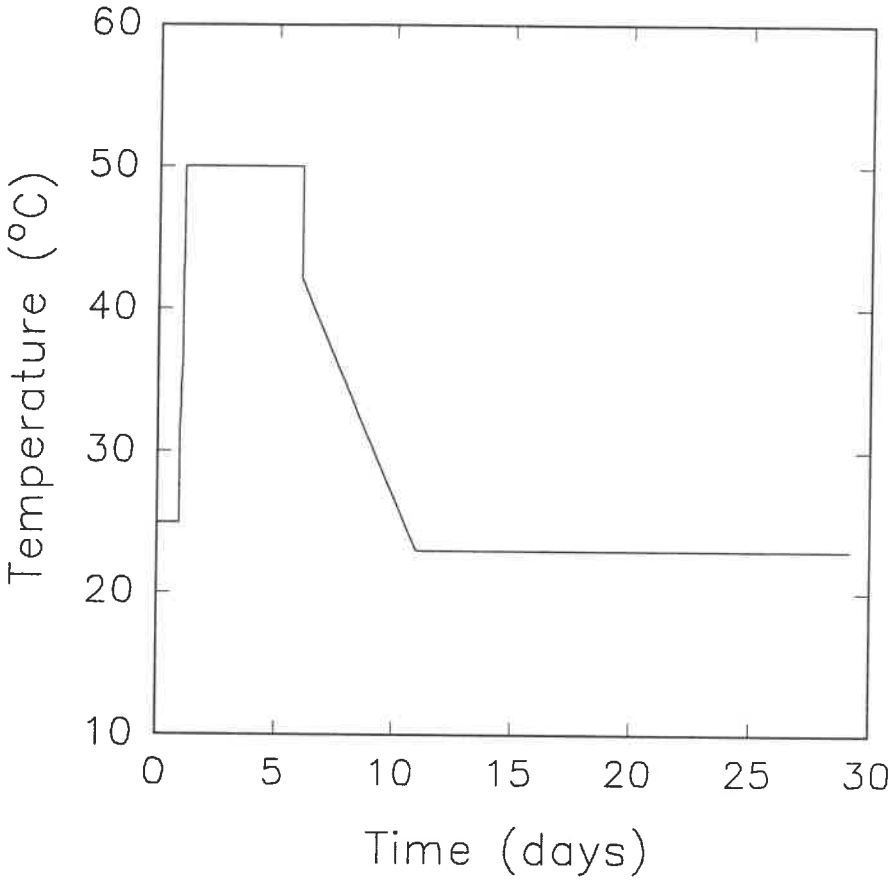


Figure 4.2. Imposed temperature profile for all experiments except those investigating the effect of temperature on MOG degradation.

30 days and followed this same imposed temperature profile except for those which investigated the effect of temperature.

The mini-composter contents were mixed twice a week and water was added to maintain the initial humidity. All of the mini-composters had an initial humidity between 50 and 60 % except those where the concentration of co-substrate was varied since the sandy soil could not absorb as much water as the co-substrate. Although there was some variation in initial pH between experiments, there was no apparent difference within an individual experiment. The only exception was the experiment investigating the effect of $CaCO_3$ and phosphate on MOG degradation.

MOG Volatilization

The percentage of MOG lost through aeration was determined using a mini-composter loaded with 29.6 % (wt/wt) soil, 39.6 % (wt/wt) alfalfa, 17.0 % (wt/wt) leaves, humidified and poisoned with mercury (3.89 % (wt/wt) Hg_2Cl) to eliminate biological activity. The MOG content of the co-compost poisoned with mercury did not change during 30 days of aeration.

Co-Substrate Addition.

An experiment was conducted to determine whether co-substrate addition stimulated hydrocarbon degradation. Five mini-composters were loaded with soil,

inoculum (90-day old co-compost) and co-substrate with the co-substrate concentration ranging from 0 to 84 % (wt(leaves and alfalfa)/wt total) (Table 1). Two controls were used. One of the mini-composters was enriched with a nutrient solution (MSM) but had no co-substrate, while another had no added nutrients or co-substrates. Only 1 % of the MOG in the well-aerated, humid soil (containing no co-substrate) had degraded after 30 days of treatment. Adding a MSM solution to the soil at the beginning of the experiment increased MOG degradation to 6 %. The percent total MOG degradation increased almost linearly from 6 % to 63 % as the quantity of co-substrate increased from 0 % (with added nutrients) to 100 % (wt(leaves and alfalfa)/wt(leaves and alfalfa and soil)) (Figure 4.3). The percent MOG of soil origin degraded increased from 6 to 43 % as co-substrate addition increased from 0 % (with added nutrients) to 73 %.

Bioaugmentation.

To determine whether the addition of inocula (bioaugmentation) would increase MOG degradation, five mini-composters were loaded with 39.4 % (wt/wt) soil, 39.3 % (wt/wt) alfalfa, 16.9 % (wt/wt) leaves, 1.5 % (wt/wt) KH_2PO_4 , and 2.9 % (wt/wt) K_2HPO_4. One received no inoculum while the other four were inoculated with either (1) microorganisms native to the contaminated soil (5 % (wt/wt)), (2) 90-day old co-compost (5 % (wt/wt)), (3) microorganisms known to degrade anthracene (5 % (wt/wt)) or (4) a mixture of all three inocula (15 % (wt/wt), 1:1:1). The % MOG degraded in the reactor with no inoculum addition (control) and the one augmented with microorganisms

Table 4.1. Composition and water content of co-substrates used to determine the effect of co-substrate addition (results shown in Fig. 4.3)

Soil (%)**	Alfalfa (%)**	Leaves (%)**	KH_2PO_4 (%)**	K_2HPO_4 (%)**	$CaCO_3$ (%)**	Inoculum (%)**	MSM* (ml)	Humidity (%)**
0	53.2	30.4	2.0	3.9	5.6	4.8	0.0	52
23.8	39.9	22.8	1.5	2.9	4.2	4.8	0.0	51
47.6	26.7	15.2	1.0	2.0	2.8	4.8	0.0	43
75.0	14.0	8.0	1.5	1.0	1.5	5.0	0.0	33
95.3	0.0	0.0	0.0	0.0	0.0	4.7	4.3	21
95.3	0.0	0.0	0.0	0.0	1.0	4.7	0.0	21

* Mineral salts medium.

** wt/wt

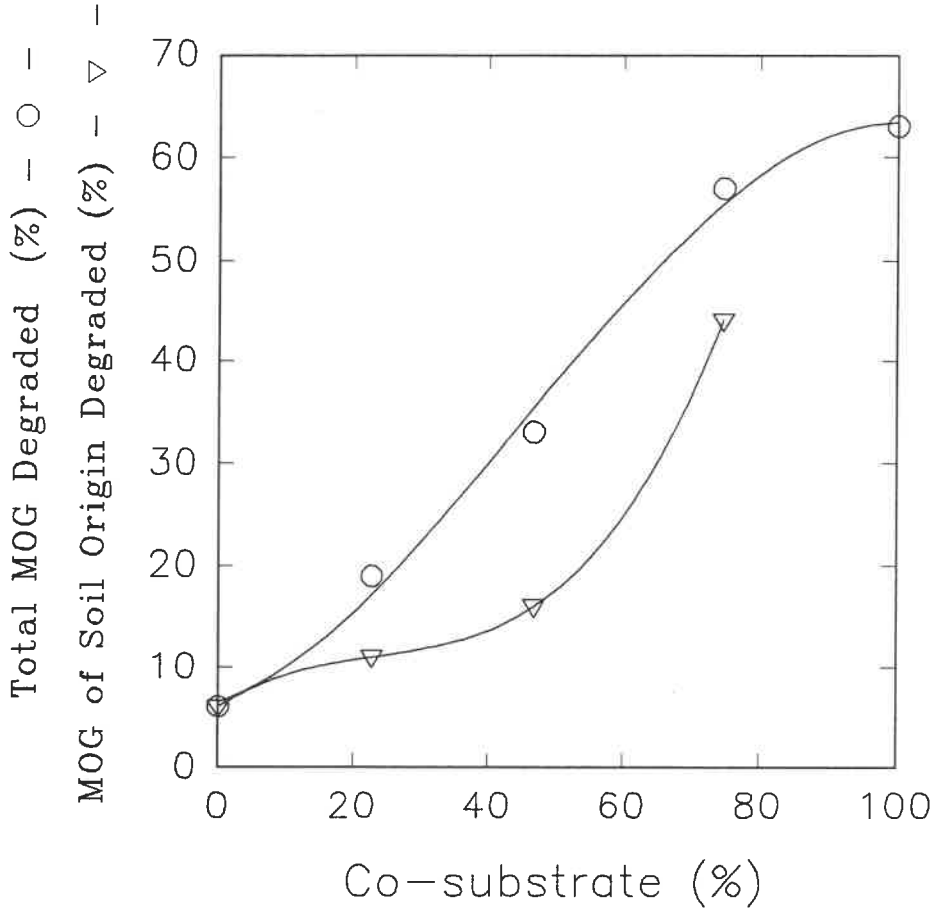


Figure 4.3. Mineral oil and grease (MOG) degradation as a function of co-substrate loading.

native to the soil was 37 % in both cases. Addition of co-compost or the PAH degrading consortium resulted in less MOG degradation than the control (28 % and 32 % respectively). Adding all three inocula simultaneously, resulted in the degradation of only 20 % of the MOG.

$CaCO_3$ and PO_4^{-3} Addition.

The influence of phosphate (PO_4^{-3}) and calcium carbonate ($CaCO_3$) on MOG degradation during co-composting was investigated. Phosphate addition changed the initial N/P ratio from 45 to 3. Nine mini-composters were loaded with 38.9 % (wt/wt) soil, 38.9 % (wt/wt) alfalfa, 17.7 % (wt/wt) leaves, and 5.5 % (wt/wt) inoculum (90-day old co-compost). Twenty-three percent of the MOG was degraded when no PO_4^{-3} or $CaCO_3$ were added to the co-compost (Figure 4.4). Adding $CaCO_3$ (4.2 % (wt/wt) at the beginning and PO_4^{-3} (1.5 % (wt/wt) $KH_2 PO_4$, 2.9 % (wt/wt) $K_2 HPO_4$) either at the beginning or at 2 weeks increased the degradation to 40 %, 35 %, and 43 % respectively. Adding $CaCO_3$ and PO_4^{-3} together was ineffective if both were added at the beginning (25 % MOG degradation), but improved results when $CaCO_3$ was added at the beginning and PO_4^{-3} two weeks later (49 % MOG degradation).

C/N Ratio.

The influence of initial co-compost C/N ratio (from 15 to 40 (wt/wt)) on the

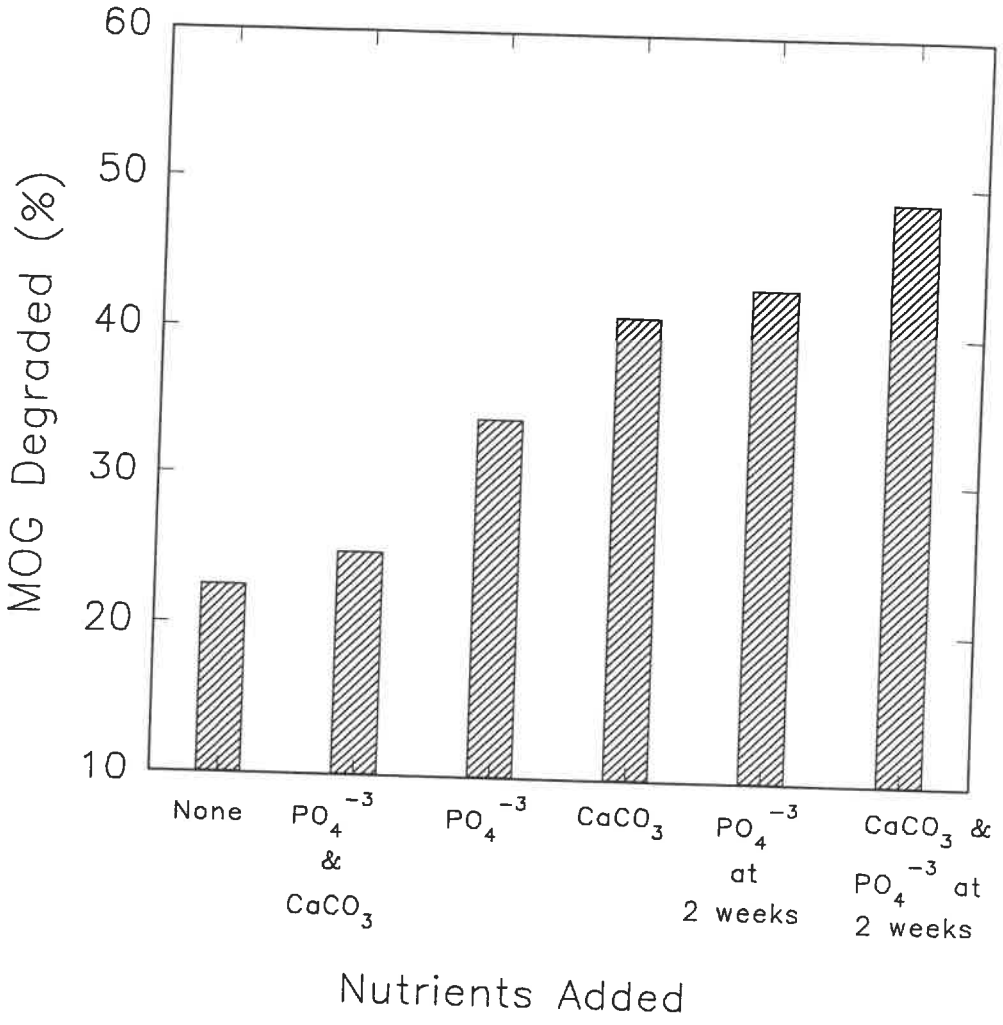


Figure 4.4. Percent mineral oil and grease (MOG) degraded as a function of the micro-elements added. All the elements were added at the beginning except for PO_4^{-3} where otherwise specified.

MOG degradation was investigated. For each of the 6 C/N ratios investigated, there was a corresponding mini-composter with uncontaminated soil in order to assess the proportion of the MOG degraded originating from the co-substrate. The co-compost composition was 37.5 % (wt/wt) clean or contaminated soil, 1.3 % (wt/wt) KH_2PO_4 , 2.5 % (wt/wt) K_2HPO_4 , 4.9 % (wt/wt) inoculum (90-day old co-compost), and 53.4 % (wt/wt) leaves and alfalfa. The ratio of alfalfa to leaves was used to vary the C/N ratio. At a C/N ratio of 15, there was 1 part alfalfa and no leaves (1/0), at 17 it was 3.9/1, at 20 it was 2.35/1, at 25 it was 0.61/1, at 30 it was 0.29/1, and at 40 it was 0/1. MOG degradation increased with decreasing C/N ratio (Figure 4.5). With hydrocarbon-contaminated soil, total MOG degradation increased from 0 % at a C/N ratio of 40 to 43 % at a C/N ratio of 15. With clean soil, the MOG (i.e. entirely co-substrate derived) degradation increased from 0 % at a C/N ratio of 40, to 50 % at a C/N ratio of 22. Degradation of MOG of soil origin increased as the C/N ratio was decreased, attaining 42 % degradation at a ratio of 15. The MOG of soil origin was calculated by assuming that the same amount of MOG of co-substrate origin was degraded in the mini-composter containing contaminated soil as in the mini-composter containing uncontaminated soil with the same C/N ratio.

Temperature.

An experiment was conducted to determine which temperatures favor MOG degradation during co-composting. The imposed temperature profile consisted of one

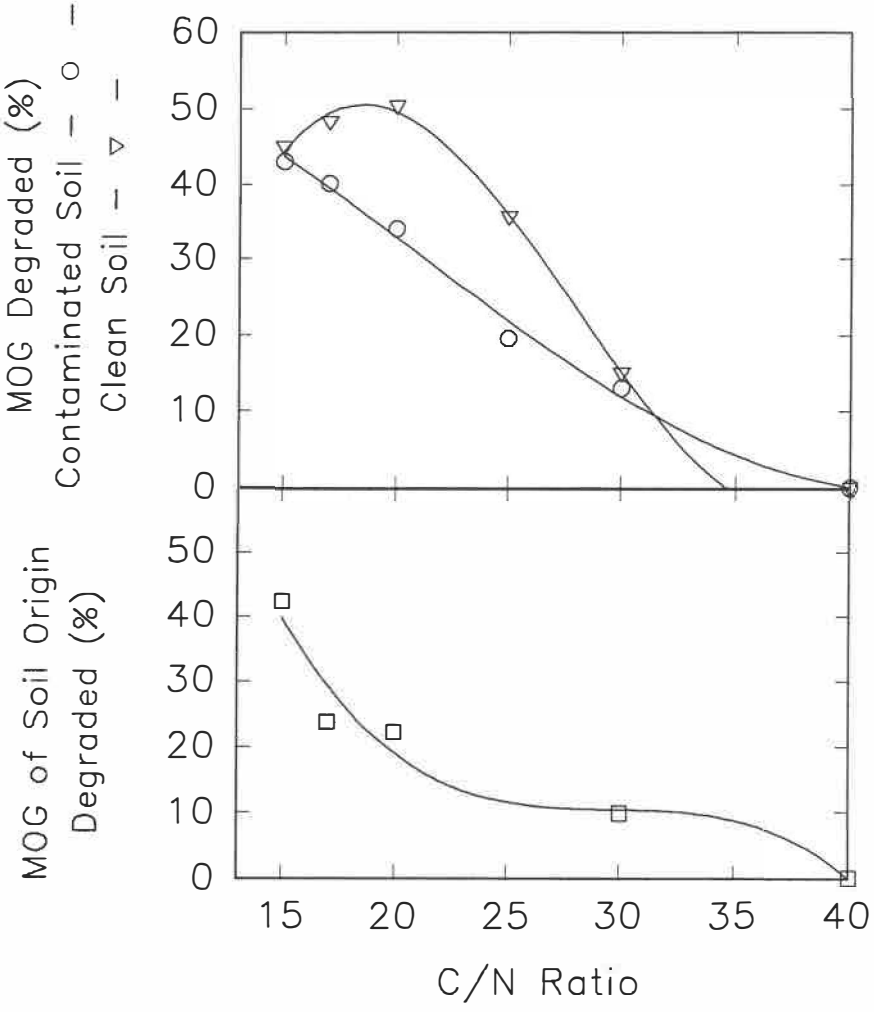


Figure 4.5. Mineral oil and grease (MOG) degradation as a function of the C/N ratio.

day at room temperature, five days at the temperature investigated, and the remaining 24 days at room temperature. Five temperatures were investigated in duplicate. They were room temperature (about 23° C), 30, 40, 50, and 60° C. Each mini-composter contained 37.3 % (wt/wt) soil, 37.3 % (wt/wt) alfalfa, 16.0 % (wt/wt) leaves, 4.0 % (wt/wt) $CaCO_3$, and 5.3 % (wt/wt) inoculum (90-day old co-compost). Although a bimodal response was obtained, the most MOG (56 %) was degraded at room temperature (Figure 4.6). MOG degradation decreased with increasing temperature from 23°C (56 %) to 40°C (33 %), then began increasing with temperature attaining 47 % degradation at 50°C. Much less MOG was degraded when the profile included a stage at 60°C (23 %). Analysis of MOG content in 120-day old co-compost showed that MOG degradation continued after the first 30 days of co-composting. MOG degradation had increased to 67 % in both the co-compost which had a plateau at 50°C, and the one at room temperature. Class separation of the MOG into its three components (aliphatic, aromatic and polar) found that the percent degradation of each of the three fractions followed the same trend as that seen with total MOG. Approximately the same amount of CO_2 (6800 mmol (kg initial dry co-compost)$^{-1}$) was produced during 30 days of co-composting for the mini-composters with a stage at 23, 30, 40 and 50° C, but a sharp decline was noted for the mini-composter with a stage at 60° C (3200 mmol (kg initial dry co-compost)$^{-1}$).

Length of the Thermophilic Phase.

This experiment was used to determine the effect of increasing the length of the

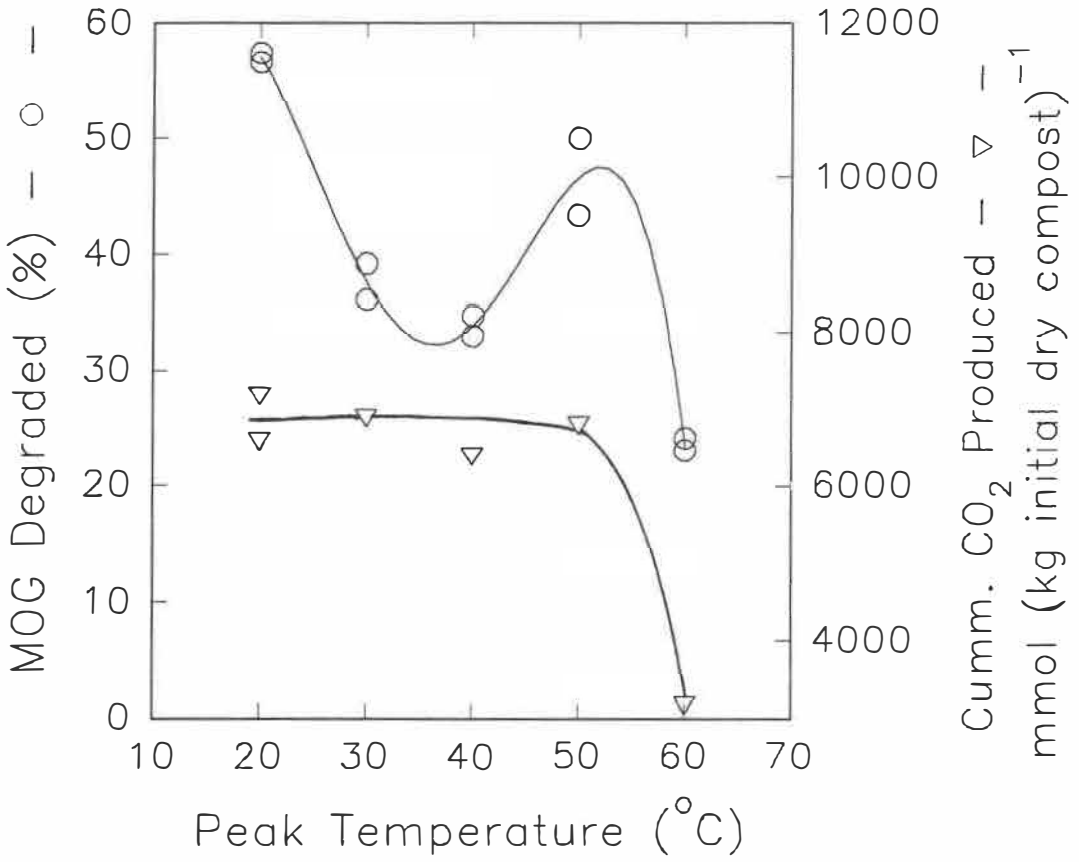


Figure 4.6. Percent mineral oil and grease (MOG) degradation and cummulative CO_2 production as a function of the temperature plateau.

thermophilic phase (length of time the temperature profile was maintained at 50°C) on MOG degradation. The imposed temperature profile was one day at room temperature, then 5, 10, 20, or 29 days at 50°C, and the remaining time at room temperature. The co-compost composition was identical to that used in the above experiment. Although all experiments lasted 30 days, MOG degradation was directly proportional to the length of the imposed thermophilic phase (Figure 4.7). Only 50 % of the MOG was degraded when the temperature was maintained at 50°C for 5 days, whereas 70 % was degraded when it was held at 50°C for 30 days. The extended time at 50°C increased degradation in all three hydrocarbon fractions, aliphatic, aromatic and polar. Analysis of MOG content in 120-day old co-compost showed a continued effect of the length of the thermophilic phase on MOG degradation. The co-compost that had been maintained at 50° C for 30 days resulted in more MOG degradation after an additional 90 days at room temperature (78 %) than the co-compost having had a short (5 days) or non-existent thermophilic phase (67 %).

DISCUSSION

The much larger surface to volume ratio of any laboratory-scale reactor (compared to a field scale process) results in a disproportionately large conductive heat loss making simulation difficult. To simulate the interior of a large compost heap (windrow), the conductive heat flow must be minimized. Two methods have generally been used to solve this problem. One allows the rate of heat generation to determine the

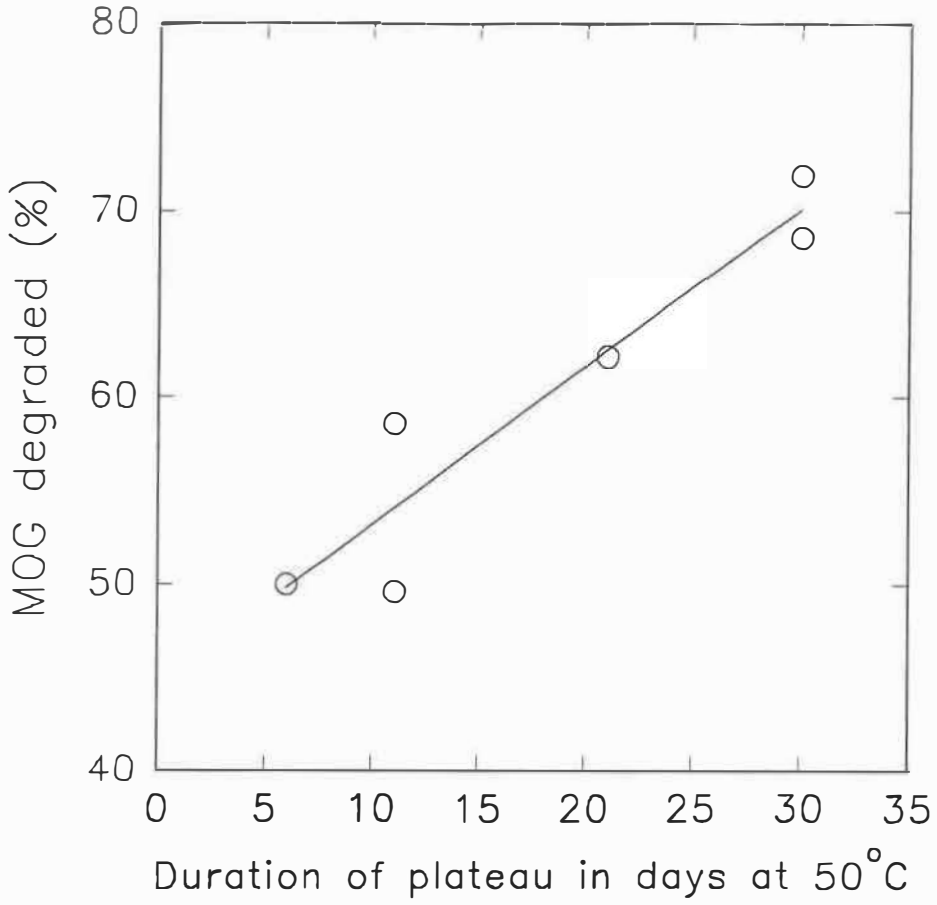


Figure 4.7. Mineral oil and grease (MOG) degradation as a function of the length of the thermophilic phase during co-composting.

temperature profile. It minimizes the conductive heat flow by adjusting the exterior wall temperature to that of the compost center since insulation is generally ineffective (Hogan *et al.*, 1989).

The other system (used in this research) forces the composters to follow a set temperature profile by placing them in a temperature-controlled environment and heating the surroundings. This system is less expensive than the first, simpler to use and assemble, and useful for preliminary optimization tests. However, it is not as effective at simulating the interior portion of a windrow as the first system described above, because the temperature profile is imposed rather than self-generated. In addition, the system in which the rate of heat generation determines the temperature profile is more complicated to operate. Furthermore, since the temperature profile changes when the initial conditions are changed, experiments investigating the effect of different parameters are more difficult to interpret due to uncertainty as to whether a given result was caused by the parameter investigated or the resulting shift in the temperature profile.

Less MOG was degraded in the mini-composters than in a larger laboratory-scale reactor with self-heating (unpublished data), demonstrating that simulation was not perfect. However, conducting experiments in one parallel run with several mini-composters minimizes error due to the heterogeneous nature of a co-compost mixture and greatly increases the number of experiments that can be conducted. For these reasons, mini-composters, with an imposed temperature profile, were used to investigate the effect of co-composting parameters on hydrocarbon degradation.

Hydrocarbons, many of which can serve as sole carbon and energy sources, are often present in too low a concentration to support an active microbial population. Furthermore, some hydrocarbons such as 5 and 6-ring PAHs (Sims and Overcash, 1983; Sims *et al*, 1986; Bossert *et al.*, 1984) cannot be used as an energy source by microorganisms and must be degraded by co-oxidation. These problems are easily overcome in co-composting as a nutrient rich amendment (co-substrate) is added to the contaminated soil. Unfortunately, co-substrate addition with the goal of enhancing biodegradation is not always beneficial as metabolism of the contaminant can be delayed through preferential utilization of the more easily degradable carbon amendments (Swindoll *et al.*, 1988).

Reports can be found in the literature citing both the positive and negative effects of co-substrate addition. For example, 4 and 5-ringed PAHs have been found to disappear more rapidly from soils amended with complex biodegradable wastes (Keck et. al, 1989). The addition of co-substrates such as yeast extract and arginine have been shown to increase mineralization of phenol and p-nitrophenol in lake waters, while glucose, adenine and propionate had an inhibitory effect (Rubin and Alexander, 1983).

In our study, the percent MOG degraded increased with increasing co-substrate addition (Figure 4.3). Although MOG content is often used in government guidelines concerning total hydrocarbon content in soils, these results could be misleading when co-substrates are added. This is because substances such as chlorophyll from leaves and alfalfa are also extracted by 1,1,2-trichloro-1,1,2-trifluorethane (Freon 113) during the determination of the MOG content (APHA, 1985). In co-composting, MOG of soil

origin would be a better indicator but it is very difficult to measure. We assumed that the MOG of co-substrate origin in each mini-composter was degraded to the same extent as in the mini-composter containing only leaves and alfalfa. However, the trend based on MOG of soil origin was similar to that obtained using total MOG degradation, signifying that the latter is a fair indicator even with the additional substances originating from the co-substrate.

In our study, bioaugmentation either had no significant effect on MOG degradation or decreased performance. Similar results have been reported for weathered crude oil treated by a landfarming process (Leavitt and Brown, 1994). Many reasons have been postulated for the poor performance of inoculum addition. Several may be applicable to the present study. For example, the inocula may have been inadequately homogenized. This could be important as the inability of some bacteria to move appreciably through soil has been cited to explain the failure of several commercially available inocula to significantly increase hydrocarbon degradation in landfarming (Compeau *et al.*, 1991). Bioaugmentation may also fail due to suppression of the added microorganisms by predators and parasites. A study on p-nitrophenol degradation in lake water found that the added inocula did not survive. However, if the protozoan population was suppressed, the added bacterium multiplied and mineralized p-nitrophenol (Ramadan *et al.*, 1990). The same study found that the problem could be solved by increasing the inoculum size. However, the cost and added manipulations of adding larger volumes of inoculum to contaminated soil render this solution unrealistic. Furthermore, the environmental conditions may be unsuitable for the added

microorganisms. Except for the compost, none of the inocula in the present study had previously been exposed to a thermophilic environment but 5 out of the first 6 days of co-composting were conducted under thermophilic conditions (temperature of 50°C). There is no obvious explanation as to why bioaugmentation seemed to decrease performance other than the possibility of negative microbial interactions (competition, predation) between added microorganisms and those already present in the co-compost. It can only be concluded that, in this case, bioaugmentation was unnecessary. An acclimatized, hydrocarbon-degrading population was already present in the contaminated soil.

The addition of PO_4^{-3} to natural wastes has been reported to increase, decrease or have no effect on the mineralization of organic chemicals (Rubin and Alexander, 1983; Swindoll *et al.*, 1988). It is known that PO_4^{-3} availability to microorganisms is dependent on pH and the presence of divalent cations such as Ca^{2+} and Mg^{2+} which bind PO_4^{-3}. Even in studies using pure cultures for the degradation of chemicals such as phenol, PO_4^{-3} and Ca^{2+} concentration, and the pH of the environment have had dramatic effects on mineralization (Robertson and Alexander, 1992). However, due to the complexity of PO_4^{-3} solution chemistry, there is no clear explanation for this, making it difficult to predict which nutrients should be added in co-composting. Changes in pH caused by $CaCO_3$ and PO_4^{-3} addition further complicates the matter. Optimization using mini-composters or some similar laboratory technique is therefore necessary. In our case, $CaCO_3$ addition at the beginning of a co-compost run followed by PO_4^{-3} addition after 2 weeks resulted in maximum MOG degradation (Figure 4.4).

One would expect low C/N ratios to favor carbon utilization and thus greater MOG degradation. This proved to be the case as percent degradation of MOG of soil origin increased as the C/N ratio was reduced from 40 to 15 (Figure 4.5). Similar results have been obtained in landfarming where a C/N ratio of 18 (lowest ratio tested) resulted in maximum oil decomposition (Rasiah *et al.*, 1991). Operating at either extreme (C/N ratio) is inadvisable. No MOG degradation was observed at a C/N ratio of 40, even though CO_2 generation analysis indicated that there was still significant microbial activity. No microbial activity occurred at C/N ratios lower than 15, possibly due to inactivation of the microbial flora by excessive ammonia production. The greatest amount of MOG of soil origin was degraded at a C/N ratio of 15. However the absence of leaves in the initial co-compost composition resulted in a muddy mixture in which aerobic conditions were difficult to maintain. A compromise solution would be a C/N ratio of 17 where MOG degradation was still high and the co-compost composition contains sufficient leaf material to provide good compost structure. The co-compost mixture should contain enough bulky material to achieve a loose, easily aerated pile.

Although there is considerable disagreement as to the optimal temperature for hydrocarbon degradation, the most rapid biodegradation of petroleum has generally been shown to be between 30 and 40° C (Bossert and Bartha, 1984). However, local environmental conditions may select a population with a different optimal temperature. A landfarming study evaluating crude oil biodegradation at 5, 13, 20, 28 and 37° C, found a rise in degradation rate from 5 to 20°C, followed by little or no increase in biodegradation at higher temperatures (Dibble and Bartha, 1979). Another landfarming study evaluating

hydrocarbon degradation at 17, 27 and 37° C, reported shorter contaminant half-lives at 27°C (Song *et al.*, 1990).

In our study, while 23°C and 50°C favored MOG degradation, less degradation was observed at 30°C and 40°C. The conclusion that more MOG is degraded at 23°C than at 40°C is unexpected as overall metabolism generally proceeds at a slower rate at lower temperatures. However, cumulative CO_2 production was not significantly different at 23, 30, 40 and 50°C. This indicates that while the hydrocarbon degrading population may have been depressed by the stage at 30 and 40°C, the activity of the total population was not. In composting, at temperatures above 40°C, the population is generally dominated by thermophilic fungi (Sterritt and Lester, 1988) which may also be active in hydrocarbon degradation. The change in microbial population would explain the increased degradation at 50°C. At 60°C the thermophilic fungi die off and are replaced by spore-forming bacteria and actinomycetes (Sterritt and Lester, 1988). The decrease in hydrocarbon degradation at this temperature can be attributed to this phenomenon but also to the lower microbial diversity found at these temperatures (McGregor *et al.*, 1981; Kuter *et al*, 1985, McKinley *et al.*, 1984). In our study, more MOG was degraded if the temperature profile was maintained at 23°C rather than imposing a 5-day plateau at 50°C (thermophilic conditions). This may be because the microflora had insufficient time to adapt to the temperature shifts. The change in temperature would be much more gradual in a windrow co-composting process. Indeed, thermophilic conditions proved to be preferable to mesophilic temperatures. Maintaining the co-compost at 50°C for 29 days resulted in more MOG degradation than that obtained at 23°C. This indicates that an in-

vessel system would be preferable to a windrow system for field-scale co-composting as the thermophilic phase could be more easily extended. However, these results disagree with a previous study on co-composting where the PAH degradation rate was higher at 35°C than at 50°C (Hogan, 1988). The result of the volatilization study negates the possibility that the increased degradation could be due to increased volatilization at 50°C.

Optimal co-composting conditions change depending on the type of soil and contaminant to be treated. Consequently, tests should always be conducted prior to pilot-scale co-composting to determine whether the contaminants are treatable by this remediation technology and what the optimal conditions are. Use of mini-composters with fixed temperature profiles is a simple, relatively inexpensive and quick way of achieving these objectives.

ACKNOWLEDGEMENTS

This research was supported by Imperial Oil, ESTAC, and FCAR-CQVB. We thank Michael Mitchell and Claude Bélanger for technical assistance, Dr. L. Lawlor (Imperial Oil research department, Sarnia, Ontario) for valuable discussions and Prof. M. A. Pickard (University of Alberta, Edmonton, Alberta) for providing the anthracene degrading consortium.

REFERENCES

1. **Bossert, I., W. M. Kachel, and R. Bartha.** 1984. Fate of hydrocarbons during oily sludge disposal in soil. Appl. Environ. Microbiol. **47:**763-767.

2. **Compeau, G. C., W. D. Mahaffey, and L. Patras.** 1991. Full-scale bioremediation of a contaminated soil and water, p. 91-109. *In* G. S. Sayler, R. Fox and J. W. Blackburn (eds.), Environmental Biotechnology for Waste treatment. Plenum, New York.

3. **Dibble, J. T., and R. Bartha.** 1979. Effect of environmental parameters on the biodegradation of oil sludge. Appl. Environ. Microbiol. **37:**729-739.

4. **Fogarty, A. M., and O. H. Tuovinen.** 1991. Microbiological degradation of pesticides in yard waste composting. Microbiol. Rev. **55(2):**225-233.

5. **Hogan, J. A., G. R. Tofoli, F. C. Miller, J. V. Hunder, and M. S. Finstein.** 1988. Composting physical model demonstration: mass balance of hydrocarbons and PCB's, p. 742-758. *In* X. C. Wu (Ed.) The International Conference on Physicochemical and Biological Detoxification of Hazardous Wastes, Vol. 2. Technomic Publication, Lancaster, Pa.

6. **Hogan, J. A., F. C. Miller, and M. S. Finstein.** 1989. Physical modeling of the composting ecosystem. Appl. Environ. Microbiol. **55:**1082-1092.

7. **Kaplan, D. L., and A. M. Kaplan.** 1982. Thermophilic biotransformations of 2,4,6-trinitrotoluene under simulated composting conditions. Appl. Environ. Microbiol. **44:**657-760.

8. **Keck, J., R. C. Sims, M. Coover, K. Park, and B. Symons.** 1989. Evidence for cooxidation of polynuclear aromatic hydrocarbons in soil. Water Res. **23:**1467-1476.

9. **Leavitt, M. E., and K. L. Brown.** 1994. Biostimulation versus bioaugmentation three case studies, p. 72-79. *In* R. E. Hinchee, B. C. Allemen, R. E. Höppel, and R. N. Miller (eds.), Hydrocarbon Bioremediation. CRC Press, Lewis Publishers, Boca Raton, Fla. U.S.

10. **McFarland, M. J., X. J. Qiu, J. L. Sims, M. E. Randolph and R. C. Sims.** 1992. Remediation of petroleum impacted soils in fungal compost bioreactors. Water. Sci. & Technol. **25(3):**197-206.

11. **McGregor, S. T., F. C. Miller, K. M. Psarianos, and M. S. Finstein.** 1981. Composting process control based on interaction between microbial heat output and temperature. Appl. Environ. Microbiol. **41:**1321-1330.

12. **McKinley, V. L., and J. R. Vestal.** 1984. Biokinetic analyses of adaptation and succession: Microbial activity in composting municipal sludge. Appl. Environ. Microbiol. **47:**933-941.

13. **Magalhães, A. M. T., P. J. Shea, M. D. Jawson, E. A. Wicklund, and D. W. Nelson.** 1993. Practical simulation of composting in the laboratory. Waste Mgmt. Res. **11:**143-154.

14. **Michel Jr., F. C., A. Reddy, and L. J. Forney.** 1993. Yard waste composting: Studies using different mixes of leaves and grass in a laboratory scale system. Comp. Sci. Util. **1:**85-96.

15. **Nakasaki, K., H. Yaguchi, Y. Sasaki, and H. Kubota.** 1992. Effects of C/N ratio on thermophilic composting of garbage. J. Ferment. Bioeng. **73:**43-45.

16. **Ramadan, M. A., O. M. El-Tayeb, and M. Alexander.** 1990. Inoculum size as a factor limiting success of inoculation for biodegradation. Appl. Environ. Microbiol. **56:**1392-1396.

17. **Rasiah V., R. P. Voroney, and R. G. Kachanoski.** 1991. Effect of N amendment on C mineralization of an oily waste. Water Air Soil Pollut. **59:**249-259.

18. **Robertson, L., and M. Alexander.** 1992. Influence of calcium, iron, and pH on phosphate availability for microbial mineralization of organic chemicals. Appl. Environ. Microbiol. **58:**38-41.

19. **Rubin, H. E., and M. Alexander.** 1983. Effect of nutrients on the rates of mineralization of trace concentrations of phenol and p-nitrophenol. Environ. Sci. Technol. **17:**104-107.

20. **Sims, R. C., and M. R. Overcash.** 1983. Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Residue Rev. **88:**1-68.

21. **Sims, R. S., D. Sorenson, J. Sims, J. McLean, R. Mahmood, R. Dupoint, J. Jurinak, and K. Wagner.** 1986. Contaminated surface soils in-place treatment techniques. Noyes, Park Ridge, N. J.

22. **Song, H., X. Wang, and R. Bartha.** 1990. Bioremediation potential of terrestrial fuel spills. Appl. Environ. Microbiol. **56:**652-656.

23. **Sterritt R. M., and J. N. Lester.** 1988. Microbiology for environmental and public health engineers. E & F.N. Spon Ltd, New York, NY.

24. **Suler, D. J., and M. S. Finstein.** 1977. Effect of temperature, aeration, and moisture on CO_2 formation in bench-scale, continuously thermophilic composting of solid waste. Appl. Environ. Microbiol. **33:**345-350.

25. **Swindoll, C. M., C. M. Aelion, and F. K. Pfaender.** 1988. Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation response of subsurface microbial communities. Appl. Environ. Microbiol. **54:**212-217.

26. **Valo, R., and M. Salkinoja-Salonen.** 1986. Bioreclamation of chlorophenol-contaminated soil by composting. Appl. Microbiol. Biotechnol. **25:**68-75.

27. **Williams, R. T., and C. A. Myler.** 1990. Bioremediation using composting. Biocycle **31:**78-82.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The main objective of this work was to determine the feasibility of co-composting to treat a soil contaminated with a weathered crude oil. The feasibility studies were carried out in an 8-liter laboratory-scale reactor and optimization studies in a series of experiments in mini-composters.

1. Co-composting is an effective treatment for soils contaminated with recalcitrant contaminants such as weathered hydrocarbons. In one experiment, eighty-three percent of the MOG and at least 73 % of the MOG of soil origin were degraded in 287 days of co-composting. These results were superior to those obtained in landfarming.

2. Although the treatment of 1 ton of contaminated soil by co-composting necessitated the addition of about 1.7 tons of co-substrate, the final mass is far less than the initial mass as a result of the degradation of the organic matter. For example, in the experimentione mentionned above, the co-compost mass was reduced to 62 % of its original mass in 287 days of co-composting.

3. A co-composting experiment with an uncontaminated sand (no hydrocarbons) demonstrated that 89 % of the MOG of co-substrate (leaves and alfalfa) origin was degraded in the first 3.5 months of co-composting. Consequently, it was concluded that MOG of co-substrate origin was easily degraded by co-composting and that almost all of the MOG remaining after 3.5 months of co-composting was of soil origin.

4. In the co-composting experiments, a higher proportion of the more assimilable substrates, such as sugars from plant materials were used at the beginning of the run. This was indicated by both the high RQ and the low ratio of the MOG degradation rate to organic degradation rate at the beginning of the run. Despite this conclusion, the highest rate of MOG degradation occurred at the beginning of co-composting treatment when microbial activity and the resulting temperature were at a maximum.

5. Mini-composters are useful because it is difficult to predict optimal co-composting parameters for a given contaminant. Optimal conditions are dependent not only on the contaminant but also on the type of soil and the indigenous microorganisms. However, co-composting in mini-reactors resulted in less MOG degraded than in the laboratory-scale reactor. Consequently, the results (from the mini-reactors) while good for purposes of comparison cannot be used to accurately predict the final extent of MOG degradation in a full-scale composting system..

6. The following was concluded during optimization tests in mini-composters:

i. The soil was limited in nutrients such as phosphate. Consequently adding a nutrient solution to the soil increased MOG degradation.

ii. Co-substrate addition also resulted in an increased microbial population allowing increased mineralization as well as co-oxidation.

iii. Bioaugmentation was found to be ineffective in co-composting. The added microorganisms could not compete effectively with the native culture. In addition, bioaugmentation was unnecessary because an acclimatized hydrocarbon - degrading population was already present in the contaminated soil.

iv. MOG degradation increased with decreasing C/N ratio. The highest % of MOG was degraded at a C/N of 15. There was no apparent MOG degradation at a C/N ratio of 40. It was concluded that a low initial co-compost C/N ratio (less than 20) results in carbon limitation and consequently promotes hydrocarbon degradation, whereas a C/N ratio of 40 results in preferential degradation of the more easily degradable co-substrates.

v. More MOG was degraded at 20°C and 50°C than at 30°C, 40°C and 60°C. Therefore, the hydrocarbon-degrading populations in the soil were most active at 23°C and 50°C. Apparently one group of hydrocarbon-degrading organisms was active at mesophilic temperatures and another at thermophilic temperatures.

vi. There was a decrease in microbial activity at 60°C. This was indicated by the decrease in CO_2 generation at this temperature.

vii. More MOG was degraded at 50°C than at 23°C if this temperature was maintained for at least 14 days. This indicates that an in-vessel system would be

preferable to a windrow system for field-scale co-composting because the thermophilic phase could be more easily extended and controlled.

5.2 Recommendations

This study has demonstrated that co-composting for the degradation of hydrocarbons is feasible as well as showing which operating conditions most affect MOG degradation. However it is important to note that a substitute should be found for the alfalfa used as a co-substrate, because this feed would be too expensive on a large scale. The substitute substrate should have a C/N ratio less than 15, contain little MOG or only that which is very easily biodegradable, and be very inexpensive (preferably free).

One of the possible disadvantages of co-composting is the added cost of co-substrates. The cost of co-substrates was estimated to be $104 per ton of soil. This does not include the cost to transport these co-substrates to the site. Unprocessed alfalfa was used in the cost estimation rather than the alfalfa (purina rabbit chow) used in this study. The price of alfalfa was $90 ton^{-1} of soil, a considerable portion of the total cost. Selecting a suitable, less costly substitute would render this process more economical.

Table 5.1. Cost of co-substrates in co-composting process.

	Purchase price ($)
Soil (1 ton)	-
Alfalfa[1] (1.126 tons)	90.00
Leaves (0.433 tons)	-
Lime[2] (0.14 tons)	14.20

[1] Price of alfalfa directly from farm. [2] Cost of activated lime. Inactivated lime should be slightly less expensive.

Once the substitute is chosen, experiments should be conducted in the laboratory-scale reactor prior to scale-up in order to determine if this new co-substrate gives acceptable results. In addition, co-composting trials should be conducted on a pilot-scale. Due to the problems encountered during MOG analysis, a new hydrocarbon analysis method such as TPH analysis should be considered.

Although this work concluded that an initial C/N ratio of 15 gave the most MOG degradation, this condition may be impossible to achieve on a pilot-scale. The larger mass may necessitate that a higher proportion of leaves be added in order to improve air permeability. Another possibility would be to add a bulking agent such as wood chips. This treatment necessitates good airflow in order to maintain aerobic conditions (necessary for oxygenases, usually the first step in the degradation of

hydrocarbons).

BIBLIOGRAPHY

ALEXANDER M. (1965). Biodegradation: Problems of molecular recalcitrance and microbial fallability. Adv. Appl. Microbiol., 7, 35-80.

ATLAS, R.M. (1975). Effects of temperature and crude oil composition on petroleum biodegradation. Appl. Microbiol., 30, 396-403.

APHA, AWWA and WPCF (1985). Standard methods for the examination of water and wastewater. 16th Edition. American Public Health Association, Washington, DC.

ATLAS, R.M. and BARTHA, R. (1980). Microorganisms and some novel pollution problems. Microbial ecology: Fundamentals and applications, Addison-Wesley, Reading, Mass., 403-418.

BACH, P.D., SHODA, M. and KUBOTA, H. (1984). Rate of composting of dewatered sewage sludge in continuously mixed isothermal reactor. J. Ferment. Technol., 62, 199-209.

BACH, P.D., SHODA, M. and KUBOTA, H. (1985). Composting reaction rate of sewage sludge in an autothermal packed bed reactor. J. Ferment. Technol., 63, 271-278.

BENOIT, P. and BARRIUSO, E. (1995). Efect of straw composting on the degradation and stabilization of chlorophenols in soil. Comp. Sci. Util., 3(3), 31-37.

BOSSERT, I. and BARTHA, R. (1984). The fate of petroleum in soil ecosystems. Petroleum Ecology, R.M. Atlas, Macmillan, New York, 435-473.

BOSSERT, I., KACHEL, W.M. AND BARTHA, R. (1984). Fate of hydrocarbons during oily sludge disposal in soil. Appl. Environ. Microbiol., 47, 763-767.

BRIEDENBACH, A.W. (1971). Composting of municipal solid wastes in the United States. PB213-478 U.S. Public Service, Rockville, MD.

BRITTON, L.N. (1984). Microbial degradation of aliphatic hydrocarbons. Microbial degradation of organic compounds, D.T. Gibson, Marcel Dekker, New York, 89-130.

BUMPUS, J.A. (1989). Biodegradation of polycyclic aomatic hdrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbial., 55, 154-158.

BUMPUS, D.A. and AUST D.A. (1986). Biodegradation of environmental pollutants by the white rot fungus *Phanerochaete chrysosporium*: Involvement of the lignin degrading system. Bioessays, 6, 166-170.

CERNIGLIA, C.E. (1981). Aromatic hydrocarbons: Metabolism by bacteria, fungi and algae. Rev. Biochem. Toxic., 3, 321-361.

CERNIGLIA, C.E., ALTHAUS, J.R., EVANS, F.E., FREEMAN, J.P., MITCHUM, R.K. and YANG, S.K. (1983). Stereochemistry and eidence for an arene oxide-NIH shift pathway in he fungal metabolism of naphtalene. Chem. Biol. Interact., 44, 119-132.

CERNIGLIA, C.E. and CROW, S.A. (1981). Metabolism of aromatic hydrocarbons by yeasts. Arch. Microbiol., 129, 9-13.

CERNIGLIA, C.E., FREEMAN, J.P. and MITCHUM, R.K. (1982). Glucoronide and sulfate conjugation in the fungal metabolism of aromatic hydrocarbons. Appl. Environ. Microbiol., 43, 1070-1075,

CERNIGLIA, C.E. and GIBSON, D.T. (1977). Metabolism of naphthalene by *Cunninghamella elegans*. Appl. Environ. Microbiol., 34, 363-370.

CERNIGLIA, C.E. and GIBSON, D.T. (1978). Metabolism of naphthalene by cell extracts of *Cunninghamella elegans*. Arch. Biochem. Biophys., 1981, 121-127.

CERNIGLIA, C.E. and GIBSON, D.T. (1980). Fungal oxidation of benzo[a]pyrene and (±)-*trans*-7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene: Evidence forthe formation of a benzo[a]pyrene 7,8-diol-9,10-epoxide. J. Biol. Chem., 255, 5159-5163.

CERNIGLIA, C.E., HEBERT, R.L., SZANISZLO, P.J. and GIBSON, D.T. (1978). Fungal transformation of naphthalene. Arch. Microbiol., 117, 135-143.

CERNIGLIA, C.E. and HEITKAMP M.A. (1989). Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. Metabolism of polycyclic aromatic hydrocarbons in the quatic environment. U. Varanasi, CRC Press, Boca Raton, 41-68.

CERNIGLIA, C.E., SUTHERLAND, J.B. and CROW, S.A. (1992). Fungal metabolism of aromatic hydrocarbons. Microbial degradation of natural products , G. Winkelmann, VCH Verlagsgesellschaft, Weinheim.

CERNIGLIA, C.E., WHITE, G.L. and HEFLICH, R.H. (1985). Fungal metabolism and detoxification of polycyclic aromatic hydrocarbons. Arch. Microbiol., 143, 105-110.

CHANYASAK, V., YOSHIDA, T. and KUBOTA, H. (1980). Chemical components in gel chromatographic fractionation of water extract from sewage sludge compost. J. Ferment. Technol., 58, 533-539.

COMPEAU, G.C., MAHAFFEY, W.D. and PATRAS, L. (1991). Full-scale bioremediation of a contaminated soil and water. Environmental Biotechnology for Waste Treatment, G.S. Sayler, R. Fox, J.W. Blackburn, Plenum, New York, 91-109.

COOKSON. JR., J.T. (1995). Optimizing microbial transformation of hazardous chemicals. Bioremediation Engineering: Design and Application, McGraw Hill, 95-163.

DAGLEY, P.J. (1988). Catabolism of aromatic compounds by microorganisms. Adv. Microbial Physiol., 6, 1-46.

DAVIS, P.J. (1988). Microbial models of mammalian drug metabolism. Dev. Ind. Microbiol., 29, 197-219.

DAVIS, M.D., GLASER, J.A., EVANS, J.W. and LAMAR, R.T. (1993). Field evaluation of the lignin-degrading fungus *Phanerochaete sordida* to treat creosote-contaminated soil. Environ. Sci. Technol., 27, 2572-2576.

DEY, S., MAITIM, T.K. and BHATTACGARYYA, B.C. (1991). Lignin peroxidase production by a brown-rot fungus, *Polyporus osteirformis*. J. Ferment. Bioeng., 72, 402-404.

DIBBLE, J.T. and BARTHA, R. (1979). Effect of environmental parameters on the biodegradation of oil sludge. Appl. Environ. Microbiol., 37, 729-739.

ENGLERT, C.J., KENZIE, E.J. and DRAGUN, J. (1993). Bioremediation of petroleum products in soil. Principles and Practices for Petroleum Contaminates Soils, E.J. Calabrese, P. T. Kostecki, Lewis Publishers, Chelsea, MI, 111-130.

FERNANDO, T., BUMPUS, J.A. and AUST, S.D. (1990). Biodegradation of TNT (2,4,6-Trinitrotoluene) by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol., 56, 1666-1671.

FERRIS, J.P., FASCO, M.J., STYLIANOPOULOU, F.L., JERINA, D.M., DALY, J.W. and JEFFREY, A.M. (1973). Monooxygenase activity in *Cunninghamella bainieri*:

Evidence for a fungal system similar to liver microsomes. Arch. Biochem. Biophys., 156, 97-103.

FERRIS, J.P., MACDONALD, L.H., PATRIE, M.A. and MARTIN, M.A. (1976). Aryl hydrocarbon hydroxylase activity in the fungus *Cunninghamella bainieri*: Evidence forthe presence of cytochrome P-450. Arch. Biochem. Biophys., 175, 443-452.

FOGARTY, A.M. and TUOVINEN, O.H. (1991). Microbial degradation of pesticides in yard waste composting. Microbiol. Rev., 55(2), 225-233.

GIBSON, D.T. (1977). Biodegradation of aromatic petroleum hydrocarbon. Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, D.A. Wolfe, Pergammon Press, New York, 36-46.

GIBSON, D.T. (1982). Microbial degradation of hydrocarbons. Toxicol. Environ. Chem., 5, 237-250.

HART, S.A. (1991). Composting potentials for hazardous waste management biological processes, Vol. 3. H.M. Freeman and P.R. Sferra. Technomic Publishing, Lancaster, PA.

HAUG, R.T. (1993). The practical handbook of compost engineering. CRT Press, Boca Raton, Fla.

HOGAN, J.A., MILLER, F.C. and FINSTEIN, M.S. (1989). Phisical modeling of the composting ecosystem. Appl. Environ. Microbiol., 55, 1082-1092.

HOGAN, J.A., TOFFOLI, G.R. MILLER, F.C. HUNTER, J.V. and FINSTEIN, M.S. (1988). Composting physical model demonstration: mass balance of hydrobarbons and PCB's. The Internation Conference on Physicochemical and Biological Detoxification of Hazardous Wastes, Vol. 2, X.C. Wu, Technomic Publication, Lancaster, Pa.

IWM SCIENTIFIC AND TECHNICAL COMMITTEE (1994). Down to earth composting - of municipal green wastes.

JENSEN, V. (1975). Bacterial flora of soil after application of oily waste. Oikas, 26, 152-158.

JERIS, J.S. and REGAN, R.W. (1973). Controlling environmental parameters for optimum composting Part III.. Compost Science, 14 (May-June), 16-22.

JOBSON, A., COOK, F.D. and WESTLAKE, D.W.S. (1972). Microbial utilization of crude oil. Appl. Environ. Microbiol., 23, 1082-1089.

KAPLAN, D.L. and KAPLAN, A.M. (1982). Thermophilic biotransformations of 2,4,6-trinitrotoluene under simulated composting conditions. Appl. Environ. Microbiol., 44, 657-660.

KECK, J., SIMS, R., COOVER, M.O., PARK, K. and SYMONS, B. (1989). Evidence for cooxidation of polynuclear aromatic hydrocarbons in soil. Wat. Res., 23, 1467-1476

KING, D.J. and WISEMAN, A. (1987). Yeast cytochrome P-448 enzymes and the activation of mutagens, including carcinogens. Mutagen Activation and Carcinogen Testing in Yeast, A. Wiseman, Ellis Horwood, Chichester, 115-167.

LAMAR T.L. and DIETRICH, D.M., (1990). In situ depletion of pentachlorophenol from contaminated soil by *Phanerochaete spp.*", Appl. Environ. Microbiol., 56, 3093-3100.

LEAVITT, M.E. and BROWN, K.L. (1994). Biostimulation verrsus bioaugmentation - three case studies. Hydrocarbon Bioremediation, R.E. Hinchee, B.C. Allemen, R.E. Höppel, R.N. Miller, CRC Press, Lewis Publishers, Boca Raton, Fla, U.S. 72-79.

MACGREGOR, S.T., MILLER, F.C., PSARIANOS, K.M. and FINSTEIN, M.S. (1981). Composting process control based on interaction between microbial heat output and temperature. Appl. Environ. Microbiol., 41, 1321-1330.

MCKINLEY, V.L. and VESTAL, J.R. (1984). Biokinetic analysis of adaption and succession: microbial activity in composting municipal sewage sludge. Appl. Environ. Microbiol., 47, 933-941.

MCMILLAN, D.C., FU, P.P., FREEMAN, J.P., MILLER, D.W. and CERNIGLIA, C.E. (1988). Microbial metabolism and detoxification of 7,12-dimetylbenz[a]anthracene. J. Ind. Microbiol., 3, 211-225.

MCMILLEN, A.J., KERR, J.M., GRAY, N.R. and FINDLAY, M. (1992). Composting of a production pit sludge. IGT Symposium on Gas, Oil and Environmental Biotechnology.

MAGALHÃES, A.M., SHEA, P.J., JAWSON, M.D., WICKLUND, E.A. and NELSON, D.W. (1993). Practical simulation of composting in the laboratory. Waste Management & Research, 11, 143-154.

MEANS, J.C., WARD, S.G., HASSET, J.J. and BANWART, W.L. (1980). Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environ. Sci. Technol., 14, 1524-1528.

MICHEL JR., F.C., REDDY, A. and FORNEY, L.J. (1993). Yard waste composting: Studies using different mixes of leaves and grass in a laboratory scale system. Comp. Sci. Util., 1, 85-96.

MILLER, F.C. (1989). Matrix water potential as an ecological determinant in compost, a substrate dense system. Microbial Ecology. 18, 59-71.

MUSTIN, M. (1987). Le Compost. Éditions François Dubusc, Paris. .

NAKASAKI, K., YAGUCHI, H., SASAKI, Y. and KUBOTA, H. (1992). Effects of C/N ratio on thermophilic composting of garbage. J. Ferment. Bioeng., 73, 43-45.

PARK, K.S., SIMS, R.C., DUPONT, R.R., DOUCETTE, W.J. and MATTHEWS, J.E. (1990). Fate of PAH compounds in two soil types: Influence of volatilization, abiotic loss and biological activity. Environmental Toxicology and Chemistry, Vol. 9, 187-195.

PERRY, J.J. (1979). Microbial cooxidation involving hydrocarbons. Microbiol. Rev., 43, 59-72.

RAMADAN, M.A., EL-TAYEB, O.M. and ALEXANDER, M. (1990). Inoculum size as a factor limiting success of inoculation for biodegradation. Appl. Environ. Microbiol., 56, 1392-1396.

RASIAH, V., VORONEY, R.P. and KACHANOSKI, R.G. (1991). Effect of N amendment on C mineralization of an oily waste. Water Air Soil Pollut., 59, 249-259.

ROBERTSON, L. and ALEXANDER M. (1992). Influence of calcium, iron, and pH on phosphate availability for microbial mineralization of organic chemicals. Appl. Environ. Microbiol., 58, 38-41.

ROBINSON, J.J. and STENTIFORD, E.I. (1993). Improving the aerated static pile composting method by the incorporation of moisture control", Comp. Sci. Util., 1(1), 12-68.

RUBIN, H.E. and ALEXANDER, M. (1983). Effect of nutrients on the rates of mineeralization of trace concentrations of phenol and p-nitrophenol. Environ. Sci. Technol., 17, 104-107.

SASEK, V., VOLFOVA, O., ERBANOVA, P., VYAS, B.R.M. and MATUCHA, M. (1993). Degradation of PCBs by white rot fungi, methylotrophic and hydrocarbon utilizing yeasts and bacteria. Biotech. Let., 15:5, 521-526.

SIMS, R. C. and OVERCASH, M. R. (1983). Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Residue Rev., 88, 1-68.

SIMS, R.S., SORENSON, D., SIMS, J., MCLEAN, J., MAHMOOD, R., DUPOINT, R., JURINAK, J. and WAGNER, K. (1986). Contaminated surface soils in-place treatment techniques. Noyes, Park Ridge, N.J.

SONG, H.G. and BARTHA, R. (1990) Effects of jet fuel spills on the microbial commu8nity of soil. Appl. Environ. Microbiol., 56, 646-651.

SONG, H., WANG, X. and BARTHA, R. (1990). Bioremediation potential of terrestrial fuel spills. Appl. Environ. Microbiol., 56, 652-656.

STERRITT, R.M. and LESTER, J.N. (1988). Microbiology for environmental and public health engineers. E & FN. Spon Ltd, New York, NY.

STROM, P.F. (1985). Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. Appl. Environ. Microbiol., 50, 899-905.

SULER, D.J. and FINSTEIN, M.S. (1977). Effect of temperature, aeration, and moisture on CO_2 formation in bench-scale, continuously thermophilic composting of solid waste. Appl. Microbiol., 33, 345-350.

SUTHERLAND, J.B. (1992). Detoxification of polycyclic aromatic hydrocarbons by fungi. J. Ind. Microbiol., 9, 53-62.

SWINDOLL, C.M., AELION, C.M. and PFAENDER, F.K. (1988). Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation of subsurface microbial communities. Appl. Environ. Microbiol., 54, 212-217.

TEXAS RESEARCH INSTITUTE (1982). Enhancing the microbial degradation of underground gasoline by increasing the available oxygen. Report of the American Petroleum Institute. Washington, D.C.

TIEN, M. and KIRK, T.K. (1985). Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. Proc. Natl. Acad. Sci. USA, 81, 2280-2284.

TRUDGILL, P.W. (1984). Microbial degradation of the alicyclic ring. Microbial degradation of orgainc compounds, D.T. Gibson, Marcel Dekker, New York, 131-180.

VALO, R. and SALKINOJA-SALONEN, M. (1986). Bioreclamation of chlorophenol-contaminated soil by composting. Appl Environ. Microbial., 25, 68-75.

VAN DEN MUNCKHOF, G.P.M. and VEUL, F.X. (1991). Production-scale trials on the decontamination of oil-polluted soil in a rotating bioreactor at field capacity. On-Site

Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment, R.E. Hinchee, R.F. Olfenbuttel, Butterworth-Heinemann, Stoneham, Mass., 241-252.

WESTLAKE, D.W.S., JOBSON, A., PHILLIPPE, R. and COOK, F.D. (1974). Biodegradability and crude oil composition. Can. J. Microbiol., 20, 915-928.

WILLIAMS, R.T. and KEEHAN, K.R. (1992). Bioremediation using composting. 1992. National Waste Processing Conference - Proceedings of the Fifteenth Biannual Conference. 429-433.

WILLIAMS, R.T. and MYLER, C.A. (1990). Bioremediaiton using composting. Biocycle, 31:11, 78-82.

YADAV, J.S. and REDDY, C.A. (1993). Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol., 59:3, 756-762.

ZIMMERMAN, W. (1990). Degradation of lignin by bacteria. J. Biotechnol., 13, 119-130.

APPENDICES

APPENDIX A

INITIAL CO-COMPOST COMPOSITION FOR THE EXPERIMENTS CONDUCTED IN THE LABORATORY-SCALE REACTOR

Table A.1. Initial co-compost composition in wt. % for the runs conducted in the laboratory-scale reactor.

Run #	Soil	Alfalfa	Paper	Leaves	Inoculum	$CaCO_3$	KH_2PO_4**	K_2HPO_4**
1	35.6	40.1	15.3	-	5.9	3.0	-	-
2	33.7	37.9	-	18.0	5.6	4.8	-	-
3	34.9	39.3	-	15.1	5.8	4.9	0.9	1.8
4	35.4	35.4	-	20.2	5.1	-	1.4	2.6
5	40.4	44.1	-	11.4	-	4.1	-	-
6	35.0 *	39.4	-	15.2	5.8	4.7	0.9	1.8
7	35.0	39.4	-	15.2	5.8	4.7	0.9	1.8

* The contaminated soil was replaced by clean sand in run 6.

**Added at $t_{3\ months}$(run 3), t_0(run 4), $t_{2.5\ months}$(run 6), $t_{2\ months}$(run 7).

APPENDIX B

OPERATING CONDITIONS AND RESULTS FOR THE EXPERIMENTS CONDUCTED IN THE LABORATORY-SCALE REACTOR

Table B.1. Operating conditions and results for co-composting run 1 (C/N=17.0, N/P=71.2).

Time (days)	pH	Dry Mass (%)	Ash (%)	MOG (ppm)	MOG degradation (%)
0	7.0	50	33.0	15569	0
2	7.99	41	37.0	13233	24.1
3	7.85	39.4	48.2	12440	40.4
7	7.94	39.4	49.3	10537	51.0
10	8.06	38.8	49.4	6304	70.7
16	7.24	37.2	49.4	6131	71.5
40	6.93	39.3	51	-	-
43	6.88	40.1	53.7	-	-

Table B.2. Operating conditions and results for co-composting run 2 (C/N=19.0, N/P=46.9).

Time (days)	pH	Dry Mass (%)	Ash (%)	Bacteria (CFU/g dry compost)	Fungi (CFU/g dry compost)	MOG (ppm)	MOG degra-dation (%)
0	7.15	48.5	42.0	$2*10^7$	$1.3*10^6$	15800	0
2	-	49.9	42.6	$2*10^{10}$	$4*10^6$	-	-
4	7.99	40.8	49.2	$2*10^{13}$	$1.2*10^6$	-	-
7	8.03	41.7	51.1	$5*10^{13}$	$4*10^7$	8200	57
8	8.10	41.6	53.9	$6*10^{13}$	$4*10^7$	-	-
11	8.10	41.3	51.2	$1.4*10^{11}$	$1.4*10^7$	-	-
20	7.80	40.0	58.7	$1.7*10^{12}$	$2*10^7$	6900	69
35	8.42	40.2	59.1	$1*10^{13}$	$4*10^7$	-	-
36	-	41.8	63.0	-	-	-	-
37	-	42.0	59.4	-	-	-	-
94	-	35.0	-	-	-	6600	70
184	-	42.0	-	-	-	5100	77

Table B.3. Operating conditions and results for co-composting run 3 (C/N=18.3, N/P=45)

Time (days)	Added (%)	pH	Dry Mass (%)	Ash (%)	MOG (ppm)	MOG degraded (%)
0	-	6.72	48	43.7	13000	0
8	alfalfa (9)	-	-	-	-	-
28	-	-	37	52	9300	39.9
48		-	38	63.8	6300	62.8
60	P					
90	P	7.7	-	-	-	-
91	-	-	40	63.8	-	-
94	-	7.2	-	-	-	-
105	-	8.0	40	65.5	-	-
108	-	-	41	66.0	5165	73.7
150	-	-	49	68.2	-	-
191	-	-	51	68.6	4677	77.1
287	-	-	51.5	70.6	3530	78.3

Table B4. Operating conditions and results for co-composting run 4 (C/N=19.7, N/P=3).

Time (days)	Added (%)	pH	dry mass (%)	ash (%)	Bacteria (CFU/g dry compost)	Fungi (CFU/g dry compost)	MOG (ppm)	MOG degra-dation (%)
0	-	6.3	44.6	41.0	$1.0*10^{7}$	$1.3*10^{5}$	17721	0
6	-	7.4	35.0	51.0	$2.8*10^{10}$	$2.8*10^{10}$	18021	19.9
9	-	7.8	39.0	55.0	$7.7*10^{11}$	$5.1*10^{10}$	20340	16.2
13	-	8.3	43.0	57.0	$4.7*10^{10}$	$1.2*10^{9}$	18741	26.1
19	H_2O (12)	8.5	43.0	58.0	-	-	-	-
21	-	-	-	-	$1.9*10^{10}$	$7*10^{8}$	11415	56.3
27	P (0.4)	7.9	43.0	9.0	-	-	-	-
31	P (1.0)	-	44.0	61.0	-	-	-	-
36	Ca (0.6)	-	43.0	61.0	-	-	-	-
43	-	7.6	44.0	64.0	-	-	9655	65.7
104	-	-	49.0	-	-	-	-	-
117	-	8.0	48.0	66.0	-	-	-	-
177	-	-	-	-	-	-	7569	77.5

Table B.5. Operating conditions and results for co-composting run 5 (C/N=17.2, N/P=45)

Time (days)	Added (%)	pH	Dry Mass (%)	Ash (%)	MOG (ppm)	MOG corrected for ash
0	-	6.85	43.9	39.1	11200	11200
10	-	7.76	40.8	53.1	9969	7358
18	-	7.88	39.6	56.9	8803	6059
29	-	7.93	38.5	60.1	8095	5475
32	KH_2PO_4 (1.4) K_2HPO_4 (2.8)	-	-	-	-	-
39	-	7.59	42.4	62.7	6171	3929
70	-	7.67	48.3	63.7	6506	4027
105	-	7.82	54.9	63.8	6070	3722

Table B.6. Operating conditions and results for co-composting run 6 (C/N=18.3, N/P=45)

Time (days)	Added (%)	Dry mass (%)	Ash (%)	MOG (ppm)	MOG corrected for ash (ppm)
0	-	50.3	40.4	4164	4164
78	KH_2PO_4 (0.9) K_2HPO_4 (1.8)	-	-	-	-
105	-	53.5	70.9	700	496

Table B.6. Continued

Time (days)	Aliphatics (%)	1-Ring aromatics (%)	2-Ring aromatics (%)	3-Ring aromatics (%)	4^+-Ring aromatics (%)	Polars (%)
0	14.9	0.7	1.0	16.2	25.9	41.3
105	-	-	-	-	-	-

Table B.7. Operating conditions and results for co-composting run 7 (C/N=18.3, N/P=45).

Time (days)	Added (%)	Dry mass (%)	Ash (%)	MOG (ppm)	MOG corrected for ash (ppm)
0	-	47.7	41.3	13369	13369
56	KH_2PO_4 (0.9) K_2HPO_4 (1.8)	-	-	-	-
105	-	42.5	63.4	7443	5100

Table B.7. Continued.

Time (days)	Aliphatics (%)	1-Ring Aromatics (%)	2-Ring Aromatics (%)	3-Ring Aromatics (%)	4^+Ring Aromatics (%)	Polars (%)
0	15.1	2.8	2.5	10.5	20.4	48.9
105	22.3	7.0	5.0	7.9	15.3	42.5

APPENDIX C

TEMPERATURE AND CO_2 GENERATION CURVES FOR EXPERIMENTS CONDUCTED IN THE LABORATORY-SCALE REACTOR

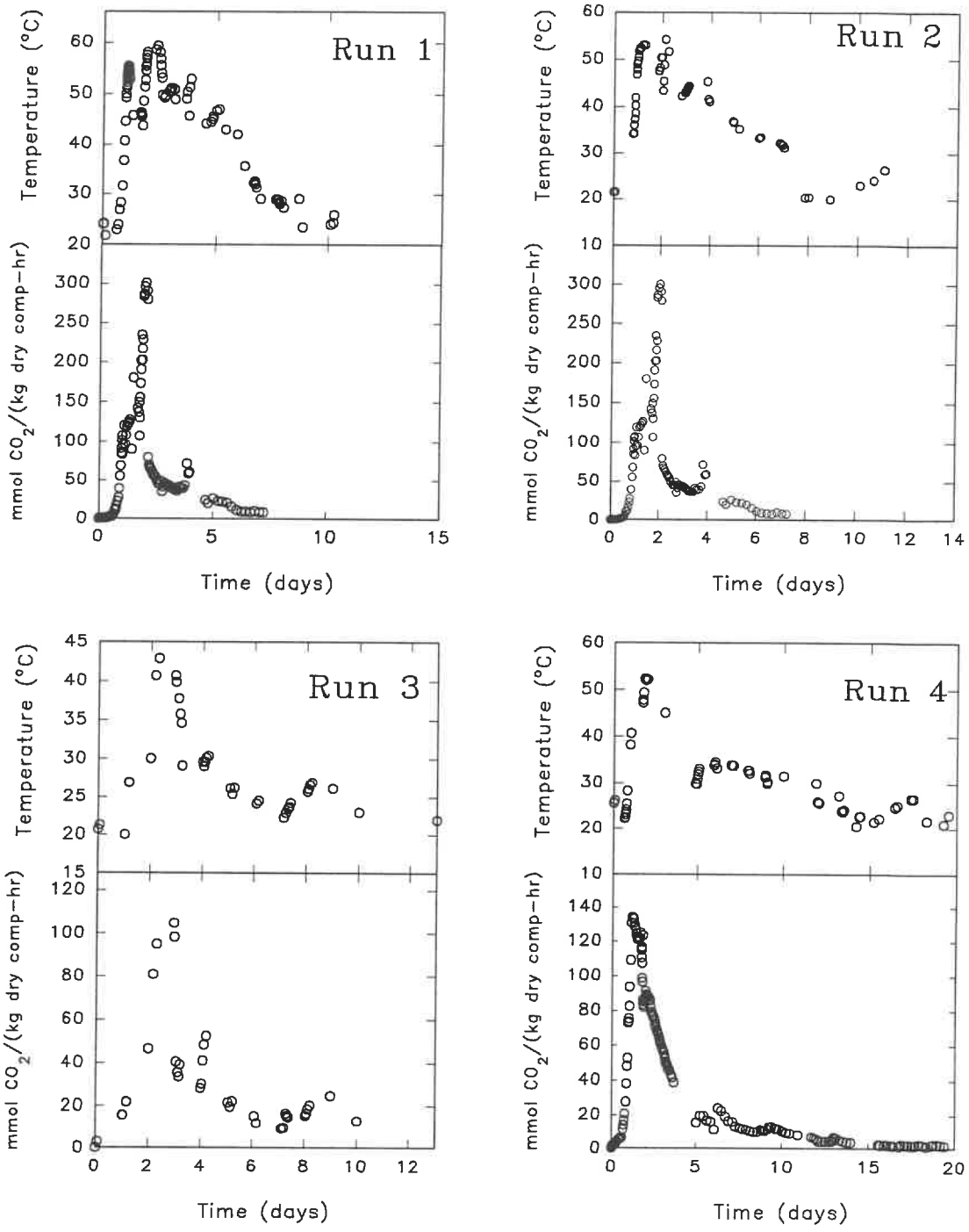
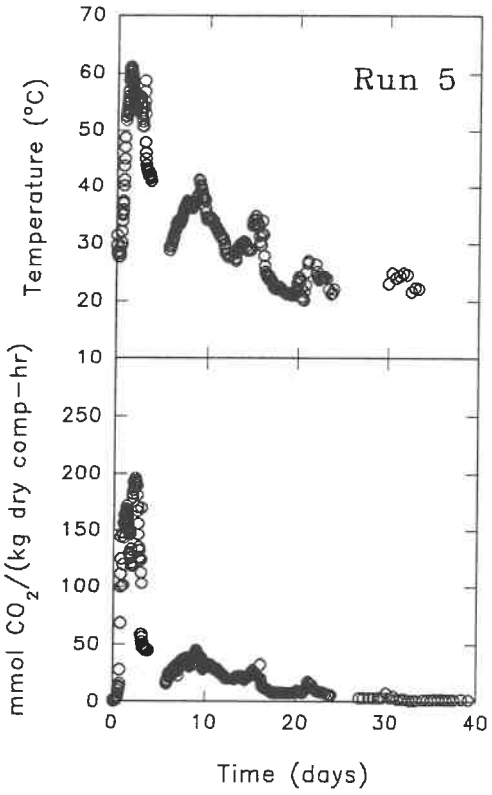
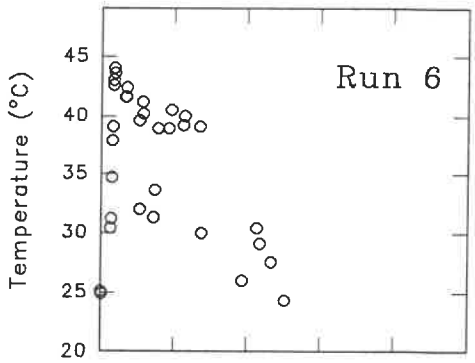


Figure C.1. Temperature and CO_2 generation curves for experiments conducted in the laboratory-scale reactor.





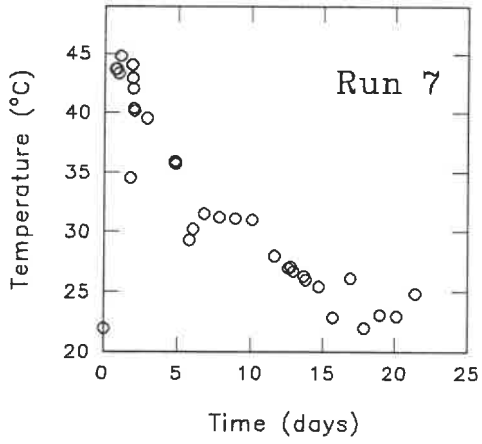


Figure C.1. (continued.)

APPENDIX D

INITIAL CO-COMPOST COMPOSITION

IN THE MINI-COMPOSTER EXPERIMENTS

Table D.1. Percent dry weight of each component in the co-substrate experiments.

	Soil	Alfalfa	leaves	inoculum *	KH_2PO_4	K_2HPO_4	$CaCO_3$
Poisoned control (3.8 % wt/wt HgCl)	41.2	41.2	17.6	-	-	-	-
100% soil	100	-	-	-	-	-	-
100% soil with nutrient	100	-	-	-	-	-	-
75% soil	75	14.0	8.0	5.0	0.5	1.0	1.5
48% soil	47.6	26.7	15.2	4.8	1.0	2.0	2.8
24% soil	23.8	39.9	22.8	4.8	1.5	2.9	4.2
0% soil	0	53.2	30.4	4.8	2.0	3.9	5.6

* Except where otherwise specified compost was used as the inoculum.

Table D.2. Percent dry weight of each component in the Ca and P addition experiments.

	Soil	Alfalfa	leaves	inoculum *	KH_2PO_4	K_2HPO_4	$CaCO_3$
No elements clean soil	38.9	38.9	16.7	5.6	-	-	-
P at t=0 clean soil	37.2	37.2	16.0	5.3	1.4	2.8	-
Ca at t=0 clean soil	37.3	37.3	16.0	5.3	-	-	4.0
No elements	38.9	38.9	16.7	5.6	-	-	-
P at t=0	37.2	37.2	16.0	5.3	1.4	2.8	-
Ca at t=0, P at t=2 wk	37.3	37.3	16.0	5.3	(1.4)	(2.8)	4.0
Ca & P at t=0, double	35.8	35.8	15.4	5.1	1.4	2.7	3.8
Ca at t=0, double	37.3	37.3	16.0	5.3	-	-	4.0
P at t=2 wk, double	38.9	38.9	16.7	5.6	(1.4)	(2.8)	-

* Except where otherwise specified compost was used as the inoculum.

Table D.3. Percent dry weight of each component in the C/N ratio experiments.

	Soil	Alfalfa	Leaves	Inoculum *	KH_2PO_4	K_2HPO_4	$CaCO_3$
C/N=15	35.4	55.6	-	5.1	1.3	2.6	-
C/N=15, clean soil	35.4	55.6	-	5.1	1.3	2.6	-
C/N=17	37.5	42.8	11.0	4.9	1.3	2.5	-
C/N=17, clean soil	37.5	42.8	11.0	4.9	1.3	2.5	-
C/N=20	37.6	37.6	16.0	4.9	1.3	2.5	-
C/N=20, clean soil	37.6	37.6	16.0	4.9	1.3	2.5	-
C/N=25	37.5	20.4	33.4	4.9	1.3	2.5	-
C/N=25, clean soil	37.5	20.4	33.4	4.9	1.3	2.5	-
C/N=30	35.4	12.6	43.0	5.1	1.3	2.6	-
C/N=30, clean soil	35.4	12.6	43.0	5.1	1.3	2.6	-
C/N=40	35.4	-	55.6	5.1	1.3	2.6	-
C/N=40, clean soil	35.4	-	55.6	5.1	1.3	2.6	-

* Except where otherwise specified, compost was used as the inoculum.

Table D.4. Percent dry weight of each component in the bioaugmentation and temperature experiments.

	Soil	Alfalfa	Leaves	Inoculum *	KH_2PO_4	K_2HPO_4	$CaCO_3$
Bioaugmentation	39.3	39.3	16.9	**	1.5	2.9	-
Temperature	37.3	37.3	16.0	5.3	-	-	4.0

* Except where otherwise specified, compost was used as the inoculum.
** The innoculi used were none for the first reactor, activated soil (5.6 %) for the second, compost (5.6 %) for the third, a consortium known to degrade hydrocarbons for the fourth (5.6 %) and all three (16 %) for the fifth.

APPENDIX E

OPERATING CONDITIONS AND RESULTS IN THE MINI-COMPOSTER EXPERIMENTS

Table E.1. Operating Conditions and results obtained in the experiments investigating the effect of co-substrate addition.

	Dry Mass (%)		Non-Volatiles (%)		pH		MOG degraded (%)
	time =0	time =1 month	time =0	time =1 month	time =0	time =1 month	
Poisoned control	49.8	48.1	40.1	40.1	6.8	-	0
100 % soil	21.4	11.1	88.0	87.5	6.52	7.03	1.0
100 % soil with nut.	20.9	15.4	87.0	87.2	6.85	6.83	5.6
75 % soil	33.0	27.6	68.6	78.4	6.32	8.03	18.7
48 % soil	43.1	42.2	57.4	67.9	6.49	8.83	33.3
24 % soil	51.1	54.5	35.5	51.3	6.37	8.90	57.0
0 % soil	52.4	61.3	20.1	33.4	6.45	9.08	62.7

Table E.2. Operating Conditions and results obtained in the experiments investigating the effect of Ca and P addition.

	Dry Mass (%)		Non-Volatiles (%)		pH		MOG degraded (%)
	time =0	time =1 month	time =0	time =1 month	time =0	time =1 month	
No elements clean soil	49.0	47.3	47.9	57.7	5.74	9.17	33.6
P @ t=0 clean soil	51.7	39.4	48.5	64.2	6.40	8.22	49.8
Ca @ t=0 clean soil	50.4	38.3	49.4	63.6	6.82	8.74	63.7
No elements	47.8	36.3	42.4	52.7	5.75	8.37	22.5
P @ t=0	50.1	37.2	42.7	55.1	6.42	8.79	34.7
Ca @ t=0, P @ t=2wks	48.4	49.8	44.1	55.0	6.61	7.60	48.9
Ca & P @ t=0	48.3	41.5	43.8	57.3	6.39	8.74	27.1
Ca & P @ t=0, double	48.3	42.6	45.2	55.2	6.35	8.76	22.4
Ca @ t=0	47.3	41.6	40.3	53.3	6.56	8.59	39.3
Ca @ t=0 double	48.4	34.9	41.8	53.7	6.56	8.14	42.7
P @ t=2wks	47.6	39.8	39.5	53.8	5.60	7.92	42.8
P @ t=2 wks double	47.8	38.7	43.1	56.3	5.55	7.87	41.4

Table E.3. Operating conditions and results obtained in the experiments investigating the effect of the initial C/N ratio.

	Dry Mass (%)		Non-Volatiles (%)		pH		MOG degraded (%)
	t=0	t=1 month	t=0	t=1 month	t=0	t=1 month	
C/N=15	59.1	37.7	38.5	56.8	6.57	8.70	43.0
C/N=15, clean soil	60.6	36.6	42.2	55.9	6.42	8.01	45.0
C/N=17	46.3	40.1	41.6	51.9	6.66	7.15	40.1
C/N=17, clean soil	48.7	41.5	49.2	59.2	6.63	7.12	48.3
C/N=20	50.1	36.1	41.9	56.0	6.42	8.79	34.0
C/N=20, clean soil	51.6	39.4	48.5	64.2	6.40	8.22	50.3
C/N=25	42.9	37.4	41.1	49.4	6.48	6.05	19.6
C/N=25, clean soil	45.7	41.2	44.1	52.4	6.59	6.04	35.6
C/N=30	48.0	33.3	38.6	50.1	6.42	7.44	13.0
C/N=30, clean soil	45.1	33.8	44.2	53.4	6.31	6.70	15.0
C/N=40	44.7	32.8	39.1	45.4	6.48	6.45	0.0
C/N=40, clean soil	44.0	34.1	45.1	49.6	6.37	6.07	0.0

Table E.4. Operating conditions and results obtained in the experiments investigating the effect of bioaugmentation.

	Dry Mass (%)		Non-Volatiles (%)		pH		MOG degraded (%)
	t=0	t=1 month	t=0	t=1 month	t=0	t=1 month	
No inoculum	47.0	45.0	41.4	52.4	6.81	7.27	36.5
Concentrated soil culture	47.0	46.0	42.9	54.6	6.88	7.55	36.5
Compost	48.9	42.5	40.9	54.6	6.90	8.25	27.7
Hydrocarbon degrading consortium	47.4	49.9	43.2	53.0	6.90	7.91	31.5
All 3 inoculi	48.5	46.4	43.4	53.2	6.90	7.89	20.4

Table E.5. Operating conditions and results obtained in the experiments investigating the effect of the temperature of the plateau.

	Dry Mass (%)		Non-Volatiles (%)		pH		MOG degrad-ed (%)
	t=0	t=1 month	t=0	t=1 month	t=0	t=1 month	
Temperature 20°C-6 days then ambient	48.7	53.9	40.8	57.4	6.80	7.95	56.5
Temp. peak= 20°C, double	48.7	46.3	40.8	57.1	6.80	7.97	57.3
Temp. peak= 30°C,	48.5	37.4	40.9	52.8	6.79	7.65	36.1
Temp. peak= 30°C, double	48.5	37.5	40.9	52.6	6.79	7.57	39.2
Temp. peak= 40°C	48.5	39.9	40.9	55.0	6.79	7.69	34.7
Temp. peak= 40°C, double	48.5	59.2	40.9	56.8	6.79	7.57	33.0
Temp. peak= 50°C	47.7	54.9	39.7	52.4	6.70		43.3
Tempe. peak= 50°C, double	48.7	56.8	40.8	55.4	6.80	8.28	50.0
Temp. peak= 60°C	48.5	33.0	40.9	56.0	6.79	7.79	24.1
Temp. peak= 60°C, double	48.5	43.6	40.9	56.3	6.79	7.88	23.0

Table E.6. Operating conditions and results obtained in the experiments investigating the effect of the length of the thermophilic phase.

	Dry Mass (%)		Non-Volatiles (%)		pH		MOG degraded (%)
	t=0	t=1 month	t=0	t=1 month	t=0	t=1 month	
Temp. peak= 50°C-6 days, then ambient	48.7	56.8	40.8	55.4	6.7	8.28	50.0
Temp. peak= 50°C-11 days then ambient	48.7	41.2	40.8	60.2	6.7	8.29	58.5
Temp. peak= 50°C-11 days, double	48.7	45.7	40.8	56.8	6.7	8.12	49.6
Temp. peak= 50°C-21 days then ambient	48.7	49.9	40.8	58.1	6.7	8.26	62.1
Temp. peak= 50°C-31 days then ambient	48.7	59.3	40.8	61.8	6.7	8.26	71.9
Temp. peak= 50°C-30 days, double	48.7	50.3	40.8	61.5	6.7	8.3	68.5

Table E.7. Operating conditions and results of miscellaneous experiments investigating the effect of temperature.

	Dry Mass (%)		Non-Volatiles (%)		pH		MOG degraded (%)
	t=0	t=1 month	t=0	t=1 month	t=0	t=1 month	
Temp. peak= 40°C-6 days, then 40°C	48.5	34.2	40.9	61.7	6.79	8.05	42.6
Temp. peak= 40°C-6 days, 40°C, double	48.5	44.1	40.9	58.3	6.79	8.12	40.8
Temp. peak= 50°C-6 days, then 38°C	49.7	33.3	40.8	57.2	6.7	-	64.4
Temp. peak= 50°C-6 days, 38°C, double	49.7	32.5	40.8	57.3	6.7	-	57.9

APPENDIX F

CO_2 GENERATION CURVES OBTAINED IN THE MINI-COMPOSTER EXPERIMENTS

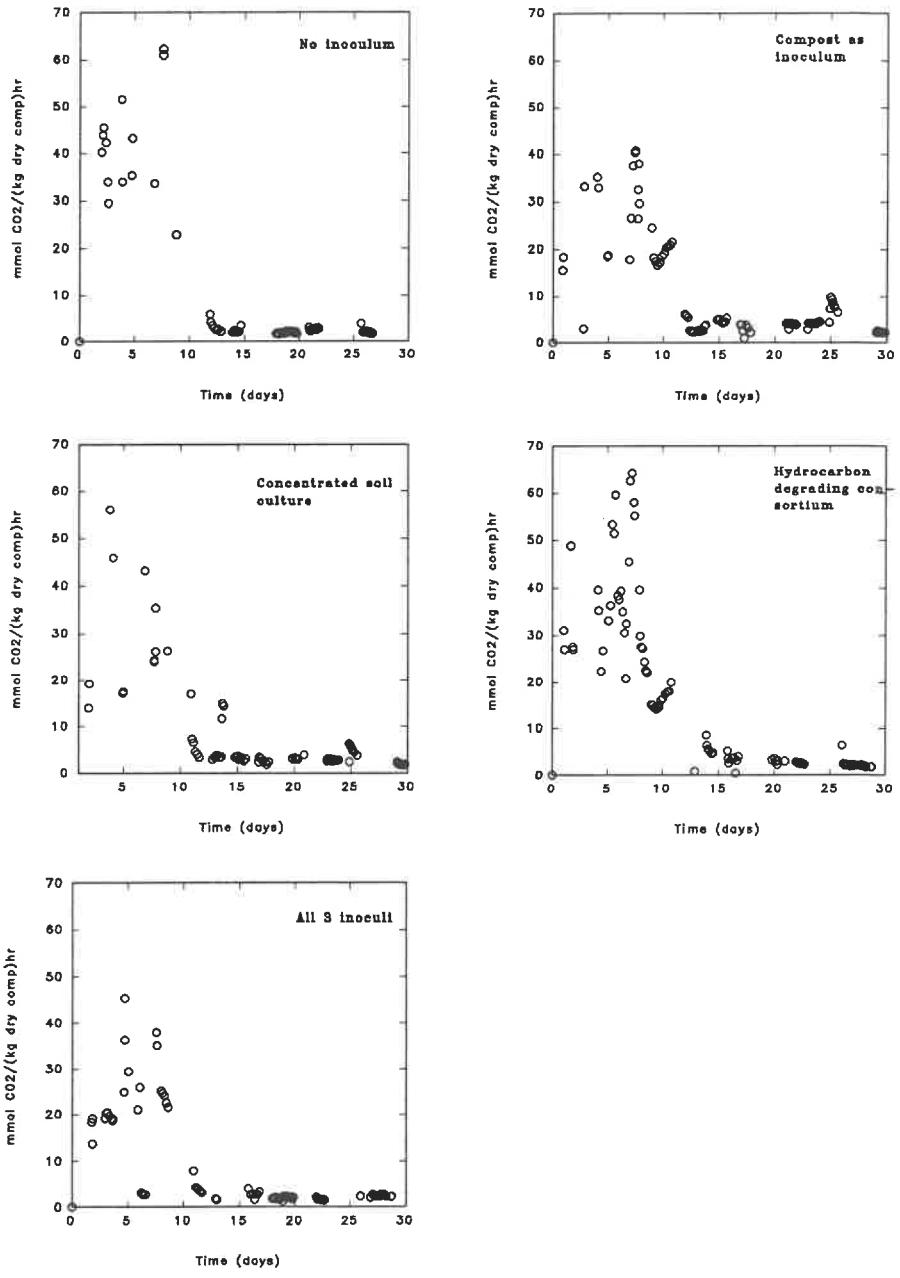


Figure F.1. CO_2 generation curves in the bio-augmentation experiments.

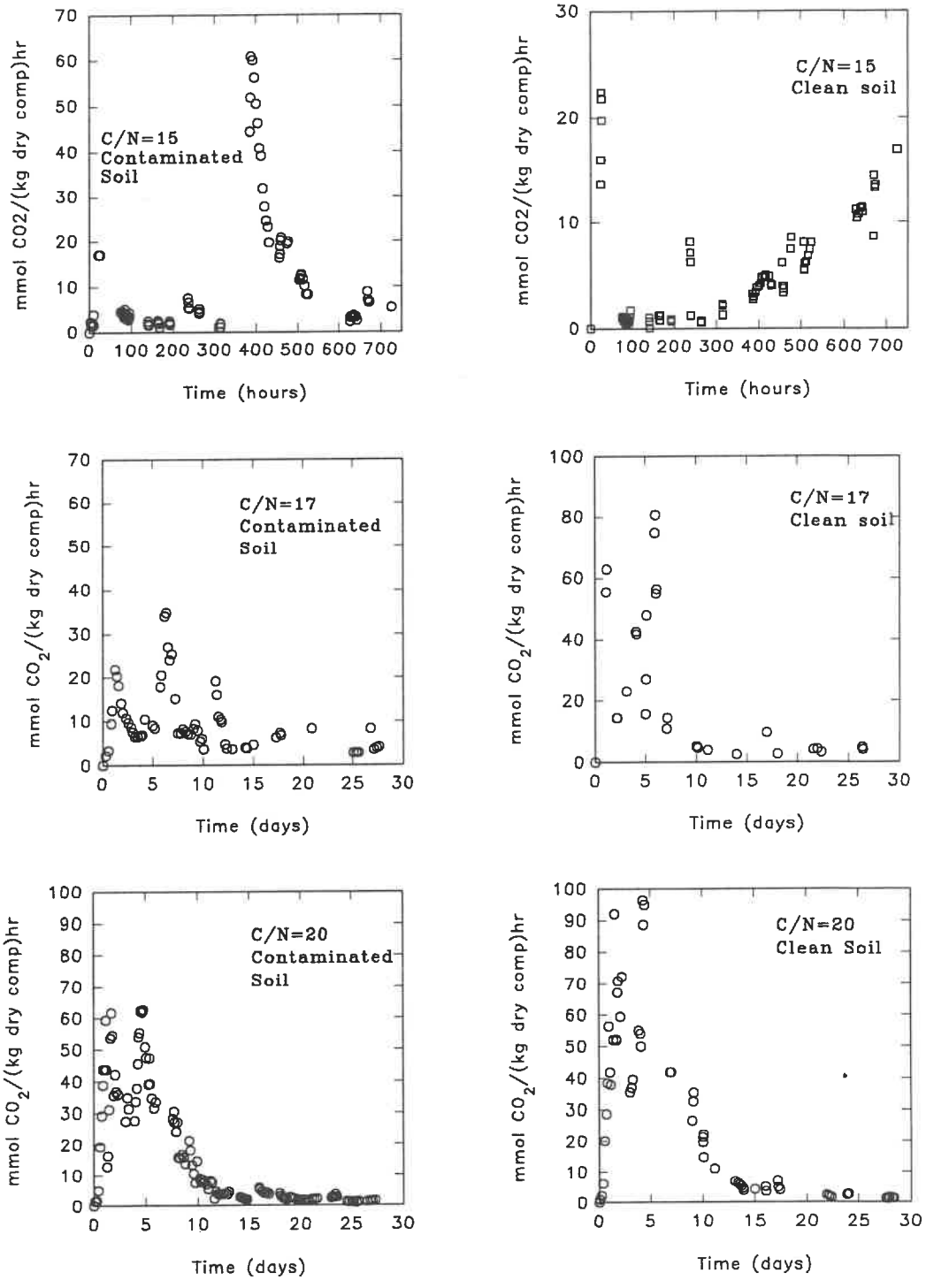


Figure F.2. CO_2 generation curves obtained in the C/N ratio experiments.

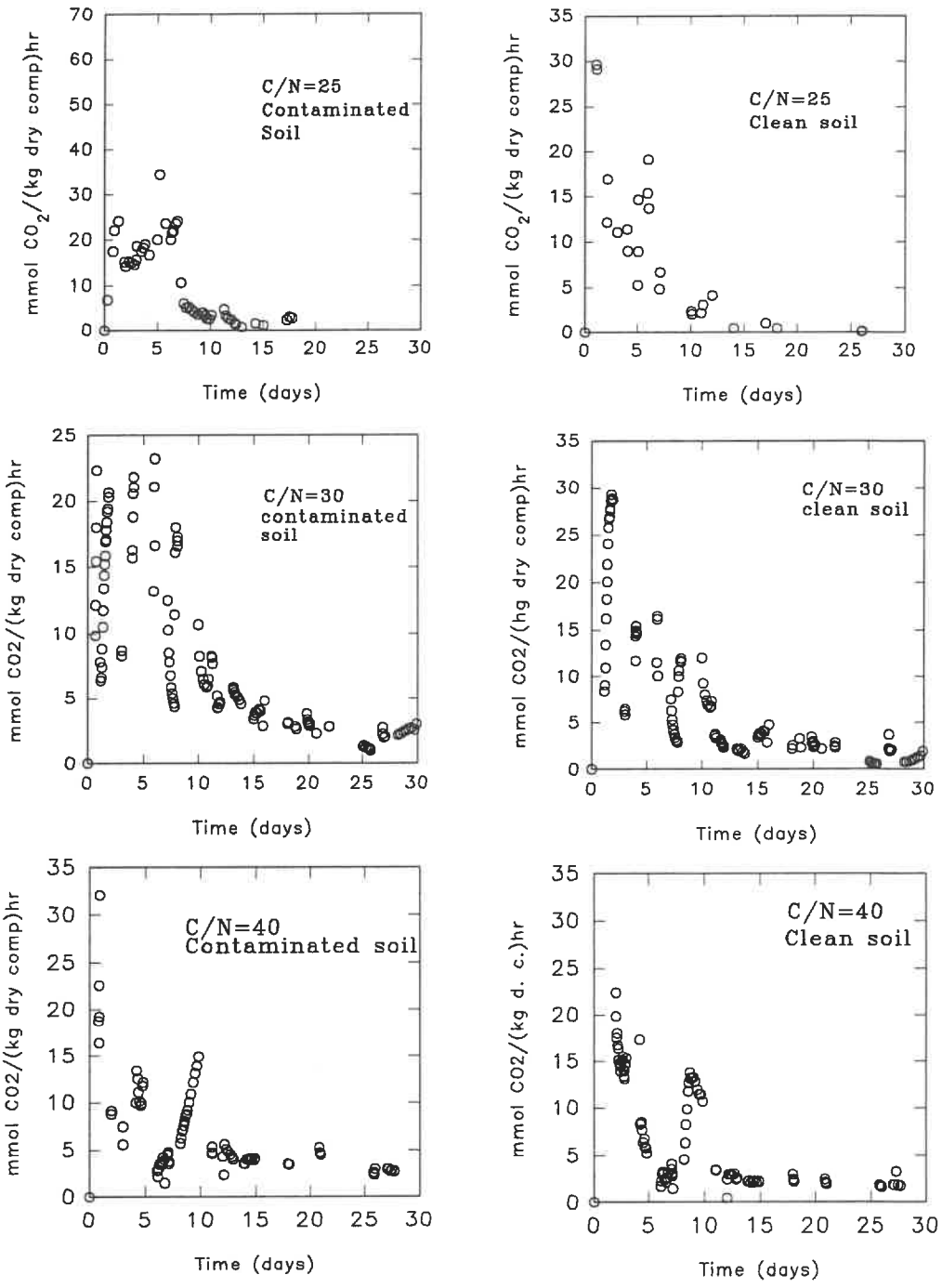


Figure F.2. (continued).

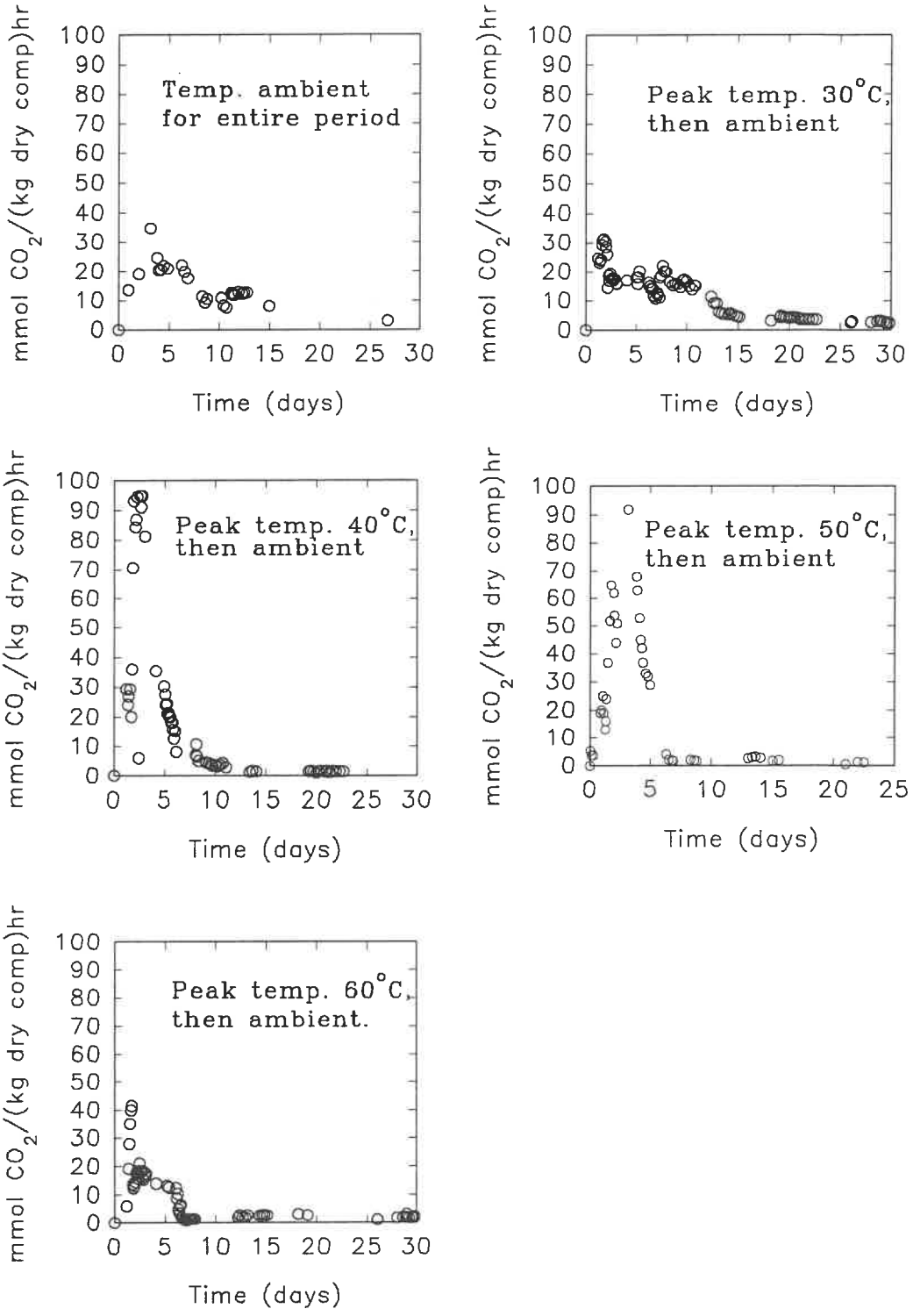


Figure F.3. CO_2 generation curves for the experiment investigating the effect of varying the temperature of the plateau.

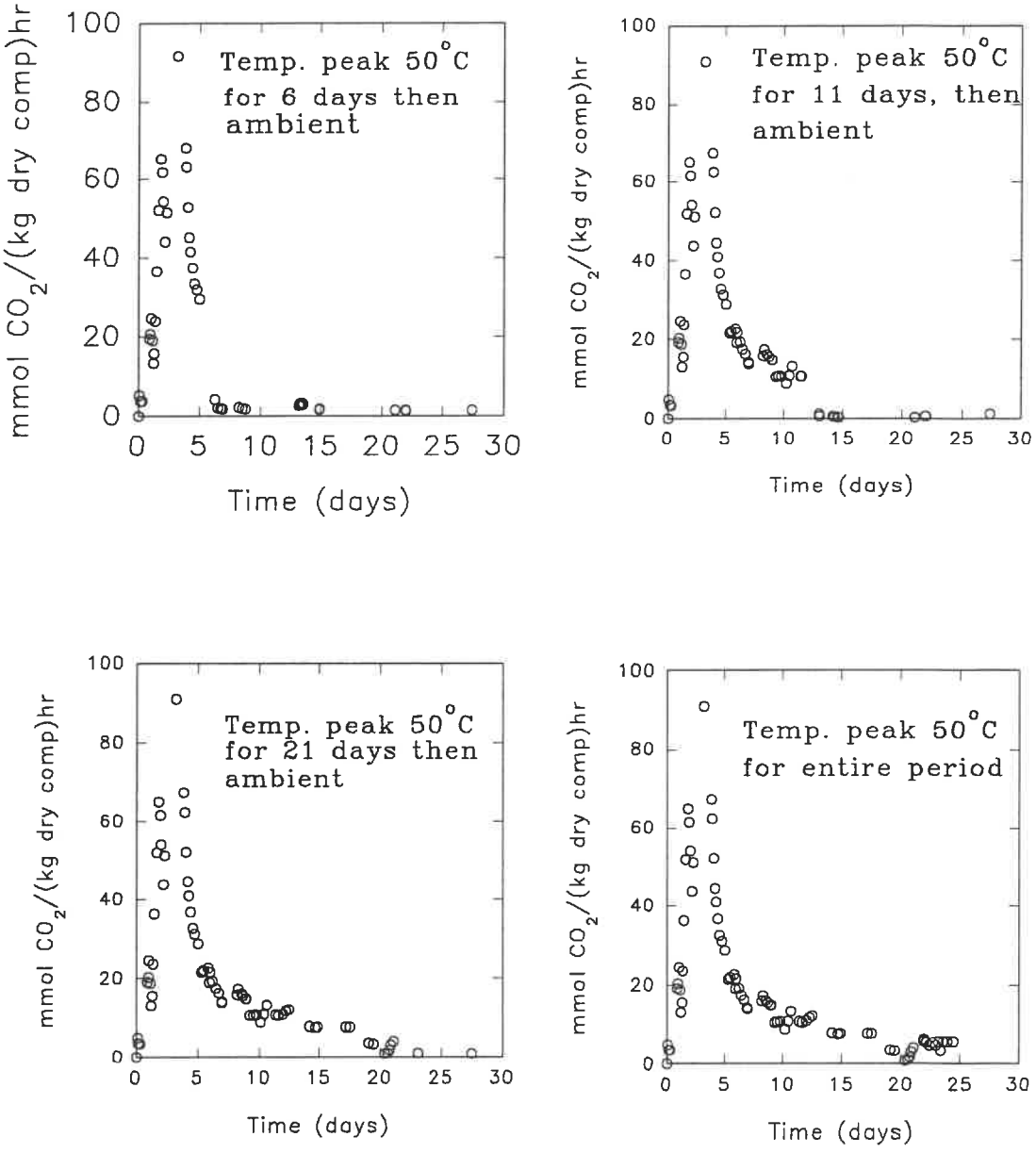
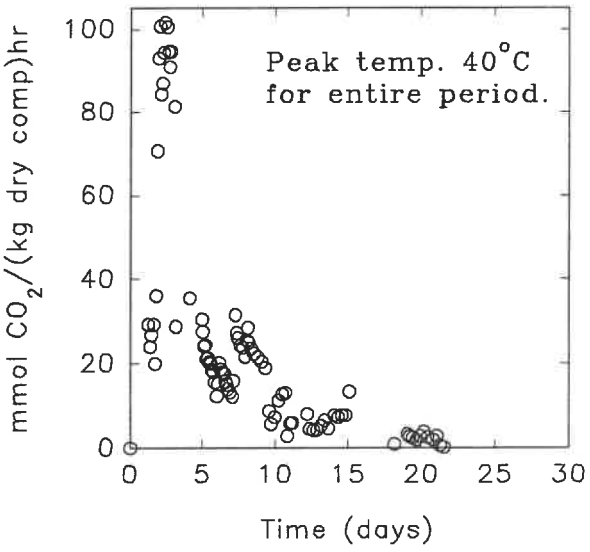


Figure F.4. CO_2 generation curves for the experiments investigating the effect of the duration of the plateau at 50°C.



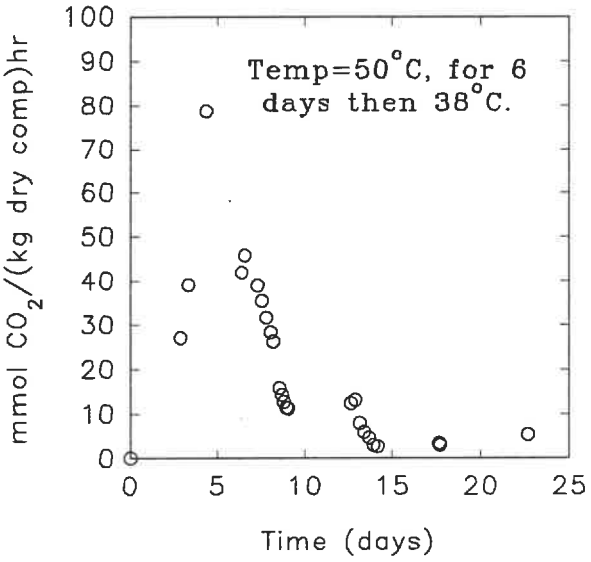


Figure F.5. CO_2 generation curves for the miscellaneous runs.

APPENDIX G

IMPOSED TEMPERATURE PROFILE IN THE MINI-COMPOSTER EXPERIMENTS

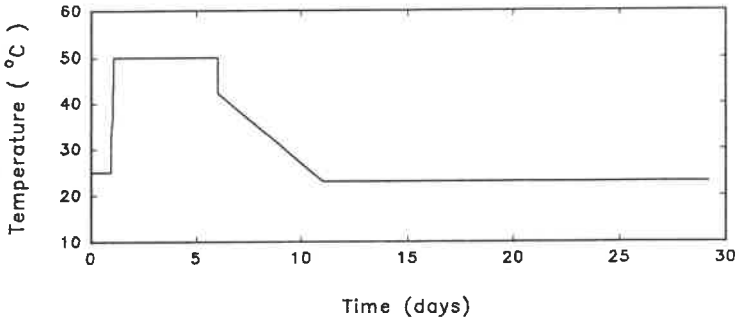


Figure G.1. Imposed temperature profile for the experiments investigating the effect of the following parameters: bioaugmentation, C/N ratio, and Ca and P addition.

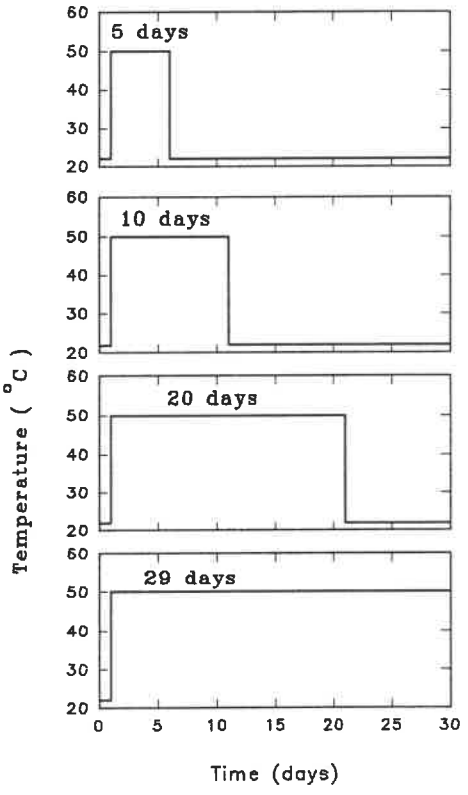


Figure G.2. Imposed temperature profile for the experiment investigating the effect of the length of the thermophilic phase.

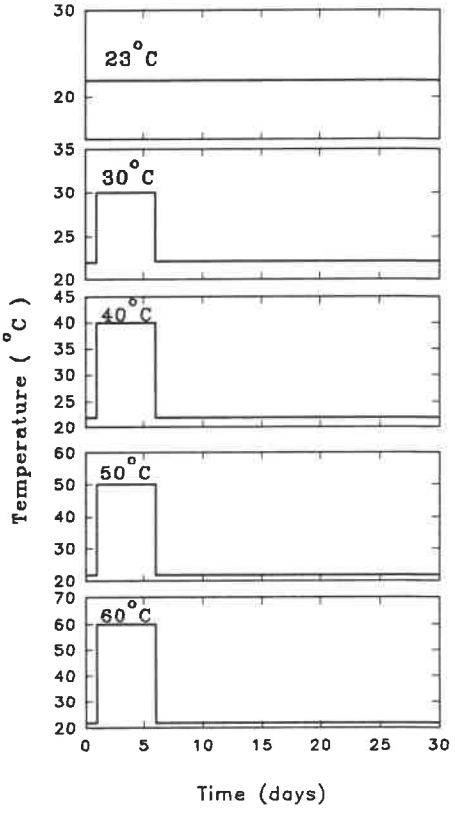


Figure G.3. Imposed temperature profile for the experiment investiogating the effect of the temperature of the plateau.

APPENDIX H

CHARACTERIZATION OF HYDROCARBON-CONTAMINATED SOIL

Particle size: 83.5 % sand (0.75mm<dp<2mm

14.7 % stones (dp>2mm)

1.8 % particles of several mm (probably oil saturated coal particles)

Non-volatiles (%): 89.5

C/N/P: 39/1/0

MOG (ppm): 17,000

No heavy metal content.

Table H.1. Fractionation of MOG components in hydrocarbon-contaminated soil.

Component	Column Chromatography (% (wt/wt))	HPLC Analysis (% (wt/wt))
Aliphatics	40	21.1
1-Ring Aromatics	-	9.2
2-Ring Aromatics	-	9.0
3-Ring Aromatics	-	9.5
4^{+}-Ring Aromatics	-	16.6
Total Aromatics	28	44.3
Polars	32	34.6

Table H.2. Boiling range distribution of extractable hydrocarbons in soil.

Percent off	Temperature (°C)
5	329
10	388
20	491
30	501
40	565
50	694