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APPENDICES

APPENDIX A

Standard curves of TCE and INTF

Standard curve of TCE

In each soil microcosm, standard curve was set as the same manner of soil microcosm. TCE stock solution was added to obtain the desired concentration (triplicate per each of concentration). The standards were analyzed similar to sample procedure.

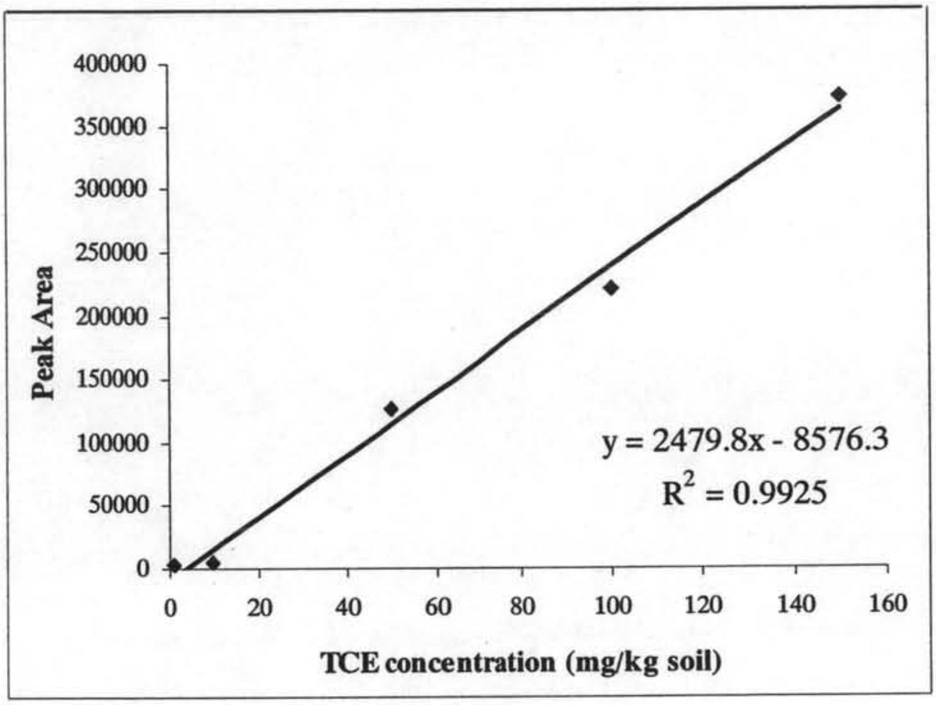

Figure A-1 Standard curves of TCE in soil

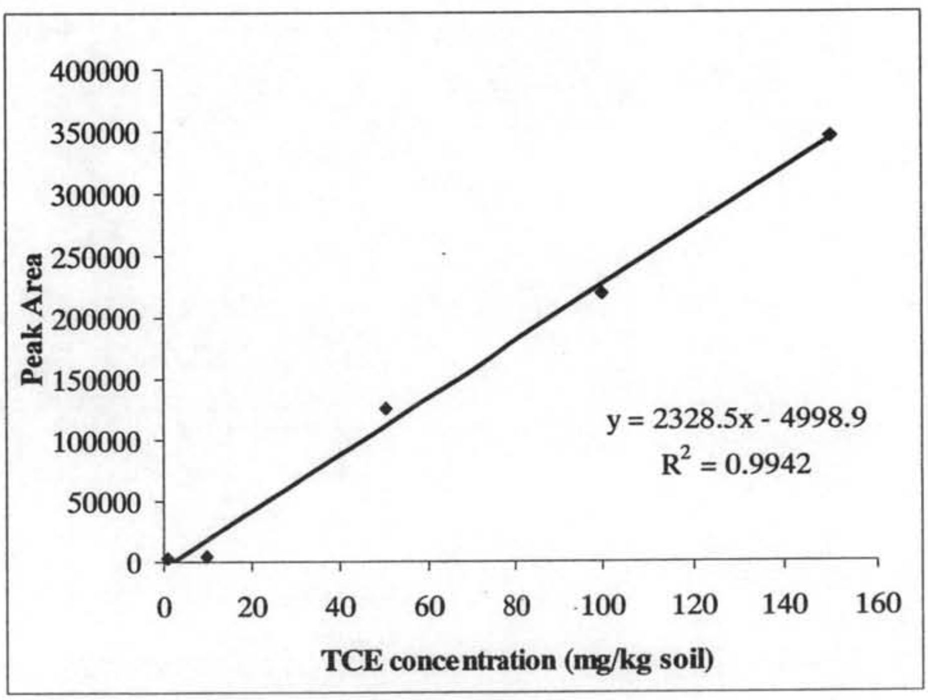

Figure A-2 Standard curves of TCE in soil containing corncob

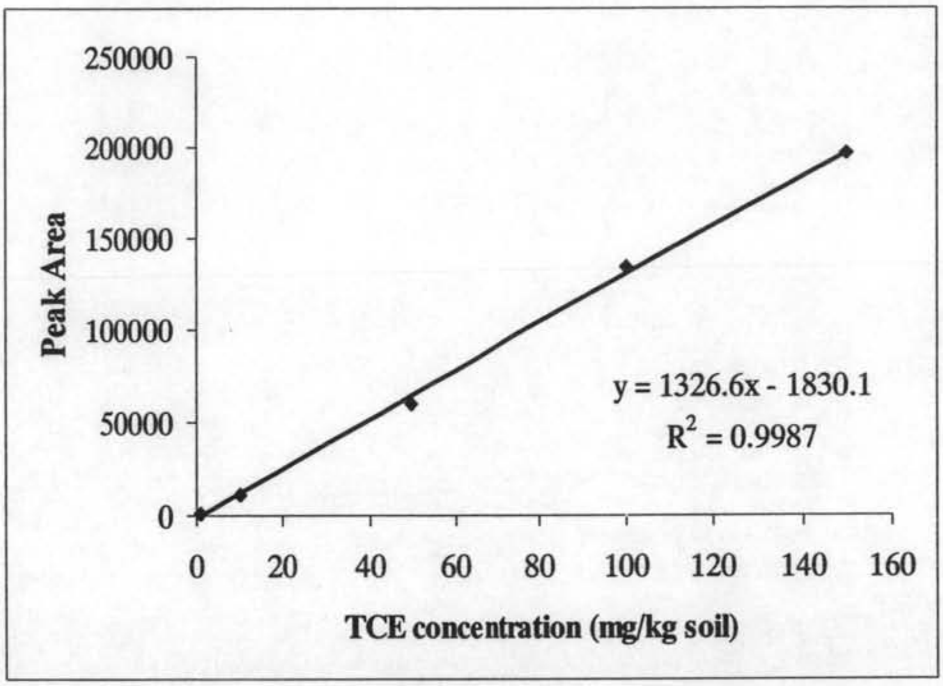

Figure A-3 Standard curves of TCE in soil containing coir

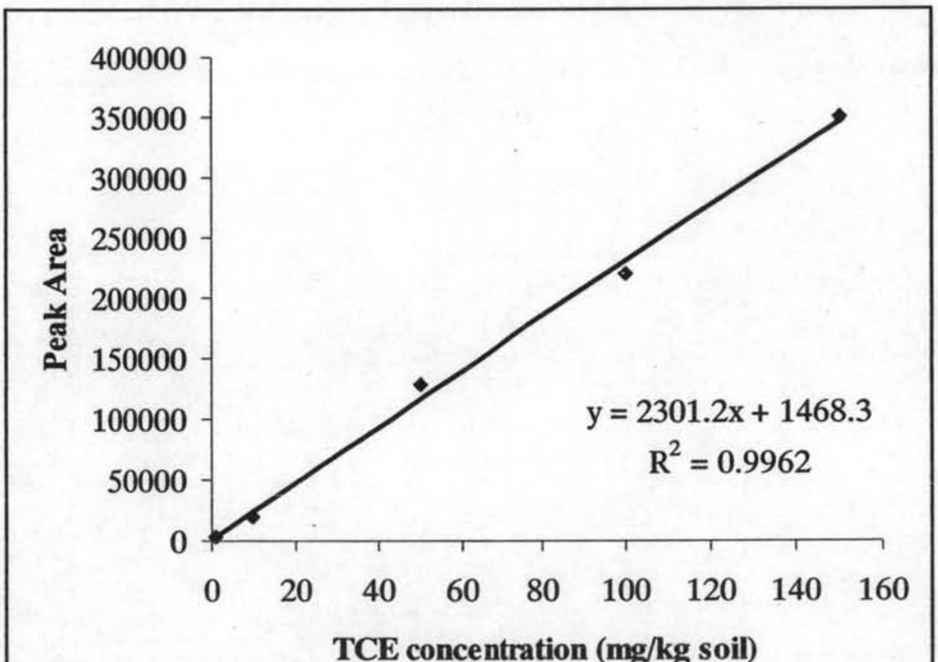

Figure A-4 Standard curves of TCE in soil containing kaffir lime peel at concentration of 50 mg/kg

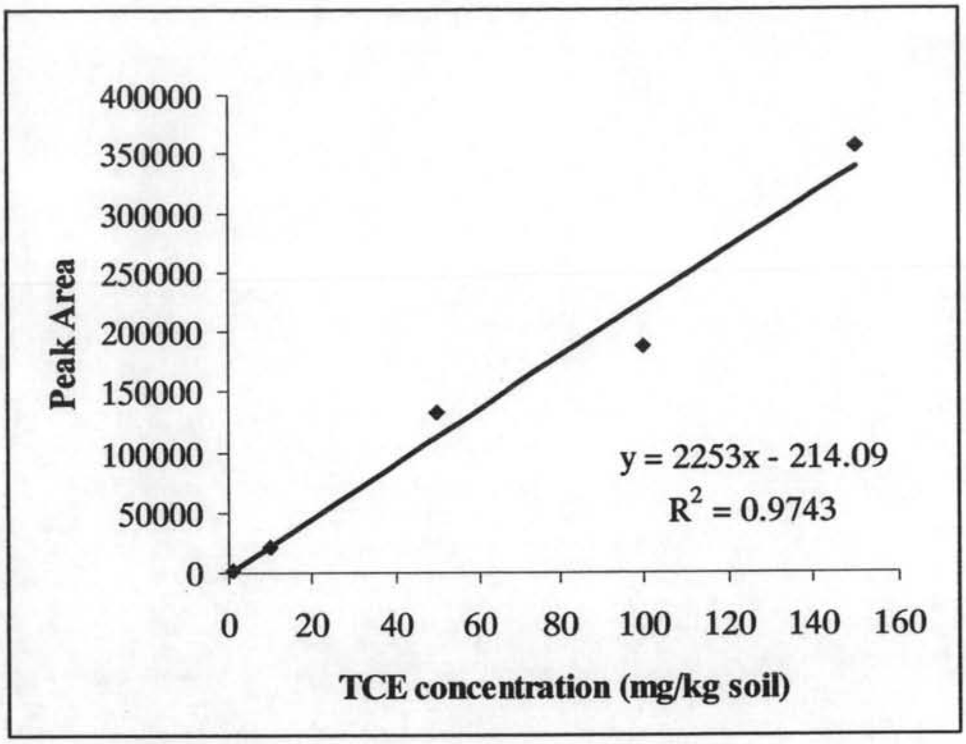

Figure A-5 Standard curves of TCE in soil containing kaffir lime peel at concentration of 100 mg/kg

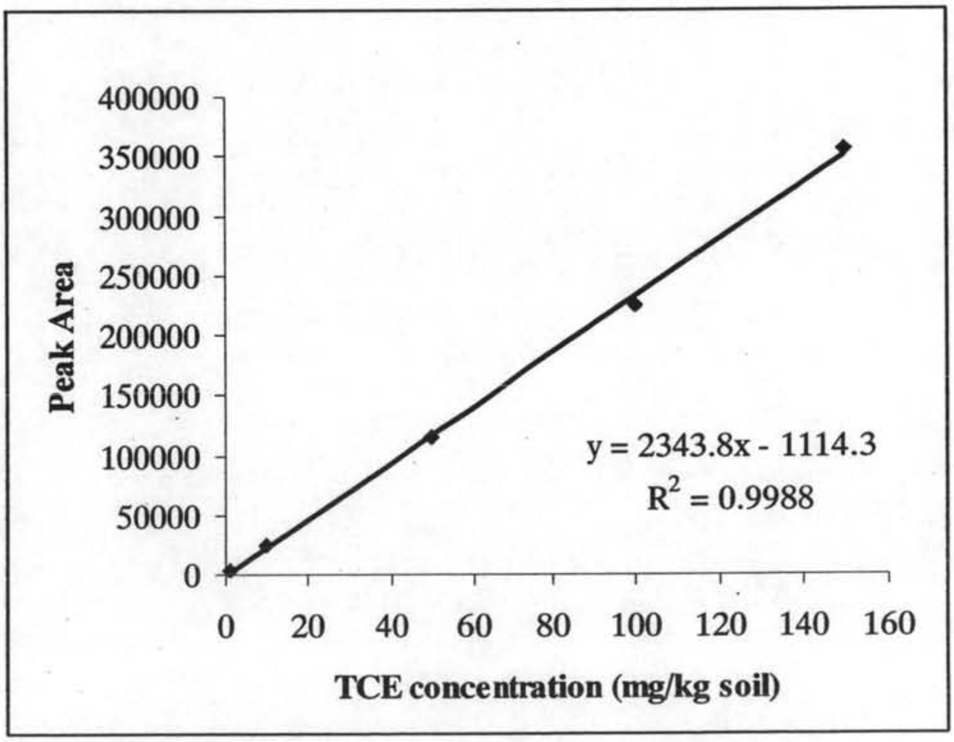

Figure A-6 Standard curves of TCE in soil containing kaffir lime peel at concentration of 150 mg/kg

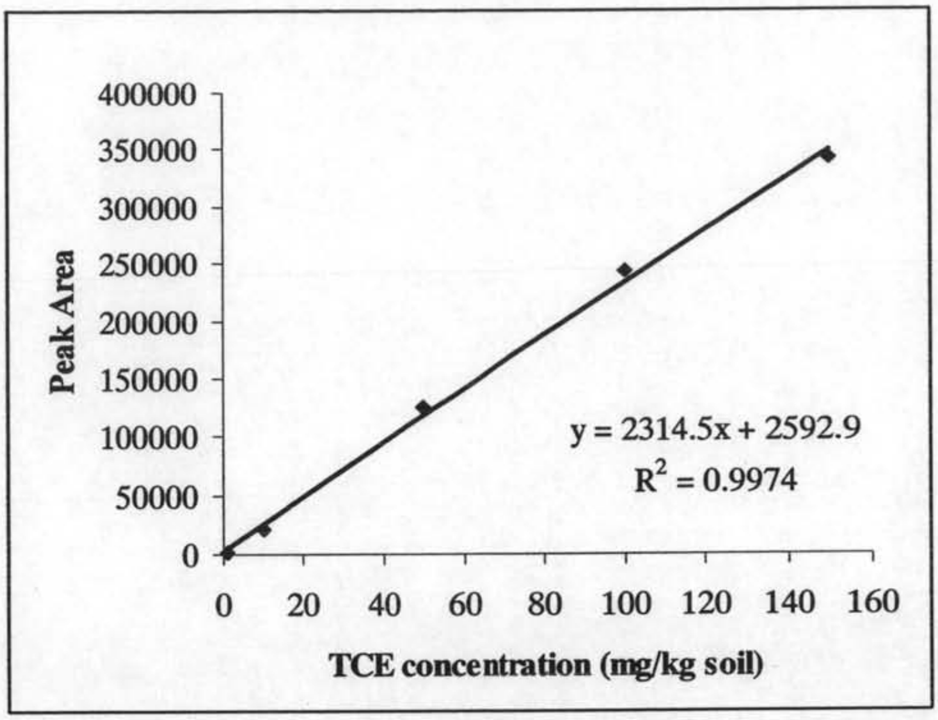

Figure A-7 Standard curves of TCE in soil containing kaffir lime peel at concentration of 250 mg/kg

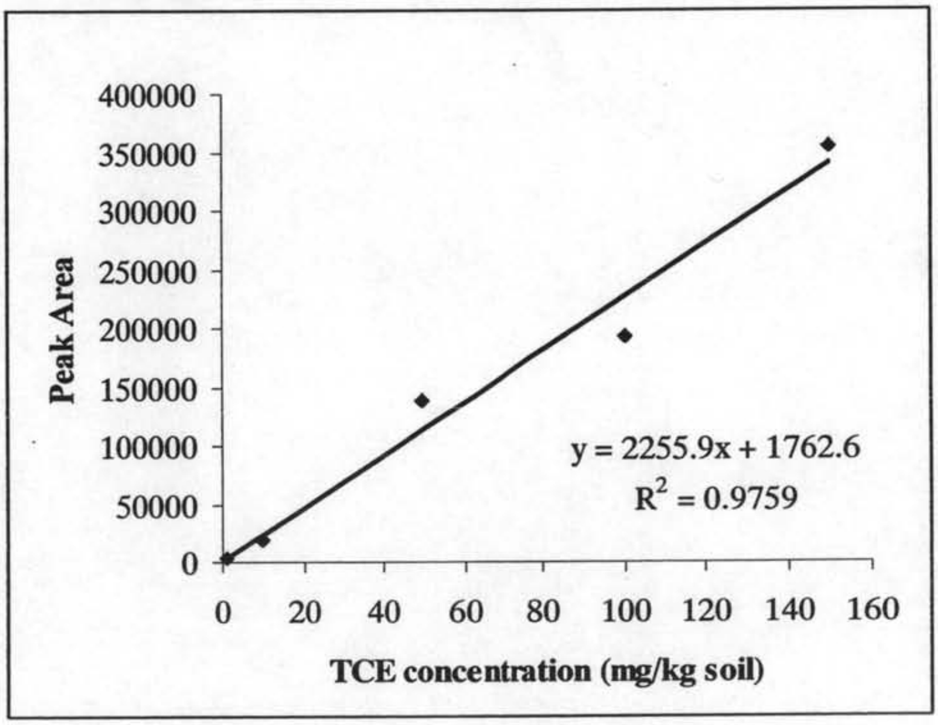

Figure A-8 Standard curves of TCE in soil adjusted C:N to 20:1 by cassava pulp

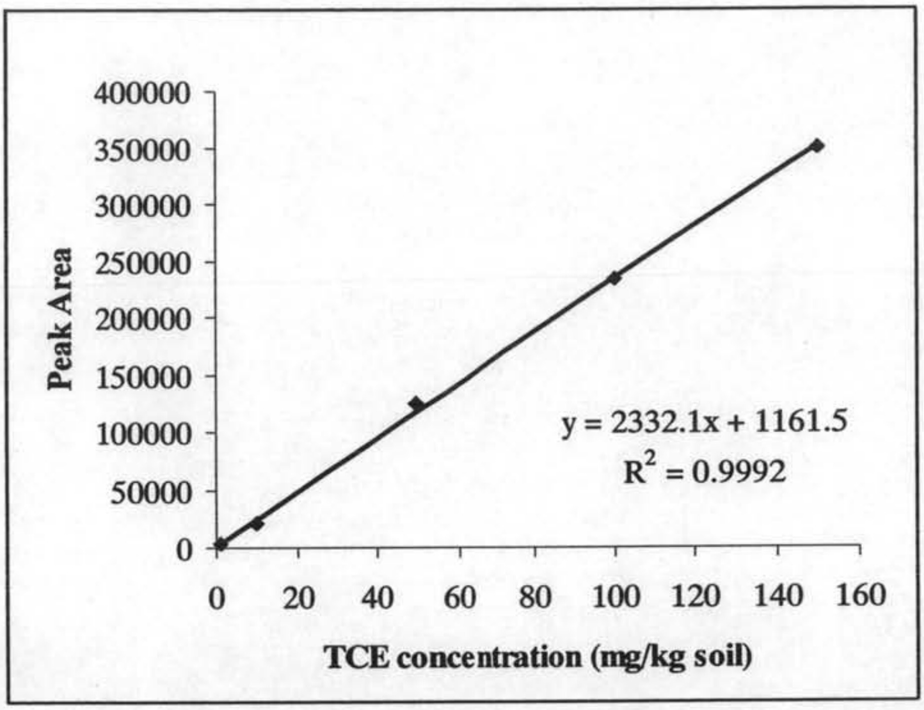

Figure A-9 Standard curves of TCE in soil adjusted C:N to 30:1 by cassava pulp

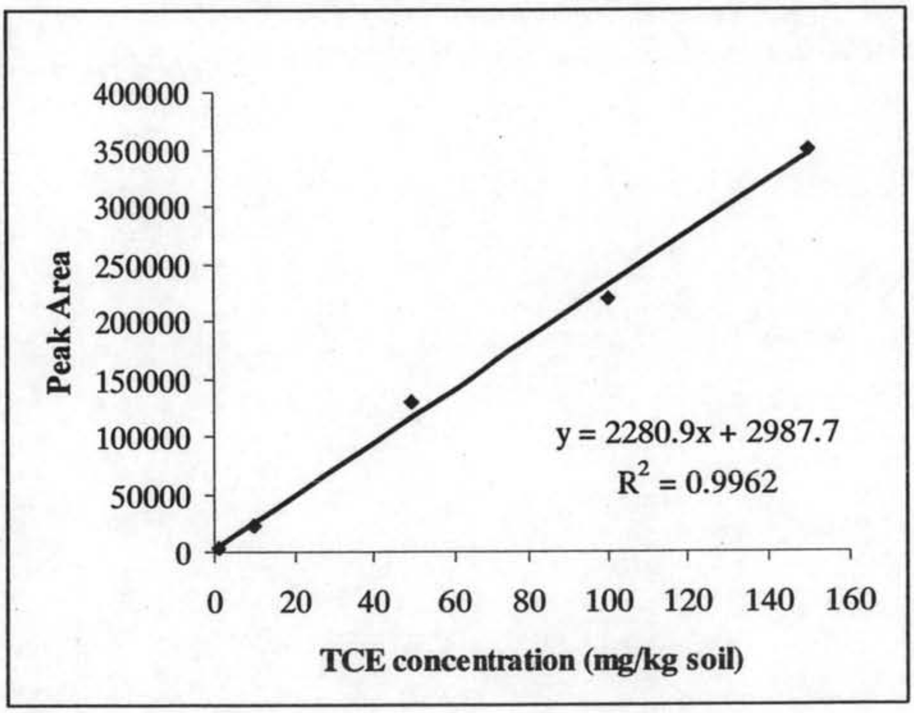

Figure A-10 Standard curves of TCE in soil adjusted C:N to 30:1 by cassava pulp

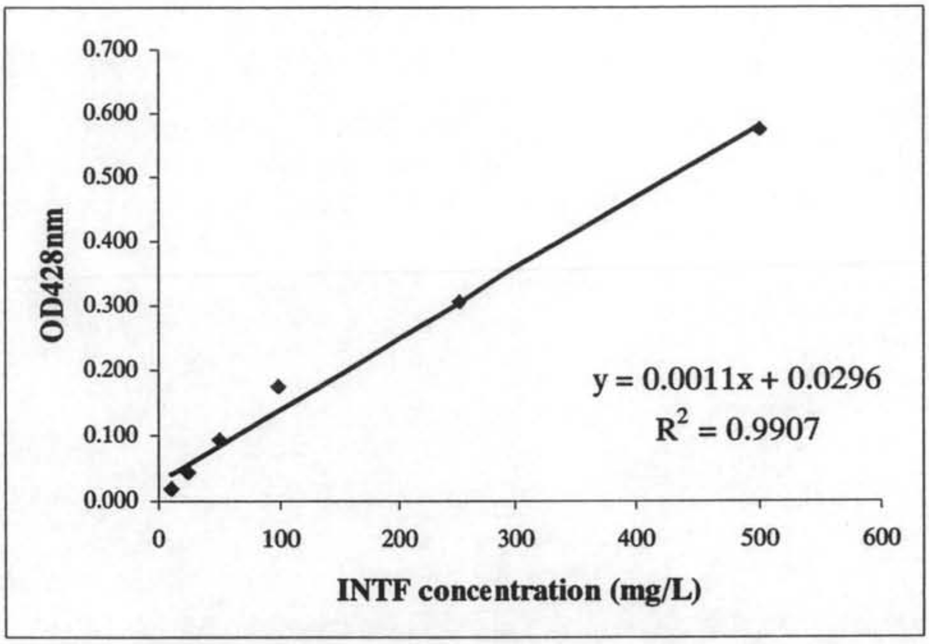

Figure A-11 Standard curve of INTF in methanol

APPENDIX B

Percent recovery of TCE in soil microcosm

Percent recovery of TCE in soil microcosm

TCE recoveries were determined for soil microcosm in triplicate. In each soil microcosm, TCE was spiked to obtained final concentration of 100 mg/kg, leaved for half of hour and then TCE recoveries were analyzed similar to sample procedures. Percent recoveries of TCE were shown in Table B-1.

Table B-1 Percent recovery of TCE in soil microcosm spiked with 100 mg/kg of TCE

Treatments	TCE concentration (mg/kg)				% Recovery
	Replication 1	Replication 2	Replication 3	Average	
S(1)	88.91	88.57	88.71	88.73	88.73±0.17(2)
S-ICC	87.34	89.14	92.28	89.59	89.59±2.50
S-ICO	105.47	113.95	114.82	111.41	111.41±5.16
S-K-50(3)	99.02	97.95	92.13	96.37	96.37±3.71
S-K-100	100.18	98.54	91.83	96.85	96.85±4.42
S-K-150	103.29	89.65	94.33	95.76	95.76±6.93
S-K-250	99.44	97.80	91.09	96.11	96.11±4.42
S-C-20(4)	98.88	89.05	80.84	89.59	89.59±9.03
S-C-30	93.78	97.08	88.02	92.96	92.96±4.58
S-C-40	95.62	98.99	89.76	94.79	94.79±4.67

(1) S=soil, ICC=immobilized on corncob, ICO=immobilized on coir, K=kaffir lime peel, C=cassava pulp

(2) Value are expressed as the mean and standard deviation of three replicates.

(3) Concentration of kaffir lime peel (mg/kg soil)

(4) C:N ratios

APPENDIX C

Degradation profiles of TCE in soil microcosms

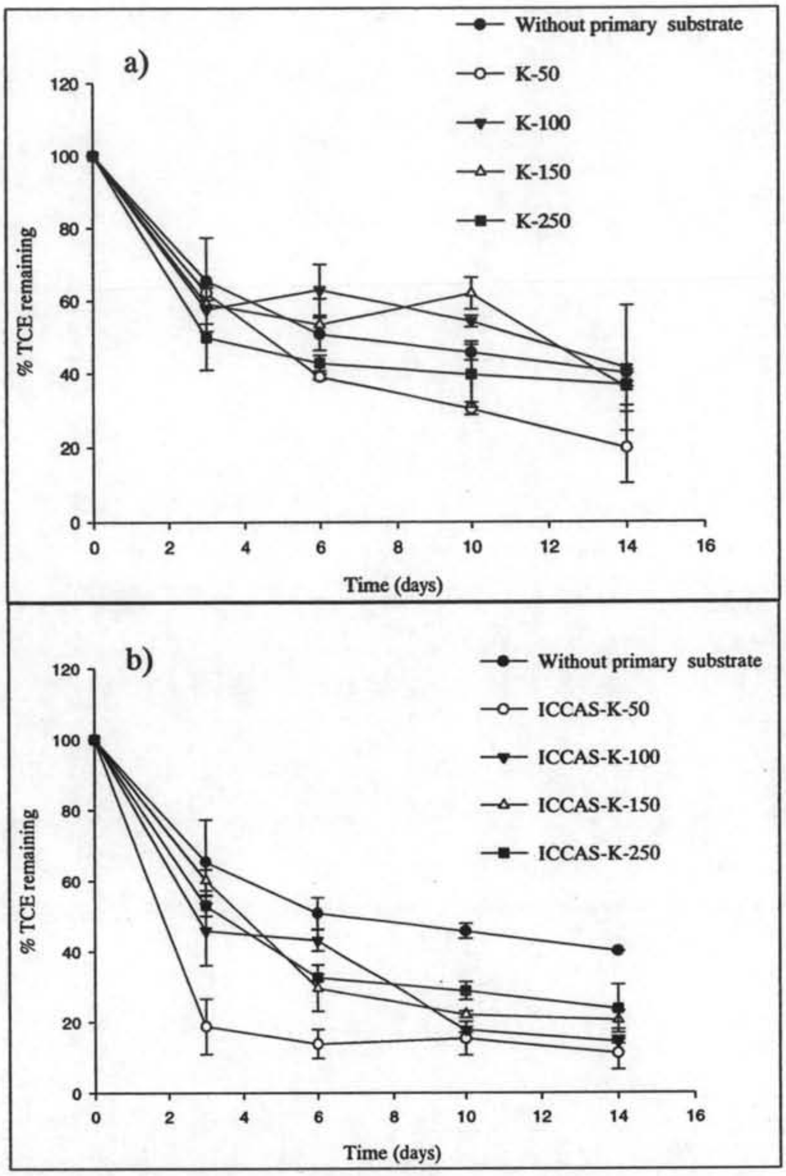

Figure C-1 Degradation profiles of TCE in soil microcosm added with various concentration of kaffir lime peel as a primary substrate (ICCAS= immobilized acclimatized activated sludge on corncob; K= kaffir lime peel)

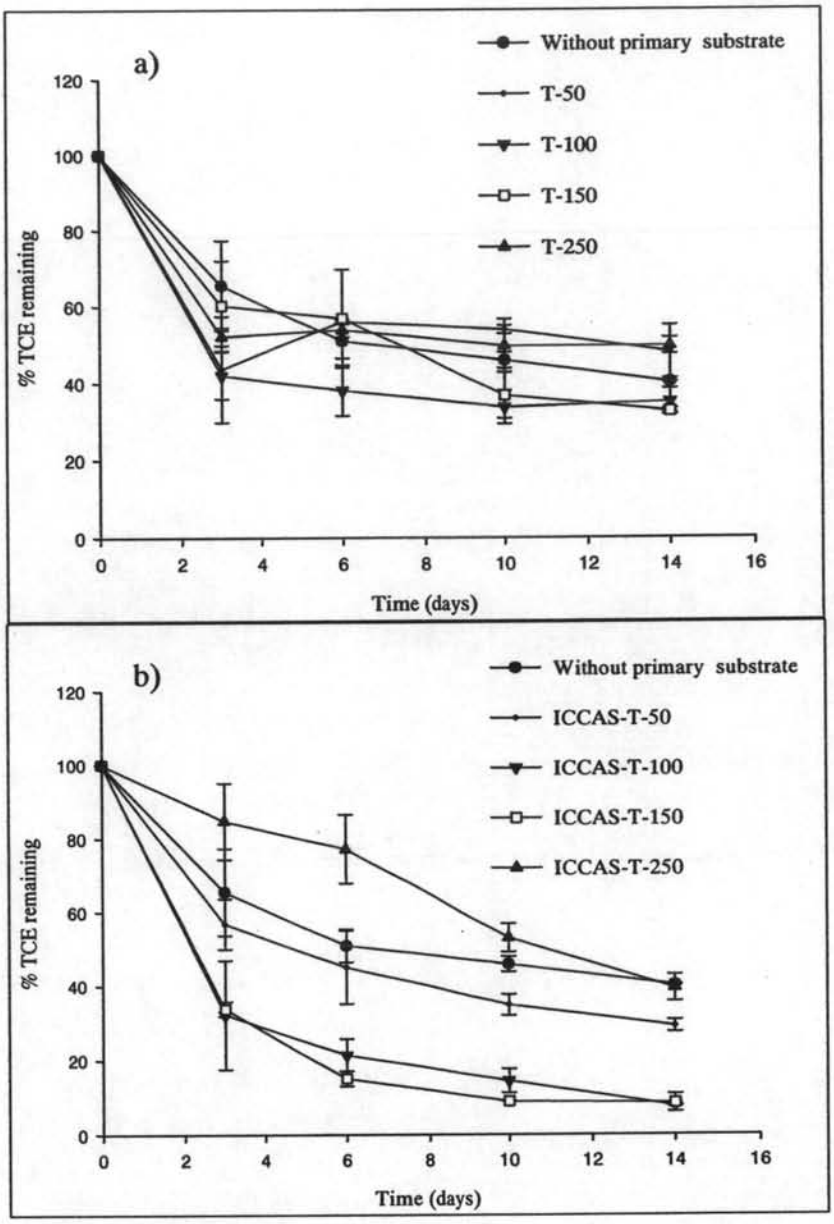

Figure C-2 Degradation profiles of TCE in soil microcosm added with various concentration of toluene as a primary substrate (ICCAS= immobilized acclimatized activated sludge on corncob; T=toluene)

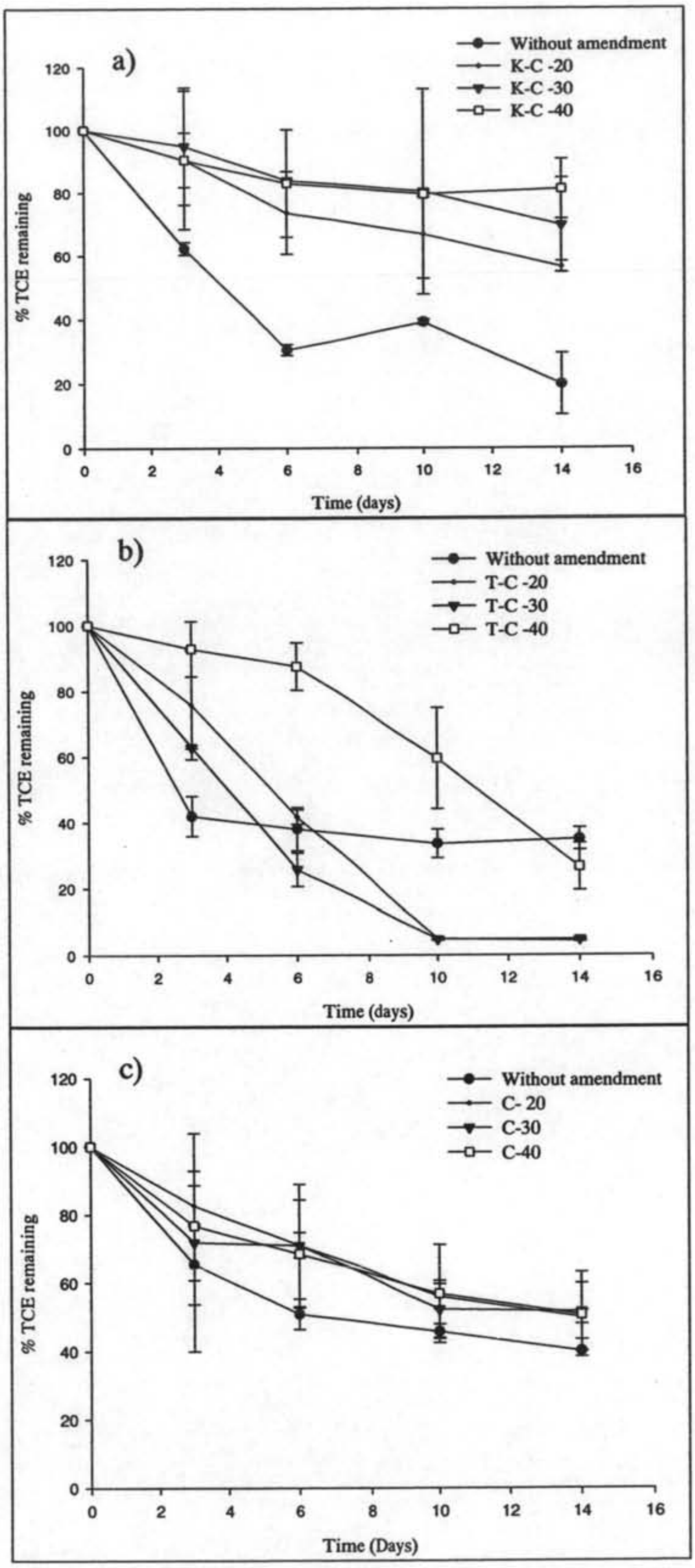

Figure C-3 Degradation profiles of TCE in soil microcosm adjusted by cassava pulp at various C:N ratios (K= 50 mg/kg of kaffir lime peel; T= 100 mg/kg of toluene; C= cassava pulps)

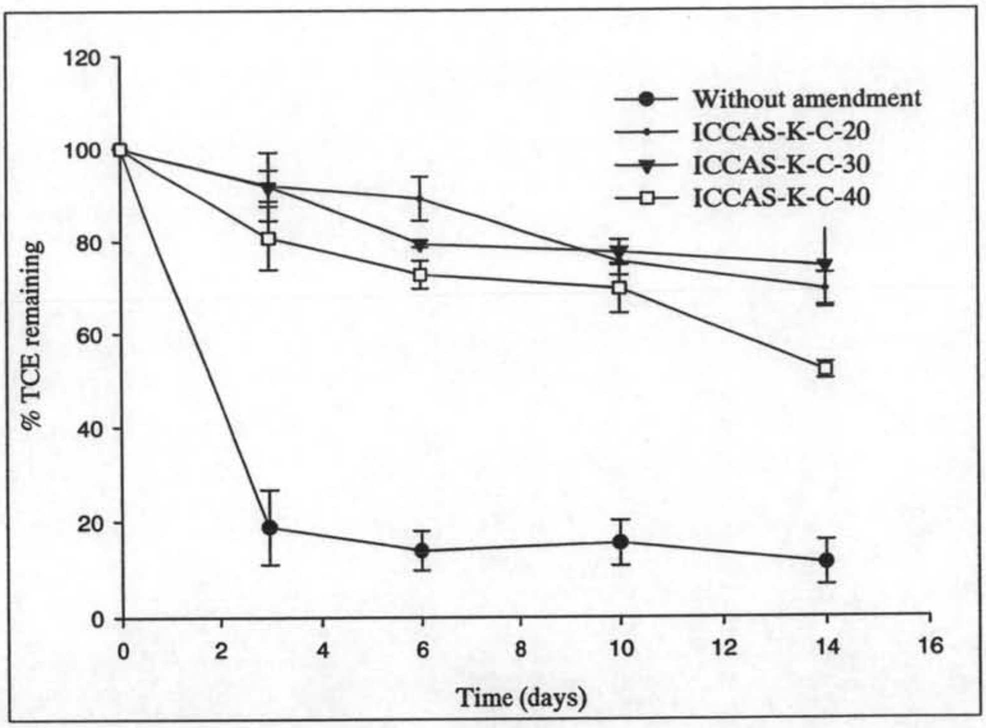

Figure C-4 Degradation profiles of TCE in soil microcosm augmented with immobilized acclimatized activated sludge on corncob and adjusted by cassava pulp at various C:N ratios (ICCAS= immobilized acclimatized activated sludge on corn cob; K= 50 mg/kg of kaffir lime peel; C= cassava pulps)

APPENDIX D

Cell number of microorganisms in the soils

Table D-1 Number of TCE degrader in soil microcosm adding with various concentrations of kaffir lime peel and toluene

Time (days)	Number of TCE degrader in soil microcosm (x 10^7 CFU/g soil)																
	(without primary substrate)	Indigenous microorganism								Immobilized acclimatized activated sludge							
		Toluene (mg/kg)				Kaffir lime peel (mg/kg)				Toluene (mg/kg)				Kaffir lime peel (mg/kg)			
		50	100	150	250	50	100	150	250	50	100	150	250	50	100	150	250
0	5.70	2.52	1.67	7.45	4.65	2.56	2.35	2.81	2.18	1.83	1.24	1.67	1.93	2.43	4.62	8.40	1.75
3	1.75	7.35	4.50	3.50	3.00	11.80	1.43	6.30	3.50	14.80	16.30	13.00	15.80	6.65	1.05	1.43	0.21
6	1.95	6.90	6.40	6.55	5.20	7.55	1.45	0.81	5.85	25.10	17.80	12.00	13.70	16.90	1.90	1.66	1.45
10	8.25	5.45	4.30	13.10	7.90	5.60	1.48	5.00	4.90	7.20	14.00	5.15	6.10	13.30	5.70	6.75	8.10
14	6.25	10.80	6.90	8.15	11.00	0.72	12.60	0.21	0.45	13.00	13.30	8.15	10.20	3.59	5.25	8.90	6.30

Table D-2 Number of TCE degrader in soil microcosm adjusted by cassava pulps at various C:N ratio

Time (days)	Number of TCE degrader in soil microcosm (x 10^7 CFU/g soil)															
	Kiffir lime peel 50 mg/kg[1]				Toluene 100 mg/kg				Without primary substrate				ICCAS+ Kiffir lime peel 50 mg/kg[2]			
	None[3]	20:1[4]	30:1	40:1	None	20:1	30:1	40:1	None	20:1	30:1	40:1	None	20:1	30:1	40:1
0	2.56	5.40	2.80	3.62	1.67	3.15	5.50	6.35	5.70	6.90	7.40	4.35	3.79	6.30	2.26	7.20
3	11.8	2.65	5.40	4.60	4.50	39.00	14.30	8.80	1.75	19.60	15.30	7.00	5.80	10.30	5.35	1.19
6	0.75	1.15	1.32	7.65	6.40	39.00	70.50	30.50	1.95	22.50	8.65	5.15	5.55	5.20	3.50	3.70
10	5.60	9.30	4.65	7.85	4.30	21.90	11.80	11.60	8.25	20.10	4.85	2.95	8.35	3.70	8.15	5.50
14	0.72	2.44	4.60	1.33	6.90	38.00	41.50	67.50	6.25	9.90	4.45	2.80	3.83	1.05	1.68	2.71

(1) As primary substrate

(2) Augmented immobilized acllimatied activated sludge

(3) Without cassava pulp

(4) C:N ratio

Table D-3 Number of bacteria in soil microcosm adjusted by cassava pulps at various C:N ratio

Time (days)	Number of bacteria in soil microcosm (x 10^7 CFU/g soil)															
	Kiffir lime peel 50 mg/kg(1)				Toluene 100 mg/kg				Without primary substrate				ICCAS+ Kiffir lime peel 50 mg/kg(2)			
	None(3)	20:1(4)	30:1	40:1	None	20:1	30:1	40:1	None	20:1	30:1	40:1	None	20:1	30:1	40:1
0	5.20	4.85	5.20	6.25	4.25	5.40	2.92	1.73	6.50	6.20	3.95	6.00	3.75	2.33	4.06	3.38
3	8.50	3.60	13.00	4.10	6.60	6.65	7.70	3.40	28.00	2.10	3.70	7.85	5.35	8.85	6.10	6.10
6	2.45	8.50	16.30	4.25	51.00	13.40	4.85	5.30	27.00	8.60	4.45	3.50	6.20	8.50	1.70	14.80
10	7.05	3.30	12.90	4.30	6.30	46.00	65.00	21.50	2.30	4.00	5.70	1.00	4.60	4.07	15.40	14.50
14	0.55	16.90	20.20	11.40	4.50	72.50	69.00	116.00	5.95	2.95	5.05	3.60	15.4	13.60	18.00	16.30

(1) As primary substrate

(2) Augmented immobilized acllimatied activated sludge

(3) Without cassava pulp

(4) C:N ratio

Table D-4 Number of fungi in soil microcosm adjusted by cassava pulps at various C:N ratio

Time (days)	Number of fungi in soil microcosm (x 10^4 CFU/g soil)															
	Kiffir lime peel 50 mg/kg[1]				Toluene 100 mg/kg				Without primary substrate				ICCAS+ Kiffir lime peel 50 mg/kg[2]			
	None[3]	20:1[4]	30:1	40:1	None	20:1	30:1	40:1	None	20:1	30:1	40:1	None	20:1	30:1	40:1
0	1.67	1.05	0.89	0.85	2.57	1.16	1.01	1.54	1.90	1.94	1.26	2.44	1.27	1.03	1.09	1.64
3	1.07	12.90	5.15	7.50	2.21	4.23	4.40	1.51	2.95	3.50	2.37	2.42	1.05	2.15	1.85	4.80
6	1.53	2.70	2.80	4.90	4.10	0.77	3.30	1.45	1.13	2.60	2.35	1.64	1.50	2.26	2.22	4.48
10	2.61	4.40	4.20	2.10	2.10	0.130	0.53	0.28	7.05	0.02	0.52	0.58	0.98	0.75	1.11	8.25
14	0.82	2.44	6.35	3.27	1.12	0.31	0.48	0.67	0.60	0.79	0.77	0.65	0.34	0.78	4.21	0.68

(1) As primary substrate

(2) Augmented immobilized acllimatied activated sludge

(3) Without cassava pulp

(4) C:N ratio

APPENDIX E

Effect of cassava pulp on stimulation of microbial activity

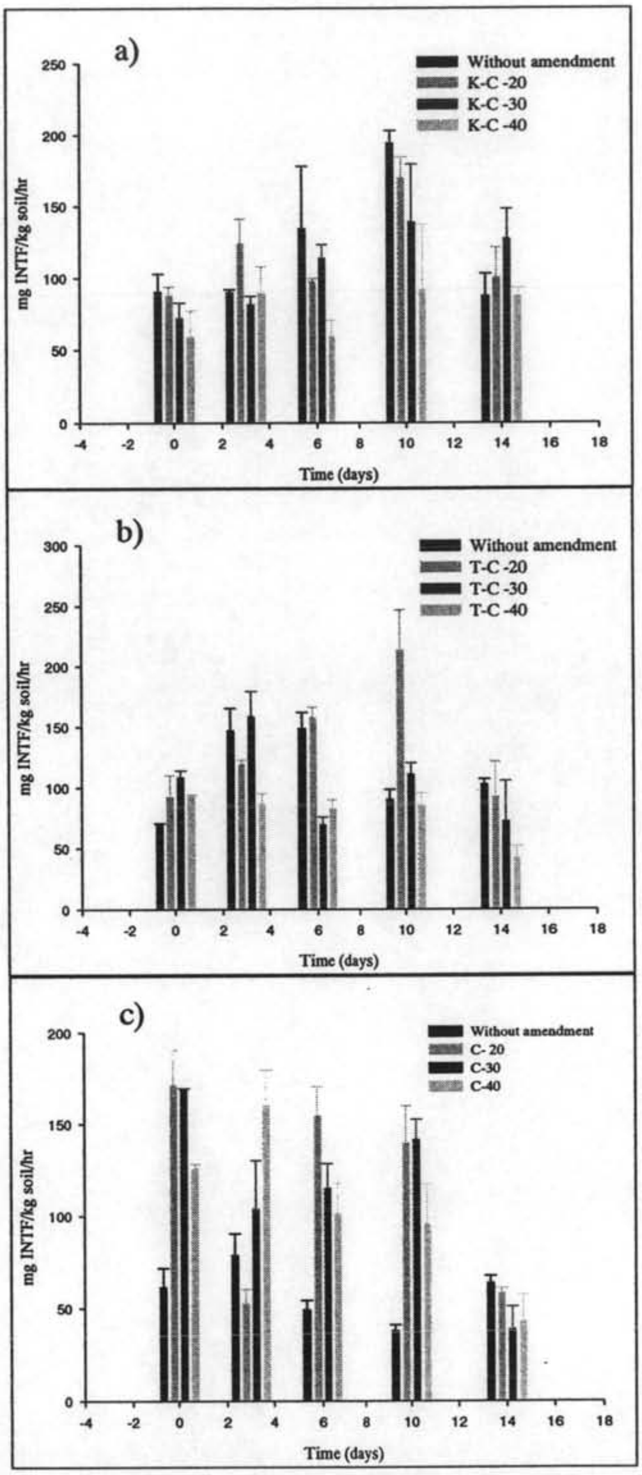

Figure E-1 Microbial activity in soil microcosm adjusted by cassava pulp at various C:N ratios (K= 50 mg/kg of kaffir lime peel; T= 100 mg/kg of toluene; C= cassava pulps)

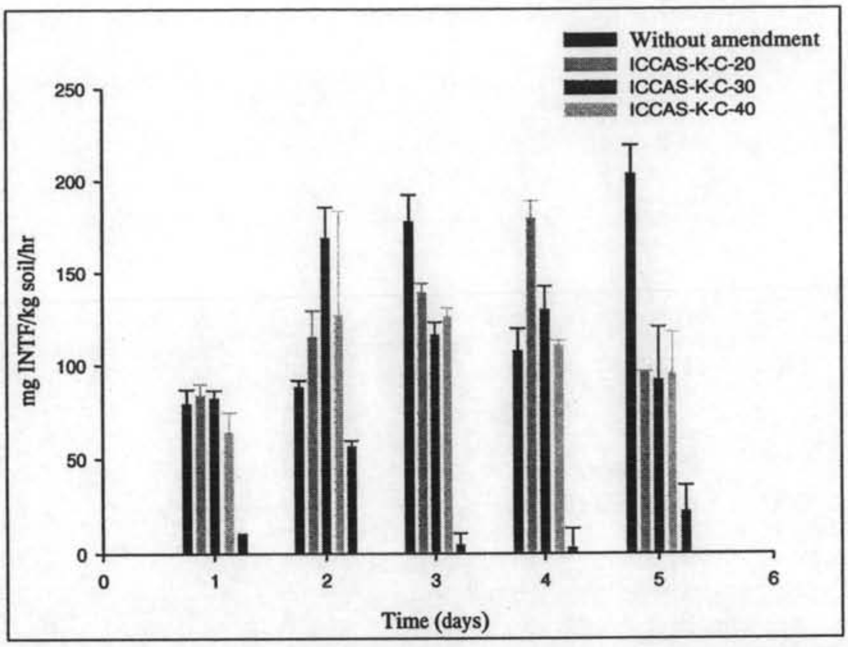

Figure E-2 Microbial activity in soil microcosm augmented with immobilized acclimatized activated sludge on corncob and adjusted by cassava pulp at various C:N ratios (ICCAS= immobilized acclimatized activated sludge on corncob; K= 50 mg/kg of kaffir lime peel; C= cassava pulps)

APPENDIX F

Calculation of carbon to nitrogen ratio (C:N) in soil microcosm

Calculation of carbon to nitrogen ration (C:N) in soil microcosm

Dry weigh of cassava pulp 1 kg..W1

Carbon content in cassava pulp =C/100 kg...C1

Nitrogen content in cassava pulp=N/100 kg..N1

Dry weight of soil X kg...W2

Carbon content in soil=C/100 kg..C2

Nitrogen content in soil=N/100 kg..N2

Calculate the results according to following equation

$$\frac{C}{N} = \frac{(W1 \times C1) + (W2 \times C2)}{(W1 \times N1) + (W2 \times N2)} \qquad (2)$$

APPENDIX G

TCE Degradation by Free Cell and Immobilized Cell of Activated Sludge and *Rhodococcus gordoniae* P3

A paper published in the proceeding of 6th National Environmental Conference, March 7-9, 2007 at Amarin Ragoon Hotel

การย่อยสลายไตรคลอโรเอธิลีนโดยเซลล์อิสระและเซลล์ตรึงของกากตะกอนจุลินทรีย์ และ *Rhodococcus gordoniae* P3

TCE Degradation by Free Cell and Immobilized Cell of Activated Sludge and *Rhodococcus gordoniae* P3

กันทิมา พุ่มมาลา[1] และ อลิศรา เรืองแสง[2,3*]

Kanthima Phummala[1] and Alissara Reungsang[2,3*]

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บทคัดย่อ

งานวิจัยนี้ทำการศึกษาประสิทธิภาพการย่อยสลายไตรคลอโรเอธิลีน (TCE) ในดินโดยใช้เทคนิคการเติมเซลล์อิสระและเซลล์ตรึงของจุลินทรีย์สายพันธุ์เดี่ยว (*Rhodococccus gordoniae* P3) และกลุ่มจุลินทรีย์จากกากตะกอนจุลินทรีย์ในระบบบำบัดน้ำเสียแบบตะกอนเร่ง (activated sludge) โดยทำการตรึงเซลล์แต่ละชนิดในซังข้าวโพดและกาบมะพร้าว ในการศึกษานี้ความเข้มข้นเริ่มต้นของสารไตรคลอโรเอธิลีนและทูโลอีนซึ่งเป็นสารตั้งต้นในการเจริญของจุลินทรีย์เท่ากับ 100 มก./กก. ดิน และ 172 มก./กก.ดิน ตามลำดับ หลังจากทำการศึกษาเป็นเวลา 21 วันพบว่า เปอร์เซ็นต์การย่อยสลายไตรคลอโรเอธิลีนในดินที่มีการเติมเซลล์อิสระของจุลินทรีย์สายพันธุ์เดี่ยว *R. gordoniae* P3 และกลุ่มจุลินทรีย์ในกากตะกอนจุลินทรีย์เท่ากับ 53.34% และ 88.15% ตามลำดับ ซึ่งสูงกว่าในดินชุดควบคุมที่ไม่มีการเติมเชื้อจุลินทรีย์ (19.96%) แสดงให้เห็นว่าเทคนิคการเติมจุลินทรีย์สามารถเพิ่มประสิทธิภาพการย่อยสลายไตรคลอโรเอธิลีนที่ปนเปื้อนในดินได้ นอกจากนี้การย่อยสลายสารไตรคลอโรเอธิลีนโดยใช้เซลล์ตรึงของจุลินทรีย์สายพันธุ์เดี่ยว (*Rhodococccus gordoniae* P3) และกลุ่มจุลินทรีย์จากกากตะกอนจุลินทรีย์ในซังข้าวโพดและกาบมะพร้าว สามารถเพิ่มประสิทธิภาพการย่อยสลายได้สูงถึง 60-98% เมื่อเปรียบเทียบกับการเติมเซลล์อิสระ (50-80%) ทั้งนี้อาจเป็นเพราะว่าโครงสร้างที่เป็นรูพรุนของวัสดุพยุงสามารถเพิ่มความสามารถในการดูดซับและการแพร่ผ่านของสารปนเปื้อนหรือสารอาหารไปสู่เซลล์ที่ถูกตรึงไว้ได้ นอกจากนี้ยังพบว่าในการทดลองวันที่ 21 ดินที่มีการเติมเซลล์ตรึงมีจำนวนจุลินทรีย์สูงกว่าในดินที่มีการเติมเซลล์อิสระ 1-15 เท่า แสดงว่าการเติมวัสดุพยุงมีผลทำให้ความพรุนของดินเพิ่มขึ้นซึ่งช่วยสนับสนุนการเจริญเติบโตของจุลินทรีย์ที่ต้องการอากาศในการเจริญและช่วยป้องกันเซลล์จุลินทรีย์จากความเป็นพิษของสารปนเปื้อนได้ เห็นได้จากจำนวนจุลินทรีย์ที่ไม่ลดลงเมื่อเวลาผ่านไป

คำสำคัญ : เทคนิคการเติมเชื้อจุลินทรีย์; การตรึงเซลล์; ไตรคลอโรเอธิลีน; จุลินทรีย์ที่มีความสามารถในการย่อยสลายสารไตรคลอโรเอธิลีน

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[1]Master degree student, International Postgraduate Program in Environmental Management (Hazardous Waste Management), Chulalongkorn University, Bangkok, 10330;

[2]Associate Professor, Research Centre for Hazardous Substance and Environmental Management, Khon Kaen University, Khon Kaen, 40002;

[3*]Associate Professor, Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, 40002;

[*]Tel : 0-4336-2121, Fax : 0-4336-2121, e-mail : alissara@kku.ac.th

Abstract

This study was conducted to investigate a TCE degradation ability of free cell and immobilized cell of TCE degrader, *R. gordoniae* P3 (P3) in comparison to mixed cultures in activated sludge (AS) augmented in soil. Corncob and coir were used as supporting materials for immobilization. Initial concentration of TCE and toluene as a primary substrate were 100 mg/kg soil and 172 mg/kg soil, respectively. After 21 days of incubation, a higher percentage of TCE degradation was obtained in soil inoculated with pure culture (P3) and mixed cultures (AS) i.e., 53.34% and 88.15%, respectively, which were higher than that in soil without inoculation (19.96%), suggesting that bio-augmentation technique improved a degradation of TCE in soil. Moreover, TCE degradation were improved by immobilized P3 and immobilized AS on corncob and coir (60-98%) comparing to free cells (50-80%) indicating that porous structure of support materials could enhance adsorption capacity and diffusion of contaminant or substrate to immobilized cells. Cells number of immobilized P3 and immobilized AS in soils were found to be 1-15 times higher than free cells in soil at Day 21. An addition of support materials might increase porosity of soil which was favorable for aerobic microorganisms and protected the cells from contaminant toxicity indicating by number of cells were not decreased overtime.

Keywords : bioaugmentation; immobilization; trichloroethylene; TCE degrader

Introduction

Trichloroethylene (TCE) is widely used in various industrial applications such as solvent to remove grease from metal parts, industrial dry-cleaning, printing, production of printing ink and paint, extraction process and textile printing, etc. TCE is a Dense Non-Aqueous Phase Liquids (DNAPLs) so it does not move with the groundwater flow but instead move downward by gravitation force through an aquifer until reaching an impermeable layer. Thus, DNAPLs can serve as a long-term source for dissolved contaminant plumes at many contamination sites [1]. TCE enters the environment via an improper management such as storage, treatment facilities and disposal due to lack of knowledge and environmental concern of the manufacturers. The contamination of TCE in environment is a serious problem because TCE is known to be a probably human carcinogenic substance [2]. Thus, the appropriate treatment technologies are required.

Bioremediation is an alternative approach to clean up the contaminated site. This technique is more attractive comparing with physical and chemical processes because it does not require the final disposal [3]. One of bioremediation treatments is bioaugmentation which is the technique that microorganism cultures are added to improve the reduction of contaminant. Microorganism is a key to a successful bioremediation. Two types of microorganisms can be added into contaminated soil i.e., mixed cultures such as activated sludge and pure culture such as TCE degrader. Main advantage of mixed cultures is their abilities to survive in a non-sterile environment [4] while the main advantage of using pure culture is a convenient to monitor during operation compare to mixed cultures. However, there is a limited information on using mixed cultures to degrade TCE.

Free cells of bacteria have been reported to successfully degrade chlorinated hydrocarbon in liquid culture [5, 6, 7]. However, in the natural condition the survival of free cells are low [8]. Immobilization is an attractive technique to solve this problem because immobilized cultures tend to have a higher level of activity and more tolerant to environmental perturbations such as pH, temperature or toxicity of contaminants [9]. Support materials could be synthetically made such as alginate, and polyvinyl alcohol and naturally available such as corncob and coir. Synthetic support materials are costly and difficult to be degraded due to a non-biodegradation characteristic. Therefore, there is an interest toward the use of natural materials such as agricultural residues to overcome this problem. Effective support materials from agricultural residues include coconut fiber [10], corncob powder [11], wheat straw and maple woodchips [9] used for bioremediation treatment.

In this work, we explored the feasibility of using mixed cultures in activated sludge to degrade TCE in soil in comparison to a pure culture i.e., TCE degrader *Rhodococcus gordoniae* P3 with the ultimate aim of application for soil treatment. One objective of the present work was to study the effectiveness of immobilization materials i.e., corncob and coir for mixed cultures and pure culture for TCE degradation and remove it efficiently from soil.

Materials and Methods

Microorganism and culture media

TCE degrader, *Rhodococcus gordoniae* P3, a gram positive aerobic bacterium isolated from petroleum-contaminated soil in Bangkok was kindly provided by Dr. Ekawan Lueprommchai of Chulalongkorn University. Aerobic activated sludge was collected from wastewater treatment plant of Lardkrabang Industrial Sector, Bangkok, Thailand. Wastewater treated at this wastewater treatment plant is from electronic part industries where organic solvents are used. Culture media was mineral salts medium (MSM) consist of (in mg/L) K_2HPO_4, 1741.6; Na_2HPO_4, 359.94; $(NH_4)_2SO_4$, 1321.3; $MgSO_4$, 120.36; $Ca(NO_3)_2$, 16.409; $Fe(NO_3)_3$, 2.419; $MnSO_4$, 0.151; $ZnSO_4$, 0.161; $CuSO_4$, 0.160; $NiSO_4$, 0.015; $CoSO_4$, 0.016; Na_2MoO_4, 0.021; adding 17 g/L agar for solid media. Toluene was used as carbon and energy sources.

Soil

Soil was collected near an abandon site at Tumbol Klang-Dong, Aumphur Pak-Chong, Nakonratchasima Province. This site has been contaminating with Volatile Organic Compounds (VOCs) such as TCE, tetrachloroethylene, xylene, toluene and 1,1,1-trichloroethylene. Soil was passed through 2 mm sieve and kept at 4°C prior the usage. Characteristics of soil were shown in Table 1.

Table 1 Characteristics of soil

Parameter	Value (Unit)
pH	8.05
EC	0.0 23 (mS/cm)
Organic matter	2.47 (%)
Total nitrogen	0.0896 (%)
Organic carbon	1.43 (%)
C:N ratio	15.97
Sand	52.5 (%)
Silt	42.5 (%)
Clay	5.0 (%)
Texture	Sandy loam

Supporting materials

Corncob and coir were obtained from Faculty of Agriculture, Khon Kaen University. Corncob and coir were shredded by knife into small pieces (approximately 0.5 x 0.5 x 0.5 cm) and passed through 0.5-1 cm sieve. After that, they were delignified by boiling in 1% NaOH for 3 hrs [12] to remove lignin which might be toxic to microorganisms and then thoroughly washed under tap water, and soaked in distilled water overnight. This process was done 2 times and kept at -20 °C prior the usage.

Cell immobilization

Seventy-five g dry wt of delignified support materials i.e., corncob and coir were put into 300 ml MSM containing 4 g/L glucose as a carbon source which were autoclaved at 121 °C for 15 min for 2 times before inoculating with 10 % (v/v) of *R. gordoniae* P3 or activated sludge. Then, 172 mg/L of toluene, a primary substrate, was added into the bottle before incubating at room temperature, shaken at 200 rpm on orbital shaker for 24 hrs. After that, these support materials were transferred into fresh MSM containing 4 g/L glucose and 172 mg/L of toluene and incubated as described previously for 2 times before harvesting by washing with sterile MSM. The numbers of microorganism in support materials were approximately 10^7 cells/g dry wt of support materials determined by viable plate count technique. These immobilized cells were used as inocula for soil microcosm study.

Soil microcosm study

Ten grams dry wt of non-sterile soil were added into a 50 mL serum bottle containing each immobilized cell (*R. gordoniae* P3 and activated sludge) with approximately 10^7 cells/g dry wt of support materials. TCE at the final concentration of 100 mg/kg soil and 172 mg/kg soil of toluene as a primary substrate were added into a serum bottle and immediately sealed with teflon-lined rubber septa and capped with aluminum cap. The bottle was incubated at room temperature in dark. TCE remained in soil was determined at Day 0, 3, 5, 7, 14 and 21 by GC-headspace technique. Numbers of TCE degrader in support materials and soil was determined by viable plate count technique.

Analysis of TCE concentration

TCE concentrations in soil microcosm were analyzed by GC-head space technique. Serum bottle containing soil sample was heated in heat box at 90°C for 30 min. Fifty µl of head space sample were taken by gas tight syringe and analyzed for TCE concentration using GC-17A Shimadzu-Flame Ionization Detector. The capillary column was 30-m Rtx-VGC with the inner diameter of 0.45-mm (Restex Inc., USA). Helium was used as carrier gas. Splitless mode was used. The injection and detector temperatures were maintained at 200 ° C. The column temperature retained at 60 ° C for 5 min and was then increased to 8 ° C/min until reached 180 ° C then hold for 2 min.

Enumeration of toluene degrading bacteria

For support material, one g wet wt of immobilized support materials were washed with sterile 0.85% NaCl for 2 times to remove soil and then blended by blender into small particles. For soil, one g dry wt of soil was mixed with 9 ml of 0.85% NaCl to make soil dilution. Then, serial 10-fold dilutions of each suspension were made and plated on MSA (Mineral Salt Agar) and incubated at room temperature in the box fumigated with toluene as a primary substrate for one week to enumerate toluene degrading bacteria. The number of colony forming units (CFU) between 30-300 colony in each plate were counted.

Results and Discussion

Degradation of TCE by free cell of *Rhodococcus gordoniae* P3 and activated sludge

This study compared the TCE degradation ability of mixed cultures in activated sludge to pure culture, i.e., *R. gordoniae* P3 (P3). Control microcosm represented the ability of indigenous microorganisms to degrade TCE and/or abiotic process affecting TCE degradation. After 3 weeks of incubation, the percentage of TCE degradation in soil microcosm inoculated with activated sludge, *R. gordoniae* P3 and without inoculum were 88%, 53% and 20%, respectively (Figure 1) indicating that mixed cultures in activated sludge and *R. gordoniae* P3 improved TCE degradation in soil microcosm. A percentage of TCE degradation in soil microcosm augmented with activated sludge was founded to be greater than in soil microcosm augmented with *R. gordoniae* P3. These may due to the fact that activated sludge contains complex microbial consortium which can be more tolerant to environment than pure strain. In addition, growth of pure strain generally requires strictly sterilized conditions and control methods, while mixed cultures used for bioaugmentation could be grown quickly and easily in the environment. Thus, the ability of mixed cultures could be better than pure culture. These explanations were supported by the work of Buitron and Gonzalez [13] on the degradation of phenol, 4-monochorophenol, 2,4-dichlorophenol and 2,4,6-trichlorrophenol by activated sludge and isolated bacteria in which the degradation rate of these phenols by activated sludge was from one to two orders of magnitude higher than pure strain isolated from activated sludge.

Degradation of TCE by immobilized *R. gordoniae* P3 and activated sludge on corncob and coir

In order to improve a survival of microorganisms in soil microcosm, we immobilized *R. gordoniae* P3 and activated sludge by corncob and coir and checked their TCE degradation abilities. Profiles of TCE degradation by immobilized *R. gordoniae* P3 and immobilized activated sludge on corncob and coir were depicted in Figure 1. After 3 weeks of incubation, TCE was degraded 94%, 98%, 94% and 60% by immobilized P3 on corncob (ICC-P3), immobilized P3 on coir (ICO-P3), immobilized activated sludge on corncob (ICC-AS) and immobilized activated sludge on coir (ICO-AS), respectively, which were higher than the percentage of removal by free cells. Results implied that immobilization technique improved the degradation of TCE comparing to free cells. A porous structure of support materials could enhance adsorption capacity and diffusion of contaminant or substrate to the immobilized cells might be responsible for this trend [11, 14]. Similar results were reported by Pattanasupong et al. [10] who founded that microbial consortium immobilized on loofa sponge and coconut fiber degraded carbendazim higher than free cells approximately 12%.

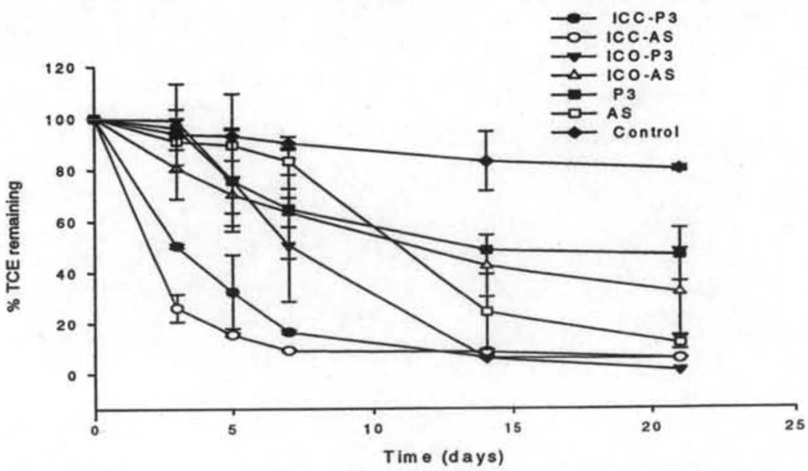

Figure 1 Degradation of TCE in soil microcosms (ICC= Immobilized cell on corncob, ICO= Immobilized cell on coir, P3= *Rhodococcus gordoniae* P3, and AS= Activated sludge)

Toluene degrading bacteria produce oxygenase which is a broad-substrate enzyme that could transform TCE into less toxic compounds. Growth of toluene degrading bacteria were used to indicate the survival of augmented bacteria in different soil microcosms as well as to represent population of TCE degrader in soil which was observed as shown in Table 2. Cell numbers of TCE degrader in soil, estimated by number of toluene degrading bacteria, were approximately 10^7-10^8 cells/g soil throughout 21 days of incubation. The cell numbers in soils inoculated with immobilized *R. gordoniae* P3 and immobilized activated sludge on corncob and coir were higher than in soil inoculated with free cells at the end of incubation (Table 2). In addition, cell numbers in support material did not markedly decreased throughout the experiment (Table 3). These results suggested that there was a growth of cells immobilized on support materials, however, the porous space in support materials was limited resulting in leakage of the cells from support materials into soil. Kumar and Das [15] reported a similar finding in which the daughter cell produced by binary fission of the immobilized of *Enterobactor cloacae* IIT-BT 08 leaked to the culture media when there was no free space on the porous support materials. In this work, we speculated that cells were immobilized on support materials by physical absorption due to electrostatic forces or by covalent binding between cell membrane and support materials. Thus, there were no barriers between cells and soil leading to a possibility of cell detachment and relocation [16].

Table 2 Cell number of TCE degrader in soil microcosms

Time (days)	In soil (x10^7 CFU/g soil)						
	Control (without inoculum)	Free cell		Immobilized cell			
		P3	AS	P3		AS	
				Corncob	Coir	Corncob	Coir
0	6.40	5.77	7.57	5.43	8.30	2.48	5.67
3	1.43	11.5	5.59	20.8	20.7	25.2	9.25
5	1.73	23.9	32.0	56.9	3.70	24.3	9.67
7	8.03	51.7	13.5	153.0	9.00	25.8	11.1
14	5.27	7.30	8.20	31.6	5.70	29.0	11.6
21	6.73	5.53	5.23	38.0	7.30	75.0	15.6

A capability of corncob and coir as support materials was evaluated. These two materials adsorbed cells and attached cells into their porous. Advantages of immobilization by adsorption method is a simple in application and no chemical reagent was used [17]. Cell survivals in each support materials were presented in Table 3. Results revealed that cell numbers in support materials did not decrease overtime of incubation. This may due to the survival and stability of cells could be improved by immobilization technique. Results implied that corncob and coir were suitable support materials to immobilize both P3 and activated sludge. Porosity of corncob and coir might provide air through the soil, thus prolong the survival and growth of bacteria [14]. Moreover, the support materials might protect cells from predation and contaminant toxicity results in a better survival of immobilized cells than free cells [18, 19].

Table 3 Cell number of TCE degrader in support materials

Time (days)	In support material (x10^7 CFU/g support material)			
	P3		AS	
	Corncob	Coir	Corncob	Coir
0	5.83	6.7	1.75	6.17
3	6.8	13.5	32.1	11.5
5	201	6.35	244	19.8
7	34	13.8	212	27.1
14	10.1	13.6	84.7	19.8
21	7.73	16.8	53.4	25.7

Conclusions

Conclusions drawn from this study were as follow:

1) Mixed cultures in activated sludge degraded TCE in soil better than *R. gordoniae* P3

2) Immobilization technique by adsorbing the cells on corncob and coir could improved cells survival resulting in the higher percentage of TCE degradation in soil compared to free cell.

3) Corncob and coir could be used as support materials to immobilized cells.

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BIOGRAPHY

Ms Kanthima Phummala graduated her B.Sc. degree in Biotechnology from Khon Kaen University in 2004. She applied to study in the Master of Science Program in Environmental Management (Interdisciplinary Program) at Chulalongkorn University and got the M.Sc. in Environmental Management (Interdisciplinary Program), Chulalongkorn University in 2006. Her email address is kanthima_phummala@yahoo.com.