REFERENCES

Abdel-El-Haleem, D. (2003) *Acinetobacter*: environmental and biotechnological applications. African J. Biotech. 2: 71-74.

Adriaens, P. and Focht, D. (1991). Cometabolism of 3,4-dichlorobenzoate by *Acinetobacter* sp. strain 4-CB1. Appl. Environ. Microbiol. 57(1): 173-179.

Alexander, M. (1994). Biodegradation and Bioremediation. New York: Academic Press.

Allende, L., Gibello, A., Fortun, A., Mengs, G., Ferrer, E. and Martin, M. (2000). 4-Hydroxybenzoate uptake in an isolated soil *Acinetobacter* sp. Curr Microbiol 40: 34-39.

Assinder, S. J. and Williams, P. A. (1990). The TOL plasmids: determinants of the catabolism of toluene and xylenes. Adv. Microbiol. Physiol. 31: 1-69.

Bachofer, R., Lingens, F. and Schafer, W. (1975). Conversion of aniline into pyrocatechol by a Nocardia sp. Incorporation of oxygen-18. FEBS Lett 50: 288-290.

Bartha, R. (1971). Fate of herbicide-derived chloroanilines in soil. J. Agric. Food. Chem. 19: 385-387.

Bergmann, J. G. and Sanik, J. (1957). Determination of trace amounts of chlorine in naptha. Anal. Chem. 29: 241-243.

Boehncke, A., Kielhorn, J., Konnecker, G., Pohlenz-Michel, C. and Mangelsdorf, I. (2003). 4-Chloroaniline [Online], Available from http://www.inchem.org/documents/cicads/cicads/cicad48.htm. (12/08/04)

Bollag, J. M., Blattmann, P. and Laanio, T. (1978). Adsorption and transformation of four substituted anilines in soil. J. Agric. Food. Chem. 26: 1302-1306.

Bonilla, M., Olivaro, C., Corona, M., Vazquez, A. and Soubes, M. (2005). Production and characterization of a new bioemulsifier from *Pseudomonas putida* ML2. J. Appl. Microbiol. 98: 456-463.

Boon, N., Goris, J., Vos, P. D., Verstraete, W. and Top, E. M. (2000). Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* strain, I2*gfp*. Appl. Environ. Microbiol. 66(7): 2906-2913.

Boon, N., Goris, J., Vos, P. D., Verstraete, W. and Top, E. M. (2001). Genetic Diversity among 3-chloroaniline- and aniline-degrading strains of the *Comamonadaceae*. Appl. Environ. Microbiol. 67(3): 1107-1115.

Broderick, J. B. and O'Halloran, T. V. (1991). Overproduction, purification, and characterization of chlorocatechol dioxygenase, a non-heme iron dioxygenase with broad substrate tolerance. Biochemistry 30: 7349-7358.

Brunsbach, F. R. and Reineke, W. (1993). Degradation of chloroanilines in soil slurry by specialized organisms. Appl. Microbiol. Biotechnol. 40: 402-407.

BUA (1995). p-Chloroaniline. Beratergremium für Umweltrelevante Altstoffe (BUA)der Gesellschaft Deutscher Chemiker. Weinheim, VCH, 171 pp. (BUA Report 153).

Cann, A. J. (2002). Growth of bacteria [Online] Available from http://www-micro.msb.le.ac.uk/Labwork/bact/bact17.htm (11/09/06)

Chauhan, S., Barbieri, P. and Wood, T. K. (1998). Oxidation of Trichloroethylene, 1,1-dichloroethylene, and chloroform by toluene/*o*-xylene monooxygenase from *Pseudomonas stutzeri* OX1. Appl Environ Microbiol 64(8): 3023-3024.

Coppo, P., Fagnoni, M. and Albini, A. (2001). Photochemical conversion of 4-chlorocatechol into 4-alkylanilines. Tetrahedron Lett. 42: 4271-4272.

Daugherty, D. D. and Karel, S. F. (1994). Degradation of 2,4-dichlorophenoxyacetic acid by *Pseudomonas cepacia* DBO1(pRO101) in a dual-substrate chemostat.. Appl Environ Microbiol 60(9): 3261-3267.

Dinkla, I. J. T. and Janssen, D.B. (2003). Simultaneous growth on citrate reduces the effects of iron limitation during toluene degradation in *Pseudomonas*. Microbiol. Ecol. 45: 97-107.

Dejonghe, W., Goris, J., Dierickx, A., Dobbeleer, V. D., Crul, K., Vos, P. D., Verstraete, W. and Top, E. M. (2002). Diversity of 3-chloroaniline and 3,4-dichloroaniline degrading bacteria isolated from three different soils and involvement of their plasmids in chloroaniline degradation. FEMS Microbiol. Ecol. 42: 315-325.

Delneri, D., Degrassi, G., Rizzo, R. and Bruschi, C.V. (1995). Degradation of trans-ferulic and p-coumaric acid by *Acinetobacter calcoaceticus* DSM 586. Biochim. Biophys. Acta. 1244: 363-367.

Dorn, E. and Knackmuss, H. J. (1978a). Chemical structure and biodegradability of halogenated aromatic compounds. Biochem. J. 174: 73-84.

Dorn, E. and Knackmuss, H. (1978b). Chemical structure and biodegradability of haloginated aromatic compounds. Substituent effects on 1,2-dioxygenation of catechol. Biochem. J. 174: 85-94.

Dulley, J. R. and Grieve, P. A. (1975). A simple technique for eliminating interference by method of protein determination. Anal. Biochem. 64: 136-141.

EC (2000). Amended proposal for a Directive of the European Parliament and of the Council amending for the nineteenth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous

substances and preparations (azocolourants), Brussels, European Commission: 8.

Emtiazi, G., Satarii, M. and Mazaherion, F. (2001). The utilization of aniline, chlorinated aniline, and aniline blue as the only source of nitrogen by fungi in water. Wat. Res. 35: 1219-1224.

Federal register. (1979). In Priority Pollutant List. (promulgated by the U.S. Environmental Protection agency under authority of Clean water Act of 1997) 44: 233.

Garst, J. E. and Wilson, W. C. (1984). Accurate, wide-range, automated, high-performance liquid chromatographic method for the estimation of octanol/water partition coefficients. I: Effect of chromatographic conditions and procedure variables on accuracy and reproducibility of the method. J. Pharm. Sci. 73: 1616–1623.

Harayama, S., Kok, M. and Neidle, E. L. (1992). Functional and evolutionary relationships among diverse oxygenases. Annu. Rev. Microbiol. 46: 565-601.

Harder, W. and Dijkhuizen, L. (1982). Strategies of mixed substrate utilization in microorganisms. Phil. Trans. R. Soc. Lond. B. Biol. Sci. 297: 459-480.

Hartmann, J., Reineke, W. and Knackmuss, H.-J. (1979). Metabolism of 3-chloro-, 4-chloro-, and 3,5-dichlorobenzoate by a *Pseudomonas*. Appl Environ Microbiol 37(3): 421-428.

Harwood, C. S. and Parales, R. E. (1996). The β-ketoadipate pathway and the biology of self-identity. Annu. Rev. Microbiol. 50: 553-590.

Helm, V. and Reber, H. (1979). Investigation on the regulation of aniline utilization in *Pseudomonas multivorans* strain An 1. Eur. J. Appl. Microbiol. Biotechnol. 7: 191-199.

Hinteregger, C., Loidl, M. and Streichsbier, F. (1992). Characterization of isofunctional ring-cleavage enzymes in aniline and 3-chloroaniline degradation by *Pseudomonas acidovorans* CA28. FEMS Microbiol. Lett. 97: 261-266.

IARC (1993). Para-chloroaniline. In: Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 57: 305-321.

Kaschabek, S. R., Kasberg, T., Muller, D., Mars, A. E., Janssen D.B. and Reineke, W. (1998). Degradation of chloroaromatics: purification and characterization of a novel type of chlorocatechol 2,3-dioxygenase of Pseudomonas putida GJ31. J Bacteriol 180: 296-302.

Kearny, P. C. and Kaufman, D. D. (1969). Degradation of herbicides. New York: Marcel Dekker.

Kearny, P. C. and Kaufman, D. D. (1975). Herbicides: chemistry, degradation and mode of action. New York: Marcel Dekker.

Kilzer, L., Scheunert, I., Geyer, H., Klein, W. and Korte, F. (1979). Laboratory screening of the volatilization rates of organic chemicals from water and soil. Chemosphere 10: 751-761.

Kloskowski, R., Scheunert, I., Klein, W. and Korte, F. (1981a). Laboratory screening of distribution, conversion and mineralization of chemicals in the soil–plant system and comparison to outdoor experimental data. Chemosphere 10: 1089-1100.

Knackmuss, H.-J. (1983). Xenobiotic degradation in industrial sewage: haloaromatics as target substrates. p. 173-190. *In* C. F. Phelps and P. H. Clarke (ed.),

Biotechnology. Biochemical Society Symposium no. 48, Biochemical Society, London.

Konopka, A., Knight, D. and Turco, R. F. (1989). Characterization of a *Pseudomonas* sp. capable of aniline degradation in the presence of secondary carbon sources. Appl. Environ. Microbiol. 55 (2): 385-389.

Lacorte, S., Perrot, M.-C., Fraisse, D. and Barcelo, D. (1999). Determination of chlorobenzidines in industrial effluent by solid-phase extraxtion and liquid chromatography with electrochemical and mass spectrometric detection. J. Chromatogr. A 833: 181-194.

Lagraga, M. P., Buckingham, P. L. and Evans, J. C. (1994). Hazardous Waste Management. New York: McGraw Hill.

Latorre, J., Reineke, W. and Knackmuss, H. J. (1984). Microbial metabolism of chloroanilines: enhanced evolution by natural genetic exchange. Arch. Microbiol. 140: 159-165.

Loidl, M., Hinteregger, C., Ditzelmuller, G., Ferschl, A. and Streichsbier, F. (1990). Degradation of aniline and monochlorinated anilines by soil-born *Pseudomonas acidovorans* strains. Arch. Microbiol. 155: 56-61.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem.193: 265–275.

Lyons, C. D., Katz, S. E. and Bartha, R. (1985). Persistence and mutagenic potential of herbicide-derived aniline residues in pond water. Bull. Environ. Contam. Toxicol. 35: 696-703.

Maltseva, O. V., Solyanikova, I. P. and Golovleva, L. A. (1994). Chlorocatechol 1,2-dioxygenase from *Rhodococcus erythropolis* 1CP kinetic and

immunochemical comparison with analogous enzymes from gram-negative strains. Eur. J. Biochem. 226: 1053-1061.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J. and Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64(2): 795-799.

Mars, A. E., Kasberg, T., Kaschabek, S. R., Agteren, M. H. V., Janssen, D. B. and Reineke, W. (1997). Microbial degradation of chloroaromatics: use of the *meta*-cleavage pathway for mineralization of chlorobenzene. J. Bacteriol. 179 (14): 4530-4537.

Mars, A. E., Kingma, J., Kaschabek, S. R., Reineke, W. and Janssen, D. B. (1999). Conversion of 3-chlorocatechol by various catechol 2,3-dioxygenases and sequence analysis of the chlorocatechol dioxygenase region of *Pseudomonas putida* GJ31. J Bacteriol 181(4): 1309-1318.

Martin, M., Mengs, G., Allende, J. L., Fernandez, J., Alonso, R. and Ferrer, E. (1999). Characterization of two novel propachlor degradation pathways in two species of soil bacteria. Appl. Environ. Microbiol. 65(2): 802-806.

Mcbride, K. E., Kenny, J. W. and Stalker, D. M. (1986). Metabolism of the herbicide bromoxynil by *Klebsiella pneumoniae* subsp. *ozaenae*. Appl Environ Microbiol 52(2): 325-330.

Müller-Wegener, U. (1982). Über die Adsorption umweltrelevanter Chemikalien in Böden. Chemie Erde 41: 175–181.

Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Yolken, R. H. (1999). Manual of Clinical Microbiology. 7 th ed. Washington, D C: ASM Press.

Nakai, C., Kagamiyama, H., Nozaki, M., Nakazawa, T. and Inouye, S. (1983). Complete nucleotide sequence of the meatapyrocatechase gene on the TOL plasmid of *Pseudomonas putida mt-2*. J. Biol. Chem. 258: 2923-2928.

Nelson, K. E., Weinel, C., Paulsen, I. T., Dodson, R. J., Hilbert, H., Martins-dos-Santos, V. A., Fouts, D. E., Gill, S. R., Pop, M., Holmes, M., Brinkac, L., Beanan, M., DeBoy, R. T., Daugherty, S., Kolonay, J., Madupu, R., Nelson, W., White, O., Peterson, J., Khouri, H., Hance, I., Chris-Lee, P., Holtzapple, E., Scanlan, D., Tran, K., Moazzez, A., Utterback, T., Rizzo, M., Lee, K., Kosack, D., Moestl, D., Wedler, H., Lauber, J., Stjepandic, D., Hoheisel, J., Straetz, M., Heim, S., Kiewitz, C., Eisen, J. A., Timmis, K. N., Dusterhoft, A., Tummler, B. and Fraser, C. M. (2002). Complete genome sequence and comparative analysis of themetabolically versatile *Pseudomonas putida* KT2440 J. Environ. Microbiol. 4: 799-808.

Nozaki, M. (1979). Oxygenase and dioxygenase. Top. Curr. Chem. 78: 145-186.

Parris, G. E. (1980). Environmental and metabolic transformations of primary aromatic amines and related compounds. Residue Rev. 76: 1-30.

Pieper, D. H., Winkler, R. and Sandermann, H. (1992). Formation of a toxic dimerization product of 3,4-dichloroaniline by lignin peroxidase from *Phanerochaete chrysosporium*. Angew chem. 104: 60-61.

Pillai, P., Helling, C. S. and Dragun, J. (1982). Soil-catalyzed oxidation of aniline. Chemosphere 11: 299-317.

Radianingtyas, H., Robinson, G. K. and Bull, A. T. (2003). Characterization of a soil-derived bacterial consortium degrading 4-chloroaniline. Microbiology 149: 3279-3287.

Reber, H., Helm, V. and Karanth, N. G. K. (1979). Comparative studies on the metabolism of aniline and chloroanilines by *Pseudomonas multivorans* strain An 1. Eur. J. Appl. Microbiol. Biotechnol. 7: 181-189.

Reece, C. (2005). Biology. 7 th ed. New York: Benjamin Cummings.

Riser-Roberts, E. (1998). Remediation of Petroleum Contaminated Soils. Boca Raton, New York: Lewis Publishers.

Rusansky. S., Avigad, R., Michaeli, S. and Gutnick, D. L. (1987). Involvement of plasmid in growth on and dispersion of crude oil by *Acinetobacter calcoaceticus* RA57. Appl. Environ. Microbiol. 53(8): 1918-1923.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Molecular Cloning: A laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.d0

Sanchez, M., Garbi, C., Martinez-Alvarez, R., Ortiz, L. T., Allende, J. L. and Martin, M. (2005). *Klebsiella planticola* strain DSZ mineralizes simazine: physiological adaptations involved in the process. Appl. Microbiol. Biotechnol 66: 589-596.

Schlomann, M. (1994). Evolution of chorocatechol catabolic pathways. Biodegradation 5: 301-321.

Schaefer, C. H., Colwell, A. E. and Dupras, E. F. (1980). The occurrence of *p*-chloroaniline and *p*-chlorophenylurea from the degradation of diflubenzuron in water and fish. Proceedings Papers of the 48th Annual Conference of the California Mosquito Vector Control Association. Fresno, CA, California Mosquito Vector Control Association: 84–89.

Schukat, B., Janke, D., Krebs, D. and Fritsche, W. (1983). Cometabolic degradation of 2- and 3-chloroaniline because of glucose metabolism by *Rhodococcus* sp. An 117. Curr. Microbiol. 9: 81-86.

Surovtseva, E. G., Vasileva, G. K., Volnova, A. I. and Baskunov, B. P. (1980). Destruction of monochloroanilines by the meta cleavage by *Alcaligenes faecalis, Dokl. Alkad. Nauk.* SSSR 254: 226-230.

Surovtseva, E. G., Ivoilov, V. S., Karasevich, Y. N. and Vasileva, G. K. (1985). Chlorinated anilines, a source of carbon, nitrogen and energy for *Pseudomonas diminuta*. Mikrobiologiya 54: 948-952.

Surovtseva, E. G., Ivoilov, V. S., Vasileva, G. K. and Belyaev, S. S. (1996). Degradation of chlorinated anilines by certain representatives of the genera *Aquaspirillum* and *Paracoccus*. Mikrobiologiya 65: 553-559.

Tropel, D., Meyer, C., Armengaud, J. and Jouanneau, Y. (2002). Ferredoxin-mediated reactivation of the chlorocatechol 2,3-dioxygenase from *Pseudomonas putida* GJ31. Arch. Microbiol. 177: 345-351.

Urata, M., Uchida, E., Nojiri, H., Omori, T., Obo, R., Miyaura, N and Ouchiyama, N. (2004). Genes involved in aniline degradation by *Delftia acidovorans* strain 7N and its distribution in the natural environment. Biosci. Biotechnol. Biochem. 68(12): 2457-2465.

Van-der-Meer, J. R., Eggen, R. I. L., Zender, A. J. B. and de-Vos, V. M. (1991). Sequence analysis of the *Pseudomonas* sp. strain P51 *tcb* gene cluster, which encodes metabolism of chlorinated catechols: evidence for specialization of catachol 1,2-dioxygenases for chlorinated substrates. J. Bacteriol. 173(1): 2425-2434.

Heesche-Wagner, K., Schwarz, T. and Kaufmann, M. (1999). Phenol degradation by an enterobacterium: a *Klebsiella* strain carries a TOL-like plasmid and a gene encoding a novel phenol hydroxylase Can. J. Microbiol./Rev. can. microbiol. 45(2): 162-171.

Walker, N. and Harris, D. (1969). Aniline utilization by a soil *Pseudomonad*. J. Appl. Bacteriol. 32: 457-462.

Worsey, M. J., Franklin, F. C. H. and Williams, P. A. (1978). Regulation of the degradation pathway enzymes coded for by the TOL plasmid (pWWO) from *Pseudomonas putida* mt-2. J. Bacteriol. 134(3): 757-764.

Wrenn, B. A. (1998). Biodegradation of Aromatic Hydrocarbons [online], Available from http://www.ence.umd.edu/~eseagren/bioAHC97.htm.

Wyndham, R. C. (1986). Evolved aniline catabolism in *Acinetobacter calcoaceticus* during continuous culture of river water. Appl. Environ. Microbiol. 51(4): 781-789.

Zaitsev, G. M. and Baskunov, B. P. (1985). Utilization of 3-chlorobenzoic acid by *Acinetobacter calcoaceticus* . Mikrobiologiia. 54: 203-208.

Zeyer, J. and Kearney, P. C. (1982). Microbial degradation of *para*-chloroaniline as sole carbon and nitrogen source. Pestic. Biochem. Physiol. 17: 215-223.

Zeyer, J., Wasserfallen, A. and Timmis, K. N. (1985). Microbial mineralization of ring-substituted anilines through an *ortho*-cleavage pathway. Appl. Environ. Microbiol. 50(2): 447-453.

Zilli, M., Palazzi, E., Sene, L., Converti, A and Borghi, M. D. (2001). Toluene and styrene removal rom air in biofilters. Process. Biochem 10: 423-429.

APPENDICES

APPENDIX A

A.1 Gram stain

A.1.1 Dye and chemical solution for bacterial staining

The widely used staining technique in bacteriology is the Gram stain developed in 1884 by Hans Christian Gram. This procedure is a differential stain method meaning that bacteria will give different results depending on their cell wail chemistry. The Gram stain divides bacteria into two groups based on their reaction to the stain. Gram positive bacteria will be purple after the last step; gram negative bacteria will be red. The size, shape and arrangement of the organisms can also be determined from a stained specimen using microscope.

1. Gram's Crystal Violet

Solution A	Crystal Violet	2	grams
	Ethyl alcohol	20	grams
	Distilled water	100	milliliters
Solution B	Ammonium oxalate	0.8	milliliters
	Distilled water	100	milliliters

Solution A and Solution B were mixed and filtrated in order to remove dregs before using.

2. Gram's Iodine

Iodine	1	grams
KI	2	grams
Distilled water	300	milliliters

KI was dissolved in distilled water and added iodine before using.

3. Gram's Alcohol

Ethyl Alcohol	98	milliliters
Acetone	2	milliliters

All of solutions were mixed before using.

4. Gram's safranin

Safranin o (2.5% solution in 95% ethyl alcohol)	10	milliliters
Distilled water	100	milliliters

All of solutions were mixed before using.

A.1.2 Gram-staining Procedure

The four steps of the Gram stain can be summarized as follows:

1. Primary stain - cover the smear with crystal violet for one minute. All bacteria will take up this dye and appear purple. Rinse off the excess dye with water.

2. Mordant - Gram's iodine is added to interact for one minute with the crystal violet. This complex will be difficult to remove from certain bacteria during the next (decolorization) step. Excess iodine is rinsed off with water.

3. Decolorization - 95% ethanol is briefly (10-20 seconds) applied to the smear followed by a water rinse.

4. Counterstain - Safranin is added for 20 seconds to dye any decolorized cells. It will not change the color of the cells that retain the crystal violet.

A.2 Biochemical test

Oxidase test

This test determines the presence of oxidase enzymes. Use only plastic or platinum loops for this test (A platinum loop may be used to transfer organisms but iron in a nichrome loop may interfere with the reaction.). Using a sterile swab, transfer a heavy inoculum of the bacteria to a slide. Place the slide on a white paper and add the oxidase reagent. Observe for a color change. A positive reaction appears pink, then maroon and finally black. Take care to avoid contact with the oxidase reagent

Catalase test

This test determines bacterial production of catalase enzymes. Place a drop of hydrogen peroxide (3% H_2O_2-reagent grade) on a microscope slide or in the concave surface of a hanging drop slide. With a sterile loop, collect a sample of 18-24 hour old pure bacterial culture. Place the loop in the hydrogen peroxide. If the test is positive, there will be immediate bubbling or foaming, and liberation of o_2 gas. Record results

TSI/H_2S production

This medium can determine the ability of an organism to utilize specific carbohydrates incorporated in basal growth medium, with or without the production of gas, along with the determination of hydrogen sulfide (H_2S) production. TSI agar contains the three sugars in varying concentrations: glucose (1X), lactose (10X), and sucrose (10X).It also contains the pH indicator phenol red. If sugar fermentation occurs, glucose will be initially used and the butt of the tube will be acidic (yellow).After glucose utilization the organism may continue to ferment the remaining sugars. If this occurs the entire tube will become acidic. Certain bacteria are unable to utilize any sugars and will breakdown the peptone present. Peptone

utilization causes an alkaline (red) shift in the medium that causes a color change from orange to red. Blackened medium is caused by hydrogen sulfide production, which changes ferrous sulfate to ferrous sulfide. In addition, splitting of the medium or presence of bubble in the butt of the tube can determine gas productionWith a sterile needle inoculate the TSI slant by stabbing to the bottom of the tube and then streaking the surface of the slant as the needle is drawn out of the tube. Screw the cap on loosely. Incubate at 20-24 °C and read after 18-24 hours

RESULTS: A = Acid; K = Alkaline; H_2S= Hydrogen sulfide produced; N = No change

Slant/Butt	Color/Reaction	Interpretation
K/N or K/A	red/orange (oxidative) or red/yellow (fermentative)	only peptone utilized or only glucose-fermented
A/A	yellow/yellow and/or sucrose-fermented	glucose, plus lactose
Gas	Splitting or bubbles	Gas production
H_2S	Black butt	Hydrogen sulfide produced

Indole Test

To determine bacterial ability to split indole from the tryptophan molecule. Certain bacteria are able to oxidize the amino acid, tryptophan, with tryptophanase enzymes to form three indolic metabolites – indole, skatole (methyl indole), and indoleacetate. Indole, when split from the tryptophan molecule, can be detected with the addition of Kovac's eagent. The reagent is not a dye or stain, but reacts with indole to produce ax AZO dye. Inoculate tryptone broth with a light inoculum from an 18 to 24 hour pure culture. Incubate 24-48 hours at 20-24 °C, at the end of 24 hours

incubation does the following: a). aseptically remove 2 ml of media and place in an empty sterile test tube. Save extra tube for 48-hour incubation, if necessary. b) Add about 5 drops of Kovac's Indole reagent to one of the tubes and agitate tube. c) If a positive reaction is observed, the test is complete. d) If the 24 hours incubated sample is negative, incubate the remaining tube for an additional 4 hours, and test again for the presence of indole with Kovac's Indole reagent

RESULTS: Positive – within 1 to 2 minutes, a cherry red ring will form at the surface of the media

Negative – No color formation I observed at the surface; the color remains that of the reagent – yellow

Variable – An orange color may develop. This indicates the presence of skatole, which may be a precursor of indole formation

Motility

This test determines I a bacterial isolate is motile by means of flagella.Place a drop of distilled water or sterile PBS onto the center of a clean microscope cover glass. Place an additional tiny drop in one corner of the cover glass (to adhere the cover glass to the depression slide when it is inverted). Incubate the center drop from a pure strain culture that is 24-48 hours old using a sterile loop. Carefully invert the cover glass and place over the concave portion of a hanging drop slide. Observe or motility using phase contrast at 400x magnification on a compound microscope. Care shoud be taken to not interpret "drift" or "Brownian motion" as motility. Record results as motile or non-motile.

If this method fails to show motility then inoculate a nutrient broth with the isolate and incubate at room temperature until growth is obtained usually 24 hours.

After incubation use a sterile loop or sterile dropper and place a drop on a clean cover glass. Place a tiny drop of distilled water in one corner of the same cover glass. Continue as above. Semi-solid motility test medium can be used. Stab the medium with a small amount of inoculum. Incubate overnight at room temperature. If the bacterial species is motile, the medium will become turbid with growth that radiates from the line of inoculum. If the bacterial species is non-motile, only the stab line will have visible bacterial growth.

Citrate Utilization

Organisms that are able to use citrate as the sole source for metabolism and growth are able to grow on Simmons citrate agar. By metabolizing citrate by the bacteria alkaline conditions are formed in the medium. The pH indicator in on Simmons citrate agar, bromothymol blue, will turn from green from acidic conditions to a royal blue when the medium becomes alkaline.

Inoculate the Simmons Citrate Agar by making a streak onto the surface of the slant with an 18 to 24 hour old pure culture. Incubate for up to 4 days at 20-24 °C.

RESULTS: Positive – growth and medium color change to a blue-green or royal blue.

Negative – little or no growth and no color change in the medium, remaining dark green.

Urease test

Inoculate the surface of a Christensen urea agar slant and incubate. A position test is indicated by a red-violet color

Nitrate reduction

To determine the ability o an organism to reduce nitrate (NO_3) to nitrite (NO_2) or further reduced products. Inoculate the Nitrate Broth with 18 to 24 hour old pure culture. Incubate 24-48 hours at 20-24 °C aerobically. After incubation add about 5 drops of α-naphthylamine and sulfanilic acid to the medium and shake gently to mix reagent. If there is no color development after addition of α-naphthylamine and sulfanilic acid, add a small amount of zinc dust.

RESULTS: Positive – Formation of a pink or red color in the medium within 1-2 minutes following the addition of α-naphthylamine and sulfanilic acid or no color development within 5-10 minutes after adding zinc dust.

Negative – No pink or red color development within 1-2 minutes following the addition of α-naphthylamine and sulfanilic acid or red color development within 5-10 minutes after adding zinc dust.

Esculin hydrolysis

To determine the ability of an organism to hydrolyze the glycoside esculin (aesculin) to esculetin (aesculetin) and glucose in the presence of bile (10 to 40%). Inoculate the surface of the bile esculin slant with inoculum from an 18 to 24 hour old pure culture. Incubate 20-24 °C for 24 to 48 hours.

RESULTS: Positive- Presence of black to dark brown color on the slant.

Negative-No blackening of the medium.

Voges-Proskauer reaction

To detect the production of acetylmethylcarbinol (actoin), a natural product formed from pyruvic acid in the course of glucose fermentation.Inoculate buffered

glucose broth with the organism and incubate at 37°C for 3 days. Add aproximately 3 ml of alpha napthol, followed by 1 ml of 40% KOH. Mix well and allow to stand for 30 minutes.

RESULTS:

Pink	VP(+)
No change	VP(-)

Malonate Utilization

A method to establish if a bacterial isolate is able to utilize sodium malonate as its only source of carbon.Inoculate malonate broth with a light inoculum from an 18 to 24 hour pure cultureIncubate 24-48 hours at 20-24 °C.

RESULTS: Positive – Light blue to deep blue color throughout the media

Negative – Color re mains the same as un-inoculated tube - green

Gelatinase Liquefaction

A test to determine bacterial production of gelatinase enzymes that liquefy gelatin.Inoculate by stabbing ½ to 1 inch deep into the nutrient gelatin media with a heavy inoculum from an 18 to 24 hour pure culture .Incubate 18 to 24 hours at 20-24°C.

RESULTS: Positive-Media is liquefied.Weak results can be visualized by rapping the tube against the palm of the hand to dislodge droplets of liquid from the media.Any drops seen are considered positive.

Negative-No liquefaction occurs in media.

Carbohydrate fermentation test

Bacteria metabolize carbohydrates by oxidative and /or fermentative pathways. Oxidation occurs in the presence of atmospheric oxygen (aerobic), whereas fermentation takes place in an anaerobic environment. Metabolism of the carbohydrate dextrose by either an aerobic or anaerobic pathway results in acid production. The resulting acidic environment causes the Brom Thymol blue pH indicator in the medium to turn from green to yellow. The presence of bubbles in the tube indicates gas production (aerogenic). If no reaction occurs, the medium can remain unchanged or become alkaline (blue at the surface). A deep butt tube (~7 ml in 16 × 125 mm) is used for this test. With a sterile needle to a small inoculums from an isolated colony and stab to the bottom of the tube. Incubate at 20-24°C for 24-48 hours. Check tubes at 24 hr for acid and/ or gas production.

RESULT: A = acid (yellow); AG = acid + gas, N = no change or alkaline

	Top of Tube	Bottom of Tube
Oxidative	A	N
Fermentative	AG or A	AG or A
Non-reactive	N	N

Decarboxylase Test (Lysine or Ornithine)

A determination of bacterial enzymatic capability to decarboxylate an amino acid to form an amine with resultant alkalinity. For each isolate to be tested, it is necessary to inoculate a decarboxylase control tube and lysine or ornithine test tube. Use light inoculum from 18 to 24 our pure culture. Add 1 to 2 ml oil overlay to each tube. Incubate 24 hours at 20-24 °C. a prolonged incubation of up to four days may be necessary.

RESULTS

Test Result	Lysine or Ornithine Tube	Control Tube
Positive	Turbidto faded purple (glucose fermented, Decarboxylase produced)	Yellow (glucose fermented)
Negative	Yellow (glucose fermented, Decarboxylase not produced)	Yellow (glucose fermented)
Negative	Purple (glucose not fermented, Decarboxylase not produced)	Purple (glucose not fermented)

Arginine Dihydrolase

This method is based on the direct measurement of the disappearance of arginine. Make a dense suspension of the bacteria in 0.033 mM phosphate buffer (pH 6.8). Purge 4 ml pf the suspension by bubbling nitrogen through the suspension for several minutes, and add 1 ml of 0.001 M L-arginine monohydrochloride. After purging again, stopper the tubes, incubate them for 2 hours, and heat them at 100 °C for 15 min. After removing the cells by centrifugation, determine the concentration of arginine in the supernatant by the method of Rosenberg *et al.* as follows. Mix 1 ml of sample with 1 ml of 3 N NaOH, 2 ml of developing solution, and 6 ml of water; read the tubes at 30 min against a blank prepared without arginine by using a colorimeter equipped with a green filter (540 nm); and compare the reading with those obtained with an uninoculated control containing arginine.

RESULTS: A positive test is indicated by the disappearance of some or all of the arginine.

APPENDIX B

Information and raw data of screening, isolation and identification

Table B-1 Comparison of bacterial growth duing pelim screening growth in different medium with and without (control) 25 ppm (0.2 mM) 4-chloroaniline (Fig4.1)

Bacteria	Bacterial growth (OD_{590})									
	0 Days		3 Days		6 Days		12 Days		18 Days	
	4CA[a]	meth[b]	4CA[a]	meth[b]	4CA[a]	meth[b]	4CA[a]	meth[b]	4CA[a]	meth[b]
4CA-2	0.019 ±0.0048	0.0980 ±0.077	0.427 ±0.067	0.211 ±0.023	0.465 ±0.056	0.223 ±0.033	0.687 ±0.055	0.245 ±0.024	0.689 ±0.055	0.246 ±0.024
4CA-16	0.097 ±0.057	0.015 ±0.0086	0.411 ±0.043	0.247 ±0.034	0.430 ±0.038	0.355 ±0.012	0.492 ±0.052	0.326 ±0.011	0.504 ±0.052	0.326 ±0.011
4CA-17	0.095 ±0.0087	0.095 ±0.097	0.311 ±0.0032	0.225 ±0.034	0.358 ±0.0033	0.265 ±0.023	0.755 ±0.0043	0.316 ±0.033	0.758 ±0.0043	0.315 ±0.033
4CA-19	0.097 ±0.0054	0.096 ±0.088	0.0272 ±0.044	0.180 ±0.034	0.0640 ±0.045	0.190 ±0.036	0.858 ±0.034	0.216 ±0.032	0.859 ±0.034	0.216 ±0.032
4CA-20	0.098 ±0.078	0.016 ±0.086	0.300 ±0.023	0.122 ±0.021	0.595 ±0.032	0.150 ±0.022	0.950 ±0.021	0.170 ±0.032	0.958 ±0.021	0.170 ±0.032

4CA[a] = 4-Chloroaniline containing mineral medium (25ppm 4-chloroaniline was provided and dissolved in methanol)
meth[b] = Without 4-chloroaniline in mineral medium (methanol used as solvent)

B.1 Determination of % remaining 4-chloroaniline and 4-chloroaniline degradation

Percentage of remaining 4-chloroaniline and percentage of 4-chloroaniline degradation were calculated from the HPLC peak area when compared to that of the standard curve (Appendix D). The following equations were used to calculate % 4-chloroaniline and 4-chloroaniline degradation

$$\% \text{ remaining 4-chloroaniline in 2 days} = 100 \times \frac{\text{Peak area reported in day-2}}{\text{Peak area reported in day-0}}$$

$$\% \text{ 4-chloroaniline degradation in 2 days} = 100 - 100 \times \frac{\text{Peak area reported in day-2}}{\text{Peak area reported in day-0}}$$

B.2 Preliminary-screening of 4-chloroaniline-degrading bacteria

Table B-2 Percentage of remaining 4-chloroaniline by resting cells of five 4-chloroaniline-degrading bacteria (Fig.4.2)

Days	Percentage of remaining 4-chloroaniline				
	4CA-2	**4CA-16**	**4CA-17**	**4CA-19**	**4CA-20**
0	100.00 ± 0.57	100.00 ± 0.45	100.00 ± 0.87	100.00 ± 0.77	100.00 ± 0.97
3	95.23 ± 1.24	87.75 ± 3.32	84.53 ± 2.57	90.51 ± 2.87	91.98 ± 3.44
6	91.93 ± 3.50	78.68 ± 3.87	65.23 ± 3.45	83.19 ± 3.21	97.05 ± 3.44
9	88.87 ± 1.77	63.63 ± 2.78	50.10 ± 4.35	75.80 ± 1.75	88.06 ± 3.53
12	48.74 ± 2.67	30.04 ± 3.51	21.80 ± 2.44	74.00 ± 2.83	77.65 ± 3.55
18	50.65 ± 3.44	24.36 ± 3.46	16.81 ± 3.56	78.24 ± 3.51	89.02 ± 3.87

APPENDIX C

Information and raw data of 4-chloroaniline-degrading bacteria by three newly bacteria isolate (*Acinetobacter baumannii* (4CA-2), *Pseudomonas putida* (4CA-16), *and Klebsiella pneumoniae* (4CA-17))

C.1 Kinetic of 4-chloroaniline transformations

Specific degradation rate

The substrate degradation kinetic in batch culture is described by simple differential equations, which are described by

$$-\frac{d\,\mathrm{S}}{d\,\mathrm{t}} = q\,\mathrm{X}$$

Where X is the cell concentration, t is the cultivation time, and q is the specific degradation rate of the substrate. Therefore, to the calculation of specific degradation rate is to determine cell concentration and the disappearance of substrate during the time indicated.

In this experiment, d S/d t is the slope, of the plot between with concentration of 4CA (mM) as (X axis) and incubation time (Y axis). The slope represents the 4-chloroaniline degradation rate during time indicated.

There are several methods to determined cell concentration such as wet cell weight, dry cell weight, viable count and etc., but they have the effect of technical error and their precision. Therefore, cell protein concentration is determined to represent cell the concentration for calculation of specific degradation rate in this test. Protein estimation is to determine the colorimetric assay of Modified Lowry method Dulley and Grieve (1975) and Lowry et al. (1951).

Thus, Specific degradation rate was determined as degradation rate (slope) divided by cell protein concentration in this experiment which was described by

$$\text{Specific degradation rate } (\text{nmol.(min.mg.protein)}^{-1}) = \frac{\text{Degradation rate (slope) (nmol.min}^{-1})}{\text{Cell protein concentration } (\Delta X)\ (\text{mg.protein})}$$

Growth rate

During the exponential (or logarathmic) growth phase, a bacterial culture mimics a first-order chemical reaction, i.e. the rate of increase of cells is proportional to the number of bacteria present at that time. The constant of proportionality, μ, is an index of the growth rate and is called the growth rate constant:

Rate of increase of cells = μ x number of cells

The value of μ can be determined from the following equation:

$$\ln N_t - \ln N_0 = \mu(t - t_0)$$

in other words:

the natural log of the number of cells at time t minus the natural log of the number of cells at time zero (t_0) equals the growth rate constant multiplied by the time interval.

For most purposes, it is easier to use $\log_{10}$ values rather than natural logs, so the above equation can be converted as follows:

$$\log_{10} N - \log_{10} N_0 = (\mu/2.303)\,(t - t_0)$$

or alternatively:

$$\mu = ((\log_{10} N - \log_{10} N_0)\, 2.303) / (t - t_0)$$

By measuring the increase in the number of cells during a certain time period, the growth rate constant (μ) can be calculated.

(http://www-micro.msb.le.ac.uk/LabWork/bact/bact17.htm quoted from Cann, 2002)

Ex. Growth rate (μ) of *Acinetobacter baumannii* (4CA-2)

$\mu = ((\log_{10} N - \log_{10} N_0)\, 2.303) / (t - t_0)$

$\mu = ((\log_{10} 11.12 - \log_{10} 3.96)\, 2.303) / (8 - 0)$

$\mu = 2.06\ day^{-1}$

$\mu = 0.086\ h^{-1}$

C.2 Degradation test of three 4-chloroanine-degrading bacteria

Table C-1 The percentage of 4-chloroaniline degradation and growth of 4-chloroaniline degraders (Fig.4.6)

Condition	4-Chloroaniline degradation (%)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
4CA-2	100.00 ± 2.50	83.25 ± 1.68	58.44 ± 5.50	48.75 ± 5.50	42.01 ± 4.40	40.00 ± 4.40	39.00 ± 1.68
4CA-16	100.00 ± 3.50	78.33 ± 4.70	67.00 ± 5.20	55.34 ± 2.90	53.94 ± 3.50	43.22 ± 3.80	39.97 ± 3.60
4CA-17	100.00 ± 2.50	64.77 ± 3.87	40.81 ± 4.80	39.00 ± 6.05	39.24 ± 4.66	38.70 ± 3.50	37.18 ± 3.87
control	100.00 ± 1.57	96.73 ± 1.57	90.04 ± 1.68	88.23 ± 1.57	87.04 ± 1.68	86.80 ± 2.50	84.25 ± 2.50
Condition	**Cell turbidity (OD590nm)**						
	0 day	**2 day**	**4 day**	**6 day**	**8 day**	**10 day**	**12 day**
4CA-2	0.321 ± 0.050	0.421 ± 0.050	0.561 ± 0.080	0.622 ± 0.070	0.872 ± 0.050	0.894 ± 0.050	0.901 ± 0.050
4CA-16	0.122 ± 0.020	0.211 ± 0.020	0.233 ± 0.110	0.282 ± 0.080	0.351 ± 0.080	0.422 ± 0.050	0.463 ± 0.080
4CA-17	0.212 ± 0.050	0.312 ± 0.080	0.322 ± 0.070	0.421 ± 0.050	0.541 ± 0.080	0.642 ± 0.060	0.682 ± 0.040
control	0.010 ± 0.005	0.010 ± 0.005	0.010 ± 0.008	0.010 ± 0.005	0.010 ±0.005	0.010 ± 0.005	0.010 ± 0.008

Table C-2 Degradation of 4-chloroanine by 4CA degraders: *Acinetobacter baumannii* (4CA-2), *Pseudomonas putida* (4CA-16) and *Klebsiella pneumoniae* (4CA-17) (Fig4.7)

Condition	4-Chloroaniline degradation (%)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
4CA-2	0.20 ± 0.02	0.16 ± 0.01	0.10 ± 0.02	0.09 ± 0.01	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.02
4CA-16	0.20 ± 0.01	0.15 ± 0.03	0.11 ± 0.01	0.10 ± 0.03	0.09 ± 0.02	0.08 ± 0.03	0.06 ± 0.01
4CA-17	0.20 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.04	0.06 ± 0.01
control	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.16 ± 0.01

Table C-3 Accumulation of chloride during 4CA degradation by *Acinetobacter baumannii* (4CA-2), *Pseudomonas putida* (4CA-16) and *Klebsiella pneumoniae* (4CA-17) (Fig4.7)

Time (days)	Concentration of Chloride (mM)			
	Acinetobacter baumannii (4CA-2)	*Pseudomonas putida* (4CA-16)	*Klebsiella pneumoniae* (4CA-17)	Control
0	0.05 ± 0.006	0.05 ± 0.008	0.05 ± 0.006	0.15 ± 0.006
4	0.07 ± 0.010	0.06 ± 0.008	0.13 ±0.010	0.19 ± 0.006
8	0.12 ± 0.006	0.09 ± 0.010	0.14 ± 0.006	0.02 ± 0.006
12	0.14 ±0.006	0.13 ± 0.010	0.14 ± 0.010	0.02 ± 0.006

Table C-4 Determination of cell protein concentration by using protein basis (using protein standard in APPENDIX F)

Cell-free extract of bacterial strain	Protein determination using Modified Lowry method (mg/ml)	Cell protein concentration (g. protein/liter)
Acinetobacter baumannii (4CA-2)	0.413 ± 0.0466	0.3868 ± 0.0439
Pseudomonas putida (4CA-16)	0.212 ± 0.0393	0.1981 ± 0.0370
Klebsiella pneumoniae (4CA-17)	0.300 ± 0.0483	0.2830 ± 0.0456

Table C-5 Effect of additional carbon and/or nitrogen source which were supplemented in addition to mineral medium containing 25 ppm 4-chloroaniline on degradation and growth of 4-chloroaniline of *Acinetobacter baumannii* (4CA-2) (Fig 4.8)

Condition	Cell turbidity (OD 590 nm)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
4CA-2 + 4CA	0.221 ± 0.050	0.321 ± 0.050	0.462 ± 0.050	0.522 ± 0.090	0.602 ± 0.090	0.654 ± 0.050	0.661 ± 0.050
4 mM succinate + 4CA	0.237 ± 0.050	0.323 ± 0.050	0.327 ± 0.080	0.382 ± 0.080	0.391 ± 0.050	0.401 ± 0.050	0.402 ± 0.050
4 mM citrate + 4CA	0.254 ± 0.030	0.351 ± 0.090	0.401 ± 0.050	0.575 ± 0.050	0.630 ± 0.030	0.660 ± 0.030	0.670 ± 0.030
4 mM NH_4Cl + 4CA	0.223 ± 0.090	0.322 ± 0.050	0.422 ± 0.080	0.591 ± 0.080	0.630 ± 0.050	0.656 ± 0.050	0.672 ± 0.050
4 mM $NaNO_3$ + 4CA	0.240 ± 0.030	0.342 ± 0.090	0.362 ± 0.050	0.381 ± 0.050	0.401 ± 0.050	0.405 ± 0.050	0.405 ± 0.050
1 *m*M aniline + 4CA	0.211 ± 0.120	0.321 ± 0.120	0.351 ± 0.080	0.372 ± 0.080	0.400 ± 0.050	0.402 ± 0.030	0.402 ± 0.050
control	0.01 ± 0.010	0.01 ± 0.010	0.01 ± 0.010	0.01 ± 0.010	0.01 ± 0.010	0.01 ± 0.010	0.01 ± 0.010
Condition	**4-Chloroaniline degradation (%)**						
	0day	**2days**	**4days**	**6days**	**8days**	**10days**	**12days**
4CA-2 + 4CA	0 ± 0	16.75 ± 1.68	44.85 ± 5.50	50.14 ± 5.50	59.42 ± 4.40	60.42 ± 4.40	61.88 ± 1.68
4 mM citrate + 4CA	0 ± 0	16.53 ± 4.40	17.79 ± 3.31	19.77 ± 3.31	23.26 ± 3.87	25.56 ± 3.87	26.75 ± 2.95
4 mM succinate + 4CA	0 ± 0	23.26 ± 4.82	38.75 ± 3.31	45.27 ± 4.40	57.95 ± 2.87	63.67 ± 2.87	64.06 ± 2.95
4 mM NH_4Cl + 4CA	0 ± 0	12.32 ± 3.35	23.44 ± 3.31	53.86 ± 4.40	64.00 ± 2.87	68.20 ± 2.95	68.21 ± 2.95
4 mM $NaNO_3$ + 4CA	0 ± 0	2.62 ± 2.32	8.24 ± 2.87	28.11 ± 4.40	28.90 ± 2.95	29.39 ± 4.40	29.54 ± 3.31
1 *m*M aniline + 4CA	0 ± 0	7.52 ± 4.21	18.26 ± 4.54	44.34 ± 4.54	44.65 ± 3.35	44.72 ± 3.35	44.74 ± 3.31
control	0 ± 0	1.45 ± 0.31	8.60 ± 2.55	10.57 ± 2.87	15.09 ± 3.31	15.34 ± 2.55	15.57 ± 3.31

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-4 Effect of additional carbon and/or nitrogen source which were supplemented in addition to mineral medium containing 25 ppm 4-chloroaniline on degradation of 4-chloroaniline in *Acinetobacter baumannii* (4CA-2) (Fig 4.9 – Fig 4.11)

Condition	4-Chloroaniline degradation (%)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
control	0 ± 0	0.75 ± 3.31	8.19 ± 2.55	10.76 ± 2.87	14.13 ± 3.31	14.90 ±2.55	15.14 ± 3.31
4CA-2+4CA	0 ± 0	19.51 ± 1.68	43.36 ± 5.50	47.98 ± 5.50	60.01 ± 4.40	60.04 ± 4.40	60.39 ± 1.68
4 mM citrate + 4CA	0 ± 0	23.22 ± 4.82	38.80 ± 3.31	45.29 ± 4.40	57.54 ± 2.87	63.70 ± 2.87	64.08 ± 2.95
8 mM citrate + 4CA	0 ± 0	3.46 ± 2.69	6.75 ± 1.79	17.15 ± 1.00	24.94 ± 1.60	25.70 ± 1.65	25.85 ± 1.77
4 mM NH_4Cl + 4CA	0 ± 0	2.33 ± 1.73	11.40 ± 1.99	16.55 ± 1.57	18.71 ± 1.36	23.40 ± 1.17	23.48 ± 1.25
4 mM NH4Cl + 4CA	0 ± 0	12.82 ± 3.35	23.46 ± 3.31	53.85 ± 4.40	64.00 ± 2.87	68.22 ± 2.95	68.33 ± 2.95
8 mM NH4Cl + 4CA	0 ± 0	12.28 ± 1.17	15.82 ± 1.87	38.82 ± 1.33	62.82 + 1.87	62.98 ± 1.72	63.15 ± 1.69
18 mM NH4Cl + 4CA	0 ± 0	17.86 ± 1.97	31.41 ± 1.97	50.13 ± 1.65	62.04 ± 1.87	62.10 ± 2.87	62.32 ± 2.87
4 mM citrate + l4 mM NH_4Cl+ 4CA	0 ± 0	23.62 ± 1.87	33.30 ± 2.87	60.66 ± 1.13	67.57 ± 1.89	68.50 ± 1.88	70.16 ± 1.56
8 mM citrate + 8 mM NH_4Cl+ 4CA	0 ± 0	8.22 ± 1.68	12.92 ± 1.46	19.80 ± 1.68	33.16 ± 1.22	33.26 ± 2.87	33.38 ± 1.68
18 mM citrate + 18 mM NH_4C l+ 4CA	0 ± 0	9.50 ± 1.87	17.51 ± 1.87	20.30 ± 1.87	23.91 ± 2.87	29.30 ± 1.41	29.35 ± 2.87

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-5 Effect of additional carbon and/or nitrogen source which were supplemented in addition to mineral medium containing 25 ppm 4-chloroaniline on growth in *Acinetobacter baumannii* (4CA-2) (Fig 4.9 – Fig 4.11)

Condition	Cell turbidity (OD590nm)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
control	0.01 ± 0.05	0.01 ± 0.05	0.01 ± 0.08	0.01 ± 0.07	0.01 ± 0.05	0.01 ± 0.05	0.01 ± 0.05
4CA-2 + 4CA	0.23 ± 0.03	0.32 ± 0.09	0.46 ± 0.05	0.51 ± 0.05	0.62 ± 0.03	0.64 ± 0.03	0.64 ± 0.03
4 mM citrate + 4CA	0.25 ± 0.03	0.35 ± 0.02	0.40 ± 0.04	0.58 ± 0.02	0.63 ± 0.02	0.66 ± 0.04	0.67 ± 0.08
8 mM citrate + 4CA	0.22 ± 0.05	0.33 ± 0.04	0.33 ± 0.04	0.34 ± 0.05	0.36 ± 0.02	0.41 ± 0.04	0.41 ± 0.04
18 mM citrate + 4CA	0.26 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.36 ± 0.01
4 mM NH_4Cl + 4CA	0.22 ± 0.09	0.32 ± 0.05	0.42 ± 0.08	0.59 ± 0.08	0.63 ± 0.05	0.66 ± 0.05	0.67 ± 0.05
8 mM NH_4Cl + 4CA	0.27 ± 0.05	0.33 ± 0.04	0.36 ± 0.04	0.47 ± 0.05	0.63 ± 0.02	0.63 ± 0.04	0.63 ± 0.04
18 mM NH_4Cl + 4CA	0.28 ± 0.03	0.30 ± 0.04	0.32 ± 0.04	0.47 ± 0.02	0.61 ± 0.02	0.61 ± 0.04	0.61 ± 0.04
4 mM citrate + l4 mM NH_4Cl+ 4CA	0.28 ± 0.05	0.50 ± 0.04	0.61 ± 0.04	0.62 ± 0.02	0.68 ± 0.02	0.69 ± 0.04	0.69 ± 0.04
8 mM citrate + 8 mM NH_4Cl+ 4CA	0.29 ± 0.03	0.42 ± 0.04	0.42 ± 0.02	0.41 ± 0.02	0.40 ± 0.02	0.39 ± 0.04	0.39 ± 0.02
18 mM citrate + 18 mM NH_4C l+ 4CA	0.290 ± 0.03	0.46 ± 0.04	0.42 ± 0.04	0.42 ± 0.02	0.42 ± 0.02	0.41 ± 0.02	0.40 ± 0.04

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-6 Effect of carbon and/or nitrogen source which were supplemented in addition to mineral medium on growth in *Acinetobacter baumannii* (4CA-2) (Fig.4.12- Fig. 4.14)

Condition	Cell turbidity (OD_{590})						
	0day	2days	4days	6days	8days	10days	12days
4 mM Citrate	0.238 ± 0.020	0.361 ± 0.040	0.348 ± 0.040	0.350 ± 0.040	0.350 ± 0.020	0.360 ± 0.040	0.360 ± 0.040
8 mM Citrate	0.230 ± 0.030	0.340 ± 0.020	0.360 ± 0.040	0.360 ± 0.020	0.360 ± 0.020	0.360 ± 0.020	0.360 ± 0.080
18 mM Citrate	0.266 ± 0.050	0.368 ± 0.040	0.347 ± 0.050	0.329 ± 0.050	0.323 ± 0.020	0.317 ± 0.040	0.314 ± 0.040
4 mM NH_4Cl	0.250 ± 0.020	0.336 ± 0.040	0.329 ± 0.040	0.318 ± 0.020	0.308 ± 0.020	0.302 ± 0.040	0.302 ± 0.040
8 mM NH_4Cl	0.250 ± 0.020	0.340 ± 0.040	0.350 ± 0.040	0.348 ± 0.040	0.340 ± 0.020	0.331 ± 0.040	0.330 ± 0.040
18 mM NH_4Cl	0.253 ± 0.030	0.352 ± 0.040	0.322 ± 0.040	0.312 ± 0.020	0.312 ± 0.020	0.310 ± 0.020	0.299 ± 0.040
4 mM Citrate + 4 mM NH_4Cl	0.222 ± 0.050	0.390 ± 0.020	0.550 ± 0.040	0.590 ± 0.020	0.583 ± 0.020	0.583 ± 0.040	0.583 ± 0.040
8 mM Citrate + 8 mM NH_4Cl	0.229 ± 0.030	0.320 ± 0.020	0.426 ± 0.020	0.416 ± 0.040	0.413 ± 0.020	0.391 ± 0.040	0.390 ± 0.020
18 mM Citrate + 18 mM NH_4Cl	0.227 ± 0.030	0.330 ± 0.040	0.448 ± 0.040	0.425 ± 0.020	0.423 ± 0.040	0.420 ± 0.020	0.410 ± 0.040

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-7 Effect of additional carbon and/or nitrogen source which were supplemented in addition to mineral medium containing 25 ppm 4-chloroaniline on degradation and growth of 4-chloroaniline of *Pseudomonas putida* (4CA-16) (Fig 4.15)

Condition	Cell turbidity (OD 590 nm)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
4CA-16 + 4CA	0.122 ± 0.050	0.211 ± 0.080	0.223 ± 0.020	0.282 ± 0.050	0.352 ± 0.050	0.422 ± 0.080	0.461 ± 0.080
4 mM succinate + 4CA	0.152 ± 0.090	0.212 ± 0.050	0.221 ± 0.050	0.311 ± 0.050	0.411 ± 0.050	0.475 ± 0.120	0.476 ± 0.080
4 mM citrate + 4CA	0.192 ± 0.050	0.224 ± 0.080	0.323 ±0.020	0.421 ± 0.050	0.452 ± 0.050	0.454 ± 0.080	0.454 ± 0.080
4 mM NH_4Cl + 4CA	0.147 ± 0.090	0.201 ± 0.120	0.212 ±0.080	0.242 ± 0.080	0.292 ± 0.050	0.353 ± 0.050	0.387 ± 0.090
4 mM $NaNO_3$ + 4CA	0.151 ± 0.050	0.211 ± 0.080	0.212 ± 0.020	0.322 ±0.050	0.365 ± 0.050	0.372 ± 0.080	0.374 ± 0.080
1 *m*M aniline + 4CA	0.189 ± 0.050	0.216 ±0.120	0.229 ±0.080	0.354 ±0.080	0.364 ± 0.120	0.389 ± 0.120	0.395 ± 0.090
control	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010
Condition	**4-Chloroaniline degradation (%)**						
	0day	**2days**	**4days**	**6days**	**8days**	**10days**	**12days**
4CA-16+ 4CA	0 ± 0	21.63 ± 4.7	40.33 ± 5.20	44.56 ± 2.90	49.68 ± 3.50	56.44 ± 3.80	60.48 ± 3.60
4 mM citrate + 4CA	0 ± 0	17.68 ± 2.87	25.31 ± 2.87	42.18 ± 2.55	58.95 ± 2.55	63.63 ± 1.57	68.31 ± 2.87
4 mM succinate + 4CA	0 ± 0	22.22 ± 1.57	29.28 ± 1.49	46.17 ± 2.55	65.16 ± 3.55	68.20 ± 2.75	68.24 ± 2.55
4 mM NH_4Cl + 4CA	0 ± 0	5.15 ± 1.55	11.52 ± 2.55	16.81 ± 3.38	19.79 ± 3.57	23.47 ± 3.57	39.25 ± 4.22
4 mM $NaNO_3$ + 4CA	0 ± 0	3.89 ± 0.51	9.16 ± 2.55	19.46 ± 3.42	22.77 ± 2.55	22.87 ± 3.38	22.97 ± 3.38
1 *m*M aniline + 4CA	0 ± 0	12.15 ± 3.31	20.14 ± 4.22	39.07 ± 3.57	40.60 ± 4.22	43.55 ± 2.55	43.63 ± 2.87
control	0 ± 0	1.45 ± 0.31	8.60 ± 2.55	10.57 ± 2.87	15.09 ± 3.31	15.34 ± 2.55	15.57 ± 3.31

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-8 Effect of additional carbon source which were supplemented in addition to mineral medium containing 25 ppm 4-chloroaniline on degradation in *Pseudomonas putida* (4CA-16) (Fig 4.16- Fig 4.17)

Condition	4-Chloroaniline degradation (%)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
control	0 ± 0	0.74 ± 3.31	8.12 ± 2.55	10.60 ± 2.87	14.14 ± 3.31	14.90 ± 2.55	15.14 ± 3.31
4CA-16 + 4CA	0 ± 0	22.03 ± 4.70	38.09 ± 5.20	45.08 ± 2.90	48.50 ± 3.50	55.27 ± 3.80	57.87 ± 3.60
4 mM citrate + 4CA	0 ± 0	22.22 ± 1.57	29.28 ± 1.49	46.17 ± 2.55	65.16 ± 3.55	68.20 ± 2.75	68.24 ± 2.55
8 mM citrate + 4CA	0 ± 0	4.31 ± 2.55	8.10 ± 2.55	16.05 ± 3.38	26.67 ± 3.57	26.69 ± 3.57	26.75 ± 4.22
18 mM citrate + 4CA	0 ± 0	9.79 ± 3.55	16.50 ± 2.55	26.06 ± 3.42	32.78 ± 2.55	32.85 ± 3.38	32.91 ± 3.38
4 mM succinate + 4CA	0 ± 0	17.68 ± 2.87	25.31 ± 2.87	42.18 ± 2.55	58.95 ± 2.55	63.63 ± 1.57	68.31 ± 2.87
8 mM succinate + 4CA	0 ± 0	5.98 ± 3.55	18.51 ± 3.57	22.91 ± 3.38	38.13 ± 3.57	39.30 ± 2.55	40.00 ± 4.22
18 mM succinate + 4CA	0 ± 0	10.27 ± 3.55	15.19 ± 2.55	19.24 ± 3.42	30.30 ± 2.55	36.11 ± 3.38	36.15 ± 3.38

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-9 Effect of additional carbon source which were supplemented in addition to mineral medium containing 25 ppm 4-chloroaniline on growth in *Pseudomonas putida* (4CA-16) (Fig 4.16 – Fig 4.17)

Condition	Cell turbidity (OD 590 nm)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
Control	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
4CA-16 + 4CA	0.23 ± 0.05	0.24 ± 0.08	0.26 ± 0.02	0.35 ± 0.05	0.35 ± 0.05	0.42 ± 0.08	0.44 ± 0.08
4 mM citrate + 4CA	0.25 ± 0.05	0.35 ± 0.08	0.40 ± 0.02	0.58 ± 0.05	0.63 ± 0.05	0.66 ± 0.08	0.67 ± 0.08
8 mM citrate + 4CA	0.23 ± 0.09	0.38 ± 0.08	0.38 ± 0.08	0.39 ± 0.08	0.40 ± 0.05	0.40 ± 0.05	0.40 ± 0.09
18 mM citrate + 4CA	0.25 ± 0.05	0.40 ± 0.08	0.42 ± 0.02	0.41 ± 0.05	0.40 ± 0.05	0.40 ± 0.08	0.40 ± 0.08
4 mM succinate + 4CA	0.24 ± 0.09	0.35 ± 0.05	0.36 ± 0.05	0.38 ± 0.05	0.39 ± 0.05	0.40 ± 0.05	0.40 ± 0.08
8 mM succinate + 4CA	0.22 ± 0.09	0.34 ± 0.04	0.34 ± 0.08	0.37 ± 0.08	0.39 ± 0.05	0.39 ± 0.05	0.40 ± 0.09
18 mM succinate + 4CA	0.25 ± 0.05	0.33 ± 0.08	0.34 ± 0.02	0.35 ± 0.05	0.37 ± 0.05	0.38 ± 0.08	0.40 ± 0.08

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-10 Effect of carbon source which were supplemented in addition to mineral medium on growth of 4-chloroaniline in *Pseudomonas putida* (4CA-16) (Fig 4.18- Fig 4.19)

Condition	Cell turbidity (OD_{590})						
	0day	2days	4days	6days	8days	10days	12days
4 mM Citrate	0.242 ± 0.050	0.521 ± 0.080	0.445 ± 0.020	0.348 ± 0.050	0.275 ± 0.050	0.272 ± 0.080	0.271 ± 0.080
8 mM Citrate	0.242 ± 0.040	0.518 ± 0.080	0.434 ± 0.080	0.422 ± 0.080	0.391 ± 0.050	0.355 ± 0.050	0.322 ± 0.040
18 mM Citrate	0.232 ± 0.050	0.502 ± 0.080	0.451 ± 0.020	0.415 ± 0.050	0.410 ± 0.050	0.388 ± 0.080	0.354 ± 0.080
4 mM Succinate	0.252 ± 0.090	0.453 ± 0.050	0.393 ± 0.050	0.387 ± 0.050	0.314 ± 0.050	0.280 ± 0.040	0.249 ± 0.040
8 mM Succinate	0.241 ± 0.040	0.425 ± 0.040	0.375 ± 0.040	0.351 ± 0.040	0.322 ± 0.050	0.312 ± 0.050	0.300 ± 0.040
18 mM Succinate	0.232 ± 0.050	0.411 ± 0.020	0.402 ± 0.020	0.323 ± 0.050	0.318 ± 0.040	0.225 ± 0.020	0.220 ± 0.040

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-11 Effect of additional carbon and/or nitrogen source which were supplemented in addition to mineral medium containing 25 chloroaniline on degradation and growth of 4-chloroaniline of *Klebsiella pneumoniae* (4CA-17) (Fig 4.20)

Condition	Cell turbidity (OD 590 nm)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
4CA-17 + 4CA	0.221 ± 0.050	0.321 ± 0.050	0.462 ± 0.050	0.522 ± 0.110	0.620 ± 0.050	0.654 ± 0.080	0.661 ± 0.080
4 mM succinate + 4CA	0.237 ± 0.050	0.323 ± 0.050	0.327 ± 0.080	0.382 ± 0.050	0.391 ± 0.050	0.401 ± 0.080	0.402 ± 0.050
4 mM citrate + 4CA	0.254 ± 0.090	0.351 ± 0.110	0.401 ± 0.050	0.575 ± 0.050	0.630 ± 0.050	0.660 ± 0.050	0.670 ± 0.050
4 mM NH_4Cl + 4CA	0.223 ± 0.050	0.322 ± 0.050	0.422 ± 0.050	0.591 ± 0.050	0.630 ± 0.050	0.656 ± 0.050	0.672 ± 0.050
4 mM $NaNO_3$ + 4CA	0.24 ±0.090	0.342 ± 0.050	0.362 ±0.080	0.381 ± 0.080	0.401 ± 0.050	0.405 ± 0.050	0.405 ± 0.050
1 *m*M aniline + 4CA	0.211 ±0.090	0.321 ± 0.120	0.351 ±0.080	0.372 ± 0.080	0.400 ± 0.050	0.402 ± 0.050	0.402 ±0.050
control	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010	0.010 ± 0.010
Condition	**4-Chloroaniline degradation (%)**						
	0day	**2days**	**4days**	**6days**	**8days**	**10days**	**12days**
4CA-17 + 4CA	0 ± 0	35.29 ± 3.87	43.66 ± 4.80	53.59 ± 6.05	57.29 ± 4.66	63.20 ± 3.50	65.30 ± 3.87
4 mM citrate + 4CA	0 ± 0	7.02 ± 1.57	12.41 ± 3.35	16.48 ± 3.35	18.83 ± 4.66	22.02 ± 3.87	30.88 ± 2.87
4 mM succinate + 4CA	0 ± 0	22.60 ± 3.35	23.06 ± 4.54	28.70 ± 4.54	29.47 ± 2.87	30.41 ± 2.87	30.43 ± 3.31
4 mM NH_4Cl + 4CA	0 ± 0	3.66 ± 0.31	6.33 ± 0.81	11.15 ± 3.57	15.77 ± 4.66	23.62 ± 3.87	24.09 ± 3.87
4 mM $NaNO_3$ + 4CA	0 ± 0	6.58 ± 2.87	7.41 ± 1.22	17.24 ±2.87	17.39 ± 3.31	23.80 ± 4.22	23.97 ± 3.31
1 *m*M aniline + 4CA	0 ± 0	17.81 ± 3.31	45.77 ± 4.66	61.69 ±2.87	65.08 ± 3.31	68.31 ± 4.22	68.38 ± 3.31
control	0 ± 0	1.45 ± 0.31	8.60 ± 2.55	10.57 ± 2.87	15.09 ± 3.31	15.34 ± 2.55	15.57 ± 3.31

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-12 Effect of additional carbon and nitrogen source which were supplemented in addition to mineral medium containing 25 ppm 4-chloroaniline to growth in *Klebsiella pneumoniae* (4CA-17) (Fig4.21)

Condition	4-Chloroaniline degradation (%)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
control	0 ± 0	0.74 ± 3.31	8.12 ± 2.55	10.60 ± 2.87	14.14 ± 3.31	14.90 ± 2.55	15.14 ± 3.31
4CA-17 + 4CA	0 ± 0	33.01 ± 3.87	42.34 ± 4.80	53.86 ± 6.05	54.97 ± 4.66	63.39 ± 3.50	64.46 ± 3.87
1 mM aniline + 4CA	0 ± 0	17.81 ± 3.31	45.77 ± 4.66	61.69 ± 2.87	65.08 ± 3.31	68.31 ± 4.22	68.38 ± 3.31
2 mM aniline + 4CA	0 ± 0	28.39 ± 3.31	44.68 ± 4.81	53.38 ± 3.57	63.19 ± 4.66	67.10 ± 3.87	67.19 ± 3.87
4 mM aniline + 4CA	0 ± 0	20.67 ± 2.87	40.25 ± 4.22	53.97 ± 2.87	54.29 ± 3.31	62.24 ± 4.22	62.32 ± 3.31
8 mM aniline + 4CA	0 ± 0	3.24 ± 3.31	8.76 ± 4.66	9.79 ± 2.87	9.94 ± 3.31	10.04 ± 3.31	10.14 ± 2.87
16 mM aniline l + 4CA	0 ± 0	1.17 ± 4.66	8.41 ± 2.87	9.20 ± 4.66	9.36 ± 2.87	9.44 ± 3.50	10.06 ± 3.50

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-13 Effect of 4-chloroaniline on growth in *Klebsiella pneumoniae* (4CA-17) (Fig 4.22- Fig 4.23)

Condition	Cell turbidity (OD 590 nm)						
	0 day	2 day	4 day	6 day	8 day	10 day	12 day
control	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
4CA-17 + 4CA	0.21 ± 0.05	0.24 ± 0.08	0.32 ± 0.07	0.44 ± 0.05	0.54 ± 0.08	0.61 ± 0.06	0.62 ± 0.04
1 mM aniline + 4CA	0.21 ± 0.09	0.32 ± 0.12	0.38 ± 0.08	0.45 ± 0.08	0.58 ± 0.05	0.65 ± 0.05	0.65 ± 0.05
2 mM aniline + 4CA	0.24 ± 0.05	0.35 ± 0.05	0.38 ± 0.08	0.48 ± 0.05	0.54 ± 0.05	0.66 ± 0.04	0.68 ± 0.05
4 mM aniline + 4CA	0.26 ± 0.04	0.35 ± 0.08	0.37 ± 0.05	0.46 ± 0.08	0.55 ± 0.05	0.56 ± 0.05	0.56 ± 0.05
8 mM aniline + 4CA	0.27 ± 0.05	0.28 ± 0.05	0.28 ± 0.05	0.28 ± 0.05	0.28 ± 0.04	0.28 ± 0.04	0.28 ± 0.05
16 mM aniline l + 4CA	0.27 ± 0.04	0.28 ± 0.05	0.28 ± 0.04	0.28 ± 0.08	0.28 ± 0.05	0.28 ± 0.05	0.28 ± 0.05
Condition	**Cell turbidity (OD_{590})**						
	0day	**2days**	**4days**	**6days**	**8days**	**10days**	**12days**
1 mM aniline	0.261 ± 0.040	0.440 ± 0.080	0.480 ± 0.080	0.502 ± 0.080	0.555 ± 0.050	0.605 ± 0.050	0.605 ± 0.050
2mM aniline	0.275 ± 0.050	0.457 ± 0.050	0.487 ± 0.080	0.529 ± 0.050	0.609 ± 0.040	0.684 ± 0.040	0.683 ± 0.080
4 mM aniline	0.251 ± 0.040	0.351 ± 0.080	0.377 ± 0.050	0.457 ± 0.080	0.484 ± 0.050	0.585 ± 0.050	0.580 ± 0.040
8 mM aniline	0.261 ± 0.050	0.268 ± 0.050	0.264 ± 0.050	0.264 ± 0.050	0.262 ± 0.040	0.261 ± 0.040	0.261 ± 0.050
16 mM aniline	0.245 ± 0.040	0.248 ± 0.050	0.249 ± 0.040	0.248 ± 0.080	0.245 ± 0.050	0.245 ± 0.050	0.245 ± 0.050

4-Chloroaniline without inoculums were maintained as abiotic control

Table C-14 Growth of 4-chloroaniline in various concentrations by 4-chloroaniline degraders (Fig. 4.24-Fig 4.27)

Conc.	Sample	Bacterial growth (OD_{590})						
		0day	2days	4days	6days	8days	10days	12days
25 ppm	4CA-2	0.233 ± 0.050	0.321 ± 0.050	0.460 ± 0.080	0.505 ± 0.070	0.620 ± 0.050	0.640 ± 0.050	0.642 ± 0.050
	4CA-16	0.232 ± 0.040	0.235 ± 0.040	0.258 ± 0.020	0.351 ± 0.050	0.352 ± 0.050	0.420 ± 0.080	0.435 ± 0.040
	4CA-17	0.214 ± 0.050	0.243 ± 0.080	0.323 ± 0.070	0.440 ± 0.050	0.535 ± 0.080	0.610 ± 0.060	0.620 ± 0.040
	control[a]	0.010 ± 0.005	0.010 ± 0.005	0.010 ± 0.008	0.010 ± 0.005	0.010 ± 0.005	0.010 ± 0.005	0.010 ± 0.008
50 ppm	4CA-2	0.212 ± 0.040	0.256 ± 0.050	0.385 ± 0.040	0.411 ± 0.040	0.415 ± 0.050	0.420 ± 0.050	0.428 ± 0.050
	4CA-16	0.223 ± 0.050	0.287 ± 0.040	0.352 ± 0.020	0.382 ± 0.050	0.400 ± 0.040	0.411 ± 0.080	0.415 ± 0.080
	4CA-17	0.224 ± 0.050	0.304 ± 0.080	0.395 ± 0.020	0.424 ± 0.050	0.455 ± 0.040	0.465 ± 0.060	0.474 ± 0.040
	control[b]	0.100 ± 0.006	0.100 ± 0.000	0.100 ± 0.008	0.100 ± 0.008	0.100 ± 0.008	0.100 ± 0.008	0.100 ± 0.004
100 ppm	4CA-2	0.232 ± 0.050	0.242 ± 0.050	0.277 ± 0.080	0.342 ± 0.070	0.376 ± 0.050	0.385 ± 0.050	0.405 ± 0.050
	4CA-16	0.231 ± 0.040	0.258 ± 0.040	0.285 ± 0.020	0.377 ± 0.050	0.387 ± 0.050	0.400 ± 0.040	0.410 ± 0.080
	4CA-17	0.226 ± 0.050	0.267 ± 0.030	0.385 ± 0.070	0.411 ± 0.050	0.421 ± 0.040	0.442 ± 0.060	0.450 ± 0.040
	control[c]	0.100 ± 0.006	0.100 ± 0.006	0.100 ± 0.008	0.100 ± 0.008	0.110 ± 0.008	0.110 ± 0.005	0.110 ± 0.008
150 ppm	4CA-2	0.224 ± 0.050	0.238 ± 0.050	0.296 ± 0.040	0.355 ± 0.040	0.369 ± 0.050	0.387 ± 0.050	0.399 ± 0.050
	4CA-16	0.212 ± 0.050	0.242 ± 0.040	0.345 ± 0.020	0.355 ± 0.050	0.387 ± 0.050	0.399 ± 0.040	0.408 ± 0.080
	4CA-17	0.223 ± 0.040	0.310 ± 0.020	0387 ± 0.040	0.399 ± 0.050	0.405 ± 0.020	0.409 ± 0.040	0.420 ± 0.040
	control[d]	0.100 ± 0.006	0.100 ± 0.008	0.100 ± 0.006	0.100 ± 0.004	0.110 ± 0.004	0.110 ± 0.005	0.110 ± 0.008
200 ppm	4CA-2	0.222 ± 0.050	0.224 ± 0.050	0.226 ± 0.080	0.228 ± 0.070	0.231 ± 0.050	0.230 ± 0.050	0.230 ± 0.040
	4CA-16	0.212 ± 0.050	0.214 ± 0.040	0.226 ± 0.020	0.228 ± 0.050	0.228 ± 0.050	0.229 ± 0.080	0.229 ± 0.080
	4CA-17	0.232 ± 0.020	0.238 ± 0.080	0.240 ± 0.040	0.241 ± 0.020	0.242 ± 0.080	0.244 ± 0.060	0.244 ± 0.040
	control[e]	0.100 ± 0.006	0.100 ± 0.006	0.100 ± 0.008	0.100 ± 0.004	0.100 ± 0.004	0.110 ± 0.006	0.110 ± 0.008

[a,b,c,d,e] Control were abiotic controls having 25 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm 4-Chloroaniline in mineral medium without bacterial culture, respectively. (4CA-2), (4CA-16) and (4CA-17) were represented for *Acinetobacter baumannii, Pseudomonas putida* and *Klebsiella pneumoniae*, respectively.

Table C-15 The percentage of remaining 4-chloroaniline in various concentrations by 4-chloroaniline degraders (Fig. 4.24-Fig 4.27)

Substrate	Sample	Percentage of degradation						
		0day	2days	4days	6days	8days	10days	12days
25 ppm	4CA-2	0 ± 0	19.51 ± 1.68	37.00 ± 5.50	47.98 ± 5.50	5.50 ± 4.40	60.04 ± 4.40	61.00 ± 1.68
	4CA-16	0 ± 0	22.03 ± 4.70	38.09 ± 5.20	45.08 ± 2.90	48.50 ± 3.50	55.27 ± 3.80	57.87 ± 3.60
	4CA-17	0 ± 0	33.01 ± 3.87	42.34 ± 4.80	53.86 ± 6.05	54.97 ± 4.66	63.39 ± 3.50	64.46 ± 3.87
	control[a]	0 ± 0	0.74 ± 3.31	8.12 ± 2.55	10.60 ± 2.87	14.10 ± 3.31	14.90 ± 2.55	15.14 ± 3.31
50 ppm	4CA-2	0 ± 0	16.54 ± 1.68	27.61 ± 2.55	31.33 ± 2.55	36.61 ± 2.87	37.56 ± 2.87	40.05 ± 1.68
	4CA-16	0 ± 0	20.43 ± 4.70	41.92 ± 5.20	42.44 ± 2.90	44.48 ± 3.50	46.54 ± 3.80	48.22 ± 3.60
	4CA-17	0 ± 0	26.76 ± 1.68	50.04 ± 2.55	50.65 ± 2.55	51.64 ± 2.55	53.22 ± 2.87	55.25 ± 1.68
	control[b]	0 ± 0	8.57 ± 1.68	12.32 ± 2.55	12.76 ± 2.87	13.39 ± 3.31	13.21 ± 2.55	14.55 ± 3.31
100 ppm	4CA-2	0 ± 0	17.88 ± 1.68	27.98 ± 2.90	31.33 ± 2.55	34.62 ± 4.40	36.63 ± 4.40	38.85 ± 1.68
	4CA-16	0 ± 0	16.63 ± 4.70	22.77 ± 1.68	28.76 ± 2.90	31.82 ± 3.50	37.55 ± 3.80	40.22 ± 3.60
	4CA-17	0 ± 0	20.22 ± 3.87	34.56 ± 2.90	37.33 ± 1.68	39.28 ± 4.66	44.56 ± 3.50	47.85 ± 3.87
	control[c]	0 ± 0	5.87 ± 3.31	9.28 ± 2.55	10.22 ± 2.87	11.75 ± 3.31	12.11 ± 2.55	12.55 ± 3.31
150 ppm	4CA-2	0 ± 0	19.76 ± 1.68	32.20 ± 5.50	32.45 ± 5.50	32.54 ± 4.40	35.34 ± 4.40	37.45 ± 1.68
	4CA-16	0 ± 0	16.54 ± 1.68	29.75 ± 5.20	30.21 ± 2.90	31.89 ± 3.50	34.33 ± 3.80	37.56 ± 3.60
	4CA-17	0 ± 0	16.11 ± 3.87	30.14 ± 4.80	32.22 ± 2.90	33.53 ± 4.66	37.22 ± 3.50	39.44 ± 3.87
	control[d]	0 ± 0	1.12 ± 3.31	2.13 ± 2.55	3.56 ± 2.87	4.61 ± 3.31	8.87 ± 2.55	10.87 ± 3.31
200 ppm	4CA-2	0 ± 0	2.87 ± 1.68	3.72 ± 2.55	5.76 ± 1.68	7.79 ± 1.87	8.66 ± 1.87	9.55 ± 1.68
	4CA-16	0 ± 0	2.11 ± 2.55	3.57 ± 5.20	4.21 ± 2.90	4.56 ± 350	5.55 ± 3.80	6.55 ± 3.60
	4CA-17	0 ± 0	4.35 ± 3.87	7.44 ± 4.80	8.22 ± 2.90	9.44 ± 2.90	9.89 ± 1.50	10.55 ± 3.87
	control[e]	0 ± 0	1.54 ± 3.31	1.72 ± 2.55	2.56 ± 2.87	3.46 ± 3.31	4.34 ± 2.55	5.65 ± 3.31

[a,b,c,d,e] Control were abiotic controls having 25 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm 4-Chloroaniline in mineral medium without bacterial culture, respectively. (4CA-2), (4CA-16) and (4CA-17) were represented for *Acinetobacter baumannii, Pseudomonas putida* and *Klebsiella pneumoniae*, respectively

Table C-16 Growth of 4-chloroaniline degraders in substrate range toward to 4-chloroanine by (Fig 4.28- Fig 4.31)

Substrate	Sample	Bacterial growth (OD_{590})						
		0day	2days	4days	6days	8days	10days	12days
Aniline	4CA-2	0.220 ± 0.050	0.250 ± 0.050	0.310 ± 0.020	0.320 ± 0.040	0.350 ± 0.050	0.400 ± 0.050	0.410 ± 0.050
	4CA-16	0.230 ± 0.050	0.290 ± 0.040	0.350 ± 0.020	0.370 ± 0.050	0.388 ± 0.040	0.410 ± 0.080	0.425 ± 0.080
	4CA-17	0.235 ± 0.040	0.100 ± 0.030	0.395 ± 0.040	0.420 ± 0.020	0.430 ± 0.080	0.435 ± 0.060	0.440 ± 0.040
	control[a]	0.006 ± 0.006	0.006 ± 0.006	0.006 ± 0.008	0.007 ± 0.004	0.007 ± 0.008	0.007 ± 0.005	0.008 ± 0.008
2-Chloroaniline	4CA-2	0.207 ± 0.020	0.345 ± 0.050	0.380 ± 0.080	0.460 ± 0.040	0.480 ± 0.050	0.510 ± 0.050	0.560 ± 0.050
	4CA-16	0.254 ± 0.050	0.334 ± 0.040	0.350 ± 0.020	0.410 ± 0.050	0.440 ± 0.040	0.480 ± 0.080	0.490 ± 0.080
	4CA-17	0.207 ± 0.050	0.350 ± 0.080	0.420 ± 0.070	0.430 ± 0.050	0.440 ± 0.080	0.460 ± 0.060	0.465 ± 0.040
	control[b]	0.006 ± 0.006	0.006 ± 0.006	0.006 ± 0.008	0.006 ± 0.004	0.008 ± 0.004	0.008 ± 0.005	0.008 ± 0.008
3-Chloroaniline	4CA-2	0.210 ± 0.050	0.250 ± 0.050	0.320 ± 0.040	0.350 ± 0.020	0.380 ± 0.050	0.410 ± 0.050	0.425 ± 0.050
	4CA-16	0.250 ± 0.020	0.270 ± 0.040	0.340 ± 0.020	0.360 ± 0.020	0.370 ± 0.050	0.390 ± 0.080	0.410 ± 0.040
	4CA-17	0.275 ± 0.050	0.358 ± 0.040	0.420 ± 0.040	0.430 ± 0.050	0.440 ± 0.040	0.450 ± 0.060	0.455 ± 0.040
	control[c]	0.007 ± 0.006	0.007 ± 0.006	0.008 ± 0.008	0.008 ± 0.004	0.010 ± 0.004	0.010 ± 0.005	0.010 ± 0.008
4-Chloroaniline	4CA-2	0.233 ± 0.050	0.321 ± 0.050	0.460 ± 0.080	0.505 ± 0.070	0.620 ± 0.050	0.640 ± 0.050	0.642 ± 0.050
	4CA-16	0.232 ± 0.040	0.235 ± 0.040	0.258 ± 0.020	0.351 ± 0.050	0.352 ± 0.050	0.420 ± 0.080	0.435 ± 0.040
	4CA-17	0.214 ± 0.050	0.243 ± 0.080	0.323 ± 0.070	0.440 ± 0.050	0.535 ± 0.080	0.610 ± 0.060	0.620 ± 0.040
	control[d]	0.010 ± 0.005	0.010 ± 0.005	0.010 ± 0.008	0.010 ± 0.005	0.010 ± 0.005	0.010 ± 0.005	0.010 ± 0.008
3,4-dichloroaniline	4CA-2	0.210 ± 0.020	0.230 ± 0.050	0.235 ± 0.080	0.242 ± 0.070	0.245 ± 0.050	0.242 ± 0.050	0.240 ± 0.050
	4CA-16	0.225 ± 0.050	0.226 ± 0.040	0.228 ± 0.020	0.230 ± 0.050	0.235 ± 0.050	0.240 ± 0.080	0.240 ± 0.080
	4CA-7	0.226 ± 0.050	0.228 ± 0.080	0.231 ± 0.070	0.231 ± 0.040	0.234 ± 0.080	0.232 ± 0.060	0.230 ± 0.040
	control[e]	0.007 ± 0.006	0.007 ± 0.006	0.008 ± 0.008	0.008 ± 0.004	0.010 ± 0.006	0.010 ± 0.005	0.010 ± 0.008

[a,b,c,d,e] Control were abiotic controls having 1mM aniline, 25 ppm (0.2mM)2-chloroaniline,, 25 ppm (0.2mM)3-chloroaniline, 25 ppm (0.2mM)4-chloroaniline, , 25 ppm (0.15mM)3,4-dichloroaniline

Table C-17 Percentage of degradation in substrate range toward to 4-chloroanine by 4-chloroaniline degraders (Fig 4.28- Fig 4.31)

Substrate	Sample	Percentage of degradation						
		0day	2days	4days	6days	8days	10days	12days
Aniline	4CA-2	0 ± 0	8.55 ± 1.68	11.25 ± 3.31	17.22 ± 5.50	21.76 ± 1.68	28.33 ± 4.40	30.11 ± 1.68
	4CA-16	0 ± 0	16.56 ± 4.70	23.87 ± 3.32	25.55 ± 2.90	32.30 ± 3.50	33.11 ± 3.80	35.66 ± 3.60
	4CA-17	0 ± 0	19.56 ± 3.87	35.05 ± 4.80	36.11 ± 2.87	38.51 ± 4.66	43.54 ± 3.50	45.97 ± 3.87
	control[a]	0 ± 0	7.78 ± 1.68	10.06 ± 2.55	10.76 ± 2.87	11.61 ± 3.31	12.45 ± 2.55	15.65 ± 3.31
2-Chloroaniline	4CA-2	0 ± 0	25.66 ± 1.68	54.02 ± 2.55	56.02 ± 5.50	59.32 ± 2.55	62.55 ± 2.87	64.78 ± 1.68
	4CA-16	0 ± 0	21.11 ± 2.87	52.18 ± 5.20	56.88 ± 2.90	58.24 ± 3.50	60.22 ± 3.80	63.00 ± 3.60
	4CA-17	0 ± 0	24.22 ± 3.87	50.44 ± 2.55	54.22 ± 1.86	55.14 ± 1.87	55.55 ± 3.50	58.85 ± 3.87
	control[b]	0 ± 0	6.33 ± 2.55	10.55 ± 2.55	14.22 ± 2.87	15.21 ± 3.31	17.22 ± 3.87	19.55 ± 3.31
3-Chloroaniline	4CA-2	0 ± 0	25.22 ± 1.68	34.03 ± 5.50	34.55 ± 5.50	35.43 ± 4.40	37.87 ± 3.60	39.59 ± 1.68
	4CA-16	0 ± 0	24.11 ± 1.68	31.56 ± 5.20	32.33 ± 2.90	33.62 ± 3.50	37.22 ± 3.80	38.66 ± 3.60
	4CA-17	0 ± 0	26.54 ± 1.68	34.84 ± 2.55	41.11 ± 2.55	49.51 ± 2.55	62.44 ± 2.87	64.87 ± 1.68
	control[c]	0 ± 0	9.55 ± 2.55	11.22 ± 2.55	13.35 ± 2.87	14.22 ± 3.31	19.22 ± 2.55	19.55 ± 3.31
4-Chloroaniline	4CA-2	0 ± 0	19.51 ± 1.68	37.00 ± 5.50	47.98 ± 5.50	5.50 ± 4.40	60.04 ± 4.40	61.00 ± 1.68
	4CA-16	0 ± 0	22.03 ± 4.70	38.09 ± 5.20	45.08 ± 2.90	48.50 ± 3.50	55.27 ± 3.80	57.87 ± 3.60
	4CA-17	0 ± 0	33.01 ± 3.87	42.34 ± 4.80	53.86 ± 6.05	54.97 ± 4.66	63.39 ± 3.50	64.46 ± 3.87
	control[d]	0 ± 0	0.74 ± 3.31	8.12 ± 2.55	10.60 ± 2.87	14.10 ± 3.31	14.90 ± 2.55	15.14 ± 3.31
3,4-dichloroaniline	4CA-2	0 ± 0	1.09 ± 1.68	2.05 ± 1.68	2.23 ± 1.68	3.46 ± 2.55	5.87 ± 1.68	8.55 ± 1.68
	4CA-16	0 ± 0	2.11 ± 2.55	3.78 ± 1.68	4.11 ± 2.90	4.87 ± 3.50	5.11 ± 3.80	5.65 ± 2.87
	4CA-17	0 ± 0	3.57 ± 3.87	5.72 ± 1.22	6.22 ± 2.55	7.89 ± 1.22	8.12 ± 3.50	9.87 ± 2.87
	control[e]	0 ± 0	1.05 ± 1.68	1.46 ± 2.55	2.44 ± 2.87	3.46 ± 3.31	4.43 ± 2.55	5.55 ± 3.31

[a,b,c,d,e] Control were abiotic controls having 1mM aniline, 25 ppm (0.2mM)2-chloroaniline,, 25 ppm (0.2mM)3-chloroaniline, 25 ppm (0.2mM)4-chloroaniline, , 25 ppm (0.15mM)3,4-dichloroaniline

APPENDIX D

The following formation is the sequence fragment of 16S rDNA sequencing of each bacterium with forward primer (63f) and reverse primer (1387r).

63f

CAGGCCTAACACATGCAAGTCGAGCGGAGAGAGGTAGCTTGCTACTGATCTTAGCGGCGGACGGGTGAGTAATGCTTAG
GAATCTGCCTATTAGTGGGGGACAACATTTCGAAAGGAATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGAT
CTTCGGACCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCT
GTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGC
GAGGAGGAGGCTACTTTAGTTAATACCTAGAGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAG
CAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGATTTACTGGGCGTAAAGCGCGCGTAGGCGGCTAATTAAGTCA
AATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCGATACTGGTTAGCTAGAGTGTGGGAGAGGATGGTAGAATTC
CAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAACACTGACGCTG
AGGTGCGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGG
GCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAAT
GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGAC
ATAGTAAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTTACATACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCCTTATTTGCCAGCGAGTAATGTCGGGAACTTTAA
GGATACTGCCAGTGACAAACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACG
TGCTACAATGGTCGGTACAAAGGGTTGCTACCTAGCGATAGGATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATTGG
AGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGCC
TTGTACACACCGGCC

1387r

Fig D-1 A partial 16s rDNA sequence (1358 bp) of *Acinetobacter baumannii* (4CA-2)

63f

CAGGCCTAACACATGCAAGTCGAGCGGATGACGGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAG
GAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGAC
CTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCC
GTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGT
TGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGC
AGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGG
ATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTC
CTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTATACTGACACTGAG
GTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAAT
CCTTGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACAT
GCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGG
AGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTG
CTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGATCGTAGTCCGGATCGCAG
TCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGGCC

1387r

Fig D-2 A partial 16s rDNA sequence (1357 bp) of *Pseudomonas putida* (4CA-16)

63f

CAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAATGT
CTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGG
GGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTGGTAGGTGGGGTAACGGCTCACCTAGGCGACG
ATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTT
CAGCGGGGAGGAAGGCCGTGAGGTTAATAACCTCGCCGATTGACCTTACCCGCAGAAAAAGCCCCGGCTAAATCCGTGC
CCGCAGCCCGCGGTAATACCGAAGGTGCCAGCGTTAATCCGAATTACCGGGCCGAAAGCCCACGCCAGCGGCCGTCCAG
TCGGATGTTAAAACCCCCCGGCTCAACCGGGGAACTGGCATTCCAAACTTGCCAGGCTAAAATCTTTGTAAAAGGGGGG
AAAATTCCCGGTGGAACCGGTGAAATGCCAAAAAGAATCTGGAGAATACCGGTGCCGAAGGCGCCCCCCTGGACAAAGA
CTGACGTTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTT
GGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAA
AACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT
GGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGT
CAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGG
AACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGC
TACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTC
CGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTT
CCCGGGCCTTGTACACACCGGCC

1387r

Fig D-3 A partial 16s rDNA sequence (1366 bp) of *Klebsiella pneumoniae* (4CA-17)

Table D-1 The blast N result of *Acinetobacter baumannii* (4CA-2) using 16S rDNA gene sequence comparison (http://www.ncbi.nlm.nih.gov/blast/)

Sequence identities (%)	Sequence accession number	Source of bacteria	References	Bacteria	Note
1) 99%	AY847284.1	Soil	Tan (unpublished)	*Acinetobacter baumannii*	Biocontrol in citrus canker
2) 99%	AJ888983.1	Soil	Dijkshoorn et al. (unpublished)	*Acinetobacter calcoaceticus*	-
3) 99%	DQ226213	Soil	Shukor and Dahalan (unpublished)	*Acinetobacter sp.*	-
4) 99%	AY823621.1	Soil	Weinitschke et al. (unpublished)	*Acinetobacter calcoaceticus*	-
5) 99%	DQ227342.1	Soil	Hong, et al. (unpublished)	*Acinetobacter* sp.	Phenol-degrading bacteria
6) 99%	DQ187381.1	Soil	Hui and Zhong (unpublished)	*Acinetobacter calcoaceticus*	-
7) 99%	DQ366106.1	Soil	Nohit et al. (unpublished)	*Acinetobacter* sp.	Oil-degrading bacteria
8) 99%	DQ366092.1	Soil	Nohit et al. (unpublished)	*Acinetobacter* sp.	Oil-degrading bacteria
9) 99%	AF500278.1	Soil	Vepritskiy et al .(unpublished)	*Acinetobacter* sp.	-
10) 99%	AB098569.1	Soil	Hiraishi et al .(unpublished)	*Acinetobacter* sp.	-

Table D-2 The blast N result of *Pseudomonas putida* (4CA-16) using 16S rDNA gene sequence comparison (http://www.ncbi.nlm.nih.gov/blast/)

Sequence identities (%)	Sequence accession number	Source of bacteria	References	Bacteria	Note
1) 99%	AY741157.1	Soil	Muthukumarasamy and Kang (unpublished)	*Pseudomonas putida*	Diazotrophic bacteria
2) 99%	AM403529.1	Soil	Wang (unpublished)	*Pseudomonas* sp.	Deep-sea sediments bacteria
3) 99%	AM184288.1	Water	Abraham et al (unpublished)	*Pseudomonas putida*	Pathogenic bacteria along River downstream
4) 99%	DQ860087.1	Soil	Dong et al. (unpublished)	*Pseudomonas* sp.	p-Nitrophenol-degrading bacterium
5) 99%	AE015451.1	Soil	Nelson et al. (2002)	*Pseudomonas putida*	-
6) 99%	AM184274.1	Water	Abraham et al.(unpublished)	*Pseudomonas putida*	Pathogenic bacteria along River downstream
7) 99%	AM184221.1	Water	Abraham et al.((unpublished)	*Pseudomonas putida*	Pathogenic bacteria along River downstream
8) 99%	DQ229317.1	Soil	Joo et al. (unpublished)	*Pseudomonas putida*	Solvent-tolerant
9) 99%	AF378011.1	Soil	Bonilla et al .(2005)	*Pseudomonas* sp.	Biosurfactant producer
10) 99%	AY741156.1	Soil	Kang and Muthukumarasamy (unpublished)	*Pseudomonas putida*	Diazotrophic bacteria

Table D-3 The blast N result of *Klebsiella pneumoniae* (4CA-17) using 16S rDNA gene sequence comparison (http://www.ncbi.nlm.nih.gov/blast/)

Sequence identities (%)	Sequence accession number	Source of bacteria	References	Bacteria	Note
1) 96%	AB114634.1	Soil	Meunchang et al.(unpublished)	*Klebsiella* sp.	nitrogen fixing bacteria
2) 95%	AF511429.1	Water	Ovesen et al. (unpublished)	*Klebsiella pneumoniae*	-
3) 96%	AJ783916.1	Soil	Kaempfer and Ruppel (unpublished)	*Klebsiella variicola*	-
4) 95%	AF130981	Soil	Drancourt et al. (unpublished)	*Klebsiella pneumoniae*	-
5) 95%	SSU32868	Soil	Palus et al. (unpublished)	*Klebsiella* sp.	Diazotrophic bacteria
6) 95%	SSU31076	Soil	Palus et al (unpublished)	*Klebsiella* sp.	Diazotrophic bacteria
7) 96%	AY517552.1	Soil	Zhu and Zhao (unpublished)	*Klebsiella* sp.	Bensulfuron-methyl Degrading bacteria
8) 96%	AB074192.1	Soil	Fukuda et al.(unpublished)	*Klebsiella* sp.	Dibenzofuran-Degrading Bacteria
9) 95%	AB004753.1	Soil	Harada et al .(unpublished)	*Klebsiella pneumoniae*	-
10) 95%	AB114637.1	Soil	Meunchang et al .(unpublished)	*Klebsiella* sp.	nitrogen fixing bacteria

APPENDIX E

Standard curve was used to analyze chromatogram of HPLC result in order to calculate the concentration of substance. The standard 4-chloroaniline was freshly prepared as a stock solution at 12.5 mM by dilution to various concentrations at 0.04, 0.1, 0.2, 0.4, 0.8 and 1.2 mM. It was filtered with 0.45 µm diameter filter and mixed with mobile phase. The separation was performed on C18 HPLC column (Inersil ODS-3 250 x 4.6 mm) and then the compound was analyzed by UV detector at a wavelength of 240 nm. The mobile phase was acetonitrile: water: phosphoric acid mixture (70:29.75:0.25 by volume) with a flow rate 1 ml/min.

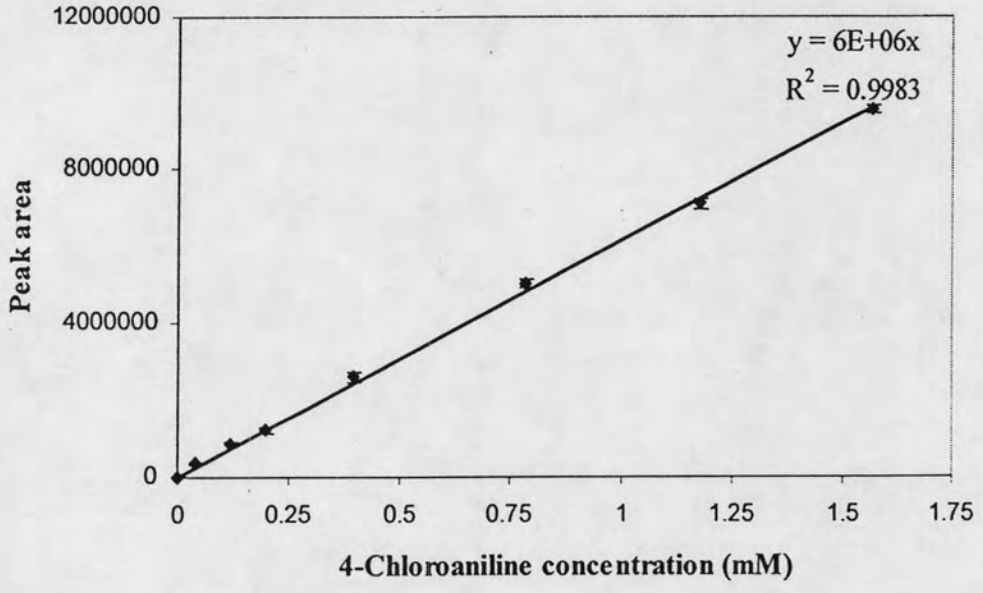


Figure E-1 4-Chloroaniline standard for calculation of 4-chloroaniline concentration

The standard 2-chloroaniline and 3-chloroaniline were freshly prepared as a stock solution at 15.7 mM by dilution to various concentrations at 0.04, 0.1, 0.2 and 0.4 mM. It was filtered with 0.45 μm diameter filter and mixed with mobile phase. The separation was performed on C18 HPLC column (Inersil ODS-3 250 x 4.6 mm) and then the compound was analyzed by UV detector at a wavelength of 240 nm. The mobile phase was acetonitrile: water: phosphoric acid mixture (70:29.75:0.25 by volume) with a flow rate 1 ml/min.

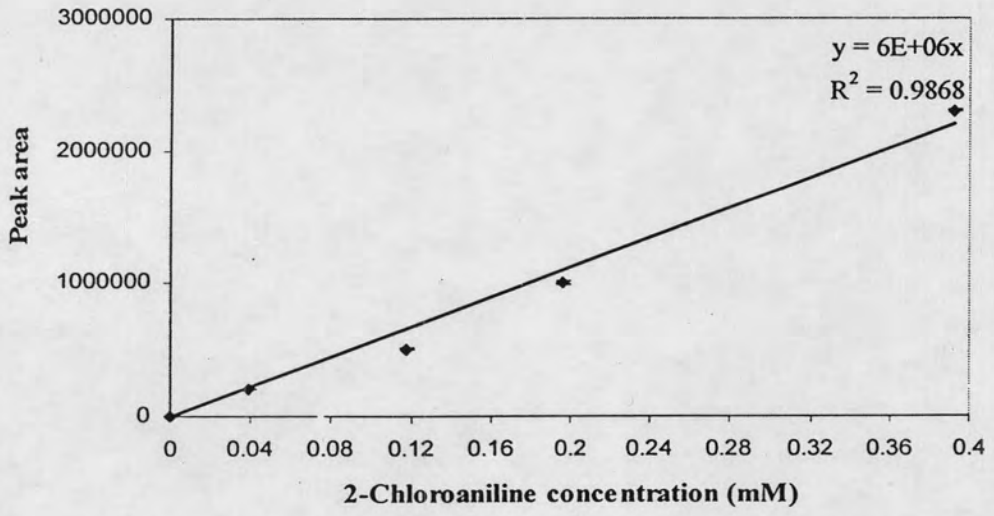


Figure E-3 2-Chloroaniline standard curve for calculation of concentration

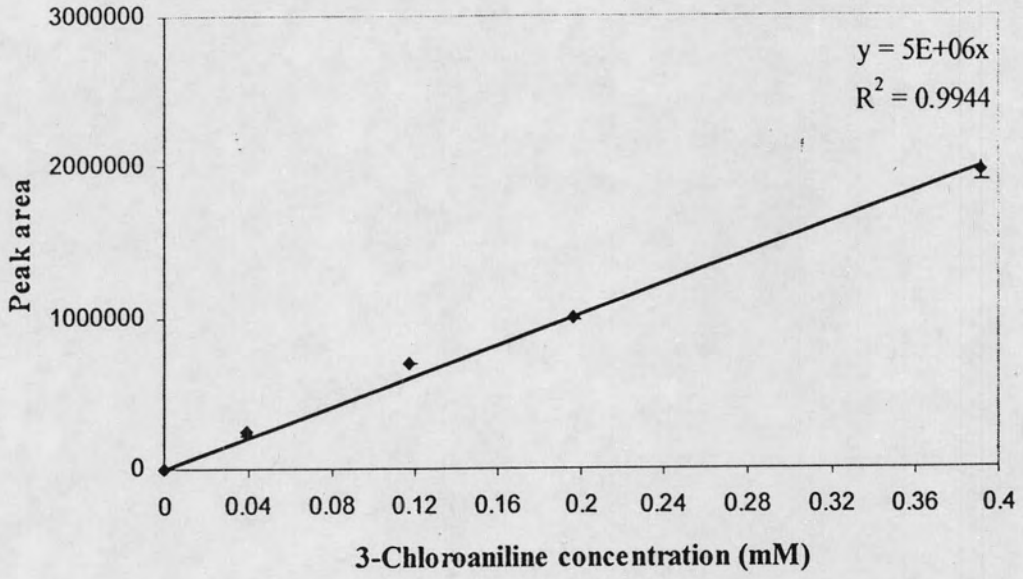


Figure E-4 3-Chloroaniline standard curve for calculation of concentration

The standard 3,4-dichloroaniline was freshly prepared as a stock solution at 3.1 mM by dilution to various concentrations at 0.04, 0.1, 0.2 and 0.4 mM. It was filtered with 0.45 μm diameter filter and mixed with mobile phase. The separation was performed on C18 HPLC column (Inersil ODS-3 250 x 4.6 mm) and then the compound was analyzed by UV detector at a wavelength of 240 nm. The mobile phase was acetonitrile: water: phosphoric acid mixture (70:29.75:0.25 by volume) with a flow rate 1 ml/min.

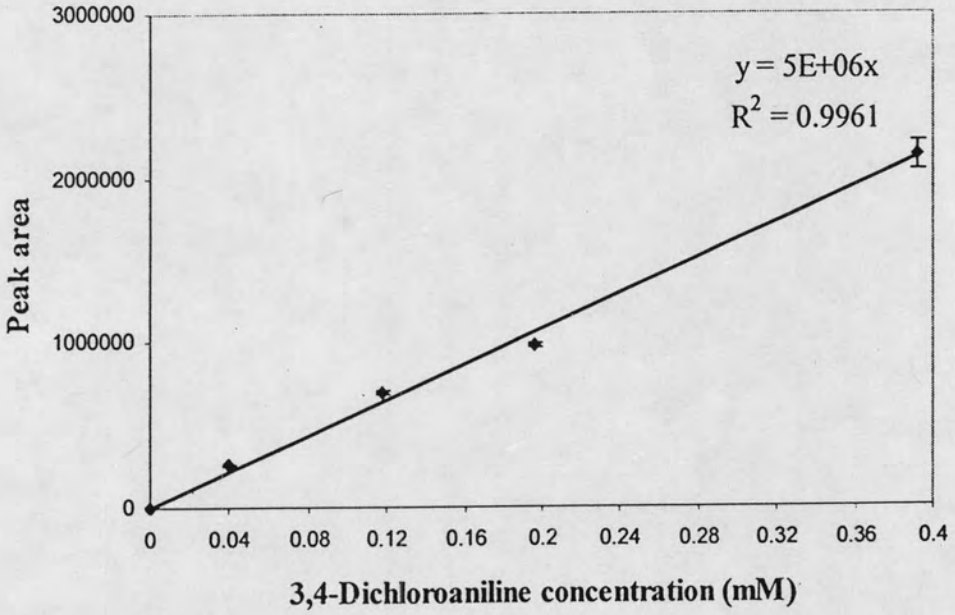


Figure E-5 3, 4-Dichloroaniline standard curve for calculation of concentration

The standard aniline was freshly prepared as a stock solution at 0.01 M by dilution to various concentrations at 0.05, 0.25, 0.5, 0.75 and 1 mM. It was filtered with 0.45 μm diameter filter and mixed with mobile phase. The separation was performed on C18 HPLC column (Inersil ODS-3 250 x 4.6 mm) and then the compound was analyzed by UV detector at a wavelength of 240 nm. The mobile phase was acetonitrile: water: phosphoric acid mixture (70:29.75:0.25 by volume) with a flow rate 1 ml/min.

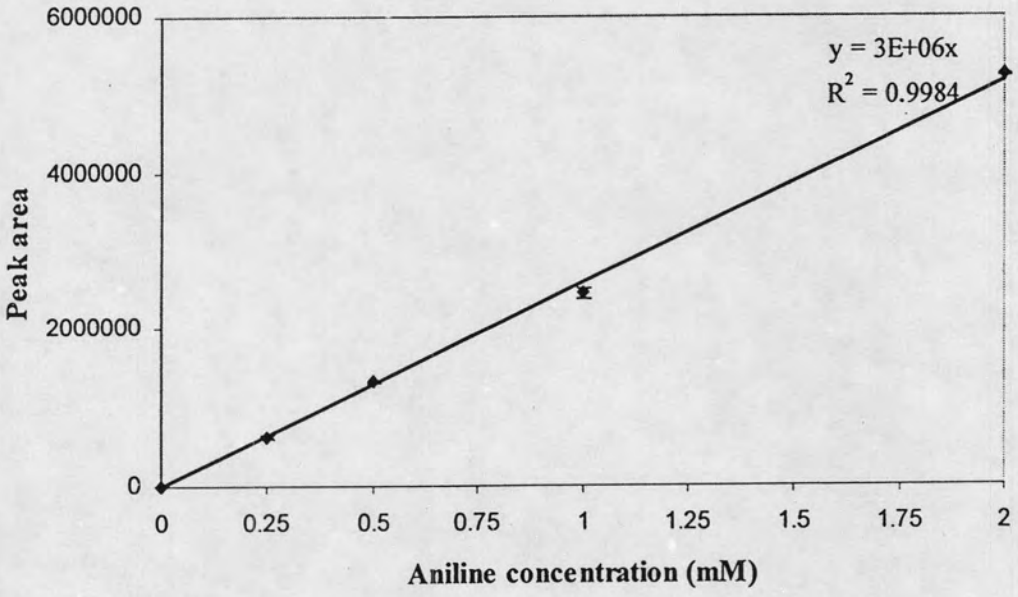


Figure E-5 Aniline standard curve for calculation of aniline concentration

APPENDIX F

Protein calibration curve

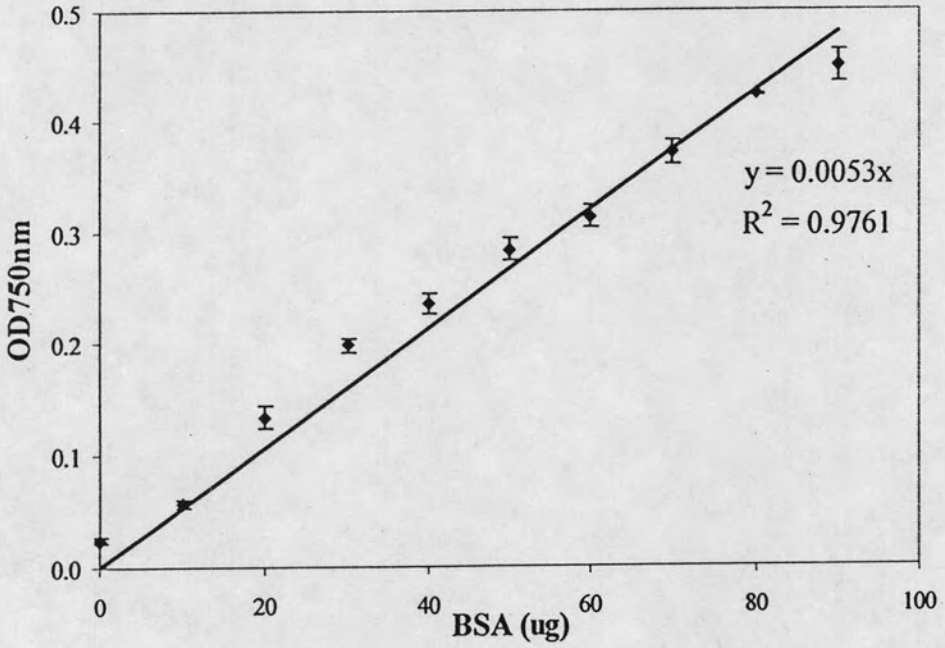


Figure F-1 Standard curve of a modified Lowry method used to determine cell protein

Principle: (Dulley and Grieve (1975) and Lowry et al., (1951))

Copper ($CuSO4.5H_2O$) + Protein $\xrightarrow[\text{Phenol reagent}]{\text{Alkaline pH}}$ Copper-protein complex (blue color)

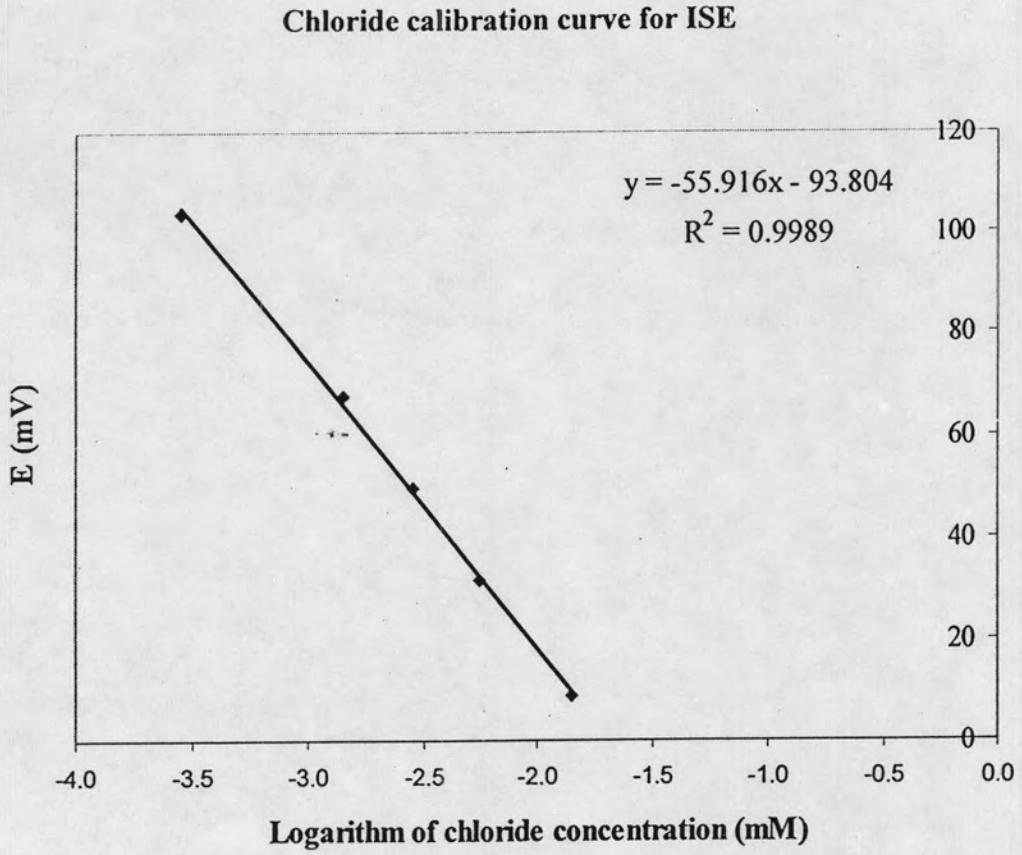


Figure F-2 Chloride calibration curve for determination of chloride concentration using ISE (ion selective electrode)

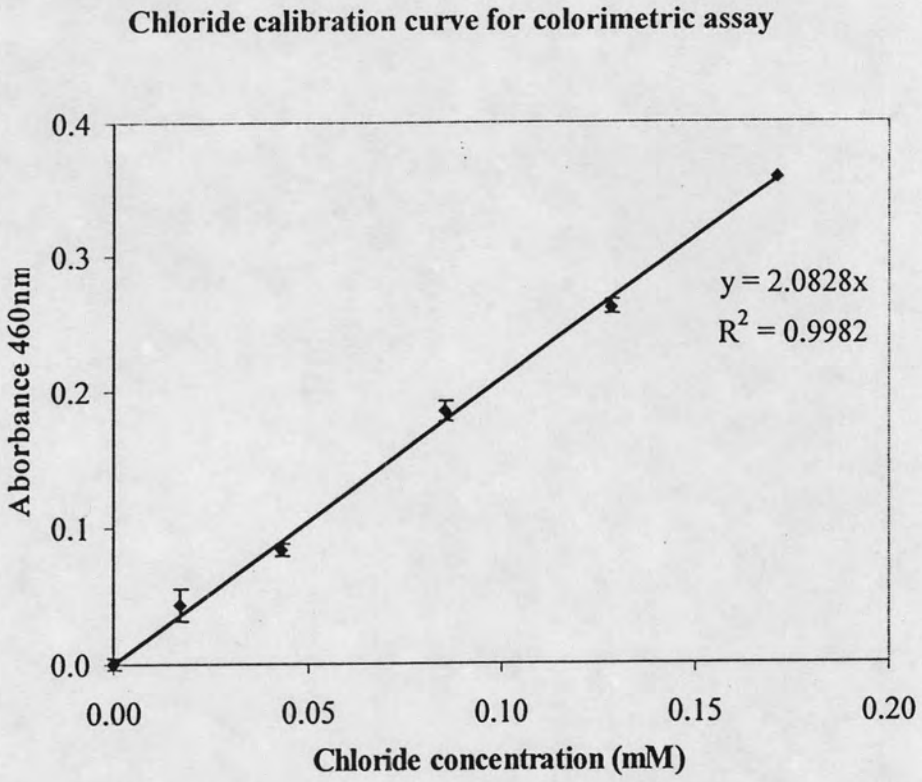


Figure F-3 Chloride calibration curve for determination of chloride concentration using colorimetric assay

Principle: (Bergmann and Sanik, 1957)

The sensitivity procedure depends upon displacement of thiocyanate ion from mercuric thiocyanate by chloride ion; in the presence of ferric ion, a highly colored ferric thiocyanate complex is formed,

$$2Cl^- + Hg(SCN)_2 + 2Fe^{+++} \longrightarrow HgCl_2 + 2Fe(SCN)^{++}$$

The color is stable and propotional to the original chloride ion concentration as shown in Fig F-4 and Fig F-5.

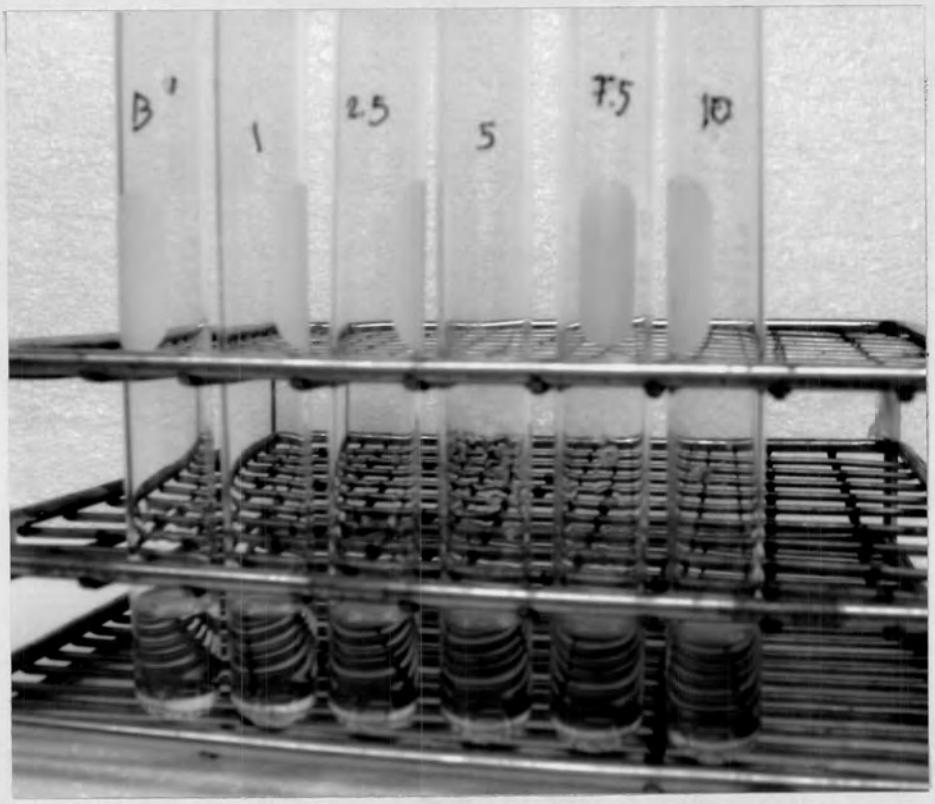


Figure F-4 Chloride standard for determination of chloride concentration using colorimetric assay

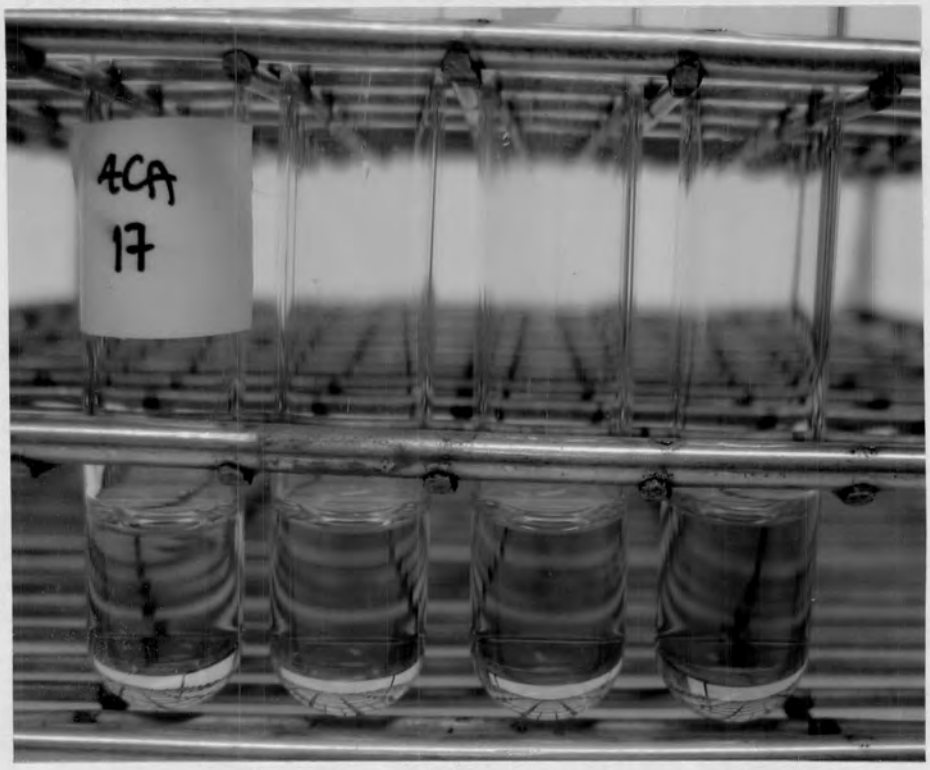
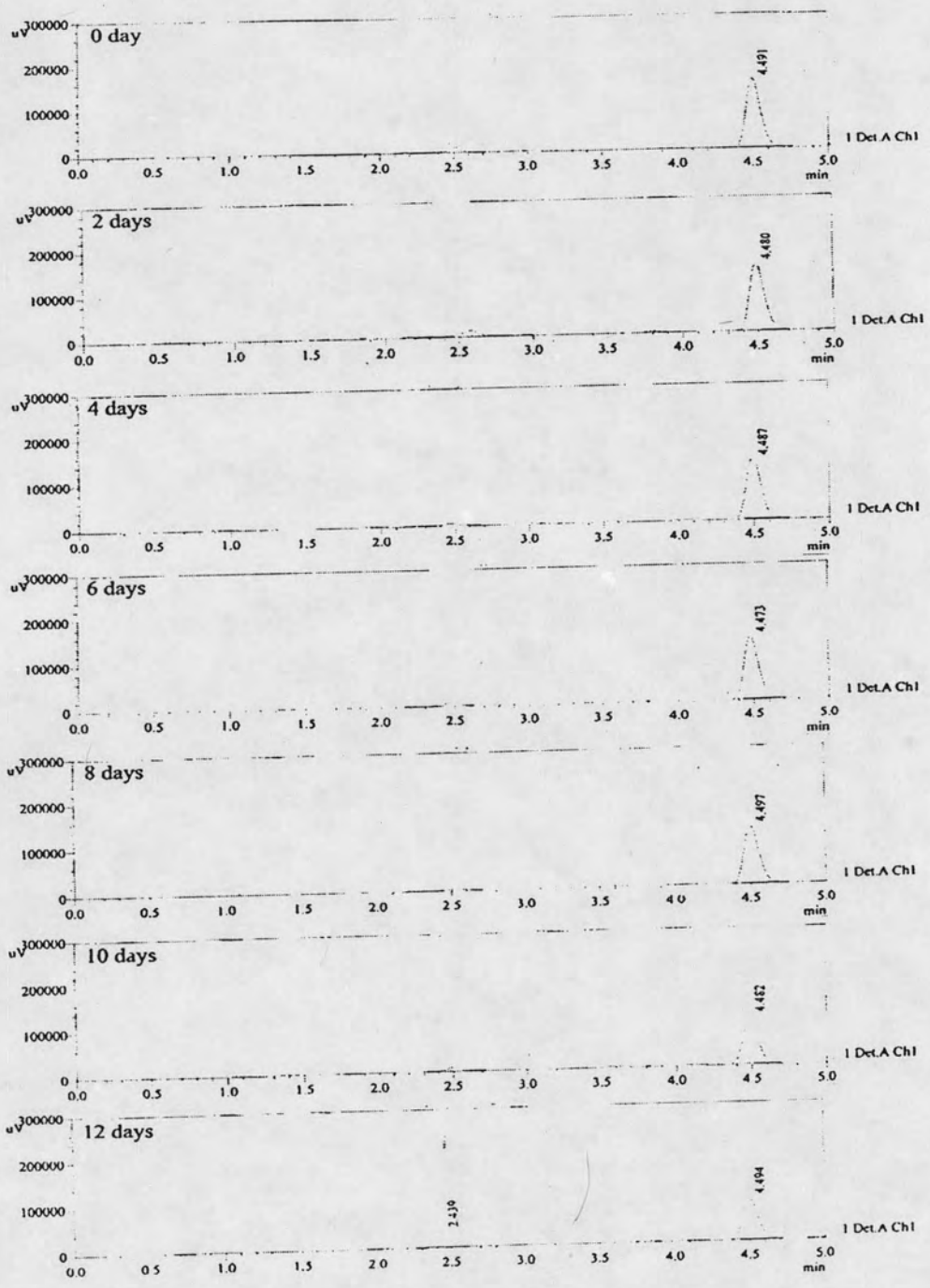


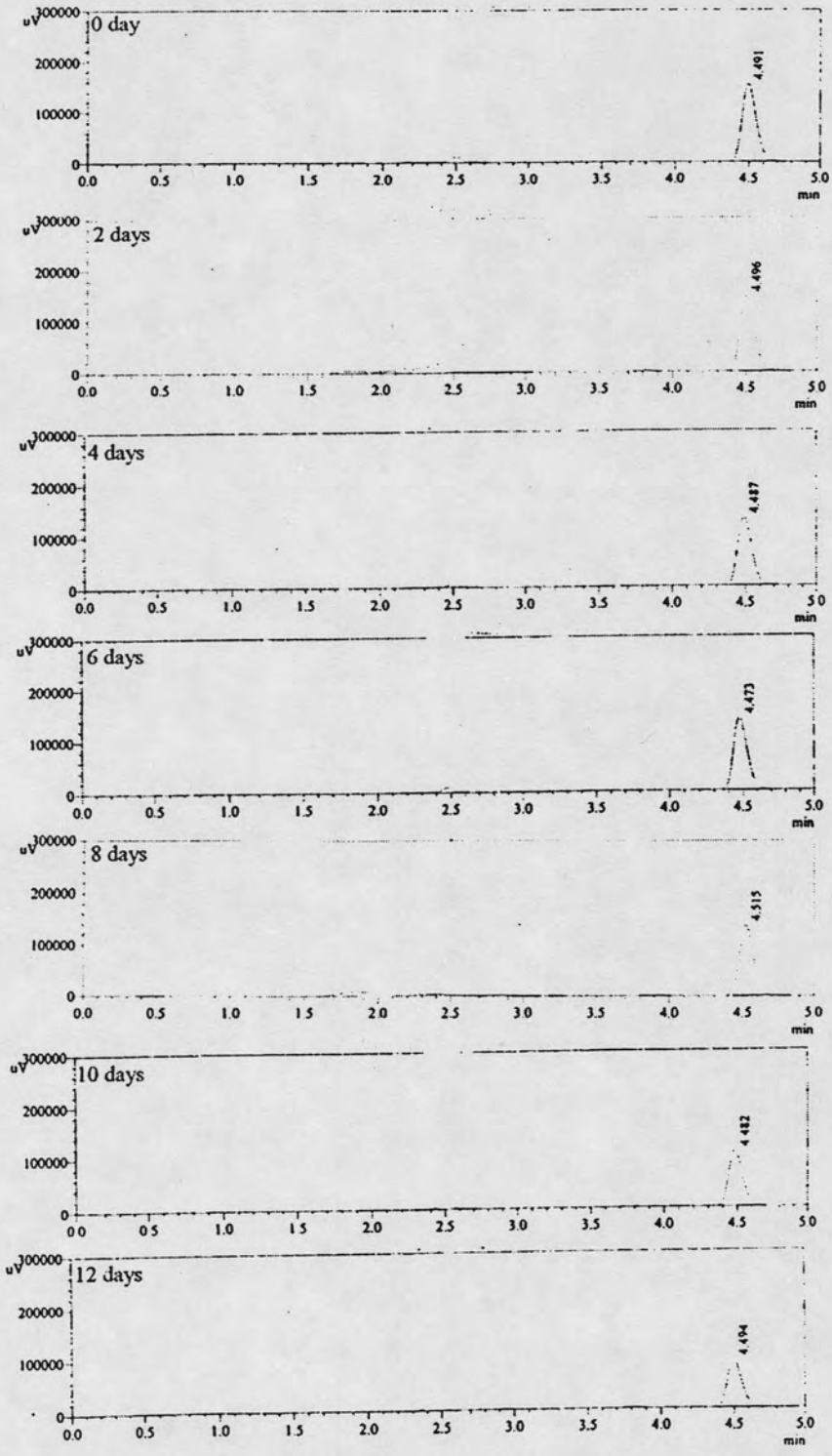
Figure F-5 Determination of chloride of (4CA-17) *Klebsiella pneumoniae* for degradation test in 0, 4, 8 and 12 days.

APPENDIX G

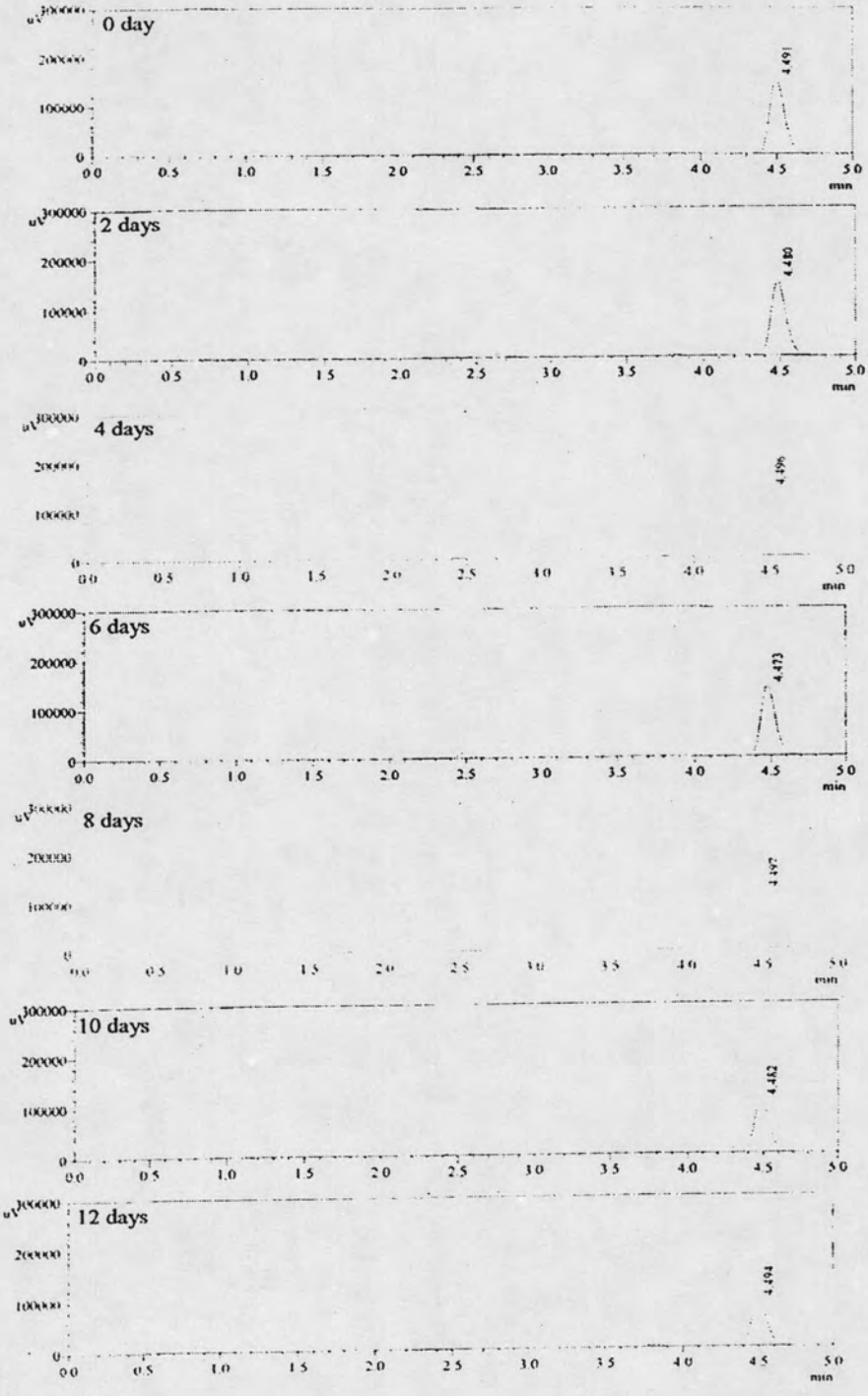
HPLC peak of 25 ppm (0.2 mM) 4-chloroaniline degradation test of *Acinetobacter baumannii* (4CA-2) , retention time of 4-chloroaniline is 4.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



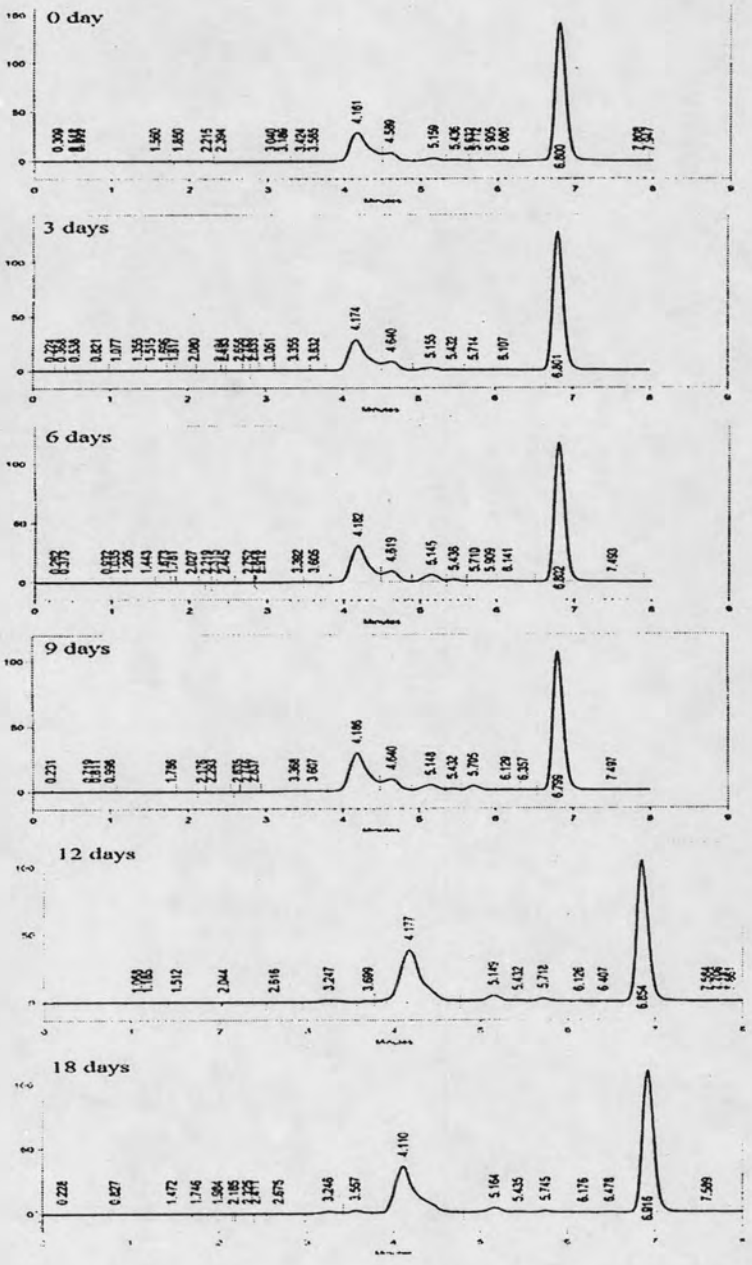
HPLC peak of 25 ppm (0.2 mM) 4-chloroaniline degradation test of *Pseudomonas putida* (4CA-16) , retention time of 4-chloroaniline is 4.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



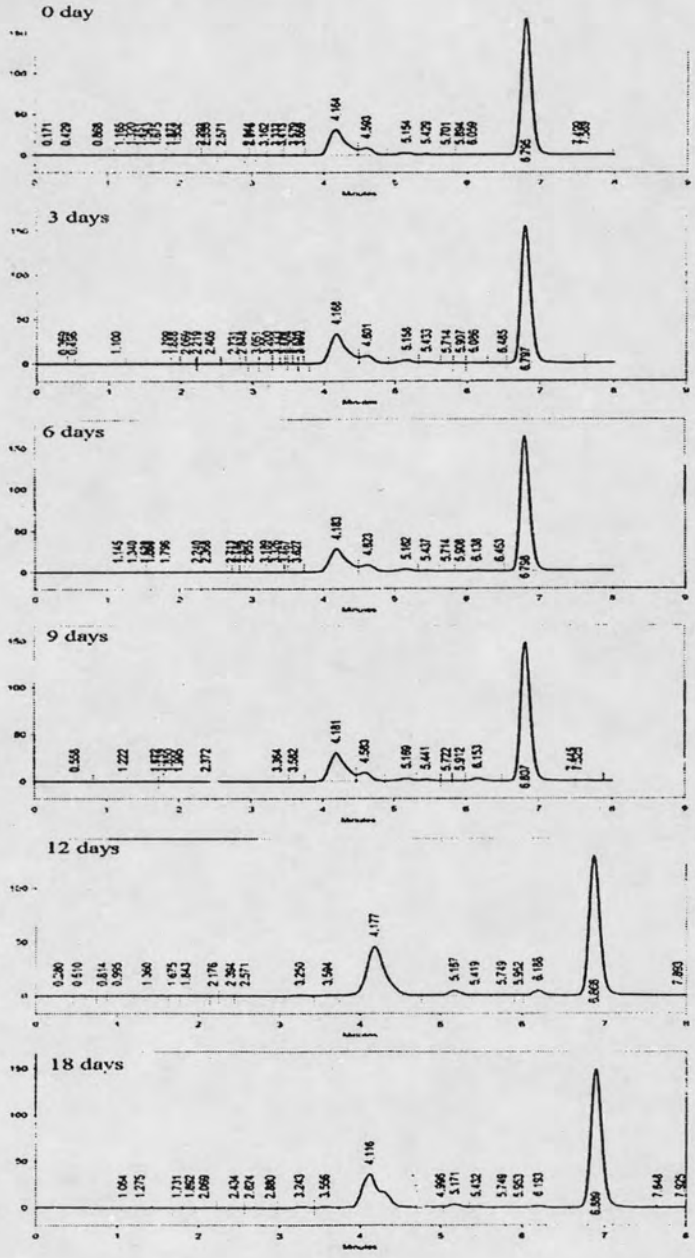
HPLC peak of 25 ppm (0.2 mM) 4-chloroaniline degradation test of *Klebsiella pneumoniae* (4CA-17) , retention time of 4-chloroaniline is 4.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



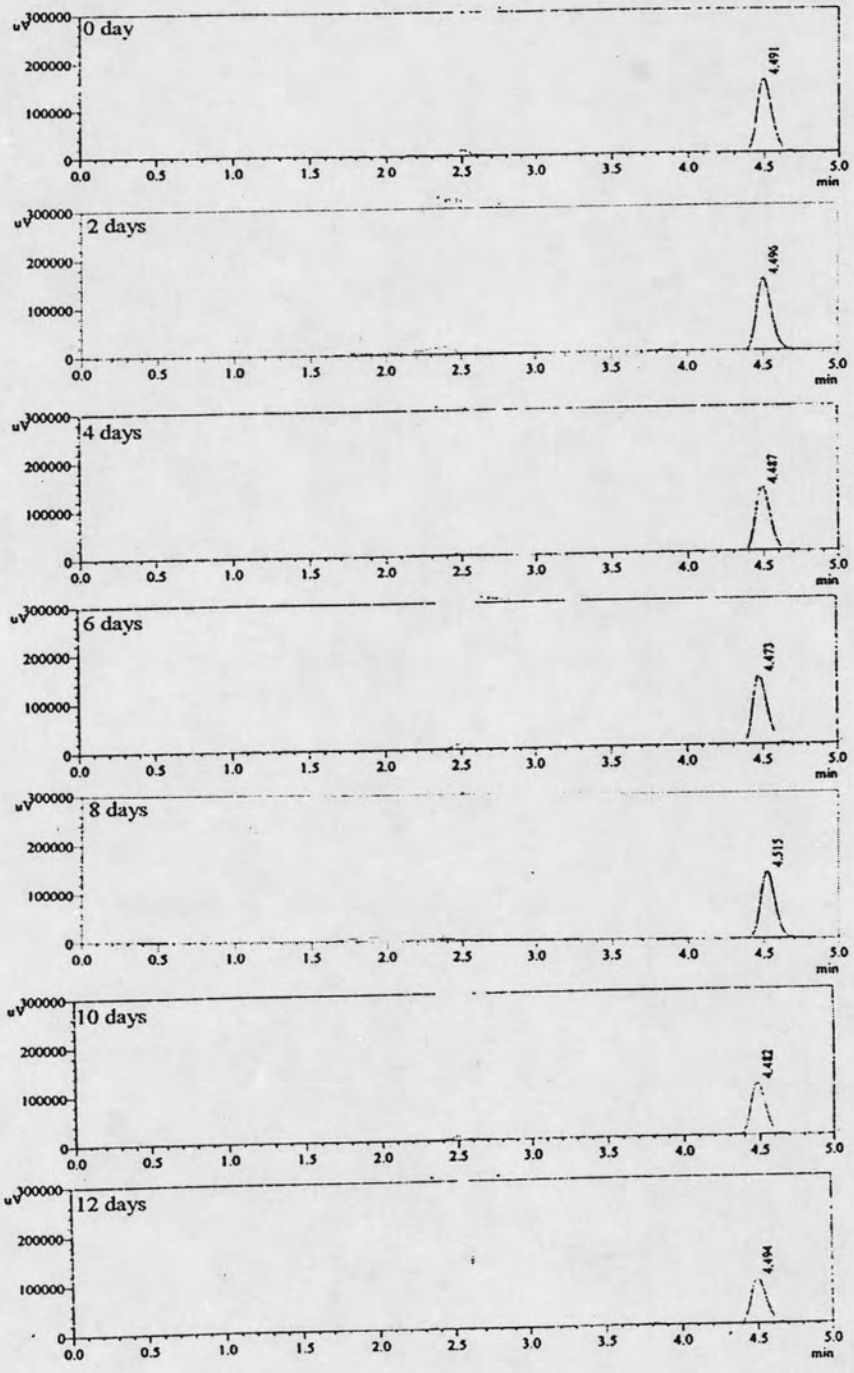
HPLC peak of 25 ppm (0.2 mM) 4-chloroaniline degradation test of 4CA-19, could grow on mineral medium contaning 4-chloroaniline but it cannot degrade 4-chloroaniline. The separation was performed on C18 HPLC column (phenomenex 250 x 4.6 mm) and then the compound was analyzed by UV detector at a wavelength of 240 nm. The mobile phase was acetonitrile: water: phosphoric acid mixture (70:29.75:0.25 by volume) with a flow rate 0.5 ml/min. The retention time of 4-chloroaniline is 6.8 min.



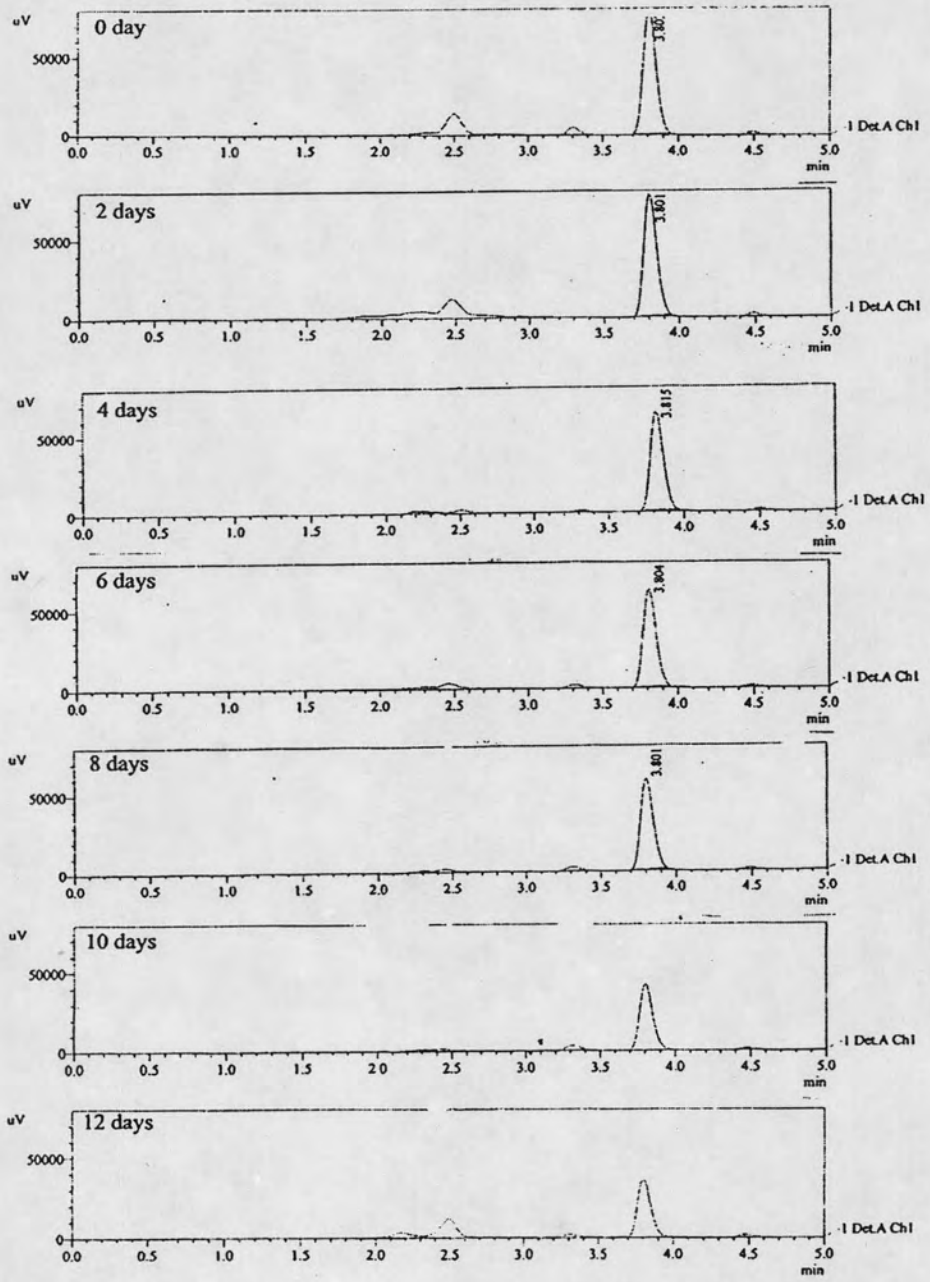
HPLC peak of 25 ppm (0.2 mM) 4-chloroaniline degradation test of 4CA-20, could grow on mineral medium contaning 4-chloroaniline but it cannot degrade 4-chloroaniline. The separation was performed on C18 HPLC column (phenomenex 250 x 4.6 mm) and then the compound was analyzed by UV detector at a wavelength of 240 nm. The mobile phase was acetonitrile: water: phosphoric acid mixture (70:29.75:0.25 by volume) with a flow rate 0.5 ml/min. The retention time of 4-chloroaniline is 6.8 min.



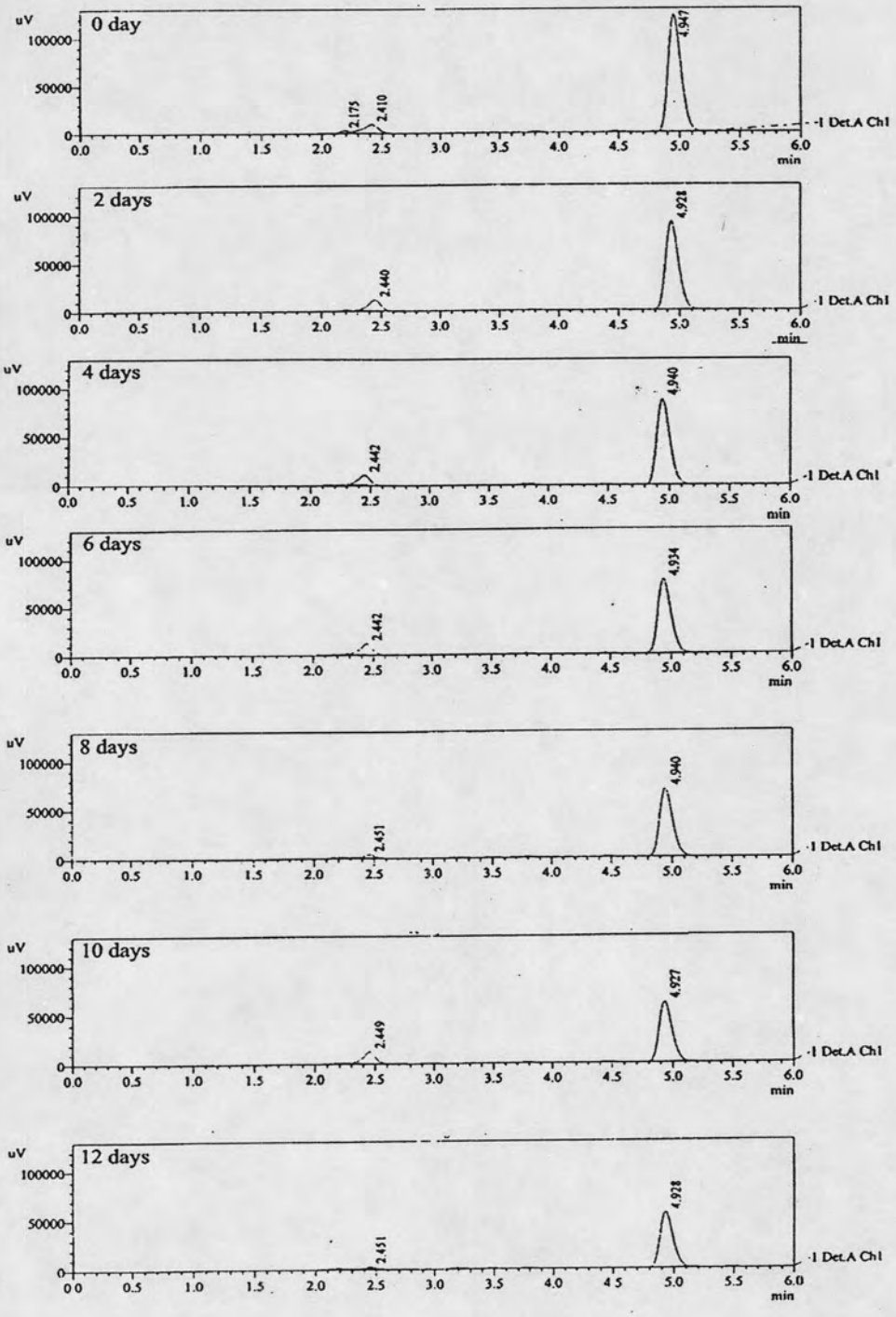
HPLC peak of 25 ppm (0.2 mM) 4-chloroaniline with 1 mM aniline in the optimum condition test of *Klebsiella pneumoniae* (4CA-17), retention time of 4-chloroaniline is 4.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



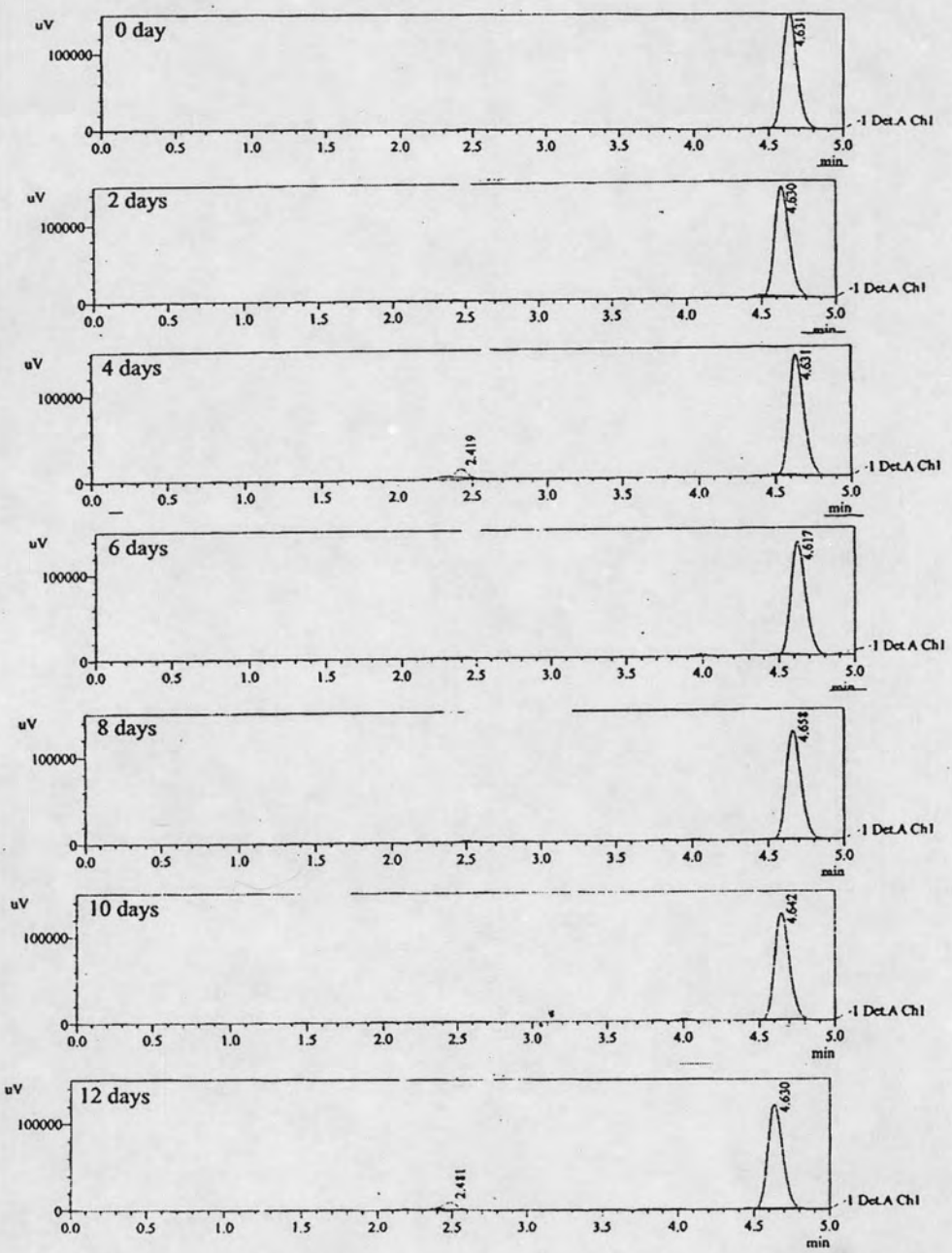
HPLC peak of 1 mM aniline degradation of *Acinetobacter baumannii* (4CA-2), retention time of aniline is 3.8 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



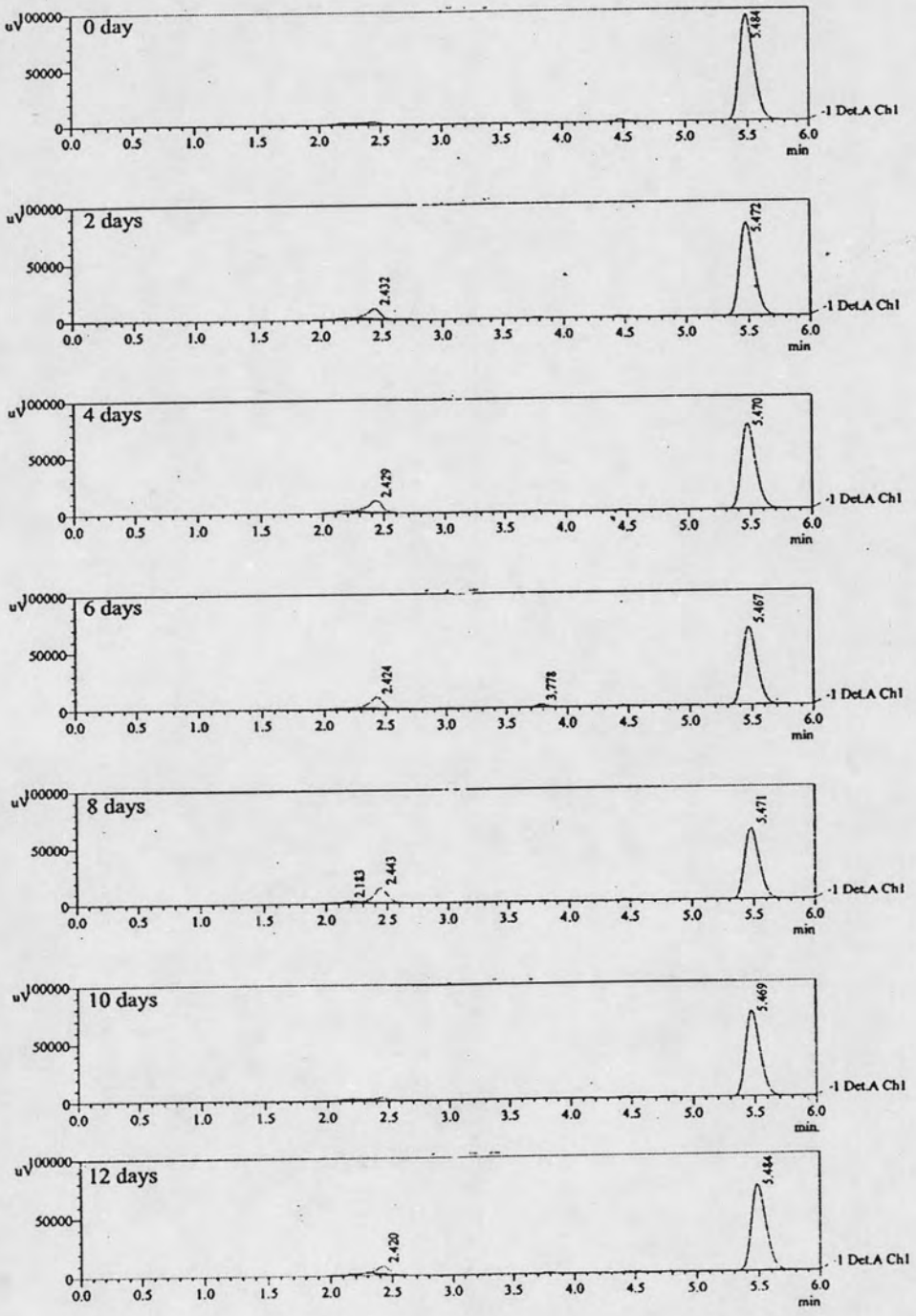
HPLC peak of 25 ppm (0.2 mM) 2-chloroaniline degradation test of *Acinetobacter baumannii* (4CA-2), reten tion time of 4-chloroaniline is 4.9 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



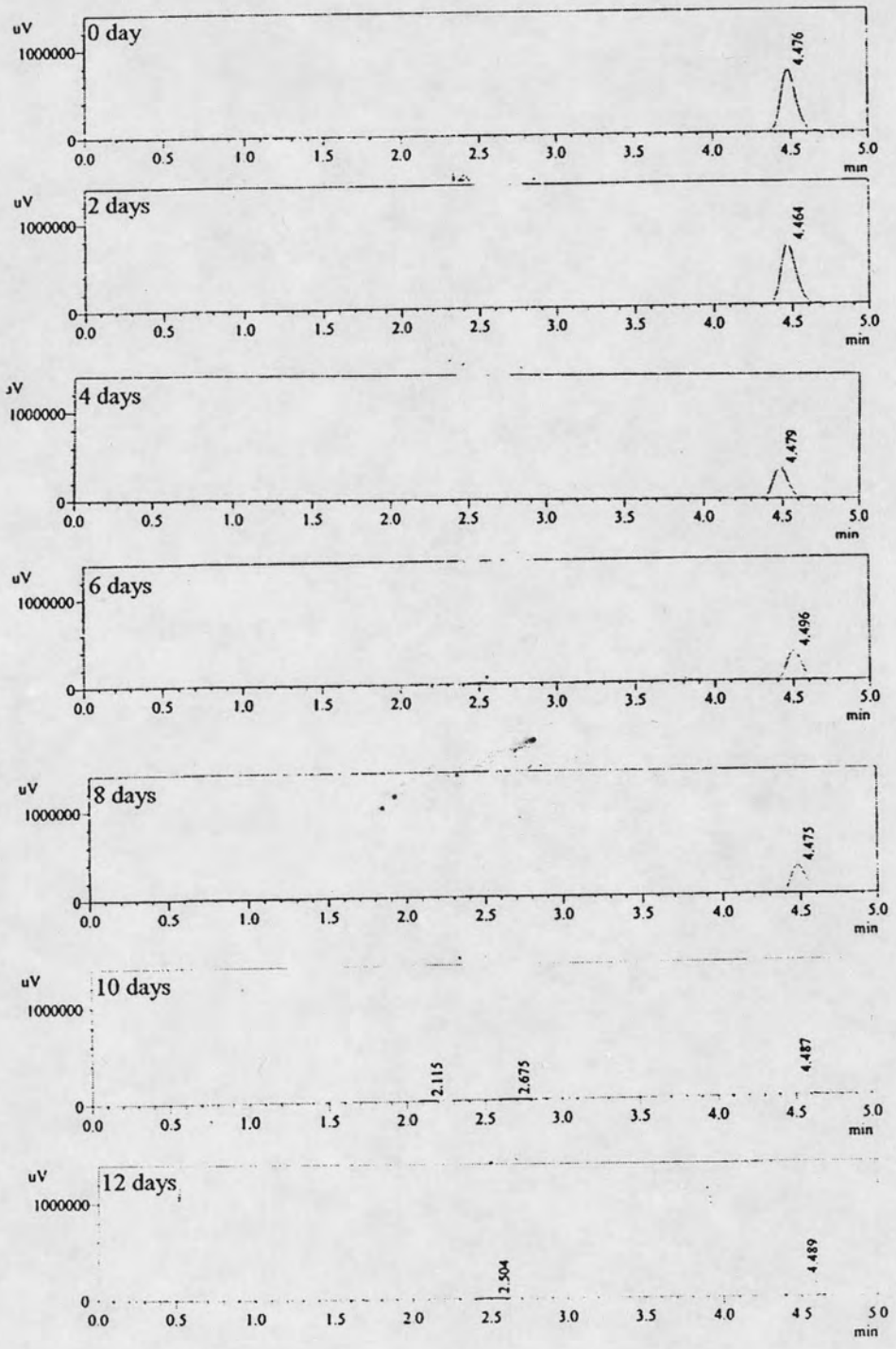
HPLC peak of 25 ppm (0.2 mM) 3-chloroaniline degradation test of *Acinetobacter baumannii* (4CA-2), reten tion time of 4-chloroaniline is 4.6 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



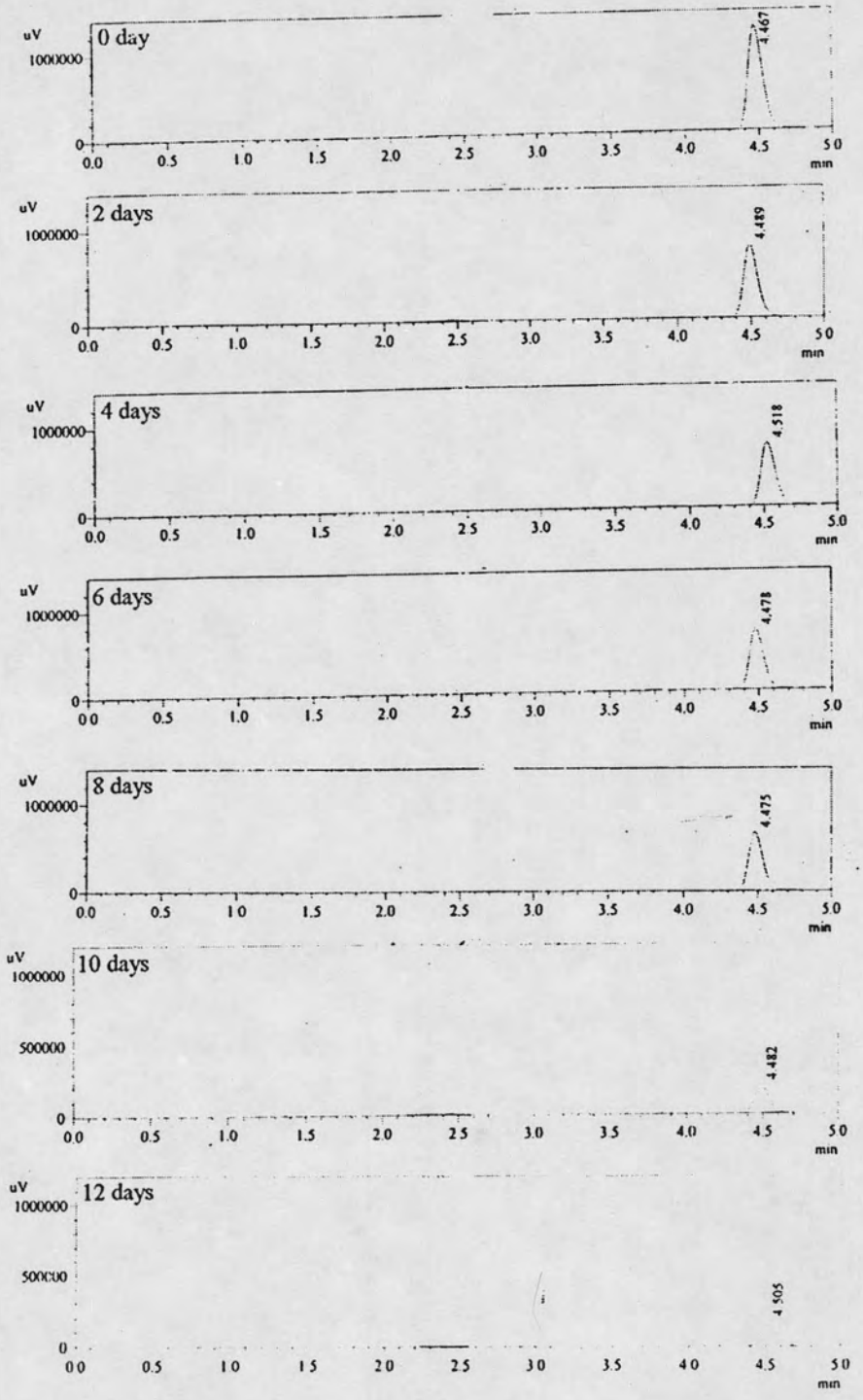
HPLC peak of 25 ppm (0.15 mM) 3,4-dichloroaniline degradation test of *Acinetobacter baumannii* (4CA-2), retention time of 4-chloroaniline is 5.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



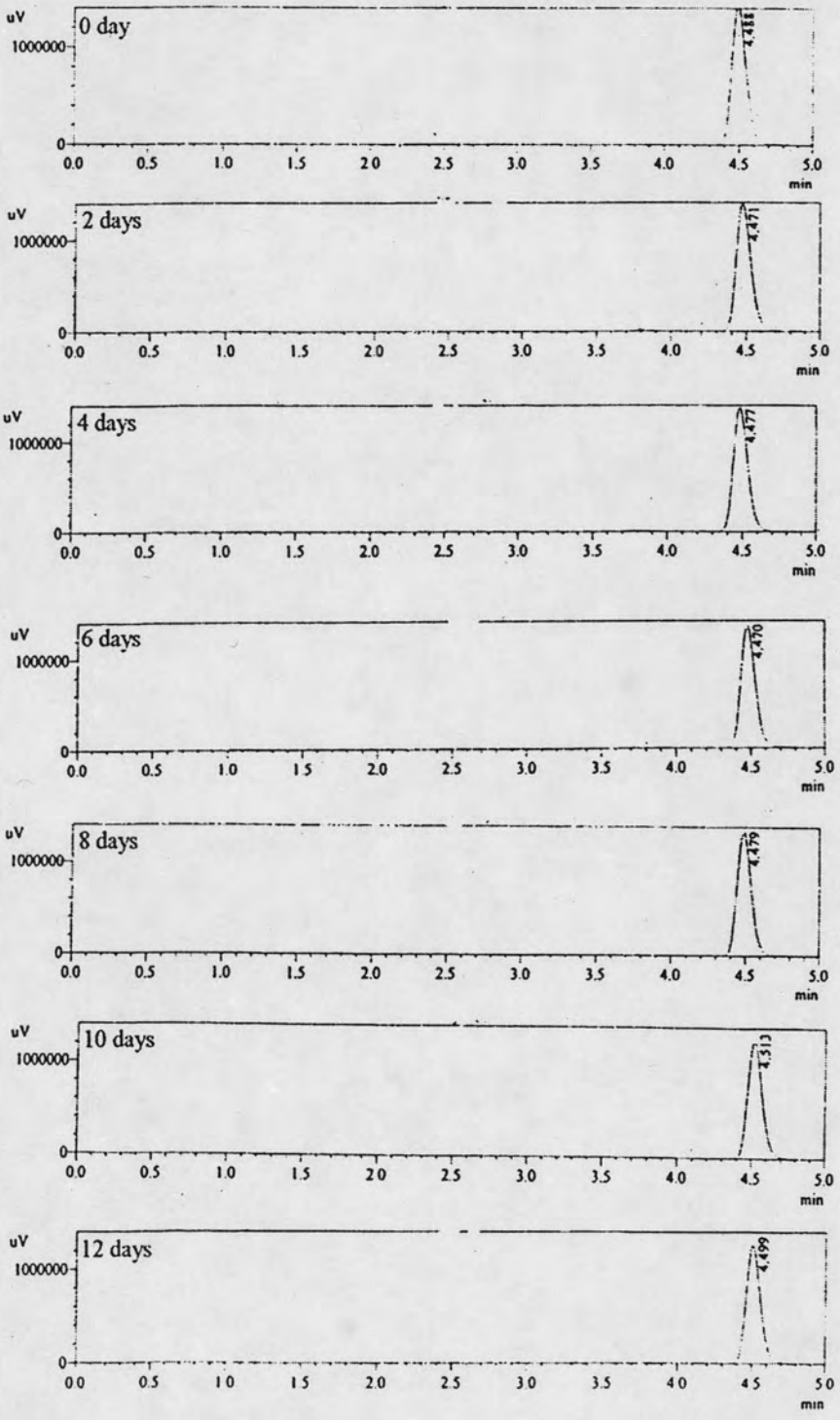
HPLC peak of 50 ppm (0.4 mM) 4-chloroaniline degradation test of *Klebsiella pneumoniae* (4CA-17), retention time of 4-chloroaniline is 4.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



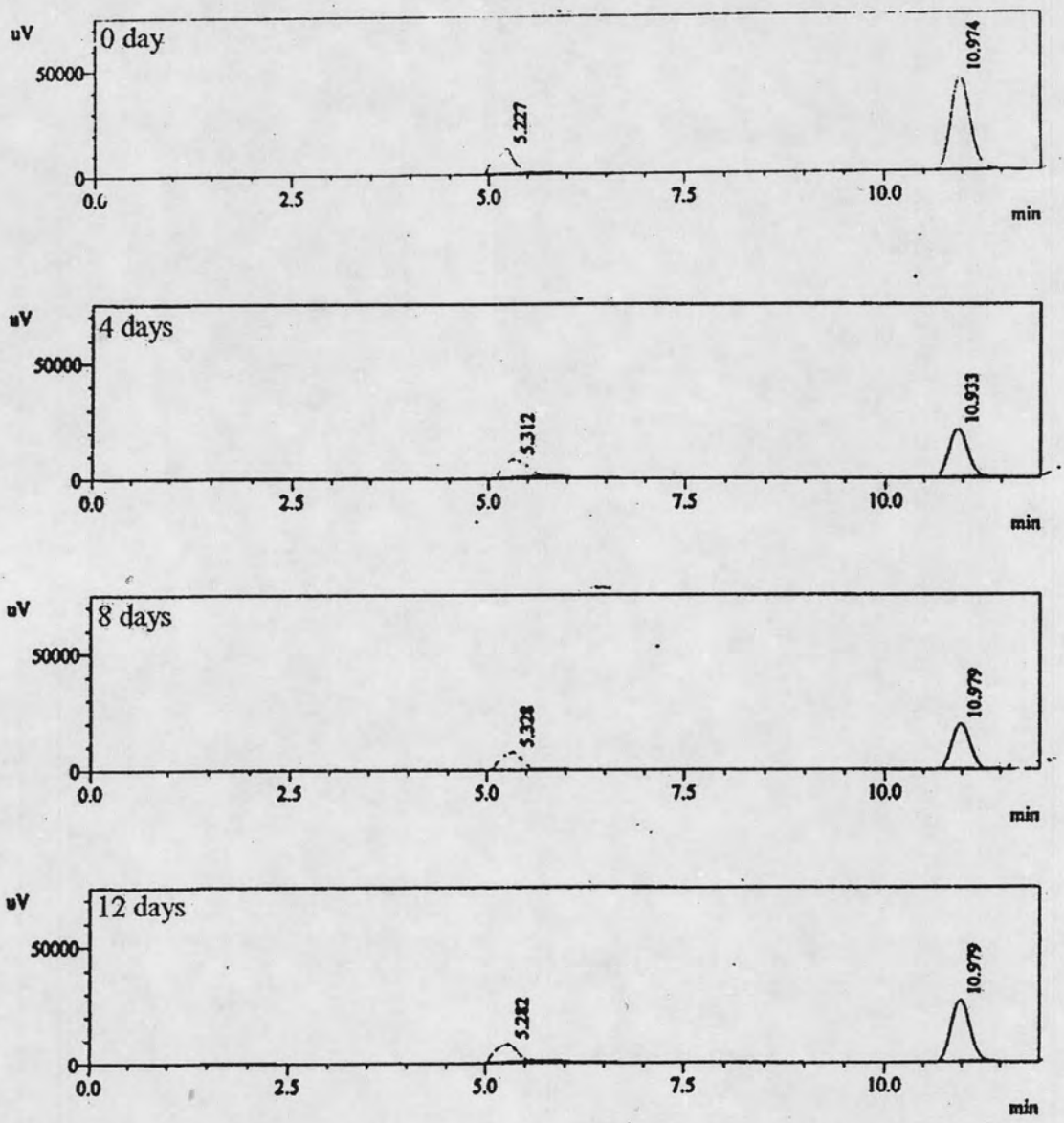
HPLC peak of 150 ppm (1.2 mM) 4-chloroaniline degradation test of *Klebsiella pneumoniae* (4CA-17), retention time of 4-chloroaniline is 4.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



HPLC peak of 200 ppm (1.6 mM) 4-chloroaniline degradation test of *Klebsiella pneumoniae* (4CA-17), retention time of 4-chloroaniline is 4.4 min determined under the specific HPLC condition as described in Chapter 3 (3.7.1).



HPLC peak of 25 ppm (0.2 mM) 4-chloroaniline degradation test of *Klebsiella pneumoniae* (4CA-17), retention time of 4-chloroaniline is 10.9 min determined under another method of HPLC condition as described in Chapter 3 (3.7.1). This condition was performed for detection of intermediate (4-chlorocatechol) (Zeyer et al., 1985).



APPENDIX H

Bacterial standard curve

4-Chloroaniline-degradaing bacteria were cultured on mineral medium containing 25 ppm (0.2mM) 4-chloroaniline. Bacterial cells were counted under a microscope and the growth was detected using a spectrophotometer at 590 nm. The slope from standard curve was used to determine growth rate as described in Appendix C.

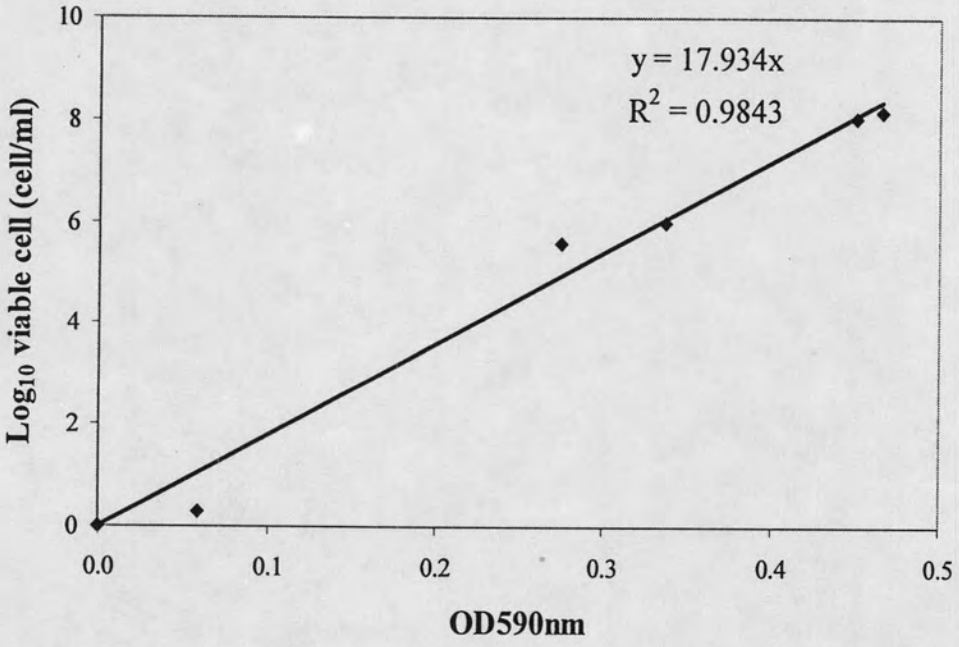


Fig H-1 Standard curve of *Acinetobacter baumannii* (4CA-2) plotted between the logarithm viable cell (cell/ml) and OD_{590}nm for growth rate determination

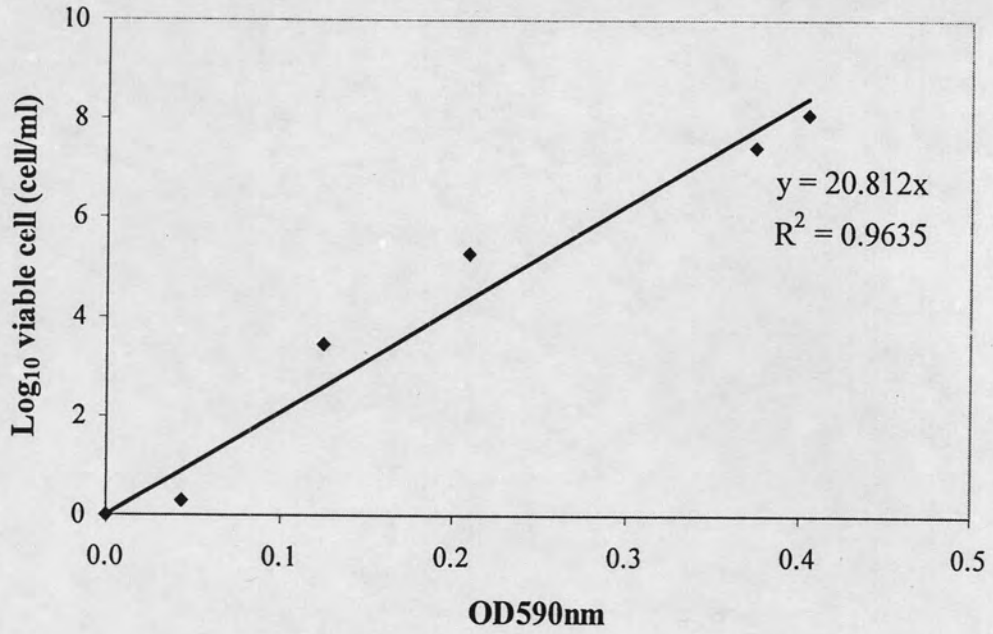


Fig H-2 Standard curve of *Pseudomonas putida* (4CA-16) plotted between the logarithm viable cell (cell/ml) and OD_{590}nm for growth rate determination

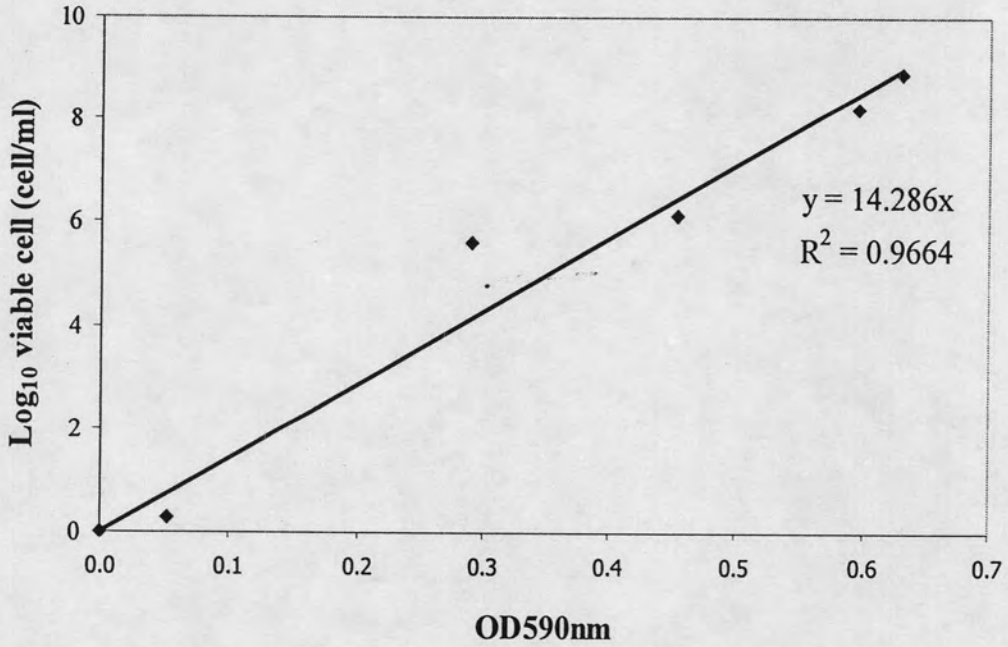


Fig H-3 Standard curve of *Klebsiella pneumoniae* (4CA-17) plotted between the logarithm viable cell (cell/ml) and OD_{590}nm for growth rate determination

BIOGRAPHY

Miss Wansiri Petchkroh was born on August 12, 1980 in Bangkok province, Thailand. She received Bachelor's Degree in Microbiology, Faculty of science, Kasetsart University in 2002. She pursued her Master degree study in the inter-Department of Environmental Management, Chulalongkorn University, Bangkok, Thailand in May 2002.