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APPENDICES

APPENDIX A

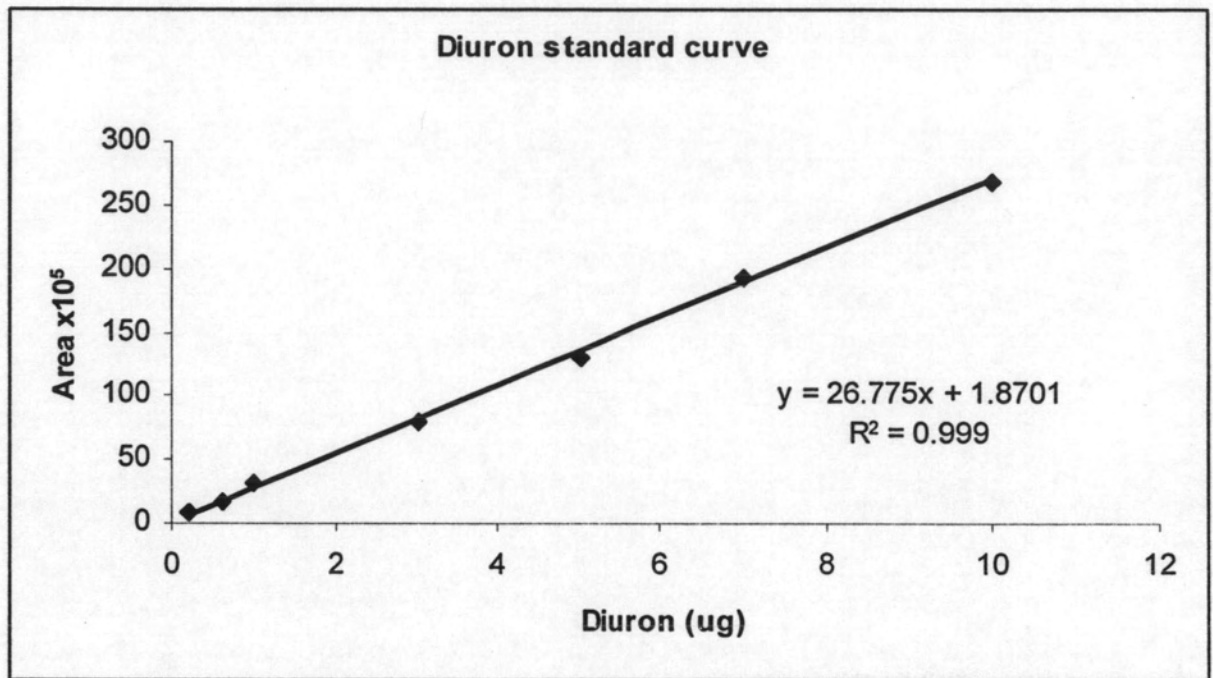


Figure A The standard curve of diuron plotted between the amount of diuron (μg) and peak area analyzed by HPLC.

The slope of standard curve was 26.775.

The amount of diuron can calculated followed the equation;

$$\text{The amount of diuron } (\mu\text{g}) = \frac{(\text{peak area} - 1.8701)}{26.775}$$

APPENDIX B

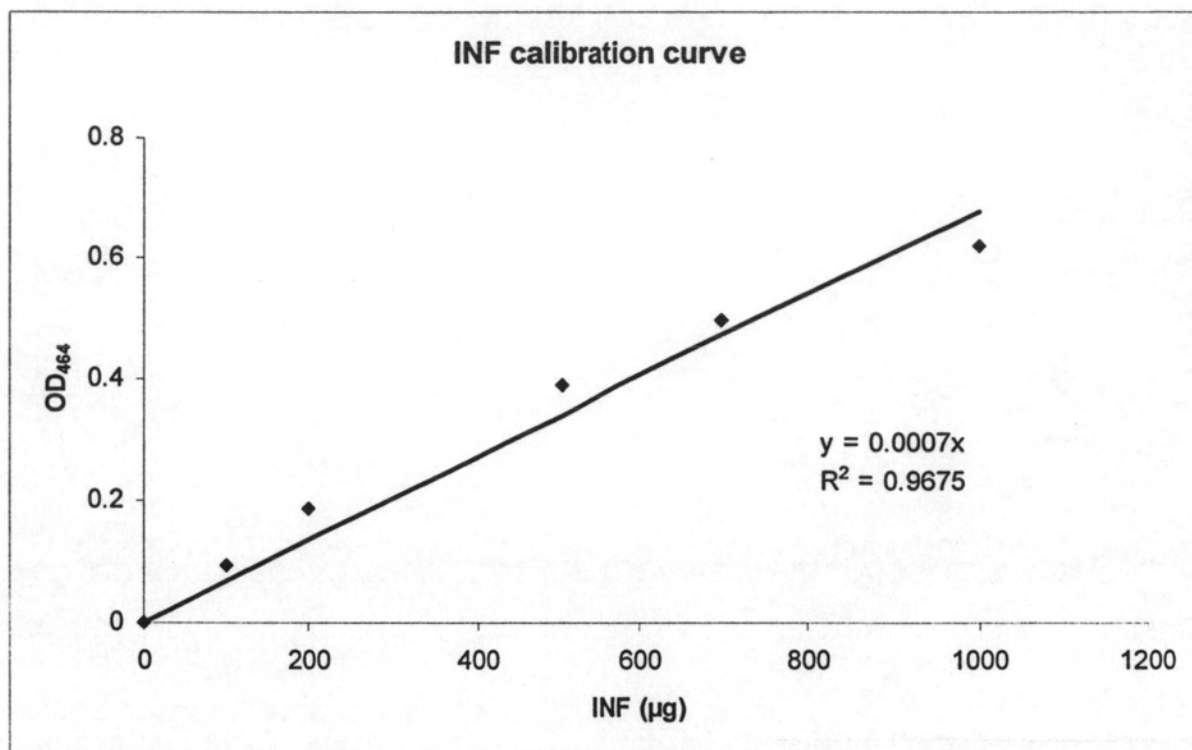
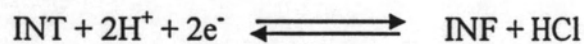


Figure B INF calibration curve for the calculation of total microbial activity (dehydrogenase activity).



The dehydrogenase activity is expressed as $\mu\text{g INF g}^{-1} \text{dwt } 2 \text{ h}^{-1}$ and calculated according the following relationship:

$$\text{INF } (\mu\text{g INF g}^{-1} \text{dwt } 2 \text{ h}^{-1}) = \frac{(S_1 - S_0)}{\text{dwt}}$$

S_1 is the INF of the test

S_0 is the INF of the control

dwt is the dry weight of 1 g moist soil

APPENDIX C

Prepare of the Glass plates for SSCP analysis

Glass plate cleaning

1. Glass plates must be meticulously clean that the plate was cleaned water and 10% detergent then washed thoroughly with deionized water to remove detergent residue.
2. Glass plate was soaked in 10% NaOH for 30 min., rinsed by deionized water and washed by 95% ethanol at the plates 3 times.

The coating glass plate was prevented the tearing gel during silver staining.

Short glass plate preparation

Treat the short glass plate with binding solution each time a gel is prepared.

1. 800 μ l Repel-Silane is add on glass plate and wipe with a tissue in one direction and then perpendicular to the first direction using gentle pressure and performed glass plate for 15 min.
2. The coated glass plate was washed by 95% ethanol.

Long glass plate preparation

1. Preparation fresh bind solution by adding 995 μ l of 95% ethanol, 5 μ l of acetic acid and 4 μ l bind-silane (the solution is fast volatile), tissue was wipe in one direction and then perpendicular to the first direction using gentle pressure and performed glass plate for 15 min.
2. The coated glass plate was washed by 95% ethanol.

Appendix D

Preparation Polyacrylamide gel electrophoresis for SSCP

40% Polyacrylamide (37.5 : 1, Acrylamide : Bis-acrylamide)

Acrylamide 37 g

N.N'-methylene-bis-acrylamide 1 g

Adjust volume to 100 ml with distilled water and filtrate with 0.45 μm

10x TBE

Tris 108 g

Boric acid 55 g

0.5 M EDTA (pH 8.0) 40 ml

Adjust volume to 100 ml with distilled water.

17.5% Nondenaturing Polyacrylamide Gel Electrophoresis

40% Polyacrylamide 17.5 ml

10X TBE 4 ml

Distilled water 19.5 ml

This solution was degassed for 15 minutes

10% Ammonium persulphate 300 μl

TEMED 30 μl

Fixative / Stop Solution, 2 litres

Glacial acetic	200 ml
Distilled water	1,800 ml

Staining solution (0.1% Silver nitrate)

Silver nitrate	1.5 g
Formaldehyde	2.25 ml

Adjust volume to 1.5 litres (this solution was freshly prepared.)

Developing Solution

Sodium Carbonate	90 g
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Adjust volume to 3 litres and divided to 1.5 litres, storage at 4°C

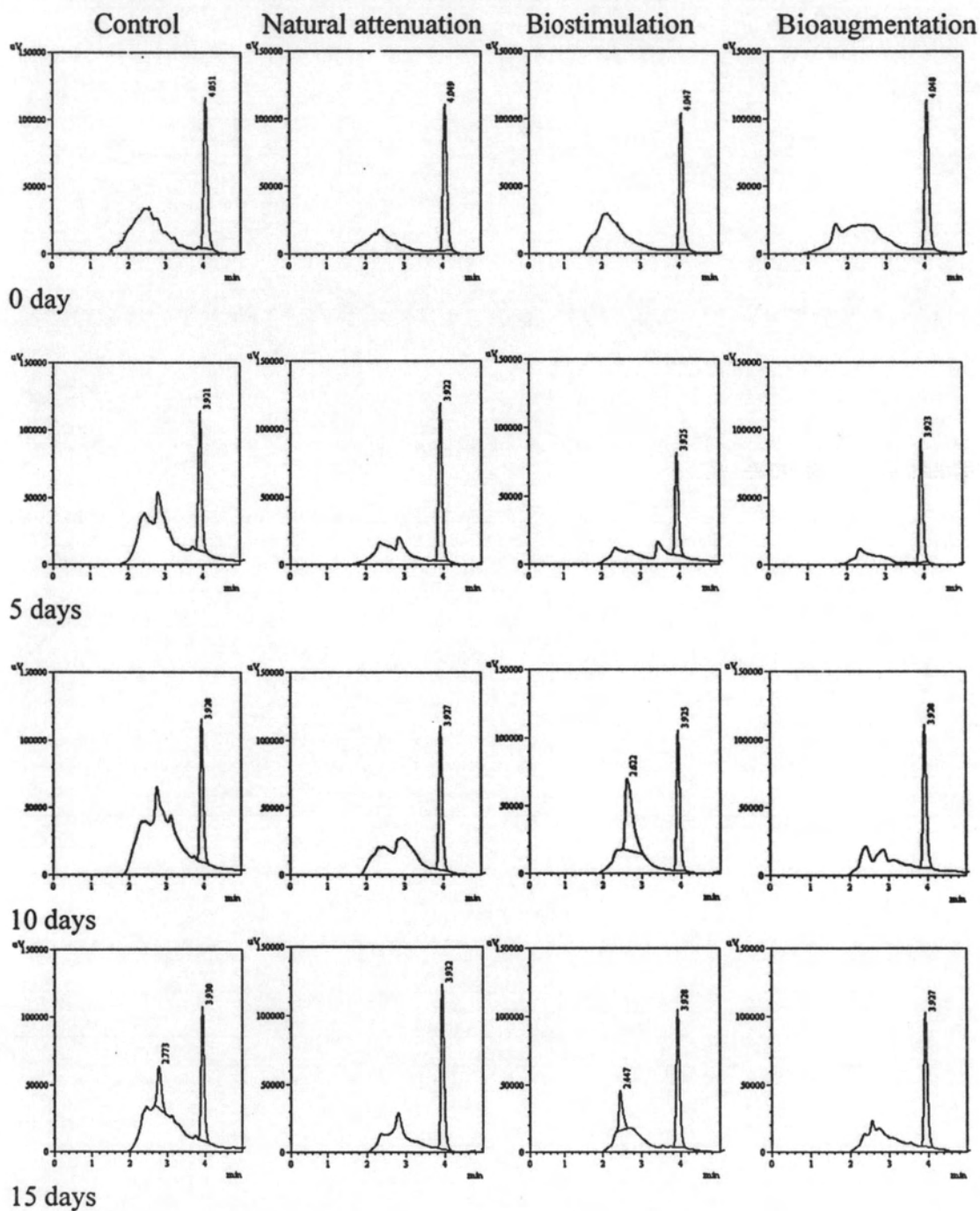
Add supplements in developing solution before used

10 mg/ml Sodium thiosulfate	300 µl
Formaldehyde	2.25 ml

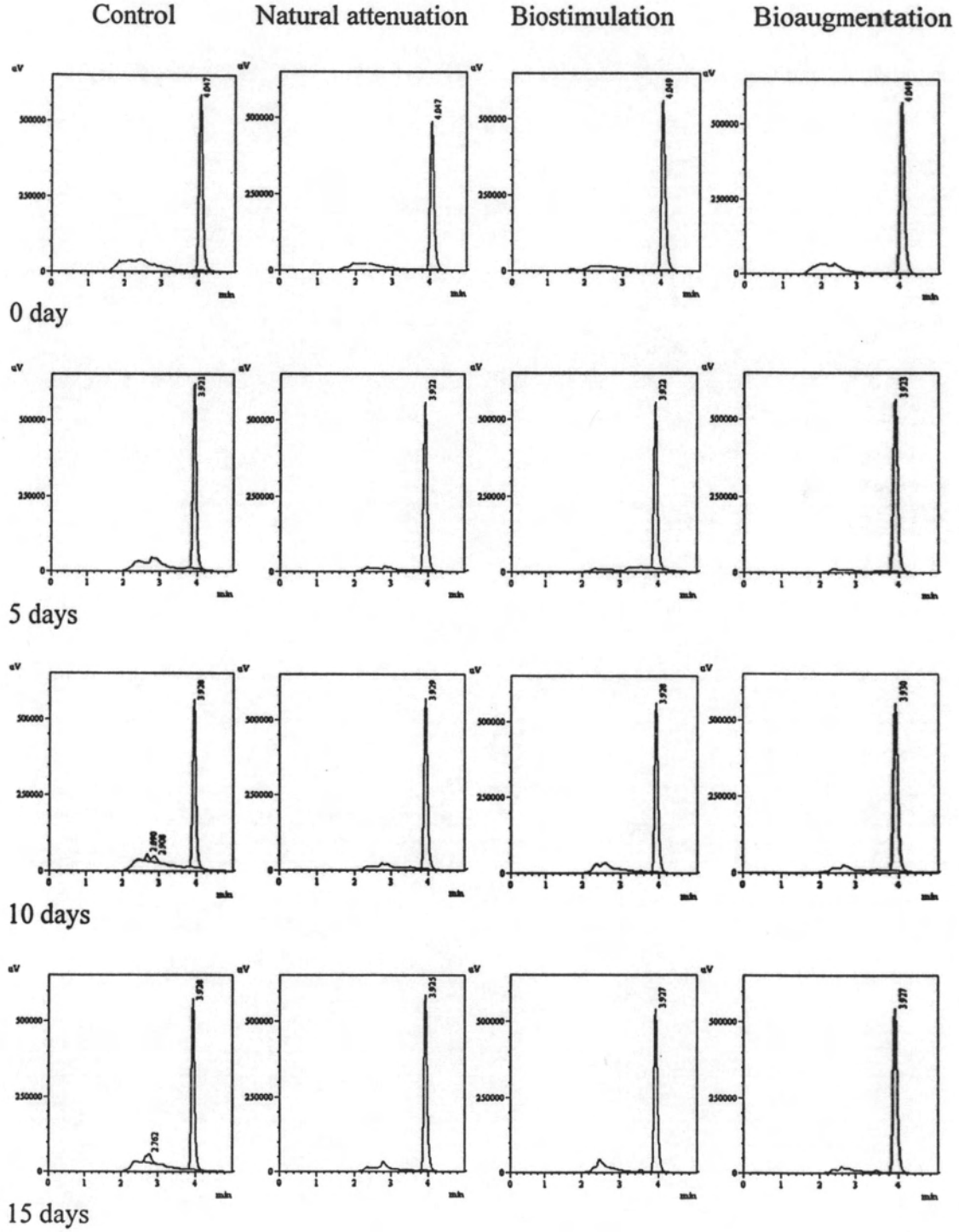
Appendix E

HPLC Chromatogram

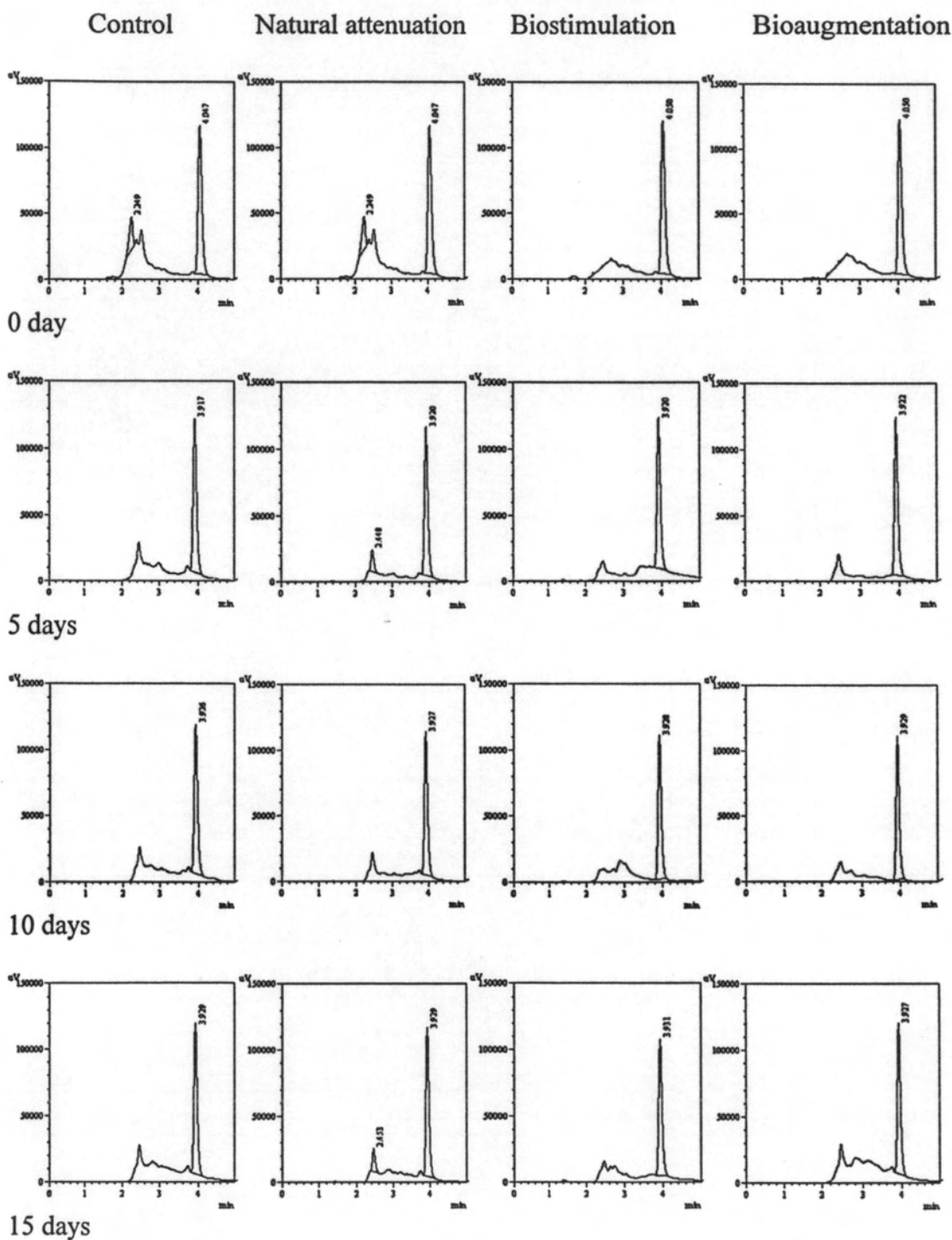
Condition B: HPLC peak in each bioremediation treatment of loam soil with 20-ppm diuron during 15 days of the incubation time. Retention time of diuron is 4.0 min determined under the specific HPLC condition as described in 3.6.2.2.



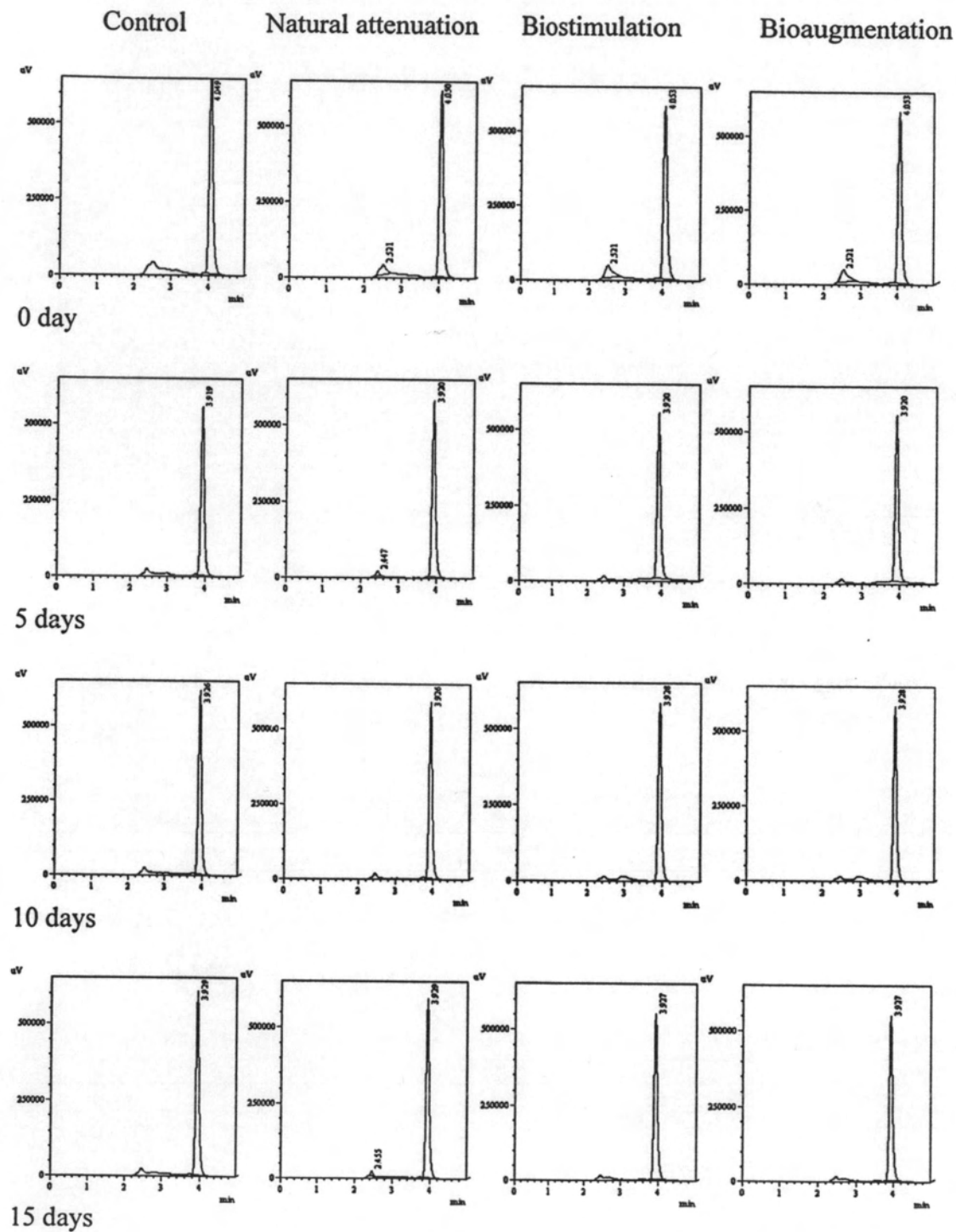
Condition B: HPLC peak in each bioremediation treatment of loam soil with 100-ppm diuron during 15 days of the incubation time. Retention time of diuron is 4.0 min determined under the specific HPLC condition as described in 3.6.2.2.



Condition B: HPLC peak in each bioremediation treatment of silty clay with 20-ppm diuron during 15 days of the incubation time. Retention time of diuron is 4.0 min determined under the specific HPLC condition as described in 3.6.2.2.



Condition B: HPLC peak in each bioremediation treatment of silty clay soil with 100-ppm diuron during 15 days of the incubation time. Retention time of diuron is 4.0 min determined under the specific HPLC condition as described in 3.6.2.2.



BIOGRAPHY

Mr. Surachai Sritampiwat was born on July 19, 1980. He graduated with the degree of Bachelor of Science in Biochemistry from Chulalongkorn University in 2003. He continued his study in the Master of Science Program in Biotechnology, Faculty of Science at Chulalongkorn University.