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Bacterial Growth Inhibition
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English Translation of Report Summary for
DuPont Chemical Solutions Enterprise
27-Jan-03

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TRANSLATION

ATOCHEM

Levallois Applications Center
Analysis - Environmental Services
Eco-toxicology Laboratory

TEST REPORT

DETERMINATION OF AN INHIBITING EFFECT ON BACTERIAL PSEUDOMONAS PUTIDA CELL GROWTH according to proposed standard ISO TC147/SC5/WG1/N111

Substance studied :

Name :

Customer reference :

Internal sample reference :

Person responsible for test :
Person carrying out test :

J. C. BOUTONNET
A. M. PETIT

Date test started :
Date report written :

22.10.91

Sample preparation

Mother solution at 20 g/l in ultra-pure water

Organism tested

Pseudomonas putida MIGULA, STRAIN Berlin 33/2 (DSM 50025)

Test conditions

Equipment :

Sterilized glassware
Perkin-Elmer spectrophotometer Lamda 5
Thermostatically controlled agitator at $21 \pm 1^\circ\text{C}$

Concentration range (each concentration is tested in triplicate) :

Vol. Substance solution (ml)	15	10	5	2.5		
Vol. Water (ml)	65	70	75	77.5		
Vol. Medium (ml)	10	10	10	10	10	10
Vol. Inoculum (ml)	10	10	10	10	10	10
Substance concentration (g/l)	3	2	1	0.5		

The flasks are incubated in a thermostatically controlled agitator

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The culture is started on 22/10/91 at 16 h and stopped on 23/10/91 at 8 h 30
Incubation duration : 16 h 30

The turbidity is measured using a spectrophotometer by determination of the optical density at a wavelength of 436 nm (ISO standard 7027 - 1984) after dilution : 10 before incubation

Results Dilution at 1/10 to effect the spectrophotometer reading

Substance concentration (mg/l)	OD in flask			Turbidity B		
(Optical density of control = 0.0037)	1	2	3	1	2	3
<u>3</u>	0.151	0.150	0.154	average = 404°/ml		
<u>2</u>	0.247	0.202	0.312	average = 587°/ml		
<u>1</u>	0.241	0.277	0.267	average = 715°/ml		
<u>0.5</u>	0.412	0.427	0.414	average = 1147°/ml		
	10 flasks			average =		
control O. D. = 0.019, 1/10	5	average 0.228				

Using the equation $H = ((B_k - B_n)/(B_k - B_0)) \times 100$

where H is percent cell growth inhibition H
B_k is the turbidity in the control flasks
B_n is the turbidity in the test flasks (after deduction of non-inoculated whites)
B₀ is the turbidity at t = 0 in the control flasks.

We obtain :

Substance concentration (mg/l)	H %
<u>3</u>	67.9 %
<u>2</u>	53 %
<u>1</u>	42.6 %
<u>0.5</u>	7.9 %

By drawing the logarithmic Gaussian curve (attached) we obtain :

CE 10 = g/l	0.47 g/l
CE 50 = g/l	1.75 g/l

By calculation (linear regression) we obtain :

CE 10 =	0.466 g/l
CE 50 =	1.76 g/l

Additional results :

The pH was measured at the start and end of the test :
At 3 mg/l, at t = 0, pH = 7.03; at t = 16 h 30, pH = 7.10
At 0 mg/l, at t = 0, pH = 6.99; at t = -- h, pH = 7.06

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Validity clauses :

The test was carried out using the protocol described in document :
ISO TC147/SC5/WG1/N111

During the test, cell growth in the control series occurred at a factor of : 12
(B0 = 10 and Bk = 1299)

The reference substance, 3,5-dichlorophenol gave under the test conditions :

CE 50 = 8.55 mg/l
Ce 10 = 0.427 mg/l