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World Journal of Microbiology & Biotechnology 15: 000–000, 1999.

Effect of C/N/P ratio and nonionic surfactants on polychlorinated biphenyl biodegradation

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Received 31 January 1999; accepted 9 June 1999

Keywords: Box–Behnken, carbon dioxide, contaminated soil, PCB, Tergitol, Triton, Tween

Summary

This work investigated whether polychlorinated biphenyl (PCB) removal from a highly contaminated soil (7000-p.p.m.) could be enhanced by manipulating the carbon to nitrogen to phosphorus (C/N/P) ratio, and by nonionic surfactant addition. A Box–Behnken statistical experimental design was used to evaluate the combined effect of surfactant type, surfactant concentration, and C/N/P ratio in a relatively short treatment period (35 days). The variable with the greatest effect on PCB degradation was the type of surfactant used. Higher PCB removal efficiencies (39–60%) were obtained with Tween 80 (compared to Tergitol NP 10 and Triton X-100). This was attributed to its lower critical micelle concentration. Higher C/N/P ratios (increased by biphenyl addition) significantly stimulated the soil heterotrophic activity without enhancing PCB removal. This suggests that nonionic surfactants have a greater potential to enhance bioremediation of PCB-contaminated soil than efforts to enhance the soil heterotrophic activity through nutrient and analogue substrate addition.

Introduction

Bioremediation holds great promise for treating soils contaminated with PCB-containing oils. Nevertheless, several variables can affect the feasibility of PCB bioremediation. Inorganic macro-nutrients, mainly nitrogen and phosphorus, must be present in sufficient quantities to satisfy the stoichiometric requirements for contaminant biodegradation and cell growth. Therefore, N and P are commonly added when the target contaminants represent a relative excess of carbon sources. Several researchers have found that adjusting the C/N/P ratio in contaminated environments (rivers, lakes, sediments and soils) can stimulate the degradation of hydrophobic petroleum hydrocarbons (Dibble & Bartha 1979; Rogers *et al.* 1993). However, the effect of manipulating the C/N/P ratio on PCB degradation has received only limited attention in the literature (Harkness *et al.* 1993).

Bioremediation of hydrophobic organic compounds, such as PCBs, can also be limited by a lack of adequate contact between microorganisms and the sorbed contaminant. One possible way of enhancing contaminant bioavailability is the addition of synthetic or microbial surfactants to increase the interfacial tension and the phase partitioning of organic compounds, enhancing their solubility (Aronstein & Alexander 1992; Oberbremer *et al.* 1990). Nonionic surfactants are commonly used to solubilize hydrophobic compounds since they are less toxic to bacteria than ionic surfactants (Swisher 1987; Volkering *et al.* 1995). In general, there is a certain surfactant concentration (called the critical micelle concentration – CMC) above which surfactants form micelles (i.e., aggregates of 10 to 200 molecules). Micelles have an important effect on the solubilization of hydrophobic compounds. Many remediation studies have dealt either with their effect on contaminant desorption and biodegradation (Abdul *et al.* 1990; Aronstein *et al.* 1991; Guha *et al.* 1998; Laha & Luthy 1992) or the practical problems of application of surfactants to soil (Van Dyke 1993; Vigon & Rubin 1989). The results of such studies, however, can be difficult to interpret because of complex interactions among soil, pollutant, surfactant, and microorganisms.

Statistical methods can provide valuable insight into how environmental conditions affect PCB degradation in complex systems. Such methods can also yield practical information on how to combine reaction variables to enhance the treatment process. This study used a Box–Behnken factorial experimental design (Box & Behnken 1960) to evaluate whether aerobic PCB

Journal : **WIBI**	SPS Article No. : **2299**	Dispatch : **22-6-2000**	Pages : **6**
PIPS No. : **238762**		☐ LE	☐ TYPESET
MS Code :		☑ CP	☑ DISK

degradation could be enhanced by manipulating the carbon/nitrogen/phosphorus (C/N/P) ratio in soil, and by surfactant addition. The type and concentration of three different nonionic surfactants were evaluated.

Materials and Methods

Soil

A sandy and lightly acidic (pH 6.3) soil from a highly contaminated site in Mexico City (ca. 7000 mg-PCBs/kg-soil) was used in this study. The PCB mixture consisting mainly of di-, tri-, tetra-, penta-, hexa- and heptachlorobiphenyl was identified as Aroclor 1260. The soil was homogenized and characterized before treatment to establish its nutritional status, textural class, pH, and ion-exchange and water-holding capacities (Jackson 1970). Organic carbon was determined by a Walkley–Black wet combustion method, total nitrogen by the Micro-Kjeldahl method (AOAC 1970), and total phosphorus by the stannous chloride method (APHA 1974). The initial C/N/P (mass) ratio, based on the indigenous carbon, nitrogen and phosphorus content, was high (2700/140/1) reflecting a deficiency of N and P. Metal content was determined by atomic absorption spectroscopy following extraction with HNO_3 and H_2O_2. Moisture content was adjusted to 20%, which was the minimum value that did not hinder soil heterotrophic activity (as measured by CO_2 evolution). Table 1 summarizes the soil characteristics.

Table 1. Contaminated soil characteristics.

	Value
Physical characteristics	
Sand, %	78
Silt, %	18
Clay, %	4
Moisture, %	4.4
Water holding capacity, %	40
Chemical characteristics	
pH	6.3
Ion exchange capacity, meq/g	55
Organic matter %	3.76*
Organic carbon, %	1.88
Nitrogen, %	0.098
Phosphorus, %	0.0007
Iron, p.p.m.	1700
Magnesium, p.p.m.	623
Manganese, p.p.m.	95
Copper, p.p.m.	13
Nickel, p.p.m.	8
Sodium, p.p.m.	1310
Potassium, p.p.m.	282
Calcium, p.p.m.	127
Lead, p.p.m.	61
Cadmium, p.p.m.	12
Chromium, p.p.m.	1
Zinc, p.p.m.	30

* Organic matter includes PCBs (7,000 mg/kg of soil)

Experimental design

A Box-Behnken experimental design (Box & Behnken 1960) was used to evaluate the effects of the C/N/P ratio and of the type and concentration of three nonionic surfactants (Table 2) on CO_2 evolution and PCB removal efficiency. Thus, three independent variables were considered at three levels.

A total of 15 soil microcosms were incubated in sealed vials for 35 days at 28 °C. Two controls were also run: sterilized soil with $HgCl_2$ at 2% (w/w), and soil without addition of surfactants or nutrients. The 17 microcosms were prepared in sealed vials (125-ml). Each vial contained 15 g of PCB-contaminated soil (20% moisture content) amended with nutrients and one of the three nonionic surfactants (Tween 80, Tergitol NP 10, or Triton X-100) (Tables 3 and 4). The C/N/P ratio was varied within a typical range used by other researchers working with hydrocarbon and PCB biodegradation in sediments (Atlas & Bartha 1973; Dibble & Bartha 1979; Harkness *et al.* 1993) (Table 4). Nitrogen and phosphorus were added as $(NH_4)_2SO_4$ and K_3PO_4, respectively, as needed to achieve C/N/P ratios of 100/10/1 or 120/10/1. Biphenyl was also added to sets 3, 7, 10, and 15 at 46.5 g/kg to increase the (mass) C/N/P ratio to 800/13/1. Surfactant type and concentration (Table 3) were selected based on positive results reported for the degradation of polycyclic aromatic hydrocarbons (Abdul & Ang 1994; Cook 1992). The selected surfactant concentrations fall below their cmCs (Table 2), which is a concentration range that has received limited attention in the PCB literature (Kile & Chiou 1989). According to the Box-Behnken experimental design (Montgomery 1991) three mid-level replicates were included to consider the experimental error (i.e., ~~sets~~ 11, 12 and 13).

CMC's
(use caps)

microcosms

Table 4

Table 2. Properties of nonionic surfactants tested.

Surfactant	Structure	Avg. MW	CMC (mg/l)	HLB	Aggregation number
Tween 80	Polyoxyethylenesorbitan monooleate	1300	13	15.5	59
Tergitol NP 10	$C_9H_{19}C_6H_4O(CH_2CH_2O)_{10.5}H$	682	36–55	13.3	100
Triton X-100	$C_8H_{17}C_6H_4O(CH_2CH_2O)_{9.5}H$	625	150	13.5	140

HLB – hydrophile-lipophile balance; CMC – critical micelle concentration; Avg. MW – average molecular weight; Aggregation number – number of molecules of surfactant needed to form a micelle.

Table 3. Factors and levels used in the Box Behnken experimental design.

Factors	x_1 Surfactant type	x_2 Surfactant dose (mg/Kg soil)	x_3 C/N/P (mass) ratio
Lower level (−1)	Tergitol NP 10	0.2	120/10/1
Base level (0)	Triton X 100	2	100/10/1
Upper level (+1)	Tween 80	3	800/13/1

Microcosms were incubated for 35 days at 28 °C, and the pH remained constant at 6.3. PCB concentrations were measured at the beginning and at the end of the experiment to calculate removal efficiencies. CO_2 evolution was measured daily to quantify heterotrophic activity. The experimental error was calculated with data from replicate microcosms 11, 12 and 13.

Regression analysis (SAS 6.01) was performed to characterize CO_2 evolution and PCB removal efficiency as a function of both qualitative variables (i.e., type of surfactant) and quantitative variables (i.e., surfactant concentration and C/N/P ratio). To facilitate the regression, the three types of surfactants were assigned coded values (−1, 0, and +1; Table 3). Following the Box Behnken (1960) experimental design approach, the same (equally-spaced) coded values were assigned to the three tested levels of the quantitative variables. Regression equations were obtained in terms of these coded values, and the corresponding response surfaces were plotted with Scientist IM 1.05 Micro Math Software.

Carbon dioxide evolution

Headspace samples (2 ml) were taken from the soil microcosms with a 5-ml gas-tight syringe. The CO_2 content of the samples was determined with a Gow Mac 550 gas chromatograph equipped with a thermal conductivity detector and an Alltech CTRI stainless steel column. The operation conditions were: 30 °C oven temperature, 30 °C injector temperature, and 125 °C detector temperature. Helium was used as a carrier gas, and the flow rate was 45 ml/min. Data were processed with Gow Mac software and integrated to obtain the cumulative CO_2 production.

PCB analysis and extraction

PCB removal was evaluated after 35 days of incubation. This corresponded to the time at which CO_2 evolution levelled off. Soil samples (2.5 g) were removed from each bottle and extracted in a Soxhlet system with hexane-ketone (3:1) during 9-h (Bellar & Lichtenber 1982). The extracts were purified through a glass column (1.5 × 30 cm) packed with Florisil mesh 60–100. The extracts were eluted with 100 ml of hexane, and 100 ml were collected and concentrated to 25 ml. Concentrated samples (1 μl) were analysed by impact electron gas chromatography/mass spectrometry (GC-MS) using a Varian Saturn 3 chromatograph with the following conditions: DB-5 capillary column (0.32 × 30 m, 5% phenyl and 95% methyl silicon), column temperature 90 °C for 3 min, 90–120 °C at 6.7 °C/min, 120–250 °C at 5.8 °C/min, and 280 °C for 15 min. Helium was used as carrier gas at a flow rate of 15 ml/min. The

Table 4. Box-Behnken experimental design and results.

Microcosm	Factor			CO_2 evolution		Overall PCB removal	
	x_1 (type)	x_2 (dose) (mg/kg)	x_3 (C/N/P)	Observed (μg/g)	Predicted (μg/g)	Observed (%)	Predicted (%)
1	Tergitol NP 10	0.2	100/10/1	1.8	2.5	11	15
2	Tergitol NP 10	2	100/10/1	2.0	2.4	35	30
3	Tergitol NP 10	2	800/13/1	16.2	16.1	19	19
4	Tergitol NP 10	3	100/10/1	2.3	1.2	43	47
5	Tween 80	0.2	100/10/1	2.2	3.3	58	57
6	Tween 80	2	120/10/1	6.1	6.2	60	57
7	Tween 80	2	800/13/1	18.7	18.4	39	42
8	Tween 80	3	100/10/1	7.2	6.4	59	59
9	Triton X-100	0.2	120/10/1	1.7	0.6	ND	-
10	Triton X-100	0.2	800/13/1	15.8	15.1	50	46
11	Triton X-100	2	100/10/1	2.2	2.2	22	25
12	Triton X-100	2	100/10/1	2.0	2.2	29	25
13	Triton X-100	2	100/10/1	2.4	2.2	22	25
14	Triton X-100	3	120/10/1	2.2	2.9	ND	-
15	Triton X-100	3	800/13/1	13.3	14.4	11	7
16	Sterile control	0	2700/13/1	0.5	-	7	-
17	No-treatment control	0	2700/13/1	1.1	-	7	-

ND = not determined.

Note: All sets were incubated for 35 days at 28 °C, pH = 6.3, 20% moisture. Nitrogen and phosphorus were added to all microcosms amended with surfactants, but not to controls. Biphenyl also was added to microcosms 3, 7, 10, and 15 to adjust the C/N/P ratio to 800/13/1.

temperature of the injector was 250 °C, and the detector was an electromultiplier. Mass range corresponded from 60 to 500 m/e, based on the fragmentation properties of the tested PCBs.

PCBs were identified on the basis of their fragmentation peaks in the GC-MS library. Identification was validated by comparison of retention times using Aroclor 1260 as a standard. PCB removal in viable treatments was determined after normalization of chromatogram peak areas for nondegradable congeners with those from control samples (Mondello 1989). Residual concentrations of these congeners were relatively constant regardless of treatment. For example, the coefficient of variation for residual concentrations of 2,2′, 3,3′, 5,6′-hexachlorobiphenyl was only 0.112%. This indicates that experimental and statistical procedures were precise and reproducible.

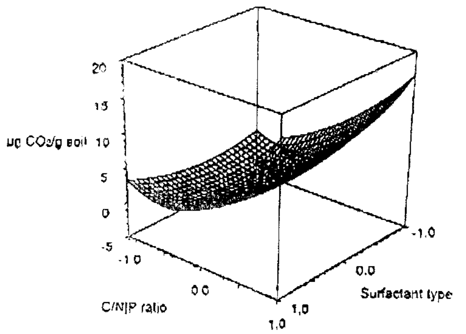

Figure 1. Surface response of CO_2 evolution in PCB-contaminated soil as a function of surfactant type and C/N/P ratio. The independent variables are qualitative; thus, their axes were coded according to the Box–Behnken experimental design (Table 3). Surfactant type code "−1" corresponds to Tergitol NP 10, "0" to Triton X-100, and "1" to Tween 80. C/N/P ratio code "−1" corresponds to 120/10/1, "0" to 100/10/1, and "1" to 100/1.6/0.125.

Results

A statistical analysis of the Box–Behnken experimental design shows that higher C/N/P ratios had a positive significant effect on CO_2 evolution, as reflected by the positive coefficient and low p-value for factor x_3 (Table 5). The type of surfactant used also had a statistically significant effect on CO_2 evolution ($p < 0.05$). The tested surfactant concentrations, however, did not have a statistically significant effect. Therefore, the response surface for the cumulative CO_2 evolution was plotted as a function of only the C/N/P ratio and surfactant type (Figure 1). After 35 days of incubation, the highest CO_2 evolution (18.7 μg-CO_2/g-soil) occurred with a C/N/P ratio of 800/13/1 and Tween 80 (Table 4, microcosm 7). The relatively high CO_2 evolution observed in this microcosm, as well as in all other microcosms prepared with the same C/N/P ratio, reflects that biphenyl addition significantly stimulated the heterotrophic activity. For reference, CO_2 evolution was 0.50 μg CO_2/g-soil in the poisoned control, and 1.11 μg CO_2/g-soil in the no-treatment control.

Following 35 days of incubation, chromatographic analysis showed a significant decrease in PCB concentration in treated microcosms. Unidentified hydrocarbons of high molecular weight were also degraded. All of the tested variables had a statistically significant effect on PCB removal, although surfactant type had a more significant effect (i.e., much lower p value) (Table 6). The highest PCB removal efficiencies were obtained with Tween 80. For example, for 0.2 mg/kg Tween 80 and a C/N/P ratio equal to 100/10/1, removal efficiencies were about 50% for dichlorobiphenyls, 70% for trichlorobiphenyls, 53% for tetrachlorobiphenyls, 54% for pentachlorobiphenyls, 56% for hexachlorobiphenyls, and 55% for heptachlorobiphenyls (Figure 2). This represents an overall (weighted-average) removal of 57% (Table 4, microcosm 5). Increasing the surfactant concentration towards the CMC generally had a positive effect on PCB removal efficiency, as shown by the positive coefficient for factor x_2 (Table 6). This enhancement was more pronounced for Tergitol NP10 than for Tween 80 or Triton X-100 (Table 4). In general, nutrient addition (lower C/N/P ratios) also increased the PCB removal efficiency, as shown by the negative coefficient for factor x_3 (Table 6).

Table 5. Regression analysis of CO_2 evolution (μg CO_2/g-soil), according to the Box–Behnken experimental design.

Term	Coefficient	T value	Significance level (p value)
Intercept	2.18	3.078	0.0275
x_1 (type)	1.51	3.474	0.0178
x_2 (dose)	0.44	1.012	0.3582
x_3 (C/N/P)	6.49	14.928	0.0001
x_1x_2	1.10	1.800	0.1317
x_1x_3	−0.39	−0.642	0.5491
x_2x_3	−0.75	−1.223	0.2757
x_1^2	1.84	2.872	0.0349
x_2^2	−0.65	−1.028	0.3510
x_3^2	6.74	10.525	0.0001

$R^2 = 0.9863$; R^2(adj.) = 0.9617; Coefficient of variation = 2.93%.

Table 6. Regression analysis of PCB removal efficiency (%), according to the Box–Behnken experimental design.

Term	Coefficient	T value	Significance level (p value)
Intercept	25.03	9.078	0.0003
x_1 (type)	13.42	7.061	0.0009
x_2 (dose)	8.44	3.143	0.0256
x_3 (C/N/P)	−7.08	−2.874	0.0348
x_1^2	11.14	3.416	0.0189
x_2^2	8.51	2.709	0.0423
x_1x_2	−7.67	−2.856	0.0356
x_2x_3	−27.83	−5.978	0.0019

$R^2 = 0.9628$; R^2(adj.) = 0.9107; Coefficient of variation = 15.47%.

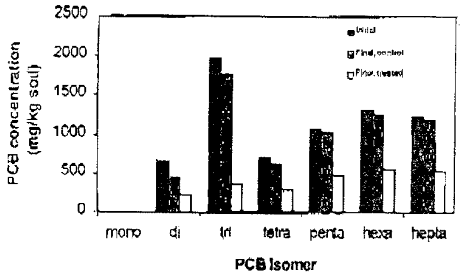

Figure 2. PCB removal from contaminated soil. Microcosms were incubated for 35 days at 28 °C, pH 6.3, and 20% moisture content. The depicted treatment was with 0.2 mg/kg of Tween 80, and a C/N/P ratio of 100/10/1.

Discussion

Longer contact times between PCBs and soil generally increase the fraction of PCBs adsorbed in the micropores and decrease the bioavailable fraction (Coates & Elzerman 1986; Harmsen 1993; Witrowsk *et al.* 1988). Thus, contaminated soil from old sites is often much more difficult to bioremediate than soil spiked with PCBs in laboratory studies. Nevertheless, this study shows that a relatively low dose of nonionic surfactant (e.g., Tween 80 at 0.2 mg/kg) can enhance PCB bioremediation in a relatively short period (35 days) at a low moisture content (20%), even for contaminated soil from an old site.

Decreasing the C/N/P ratio by nutrient addition enhanced PCB removal. This corroborates the prevailing notion that adding nutrients can enhance bioremediation. Nevertheless, other researchers have found that petroleum hydrocarbon biodegradation could be hindered by nutrient addition. Apparently, N and P addition can have detrimental effects on bioremediation if it inhibits microorganisms that were adapted to the oligotrophic soil environment (Morgan & Watkinson 1992) or if it stimulates the degradation of carbon compounds other than the target pollutants (Entry *et al.* 1993). This suggests that nutrient addition should be considered for biostimulation approaches only when indigenous nutrient levels are stoichiometrically limiting.

Higher C/N/P ratios corresponded to higher heterotrophic activity of the soil, as indicated by higher CO_2 evolution (Figure 1). This is consistent with the paradigm that, when environmental carbon/nutrient ratios are higher than those found in microbial biomass, CO_2 evolution may increase as a lower percentage of the metabolized carbon is incorporated into cell material (Amador & Jones 1993; Bosatta & Berebse 1984).

Biphenyl, which has been used previously as a primary substrate to stimulate (aerobic) PCB cometabolism (Kohler *et al.* 1988), stimulated CO_2 evolution (Table 4, microcosms 3, 7, 10, and 15). Biphenyl addition, however, decreased the PCB removal efficiency, as observed in treatments with 2 mg/kg of either Triton X-100 (microcosms 3) or Tween 80 (microcosm 7). This reflects that a higher heterotrophic activity does not necessarily correspond to enhanced degradation of the target pollutants. Whether the decrease in PCB removal efficiency resulting from biphenyl addition was due to metabolic or population shifts, competitive inhibition, or exacerbation of the oxygen demand, was not determined.

Differences in efficacy of the tested surfactants can be explained, in part, by differences in their corresponding CMCs (Table 2). The CMC for nonionic surfactants increases with the ethylene oxide content and it is inversely related to the efficiency of solubilization of the hydrophobic material (Irkin *et al.* 1989; Larson & Maki 1982). The higher PCB removal with Tween 80 (polyoxyethylene sorbitan monooleate) is consistent with the lower CMC for this surfactant (13 mg/l) relative to Triton X-100 (150 mg/l) and Tergitol NP 10 (37–55 mg/l). The tested surfactant concentrations (1–16 mg/l in solution) were considerably lower than the CMCs for Triton X-100 and Tergitol NP 10, but not for Tween 80. This suggests that Tween 80 enhanced PCB solubilization (and thus, biodegradation) to a greater extent than the other surfactants under the tested conditions. Incidentally, Tween 80 was shown in separate experiments to support the growth of mixed cultures (data not shown). Thus, the possibility that surfactant addition could have also affected the number and activity of PCB degraders cannot be excluded.

Conclusion

PCB bioavailability must be considered to comprehend the effect of adding nutrients or analogue substrates on bioremediation. This work suggests that adding nonionic surfactants has a greater potential to enhance PCB degradation than manipulating the C/N/P ratio. Nevertheless, treatability studies are recommended to avoid detrimental effects associated with microbial toxicity and reduced bioavailability of PCBs partitioning into the surfactant phase if relatively high surfactant concentrations are used.

Acknowledgements

Funding for this project was provided by IMP, FIES 95-105-VI and CONACYT. We are grateful to C. Anguis-Terrazas, and Loring Nies for helpful discussions.

References

AOAC. 42.014. 1970 *Official Methods of Analysis*. 11th ed., Washington D.C., Association of Official Analytical Chemists.

Abdul, A.S., Gibson, T.L. & Rai, D.N. 1990 Selection of surfactants for the removal of petroleum products from sandy aquifers. *Ground Water* 28, 920–926.

Abdul, S.A. & Ang, C. 1994 *In situ* surfactant washing of polychlorinated biphenyls and oils from a contaminated field site phase II pilot study. *Ground Water* 82, 727–724.

Amador, J.A. & Jones, R.D. 1993 Nutrient limitations on microbial respiration in peat soils with different total phosphorus content *Soil Biology & Biochemistry* **25**, 793–801.

APHA *Standard Methods for the Examination of Water and Wastewater* 1974. 13th ed Washington D.C., American Public Health Association.

Aronstein, B.A., Calvillo, Y.M. & Alexander, M. 1991 Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. *Environmental Science and Technology* **25**, 1728–1731.

Aronstein, B.N. & Alexander, M. 1992 Surfactants at low concentrations stimulate the biodegradation of sorbed hydrocarbons in samples of aquifer sands and soil slurries. *Environmental Toxicology and Chemistry* **11**, 1227–1233.

Atlas, R.M. & Bartha, R. 1973 Stimulated biodegradation of oils slicks using oleophilic fertilizers. *Environmental Science and Technology* 7, 538–541.

Bellar, T.A. & Lichtenber, J.J. 1982 The determination of polychlorinated biphenyls in transformer fluid and waste oils. EPA/600/4-81/045; U.S. Environmental Protection Agency: Cincinnati, OH.

Bosatta, E. & Berebse, F. 1984 Energy of nutrient regulation of decomposition: Implication for the mineralization–immobilization response to perturbations. *Soil Biology and Biochemistry* **16**, 63–67.

Box, G.E.P. & Behnken, D.W. 1960 Some new three level designs for the study of quantitative variables. *Technometrics* **2**, 455–476.

Coates, J.T. & Elzerman, A.W. 1986 Desorption kinetics for selected PCB congeners from river sediments. *Journal of Contamination and Hydrology* **1**, 191–210.

Cook, C. 1992 Surfactant-enhanced solubilization of tetrachloroethylene and degradation products in pump and treat remediation In *Transport and Remediation of Subsurface Contaminants*. pp. 149–158. American Chemical Society. ISBN 0-84122223-1

Dibble, J.T. & Bartha, R. 1979 Effect of environmental parameters on the biodegradation of oil sludge. *Applied Environmental and Microbiology* 37, 729–739.

Entry, J.A., Mattson, K.G. & Emmingham, W.H. 1993 The influence of nitrogen on atrazine and 2.4-dichlorophenoxyacetic acid mineralization in grassland soils. *Biology of Fertilizers and Soils* **16**, 179–182.

Guha, S., Jaffé, P.R. & Peters, C.A. 1998 Bioavailability of mixtures of PAHs partioned into the micellar phase of a non-ionic surfactant. *Environmental Science and Technology* **32**, 2317–2324.

Harkness, M.R., McDermott, J.B., Abramowitz, D.A., Salvo, J.J., Flanagan, W.P., Stephens, M.L., Mondello, F.J., May, R.L., Lobos, J.H., Carroll, K.M., Brennan, M.J., Bracco, A.A., Fish, K.M., Wagner, G.L., Wilson, P.R., Dietrich, D.K., Lin, D.T., Morgan, C.B. & Gately, W.L. 1993 *In situ* stimulation of aerobic PCB biodegradation in Hudson River sediments. *Science* **259**, 503–507.

Harmsen, J. 1993 *Integrated Soil and Sediment Research* Dordrecht, The Netherlands, Kluwer Academic Publishers. ISBN 0-79232321-1.

Irkhin, A.I., Kondrashenko, T.N. & Puchkiow, E.O. 1989 Sensitivity of *Escherichia coli* cell membranes to different classes of detergents (in Russian). *Mikrobiologiya* 58, 217–221.

Jackson, M.L. 1970 *Análisis de suelos* Iberoamericana: México (In Spanish).

Kile, D.E. & Chiou, C.T. 1989 Water solubility enhancements of DDT and trichlorobenzene by some surfactants below and above the critical micelle concentration. *Environmental Science and Technology* **23**, 832–838.

Kohler, H.P.E., Kohler-Staub, D. & Focht, D.D. 1988 Cometabolism of polychlorinated biphenyls enhanced transformation of Aroclor 1254 by growing bacterial cells. *Applied and Environmental Microbiology* 54, 1940–1945.

Laha, S. & Luthy, R.G. 1992 Effects of non-ionic surfactants on the solubilization and mineralization of phenanthrene in soil-water systems. *Biotechnology and Bioengineering* **40**, 1367–1380.

Larson, R.J. & Maki, A.W. 1982 *Aquatic Toxicology and Hazard Assessment*. Sixth Symposium American Society for Testing materials: Philadelphia, PA.

Mondello, F.J. 1989 Cloning and expression in *Escherichia coli* of *Pseudomonas* strain LB400 genes encoding polychlorinated biphenyl degradation. *Journal of Bacteriology* **17**, 1725–1732.

Morgan, P. & Watkinson, R.J. 1992 Factors limiting the supply and efficiency of nutrient and oxygen supplements for the *in situ* biotreatment of contaminated soil and groundwater. *Water Research* 26, 73–78.

Montgomery, D.C. 1991 *Design and analysis of experiments* 3rd edn John Wiley & Sons Inc. (Ed.) USA. ISBN 0-471-52000-4.

Oberbremer, A., Muller-Hurtig, R. & Wagner, F. 1990 Effect of the addition of microbial surfactants on hydrocarbon degradation in a soil population in a stirred reactor. *Applied Microbiology and Biotechnology* **32**, 485–489.

Rogers, J.A., Tedaldi, D.J. & Kavanaugh, M.C. 1993 A screening protocol for bioremediation of contaminated soil. *Environmental Progress* **12**, 146–156.

Swisher, R.D. 1987 Surfactants Biodegradation. *Surface Science Series* **18**, 415–517.

Van-Dyke, M.I., Gulley, S.L., Lee, H. & Trevors, J.T. 1993 Evaluation of microbial surfactants from recovery of hydrophobic pollutants from soil. *Journal of Industrial Microbiology* **11**, 163–170.

Vigon, B.W. & Rubin, A.J. 1989 Practical considerations in the surfactant-aided mobilization of contaminants in aquifers. *Journal of Water Pollution Control Federation* **61**, 1233–1240.

Volkering, F., Breure, A.M., van-Andel, J.G. & Rulkens, W.H. 1995 Influence of non-ionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons. *Applied and Environmental Microbiology* **61**, 1699–1705.

Witrowsk, P.J., Jafre, P.R. & Ferrara, R.A. 1988 Sorption and desorption dynamics of Aroclor 1242 to natural sediments. *Journal of Contamination and Hydrology* 2, 249–269.