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The effect of rhizosphere on growth of *Sphingomonas chlorophenolica* ATCC 39723 during pentachlorophenol (PCP) biodegradation in batch culture and soil

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Resumo

O efeito da rizosfera sobre o crescimento de *Sphingomonas chlorophenolica* ATCC 39723 durante a biodegradação de pentaclorofenol em cultura e no solo. Estudos da influência da rizosfera sobre o crescimento de *Sphingomonas chlorophenolica* durante a biodegradação de Pentaclorofenol (PCP) com cultura pura e em solo foram realizados. Utilizou-se um meio mineral com e sem exudatos de rizosfera extraídos de trigo. No solo, os experimentos foram realizados na presença e ausência de plantas. As concentrações de PCP foram determinadas através de Análises de Alta Performance de Cromatografia Líquida. Enumeração bacteriana de *S. chlorophenolica* foi realizada cultivando-se no Meio Mineral MSM. Resultados indicam que os exudatos de rizosphera estimularam o crescimento de *S. chlorophenolica* em concentrações de 50 e 80mg L^{-1} PCP, assim como a capacidade degradadora do organismo em concentração de 80mg/kg solo peso seco. O pentachlorophenol teve um efeito adverso sobre o crescimento de *S. chlorophenolica*. A introdução de *S. chlorophenolica* em um solo argiloso com plantas proporciou degradação mais rápida quando comparada ao solo inoculado sem plantas. Houve aumento na população de *S. chlorophenolica* na rizosfera do que no solo somente. Este estudo mostrou que a presença do inóculo *S. chlorophenolica* melhorou a degradação de PCP em um solo argiloso indicando o potencial para tratamento em condições ambientais.

Unitermos: pesticidas, rizoremediação, exudatos de rizosfera

Abstract

Studies on the influence of the rhizosphere on the growth of *Sphingomonas chlorophenolica* during Pentacholophenol (PCP) degradation in batch culture and in soil were carried out. In batch culture, a basal minimal medium with or without rhizosphere exudates extracted from winter wheat was used. In soil systems, degradation experiments were performed in the presence and absence of plants. Measurements of PCP concentrations were made using high performance liquid chromatography analysis (HPLC). Bacterial analyses of *S. chlorophenolica* were carried out by plating on MSM medium. The results showed that the rhizosphere exudates stimulated the growth of the cells of *S. chlorophenolica* at concentrations of 50 and 80mg kg dry wt soil $^{-1}$ as well as stimulating

the ability of *S. chlorophenolica* to degrade PCP at a concentration of 80mg Kg dry wt soil $^{-1}$. In addition, pentachlorophenol had an adverse effect on the growth of *S. chlorophenolica*. The introduction of *S. chlorophenolica* into the loamy soil with plants showed a faster degradation when compared to the inoculated soil without plants. There was a significant increase of *S. chlorophenolica* in the roots in comparison to those in the soil. This study showed that the presence of the inoculum *S. chlorophenolica* enhanced the PCP degradation in a loamy soil and it indicates the potential for a treatment process under a appropriate environmental conditions such as there present in soil systems.

Key words: pesticides, rhizoremediation, rhizosphere exudates

Introduction

Pentachlorophenol is a major industrial chemical, and as a biocide it is used worldwide. It is extensively used as a wood preservative as well in a wide variety of agricultural and industrial applications. *Sphingomonas chlorophenolica* ATCC 39723 (Saber and Crawford, 1985), a Gram-negative bacterium, is a well-characterized PCP-degrader (Crawford and Mohn, 1985; Brown et al., 1986; Steiert and Crawford, 1985 and 1986; Steiert et al., 1987). Many bacterial species, most of which are isolated from soil, such as *Pseudomonas* SR3 (Resnick and Chapman, 1994), *Flavobacterium* (Saber and Crawford, 1985) and *Arthrobacter* (Stanlake and Finn, 1982), have been shown to actively express the ability to degrade PCP in culture.

To assess the contribution of the rhizosphere to the growth of *S. chlorophenolica,* rhizosphere exudates were extracted from winter wheat. Plants, such as ryegrass (Hodge et al., 1998) and maize (Radehaus and Schmidt, 1992; Benizri et al., 1995; Yoshitomi and Shann, 2001) have been used for rhizosphere exudate extraction. The exudate is associated with an additional carbon source, which may enhance the ability of the organism to degrade target compounds. In addition, exudates also have the potential to selectively speed the growth and performance of microorganisms capable of potential degradation in the rhizosphere (Hedge and Fletcher, 1996). The addition of a supplementary source of carbon may also serve to protect cells from the toxic impact of pollutants (Topp et al., 1988).

Under laboratory conditions, cells of *S. chlorophenolica* were grown in a basal minimal medium with or without rhizosphere exudates and a range of 20 to 150mg L^{-1}concentrations of PCP in liquid culture. Studies have demonstrated the growth of *S.* (*Flavobacterium*) *chlorophenolica* in low (40mg L^{-1}) (Saber and Crawford, 1985) and high concentrations of PCP (100 to 150mg L^{-1}) in pure culture (Rattray et al., 1995; Topp et al., 1988). In a soil system, soil artificially contaminated with PCP was inoculated with *S. chlorophenolica*. Bioaugmentation (the addition of pollutant-degrading microorganisms) is most likely to succeed in environmental cleanup when the introduced organisms are active and competitive. In soil systems, physicohemical factors and biological parameters may affect the performance of the inoculum. Organisms such as *Flavobacterium* sp., *Arthrobacter* and *S. chlorophenolica* RA2 were investigated for their ability to degrade PCP-contaminated soils (Edgehill and Finn, 1983; Crawford and Mohn, 1985; Saber and Crawford, 1985; Edgehill, 1994; Miethling and Karlson, 1996). The inoculation of soil by microorganisms able to mineralize chlorophenol compounds has been shown to be useful for soil decontamination (Salkinoja-Salonen et al., 1989). It has been proposed that the plant rhizosphere-rhizosplane may facilitate the enhancement of microbial degradation of hazardous compounds in soils (Walton and Anderson, 1990). The effect of plant roots in the rhizosphere has been shown to select microbial communities capable of dissipation of organic pollutants (Reilley et al., 1996). In addition, plant roots improve the humification and adsorption of pollutants, thereby increasing their bioavailability (Gunther et al., 1996). The objective of this study was to assess the influence of rhizosphere exudates on the ability of *S. chlorophenolica* to degrade PCP in batch culture and in soil systems. Growth rates and specific degradation rates in batch culture in the presence and absence of a rhizosphere are presented. The PCP degradation by *S. chlorophenolica* in soil in the presence and absence of plants (winter wheat) and the enumeration of population counts of *S. chlorophenolica* in soil and roots are shown.

Material and Methods

Growth conditions

Cells of *S. chlorophenolica* ATCC 39723 were grown in light-protected 250ml flasks containing 100ml of a mineral salt liquid medium (MSM) (0.65g of K_2HPO_4; 0.19g of KH_2PO_4; 0.10g of $MgSO_4.7H_2O$; 0.5g of $NaNO_3$; and 4g L^{-1} of sodium glutamate ($C_5H_8NO_4Na$)) on an orbital shaker (200 rpm) at 25°C. The pH was adjusted to 7.3-7.4 prior to autoclaving and 2ml L^{-1} of filter-sterilized 0.01M $FeSO_4$ solution was added. When the cell culture was in the mid-logarithmic phase of growth (O.D. 0.5), cells were induced for PCP metabolism by the addition of PCP from a stock solution at a final concentration of 50mg L^{-1} (Stanlake and Finn, 1982). Growth was monitored in a Cecil Instruments Spectrophotometer at 560nm. In order to monitor PCP degradation in batch culture, 1ml of medium was centrifuged (6,000g x 3min) and absorbance was measured at 320nm. When 80% of PCP had been degraded, a 1ml aliquot was harvested by centrifugation (6,000g x 20min), washed once in MSM without glutamate, suspended in MSM without glutamate, and then used as an inoculum for experiments (O.D. 0.7). A control without glutame in the same conditions was carried out Three determinations were made and all experiments were conducted using triplicate samples.

Calculation of the growth rate

The growth rates were determined by calculating the gradient of a mid-exponential growth phase measured by absorbance at 560nm as a function of time (Schlegel, 1993).

Rhizosphere extraction

The rhizosphere extraction was carried out in sterilized microcosms with a 0.25 strength Hoagland's nutritient solution (Hoagland and Arnon, 1950). Winter wheat (*Triticum aestivum*) seeds were sterilized in a 2% (v/v) sodium hypochlorine solution for 20min, washed for 30min with sterilized water, and incubated at 25°C to enable germination on Petri dishes (15cm diameter) containing moist autoclaved Whatman nº 44 filter paper (Rattray et al., 1995). The seeds were then transferred using forceps to sterilized microcosms. Black glass beads (5mm) (350g) were placed in the bottom chamber of the microcosms and 200ml of a 0.25 strength Hoagland's nutrient solution was added. The germinated seeds were placed in the bottom chamber and the microcosms were incubated in a controlled environmental chamber (Fitotron PG 660, Sanyo Gallenkamp, Leicester, U.K.) with a 12h diurnal cycle at a constant temperature of 18°C and 85% relative humidity. The exudate material was collected in the lower chamber of the microcosm after 24h. The exudate was removed using a sterile 50ml syringe, after which was transferred aseptically to a 500ml sterile Duran bottle and kept at 4°C until use (Hodge et al., 1998). On each sampling date, the sterility of the exudate fluid was verified by pipeting aliquots (1ml) onto nutrient agar plates. No growth was detected in any sample. The total organic carbon content in each of the exudate samples was determined using a total organic carbon analyzer (LabToc C, Pollution & Process Monitoring, Kent, England) with UV digestion and infrared detection.

PCP degradation experiments in batch culture

Sphingomonas chlorophenolica ATCC 39723 was grown in 250-ml light protected Duran bottles containing 100ml of MSM medium and 100ml of MSM with rhizosphere extracts (MSM+RE) (1:1 v/v) on an orbital shaker at 200 rpm (25°C). Induced cells of *S. chlorophenolica* were used as the inoculum (1%) for degradation experiments in MSM and MSM+RE (1:1, v/v) containing PCP concentrations ranging from 20 to 150mg L^{-1}, added from the stock solution. PCP stock solution (10g L^{-1}) was prepared in a 250ml Duran bottle by adding 1g of PCP with 99% purity (Sigma Chemical Co, USA) to 100 ml 1.0 M NaOH to optimize solubility. The pH value was adjusted to 7.5 using 1.0 M H_3PO_4. Three determinations were made and all experiments were carried out using triplicate samples.

Calculation of specific degradation rates

The rate of PCP removal within a specified incubation period was divided by the mean of the viable cell counts determined during the incubation period. The specific degradation rates were estimated as pmol/new cells/h.

PCP degradation experiments in soil

Soil was collected from Boyndie (Boyndie series), northern Scotland, U.K. A description of the soil is given in Table 1.

TABLE 1: Boyndie soil description according to its characteristics.

Texture	Loamy sandy
pH H_2O (1:1)	6.6
Cation exchange capacity (CEC)	2.11
Total Organic C (%)	2.54
Total Organic N (%)	0.09
Sand content (%)	80
Clay content (%)	6.5

The water content of the non-sterile Boyndie soil was adjusted to 35% of its water holding capacity (WHC) using deionized water. The total WHC (50%) of the wet soil was determined on the basis of oven dry weight at 105°C for 24-48h. The soil was spiked with PCP (100mg kg^{-1} dry wt soil), mixed thoroughly for 30min, and then added to plastic pots containing 350 +/- 5g dry wt soil. The treatments applied to the soil are described in Table 2.

TABLE 2: Treatments applied to soil spiked with PCP (100 mg kg^{-1} dry wt soil).

C	Soil without PCP, plant and inoculum (control)
P	Soil without PCP plus plant
I	Soil without PCP plus inoculum
PCP	Soil spiked with PCP
PCP+I	Soil plus PCP plus inoculum
PCP+P	Soil plus PCP plus plant
PCP+I+P	Soil plus PCP plus inoculum plus plant

A single one-two days old seedling was transplanted to each pot filled with moist soil and over a periodo of 15 days, the water content of the non-sterile soil was maintained daily at 35% of its WHC by irrigating the pots with distilled water. Five replicates of each treatment were incubated in a controlled environmental growth chamber (Fi-totron PG 660, Sanyo Gallenkamp, Leicester, U.K.) with a 12h diurnal cycle at a constant temperature of 18°C and 85%. Sampling was destructive.

Winter wheat seeds

Winter wheat seeds (*Triticum aestivum*) were sterilized in a 2% (v/v) sodium hypochlorite solution for 20min, washed for 30min with sterilized water and incubated at 25°C for germination in Petri dishes (15cm diameter) containing moist autoclaved Whatman nº 44 filter paper (Rattray et al., 1995). After 48h, the germinated seeds were planted in plastic pots containing 350 +/- 5g dry wt soil.

Microbial analysis

Enumeration of *S. chlorophenolica* was carried out by culturing on MSM agar medium for *S. chlorophenolica* ATCC 39723: (0.65g of K_2HPO_4; 0.19g of KH_2PO_4; 0.10g of $MgSO_4.7H_2O$; 0.5g of $NaNO_3$; and 4g L^{-1} of sodium glutamate ($C_5H_8NO_4Na$). The pH was adjusted to 7.3-7.4 prior to autoclaving and 2ml L^{-1} of a filter sterilized 0.01M $FeSO_4$ solution was added. Each sample was analyzed in five replicates. The dilution series were plated on MSM medium and incubated at 25°C. Bacterial counts were made between 24 and 48h after inoculation.

Extraction and determination of PCP

Aliquots of 1ml of the bacterial suspension and soil solution were mixed with 1ml of methanol (v/v) and then centrifuged (6,000g x 3min). High performance liquid chromatography analysis (HPLC) was carried out using a binary pump system (Spectra system P200, Thermo Separation products), an automated sample injector (AS 3000), and an Alltech C18 column (250mm x 4.6mm (SN: 1456-98)). The

detection was carried out at 210nm (spectrum system UV 1000). The mobile phase was 70/30 (acetonitrile with 0.1% acetic acid water/methanol). PCP concentrations were calculated on the basis of peak area measurements by comparison with external standards of pure PCP prepared with methanol. All experiments were carried out using triplicate samples.

Statistical analysis

Statistically significant differences between treatments were evaluated by T-test for batch culture experiments and two-way analysis of variance (ANOVA) by General Linear Model for soil experiments, using a Minitab version 13.1 software package for Windows 98. The significance levels were quoted at a 95% confidence level (P= 0.05).

Results

Growth of *S. chlorophenolica* ATCC 39723 in the absence and presence of PCP

Table 3 shows the growth rates of *S. chlorophenolica* grown in the minimal basal medium with rhizosphere (MSM+RE) or without rhizosphere (MSM) exudates in the presence and absence of PCP. In the absence of PCP, significantly higher growth rates were obtained when compared to the cells grown in the presence of PCP (P≤0.05). The growth rates obtained in the absence of PCP were 0.91 and 0.83h^{-1} for MSM and MSM+RE, respectively. A decline in the growth rate was noted as the PCP concentration increased. In the presence of PCP, a significantly greater growth rate was obtained at 20mg L^{-1} PCP in MSM medium, but for the medium amended with rhizosphere exudates (MSM+RE), the highest growth rate was at the 80mg L^{-1} level of PCP (P≤0.05). At the lowest concentration (20mg L^{-1} PCP), a significantly greater growth rate (P≤0.05) was obtained in MSM (0.61 h^{-1}) than in MSM+RE (0.44 h^{-1}). At concentrations of 50 and 80 mg l^{-1} PCP, significantly greater growth rates were obtained in MSM+RE (P≤0.05). As the concentration increased (100 mg L^{-1} PCP), a significantly greater growth rate in MSM was observed when compared to MSM+RE (P≤0.05). The lowest growth rate was obtained at the highest concentration (150mg L^{-1} PCP) and the growth rates were similar in both media (P³0.05). In the absence of glutamate (control) there was no growth at all.

TABLE 3: Growth rates (h^{-1}) of *S. chlorophenolica* grown in MSM and MSM+RE in the presence and absence of PCP. Standard deviation in parentheses.

PCP concentration (mg L^{-1})	MSM (h^{-1})	MSM+RE (h^{-1})
0	0.83 (±0.04) *	0.91 (±0.07) *
20	0.61 (±0.02) *	0.44 (±0.02)
50	0.39 (±0.04)	0.55 (±0.03) *
80	0.49 (±0.02)	0.63 (±0.04) *
100	0.51 (±0.01) *	0.35 (±0.01)
150	0.24 (±0.01)	0.23 (±0.01)

* Significantly different statiscally (P£0.05).

PCP degradation experiments in batch culture

Table 4 shows the specific degradation rates at different PCP concentrations. In MSM, the highest specific degradation rate was obtained at 80 and 100mg L^{-1} PCP (P≤0.05). In MSM+RE medium, the highest specific degradation rate was obtained at 80 (P≤0.05). At concentrations of 80mg L^{-1} PCP, degradation was significantly more effective in the presence of rhizosphere exudate (MSM+RE) than in MSM ((P≤0.05). At the lowest concentration (20mg L^{-1} PCP) and at higher concentrations (100 and 150mg L^{-1} PCP), degradation was shown to be more effective in MSM than in MSM+RE (P≤0.05). It was noted that the specific degradation rates decreased towards higher PCP concentrations in both media.

TABLE 4: Specific degradation rates (pmol PCP/nev cfu/h) in MSM and MSM+RE media a different concentrations of PCP. Standar deviation in parentheses.

PCP concentration (mg L^{-1})	Specific degradation rates (pmol PCP/ new cfu/h)	
	MSM	MSM+ RE
20	0.7 (±0.2) *	0.05 (±0.01)
80	335 (±13.2)	465 (±13.0) *
100	313 (±2.0) *	17.8 (±0.3)
150	3.64 (±0.14) *	1.70(±0.43)

* Significantly different statiscally (P≤0.05).

PCP degradation in soil

Figure 1 shows the PCP degradation experiments in the loamy (Boyndie) soil in the presence and absence of winter wheat (*Triticum aestivum*). The PCP degradation was monitored for 3 weeks. In the presence of the inoculum alone (PCP+I), PCP had become degraded in only 2 weeks, and about 20mg kg^{-1} dry wt of PCP remained in the soil at the end of the experiment. In the presence of the plant alone, after 3 weeks of the experiment, about 60mg kg^{-1} dry wt of PCP remained in the soil. When the soil was inoculated with *S. chlorophenolica* (PCP+I+P) in the presence of plant, PCP was degraded quickly during the first week, remaining at about 20mg kg^{-1} dry wt of PCP at the end of the experiment. With plant, soil and inoculum (PCP+I+P), the PCP degradation was significantly enhanced (P≤0.05) when compared with that of the plant alone (P). At the end of the experiment, about 20mg kg^{-1} dry wt of PCP remained in the case of the inoculum alone (PCP+I) and in the case of the inoculum and plant (PCP+I+P)($P^3$0.05).

Enumeration of *S. chlorophenolica* in soil and roots

Figure 2 shows the total cfu g^{-1}dry soil wt of *S. chlorophenolica* in Boyndie soil and roots. After 20 days, for the PCP, inoculum and plant (PCP+I+P), there was a significant increase of *S. chlorophenolica* in the roots when compared to those in the soil (P≤0.05).

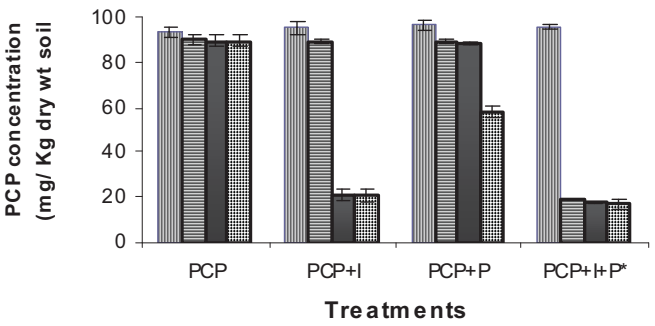

FIGURE 1: PCP degradation in a Loamy sandy soil containing initially 100mg kg^{-1} PCP under different treatments after 0 (||), 6 (=), 13 (■) and 20 days (♦). Mean of five replicates. Vertical bars indicate standard error of the mean. * Significantly different statistically (P≤0.05).

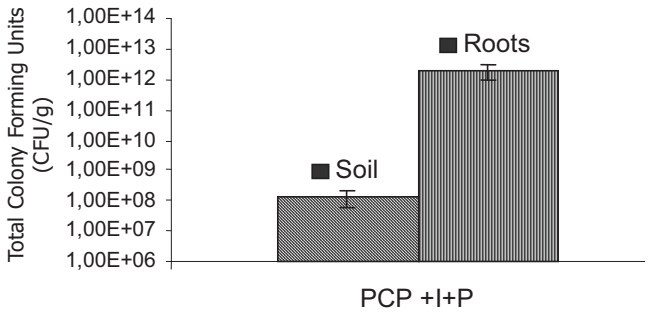

FIGURE 2: Total Colony Forming Units (cfu/g) of *S. chlorophenolica* in soil and roots after 20 days of experiment. Vertical bars indicate standard error of the mean.

Discussion

Sodium glutamate was added to a minimal medium as a carbon source to increase the rate of cell growth and PCP degradation. During the induction process, when a concentration of 50mg L^{-1} PCP was used, a higher growth rate was obtained in MSM+RE (0.55h^{-1}) than in MSM (0.39h^{-1}). Rhizosphere extracts were added to the minimal medium with an input of about 5.7mmol of carbon. The addition of a readily utilizable carbon source as glucose has been shown to stimulate the growth rate of *S.* (*Flavobacterium*) *chlorophenolica* (Topp and Hanson, 1990) when cells are grown under glucose, ammonium or sulphate limitation. The rhizosphere added to a minimal medium as MSM stimulates the viability of the cells of *S. chlorophenolica* at concentrations of 50 and 80mg L^{-1} PCP. In kinetic studies of PCP degradation, Gu and Korus (1995) obtained a specific growth rate of 0.12h^{-1} for *S. chlorophenolica* (*Flavobacterium* sp.)

when PCP concentration was about 140mg L^{-1}. In this present study, the presence of a carbon source as glutamate significantly stimulated the growth of *S. chlorophenolica*, since there was no growth in the absence of glutamate. When PCP was used as a sole source of carbon, Radehaus and Schmidt (1992) obtained a specific growth rate of 0.05 h^{-1} for *Pseudomonas* RA2 at 150mg L^{-1} PCP, and Edgehill and Finn (1982) obtained a maximum growth rate of 0.15h^{-1} at 135mg L^{-1} PCP for *Arthrobacter*.

The growth rates of cells of S. *chlorophenolica* grown on glutamate, without PCP, were higher when compared to the growth rates of cells grown on glutamate in the presence of PCP. The presence of glutamate greatly stimulated the growth of *S. chlorophenolica*. Topp et al. (1988) showed that when PCP-induced cells of *S. chlorophenolica* (*Flavobacterium*) were inoculated with 50mg L^{-1} PCP as the only carbon source, 90% of the cells lost their viability, although PCP had been degraded after 90h. Furthermore, Gu and Korus (1995) demonstrated that the cell death of *S. chlorophenolica* (*Flavobacterium*) was caused by PCP. In addition, Topp et al. (1988) suggested that an available carbon source could facilitate PCP removal by *S. chlorophenolica* (*Flavobacterium*) attenuating the toxicity of PCP. In this present study, it was observed that there was an adverse effect of PCP on growth of *S. chlorophenolica*. Similar effects have been noted by other researchers on the growth of *Pseudomonas* RA2 (Radehaus and Schmidt, 1992), *Arthrobacter* (Edgehill and Finn, 1982) and a PCP-degrading population in a mixed culture (Klecka and Maier, 1985).

An induction process was used to ensure activation of the enzymatic capability of *S. chlorophenolica* to degrade PCP. The degradation experiments showed the ability of induced cells of *S. chlorophenolica* to degrade PCP in batch culture. The highest specific degradation rate was obtained at 80mg L^{-1} PCP in both media, and it decreased considerably below 50 and above 100mg L^{-1} PCP. In kinetic studies of PCP degradation by *S. chlorophenolica* (*Flavobacterium* sp.), Gu and Korus (1995) observed that the maximum PCP degradation rate was obtained at 60mg L^{-1} PCP. Resnick and Chapman (1994) demonstrated that induced cells of *Pseudomonas* SR3 showed maximal rates of PCP removal at 100mg L^{-1} PCP, and degradation rates were lower at concentrations below 50 and above 150mg L^{-1} PCP. In the present study, it was noted that the specific degradation rates decreased as the PCP concentration increased. In the absence of rhizosphere exudates (MSM), the specific degradation rates decreased above 100mg L^{-1} PCP. In the presence of rhizosphere exudates (MSM+RE), the specific degradation rates decreased above 80mg L^{-1} PCP. Wittmann et al. (1998) also found that the degradation rates of *S. chlorophenolica* RA2 decreased towards higher PCP concentrations.

The degradation experiments with rhizosphere exudates showed that an induced cell suspension of *S. chlorophenolica* was able to degrade PCP. In a range of concentration of 20 to 80mg L^{-1} PCP, PCP was degraded in about 2-4h, while at higher concentrations (100 to 150mg L^{-1} PCP), PCP was degraded in about 12h. Using glucose as a supplementary source of carbon, Radehaus and Schmidt (1992) found that *Pseudomonas* RA2 can simultaneously mineralize glucose and PCP, but glucose had no effect on the kinetics of PCP mineralization. In our study, rhizosphere exudates had an effect on PCP degradation rates, especially at concentrations of 50 and 80mg L^{-1} PCP. At concentrations of 100 and 150mg L^{-1} PCP, degradation was more effective in the absence of rhizosphere exudates. The content of C in the rhizosphere extract was about 5.7mMol and about 21mMol from glutamate. There have been previous reports (Topp et al., 1988; Topp and Hanson, 1990) about the amendment of the basal medium with glucose or glutamate that enhanced PCP biodegradation by *S. chlorophenolica* (*Flavobacterium*). The minimal medium used in this study supplemented with rhizosphere, which contains readily assimilable organic substrates such as exudates, lysates and mucilage (Lynch and Whipps, 1990) and glutamate (Gu and Korus, 1995; Topp et al., 1988; Topp and Hanson, 1990), did enhance PCP biodegradation by *S. chlorophenolica* at lower concentrations.

The inoculated loamy sandy (Boyndie) soil with plant showed a faster PCP degradation (1 week) against 2 weeks in the inoculated soil without plant. At the end of

the experiment the same amount of PCP (20mg kg soil^{-1}) remained in the soil in both treatments. The ability of *S. chlorophenolica* to degrade PCP in soil was demonstrated by Crawford and Mohn (1985) where PCP (£100mg L^{-1}) was mineralized within one week of application in loam, clay and sandy soil. In the present study, the introduction of *S. chlorophenolica* into the loamy soil with plant significantly increased PCP degradation in comparison to non-inoculated soil without plant. There are several reports of the rhizoremediation of contaminated soils with organic compounds such as herbicides (Anderson et al., 1994), 2,5-dichlorobenzoate (Crowley et al., 1996), trichloroethylene (Walton and Anderson, 1990), 2,4,5-tri chlorophenoxyacetic acid (Boyle and Shann, 1998) and polycyclic aromatic hydrocarbons, such as pyrene (Liste and Alexander, 2000). In this study, an enhancement of PCP degradation was observed in the inoculated loamy soil with plant. In the non-inoculated loamy soil with plant, about 60mg kg soil^{-1} of PCP remained in the soil, against 20mg kg soil^{-1} in the inoculated soil with plant. Plants acted as a vector, enabling the degrader to reach its target: PCP. Although the plant-bacteria interactions in contaminated soils are not well understood, Walton and Anderson (1990) postulated that the root-soil-microbe combination has evolved to the mutual benefit of many plant and microbial species.

The population numbers of *S. chlorophenolica* were higher in the roots when compared with the soil, suggesting a stimulatory effect of the rhizosphere on the microbial growth. Grayston et al. (1998) observed a stimulatory effect of the rhizosphere on microbial growth, particularly in the pseudomonad proliferation. The microbial growth in the rhizosphere is stimulated by the utilization of rhizodeposition products by the microbial population (Lynch and Whipps, 1990). Haby and Crowley (1996) have suggested it that rhizodeposition may enhance the population numbers of 3-chloro-benzoate degraders by cometabolic growth or linked metabolism. The survival and activity of indigenous soil microorganisms and inoculated organisms in the rhizosphere soil has been well reported in the literature. Reynolds et al. (1999) noticed higher microbial numbers of indigenous population degraders of petroleum hydrocarbons in the rhizosphere of Bahia grass, in the vegetated rather than in the non-vegetated soil. Also, Alvey and Crowley (1996) observed a higher population number of an atrazine-mineralizing bacterial consortium in a maize planted soil when compared to the unplanted soil.

Our study showed that rhizosphere extract stimulated the growth of *S. chlorophenolica* ATCC 39723 at lower concentrations but not at higher concentrations. The rhizosphere exudates had a significant effect on the ability of *S. chlorophenolica* ATCC 39723 to degrade PCP at a concentration of 80mg L^{-1}PCP in batch culture. In addition, PCP had an adverse effect on the growth of *S. chlorophenolica* ATCC 39723 as the concentration increased. In the soil experiments, it has been demonstrated that the PCP degradation is enhanced in the rhizosphere. The plant acted as a vector to the inoculum in order to reach the target compound PCP, with an increase of population number in the rhizosphere, although this relationship between soil organisms, plant and soil is not completely clear.

This study was conducted under favorable laboratory conditions and these results indicate the potential for a treatment process under environmental conditions such as the use of soil systems. The success of bioaugmentation will depend on the competitiveness and activity of the specific cultures when exposed to environmental conditions.

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