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Ecotoxicological Assessment and Effects of Physicochemical Factors on Biofilm Development in Groundwater Conditions

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A new concept in groundwater containment is the development of a biobarrier formed by the biostimulation of a microbial population and exopolysaccharides (EPS) production. Although the efficiency of biofilms to clog porous media and biotransform contaminants suggests their application in aquifers, work on groundwater conditions has to be performed to confirm the potential of this technology. To determine the feasibility and the ecotoxic potential of biobarrier development, a groundwater indigenous population was inoculated in semicontinuous reactors. The effects of aeration, carbon source, feeding rate, C:N:P ratio, calcium ions, and pentachlorophenol (PCP) on biomass, EPS, and biofilm production was studied by a statistical design. The ecotoxic effect of soluble microbial products (SMPs) was assessed using two biotests: the *Selenastrum capricornutum* and Microtox (*Vibrio fischeri*) tests. Results showed that the carbon source type and its feeding rate had a significant impact on biofilm development leading to a maximum biofilm thickness of 250 μm after only 144 h. The ecotoxicological assessment revealed that *S. capricornutum* growth inhibition reached 80% while 90% of *V. fischeri* bioluminescence was affected by the presence of SMPs. Also, a toxic response was induced by the presence of PCP. These results showed the potential use of an indigenous microbial population and suggested that the ecotoxic potential has to be taken into account for in situ biobarrier development.

Introduction

Microbial barriers formed with microorganisms and exopolymeric matter produced in an aquifer offer an excellent potential as a means to prevent the spreading of a contaminant plume (*1, 2*). The indigenous microbial population of groundwater usually contains exopolysaccharide (EPS) producers, which could be stimulated to produce such compounds by injection of nutrients and electron acceptors.

The physicochemical conditions will affect the microorganisms' growth, EPS production, and biofilm density and thickness in the aquifer (*3*). Very few experiments have been performed on biobarrier development at groundwater temperature in darkness. When dealing with a mixed microbial population, the use of in situ temperature (10 °C in Québec) is important in order to promote the development of the appropriate class of microorganisms. Psychrophilic and psychrotrophic organisms have an optimum enzyme production and transport system at low temperature (*4*). The transport of microorganisms from the bulk to the porous or fractured media is a key step for biobarrier development (*3*).

In situ application of a biobarrier for the control of groundwater movement has not been reported in the literature. The concept of the biobarrier has been studied on lab-scale under two approaches: (i) a bioaugmentation with either a pure culture or a microbial consortium and (ii) a biostimulation of the indigenous population. Bioaugmentation was achieved by injecting starved cells in a porous media followed by nutrient stimulation, resulting in production of a plugging biofilm (*5*). Such an approach has been studied on small scale models (one-dimensional glass beads or sandstone cores) and larger scale models (three-dimensional reservoirs and natural reservoir cores) using ultramicrobacteria (UMBs) formed from oil-well water isolates (*6*). Results showed that when the matrix was flooded with nutrients, injected UMBs penetrated and returned to their full vegetative size and their normal level of EPS production. The permeability of the matrix was reduced by at least 99% within 30 days.

Biostimulation for lab-scale biobarrier development is achieved by stimulating indigenous bacteria to produce EPS, which eventually will clog the pore space. Flow-through experiments were developed for continuous recirculation of natural groundwater (*1*). Results showed that 1-L gravel and sand columns were biofouled up to 95% in about 90 days for gravel and 21 days for sand at room temperature. Another laboratory study used aquifer material contaminated by BTEX (benzene, toluene, ethylbenzene, and xylenes) to measure the production of EPS and the effect on hydraulic conductivity in the presence of different electron acceptors and carbon sources. Columns studies (0.5 m × 80 mm) showed that atmospheric O_2 resulted in the largest production of EPS while the presence of H_2O_2 limited the production of EPS. Hydraulic conductivity decreased from an initial value of 0.33 to 0.023 m/day after 390 h (*7*).

Ecotoxicological assessments are increasingly used in conjunction with site remediation technologies to ensure that soil and groundwater matrixes are not only decontaminated but also detoxified (*8*). The presence of intermediate degradation products and the formation of microbial compounds such as soluble microbial products (SMPs) is also believed to increase the ecotoxic potential (*9, 10*). An ecotoxicological assessment of SMPs has not yet been conducted in the prospect of in situ biobarrier as a containment technology.

The objectives of this study were first to determine physicochemical conditions for the formation of biofilm on a ceramic in groundwater using an indigenous microbial population at 10 °C in darkness and, second, to assess the ecotoxic potential of the biofilm and SMPs produced.

Methodology

Natural and Synthetic Groundwater. Noncontaminated natural groundwater was obtained from an observation well (Centre Environnemental Saint-Michel, Montreal, Canada). A purge of 3 well volumes was conducted before water samples were collected. The groundwater sample provided

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TABLE 1. Physicochemical Conditions for the Optimization of Biofilm Development on a Porous Media in Synthetic Groundwater

condition	designation	level (−)	level (+)
carbon source feeding rate	A	2 mg m^{-2} min^{-1}	20 mg m^{-2} min^{-1}
aeration	B	agitation 200 rpm	sparging 4.2 cm^3/s
carbon source	C	saccharose	molasses
PCP concn	D	0 mg/L	10 mg/L
calcium ion concn	E	0 mg/L	100 mg/L
C:N:P ratio	F	50:10:1	200:10:1

the biomass used for biofilm development. Also, a chemical analysis of the groundwater was performed to prepare a synthetic groundwater with the following composition (mg/L): Na_2CO_3 (679), NaCl (617), $MgSO_4$ (19), $CaCO_3$ (15), K_2HPO_4 (11), KNO_3 (6), and $FeCl_2$ (1) (pH ≅ 8.3). The synthetic water was sterilized at 121 °C and 103.4 kPa (20 min).

Microbial Enrichment. Indigenous microorganisms were cultured in an Erlenmeyer flask (1 L) enriched with black strap molasses (5 g/L) (West Indies Molasses, Montreal, Canada) shaken at 200 rpm and placed at 10 °C in the darkness until the microbial concentration reached 10^7 heterotrophic microorganisms/mL. Heterotrophic microorganisms concentration was determined by the most probable number (MPN) method (*11*). Carbohydrate concentration was determined by the phenol−sulfuric acid method (*12*). The culture was maintained at −20 °C with glycerol (15 g/L) before utilization (*13*). Stock cultures were resuspended in fresh synthetic groundwater (1 mL of culture/500 mL of synthetic groundwater).

Biofilm Development Apparatus. Experiments were conducted in semicontinuous reactors (1 L) to which a ceramic coupon (2.5 × 15 cm) was attached at the top (*13*). This simple system was chosen to achieve a large number of physicochemical conditions, to duplicate, and also to measure the effects of byproducts accumulation on biomass, EPS, and biofilm development. Ceramic was used to simulate a rock surface (Ceramica Industriale, Casalgrande, Italy). Its geochemical composition was 62.6% SiO_2, 0.7% TiO_2, 18.9% AlO_3, 1.3% CaO, 2.9% MgO, 1.9% Na_2O, 4.0% K_2O, and 7.0% Fe_2O_3. The reactors were placed at 10 °C in the darkness to represent aquifer conditions and to prevent photolysis of PCP (*14*). Reactors were filled with synthetic groundwater (500 mL) and inoculated with 1 mL of the microbial culture. Air bubbles were isolated from the ceramic coupon to prevent biofilm sloughing. The physicochemical conditions tested are presented in Table 1.

Carbon Sources, Ions Enrichment, and Model Contaminant. Two carbon sources were tested: saccharose and black strap molasses (Sucre Lantic and West Indies Molasses, Montreal, Canada). The carbon source was fed once a day with a syringe at a rate of 20 mL/d [concentrations 4.09 and 40.9 g/L for levels (−) and (+), respectively]. Since divalent ions are known to improve biofilm texture, calcium chloride solution (100 mg/L) was injected at the beginning of the test (*1*). Sodium pentachlorophenate (NaPCP, 10 mg/L) was added to measure the effect of a soluble contamination on biofilm development. Since many studies have shown that intensive use of PCP as a herbicide, as an insecticide, and for the protection and preservation of wood products, this compound was chosen as a model to measure the effect of such a contamination on biofilm development (*14*).

Determination of the Physicochemical Conditions Effects. A Plackett−Burman statistical design was used to determine the significant effects of physicochemical conditions on biomass growth and biofilm development (*15*) (Table 1). Each of the six physicochemical conditions tested was maintained at two levels, those being (−) and (+). The complete experimental design was divided in two sets of eight trials: a basic design and a reflected design (Table 2).

TABLE 2. Eight-Run Basic[a] Experimental Design To Determine the Effects of Physicochemical Conditions on Biomass Growth and Biofilm Development[b]

	conditions					
trials	A	B	C	D	E	F
1	−	−	−	−	−	−
2	−	−	−	+	+	+
3	−	+	+	+	+	−
4	−	+	+	−	−	+
5	+	+	−	−	+	+
6	+	+	−	+	−	−
7	+	−	+	+	−	+
8	+	−	+	−	+	−

[a] In the eight-run reflected design, levels are interchanged from (−) to (+) and vice-versa. [b] The combination of both statistical designs (16 runs) yields a resolution IV design. Main effects are separated from two-factor interactions.

In the reflected block, levels (−) became (+) and vice-versa. Each trial was performed in duplicate for a total of 32 reactors.

The physicochemical effects were measured on six variables: suspended biomass, suspended EPS, biofilm thickness, pH, oxydoreduction potential (ORP), and carbon source concentration. A volume of 20 mL of the inoculated groundwater was sampled everyday over 192 h. After this time, PCP residual concentration was determined by high-performance liquid chromatography (HPLC) (*16*). Biomass and EPS concentrations were determined on a dry weight basis as described by Hacking et al. (*17*). Biofilm thickness was evaluated by microscopy with correction for light refraction (*18*). Specific probes were used to measure pH and ORP (*11, 19*). The phenol−sulfuric acid method was used to measure carbohydrate concentration (*12*).

Evaluation of SMP Production. Chemical oxygen demand (COD) tests were conducted to evaluate the production of SMPs by a closed reflux colorimetric method (*20, 11*). $COD_{particulate}$ was obtained by subtracting the $COD_{filterable}$ from the COD_{total}. $COD_{particulate}$ came from suspended biomass and EPS. $COD_{filterable}$ came from SMPs, PCP, and the carbon source. $COD_{filterable}$ was obtained from filtered culture sample (Hydrophilic PVDF filters of 0.45 μm, Millipore Millex-HV, Bedford, MA).

The concentration of SMP (COD_{SMP}) was obtained by subtracting the COD of soluble components from the filtered COD (eq 1).

$$COD_{SMP} = COD_{filterable} - (COD_{molasses} + COD_{PCP}) \qquad (1)$$

The COD of molasses and of PCP were calculated theoretically using 0.5863 g of COD/g for molasses and 0.499 g of COD/g for Na−PCP. SMPs were assumed to include compounds resulting from the bacterial metabolism and intermediate compounds of PCP degradation.

Ecotoxicological Assessment. Evaluation of the ecotoxic potential was conducted on synthetic groundwater and biofilm sampled from the reactors with molasses fed at 20 mg m^{-2} min^{-1}, sparging aeration (4.2 cm^3/s), calcium ions (100 mg/L), and a C:N:P ratio of 50:10:1. Biofilm samples

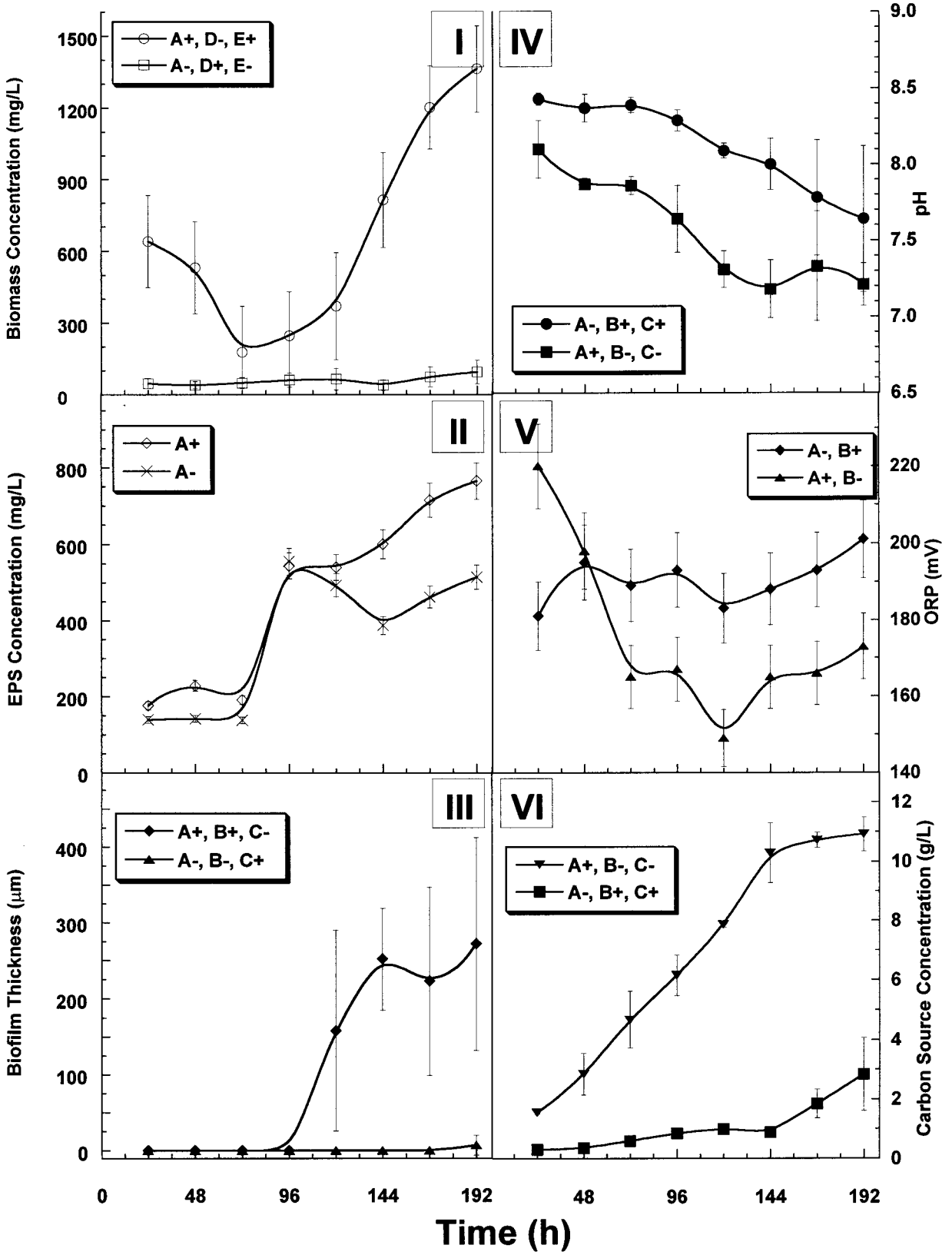

FIGURE 1. Microbial growth and biofilm development in groundwater conditions in semicontinuously fed reactors. Conditions not statistically different were grouped together (α = 95%). Table 1 describes conditions tested.

were collected from the ceramic coupons after they were placed in 500 mL of saline solution (0.85% NaCl) and sonicated for 2 min at minimal power (*21*). Growth inhibition of the green algae *Selenastrum capricornutum* was measured as described in the method suggested by Environnement Canada (*22*). The statistical method used was an interpolated linear method with a confidence coefficient of $\alpha = 95\%$. Due to the large variations in inhibition results obtained from the diluted samples, only the nondiluted samples (100% v/v) were considered. Results of the algal growth inhibition test were expressed in percentage. The bioluminescence inhibition of *Vibrio fischeri* test (Microtox) was conducted according

TABLE 3. Significant Effects of Physicochemical Factors on Microbial Production Variables over 192 hs (α = 95%)

variables	conditions	effect[a] (%)	$p > F$
suspended biomass concn	A	$+77.7 \pm 8.7$	0.0001
	D	-21.8 ± 8.7	0.0131
	E	$+17.2 \pm 8.7$	0.0500
suspended EPS concn	A	$+16.3 \pm 6.2$	0.0094
biofilm thickness	A	$+90.4 \pm 14.0$	0.0001
	B	$+69.9 \pm 14.0$	0.0001
	C	$+69.2 \pm 14.0$	0.0001

[a] Signs + and − are related to the condition effect on the variable when level (−) is changed for level (+).

to the method suggested by Le Bureau de normalization du Québec (*23*). Results are given as sample concentrations needed to inhibit the bioluminescence of 20% and 50% of a *Vibrio fischeri* population (IC_{20} and IC_{50}).

Results and Discussion

Effects of Physicochemical Conditions on Microbial Growth and Biofilm Development. The physicochemical conditions studied significantly influenced the microbial growth and biofilm development. The minimum and maximum effects of the significant conditions on biomass concentration, EPS concentration, biofilm thickness, pH, ORP, and carbon source concentration over 192 h are shown in Figure 1. This implies that all the results obtained with the Plackett−Burman experimental design were between those minima and maxima. The growth of indigenous groundwater microorganisms showed a typical batch growth curve (*3*) (Figure 1-I). The suspended EPS concentration curve resembled a logistic curve such as those obtained in batch cultures, and the biofilm thickness increased following a sigmoidal curve (Figure 1-II and 1-III) (*3*). The global effects of the physicochemical conditions on microbial production over 192 h are given in Table 3. These results, expressed in percentages, show the effect on suspended biomass, suspended EPS, and biofilm thickness of changing the condition level from (−) to (+) (α = 95%) (Table 1).

The type of carbon source and the feeding rate had the most significant effect on biomass growth, EPS production, and biofilm development (Table 3). Biofilms, when molasses was used as the carbon source, were 70% thicker than biofilms developed in saccharose-fed reactors (Table 3) ($p = 0.0001$). The carbon source played several roles in the system. First, it promoted the growth of the microbial population and conditioned the ceramic surface before cell attachment, which is a key step in biofilm development (*24*). The molasses composition probably contributed to an effective conditioning of the ceramic. Inorganic salts (≅8%) and amino acids (≅2%) in molasses could adsorb onto ceramic by ionic interactions (*3*). Second, divalent ions in molasses (≅3%) could have promoted the irreversible cell adhesion onto the ceramic (*25*). Third, the molasses offered more interactions between cells, EPS, and the surface with hydrogen, hydrophobic, and ionic bonding (*26*, *27*). Saccharose, which contained less than 0.02% of ashes and no trace of amino acids, was probably not effective in conditioning the ceramic surface and promoting cell attachment. Although some bacteria are stimulated to produce more EPS in nitrogen starvation condition, it has been shown that the response was not a general rule (*28*). That could explain the finding that no significant effect was measured between C:N:P ratios of 50:10:1 and 200:10:1 ($p > 0.05$).

A high carbon source feeding rate (20 mg m^{-2} min^{-1}) resulted in the production of 78% more suspended biomass than at low rate (2 mg m^{-2} min^{-1}) on average during the 192 h ($p = 0.0001$). This large production of biomass also contributed to a decrease in pH due to the production of organic acids (Figure 1-IV). Also, carbon sources accumulated in the water at concentrations up to 10.9 g/L after 192 h (Figure 1-VI). Meanwhile, the production of suspended EPS and the development of the biofilm on ceramic at 20 mg m^{-2} min^{-1} were 16% and 90%, respectively, more important than at 2 mg m^{-2} min^{-1} ($p = 0.0094$ and 0.0001, respectively). ORP was higher (195 mV in average) at a low feeding rate and when air was sparged rather than dissolved by agitation (Figure 1-V). The effect of a high substrate loading rate is well-known to contribute to the formation of a thick and uniform biofilm (*29*). But under such conditions, the sloughing of the biofilm into the bulk could be more important than with a low feeding rate. Such biofilm detachment explains in part the increase in the suspended EPS concentration between 144 and 156 h, which corresponded to a noticeable decrease in biofilm thickness (Figure 1).

The type of aeration system used in the reactors influenced the dissolved oxygen concentration and water turbulence. On average, air sparging resulted in a biofilm 70% thicker than with agitation at 200 rpm (Table 3) ($p = 0.0001$). However, a closer analysis of the biofilm development revealed that a biofilm was visible after 24 h with agitated water (1.5 μm) as compared to 96 h with air sparging (1.1 μm). In addition, the suspended biomass and EPS concentrations were not significantly affected by the type of aeration ($p > 0.05$). Those results indicate that the dissolved oxygen concentration was not a limiting factor in the reactor but the water turbulence would have played a key role in the development of the biofilm. It is well-known that transport controls the rate of initial deposition on the surface (*30*). A possible explanation for the longer lag period for biofilm development with air sparging is the increase in the hydrodynamic forces. A visual observation of the system under air sparging and agitation showed a difference in the water velocity. Such difference could have improved the lift force that generated a normal force to the ceramic. As reported by Characklis and Marshall (*3*, *30*), the desorption of cells weakly adsorbed to the ceramic could have been greater when the water velocity increased. In the same way, the overall thicker biofilm with air sparging could be explained in part by an increase in downsweep forces. After the conditioning and the irreversible adsorption of cells on the ceramic, greater drag and lift forces would improve the mass transfer from the bulk into the biofilm leading to a thicker film (*3*). Moreover, Marshall (*31*) reported that an increase in water turbulence led to a thicker biofilm due to (i) a preferential attachment by certain species, (ii) a microbial physiological response to environmental stress, and (iii) the squeezing of loosely bound water from the film by the fluid pressure force. The fact that the oxygen concentration was not limiting to the growth of the indigenous microorganisms could be that mixed microbial population from groundwater usually contains a large fraction of facultative aerobic bacteria (*32*).

Enrichment with calcium chloride (100 mg/L) contributed to an increase in the microbial population ($p = 0.0131$), which suggested that the number of microorganisms in the biofilm also increased (*33*). The concentration of calcium ions in the synthetic groundwater was probably not optimum for the development of the suspended biomass. An increase in the microbial population in biofilms is associated with a densification of the biofilm rather than an increase in thickness (*34*). The biofilm mass was not measured, but it would not have been surprising that it had increased with the calcium enrichment due to a higher biomass content and EPS gelation (*35*).

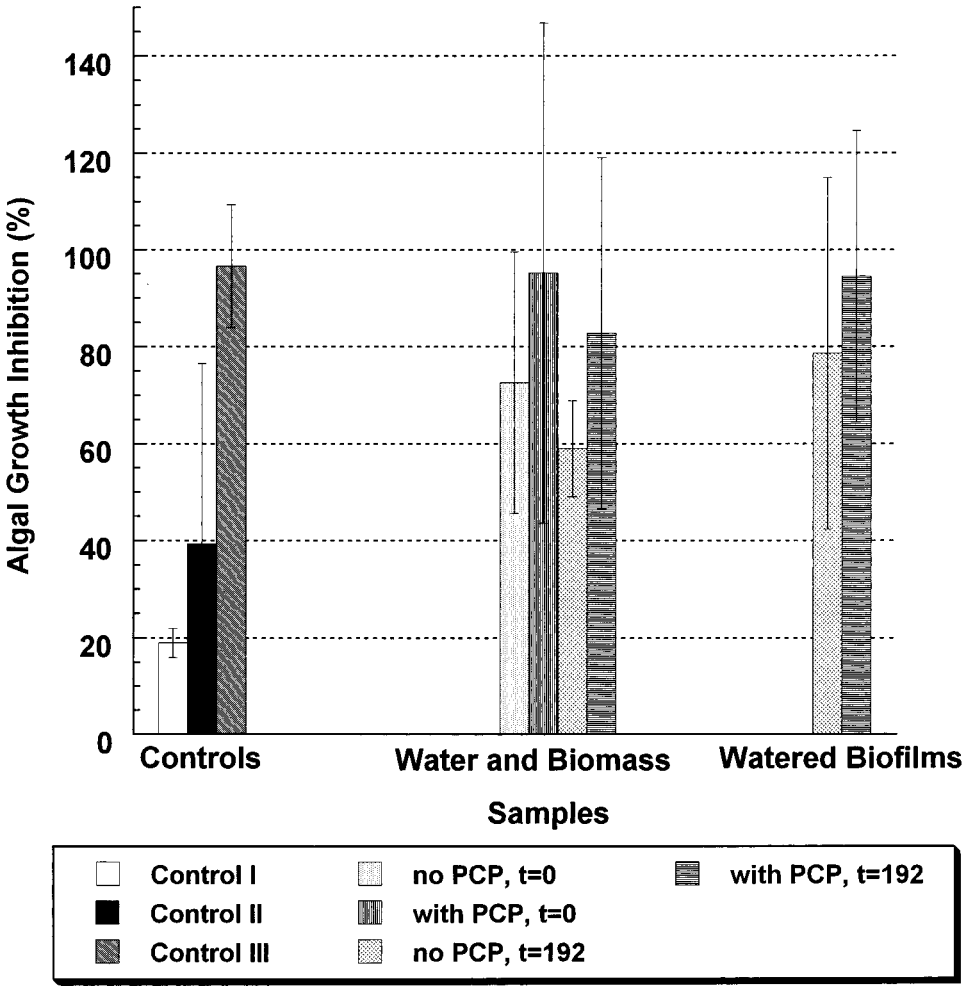

FIGURE 2. Growth inhibition of *Selenastrum capricornutum* submitted to different synthetic groundwater and biofilm samples. Physicochemical conditions in the reactor were molasses fed at of 20 mg m^{-2} min^{-1}, sparging aeration (4.2 cm^3/s), addition of calcium ions (100 mg/L) and a C:N:P ratio of 50:10:1. Controls: I, synthetic groundwater; II, synthetic groundwater and molasses (3.2 mg/L); III, saline solution (0.85%).

PCP addition (10 mg/L), to simulate groundwater contamination, affected microbial growth by decreasing the population on average by 22% (Table 3) ($p = 0.0131$). A decrease in PCP concentration from 10 mg/L down to zero was also observed in some cases, which indicated the presence of PCP degraders. Biodegradation of PCP either by a microbial consortium from a PCP-contaminated soil or a biofilm developed with pure culture bacteria has been reported at high concentration such as 500 and 366 mg/L, respectively, with lab-scale experiments (*36, 37*). The 10 mg/L PCP addition did not influence significantly either EPS production or biofilm thickness over 192 h ($p > 0.05$). No assumptions of the biofilm fixed biomass viability can be made because of the resistance mechanisms for penetration of toxic substances such as PCP. Those mechanisms include EPS binding, chemical reaction with EPS, uptake of the PCP by cells, and catalytic detoxification of PCP in the biofilm (*38*).

SMPs Accumulation and Ecotoxicological Assessment. The green algae (*S. capricornutum*) and Microtox (*V. fischeri*) tests are known to be very sensitive, and they are widely used for water toxicity assessment (*39, 40*). The algal growth inhibition results, when submitted to non diluted groundwater samples at different period of the microbial development, are shown in Figure 2. The Microtox test results are presented as the concentration of the sample needed to inhibit 20% and 50% of the bioluminescence (IC_{20} and IC_{50}). IC_{20} is considered as the threshold of detection, and IC_{50} is the reference measurement used in ecotoxicology (Table 4). As the presence of SMPs in water was suspected to increase the toxicity, COD was measured to evaluate the production over 192 h. Controls I and II, which consisted of synthetic groundwater with and without molasses (3.2 mg/L) respectively, were moderately toxic (algae growth inhibition = 39.3% and Microtox $IC_{20,15\ min} = 45.1\%$) (Figure 2 and Table 4). Addition of the microbial indigenous culture to synthetic groundwater and molasses did not significantly affect bioluminescence of *V. fischeri*, but *S. capricornutum* growth was inhibited to 72.6% (Table 4 and Figure 2).

Selenastrum capricornutum growth showed an inhibition of up to 80.4% on average when algae were exposed to inoculated synthetic groundwater or saline watered biofilm samples, whether or not PCP (10 mg/L) was present (Figure 2). Standard deviations were so wide that no significant difference was detected between the inhibition results of groundwater and saline-watered biofilm samples ($\alpha = 95\%$). Competition for nutrients between microorganisms and algae could explain the inhibitory effect on algae growth when the microbial population was added. The presence of SMPs after 192 h, especially abundant when PCP was added to water ($COD_{SMP} = 8427$ mg of O_2/L), did not affect more the growth of the algae (Figure 2). SMPs might have provided nutrients for algae growth (*41*). Results also showed that control III (0.85% NaCl) had a repression effect on algal growth. The toxicity of the saline solution on the green algae probably interfered with the possible ecotoxic effect of biofilm dispersed in the solution.

TABLE 4. Bioluminescence Inhibition of *Vibrio fischeri* Exposed to Water and Biofilm Samples for Different Inhibition Concentrations and Time Exposures[a]

sample		$IC_{20} \pm CC$ (%)		$IC_{50} \pm CC$ (%)	
		5 min	15 min	5 min	15 min
controls	I	>49.5	>49.5	>49.5	>49.5
	II	47.4 ± 0.6	45.1 ± 10.4	>49.5	>49.5
groundwater & microbial population	no PCP, $t = 0$	45.3 ± 14.8	41.8 ± 1.5	>49.5	>49.5
	with PCP, $t = 0$	4.5 ± 0.8	2.9 ± 0.3	13.3 ± 1.2	8.4 ± 0.9
	no PCP, $t = 192$ h	21.3 ± 0.7	20.4 ± 2.7	42.5 ± 3.7	30.8 ± 1.4
	with PCP, $t = 192$ h	5.2 ± 0.7	4.2 ± 0.8	15.6 ± 1.2	11.0 ± 1.2
biofilms	no PCP, $t = 192$ h	>49.5	>49.5	>49.5	>49.5
	with PCP, $t = 192$ h	>49.5	>49.5	>49.5	>49.5

[a] IC, inhibition concentration; CC, confidence coefficient; control I, synthetic groundwater; control II, synthetic groundwater and molasses (4.09 mg/L). Physicochemical conditions in the reactor were molasses as carbon source fed at a rate of 20 mg m^{-2} min^{-1}, sparging aeration (4.2 cm^3/s), addition of calcium ions (100 mg/L), and a C:N:P ratio of 50:10:1.

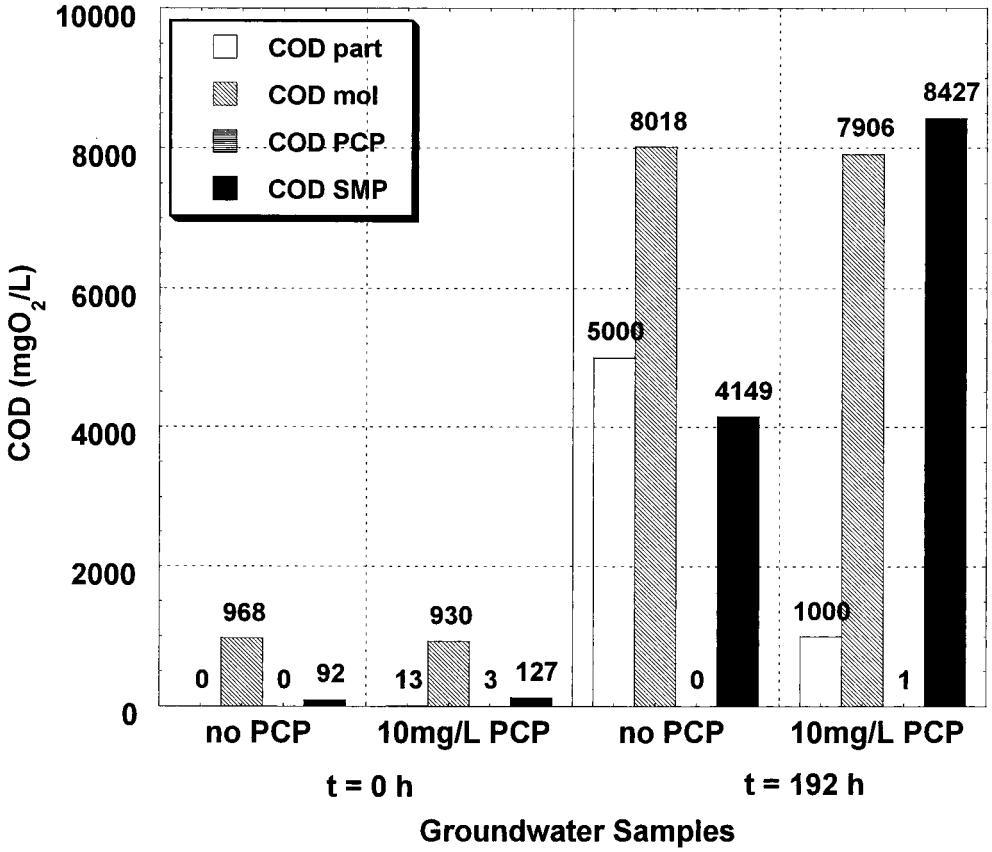

FIGURE 3. COD values of culture constituents for the evaluation of SMP production. Physicochemical conditions in the reactor were molasses fed at of 20 mg m^{-2} min^{-1}, sparging aeration (4.2 cm^3/s), addition of calcium ions (100 mg/L), and a C:N:P ratio of 50:10:1.

Microtox IC_{20} results revealed that addition of PCP to the culture ($t = 0$ h) allowed IC_{20} to decrease by 12.2-fold (Table 4). Even if the PCP concentration considerably decreased between 0 and 192 h (from 10.0 to 1.6 mg/L), bioluminescence inhibition was not significantly different. Phenolic compounds are known to disrupt cell membranes, resulting in the leakage in solution of nucleic material, proteins, and other organic material (*42*). The intermediary metabolites of PCP degradation, the release of endotoxins, and the lysis of bacteria are also SMPs that may have contributed to the inhibition of *V. fischeri* bioluminescence (Figure 3). Cultivation in a semibatch reactor under aerobic conditions is known to promote the accumulation of SMPs (*10*). Moreover, cultivation with a high concentration of glucose results in the release of organic compounds into the medium (*43*). In water with no PCP addition, the COD_{SMP} increased from 92 to 4149 mg/L in 192 h. Such production might explain the fact that bioluminescence of *V. fischeri* was reduced by half when exposed to the culture at times 0 and 192 h (Table 4). Biofilms that were detached from the ceramic in a saline solution were nontoxic to *V. fischeri,* possibly because the organic compounds were too diluted.

In conclusion, the stimulation of an indigenous microbial population to produce EPS and biofilms was effective in groundwater conditions, at 10 °C in the darkness. In this oligotrophic milieu, the type of carbon source and the feeding rate significantly influenced the speed and the magnitude of biofilm development, suggesting a large contribution of ionic, hydrophobic, and hydrogen bonding interactions between the solid surface, microbial cells, and EPS. The resistance of biofilm to the presence of PCP (10 mg/L) supports the concept of using a biobarrier for contaminated groundwater containment. The relative ecotoxicity revealed by the green algae growth and marine bacteria bioluminescence inhibition

tests indicated that compounds such as SMPs could possibly be adverse to the ecosystem if accumulated on a local basis. Future investigation on biobarrier development should focus on (i) fractured media hydrodynamics, (ii) accumulation and/or biodegradation of contaminants in the biobarrier, and (iii) comprehension of the ecological significance of SMPs production in groundwater environment.

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