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Remediation of phenol-contaminated soil by a bacterial consortium and *Acinetobacter calcoaceticus* isolated from an industrial wastewater treatment plant

S.M. Cordova-Rosa, R.I. Dams, E.V. Cordova-Rosa, M.R. Radetski, A.X.R. Corrêa, C.M. Radetski *

Universidade do Vale do Itajaí, Centro de Ciências Tecnológicas da Terra e do Mar, 88302-202, Itajaí, SC, Brazil

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ABSTRACT

Time-course performance of a phenol-degrading indigenous bacterial consortium, and of *Acinetobacter calcoaceticus* var. *anitratus*, isolated from an industrial coal wastewater treatment plant was evaluated. This bacterial consortium was able to survive in the presence of phenol concentrations as high as 1200 mg L^{-1} and the consortium was more fast in degrading phenol than a pure culture of the *A. calcoaceticus* strain. In a batch system, 86% of phenol biodegradation occurred in around 30 h at pH 6.0, while at pH 3.0, 95.2% of phenol biodegradation occurred in 8 h. A high phenol biodegradation (above 95%) by the mixed culture in a bioreactor was obtained in both continuous and batch systems, but when test was carried out in coke gasification wastewater, no biodegradation was observed after 10 days at pH 9–11 for both pure strain or the isolated consortium. An activated sludge with the same bacterial consortium characterized above was mixed with a textile sludge-contaminated soil with a phenol concentration of 19.48 mg kg^{-1}. After 20 days of bioaugmentation, the remanescent phenol concentration of the sludge-soil matrix was 1.13 mg kg^{-1}.

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1. Introduction

The ecotoxicity of phenols and related compounds means that they may interfere with the ecosystem equilibrium and consequently, affect biogeochemical pathways of organic matter and nutrient recycling. Thus, the intentional environmental release of these antimicrobial compounds should be avoided [1,2]. Industrial wastewater treatment plants usually employ microbial oxidative processes, and phenolic compounds can inhibit the microbial growth rate, even in the case of species which are capable of metabolically utilizing these compounds as a substrate for growth. In this sense, optimization of degradation condition is fundamental to use this bio-oxidative process in industrial and environmental applications.

Of the various techniques available for the removal of phenols, biodegradation is an environmentally friendly and cost-effective method and it has been extensively studied using pure and mixed cultures [3–6]. Many species have been isolated and characterized as phenol-degrading organisms [7,8], such as *Pseudomonas* species [9,10], *Serratia marcescens* [11], *Bacillus subtilis* [12], *Bacillus brevis* [7], and *Candida tropicalis* [13]. *Acinetobacter calcoaceticus* is a Gram-negative aerobic bacteria with a high ability to utilize phenol as the sole source of carbon and energy [14,15]. Thus, phenolic compounds can be degraded by acclimated activated sludge [16], by a microbial consortium in bioreactors [17], in fluidized bed reactors [5], and in fiber membrane bioreactors [4].

On the other hand, methodologies to treat contaminated soils must be cost-effective and environmental friendly. Among remediation methodologies, bioaugmentation is an attractive and innovative remediation technology [18]. An interesting application of bioaugmentation is the use of the microbes present in certain industrial wastewaters to treat contaminated soil matrixes [19]. Further than an investigation of the ability of a bacterial consortium, and *A. calcoaceticus* alone, to degrade phenol as the sole source of carbon and energy, the main objective of this study was to assess the applicability of this process in an environmental problem concerning soil remediation. Thus, an indigenous mixed culture obtained from an industrial coal wastewater treatment plant containing phenolic compounds was adapted under laboratory conditions and used as an inoculum in bioreactor degradation experiments. From the mixed culture, bacterial strains were isolated and tested for their individual phenol degradation ability. The results for the time-course performance of *A. calcoaceticus* and of the bacterial consortium in the degradation of phenol under different pH conditions and wastewater dilutions, are here presented. After this investigation, an environmental application of the activated sludge with the same bacterial consortium was carried out to assess the efficacy of this consortium in the remediation of a textile

* Corresponding author. Fax: +55 47 3341 7970.
E-mail address: radetski@univali.br (C.M. Radetski).

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sludge-contaminated soil containing phenolic compounds. Therefore, our investigation question was: introduced microorganisms can survive in a new environment and increase contaminant degradation?

2. Material and methods

2.1. Microorganism growth conditions

A bacterial consortium was obtained from a coal mine wastewater treatment plant containing phenolic compounds. The culture was adapted under laboratory conditions in batch reactors containing 100 mL of Trypticase Soy Broth (of 2.5 g L^{-1}) (TSB) (Merck, Germany) for 30 days. Aliquots were then taken (1 mL), filtered through a 0.45-μm Millipore filter, washed in distilled water and the bacterial suspension was denominated as the mixed culture used as the inoculum in the bioreactor degradation experiments.

2.2. Identification and isolation of bacterial strains from the mixed culture

For the isolation and identification of the bacterial strains, the method of dilution in Petri dishes was adopted [20]. The identification of the bacterial strains was carried out using the API 20 NE system (BioMérieux, France).

2.2.1. Bacterial strains

The organism *A. calcoaceticus* was isolated from coal mine wastewater containing a high concentration of phenolic compounds [20]. For the culture on a solid medium, a Trypticase Soy Broth (TSB) (Merck, Germany) was solidified by the addition of 1.5% (w/v) Agar (Oxoid, UK) and kept at 4 °C [20].

2.2.2. A. calcoaceticus growth conditions

Cells of *A. calcoaceticus* were grown in 250 mL flasks containing 100 mL of Trypticase Soy Broth (of 2.5 g L^{-1}) (TSB) (Merck, Germany) and 0.5 g L^{-1} phenol (for phenol metabolism induction) on an orbital shaker (200 rpm) at 25 °C, until the cell culture was in the mid-logarithmic phase of growth (OD = 0.5). Bacteria growth was monitored using a Cecil Instruments Spectrophotometer at 540 nm. In order to monitor phenol degradation in batch cultures, 1 mL of the medium was centrifuged (6000 × *g*, 3 min) and the absorbance was measured at 270 nm. When 80% of the phenol content had been degraded, the cells (1 mL) were harvested by centrifugation (6000 × *g*, 20 min), washed once in TSB without phenol, suspended in TSB without phenol and used as an inoculum for degradation experiments in a batch cultures (OD = 0.7).

2.3. Phenol biodegradation by a mixed culture in a bioreactor in a continuous and in a batch system

2.3.1. Continuous system

The reactor consisted of a 4.7-L acrylic tank with air injection, a filter and a peristaltic pump to supply the mineral salt medium (0.15 g L^{-1} NH_4Cl; 0.19 g L^{-1} $CaCl_2 \cdot 2H_2O$; 0.19 g L^{-1} NaCl; 0.4 g L^{-1} $KNaC_4H_4O_6 \cdot 4H_2O$; 0.75 g L^{-1} KH_2PO_4; 0.23 g L^{-1} $ZnSO_4 \cdot 7H_2O$; 0.6 g L^{-1} $NaNO_3$; 0.16 g L^{-1} $MgSO_4 \cdot 7H_2O$) [21]. Phenol (800–1200 mg L^{-1}) as the sole source of carbon and the inoculum (140 mL) at an initial flow velocity of 2 L day^{-1} (which was increased to 5 L day^{-1} after 25 days of the experiment) were added to the reactor. At the beginning, the work volume was 4.0 L and the temperature was 25 °C. The bacterial suspension was prepared using 140 mL of the inoculum (1600 mg L^{-1} dry weight biomass), tap water and mineral salt solution. The initial feed was maintained at 0.34 mL min^{-1} and increased by 10% per day until reach the final work volume of 4.7 L. Aliquots were withdrawn from the reactor for the determination of phenol concentration (1 mL) and biochemical oxygen demand (10 mL) and for the pH control. To quantify phenol volatilization, a control tank without bacteria inoculum was carried out in parallel.

2.3.2. Batch system

In the batch system the conditions were the same as in the continuous system. The inoculum used was the mixed culture withdrawn from the bioreactor. The peristaltic pump supplied the mineral salt medium (same composition as that described in Section 2.3.1), containing 500 mg L^{-1} of phenol and 20 mL of a nutrient solution of the following composition (15.76 g L^{-1} $(NH_4)_2SO_4$; 4.66 g L^{-1} $(NH_4)H_2PO_4$; 1.36 g L^{-1} KH_2PO_4) [21]. Aliquots (1 mL) were taken and phenol concentration determined. In order to verify the effect of pH on the velocity of biodegradation, in one experiment the mineral salt medium was neutralized using NaOH to give a pH of 6.0; while in another experiment the pH was maintained at 3.0. As mentioned above, control tanks without bacteria inoculum were carried out in parallel to assess phenol volatilization.

2.4. Phenol biodegradation by A. calcoaceticus and a mixed culture in a coke gasification wastewater

Phenol degradation by *A. calcoaceticus* or the mixed culture was tested in coke gasification wastewater from a ceramic industry containing high concentrations of phenolic compounds, including phenol, cresols and polyphenols. Twenty milliliters of the inoculum were added to the 500 mL Erlenmeyer flasks containing 200 mL of a non-sterilized 35, 18 and 12% (v/v), coke phenolic wastewater (diluted in Milli-Q water) and 2.5 g L^{-1} TSB, in triplicate. In order to check the biodegradation by the organisms in the coke phenolic wastewater, the control flasks received no inoculum. Flasks were kept on an orbital shaker at 25 °C for 24 h. Aliquots were removed for the determination of phenol (1 mL) and BOD concentration (10 mL), and for optical density (OD) measurements. Three non-inoculated controls were carried out to assess the participation of wastewater autochthonous flora in the phenol biodegradation.

2.5. Phenol biodegradation by A. calcoaceticus and a mixed culture with phenol as sole source of carbon

Phenol biodegradation by *A. calcoaceticus* or the mixed culture was tested using phenol as a sole carbon source. Twenty milliliters of each inoculum (*A. calcoaceticus* or the mixed culture) were added to five 500 mL Erlenmeyer flasks, each containing 200 mL of a nutrition medium made up of 400 mg L^{-1} phenol, 3.2 mL of nutrient solution (as described in Section 2.3.2) and 0.6 mL of mineral salt medium (as described above). To quantify phenol volatilization, flasks without bacteria inoculum were carried out in parallel. Flasks were kept on an orbital shaker at 25 °C for 24 h. Aliquots (1 mL) were removed for phenol concentration determination. The experiment was carried out in triplicate.

2.6. Phenol concentration determination

Aliquots (1 mL) of the bacterial suspension were filtered through a 0.45-μm Millipore FG filter and centrifuged (6000 × *g*, 3 min). Phenol content in the filtrated supernatant was quantified by high performance liquid chromatography (HPLC), using a binary pump system (Spectra system P200, Thermo Separation products), an automated sample injector (AS 3000) and a reverse-phase column (SUO with a length of 250 mm × 4.6 mm (SN: 1456-98). The

detection was carried out at 270 nm (Spectrum System UV 1000). The mobile phase was composed of methanol and water (1:1) [5]. Sludge-soil matrix samples were analyzed for phenol concentration by mixing, under agitation, 5 g of solid sample (dry weight basis) with 15 mL of NaOH solution (pH 13). After filtration and pH adjustment (pH 7), the filtrate supernatant was analyzed as described above. Phenol concentrations were calculated on the basis of peak area measurements by comparison with an external standard of known concentration of pure phenol prepared with methanol. All experiments were carried out in triplicate.

2.7. Contaminated soil and remediation design

The contaminated soil used in the experiments was classified as dystrophic humic cambisol with the following physico-chemical characteristics: pH 4.9; organic matter = 3.6%; apparent density ($g\,cm^{-3}$) = 1.098; clay = 30.8%; sand = 34.8%; silt = 34.4%. As this soil showed some eroded characteristics in the nature, an amendment with textile sludge contaminated with phenolic compounds was used to restore your fertility/integrity. To test bioaugmentation efficacy, a 1:20 volumetric ratio of activated sludge:contaminated soil was mixed and allowed to biodegrade in PVC basins under natural conditions for 15- and 20-day periods. The mixture was homogenized daily to improve aeration. Accumulated rainwater was naturally evaporated and there was no loss of contaminants by lixiviation. Remediation experiments by bioaugmentation were carried out in triplicate and control basins were performed without activate sludge addition to assess textile sludge autochthonous flora participation in the phenol biodegradation or phenol volatilization. This parallel participation of sludge autochthonous flora in the bioaugmentation was taken in account when results were interpretated.

2.8. Other analytical methods

2.8.1. Optical density (OD) measurement

At time intervals of 1 h, aliquots (1 mL) were taken of the bacterial suspension, harvested by centrifugation (6000 × g, 5 min) and the supernatant was collected in order to measure absorbance using a Cecil Instruments Spectrophotometer at 540 nm.

2.8.2. Biochemical oxygen demand (BOD)

Aliquots (10 mL) were taken of the bacterial suspension, harvested by centrifugation (6000 × g, 5 min) and the supernatant was collected in order to measure BOD according to a previously published methodology [22].

2.8.3. pH control

To maintain the pH between 3.0 and 4.0, a 0.025N NaOH solution was added to the bioreactor at a rate of 68.5 $mL\,g^{-1}$ phenol consumed.

3. Results and discussion

3.1. Phenol biodegradation by the mixed culture in bioreactor—batch system

The profile of phenol biodegradation by the mixed culture in the bioreactor in a batch system starting with two different pH values is shown in Fig. 1. Phenol volatilization in the control tank was negligible.

At pH 6.0, 86.5% of phenol biodegradation occurred in around 27 h, while at pH 3.0, 95.2% of phenol biodegradation occurred in a much faster time of 8 h. These pH values were chosen because they

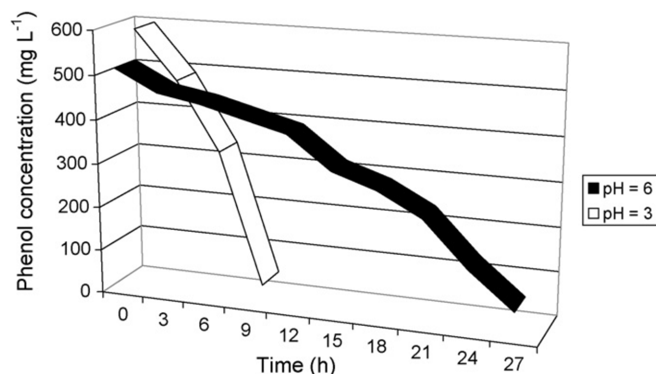

Fig. 1. Time-course phenol biodegradation by the mixed culture in the batch system bioreactor at initial pH 3.0 and 6.0.

are predominant in the influent industrial wastewater studied and it was observed that the mixed culture was able to survive at very acidic pH. At an acidic pH (3.0) the biodegradation rate was around 61.2 mg phenol $L^{-1}\,h^{-1}$, while at pH 6.0, the biodegradation rate was 15.3 mg phenol $L^{-1}\,h^{-1}$. Along the experiment, it was observed that pH value lowered as phenol biodegradation ratio increased, which is an indicative that pH had a significant effect on the rate of phenol biodegradation. In this regard, it has been reported that phenol degradation in model wastewater by aerobic bacterial was very effective at a low pH [23], while other studies have shown that pure *Pseudomonas putida* could not efficiently resist to the pH modification [24].

3.2. Phenol biodegradation by the mixed culture in a bioreactor—continuous system

A closed batch-culture system is not appropriate to study some microbial kinetic parameters as they occur in the environment and therefore the classical continuous-culture system was used to understand microbiological behaviour under dynamic conditions. Thus, the profile of phenol biodegradation by the mixed culture in the bioreactor in a continuous system is shown in Fig. 2. In this experiment, the bioreactor was supplied initially with 800 $mg\,L^{-1}$ of phenol (69.00 $mg\,h^{-1}$) and 1920 $mg\,L^{-1}$ of BOD. It was observed that the system was able to withstand the high phenol concentration for 20 days of the experiment, and all of the phenol and its metabolic products that entered the bioreactor were consumed by the mixed culture.

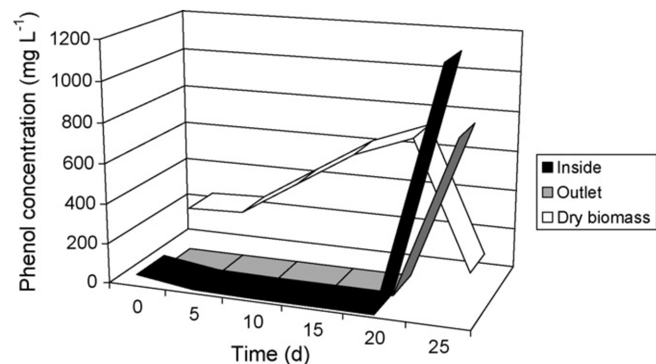

Fig. 2. Inside and outlet phenol concentration in the bioreactor (continuous system) for 25 days of the experiment, starting with 800 $mg\,L^{-1}$ of phenol (at a rate of 69.1 $mg\,h^{-1}$). After 20 days of the experiment the phenol addition was increased to 1200 $mg\,L^{-1}$ (at a rate of 362 $mg\,h^{-1}$).

Table 1
Description of kinetics data of the phenol degradation in continuous system for the mixed culture under pH control (3.0) during 22 days

Parameter	First steady-state (0–7 days)	Second steady-state (7–15 days)	Third steady-state (15–19 days)	Fourth steady-state (19–22 days)
Inlet/inside				
HRT (h)	54	31	19	13
Flow rate (L day^{-1})	2.07	3.60	5.76	8.64
Phenol concentration (mg L^{-1})	800	950	980	1254
Load ratio (mg h^{-1})	69.13	152	247	362
Outlet				
Phenol concentration (mg L^{-1})	0	0	0	0
COD (mg L^{-1})	80	70	80	-
Dry biomass (mg L^{-1})	234	527	522	530
Kinetics and stoichiometric data				
$\mu = D$ (h^{-1})	0.018	0.032	0.051	0.077
Rx (mg dry biomass L^{-1} h^{-1})	4.3	16.8	26.1	40.6
$Y_{x/s}$ (mg dry biomass mg phenol^{-1})	0.293	0.527	0.522	0.530
Rs (mg phenol L^{-1} h^{-1})	14.7	32.0	50.0	76.6
Load factor (mg phenol mg dry biomass^{-1} day^{-1})	1.51	1.47	2.42	3.49

Haldane kinetic model was used to predict biodegradation parameters.

After 20 days, there was a reduction of 96% in the BOD value and 97.5% in the phenol value in relation to the initial concentrations and the mixed culture was able to survive in the presence of this high phenol concentration. Haldane kinetics model was used to describe the dependence of the specific growth rate (μ) on the concentration of the substrate (phenol) and some data of this experiment are shown in Table 1, where HRT of three to six times were considered to assess steady-states characteristics. As load ratio increased, dry bacterial biomass increased, which can be explained by a microorganism selection, since conversion factor value ($Y_{x/s}$) started with 0.293 and reached 0.527 mg dry biomass mg phenol^{-1}. Once the degradation process had become relatively stable, the process was inhibited through a pH modification to around 2 U (at 22 days). Thus, pH control must be realized to the maintenance of phenol optimized biodegradation ratio. Also, it appears from Table 1 that $Y_{x/s}$ is the most important factor to control mixed culture degradation characteristics. Other authors have studied the effect of the adaptation of mixed cultures on phenol biodegradation. The ability of a mixed culture to remove phenol completely in a membrane bioreactor, when initial concentrations ranged from 500 to 3000 mg L^{-1}, has been previously reported [25]. Likewise, a phenol-degrading bacterial consortium obtained from an activated sludge was able to completely degrade phenol initially present at a concentration of 1700 mg L^{-1} [26].

3.3. *Identification of bacterial strains in the mixed culture from the bioreactor*

During the degradation experiments in the bioreactor aliquots were taken for identification and isolation of the bacterial strains. The isolated colonies were identified using the API 20 NE system [20] as *Pseudomonas alcaligenes*, *P. mesophilicas* and *A. calcoaceticus* var. *anitratus*, which was the predominant strain.

3.4. *Phenol biodegradation in the batch system by A. calcoaceticus and the mixed culture with phenol as the sole source of carbon*

The profile of the phenol biodegradation by the mixed culture and *A. calcoaceticus* in the bioreactor in the batch system is shown in Fig. 3.

After 30 h of biodegradation, the mixed culture degraded 86% of the initial phenol concentration, while *A. calcoaceticus* degraded 48.5% of the phenol in the same period. Both, the mixed culture and *A. calcoaceticus* were able to totally degrade the phenol, after 39 and 60 h of experiment, respectively. It seems that the microbial consortium is more fast in degrading phenol than the pure culture, which could be due to the synthrophy phenomenon on the intermediates biodegradated. In a similar previous study it was found that a microbial consortium (including *P. putida*) was able to degrade phenol concentrations as high as 2000 mg L^{-1} in a two phase partitioning reactor when compared to the performance of pure culture of *P. putida* [17]. Other interesting results were obtained with immobilized and free cells of a phenol-degrading *Acinetobacter* sp. strain W-17, isolated from a wastewater treatment plant. These immobilized *Acinetobacter* cells completely degraded 500 mg phenol L^{-1} in 40 h, while the free cells required 120 h to perform the phenol degradation [27,28].

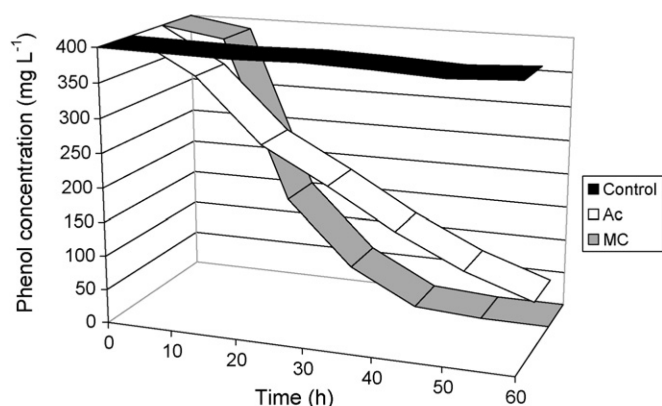

Fig. 3. Phenol biodegradation by *A. calcoaceticus* (Ac) and a mixed culture (MC) with phenol as the sole source of carbon (400 mg L^{-1}) after 60 h in a batch culture. Control without bacterial inoculum.

Table 2
Composition/characteristics of the phenolic coke gasification wastewater (mg L^{-1})

Component	Value
Phenol	1,000–3,000
CN^-	30–100
SCN^-	100–800
Cresols (o, m, p)	200–600
BOD	3,500–15,000
COD	5,000–20,000
pH	9–11
Total ammonium	2,000–4,000

Table 3
BOD and absorbance measurements during 10 days of biodegradation of a coke gasification wastewater inoculated with the mixed culture

Time (days)	Mean values BOD (mg L^{-1}) and Absorbance (540 nm) of coke gasification wastewater inoculated with mixed culture					
	BOD (35%, v/v)	Absorbance	BOD (18%, v/v)	Absorbance	BOD (12%, , v/v)	Absorbance
0	1267	0.285	790	0.490	550	0.220
2	1190	0.378	785	0.495	550	0.250
4	1186	0.220	711	0.575	565	0.275
8	1166	0.257	632	0.595	570	0.350
10	1037	0.325	474	0.605	235	0.650

Wastewater samples were diluted in Milli-Q water to 35, 18, and 12% (v/v).

3.5. Phenol biodegradation by the mixed culture and A. calcoaceticus in a coke gasification wastewater

The mixed culture and isolated *A. calcoaceticus* were tested for their ability to degrade phenolic compounds in a coke gasification wastewater from a ceramics factory, containing high concentrations of phenolic compounds, such as phenol, cresols, polyphenols and cyanides (Table 2).

In Table 3, the BOD and OD measurements for the coke gasification wastewater inoculated with the mixed culture after 10 days of experiment are shown.

No absorbance evolution implies growth inhibition, and in this sense, the higher concentration of diluted wastewater (i.e., 35%) showed a poor reduction in the BOD value (18%). When the coke gasification wastewater was diluted to 18 and 12% (v/v), there was a significant reduction of 40 and 57% in the BOD values, and the absorbance values increased by 24 and 50%, respectively. In addition, when the not diluted coke gasification wastewater was inoculated with *A. calcoaceticus*, there was 100% of bacterial growth inhibition even after 10 days of the experiment and the BOD and absorbance values did not significantly changed during this period, probably due to the bactericide composition of the wastewater (i.e., pH and toxics). In this regard, two experiments were carried out to determine the toxic component(s) of the industrial wastewater: (i) inoculation of bacterial culture in a culture medium at pH 10.00, and (ii) wastewater pH adjustment to pH 6.00. In both experiments, there was 100% bacterial growth inhibition, indicating that *A. calcoaceticus* is sensitive to pH and toxic compounds. To avoid wastewater biodegradation inhibition, different processes have been proposed for the removal of phenol from coke gasification wastewater: solvent extraction [29]; acidification and demulsion treatment [30]. Also, an integrated enzymatic treatment system, which includes *Coprinus cinereus* peroxidase is being developed to remove phenolic compounds from wastewaters at pH 7.00 [31].

3.6. Remediation of textile sludge-contaminated soil

There are many interesting bioremediation methodologies to treat phenol-contaminated soils [31–33]. In order to reduce cost remediation (or even sludge treatment), we studied an alternative cost-effective methodology to treat a phenol-soil contaminated matrix by simple wastewater bacterial cultivation under natural conditions, i.e., natural bioaugmentation. In this regard, in the study here reported, addition of a raw bacterial consortium present in the activated sludge of a coal wastewater treatment plant to the textile sludge-contaminated soil showed high microbiological efficiency in the degradation of phenolic compounds (Table 4).

It is clear from Table 4 that the inoculated bacterial consortium of the activated sludge is very effective in promoting phenol degradation, since 15 days of treatment was sufficient to decrease the phenol concentration by 73.5%. In the additional treatment period from 15 to 20 days, the phenol concentration decreased by 81.1%,

Table 4
Phenol concentration in the textile sludge-contaminated soil after 15 and 20 days of microbiological treatment

Treatment time (days)	Phenol concentration $X \pm \sigma$ (mg kg^{-1})		Degradation efficiency (%)	
	Control soil	Inoculated soil[a]	Control soil	Inoculated soil[a]
0	19.48 ± 1.96	19.48 ± 1.96	–	–
15	17.52 ± 0.82	3.19 ± 0.45	10.1	83.6
20	16.93 ± 0.60	1.13 ± 0.20	13.1	94.2

[a] Without bacterial inoculum.

taken in account the degradation promoted by textile sludge and soil autochthonous flora. In this period, the residual phenol was mineralized more slowly than during the first 15 days of treatment, probably because the majority of this substance is sorbed strongly to the particles present in the sludge and therefore the molecules are not readily bioavailable. This matrix adsorption effect has been previously noted in relation to other organic contaminants [34,35]. The participation of the textile sludge (or soil) autochthonous flora on the phenol biodegradation was significant, but in much lesser extention (approximatelly 11%) than the participation of the inoculated bacterial consortium. In a previous study [36], we showed that phenolic compounds were aerobically degraded after 4 months of natural stabilization by the textile sludge-soil autochthonous flora. It must be remembered that volatilization could be contributed to the phenol disappearance in the control basins. Thus, addition of coal wastewater activated sludge to the contaminated soil, i.e., the simple bioaugmentation, was shown to be an effective method for the treatment of this phenol-contaminated soil.

4. Conclusions

A mixed bacterial culture and isolated *A. calcoaceticus*, obtained from a coal wastewater treatment plant containing high phenolic compounds concentration, showed the ability to degrade phenol in both continuous and batch systems. The isolated colonies of the mixed culture were identified as *Pseudomonas alcaligenes*, mesophilic *Pseudomonas* and *A. calcoaceticus* var. *anitratus*. The mixed culture was able to survive in the presence of a phenol concentration as high as 1200 mg L^{-1} and promote its degradation. The mixed culture and *A. calcoaceticus* were able to degrade high phenol concentration after 8 and 27 h of experiment, respectively, with an initial phenol concentration around 550 mg L^{-1}. After 20 days, the mixed culture in the bioreactor with a continuous system was able to reduce values of BOD by 96%, and those of phenol by 97.5% from the initial concentration of 1200 mg L^{-1}. The pH had a significant effect on the velocity of phenol degradation in the batch reactor. Under acidic pH conditions (3.0) the biodegradation rate was around 61.2 mg phenol L^{-1} h^{-1}, while at pH 6.0, the biodegradation rate was 15.3 mg phenol L^{-1} h^{-1}. In the batch-culture experiments with coke gasification wastewater, the

pH (9–11) and the high toxicity of the cyanide compounds inhibited greatly the phenol degradation. In an environmental application of the activated sludge from the industrial coal wastewater treatment plant it was observed that inoculated bacterial consortium survive in the new environment and that after 15 days of microbiological degradation, a textile sludge-contaminated soil showed a decrease in phenol concentration from 19.48 to 3.19 mg kg^{-1}, and to 1.13 mg kg^{-1} after 20 days of microbiological treatment. Further studies must be carried out to assess the biodegradability potential of this mixed culture to treat different phenolic compounds present in other contaminated soils.

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