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Application of two bacterial strains for wastewater bioremediation and assessment of phenolics biodegradation

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The use of native bacteria is a useful strategy to decontaminate industrial effluents. In this work, two bacterial strains isolated from polluted environments constitutes a promising alternative since they were able to remove several phenolic compounds not only from synthetic solutions but also from effluents derived from a chemical industry and a tannery which are complex matrices. *Acinetobacter* sp. RTE1.4 showed ability to completely remove 2-methoxyphenol (1000 mg/L) while *Rhodococcus* sp. CS1 not only degrade the same concentration of this compound but also removed 4- chlorophenol, 2,4-dichlorophenol and pentachlorophenol with high efficiency. Moreover, both bacteria degraded phenols naturally present or even exogenously added at high concentrations in effluents from the chemical industry and a tannery in short time (up to 5 d). In addition, a significant reduction of biological oxygen demand and chemical oxygen demand values was achieved after 7 d of treatment for both effluents using *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1, respectively. These results showed that *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 might be considered as useful biotechnological tools for an efficient treatment of different effluents, since they showed wide versatility to detoxify these complex matrices, even supplemented with high phenol concentrations.

Keywords: wastewater; biotreatment; phenolic compounds; bacteria; bioremediation

1. Introduction

Currently, there is a global interest to avoid or reduce environmental pollution. However, many industrial processes are inherently polluting and produce effluents difficult to treat with conventional strategies. In addition, due to high operating costs, some industries release their effluents containing high concentrations of persistent and toxic compounds without previous treatment or with inadequate or incomplete decontamination processes.[1,2]

In particular, a variety of industries such as those which produce dyes, resins, plastics, soaps and detergents, as well as those related with the paper bleaching process, oil refineries, pesticides, disinfectants, fungicides and bactericides production, among others, are often sources of contamination with phenolic compounds, in concentrations ranging from 0.1 to 1000 mg/L.[3,4] Due to their high toxicities, phenol and its derivatives have been listed as priority pollutants by different regulatory agencies, such as the US Environmental Protection Agency (US EPA) [5] and the Agency for Toxic Substances and Disease Registry (ATSDR).[6] Therefore, remediation of wastewaters containing phenols is of great concern for a safe environment.

Among the different alternatives to treat effluents before their release into the environment, the biotreatment using specific bacteria with ability to degrade toxic compounds represents a simple and economical choice.[7,8] In this context, biodegradation has been used to remediate effluents contaminated with phenols from different industries, such as olive oil,[9] cork production,[10] coke gasification,[11] wood laminate manufacturing [1] and tanneries.[12] However, to our knowledge, there are no many studies related to microbial degradation of effluents derived from chemical and leather industries, containing phenolic compounds. Thus, it is important to explore the possibility of using efficient degraders to treat these effluents in order to reduce their potential environmental impact.

We have recently isolated two bacterial strains, belonging from *Acinetobacter* and *Rhodococcus* genera, from industrial effluents and polluted sediments. They showed high ability to degrade phenol in synthetic media.[13,14] Despite the ability of some bacterial strains to degrade

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different compounds in synthetic media, frequently they are not able to remove toxic compounds from effluents due to an inadequate pH or possible inhibitory effect of some wastewater components on microorganism's growth and/or catabolic activity.[10] In this sense, it has been described that phenolic compounds may often cause the breakdown of wastewater treatments by inhibition of microbial growth due to their toxicity.[15–17] Thus, it is relevant to establish the capability of bacterial strains to remove pollutants not only in a synthetic media but also in a real wastewater.

Therefore, with the aim to explore the bioremediation potential of the above-mentioned bacterial strains, tolerance and degradation of phenols contained in mineral media (MM) and in effluents derived from a chemical industry and from a tannery were studied. The variation of chemical oxygen demand (COD) and biological oxygen demand (BOD) values was also evaluated in order to analyse the removal process efficiency.

2. Materials and methods

2.1. Microorganisms and culture conditions

In the present work, *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1, previously isolated from an effluent derived from a chemical industry and from tannery sediments, respectively, were used.[13,14] Bacteria were routinely grown on TY agar medium [(g/L): 5 tryptone; 3 yeast extract; 0.65 $CaCl_2$; 13 agar] and kept at 4°C. For the different experiments described below, inoculums were prepared by growing the strains in TY liquid medium.

2.2. Biodegradation experiments of phenolic compounds

Biodegradation of different phenolic compounds such as 2-methoxyphenol (guaiacol), 2,4-dichlorophenol (2,4-DCP), 4-chlorophenol (4-CP) and pentachlorophenol (PCP) was evaluated.

For that, the tolerance of both bacteria to these compounds was initially established in agar plates containing MM supplemented with the contaminants. Then, biodegradation of pollutants was carried out. The experiments using *Acinetobacter* sp. RTE1.4 were performed in mineral media (MM9) [(g/L): 2.8 Na_2PO_4H; 9 KPO_4H_2; 2.5 NaCl; 1 NH_4Cl] supplemented with guaiacol (100–1000 mg/L) and 4-CP (50–250 mg/L) while those developed using *Rhodococcus* sp. CS1 were performed in MM [(g/L): 0.3 $MgSO_4$; 0.01 $FeSO_4$; 0.5 NaCl; 3 NH_4Cl; 0.01 $CaCl_2$; 1.5 K_2PO_4H; 0.5 KPO_4H_2] plus guaiacol (100–1000 mg/L), 4-CP (25–100 mg/L), 2,4-DCP (25 and 50 mg/L) and PCP (2.5 and 5 mg/L).

For all experiments, erlenmeyers flasks containing 30 mL of MM by triplicate, were inoculated (10% V/V) with both bacteria at late exponential phase and incubated at 28 ± 2°C on an orbital shaker at 100 rpm. Samples (2 mL) were taken at different time intervals and tested for residual phenols concentration, as it is described later (Section 2.8). Non-inoculated controls were evaluated in each assay in order to check total phenols concentration (abiotic control).

2.3. Collection and characterization of wastewaters

Two kinds of wastewaters, coincidently with those from which *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 had been initially isolated were used in this work. One of the samples belongs to a chemical industry located in Río Tercero, Córdoba province, Argentina (32° 9′ South latitude and 64° 6′ West longitude) and whose effluents are discharged into Ctalamochita River. The second wastewater was obtained from a leather industry located in Elena, Córdoba, Argentina (32° 34′ South latitude and 64° 23′ West longitude), previous to its discharge into El Barreal river. The effluents were arbitrarily designed as chemical industry effluents (CIE) and tannery effluents (TE), respectively.

Effluent samples (2–4 L) were collected from the discharge channel. Three sampling of both effluents was carried out in order to analyse possible variations during the different seasons. TE was filtered prior to perform the experiments due to its high turbidity. The effluents were not sterilized to avoid physicochemical changes of their components.

Both wastewaters were characterized according to procedures described in the standard methods.[18] The analysis of organoleptic characteristics and different physicochemical parameters of the effluents (COD, BOD, pH, temperature and turbidity) were determined. Total phenols content was estimated by a spectrophotometric assay [19] that is explained below.

2.4. Bacterial tolerance to effluents

First, tolerance was evaluated through the ability of both bacterial strains to grow in agar plates containing pure and diluted effluents (25%, 50% and 75% V/V). The plates were streaked with a fresh culture of *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 and incubated at 28 ± 2°C for 7 d. Growth of the bacteria was determined visually as positive or negative. These experiments were done by duplicate.

Later, the ability of the strains to grow in the same liquid effluents was also evaluated monitoring cell growth by turbidity measurements. Erlenmeyers flasks containing 30 mL of pure effluents were inoculated (10% V/V) with each bacterial strain and stirred on an orbital shaker at 100 rpm and 28 ± 2°C. For each effluent, a non-inoculated control was incubated simultaneously under the same conditions in order to check the growth of native microorganisms. Samples (2 mL) were collected periodically until 24 h to determine optical density at 620 nm (OD_{620nm}) as an estimation of growth. Adequate blanks, containing non-inoculated sterilized effluents were performed.

2.5. Phenols biodegradation assays in effluents

Phenols degradation by *Rhodococcus* sp. CS1 was tested in TE containing high concentration of these pollutants. In the same way, the capability of *Acinetobacter* sp. RTE1.4 to degrade phenols contained in CIE was evaluated, when these pollutants were detected in the effluents.

For these assays, erlenmeyers flasks containing 30 mL of effluent, in triplicate, were inoculated (10% V/V) with both bacteria and incubated at $28 \pm 2°C$ on an orbital shaker at 100 rpm. Culture samples (2 mL) were taken every hour and tested for residual phenols concentration, as it is described later (Section 2.7). Non-inoculated effluents, incubated under the same conditions, were considered as controls.

2.6. Effect of phenol addition on the bacterial degradation efficiency

With the aim to establish the capability of *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 to degrade high phenol concentrations, the effluents were supplemented with this pollutant.

The assays were performed similarly to those described in Section 2.5; however, in this case, 200 and 600 mg/L of phenol were added to CIE whereas 200 and 1000 mg/L of the contaminant were added to TE. Non-inoculated controls were also performed. Growth and phenol degradation ability in all conditions were evaluated.

2.7. BOD and COD determinations

To evaluate wastewater remediation produced by the studied microorganisms, BOD and COD were determined using standard methods (5-day BOD and dichromate oxygen demand methods, respectively). Analyses were carried out by IACA Laboratory (Bahía Blanca, Argentina). For this, CIE plus 600 mg/L of phenol were inoculated with *Acinetobacter* sp. RTE1.4 whereas TE supplemented with 1000 mg/L were inoculated with *Rhodococcus* sp. CS1. BOD and COD were determined before inoculation and after 7 d of inoculation.

2.8. Phenols determination

Phenol and their derivatives, guaiacol, 4-CP and 2,4-DCP, were determinated following the spectrophotometric method described by Wright and Nicell.[19] Aliquots of 100 µl of each sample, previously centrifuged (10,000 rpm, 5 min), were mixed with 100 µl of 4-aminoantipyrine (20.8 mM), 100 µl potassium ferricyanide (83.4 mM) and 700 µl of sodium bicarbonate (0.25 M pH 8.4). After five minutes, the absorbance of the coloured compound formed was determined at 510 nm, which was proportional to phenol concentration in the range of $0–10^{-4}$ M. PCP was determined through gas chromatography by IACA Laboratory (Bahía Blanca, Argentina).

2.9. Statistical analysis

Statistical analysis was performed using STATISTICA 7.1 software package. All data were analysed using analysis of variance. In all cases $p \leq .05$ was statistically significant. The Dunnett test was used for comparing several treatment groups with a control.

3. Results and discussion

3.1. Phenolic compounds biodegradation

The tolerance of *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 to guaiacol, 4-CP, 2,4-DCP and PCP was previously determined evaluating their growth in agar plates.[13,14] *Acinetobacter* sp. RTE1.4 was able to grow in the presence of guaiacol and 4-CP while *Rhodococcus* sp. CS1 grew in the presence of all the studied phenols. Then, biodegradation of these phenolic compounds by both bacteria was evaluated. In these experiments, the abiotic controls showed that phenolic compounds reduction by evaporation was not significant (0–7%).

When *Acinetobacter* sp. RTE1.4 was inoculated in MM media containing guaiacol or 4-CP, this strain was able to efficiently degrade up to 1000 mg/L of guaiacol in 16 d while it could not degrade 4-CP at the assayed concentrations after 5 d (Figure 1). Few studies on *Acinetobacter* species degrading phenolic compounds are available. In this sense, Kim and Hao [20] and Hao et al. [21] demonstrated

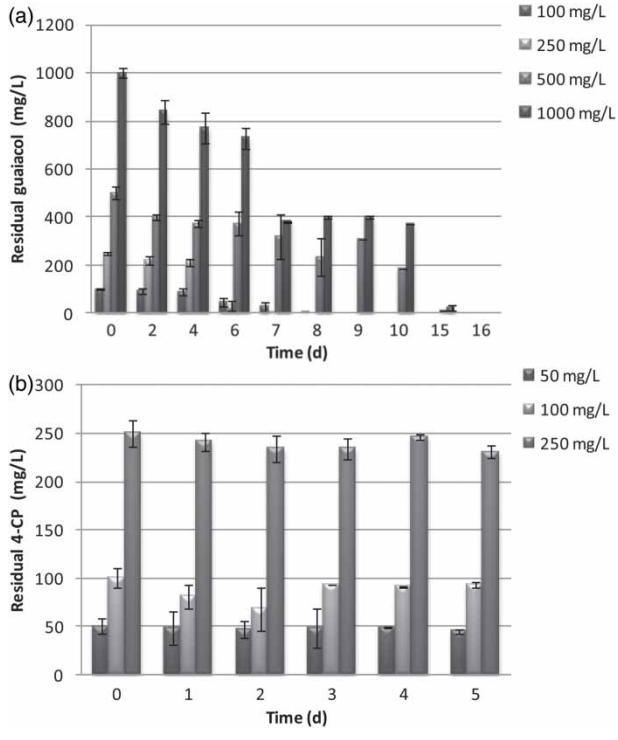

Figure 1. Guaiacol (a) and 4-CP (b) biodegradation by *Acinetobacter* sp. RTE1.4.

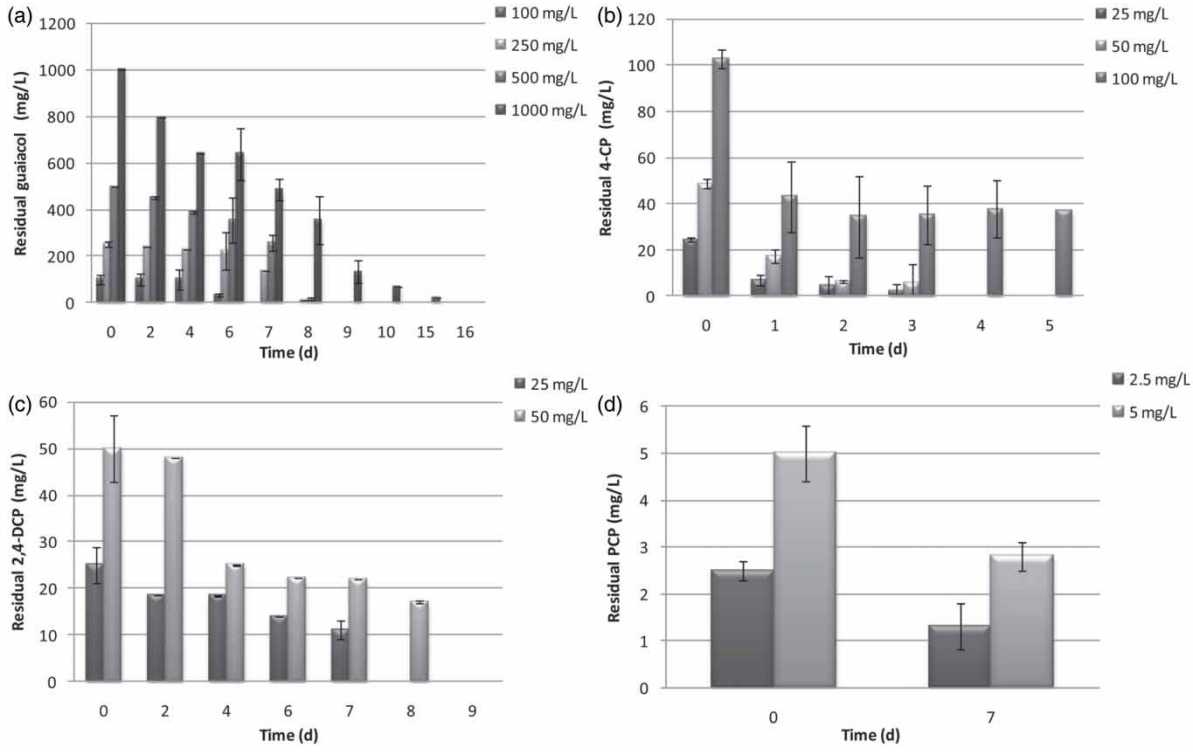

Figure 2. Guaiacol (a), 4-CP (b), 2,4-DCP (c) and PCP (d) biodegradation by *Rhodococcus* sp. CS1.

that *Acinetobacter* strains consumed 3-CP y 4-CP but this process occurred only in co-metabolism with phenol.

Figure 2 shows phenolic compounds biodegradation by *Rhodococcus* sp. CS1. It was able to completely degrade 1000 mg/L of guaiacol, 50 mg/L of both 4-CP and 2,4-DCP after 16, 5 and 9 d, respectively. The time required for complete degradation of all tested phenols increased as a function of the initial concentration of the pollutant. In addition, it was able to degrade PCP (5 mg/L) with a 44% of removal efficiency after 7 d. Only 3–7% of the removal was detected in abiotic controls.

These results demonstrated that *Rhodococcus* sp. CS1 was able to tolerate and metabolize different phenolic compounds, including methoxy- and CPs, which are known to be inhibitory to microbial growth. In particular, the biodegradability of CPs depends on the number and position of halogens in the aromatic ring. Among the CPs, PCP is expected to be recalcitrant to aerobic biodegradation due to its high degree of chlorination. Despite this, *Rhodococcus* sp. CS1 was able to use this compound as carbon and energy source. Similar results were obtained by Goswami et al. [22] which determined that *Rhodococcus erythropolis* M1 degraded up to 100 mg/L of 2-CP after 45 d. This strain was also able to degrade 4-CP and 2,4-DCP but only after benzoate induction. In addition, *Rhodococcus* strains have shown ability to biodegrade nitro- and chlorophenolic compounds such as *p*-nitrophenol, 2,4-dinitrophenol and 2,4,6-trichlorophenol.[23–25] However, our results are relevant because as far as we know this is the first report exploring the potential of a *Rhodococcus* strain to remove PCP and guaiacol from aqueous solutions.

It is important to remark the higher capability showed by *Rhodococcus* sp. CS1 compared with *Acinetobacter* sp. RTE1.4 for phenols biodegradation. However, *Acinetobacter* sp. RTE1.4 could degrade phenol and guaiacol and it also showed tolerance to different phenols. These results suggest that both *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 could be used to treat effluents contaminated with different phenolic compounds.

3.2. Characterization of the collected wastewaters

In order to evaluate the ability of both bacteria to grow and remediate natural effluents, samples derived from a chemical industry and a tannery were collected at different seasons and characterized. The values of the main

Table 1. Organoleptic and physicochemical characteristics of effluents derived from a chemical industry and from a tannery.

	Effluent	
Parameter	Chemical industry	Tannery
pH	7.5 ± 0.3	10.3 ± 2.1
Total phenols (mg/L)	0.3 ± 0.15	11.7 ± 1.1
Temperature (°C)	24.8 ± 2.3	21.0 ± 6.3
Turbidity	Low	High
Colour	Uncoloured	Whitish
Odour	Strong and irritable	Putrefied

Note: Reference values are the average of three measures.

physicochemical parameters of these effluents are shown in Table 1.

The pH of effluents from the chemical industry was near to the neutrality in all samples. However, TE were always alkaline and they reached values as high as 12, as it was also mentioned by Durai and Rajasimman.[26] These pH values exceed the acceptable limits (between 5.5 and 10.0) indicated by the law of hazardous wastes.[27]

In the CIE, which were obtained in different samplings, low levels of phenols (0.2–0.4 mg/L) or even no phenol were detected. By contrast, in TE high phenols concentrations were found in the different collected samples (10.8–12.5 mg/L). Phenols concentrations detected in both effluents exceeded the guideline values given by the abovementioned law and those recommended by US EPA [5] and ATSDR.[6]

From the analysis of pH and phenols concentrations, it is possible to assume that both effluents could be hazardous for ecosystems in which they are discharged. Moreover, the turbidity, colour and odour could indicate the presence of potentially toxic compounds. Therefore, these wastewaters need to be efficiently treated before its release in natural water bodies. In this sense, wastewater bioremediation using bacteria isolated from industrial effluents and adapted to degrade phenol could be an effective tool for this purpose.

3.3. *Bacterial tolerance to effluents*

Both strains showed ability to grow in plates containing pure effluents, reaching the highest growth after three days of incubation.

Then, tolerance of each bacterial strain was evaluated monitoring cell growth in erlenmeyers containing liquid pure effluents. *Acinetobacter* sp. RTE1.4 grew in CIE reaching high values of absorbance from 2 h of incubation (Figure 3(a)). When *Rhodococcus* sp. CS1 was inoculated in TE, the absorbance values reached the highest values at 4 h of incubation (Figure 3(b)). In non-inoculated controls low or no growth was observed. Thus, both bacteria showed high tolerance to these effluents indicating that they are able to use the organic matter as carbon and energy sources, allowing the microorganisms to efficiently increase their biomass. These results are promising, taking into account that the inhibition of microbial growth due to the bactericide composition of some wastewaters (i.e. high or low pH and presence of toxic compounds) has been described, producing a consequent reduction in wastewater remediation.[11,28–30] Since this chemical industry produce sodium hypochlorite (among other chemicals) the presence of this compound in the effluent could affect the bacterial growth due to their bactericide properties. However, *Acinetobacter* sp. RTE1.4 was able to grow in this effluent. On the other hand, Durai and Rajasimman [26] described that the presence of chromium and sulfides in TE could produce biodegradation inhibition, due to their antibacterial activity. TE collected in the present

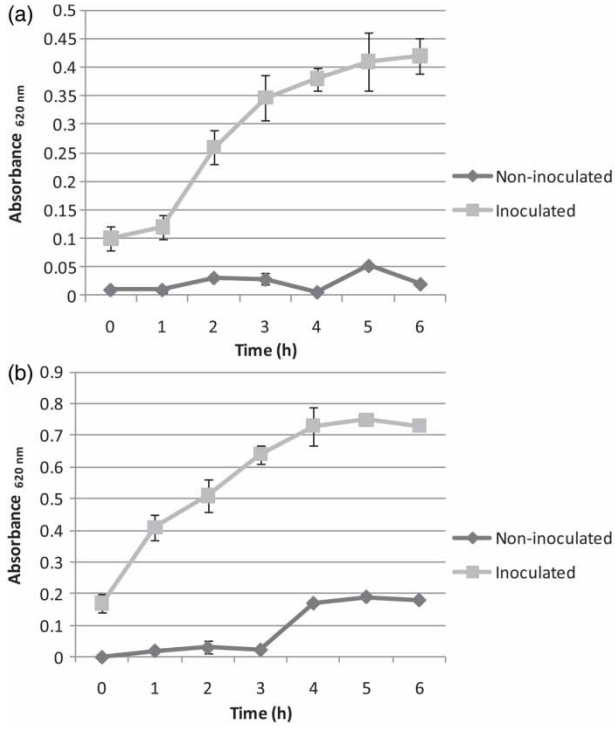

Figure 3. Growth of *Acinetobacter* sp. RTE1.4 inoculated in a CIE (a) and *Rhodococcus* sp. CS1 inoculated in a TE (b). Non-inoculated cultures media were used as abiotic controls.

work had chromium in concentrations between 0.01 and 0.45 mg/L and high pH values. However, *Rhodococcus* sp. CS1 tolerated this effluent, which is of great concern from an environmental and biotechnological point of view. However, it is important to remark that despite a microorganism can tolerate a xenobiotic it does not involve that it is able to degrade it. Thus, the evaluation of the degradation of phenols in industrial effluents by these bacteria was carried out.

3.4. *Phenols biodegradation assays*

The capability of *Acinetobacter* sp. RTE1.4 to degrade phenols in CIE was evaluated (Figure 4(a)). The strain completely degraded phenols after 8 h of incubation while in the non-inoculated controls only 16.7% of phenols were removed ($p < .05$). On the other hand, when degradation of phenolic compounds from TE by *Rhodococcus* sp. CS1 was tested, complete degradation was observed after 6 h while native microorganisms removed only 18.3% of total phenols contained in these effluents ($p < .05$) (Figure 4(b)).

It is important to note that both bacteria showed high capability to biodegrade phenols naturally contained in the effluents after few hours whereas the native population could not produce any significant reduction in phenolics concentration. The ability to remove phenol from industrial effluents has also been described for other bacterial genera. For instance, two *Pseudomonas* strains (*P. aeruginosa* and *P. fluorescens*) completely degraded phenol (30 mg/L)

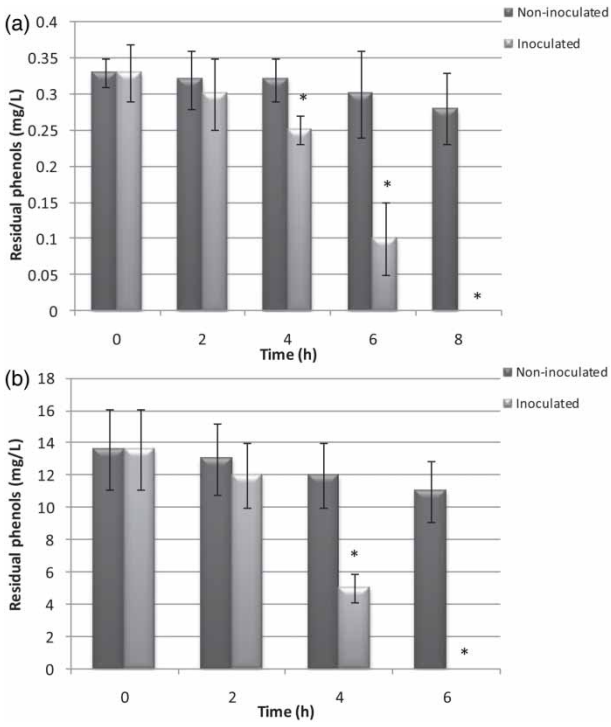

Figure 4. Degradation of total phenols from CIE by *Acinetobacter* sp. RTE1.4 (a) and from TE by *Rhodococcus* sp. CS1 (b) compared with native microorganisms from the effluents. (∗) represents significant statistic differences with non-inoculated controls ($p < .05$).

contained in a petroleum refinery effluent in 60 and 84 h, respectively, under batch conditions.[31] On the other hand, Nair et al. [32] observed that *Alcaligenes* sp. removed 99% of phenol contained in an effluent from a paper industry, while Omer [33] showed high phenol degradation from olive mill effluent (diluted at 50% and 30%) after 25 days of treatment using a bacterial mixture constituted by *Azotobacter vinelandii*, *Pseudomonas putida* and *P. fluorescens*. In the last years, several bacterial strains have been used to remediate tannery wastewater, however, in these works the specific removal of phenols was not evaluated.[34,35]

3.5. *Determination of bacterial degradation efficiency using effluents supplemented with phenol*

In order to analyse if these strains could be capable to degrade higher phenol concentrations than those detected in the effluents, two phenol concentrations were added to the studied effluents and their degradation was evaluated.

Table 2 shows that total degradation of 200 mg/L phenol by *Acinetobacter* sp. RTE1.4 from CIE and by *Rhodococcus* sp. CS1 from TE was reached after 3 d. In general, for the complete degradation of higher phenol concentrations (600–1000 mg/L), approximately 4–5 d were needed (Table 2). Thus, similar efficiencies for phenol degradation by both bacteria were obtained in spite of the differences in phenol concentrations and composition of each effluent.

Table 2. Phenol degradation of CIE and TE supplemented with high phenol concentrations by *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1.

Procedence of the effluent	Strain	Phenol (mg/L)	Days for complete degradation ($X \pm$ SD)
Chemical industry	*Acinetobacter* sp. RTE1.4	200	3 ± 1
		600	4 ± 2
	Rhodococcus sp. CS1	200	3 ± 2
		1000	4 ± 2
Tannery	*Acinetobacter* sp. RTE1.4	200	3 ± 0
		600	5 ± 2
	Rhodococcus sp. CS1	200	3 ± 3
		1000	5 ± 2

These results could indicate that *Acinetobacter* sp. RTE1.4 would be suitable for biotreatment of CIE containing phenol concentrations as high as 600 mg/L and *Rhodococcus* sp. CS1 for the treatment of TE containing up to 1000 mg/L.

The potential of both bacteria for phenol degradation in a different effluent from which they were isolated was evaluated inoculating *Acinetobacter* sp. RTE1.4 in TE and *Rhodococcus* sp. CS1 in CIE (Table 2). The average values showed that the efficiency and rate of phenol degradation of both bacteria did not change. However, as it could be seen from the table, the time required for a complete degradation of phenol was variable and dependent on each particular sample, because the effluent composition changes over time with the industrial process, even daily, affecting the efficiency of bacterial degradation.

The success of the application of a microbial treatment depends, to a large extent, on how favourable is the target environment to microorganism survival. Despite industrial effluents usually represent a hostile environment; the studied bacteria were able to remediate phenol in these wastewaters. Thus, these results are very interesting because they showed that both strains are capable of degrading the target pollutant in effluents derived from different industries, even in those whose composition would not be the same from which these microorganisms were first isolated. Therefore, they could be efficiently applied for the treatment of different wastewaters containing phenols.

To our knowledge, there are only few studies describing the use of *Acinetobacter* and *Rhodococcus* species for biotreatment of effluents contaminated with phenol. For instance, Cordova-Rosa et al. [11] obtained negative results when they used *Acinetobacter calcoaceticus* for the degradation of phenols in coke industry effluents while Begoña Prieto et al. [36] demonstrated that the high capability of *R. erythropolis* UPV-1 to biodegrade phenol from a resin industry effluents but using immobilized cells. Therefore, the results presented in this study are relevant because they demonstrate the potential of *Acinetobacter*

sp. RTE1.4 and *Rhodococcus* sp. CS1 to completely biodegrade not only natural concentrations of phenols contained in these two effluents but also, increased concentrations up to 1000 mg/L. The performance of these strains showed their high resistance and adaptability to recalcitrant conditions. This allows proposing them as excellent candidates to treat different effluents with variable characteristics.

3.6. *BOD and COD determinations*

BOD and COD values were determined before and after bacterial treatment of effluents collected in the third sampling and supplemented with phenol (600 and 1000 mg/L), in order to show wastewater remediation. Effluents without treatment were used as controls.

CIE treated with *Acinetobacter* sp. RTE1.4 showed 81% reduction in BOD and COD values compared with values recorded before treatment (Figure 5(a)). Similarly, a 62% reduction was observed in the same parameters in TE after treatment with *Rhodococcus* sp. CS1 (Figure 5(b)). From these results, it is clear that both bacterial strains are very effective to remediate wastewaters, since treatments during 7 d were enough to significantly decrease BOD and COD at values below 500 and 1500 mg/L, respectively. Although the registered values are yet above the suggested limits for effluent release in natural water bodies,[37,38] it is important to consider that the end point of our experiments was established when phenol was not detected in the samples. Probably, the degradation of other toxic compounds would require more time, taking into account that these wastewaters are highly complex and are characterized by high content of organic as well as inorganic compounds. Thus, lower BOD and COD values could be expected if the experiment would be carried out for a long time.

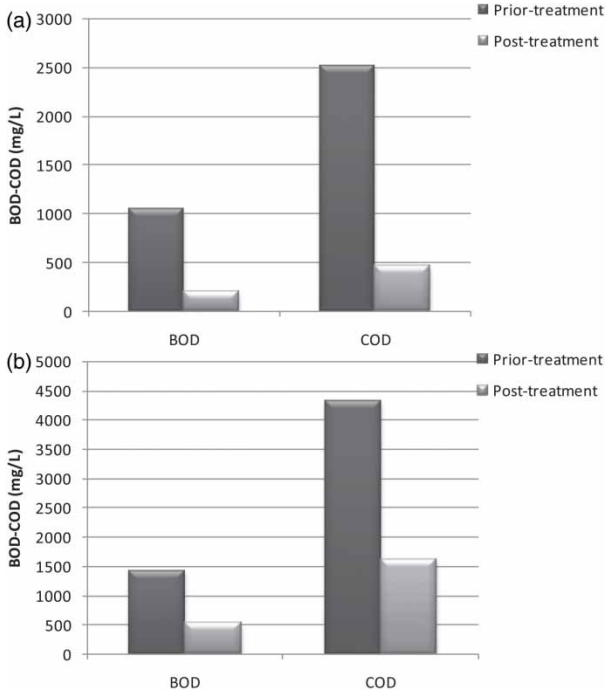

Figure 5. BOD and COD values prior-and post-treatment of effluents from the CIE containing 600 mg/L of phenol, treated with *Acinetobacter* sp. RTE1.4 (a), and from a TE containing phenol 1000 mg/L, treated with *Rhodococcus* sp. CS1 (b). (∗) represents significant statistic differences with non-treated controls ($p < .05$).

The effective reduction of BOD and COD is a key aspect in the treatment of industrial effluents. However, an adequate reduction is not always achieved because removal efficiency could be affected by the variation in organic loading rates, the presence of heavy metals such as chromium, sulfides, toxic chemicals, among other recalcitrant pollutants.[26] For example, Jayachandran et al. [39] found that the COD value of an effluent from the latex industry treated during 8 d with *Acinetobacter* sp. BTJR-10 was reduced from 22,000 to 8800 mg/L, which are still so far of the recommend values. In contrast, Rosli [40] obtained around 60% of COD reduction in a textile industry effluent with 600 mg/L initial COD after 5 d of treatment using two *Acinetobacter* strains. In this sense, the results obtained in this work, with *Acinetobacter* sp. RTE1.4, were interesting in terms of COD reduction, indicating the high potential of this strain for bioremediation.

In relation to tannery wastewater, its aerobic degradation has been described using different microorganisms [8,41–43] reaching considerable reductions in COD and BOD even near to the values obtained with *Rhodococcus* sp. CS1 in this study. Despite, considerable reductions of BOD and COD values have been obtained using different strains of the genus *Rhodococcus* to treat effluents from resin industries and household effluents,[44,45] no references have been found describing the remediation of tannery effluents with bacterial strains belonging to this genus.

4. Conclusion

Acinetobacter sp. RTE1.4 showed ability to degrade phenol and guaiacol while *Rhodococcus* sp. CS1 could degrade these compounds and also 4-CP, 2,4-DCP and PCP as sole carbon sources. Moreover, both bacteria grew in pure industrial effluents derived from a chemical industry and a tannery indicating their high tolerance to contaminated wastewaters. Phenols naturally present in such effluents were degraded in short time by these strains. Furthermore, when phenol was exogenously added to these effluents, both microorganisms could also degrade it with high efficiency compared with native bacteria. An important behaviour was that *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 were able to degrade phenols in both effluents, which showed the wide versatility of these microorganisms for the biotreatment of effluents from different sources and even different from which they were isolated. Moreover, a significant reduction of BOD and COD values of these

effluents were achieved after 7 d of treatment with *Acinetobacter* sp. RTE1.4 as well as with *Rhodococcus* sp. CS1, demonstrating the potential of these bacteria for wastewater remediation.

In summary, *Acinetobacter* sp. RTE1.4 and *Rhodococcus* sp. CS1 might be considered as useful biotechnological tools for the treatment of different effluents contaminated with phenols.

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