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Resorcinol degradation by a *Penicillium chrysogenum* strain under osmotic stress: mono and binary substrate matrices with phenol

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Abstract A phenol-degrading *Penicillium chrysogenum* strain previously isolated from a salt mine was able to grow at 1,000 mg l^{-1} of resorcinol on solid medium. The aerobic degradation of resorcinol by *P. chrysogenum* CLONA2 was studied in batch cultures in minimal mineral medium with 58.5 g l^{-1} of sodium chloride using resorcinol as the sole carbon source. The fungal strain showed the ability to degrade up to 250 mg l^{-1} of resorcinol. Resorcinol and phenol efficiency degradation by *P. chrysogenum* CLONA2 was compared. This strain removes phenol faster than resorcinol. When phenol and resorcinol were in binary substrate matrices, phenol enhanced resorcinol degradation, and organic load decreased with respect to the mono substrate matrices. The acute toxicity of phenol and resorcinol, individually and in combination, to *Artemia franciscana* larvae has been verified before and after the bioremediation process with *P. chrysogenum* CLONA2. The remediation process was effective in mono and binary substrate systems.

Keywords Resorcinol · Phenol · Halotolerant strain · Detoxification · Biodegradation

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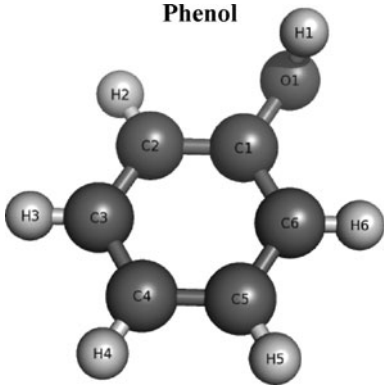
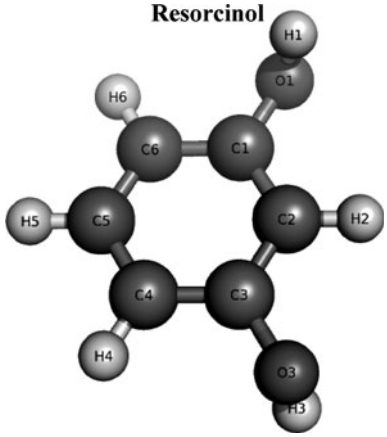
Introduction

Phenol and its derivates are well known as important environmental pollutants due their widespread occurence in nature and in several industrial wastewaters (Table 1) (Keith and Telliard 1979). Phenolic compounds are highly toxic to aquatic organisms and most of them have been recognized as carcinogens and genotoxicants (do Ceu Silva et al. 2003). Phenol has already been characterized as a major toxic pollutant; however, very little data is available about the toxicity of other phenolic compounds such as resorcinol (Kahru et al. 2000).

Resorcinol has two hydroxyl groups in the *meta* position of the phenol ring. It has been used as raw material for chemical industries and also as a solvent. It is also employed in cosmetic products as a disinfectant and exfoliating agent and therapeutically in the treatment of human acne. Several studies support the idea that multiple daily applications of resorcinol on skin could be responsible for adverse thyroid gland effects in animals and humans (Welsch 2008). Acute intoxication with resorcinol is mainly due to oral ingestion, being characterized by several symptoms like vomiting, diarrhea, nausea, methemoglobinemia, hepatic problems, pulmonary edema and depression of the central nervous system (Othmer 1995).

Resorcinol can be currently removed from waste streams by conventional methods such as physical, biological or chemical processes. Examples include activated carbon adsorption (Rodriguez et al. 2008;

Table 1 Phenol and resorcinol sources

Phenolic compound	Source	References
Phenol 	Pulp and paper mills	(Kumaran and Paruchuri 1997)
	Petrochemicals and petroleum	(Ahmaruzzaman 2008)
	Basic organic chemical manufacture	(Ahmaruzzaman 2008)
	Coal refining	(Kumaran and Paruchuri 1997)
	Tannery	(Kumaran and Paruchuri 1997)
	Textiles	(Kangsepp et al. 2009)
	Pharmaceuticals	(Busca et al. 2008)
	Pesticide	(Ahmaruzzaman 2008)
	Olive oil industry	(Ena et al. 2009)
	Cigarettes	(Rustemeier et al. 2002)
Resorcinol 	Petrochemicals	(Kumaran and Paruchuri 1997)
	Basic organic chemical manufacture	(Kumaran and Paruchuri 1997)
	Coal refining	(Kahru et al. 2002)
	Pharmaceutical	(Welsch 2008)
	Cigarettes	(Rustemeier et al. 2002)

Bayram et al. 2009; Blanco-Martinez et al. 2009), anoxic biodegradation (Godbole and Chakrabarti 1991), anaerobic biodegradation (Latkar and Chakrabarti 1994; Ngugi et al. 2005), aerobic biodegradation (Gaal and Neujahr 1979; Ngugi et al. 2005), and abiotic degradation catalyzed by birnessite (Chang Chien et al. 2009). Recently, there has been an increasing interest in the application of new technologies, namely advanced oxidation processes (AOPs), as a possible alternative method for remediation of xenobiotics pollution. These processes usually involve UV/Fenton's reagent, UV/H_2O_2, UV/O_3 or photocatalysis mediated by the use of metal oxide semiconductors like TiO_2, SnO_2, ZnO among others for the oxidative degradation of aromatic hydrocarbons including resorcinol (Lam et al. 2005; Nasr et al. 2005; Azevedo et al. 2006; Mahamuni and Pandit 2006; Yao et al. 2006; Comninellis et al. 2008; Bayram et al. 2009; Chang Chien et al. 2009; Pardeshi and Patil 2009; Jiang et al. 2010). However, all these processes have inherent limitations such as high cost of operation, generation of highly reactive radicals, low rates of degradation, low mineralization, corrosion instability or severe operating conditions (Lam et al. 2005; Comninellis et al. 2008; Yao et al., 2006; Jiang et al., 2010). Yao et al. used hydrogen peroxide as oxidizer and an enzyme from *Serratia marcescens* AB90027 to remove phenolic compounds. The degradation of phenol and resorcinol was limited; nevertheless, some phenolic compounds were completely degraded by this process (Yao et al. 2006). Pardeshi and Patil investigated the role of ZnO in the photocatalytic degradation of resorcinol; while resorcinol solutions of lower concentration were completely

mineralized, when 100 mg l^{-1} of resorcinol solution was irradiated two oxidation intermediates were detected, 1,2,4-trihydroxy-benzene and 1,2,3-trihydroxy-benzene (Pardeshi and Patil 2009). The use of boron-doped diamond anodes for the treatment of aqueous wastes polluted with the three dihydroxybenzenes showed to be effective, since complete mineralization is achieved (Nasr et al. 2005); however, when 94.1 mg l^{-1} of phenol was treated by this process only 78% of xenobiotic was eliminated (Jiang et al. 2010). Ozonation is a complex and expensive method for the treatment of phenol in contaminated waters that it is only effective in low salinity media (2 g l^{-1} of sodium chloride), whilst at higher salinity levels of 50 g l^{-1} of sodium chloride, highly toxic compounds are produced (Azevedo et al. 2006). Sonication for phenol degradation in the presence of salt has proved to be an attractive process in terms of costs and operating conditions, however several intermediates, including resorcinol are generated (Mahamuni and Pandit 2006).

Literature surveys show that there are a limited number of microorganisms, especially bacteria, able to aerobically degrade resorcinol (Godbole and Chakrabarti 1991; Latkar and Chakrabarti 1994; Kurtz and Crow 1997; Ngugi et al. 2005). The salinity in most industrial effluents is an inhibiting factor that can be even more toxic or stressful than the phenolic compounds (Kargi and Dinçer 1999). The use of salt-adapted microorganisms that are tolerant to high salinities and that remove pollutants from saline effluents may prevent the necessity of salt removal by costly physicochemical methods (Lefebvre and Moletta 2006). Phenol degrading microorganisms, such as *Halomonas* sp., *Candida tropicalis*, *Alcaligenes faecalis* and *Penicillium chrysogenum* able to degrade high amounts of phenol under osmotic stress have been described (Hinteregger and Streichsbier 1997; Bastos et al. 2000; Maskow and Kleinsteuber 2004; Leitão 2009). The evaluation of the chemical and ecotoxicological data allowed prediction of the effect of the raw effluent on the treatment plant and the impact of the final treated effluent on the receiving water (Guerra 2001).

As far as we know this is the first report wherein the ability of a fungal strain to aerobically remove resorcinol in a saline environment is described. The aim of this study is to assess the efficiency of a *Penicillium chrysogenum* strain (Leitão et al. 2007) in removal of phenol and resorcinol at high salinity (58.5 g l^{-1} of sodium chloride). The decrease of toxicity has been tested using *Artemia franciscana*, a marine organism of trophic level 2.

Materials and methods

Strain

P. chrysogenum CLONA2, identified according to morphological and biochemical characteristics, was isolated from a salt-mine in Algarve (Portugal), as described previously. Isolation and enrichment procedures were carried out in a medium containing 58.5 g l^{-1} of sodium chloride and the strain showed the ability to degrade phenol in mineral medium (Leitão et al. 2007).

Chemicals

The phenol and resorcinol used in this study were of chromatographic grade (purity ≥99%), and were obtained from Sigma-Aldrich (St. Louis, USA). HPLC acetonitrile was obtained from Lab-Scan (Dublin, Ireland). All other reagents are of analytical-reagent grade and obtained from Riedel-de Haën (Seelze, Germany). Water purified by a Mili-Q system was used in all the experiments and nutrient agar (NA) was purchased from Difco (Detroit, USA).

Resorcinol and phenol effect on growth in solid medium

To investigate the effect of resorcinol and phenol on *Penicillium* growth, Czapek Dox Agar (CDA) without sucrose and supplemented with 5.9% NaCl was used. Agar plates containing 0, 50, 100, 200, 300, 500 and 1000 mg l^{-1} of phenolic compounds were incubated at 25 ± 1°C for 7 days. Apical extension rates were determined according to the method of Ivey et al. (1996).

Inoculum development

The strain was maintained at 4°C on nutrient agar (NA) plates. Precultures of cells were routinely aerobically cultivated in 100 ml of sterile complex

medium (MC) for 3 days. The MC composition was as follows (per liter): 30.0 g glucose, 3.0 g $NaNO_3$, 0.5 g $MgSO_4 \cdot 7H_2O$, 10 mg $NH_4Fe(SO_4)_2 \cdot 12H_2O$, 1.0 g K_2HPO_4, 5.0 g Yeast extract, 58.5 g NaCl and pH was adjusted to 5.6–5.8 with 5 mol l^{-1} HCl.

All liquid cultures in these experiments were incubated at 25 ± 1°C in an INNOVA 4000 Incubator Shaker (New Brunswick Scientific, New Jersey, USA) operating at 160 rpm in the dark in order to avoid photo-degradation of aromatic compounds.

Resorcinol and phenol biodegradation test

The efficiency of resorcinol and phenol degradation by the fungal strain was investigated by inoculating 10% of *Penicillium* preculture in 250 ml Erlenmeyer flasks containing 50 ml of mineral medium with different concentrations of aromatic compounds. The mineral medium (MMFe) had the following composition (per liter): 1.0 g K_2HPO_4, 1.0 g $(NH_4)_2SO_4$, 200 mg $MgSO_4 \cdot 7H_2O$, 33 mg $FeCl_3 \cdot 6H_2O$, 100 mg $CaCl_2$, 58.5 g NaCl. The pH was adjusted to 5.6–5.8 with 5 mol l^{-1} HCl. All experiments were carried out with 275 mg l^{-1} of phenolic compounds except when especially clarified. Three replicates were used per test of aromatic compound concentration. Uninoculated control flasks (duplicates) were incubated and aerated in parallel as negative controls of the experiment. Growth, phenol and resorcinol concentrations were monitored up to an incubation time of 96 h.

Analytical methods

Microbial dry biomass was estimated gravimetrically by the method described by Gunther et al. 1995. Resorcinol and phenol concentrations were quantified by High Performance Liquid Chromatography (L-7100 LaChrom HPLC System, Merck), equipped with a quaternary pump system, and L-7400 UV detector according to a previously published method (Leitão et al. 2007). Aromatic compounds could be separated and concentrations estimated within 10 min.

For better comparison of resorcinol and phenol biodegradation efficiency, aromatic degradation rate (ADR) was determined by the following equation:

$$\mathrm{ADR} = \frac{\Delta S}{\Delta t}$$

Kinetic data for the aromatic compound degradation could be fitted according to the Monod model:

$$V = V_{\max} \frac{S}{K_S + S}$$

where: V is the specific aromatic compound degradation rate, $V_{\max}$ the maximum specific aromatic compound degradation rate, K_S the half saturation constant, ΔS the decrease substrate concentration, Δt is the elapsed time.

The increment of biomass was assumed to be not significant when phenolic compounds were used as sole carbon and energy source.

The organic carbon of the aromatic compound batch culture was estimated by determining the decay of the dissolved organic carbon (DOC) measured after filtration (Millipore 0.2 μm nylon filter) using a TOC analyzer (TOC 5000, Shimadzu).

A respirometric BOD method was used to monitor the biodegradation of phenolic compounds over 5 days, using a OxiTop® measuring system (WTW, Germany) to monitor the drop of gas pressure in the closed system, as a consequence of oxygen consumption during biodegradation and entrapment of produced carbon dioxide in a basic solution. All experiments were performed in reactors consisting of measuring heads and glass bottles (510 ml nominal volume) with a carbon dioxide trap (approximately 0.3 g of NaOH was added in each trap) with 164 ml of sample volume (MMFe with 10% of inoculum supplemented with 275 mg $l^{-1}$of phenolic compounds) incubated at 20.0 ± 1°C in darkness. Decrease in headspace pressure inside the reactor was continuously and automatically recorded. Controls were maintained for all experiments, with 164 ml of MMFe with 10% of inoculum and without phenolic compounds, and others with 164 ml of uninoculated medium. Three individual experiments were performed, samples were done in triplicate and controls in duplicate.

Acute toxicity test

A 24-h LC50 bioassay was performed using instar II-III larvae of the brine shrimp *A. franciscana* according to the standardized ARTOXKIT M™ procedure

using the instructions from the manufacturer. A reconstituted sample of normal seawater salinity (35‰) prepared from the kit reagents was used as the hatching medium for the cysts and the dilution medium for the toxicant dilution series (ASTM Standard Guide E1440-91).

A reference test with $K_2Cr_2O_7$ was regularly performed as a sensitivity control of the test population (Persoone 1992).

Mono and binary systems

Experimental studies were carried out with Erlenmeyer flasks as batch reactors. A sample of 50 ml was taken in each 250 ml Erlenmeyer flask. Each sample contained MMFe medium with different concentrations of resorcinol, phenol, or a mixture of both as a carbon source. The Erlenmeyer flasks were maintained at 25 ± 1°C and shaken at 160 rpm, until concentrations of aromatics were less than 2 mg l^{-1}.

Results and discussion

Effect of the resorcinol and phenol on *Penicillium* growth in solid medium

Experiments on solid medium were performed to determine to what extent growth of *P. chrysogenum* CLONA2 was affected by the presence of resorcinol and phenol. Parallel assays were carried out without the addition of phenolic compound (Fig. 1). When the fungal strain was inoculated into medium with 50 mg l^{-1} of resorcinol, slight growth was observed. The results show that for all investigated resorcinol concentrations only the sample treated with 1,000 mg l^{-1} was characterized by a clear decrease of fungus growth. Therefore, the resorcinol system is characterized by a near-constant apical extension rate. When *P. chrysogenum* CLONA2 was inoculated in the presence of phenol, then a negative effect on its growth was observed. As phenol concentrations increased, then growth (apical extension) decreased. An inhibition of 26.3, 78.6 and 92.8% was observed on fungal strain growth, with 100, 200 and 300 mg $l^{-1}$of phenol, respectively, as compared to control medium without phenol. Concentrations of phenol higher than 300 mg l^{-1} (500 and 1000 mg l^{-1}) completely inhibited fungal growth (Fig. 1). These results showed that

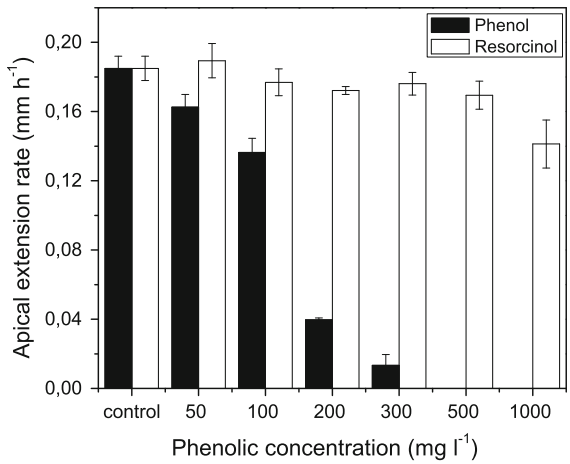


Fig. 1 Comparison of the apical extension rates (mm h^{-1}) of *P. chrysogenum* CLONA2 in CDA medium without sucrose and supplemented with different concentrations of resorcinol and phenol

phenol has a more toxic effect than resorcinol. Recently, a study on the toxic effects of dihydroxyphenolic compounds (resorcinol, catechol, and hydroquinone) on soil microbial activity reported that addition of high concentrations of these compounds to the soil samples resulted in low microbial counts (Chen et al. 2009).

Resorcinol biodegradation

Batch cultures with resorcinol as sole carbon source were performed in order to determine its effect on *P. chrysogenum* CLONA2 growth and xenobiotic removal capacity in liquid media (MMFe) (Fig. 2). Since no abiotic loss of resorcinol was detected in controls, the decrease of resorcinol concentration in presence of fungal cultures can be entirely attributed to biodegradation. The results demonstrate that *Penicillium* can utilize resorcinol as the sole carbon and energy source. Meanwhile, no significant increase of biomass was observed during the first 80 h of culture, suggesting that in experiments with 275 mg l^{-1} of resorcinol no correlation exists between the decrease of resorcinol concentrations in culture and the production of biomass. After 80 h of incubation an increase in biomass was observed with a value of 1.63 g l^{-1} at 100 h of culture. This may indicate that the fungus firstly conducts all the energy for the production of resorcinol degradation enzymes. During this time no significant growth was observed

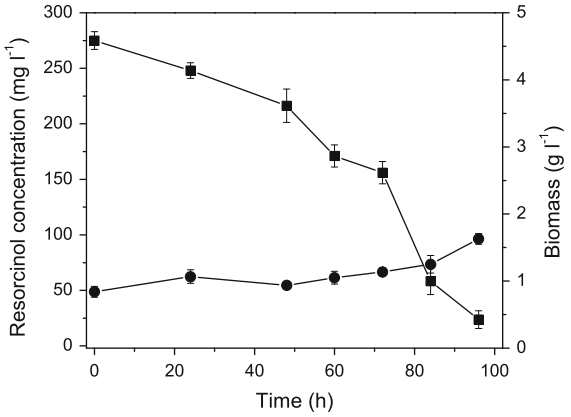


Fig. 2 Resorcinol removal and *P. chrysogenum* CLONA2 growth in mineral medium with 275 mg l^{-1} of resorcinol and 58.9 g l^{-1} of sodium chloride: *filled squares* indicate resorcinol concentration; *filled circles* indicate biomass concentration

and biomass concentration remains constant. It seems unlikely that the use of the available energy for maintaining cellular integrity would be the explanation for these biomass values. Similar results of no growth were described for phenol biodegradation processes (Godsy et al. 1992; Leonard and Lindley 1998). On the other hand, there are microorganisms that are able to use phenol for growth but not other phenolic compounds. For instance, *Arthrobacter* sp. AG31 a cold tolerant bacterium and *Pseudomonas putida* DSM6414, a mesophilic microorganism, are also not able to utilize resorcinol for growth; however, are able to utilize phenol, amongst other aromatic hydrocarbons (Margesin et al. 2004).

It has been previously reported that BOD method is reliable and suitable for small-scale studies of xenobiotic degradation because it continuously measures the biological oxygen demand (Fialová et al. 2004). BOD experiments were carried out in MMFe medium supplemented with resorcinol and phenol. The assays were performed according to manufactures instructions. The results are presented with resorcinol and phenol (Table 2). During the first 2 days of incubation under both conditions there was a clear lag-phase. In the assay performed with resorcinol as the sole carbon and energy source, the lag-phase was longer than with phenol. It is known that during the lag-phase, inducible enzymes are synthesized and in this period xenobiotic concentration decreases slowly (Kamath and Vaidyanathan 1990). This is in agreement with the profile of resorcinol degradation by *Penicillium* strain

Table 2 Respirometric assay with resorcinol or phenol as the sole carbon source during 8 days of incubation

Aromatic compound (275 mg l^{-1})	Time (d)					
	1	2	3	4	5	8
Resorcinol BOD[a]	23	56	120	190	240	340
Phenol BOD[a]	36	80	160	230	300	420
Control[b] BOD[a]	65	125	160	180	195	240

[a] Biochemical oxygen demand at time *t* (mg l^{-1})

[b] Without addition of aromatic compound

presented in Fig. 2. After the lag-phase an increase in oxygen consumption is observed. At fourth day of incubation, oxygen consumption is higher in cultures supplemented with phenol and resorcinol than in the controls without phenolic compounds. Fialová et al. (2004) mention a "substrate stress" phenomenon characterized by increased oxygen consumption for the maintenance of cellular functions when microorganisms are in the presence of substrates like phenols. It is seen that in phenol assays oxygen consumption rates are higher than in resorcinol, presumably because phenol biodegradation is accompanied by an increase in the phenol catabolism or because this compound is more readily removed and consequently the respiration rate is higher.

Decrease of organic load

The results of batch cultures with resorcinol or phenol as the sole carbon source in mineral medium (MMFe), dissolved organic carbon, ADR efficiency, and pH after 84 h, are summarized in Table 3. The ADR efficiency is higher in cultures with phenol than with resorcinol. The phenol cultures also show strongest decrease of dissolved organic carbon, demonstrating the ability of the strain to remediate phenol contamination. Reductions of dissolved organic carbon from 275 to 83.1 mg l^{-1} are observed for resorcinol cultures. No significant differences in final pH of both cultures were observed. The presented data suggest that *P. chrysogenum* CLONA2 is more efficient in the biodegradation of phenol than of resorcinol. This result is unexpected as the inhibitory effect on fungus growth was higher for phenol than resorcinol; despite the higher solubility of resorcinol in water as compared to phenol. Presumably, resorcinol degradation may be inducible. Indeed, the ascomycetous yeast species, *Candida*

Table 3 Values of different parameters from resorcinol and phenol degradation batches after 84 h of incubation

Compound	Initial concentration, S_0 (mg l^{-1})	Organic carbon (mg l^{-1})	η[a]	pH
Resorcinol	275	83.1	69.8	5.93
Phenol	275	64.1	76.7	5.95

[a] ADR efficiency

Table 4 Kinetic parameters in Monod-Michaelis Menten equation for resorcinol and phenol degradation

Phenolic compound	S_0 (mg l^{-1})	K_S (mM)	R
Resorcinol	50–300	11.27	0.9907
Phenol	50–300	2.66	0.9945

tropicalis HP15, was reported to metabolize phenol via the β-ketoadipate pathway by an inducible enzyme system (Krug et al. 1985).

Kinetic parameters

To further elucidate that phenol is more easily degraded than resorcinol by *P. chrysogenum* CLONA2, kinetics parameters for resorcinol and phenol biodegradation were estimated from experiments carried out with different initial concentration of both compounds, ranging from 50 to 300 mg l^{-1}. Several mathematical models are available for calculating biodegradation rates. The Monod-Michaelis Menten, assuming that the biomass stays constant, provides the half saturation constant (Table 4). As shown, a higher half saturation constant is observed for resorcinol as substrate indicating that CLONA2 strain prefers phenol to resorcinol as substrate. In previous studies we have argued that *P. chrysogenum* CLONA2 degrades phenol by a catabolic pathway involving sequential transformations via catechol and hydroquinone (Leitão et al. 2007). Structurally, catechol, hydroquinone and resorcinol are similar; they only differ in the relative positions of the hydroxyl groups within the benzene ring. Yao et al. (Yao et al. 2006) reported that the position of hydroxyls of phenolic compounds is an important factor for the biodegradation process. Catechol and hydroquinone can be readily converted to benzoquinones, while the degradation of resorcinol needs an additional hydroxylation process or hydroxyl transfer (Yao et al. 2006).

Mono and binary substrate systems

Industrial wastewater generally contains a complex mixture of compounds, some of them structurally related. In Table 1 several sources of both phenol and resorcinol residues are presented. Therefore, the removal capacity of resorcinol and phenol by *P. chrysogenum* CLONA2 in binary substrate systems was analyzed. Experiments were performed in mineral medium (MMFe) with different concentrations of both compounds (200 mg l^{-1} of phenol, 200 mg l^{-1} of resorcinol, and 100 mg l^{-1} of phenol and resorcinol). Figure 3 shows that degradation of 200 mg l^{-1} of phenolic compounds is faster when resorcinol and phenol are simultaneously present in culture media. Hamed et al. (2003) reported a study of substrate interactions during the biodegradation of benzene, toluene and phenol mixtures. The presence of phenol increased the biodegradation time of benzene; meanwhile the presence of benzene decreased the biodegradation times of phenol. In their study, phenol biodegradation started when benzene was consumed completely. According to the authors higher cell concentrations relative to the initial, helped the microorganism to degrade phenol faster in binary system than when it was the sole carbon source. In the present work, biodegradation of resorcinol starts prior to complete degradation of phenol (Fig. 4) and significant cell growth in the phenol and resorcinol mixture was not observed. The enhance of the phenolic degradation in this binary system could be related to the presence of an enzymatic system able to use phenol and resorcinol as substrates.

HPLC analysis of culture extracts of the fungal strain grown on MMFe supplemented with 200 mg l^{-1} of resorcinol and 100 mg l^{-1} of phenol and resorcinol indicates that initially resorcinol removal was delayed for binary substrate as compared to mono substrate (Fig. 4). The percentage of resorcinol removal with respect to initial resorcinol concentration was about 2.5-fold higher in resorcinol than in resorcinol and phenol supplemented cultures at 24 h of fermentation. In the binary substrate system phenol was removed first, and after 24 h, the differences were more evident between the removal rates of both phenolic compounds (Fig. 4). These results are in

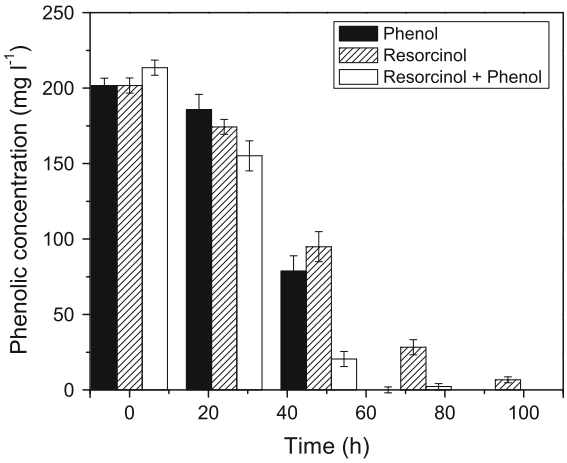


Fig. 3 Aerobic degradation of 200 mg l^{-1} of resorcinol, 200 mg l^{-1} of phenol, and 100 mg l^{-1} of resorcinol plus 100 mg l^{-1} of phenol by *P. chrysogenum* CLONA2

agreement with the kinetic data. A residual concentration around 1.0 mg l^{-1} of resorcinol was observed after 96 h of incubation in all systems. The summary of phenolic compound degradation for mono and binary substrate systems at 48 h is represented in Table 5. Data show that the percentage of phenolic compounds degraded increases with higher initial concentration of resorcinol, and that the opposite happens with phenol. The percentage of resorcinol and phenol degradation for binary substrate systems is higher than for mono substrate systems. The efficiency to remove the organic load is almost twice that of the resorcinol mono systems.

Ecotoxicological test

In order to evaluate *Penicillium* degradation efficiency on detoxification, toxicity assays using *A. franciscana* were performed. According to Kahru et al. (2000), crustacean and/or photobacterial tests (*Photobacterium phosphoreum*, Microtox, *Daphnia magna*, *Thamnocephalus platyurus*, *Tetrahymena thermophila*, *Selenastrum capricornutum*) were most sensitive and exhibited complementary sensitivity patterns. In their study, results obtained with Algaltoxkit and Protoxkit, commercial available kits, were less sensitive towards resorcinol contamination (Kahru et al. 2000). As the present study was done in saline environment, for this assay, *A. franciscana* was the organism selected. *A. franciscana* can be a useful

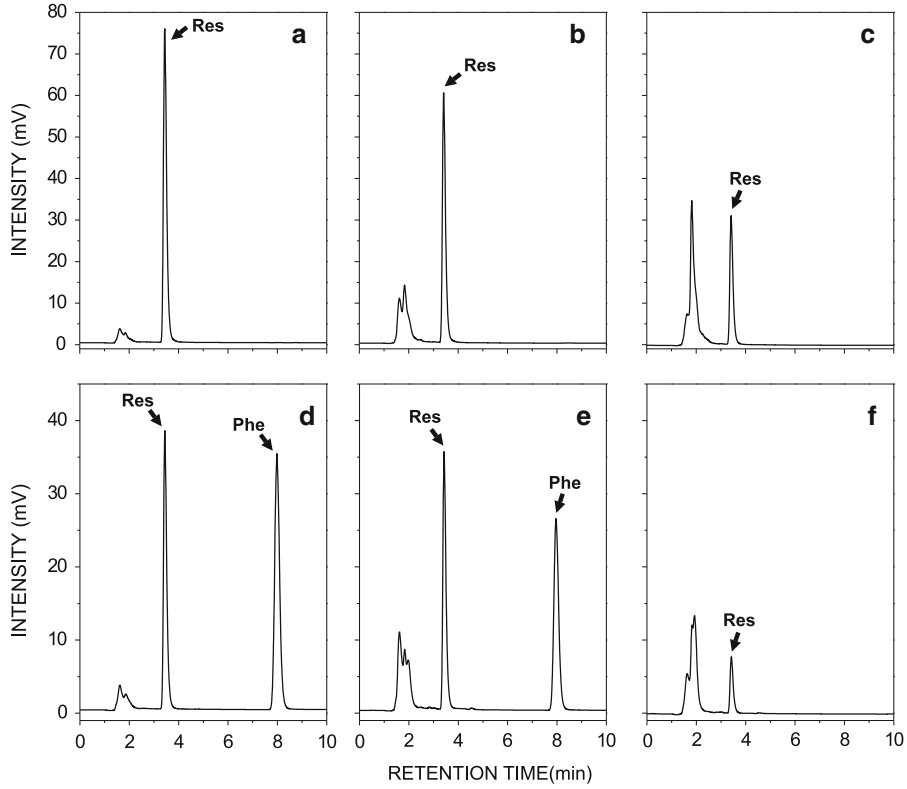


Fig. 4 HPLC analysis of culture broths at 0 h (*Panels* a and d), 24 h (*Panels* b and e), and 50 h (*Panels* c and f) of *P. chrysogenum* CLONA2 containing 200 mg l^{-1} of resorcinol (*upper panels* a, b and c) or mixture of 100 mg l^{-1} of resorcinol plus 100 mg l^{-1} of phenol (*lower panels* d, e and f). The resorcinol and phenol peaks (identified by internal controls) are represented by *arrows* with the labels Res and Phe respectively

Table 5 Effect of mono and binary substrate systems on resorcinol and phenol degradation at 48 h of culture

Compound	Organic carbon[a] (mg l^{-1})	Concentration aromatic compound[a] (mg l^{-1})	Degradation[a] (%)	Degradation[b] (%)
Resorcinol	78.8	71.0	60.4	41.2
Phenol	67.2	53.7	71.3	96.0
Resorcinol/Phenol	42.6	Resorcinol—24.0 Phenol—4.0	Resorcinol—72.6 Phenol—95.8	n.d.

[a] From a batch with starting concentration of 200 mg l^{-1} of aromatic compound

[b] From a batch with starting concentration of 100 mg l^{-1} of aromatic compound

n.d. not determined

Table 6 Biological effects of phenolic compounds on *P. phosphoreum*, *D. magna*, *T. platyurus* and *A. franciscana*

Compound	Microtox[a] 5 min EC50 (mg l^{-1})	*D. magna* 48-h LC50 (mg l^{-1})	*T. platyurus*[b] 24-h LC50 (mg l^{-1})	*A. franciscana* 24-h LC50 (mg l^{-1})
Resorcinol	264	0.2–910		12.0 (6.5–14.0)
Phenol	42.0	8.3–520		28.0 (27.0–32.0)

[a] Kaiser and Palabrica (1991)

[b] Kahru et al. (2000)

tool in assessing the toxicity of brackish effluents (Guerra 2001).

High average levels of toxicity expressed by LC50 24-h were found in cultures containing phenol and resorcinol during the initial part of the batch tests. Despite the high percentage of removal of resorcinol, samples taken from the batch at 72 h were toxic. At this time toxicity was not detected in the phenol batch and in the batch with phenol/resorcinol mixture.

There is a relation between the biodegradability of phenolic compounds and their toxicity. From Table 6, it is evident that phenol is less toxic compound than resorcinol, and that there is a relationship between biodegradability and toxicity. The LC50 value obtained with *A. franciscana* is within the intervals of values reported by Kahru et al. (Kahru et al. 2000).

Conclusions

In this research the resorcinol removal efficiency of *Penicillium* strain under high saline conditions was studied. *P. chrysogenum* CLONA2 showed the ability to degrade resorcinol as the sole carbon and energy source under osmotic stress. A comparison of this strains capacity to remove resorcinol or phenol showed that the degradation of resorcinol was inferior to that of phenol. Our findings indicate that mixtures of resorcinol and phenol are more toxic for *A. franciscana* than the individual phenolic compounds. Nevertheless, the percentage of resorcinol and phenol degradation on binary substrate systems is higher than for mono substrate, evidenced by a more significant reduction of organic load. More studies need to be done in order to see if the potential for resorcinol detoxification on solid matrices differs from liquid ones, as *P. chrysogenum* CLONA2 showed tolerance and growth at resorcinol concentrations up to 1000 mg l^{-1} on solid substrate. In summary it has been shown that *P. chrysogenum* CLONA2 may find application in remediation of phenol and resorcinol contaminated saline water.

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