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Biosorption of organochlorine pesticides using fungal biomass

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***Cladosporium* strain AJR^{3}18,501 was tested for its ability to sorb the organochlorine pesticide (OCP) *p,p′*-DDT from aqueous media. When *p,p′*-DDT was added to distilled water, ethanol or 1-propanol solutions in excess of its solubility, *p,p′*-DDT was sorbed onto the fungal biomass. Increasing the amount of *p,p′*-DDT in solution by changing the medium composition increased sorbent uptake: *p,p′*-DDT uptake by the fungal biomass was 2.5 times greater in 25% 1-propanol (17 mg of *p,p′*-DDT g^{-1} dry weight fungal biomass) than in distilled water. When *p,p′*-DDT was dissolved in 25% 1-propanol (12 mg l^{-1}), rapid *p,p′*-DDT sorption occurred during the first 60 min of incubation. *p,p′*-DDT in solution was reduced to 2.5 mg l^{-1} with the remaining *p,p′*-DDT recovered from the fungal biomass. A number of environmental parameters were tested to determine their effect on *p,p′*-DDT biosorption. As arsenic (As) is prevalent at DDT-contaminated cattle dip sites, its effect on *p,p′*-DDT uptake was determined. The presence of As [As(III) or As(V) up to 50 mg l^{-1}] did not inhibit *p,p′*-DDT uptake and neither As species could be sorbed by the fungal biomass. Changing the pH of the medium from pH 3 to 10 had a small effect on *p,p′*-DDT sorption at low pH indicating that an ion exchange process is not the major mechanism for *p,p′*-DDT sorption. Other mechanisms such as Van der Waals forces, chemical binding, hydrogen bonding or ligand exchange may be involved in *p,p′*-DDT uptake by *Cladosporium* strain AJR^{3}18,501.**
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Introduction

1,1,1-Trichloro-2,2-*bis*-(*p*-chlorophenyl)ethane (DDT) is an organochlorine pesticide (OCP) that was used extensively during the Second World War to control insect typhus and malaria vectors. After the war, DDT continued to be used as a residual spray for the eradication of malaria and as a delousing dust for typhus control as well as to control hundreds of insect pests associated with agricultural practices [17]. In 1972, DDT was banned from use in the United States due to the organochlorine exhibiting toxic effects towards nonpest invertebrates and the persistence of the compound in soils and aquatic sediments [6]. In addition, significant quantities of DDT were found to accumulate in various plant and animal tissues [7,8,24,25]. As a consequence, there were increasing concerns about the accumulation of the organochlorine in the food chain and the possible effects this may have on human health. In developed countries, the use of DDT was progressively restricted or phased out; however, DDT is still being used today in a number of developing countries.

In tropical and subtropical regions of Australia, DDT was used extensively between 1957 and 1962 for the eradication of cattle and sheep ticks. As a consequence of the dipping and disposal practices, soil surrounding the dip sites was contaminated with DDT [5]. Although the use of DDT at cattle and sheep dip sites ceased almost 40 years ago, the pesticide still persists in these soils today [16]. In addition, these sites contain significant quantities of arsenic (up to 3000 mg kg^{-1}) [5,19] as a consequence of tick eradication methods prior to DDT use. The remediation of dip sites comes as a direct response to the encroachment of residential development close to old dip sites, which has raised many questions about the human safety factor.

The remediation of DDT-contaminated soil has met with a number of problems. Physicochemical remediation processes, such as thermal destruction, may be used for soil cleanup; however, these techniques are prohibitively expensive. Bioremediation of DDT-contaminated soils has been unsuccessful due to the recalcitrant properties of the compound i.e., low aqueous solubility, high hydrophobicity, high degree of chlorination. When degradation does occur, DDT degradation rates are extremely slow and the resultant transformation products (i.e., DDD and DDE) are more toxic and recalcitrant than the parent compound [1,15]. Recently Juhasz and Smith [12] demonstrated the effectiveness of co-solvent washing on the desorption of DDT from contaminated soil. Co-solvents such as ethanol and 1-propanol enhanced the solubility of DDT and remove the OCP from a number of soil types [12]. The combination of co-solvent washing and biosorption may offer an attractive alternative for the remediation of DDT-contaminated soils.

Biosorption is a process where biological material is used to remove (adsorb/absorb) contaminants from waste streams. Biosorption has been used as an alternative technology for removing toxic heavy metals from waste effluents [23]; however, its use for removing organic contaminants from waste streams has received less attention. For the remediation of OCP-contaminated soil, the first step in the process would be to provide the contaminant in an available form for the biosorbent. This may involve a soil-washing process that utilises surfactants or co-solvents to solubilise the OCPs. The soil-wash solutions may then be passed through biological filters containing the biosorbent for the removal of the OCPs from solution. Biosorption offers many advantages over conventional remediation options. The process is rapid, has no nutritional requirements and DDT transformation products are not generated. In addition, a low operating cost is associated with the

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production of biomass and the co-solvent or surfactant washing solutions may be recycled.

Recently, *Cladosporium* strain AJR[3]18,501, isolated from DDT-contaminated soil, was shown to possess the ability to biosorb *p,p′*-DDT [9]. The experiments outlined in this paper were performed in order to determine the effect of potential soil-wash solutions and environmental parameters (such as pH and arsenic) on *p,p′*-DDT biosorption by strain AJR[3]18,501.

Materials and methods

Chemicals

p,p′-DDT and pentachloronitrobenzene (PCNB) were purchased from Aldrich Chemical (Sydney, Australia). All other chemicals and media were purchased from Sigma Chemical (Sydney, Australia). All of the solvents and chemicals were instrument grade reagents.

Stock solutions and media

Stock solutions of *p,p′*-DDT were prepared in dimethylformamide (DMF) at a concentration of 10 or 50 mg ml^{-1} and stored in the dark at 4°C. PCNB was prepared in dichloromethane (DCM) at a concentration of 1 mg ml^{-1} and stored in the dark at 20°C. Potato dextrose broth (PDB) and agar (PDA) were prepared according to the manufacturers instructions (Sigma).

Preparation of fungal inocula

The source of *Cladosporium* strain AJR[3]18,501 and the isolation procedure used has been described elsewhere [11]. Fungal inocula were prepared by growing *Cladosporium* strain AJR[3]18,501 on PDA at 25°C for 7 days. Following growth, plates were flooded with PDB (20 ml) and gently agitated to suspend the fungal spores. Aliquots of the spore suspension (10 ml) were used to inoculate 400 ml of PDB. Incubation was performed in a shaking incubator at 25°C and 150 rpm. After three days, fungal biomass was collected by filtration (Whatman No. 2 filter paper) and washed twice in sterile phosphate buffer (pH 7). Collected biomass was prepared for biosorption studies as outlined by Juhasz and Naidu [11]. Briefly, collected biomass was air dried overnight at room temperature before particle size separation. Mycelial balls were separated into two size classifications (<2.0 but >1.4 mm and <1.4 but >0.5 mm in diameter) by sieving the mycelia sequentially through 2-mm and 1.4-mm sieves. The larger mycelia size fraction (<2.0 but >1.4 mm in diameter) was used for biosorption studies.

Biosorption experiments

Biosorption experiments were performed to determine the extent of *p,p′*-DDT adsorption to fungal biomass. Fungal mycelia (100 mg) were added to sterile serum bottles (30 ml) to which sterile distilled water (10 ml) was added. Serum bottles were sealed with neoprene stoppers and crimped with an aluminium lid. *p,p′*-DDT was injected into the bottles to achieve a final concentration ranging from 10 to 375 mg l^{-1}. In these experiments, the concentrations of *p,p′*-DDT added to the distilled water were far in excess of its aqueous solubility. To enhance the amount of *p,p′*-DDT in the aqueous phase, further experiments were conducted using dilute primary alcohols as the supporting medium. Experiments were conducted as described above; however, fungal biomass was added to serum bottles containing 25% or 50% ethanol or 1-propanol and *p,p′*-DDT. Control cultures consisted of uninoculated *p,p′*-DDT-containing media as well as *p,p′*-DDT-containing media inoculated with killed fungal biomass (autoclaved three times at 121°C for 20 min on three successive days). All cultures were prepared in triplicate for each sample point. Cultures were incubated at 25°C by shaking at 150 rpm in the dark for up to 5 days. At each sampling time point, whole cultures were sacrificed for organochlorine extraction and analysis.

Effect of pH and arsenic on p,p′-DDT biosorption

The effect of pH and the presence of As in solution were tested to determine whether these parameters affected *p,p′*-DDT sorption by the fungal biomass. pH significantly influences the sorption of metal cations [14,26,27]; however, few studies have determined its influence on organic compound sorption. Since As is prevalent in cattle dip site soil, its influence on *p,p′*-DDT biosorption was also studied. Biosorption experiments were prepared in the same manner as described above with the exception of the supporting medium. The effect of pH on *p,p′*-DDT biosorption was evaluated by testing fungal *p,p′*-DDT uptake at pH values between 3 and 10. Phosphate buffers (pH 3.0, 5.5, 7.0, 8.5 and 10.0) were used as the medium to which *p,p′*-DDT was added at a concentration of approximately 80 mg l^{-1}. Cultures were incubated for 50 h before determination of the distribution of *p,p′*-DDT between the medium and the biomass.

The effect of As on *p,p′*-DDT biosorption was investigated by using distilled water containing either As(III) ($NaAsO_2$) or As(V) (Na_2HAsO_4) salts as the medium to which approximately 80 mg l^{-1} of *p,p′*-DDT was added. Arsenic was added at concentrations ranging from 1 to 50 mg l^{-1}. Cultures were incubated for 24 h before determination of the distribution of *p,p′*-DDT between the medium and the biomass. In addition, As concentrations in the medium were determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Extraction of p,p′-DDT from fungal cultures

Extraction of *p,p′*-DDT from fungal cultures was performed according to the method outlined by Juhasz and Naidu [11]. Briefly, DCM was used as the extracting solvent while PCNB (1 mg ml^{-1} in DCM) was used as the internal standard. Fungal mycelia were separated from culture fluid by filtration through a stainless steel sieve (0.5-mm grid size) containing glass wool. The two phases (aqueous and mycelia phases) were extracted separately. *p,p′*-DDT was extracted from the aqueous phase with DCM (5 ml) after the addition of PCNB (100 μl). *p,p′*-DDT from fungal mycelia was extracted ultrasonically using a Misonix ultrasonic processor (Farmingdale, NY) after HCl digestion for 6 h at 60°C. DCM extracts (50–150 μl) were transferred to brown glass sample bottles (2.0 ml), diluted appropriately and stored at −20°C until analysed by gas chromatography (GC).

Analytical procedures

GC analysis of *p,p′*-DDT DCM extracts and OCP standards was performed on a Perkin-Elmer chromatograph (San Jose, CA) equipped with an electron capture detector (GC-ECD), using a DB-5 narrow-bore column (30 m×0.25 mm ID; J & W Scientific, Folsom, CA). The oven temperature was programmed at 200°C for 1 min, followed by a linear increase of 10°C min^{-1} to 250°C, holding at 250°C for 4 min. The injector temperature was 300°C and the detector temperature was 260°C. Arsenic in solution was determined by ICP-OES with a limit of quantification of 50 $\mu g\ l^{-1}$.

Calculation of p,p′-DDT recovery and sorbate uptake

The amount of *p,p′*-DDT recovered from fungal cultures (live or autoclaved) was calculated by adding *p,p′*-DDT concentrations detected in the aqueous phase and mycelial phase (the sum of *p,p′*-DDT concentrations from glass wool and sieve fractions). Percentage *p,p′*-DDT recovery was calculated with reference to concentrations detected in uninoculated *p,p′*-DDT media. The results shown are the average of three individual samples.

Sorbate uptake was calculated using Eq. (1):

$$q = \frac{V(C_i - C_f)}{S} \quad (1)$$

where q=sorbate uptake, V=volume of liquid (l), C_i=initial concentration of *p,p′*-DDT in the medium, C_f=final concentration of *p,p′*-DDT in the medium (mg l^{-1}), and S=sorbent amount (g) [26]. C_i and C_f represent the sum of dissolved and solid phase *p,p′*-DDT concentrations in the medium.

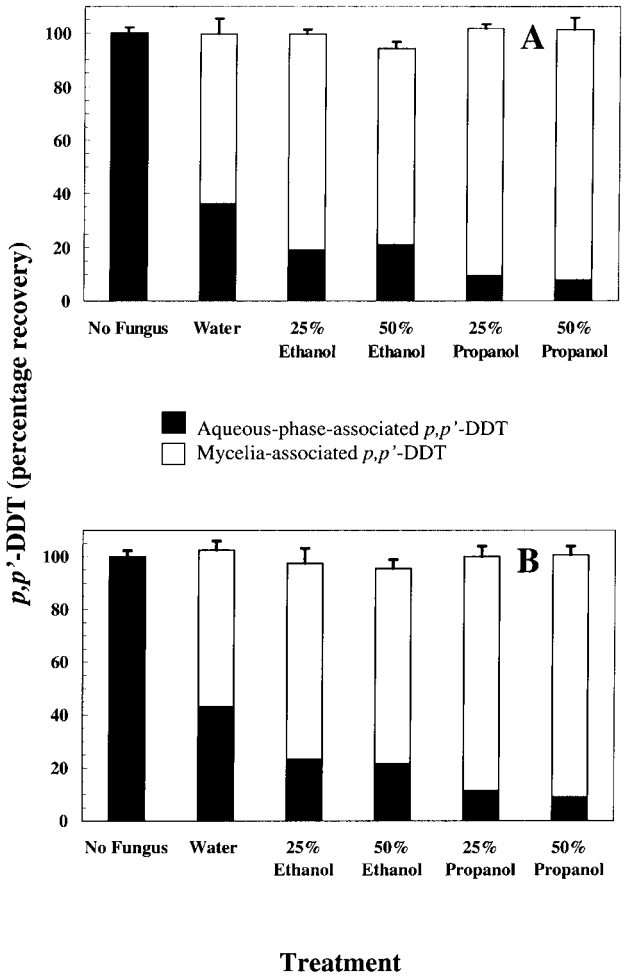

Figure 1 Percentage recovery of *p,p′*-DDT from culture fluid and fungal biomass after incubation of *Cladosporium* strain AJR^{3}18,501 in distilled water containing 90 mg l^{-1} of *p,p′*-DDT. Aqueous-phase-associated *p,p′*-DDT and mycelia-associated *p,p′*-DDT from live (A) and killed (B) biomass cultures were determined after 50 h. Percentage recovery of *p,p′*-DDT in inoculated cultures was calculated relative to the amount recovered from the uninoculated control medium (100%).

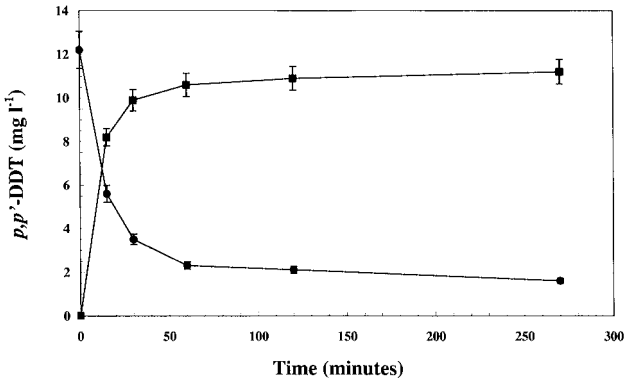

Figure 2 Biosorption of *p,p′*-DDT from 25% 1-propanol by *Cladosporium* strain AJR^{3}18,501. The concentration of *p,p′*-DDT associated with the aqueous phase (●) and mycelia (■) was determined after dichloromethane extraction and GC-ECD analysis. The concentration of *p,p′*-DDT in uninoculated controls did not change over the incubation period.

Scanning electron microscopy

Cultures containing fungal biomass were diluted and filtered onto polycarbonate Nucleopore™ membranes (Whatman). The membranes were mounted onto aluminium mounts using double-sided tape and evaporatively coated with 20 nm of carbon to provide electrical conductivity and maximum phase contrast for the backscattered electron signal. The samples were placed into a Cambridge Instruments Steroscan S250 Mk 3 scanning electron microscope (SEM) for further examination using a primary electron beam energy of 20 KeV. Imaging was performed using the secondary electron signal or the backscattered electron signal for information about surface topography or composition and phase, respectively. Characteristic X-ray signals were also collected at selected positions for qualitative energy dispersive X-ray (EDX) analysis using a Link system AN 10000 energy-dispersive X-ray system. Some areas were imaged using the characteristic X-ray signal to obtain an image of the elemental composition.

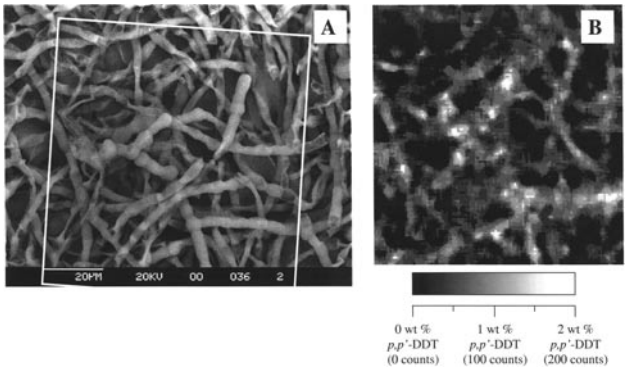

Figure 3 Scanning electron micrograph of *Cladosporium* strain AJR^{3}18,501 biomass (A) after incubation in 25% 1-propanol containing *p,p′*-DDT. *p,p′*-DDT was supplied solely (dissolved) in 1-propanol (12 mg l^{-1}). The distribution of *p,p′*-DDT was determined by probing for chlorine using a voltage energy of 20 keV (B). The white colouration indicates the distribution of chlorine on *p,p′*-DDT-treated fungal biomass. Colouration (i.e., chlorine) was not observed on the fungal biomass without prior exposure to *p,p′*-DDT.

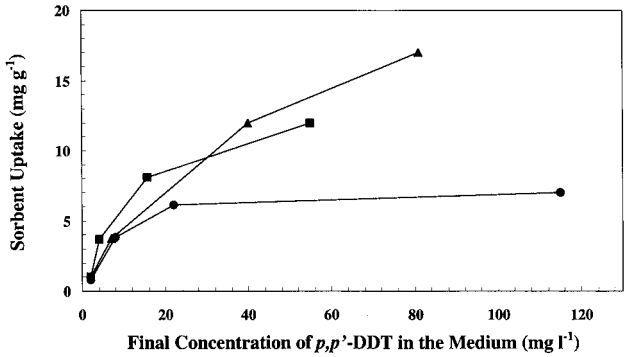

Figure 4 Biosorption isotherm plots for *p,p′*-DDT uptake by live fungal biomass in distilled water (●), 25% ethanol (■) and 25% 1-propanol (▲). Sorbate uptake is plotted against the residual *p,p′*-DDT concentration associated with the medium after 50 h incubation.

Results

Biosorption of p,p′-DDT

The ability of *Cladosporium* strain AJR[3]18,501 to sorb OCPs was assessed by adding dried biomass of strain AJR[3]18,501 to media containing *p,p′*-DDT. Different medium types were used, which varied with the amount of *p,p′*-DDT in solution. *p,p′*-DDT in solution followed the order: distilled water < ethanol < 1-propanol. Over the incubation period (50 h) there was no significant change in the concentration of *p,p′*-DDT in any of the uninoculated media (Figure 1). However, in cultures inoculated with live fungal biomass, significant amounts of *p,p′*-DDT were removed from the medium and recovered from the fungal mycelium (Figure 1A). Approximately 63% of *p,p′*-DDT added to distilled water (55.7 mg l^{-1}) was recovered from the fungal mycelium after 50 h. In addition, DDD and DDE could be removed from the medium and recovered from the fungal biomass (data not shown). Greater amounts of OCPs were recovered from fungal mycelium when low molecular weight primary alcohols were used as the medium. In addition, increasing the carbon chain length of the alcohol (from ethanol to 1-propanol) increased *p,p′*-DDT biosorption. After 50 h, 80% and 92% of *p,p′*-DDT was removed from 25% ethanol and 1-propanol media, respectively. However, increasing the concentration of co-solvent from 25% to 50% had little effect on increasing the uptake of *p,p′*-DDT by the fungal biomass.

Appreciable amounts of *p,p′*-DDT were also removed from the media by killed fungal biomass; however, greater amounts of the pesticide were recovered from live mycelium compared to killed mycelium (Figure 1B).

Although the above experiments demonstrated the potential of fungal biosorption for the removal of *p,p′*-DDT from dilute alcohol solutions, the concentration of *p,p′*-DDT in these solutions was still in excess of its solubility. Additional biosorption experiments were performed in 25% 1-propanol with *p,p′*-DDT supplied solely (dissolved) in the aqueous phase (12 mg l^{-1}). The rapid decrease in the concentration of *p,p′*-DDT in the medium corresponded to an increase in biomass-associated *p,p′*-DDT. After 60 min, the concentration of *p,p′*-DDT remaining in the aqueous phase decreased from 12 mg l^{-1} to approximately 2.5 mg l^{-1} while the remaining *p,p′*-DDT was recovered from the mycelia (Figures 2 and 3). Further incubation resulted in a decreased rate of *p,p′*-DDT uptake: after 270 min, 1.6 mg l^{-1} of *p,p′*-DDT remained in solution.

p,p′-DDT sorption isotherms

As the quality of any sorbent material is judged according to the amount of sorbate it can immobilise, *p,p′*-DDT uptake by *Cladosporium* strain AJR[3]18,501 was determined per unit weight of biomass. Sorption isotherms were plotted by first determining the sorbate uptake (q) according to Eq. (1). Sorbate uptake was then plotted against the final concentration of *p,p′*-DDT associated with the aqueous phase (C_f). Figure 4 illustrates the sorption isotherms for *p,p′*-DDT using live *Cladosporium* biomass when inoculated into distilled water, 25% ethanol and 25% 1-propanol media. Sorbate uptake varied significantly among the three medium types. Greatest *p,p′*-DDT uptake (at the concentrations tested) occurred in the 25% 1-propanol medium (17 mg g^{-1}) followed by 25% ethanol (12.2 mg g^{-1}). *p,p′*-DDT uptake from the distilled water medium was 2.4 times less than that from the 1-propanol medium. Although the extent of *p,p′*-DDT uptake was greatest when 25% 1-propanol was the supporting medium, at low *p,p′*-DDT concentrations, greater amounts of the organochlorine were partitioned onto the fungal biomass when *p,p′*-DDT was supplied in 25% ethanol (Figure 4).

Effect of pH on p,p′-DDT biosorption

The uptake of metal ions by biomass is strongly influenced by the pH of the system: ion uptake decreases significantly as the pH drops from 6.0 to 2.5. In order to assess the effect of pH on *p,p′*-DDT biosorption, fungal biomass was added to phosphate buffers of varying pH (pH 3–10) containing *p,p′*-DDT. The pH of the

Table 1 Effect of pH on the biosorption of *p,p′*-DDT by *Cladosporium* strain AJR[3]18,501

pH of medium	Concentration of *p,p′*-DDT associated with the medium and mycelium						
	Uninoculated: medium (mg l^{-1})	Killed biomass:			Live biomass:		
		Medium (mg l^{-1})	Mycelia (mg g^{-1})	% *p,p′*-DDT recovered[a]	Medium (mg l^{-1})	Mycelia (mg g^{-1})	% *p,p′*-DDT recovered[a]
3.0	74.9±3.2	34.3±2.4	3.7±0.4	95.2	22.7±1.2	5.3±0.2	100.7
5.5	68.1±0.5	16.7±1.4	4.9±0.2	97.2	13.9±3.9	6.4±0.8	115.0
7.0	74.2±4.7	18.5±1.7	5.6±0.7	100.9	15.9±2.8	6.3±0.3	106.5
8.5	79.3±2.0	21.9±1.7	5.3±0.4	94.1	12.1±1.2	5.9±0.1	89.5
10.0	76.6±3.7	24.7±0.7	4.9±0.5	96.2	14.1±1.7	6.5±0.7	103.5

[a]The percentage *p,p′*-DDT recovered from cultures inoculated with *Cladosporium* strain AJR[3]18,501 was calculated using the amount detected in the corresponding uninoculated medium after 24 h incubation.

Table 2 Effect of arsenic species on the biosorption of *p,p′*-DDT by *Cladosporium* strain AJR[3]18,501

Arsenic species	Concentration (mg l^{-1})	Concentration of *p,p′*-DDT in:		% *p,p′*-DDT recovered[a]
		Medium (mg l^{-1})	Mycelia (mg g^{-1})	
–	0	30.5±0.6	4.9±0.1	97.9
III	1	30.7±5.3	4.4±0.1	92.5
III	5	36.2±3.6	4.5±0.3	100.0
III	10	34.2±1.2	4.1±0.1	93.6
III	25	36.3±0.7	4.4±0.2	99.4
III	37.5	34.9±0.7	4.5±0.2	98.6
V	1	31.2±1.6	4.6±0.3	95.4
V	5	35.0±2.8	4.1±0.1	93.8
V	10	30.6±2.0	4.6±0.2	94.3
V	25	31.0±3.8	5.0±0.3	100.2
V	37.5	24.6±5.1	5.1±0.1	93.4

[a]The percentage *p,p′*-DDT recovered from cultures inoculated with *Cladosporium* strain AJR[3]18,501 was calculated using the amount detected in uninoculated media after 24 h incubation (80.9±1.1 mg l^{-1}).

medium had some effect on *p,p′*-DDT biosorption by strain AJR[3]18,501 (Table 1). Although *p,p′*-DDT biosorption occurred over the range of pH tested, *p,p′*-DDT uptake was slightly inhibited at pH 3.0: 37.0±4.5 and 52.7±1.8 ppm of *p,p′*-DDT was recovered from killed and live fungal biomass after 50 h, respectively, compared to 56.4±6.6 and 63.1±2.9 ppm at pH 7.0.

Effect of arsenic on p,p′-DDT biosorption

At a number of DDT-contaminated sites in Australia, As is also prevalent at high concentrations. Potentially, As species could inhibit *p,p′*-DDT uptake through competition for binding sites on the fungal biomass. To determine the effect of As on *p,p′*-DDT uptake, biosorption experiments were prepared with arsenate [As(V)]or arsenite [As(III)] in the medium. The presence of As(III) or As(V) in the medium did not affect *p,p′*-DDT biosorption by strain AJR[3]18,501 (Table 2). Varying the concentration of the As species from 1 to 37.5 mg l^{-1} also did not influence the extent of *p,p′*-DDT biosorption over the incubation period. Arsenic could not be sorbed by strain AJR[3]18,501 (Figure 5). Adjusting the pH of the medium between 3 and 10 did not promote As uptake by the fungal biomass (data not shown).

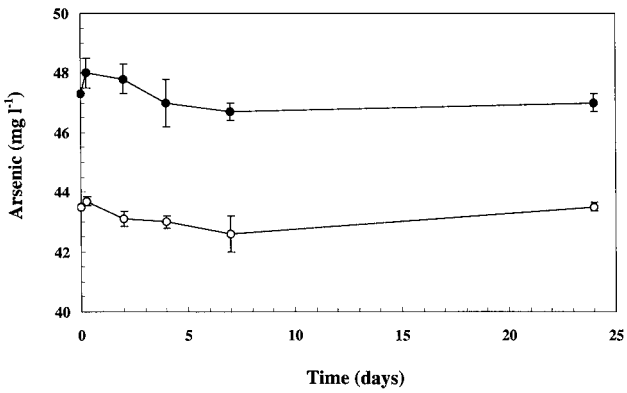

Figure 5 Biosorption of As(III) (○) and As(V) (●) by *Cladosporium* strain AJR[3]18,501. The concentration of arsenic in the medium was determined by ICP as described in Materials and methods.

Discussion

The potential use of fungal biomass for the removal of contaminants from wastewater has been recognised for some time. Since the early experiments of Zajic and Chiu [27], advancement of the utilisation of fungal biomass as biosorbents for heavy metal ions has led to the application of fungal biosorption processes for the removal of metal ions from industrial waste effluents [14]. Numerous publications have reviewed the potential of various biosorbents to sorb a variety of metal ions and the mechanisms involved in metal ion uptake [13,14,26]. Although much is known about metal ion biosorption, little information has been published on fungal biosorption of organic compounds.

Previously, strain AJR[3]18,501 was shown to sorb *p,p′*-DDT from a liquid medium when the pesticide was supplied in excess of its aqueous solubility [9]. The experiments described in this paper confirmed the ability of strain AJR[3]18,501 to sorb *p,p′*-DDT, in addition to toxic and recalcitrant DDT transformation products (DDD and DDE) [11], which may be produced from the biological degradation of the OCP. Transformation of *p,p′*-DDT was not observed during sorption; however, previous research demonstrated that strain AJR[3]18,501 was capable of transforming *p,p′*-DDT to DDD during extended incubation periods (12 days) [10]. Although transformation of *p,p′*-DDT was observed previously [10], DDD was not detected in the aqueous phase; however, it was detected sorbed to the fungal biomass. In this study, the contact time between *p,p′*-DDT and the fungal biomass was not sufficient for degradation to occur; however, if degradation was to occur, it is likely that the products formed would partition onto the fungal biomass and not in the medium.

Although an earlier study [9] demonstrated the potential of strain AJR[3]18,501 to biosorb OCPs, one limitation in the experiments was the low solubility of the OCPs in the media used. Removal of OCPs from contaminated soil with a soil-washing process utilising water as the wash phase would be unsuccessful as it would remove only microgram to milligram quantities of OCPs per litre due to the low solubility of these compounds in water. In order to increase the effectiveness of the soil-washing process, co-solvents, such as simple low molecular weight primary alcohols (e.g., ethanol or 1-propanol), could be used as the wash phase as these co-solvents can successfully solubilise OCPs [4,12].

Co-solvents such as these have been shown to enhance the solubility of hydrophobic organic compounds through the decrease in interfacial tension between the compounds and the aqueous solution and the preferential partitioning of the co-solvent into the hydrophobic organic phase [3]. An increase in the compound's solubility occurs due to a decrease in the hydrophobicity of the organic compound. *p,p′*-DDT biosorption was enhanced by supplying the OCP in ethanol or 1-propanol media indicating the suitability of these soil wash solutions for biosorption purposes. Although *p,p′*-DDT uptake was greater when the OCP was supplied at low concentrations in 25% ethanol, the fungal biomass accumulated more *p,p′*-DDT when it was supplied in 25% 1-propanol: maximum uptake was approximately 40% greater when *p,p′*-DDT was supplied in 1-propanol compared to ethanol. The enhanced uptake of *p,p′*-DDT from 1-propanol is probably a response to the increase in solubility of the compound in 1-propanol compared to ethanol. It is well recognised that increasing the carbon chain length of the co-solvent increases the solubility of the hydrophobic organic compound, as the longer carbon chain decreases the influence of the polar group on the partitioning of *p,p′*-DDT into the solution [2,18]. Increasing the volume fraction of the co-solvent (i.e., from 25% to 50%) also results in an increase in the solubility of organic compounds [12,20,21]. However, when the co-solvent volume fraction of ethanol and 1-propanol were increased from 25% to 50%, a corresponding increase in *p,p′*-DDT uptake was not observed. Presumably, saturation of *p,p′*-DDT sorption sites on the fungal biomass occurs which inhibits further *p,p′*-DDT uptake even though high concentrations of *p,p′*-DDT are still in solution. This limitation may be overcome by increasing the biomass to co-solvent solution ratio.

The efficacy of a biosorption process may be influenced by the presence of other contaminants in the waste stream. At a number of DDT-contaminated sites in Australia (cattle and sheep dip sites), As is also present at high concentrations [5,16,19]. Potentially, As species may compete for binding sites on the fungal biomass, thereby decreasing the *p,p′*-DDT uptake efficacy of strain AJR^{3}18,501. Biosorption of DDT by strain AJR^{3}18,501 was not affected by the presence of either As species [As(III)] or [As(V)]), which probably reflected the inability of the biomass to sorb these metal anions. Although it has been shown that As sorption is strongly influenced by pH (As[V] is sorbed to a greater extent at low pH while As[III] sorption increases with increasing pH) [22], changing the pH of the medium did not facilitate As sorption by *Cladosporium* stain AJR^{3}18,501.

The uptake of metal ions by most biomass types decreases dramatically as the pH of the system decreases from pH 6.0 to 2.5 [26]. The dependence on pH has been linked to an ion exchange process between protons on the biomass and the heavy metal ions, where weakly acidic carboxyl groups associated with cell wall constituents are the probable sites of ion exchange. Such a dramatic dependence on pH was not observed for *p,p′*-DDT biosorption, indicating that other mechanisms, such as van der Waals forces (dispersion/London forces), chemical binding, hydrogen bonding or ligand exchange may be involved in DDT uptake by strain AJR^{3}18,501.

The combination of co-solvent soil washing for the cleanup of DDT-contaminated soil and biosorption for the removal of DDT from soil-wash solutions offers many advantages over conventional remediation technologies. A low operating cost is associated with the production of the biomass while no nutritional requirements are needed during the biosorption process. Unlike other biological remediation strategies, the process is rapid and DDT transformation products are not produced, although if these compounds are present in the soil-wash solution, they may be removed by the fungal biomass. The DDT-laden biomass may be disposed of by incineration or chemically treated to dechlorinate the organochlorine and reused in subsequent sorption processes. Finally, soil-wash solutions may be recycled, which will result in a more economical remediation process.

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References

1 Aislabie JM, NK Richards and HL Boul. 1997. Microbial degradation of DDT and its residues — a review. *N Z J Agric Res* 40: 269–282.
2 Augustijn DCM, RE Jessup, PSC Rao and AL Wood. 1994. Remediation of contaminated soils by solvent flushing. *J Environ Eng* 120: 42–57.
3 Brandes D and KJ Farley. 1993. Importance of phase behaviour on the removal of residual DNAPLs from porous media by alcohol flushing. *Water Environ Res* 65: 869–878.
4 Chiou CT, DW Schmedding and M Manes. 1982. Partitioning of organic compounds in octanol–water systems. *Environ Sci Technol* 16: 4–10.
5 DIPMAC Report. 1992. Report on the Management of Contaminated Waste at Cattle Dip Sites in Northeastern New South Wales. NSW Government, Sydney, Australia.
6 Dunlap TR. 1981. DDT — Scientists, Citizens and Public Policy. Princeton University Press, Princeton, NJ.
7 Gilbert WS, G Singh and N Ahmad. 1990. Pesticide Contamination of Birds and Fish in the Namoi Valley. Technical Bulletin 40. NSW Agriculture and Fisheries, Orange, NSW, Australia.
8 Hunnego JN and DL Harrison. 1971. Metabolism of DDE, TCE and DDT in sheep. *N Z J Agric Res* 14: 406–416.
9 Juhasz AL and R Naidu. 1999. Biosorption of DDT from Waste Waters Using Fungal Biomass. *2nd International Conference on Contaminants in the Soil Environment in the Australasia-Pacific Region. New Delhi, India, December 1999.* Indian Network for Soil Contamination Research, New Delhi, India, pp. 376–377.
10 Juhasz AL and R Naidu. 1999. Apparent degradation of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) by a *Cladosporium* sp. *Biotechnol Lett* 21: 991–995.
11 Juhasz AL and R Naidu. 2000. Extraction and recovery of organochlorine pesticides from fungal mycelia. *J Microbiol Methods* 39: 149–158.
12 Juhasz AL and E Smith. 2000. Remediation of DDT-contaminated soil using a two phase soil washing biosorption process. *Towards Better Management of Wastes and Contaminated Sites in the Australasia-Pacific Region, Adelaide, Australia, May 2000.* CSIRO Land and Water, Adelaide, Australia, pp. 53–56.
13 Kapoor A and T Viraraghavan. 1997. Fungi as biosorbents. In: Wase J and C Forster (Eds.), Biosorbents for Metal Ions. Taylor and Francis, Bristol, VT, pp. 67–85.
14 Kratochvil D and B Volesky. 1998. Advances in the biosorption of heavy metals. *Trends Biotechnol* 16: 291–300.
15 Lal R and DM Saxena. 1982. Accumulation, metabolism and effects of organochlorine insecticides on microorganisms. *Microbiol Rev* 46: 95–127.
16 McDougall KW. 1997. Arsenic and DDT residues at cattle tick dip sites in NSW. *Land Contam Reclam* 5: 323–328.
17 Metcalf RL. 1973. A century of DDT. *J Agric Food Chem* 21: 511–519.

18 Morris KR, R Abramowitz, R Pinal, P Davis and SH Yalkowsky. 1988. Solubility of aromatic pollutants in mixed solvents. *Chemosphere* 17: 285–298.
19 Naidu R, J Smith, LH Smith, KG Tiller and KW McDougall. 1995. Arsenic/DDT Residues at Cattle Tick Contaminated Sites: Preliminary Investigation. Technical Report No. 60 CSIRO Division of Soils, Adelaide, Australia.
20 Rao PSC, AG Hornsby, DP Kilcrease and P Nkedi-Kizza. 1985. Sorption and transport of hydrophobic organic chemicals in aqueous and mixed solvent systems: model development and preliminary evaluation. *J Environ Qual* 14: 376–383.
21 Rao PSC, LS Lee and R Pinal. 1990. Cosolvency and sorption of hydrophobic organic compounds. *Environ Sci Technol* 24: 647–654.
22 Raven KP, A Jain and RH Loeppert. 1998. Arsenite and arsenate adsorption on ferrihydrite: kinetics, equilibrium and adsorption envelopes. *Environ Sci Technol* 32: 344–349.
23 Schiewer S and B Volesky. 1997. Ionic strength and electrostatic effects in biosorption and protons. *Environ Sci Technol* 31: 1863–1871.
24 Smelt JH, LGHT Tuinstra, CAAA Maenhout and W Luten. 1975. Uptake of DDT and related compounds from soils into forage crops. *Agro-Ecosystems* 2: 133–143.
25 Voerman S and AFH Besemer. 1975. Persistence of dieldrin, lindane and DDT in a light sandy soil and their uptake by grass. *Bull Environ Contam Toxicol* 13: 501–505.
26 Volesky B. 1990. Biosorption of Heavy Metals. CRC Press, Boca Raton, FL.
27 Zajic JE and YS Chiu. 1972. Recovery of heavy metals by microbes. *Dev Ind Microbiol* 13: 91–100.