Removal of Aroclor 1254 by the white rot fungus *Coriolus versicolor* in the presence of different concentrations of Mn(IV)oxide

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1. Introduction

Polychlorinated biphenyl (PCB) is a generic name for a group of compounds derived from biphenyl by the substitution of one to 10 atoms of hydrogen with atoms of chlorine. The first PCB was synthesized in 1881 by Schmidt and Schultz and PCBs have been produced commercially since 1929. From the latter half of the 1950s, production increased and reached a peak at the end of the 1960s. After the discovery of their widespread environmental contamination in the 1970s, production decreased, but significant quantities are still in use (Tanabe, 1988). Properties like chemical and thermal stability, low or no flammability, high permittivity and low vapour pressure at ambient temperature, assured that PCBs have a variety of applications. The products are used as heat-transfer fluids, dielectrics for capacitors and transformers, hydraulic fluid, lubricants, additives in plastics and dyes, etc. (Lang, 1992). PCB structures are similar to that of DDT, as are their behaviour in the environment with regards to persistence and potential for biological magnification (Hackman, 1978).

The ability of the white rot fungi to biodegrade a range of persistent toxic environmental pollutants has potential for bioremediation of PCB. The lignin degrading system that plays a role in the biodegradation of environmental pollutants depends on a group of heme-containing peroxidases, secreted to function in the extracellular environment of the fungi (Barr and Aust, 1994a). The mechanism of the white rot fungi lignin-degrading system is free-radical-based. The heme-containing enzymes responsible for the formation of the free-radical-based enzyme system are produced by the fungi in response to nutrient limitation, e.g. nitrogen, carbohydrates or sulphur. The enzyme system includes H_2O_2-generating oxidases as well as H_2O_2-requiring peroxidases. The two prominent enzymes are lignin peroxidase (LiP) and manganese(II)-dependent peroxidase (MnP) (Barr and Aust, 1994b).

C. versicolor was selected as test organism, because preliminary experiments indicated that under nitrogen-limiting growth conditions, in the presence of MnO_2, the fungus was able to produce detectable levels of Mn-dependent peroxidase (MnP). *C. versicolor* can resist the toxic effect of high concentrations of certain xenobiotics such as pentachlorophenol (PCP) and PCB (Alleman et al., 1992; Eaton, 1985). Mn(II)-dependent peroxidase has been found in ligninolytic cultures of *P. chrysosporium,* and similar Mn(II)-dependent peroxidases have been found in *C. versicolor.* Extracellular lignin peroxidases, as assayed by the oxidation of veratryl alcohol to veratrylaldehyde has also been found in *C. versicolor.* This fungus also produces the secondary metabolite veratryl alcohol, which induces the ligninolytic activity in the fungus (Waldner et al., 1988).

MnP is an H_2O_2-dependent heme glycoprotein of $M_r \approx 46,000$ which, like LiP, exists as a series of isozymes (Brown et al., 1991). The heme iron in the LiP or MnP is in the ferric state, also referred to as the "resting" or "native" state. H_2O_2 oxidizes the heme by two electrons, to a form of the enzyme known as compound I. Compound I is reactive towards a variety of reducing chemicals. Compound I oxidizes such reducing chemicals to free radicals, which results in the formation of compound II. Manganese(II)-dependent peroxidase differs from LiP in that Mn^{+2} serves as the reducing agent for compounds I and II (Barr and Aust, 1994a). Mn(III) is a non-specific oxidant which in turn oxidizes organic compounds (Gold and Glenn, 1988). MnP shows oxidase activity, producing H_2O_2 by the oxidation of reduced substrates like NAD(P)H, glutathione (GSH), dithiothreitol (DTE), and dihydroxymaleic acid (Paszynczynski et al., 1988).

Manganese takes part in the mineralisation of synthetic lignins by the white rot fungi both as an active mediator for manganese peroxidase and as regulator of Mn(II)-dependent peroxidase, lignin peroxidase, and laccase production (Kerem and Hadar, 1993). Mn has a potent inducing effect on the expression of MnP in white rot fungi, while it is also an essential cofactor for the proper functioning of the MnP protein. Mn(II) addition can decrease LiP titers in white rot fungi (Bonnarme and Jeffries, 1990; Kern, 1989; Mester et al., 1995; Van der Woude et al., 1993). The inhibition of LiP activity may be attributed to related products generated from Mn(II). One candidate known to deactivate LiP is Mn(III) (Mester et al., 1995).

Addition of solid manganese(IV)oxide to cultures of *P. chrysosporium* at the onset of ligninolytic activity, improved production, enzymatic activity, and stability of the ligninase produced (Kern, 1989).

The objective of this study was to determine the effect of different concentrations of MnO_2 on the degree of Aroclor 1254

biodegradation by the white rot fungus, C. *versicolor*.

2. Materials and methods

2.1. Microorganisms

The white rot fungus species C. *versicolor* (CSIR WR 323) was obtained from the CSIR, Pretoria, SA. The organism was maintained on 3% Malt Extract Agar at 29°C, and subcultured every 30-60 days, but not longer than 6 months. Every 6 months subcultures were made from the original culture. The inoculated fungus was incubated at 29°C for 4-6 days before being used as the inoculum. The experiments were inoculated with 3 x 4 mm diameter mycelium-agar plugs.

2.2. Culture media

The nitrogen-limited medium used was composed of 0.2 mM $MgS0_2$-$7H_20$; 1.4 mM KH_2P0_4; 0.068 mM $CaCl_2$; 1 mg l^{-1} thiamine; 29.21 mM sucrose; 0.1% peptone, and 1 ml $^{-1}$ Tween 80. The medium was unbuffered and all the components were added before autoclaving (15 min at 121°C). The initial pH of the medium was approximately 6.

2.3. Cultivation conditions

Aliquots (20 ml) of medium were placed in 150-ml Erlenmeyer flasks. Each flask was loosely plugged with cotton wool for passive aeration. The cultures were established by transferring three agar-mycelial plugs obtained from the marginal mycelia of a C. *versicolor* culture grown on 3% Malt Extract Agar, to the flasks containing the medium. After inoculation the fungus was incubated in a gyratory incubator, 29°C at 113 rpm. The cultures were incubated in the dark, for a total of 36 days. Four days after inoculation, 2 mM veratryl alcohol (Sigma) was added to each flask. Three concentrations of $Mn0_2$ were used to determine the effect on biodegradation, 17.25, 34.5, and 57.5 mM. (The concentrations given are for $Mn0_2$ and not the free ion). Aroclor 1254 (15 mg 20 ml^{-1}) was added to all the flasks. To avoid any adverse effects on cell growth, Aroclor was added to late stationary, or early exponential growth phase cultures. Before addition, the Aroclor was dispersed with 12.12 μl Tween 80 detergent, in 500 μl sterile medium. Control flasks with no biomass were analysed on day 36.

2.4. Aroclor purity

Aroclor 1254 was obtained from Electrical Supply Company (ESKOM) in South Africa. The Aroclor 1254 sample used was taken from an askarel transformer, and filtered through a # 40 Whatman filter paper, after it was dissolved in equal quantities of CP-grade n-hexane. After filtration, the hexane and Aroclor were separated with a Büchi apparatus. Purity of the sample Aroclor 1254 was assessed by gas chromatography (GC) and an electron capture detector. The sample was compared with a 100% pure Electrical Grade Aroclor 1254 (Monsanto Co., St. Louis) to determine the sample purity.

2.5. Analytical procedures

2.5.1. PCB analysis

During the experiment, hexane extractable Aroclor 1254 remaining in the cultures was extracted from the culture medium and quantitated using a GC with an electron capture detector (GC-ECD). The total contents of duplicate flasks were taken in order to determine the Aroclor 1254 degradation. This was done every 5-8 days for 36 days. The residual hexane extractable Aroclor in the culture medium of each flask was extracted three times with equal quantities of hexane and filtered through Na_2S0_4 to remove excess water. The hexane and Aroclor was then separated with a Büchi apparatus and the Aroclor redissolved in 10 ml HPLC-grade hexane. The Aroclor degradation was analyzed with GC-ECD (Hewlett Packard 5890A gas chromatograph coupled with a HP 3394A integrator). The analysis was done at the South African Bureau of Standards, Pretoria. The column was 500 mm x 2 mm glass, and was packed with 3% OV-101. The parameters were: flow rate, 30 ml min^{-1} Nitrogen; column temperature, 170°C; attenuation, 4 or 5 depending on the concentration of PCB; and chart speed, 1 cm min^{-1}. The total retention time for Aroclor 1254 was ≈ 270 s. Ten Peaks, designated Pl-10, could be seen when a 2 ng Aroclor 1254 Standard was injected and eluted. Peaks 4 to 10 were used to determine the Aroclor concentration. A standard curve using the peak heights of concentrations 0.5, 1.0, 1.5, and 2.0 ng Aroclor was drawn up to determine the Aroclor 1254 concentration.

For the GC-MS study a Hewlett Packard 5972 Series Mass Selective Detector was used. GC-MS analysis of Aroclor 1254 was performed at 70 eV with a 30 m HP-5 fused silica capillary column with a internal diameter of 0.25 mm. Full scan mode with mass range 50-450 amu was used. The oven temperature was programmed from 60°C (5 min) @ 12°C min^{-1} to 300°C (7 min).

2.5.2. Enzyme assays

Manganese(II)-dependent peroxidase was determined spectrophotometrically (Cary, IE Spectrophotometer) at 25°C under the conditions described previously (Paszynczynski et al., 1988; Pelaez et al., 1995). For the enzyme assay, culture medium from the C. *versicolor* culture was utilised. The MnP was assayed as described previously using phenol red as substrate and monitoring the disappearance of the substrate at 610 nm (Pelaez et al., 1995). Reaction mixtures contained 100 mM sodium tartrate (pH 5), 0.001% phenol red, 0.1 mM $MnSO_4$, and up to 500 μl of culture medium in a total volume of 1 ml. Assays were initiated by the addition of 0.1 mM hydrogen peroxide. Phenol red was used for MnP activity assay, because of the higher selectivity of this substrate (ε_{610} = 4460 M^{-1} cm^{-1}) (Pelaez et al., 1995). International units of enzyme activity (nmol min^{-1}) were used.

2.5.3. H_2O_2 production, biomass and pH determination

H_2O_2 accumulation in the culture medium was determined with a diagnostic kit from Merck (Reflectoquant method 304). The biomass was separated from the culture fluid by filtration, (pre-weighed Whatman filter papers), and dried for 24 h at 65°C in an oven. The dried biomass and filterpaper was left for 10 min to stabilise, and weighed. The pH of the culture medium in each flask was determined with a Beckman Φ34 pH meter.

In all experiments, the measurements were carried out with at least duplicate parallel cultures. The reported values are means.

3. Results

3.1. Fungal growth in liquid medium

C. versicolor cultured in an agitated nitrogen-limited medium for 36 days at 29°C formed 3-10 mycelial pellets (3-8 mm diameter) in the flasks. The agar plugs used as inoculum served as an immobilisation matrix, around which the most prominent mycelial pellets formed. In some cultures, the pellets aggregated and formed a mycelial mass with a diameter between 20 and 50 mm.

The production of small amounts of extracellular polysaccharides was observed as white-hairy growth on the pellets as was also found by Dosoretz et al., 1990. This was noted in the 4 days period before the addition of Aroclor, MnO_2 and veratryl alcohol. After addition of Aroclor, the production of additional extracellular polysaccharides was no longer noticeable. Some of the cultures that received lower concentrations (17.25 mM), or no MnO_2 at all, formed extracellular polysaccharides after the initial 4 days growth period. The onset of secondary metabolism was determined by the appearance of the characteristic brown-black colouration in the medium, which typically occurred 5-6 days after inoculation (Bonnarme and Jeffries, 1990; Dosoretz et al., 1990). Cultures that did not receive MnO_2 on day 4 never changed colour to brown-black, but the media, and the mycelial pellets turned a yellow colour. In the cultures that received no MnO_2, the yellow colour was taken as an indication of the onset of secondary metabolism. The darkening of the mycelia to the brown-black colour was therefore dependent on the addition of MnO_2. Cultures with higher (34.5, and 57.5 mM) concentrations of added MnO_2 turned a darker brown-black colour.

Immediately after the addition of Aroclor, the dispersed Aroclor turned the medium a milky white colour. After 3-4 d, the Aroclor was observed as a droplet either on the surface of the medium or on the bottom of the flask.

3.2. Aroclor purity

Fig. 1 shows the GC-ECD profile of the SABS Aroclor 1254 standard compared to the Aroclor 1254 sample obtained from ESKOM. The ECD profile of the Aroclor 1254 sample (ESKOM) corresponded to the Aroclor 1254 SABS standard. The same peaks were observed in the ESKOM sample, but in different ratios to the profile of the 100% pure SABS standard. Notable differences between the SABS standard and the ESKOM sample were peaks 2 and 6. Both of these peaks were more pronounced in the ESKOM sample. There was also a notable difference in peaks 7, 8, and 9 of the ESKOM sample and the corresponding peaks in the SABS standard. The GC-ECD analysis indicated that the ESKOM sample was 82.5% pure, compared to the 100% pure SABS standard. The peak height of peaks 4-10 was used to determine the purity since these were the most prominent peaks.
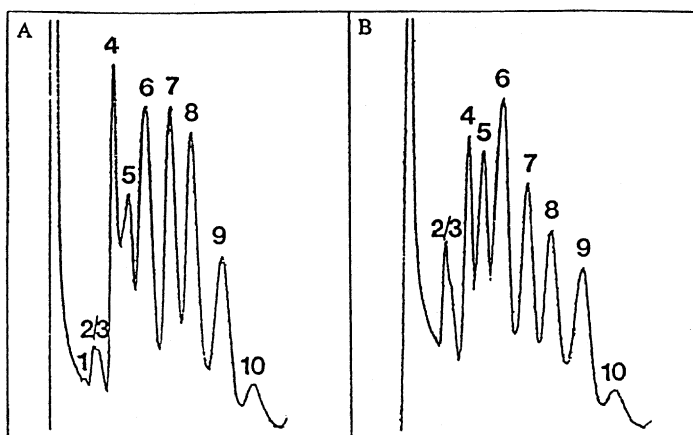
Fig. 1. GC-ECD profile of (A) 1 ng Aroclor 1254 SABS Electrical Grade Standard (Monsanto Co., St. Louis) compared to the (B) 1 ng Aroclor 1254 sample (ESKOM).

3.3. Aroclor bio degradation

3.3.1. Electron capture analysis

Fig. 2 shows the degradation profile of hexane extractable Aroclor 1254 by an agitated nitrogen-limited culture of *C. versicolor* with different concentrations of MnO_2 added after 4 days of incubation. This study showed a relationship between the concentration of MnO_2, and the rate of biodegradation. All the cultures including those that received no MnO_2 were able to degrade the Aroclor 1254. However, it was found that higher concentrations (34.5 and 57.5 mM) of MnO_2 in the cultures were responsible for a greater degree of degradation. With an initial concentration of 750 mg l^{-1} Aroclor, the cultures containing 57.5 mM MnO_2 were able to degrade the Aroclor to levels below 90 mg l^{-1}. Cultures that received 34.5 mM MnO_2 also degraded Aroclor 1254 to levels below 90 mg l^{-1}. However, the rate of degradation was marginally slower. Cultures containing 17.25 mM MnO_2, were only able to remove ca. 60% of the Aroclor. The cultures that received no MnO_2, removed the Aroclor with a similar rate than the 57.5 and 34.5 mM MnO_2 cultures. During the first 10 days of incubation more than 75% of the Aroclor was removed in cultures containing 57.5, 34.5 and 0 mM MnO_2.

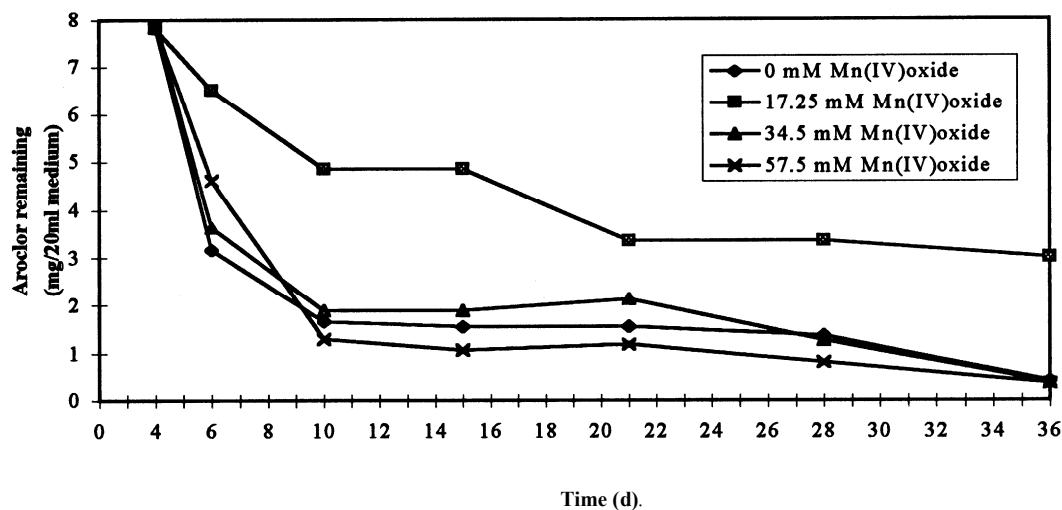The ECD profile of the residual Aroclor corresponded to the Aroclor profile obtained before the start of degradation.

Time (d).

Fig 2. Effect of Mn on the time and course of Aroclor biodegradation by ligninolytic cultures of *C. versicolor.*

3.3.2. Mass selective analysis

The GC-MS analysis of the undegraded sample revealed four groups of isomers that could be positively matched with a >90% probability. These isomers were tri-, tetra-, penta-, and hexachlorobiphenyls. They were present in the ratios 1:7:15:6, respectively. The GC-MS profile of the Aroclor 1254 sample showed that no single peak (except the trichlorobiphenyl) disappeared completely, but a similar decrease in all the peaks was observed. The typical Aroclor 1254 profile was still recognisable after 36 days of incubation.

It was noted that the higher chlorinated isomers showed more resistance towards biodegradation by the enzymes of the fungus *C. versicolor* (Table 1). The GC-MS results positively matched the peak resulting from l,l′-biphenyl, 2,2′,6-trichloride in the undegraded sample, but after 36 days of degradation, this peak could not be observed anymore. The penta-, and hexachlorobiphenyls were degraded at a slower rate than tri-, and tetrachlorobiphenyls in the cultures with MnP (57.5 mM) or LiP (no MnQ_2) (Table 1).

3.4. Enzyme activity

No MnP activity was found within the 4 day growth period before the addition of $Mn0_2$ and Aroclor. In the cultures containing 17.25, 34.5, and 57.5 mM $Mn0_2$, MnP was assayed 48 h after the addition of the $Mn0_2$. Enzyme activity increased reaching a maximum on day 13, after which activity decreased over several weeks (Fig. 3). The cultures that did not receive $Mn0_2$ showed no MnP activity during the 35 days growth period. An increase in MnP activity was noted after 23 days of incubation which reached a peak on 28 days. The culture that received 34.5 mM peaked on day 32.

3.5. H_20_2 production, biomass and pH determination

The effect of different concentrations of $Mn0_2$ on the production of H_20_2 by the cultures is shown in Fig. 4. There was a relationship between the concentration of H_2O_2 produced and the concentration of $Mn0_2$. The higher the $Mn0_2$ concentration, the higher the H_2O_2 production by *C. versicolor*. The production of H_2O_2 by *C. versicolor* began after 72 h of incubation and continued for all but the 0 mg cultures up to the end of the 35 days incubation period. The H_2O_2 production in the culture medium reached at maximum 10 days after inoculation. All the cultures except the 0 mg culture showed an increase in the production of H_2O_2 after 17 days of incubation. This coincided with an increase in the medium pH and MnP activty of all the cultures.

The effect of different concentrations of $Mn0_2$ on the pH of the culture medium is shown in Fig. 5. All the cultures showed an increase in medium pH after 15 days of incubation. This increase leveled off after 28 days of incubation. The pH of the cultures that received $Mn0_2$ remained between 4.5 and 5.5 for most of the incubation period. The pH of cultures that did not receive $Mn0_2$ was approximately 4. The pH decreased to this level from day 4 and reached a pH of 4 on day 6.

Table 1
Total ion chromatogram of ESKOM sample Aroclor 1254 before and after 36 d of degradation by the white rot fungus *C. versicolor* grown on
nutrient nitrogen-limited medium

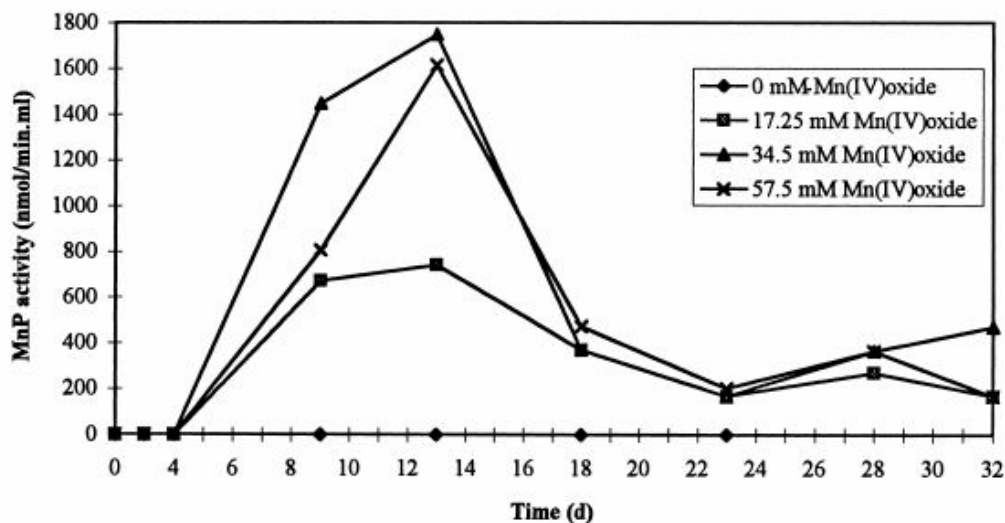| Congener	0 day of incubation	36 days of incubation	
	Undegraded sample	1.15 mM $Mn0_2$	0 mM $Mn0_2$
Σ Area % trichlorobiphenyl	0.104	0.00	0.00
Σ Area % tetrachlorobiphenyl	5.672	2.147	3.421
Σ Area % pentachlorobiphenyl	63.64	61.285	68.54
Σ Area % hexachlorobiphenyl	12.52	17.296	18.052

Fig. 3. Effect of Mn on Manganese(II)-dependant peroxidase activity of a ligninolytic culture of *C. versicolor.*

Fungal growth, measured as dry mass, is presented in Fig. 6. There seemed to be little variation in bio-mass of cultures with different concentrations of MnO_2. During the first 4 days of incubation, vigorous fungal growth occurred, achieving 50% of its maximal mass during this period. The accumulation of biomass continued up to day 4, after which it gradually entered the stationary phase. The biomass reached a maximum on day 15 and a decrease in biomass was observed after a 15 days incubation period.
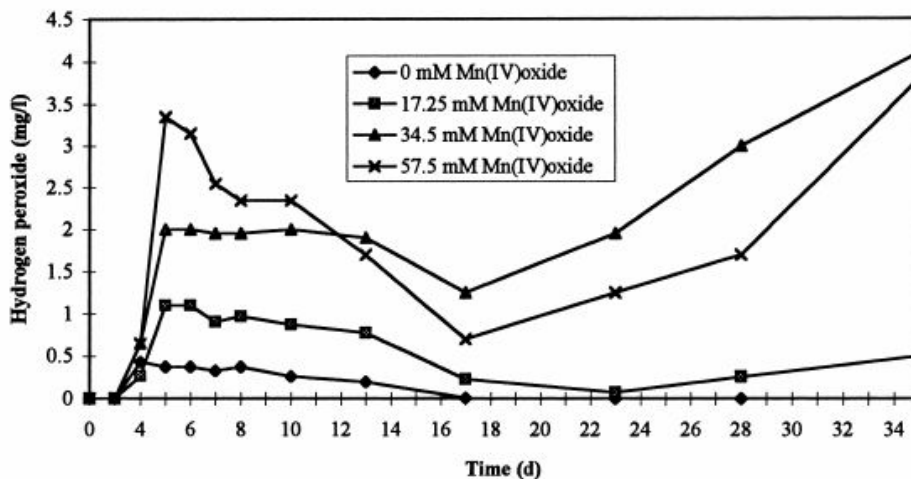

Fig. 4. Effect of Mn on hydrogen peroxide production in the extracellular fluid of a ligninolytic culture of *C. versicolor.*
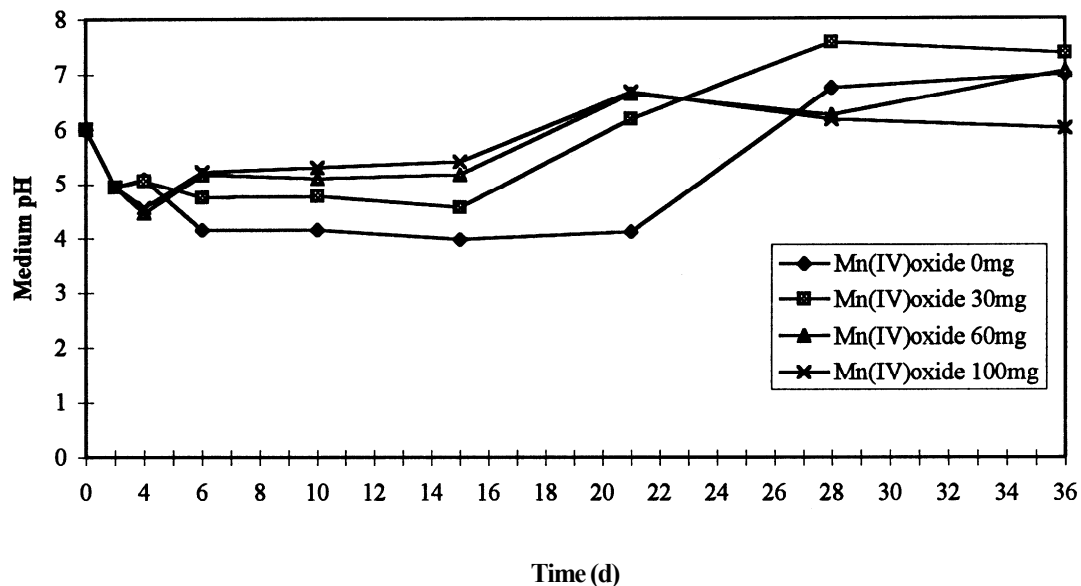
Fig. 5. Effect of Mn on the pH in the extracellular fluid of a ligninolytic culture of *C. versicolor*.

4. Discussion

4.1. Fungal growth in liquid medium

The fungus converted to a ligninolytic culture after the nitrogen source became limiting. The conversion to secondary metabolism was indicated by the appearance of the brown-black colour, characteristic of ligninolytic pellets of the white rot fungi (Dosoretz et al., 1990). In cultures that received no MnO_2, no indication of the onset of secondary metabolism could be observed. No deposition of MnO_2 took place because the basal medium contained no Mn. The brown-black colour of the mycelial pellets in the cultures that received MnO_2 was caused by the deposition of amorphous MnO_2. Secondary metabolism in all the cultures was also indicated by enzyme activity, H_2O_2 production, when the cell reached the stationary phase.
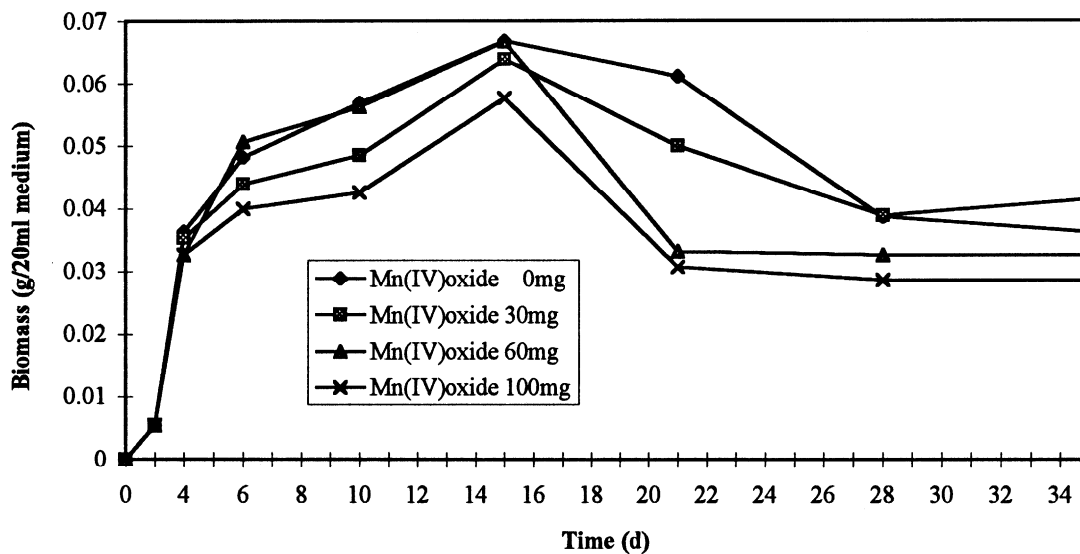

Fig. 6. Effect of Mn on the biomass accumulation of *C. versicolor*.

4.2. Aroclor purity

The ESKOM sample corresponded 82.5% to the SABS standard. The dispersion of the sample Aroclor 1254 by the addition of Tween 80 might play a role in the biodegradation of the Aroclor. This might be attributed to the accessibility of the enzymes to the Aroclor. The Aroclor molecules, due to their hydrophobic nature, were attracted to each other and approximately 3-4 days after addition of the PCB all the Aroclor was accumulated either on the surface of the medium or on the bottom of the flasks. The dispersed Aroclor might be more susceptible to biodegradation than the accumulated Aroclor because the enzymes can react more freely with Aroclor when dispersed. Another effect of Tween 80 on the biodegradation of the Aroclor 1254 by *C. versicolor* could possibly be to protect the enzymes from being mechanically inactivated due to agitation of the cultures (Haapala and Linko, 1993; Lestan et al., 1994; Venkatadri and Irvine, 1990).

4.3. Aroclor biodegradation

A relationship between the concentration of MnO_2 and the rate and extent of Aroclor degradation was observed. There is also a relationship between MnP production, H_2O_2 production and the concentration of MnO_2. Accumulation of MnP in the culture medium of nitrogen-limited cultures of *C. versicolor* was dependent on the presence of Mn. It has previously been shown that Mn(II) plays an important role in regulating the production of lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) formed by the white rot fungi (Bonnarme and Jeffries, 1990; Brown et al., 1990; Van der Woude et al., 1993). Therefore, it is possible that manganese also regulates the overall mineralisation of target compounds like persistent recalcitrant chemicals and in this case, Aroclor (Bonnarme and Jeffries, 1990).

Perez and Jeffries (1992) and Kern (1989, 1990), also found that Mn(II) induced MnP and that simultaneous repression of LiP was due to the presence of soluble complexes of simple organic acids. The higher concentrations of Mn ions, provided by solid MnO_2, indicated a higher production of MnP in our study for all trials, excepting the culture containing 57.5 mM MnO_2. The maximum concentration MnP inducable by utilising MnO_2 seemed to be at 34.5 mM. Concentrations higher than 34.5 mM MnO_2, did not further enhance the activity of MnP.

With MnP activity levels >807 nmol min"[1] for the cultures containing 34.5 mM MnO_2 and above, during the initial 10 d, it is unlikely that LiP played a significant role in the biodegradation of Aroclor. Only soluble Mn(II) ions can stimulate Mn peroxidase and it follows that the Mn(II) concentration is necessary to stimulate MnP during the 6 days period after the addition of Aroclor most probably repressed LiP activity.

All the cultures with the exception of the culture containing 0 mM solid MnO_2, precipitated MnO_2 after approximately 48 h of incubation. The precipitation of MnO_2 in the pellets most probably preceded the formation of LiP, as observed by Perez and Jeffries (1992). This implies that the period prior to LiP formation would be dominated by the presence of MnP (Fig. 4). It was deduced that during the first 48 h after the addition of Aroclor and MnO_2, the Mn(II)-dependent peroxidases were most probably the dominant enzymes in all the cultures except the one that received no MnO_2. No MnP enzyme activity could be detected in the latter culture at anytime during the 36 days incubation period. This was explained by the absence of Mn in the basal medium used for the cultures. The primary degradation inducer was most probably LiP formed in the absence of Mn(II).

Indications are that MnP played an active role in the biodegradation of Aroclor 1254 for the cultures that received MnO_2. The results indicated that ca. 50% of the Aroclor was degraded within this period. For the cultures that received higher concentrations, or no MnO_2 at all. MnP was detectable in significant concentrations during this period. The decrease in MnP activity was most probably due to the precipitation of Mn(II) as insoluble MnO_2. Another interesting observation was the slight increase in MnP activity after 18 days of incubation. This might be explained by the fact that MnP-generated Mn(III) can be stabilized by using organic acids as chelators. MnP is stimulated by chelators in the presence of Mn(II) (Perez and Jeffries, 1992). The chelator that might be responsible for the Mn-complex formation is probably oxalate. Dutton et al. (1993) found that oxalate accumulated in the medium of white rot fungi as a response to the change in pH of the medium, which became too basic to permit oxalate decarboxylase activity. They also observed oxalate accumulation in *C. versicolor*. A change in medium pH was also observed in this study. The medium pH began to increase on day 15 and on day 21 the pH was approximately 6.78. During this increase it was possible for the fungus to accumulate oxalate (Dutton et al. (1993). The accumulated oxalate might in turn have stabilized MnP-generated Mn(III). Another factor that could have influenced the LiP activity during the initial biodegradation of Aroclor was the concentration ($\approx$3 mg 1^{-1}) of H_2O_2 produced. The pH (4.5-5.5) indicated that MnP rather than LiP could have been the primary inducer of initial degradation as the optimum pH for lignin peroxidase activity was approximately 4.5.

Mn(II) itself is not toxic to LiP in vitro at concentrations below 1000 μM, therefore the toxic effect of Mn(II) addition is most likely related to a product generated from Mn(II). One obvious candidate is Mn(III), which is known to deactivate LiP (Mester et al., 1995).

A study by Brown et al. (1990), showed that addition of Mn to a cell free culture medium of *P. chrysosporium* grown in the absence of Mn does not restore MnP activity, indicating that the role of Mn is not as an activator of the enzyme. It was

also shown that MnP is detectable only in extracts of cells grown in the presence of Mn. That suggested that Mn regulates the synthesis of the MnP protein rather than its secretion.

It was noted that the level of Mn(II) had no effect of the growth of *C. versicolor,* as no significant difference in biomass accumulation could be seen in cultures containing the different concentrations of MnO_2. In studies done previously on the linear growth rates and accumulated biomass production of *Phanerochaete chrysosporium,* it was found that Mn(II) had no significant effect on the growth of the fungus (Brown et al., 1990).

Unlike the complete suppression of LiP and glyoxal oxidase (GLOX), the formation of MnP was more affected by the C/N ratio than by the oxygen level. The synthesis of MnP was partially repressed by the carbon starvation arising from a low C/N ratio, unlike that of LiP (Rothschild et al., 1995). This was observed in the accumulated biomass and MnP activity. The biomass accumulation levelled off around day 15 coinciding with the decrease in the MnP activity observed on day 18 in all but the 0 mg control. Studies done in the last 10 years indicated that white rot fungi have the potential to biodegrade the highly toxic PCB and PCB-like (furans and dioxins) group (Bumpus et al., 1987; Bumpus and Aust, 1987; Valli et al., 1992). These studies concentrated on *P. chrysosporium* as a model organism and lignin peroxidase as model enzyme. The study of the mineralization of PCB by Eaton (1985) indicated that other ligninolytic fungi (C. *versicolor, Phlebia brevispora, Funalia gallica)* bio-degraded the chlorinated biphenyls after the onset of secondary metabolism.

The results of our study indicated that the white rot fungus, C. *versicolor,* was able to degrade Aroclor 1254 during the 36 days incubation period in a nutrient nitrogen-limited medium, while low concentrations of PCBs (<20 nM) could be degraded by *P. chrysosporium* (Bumpus et al., 1987, 1988) higher concentrations (750 ppm) could be degraded equally effectivly by the white rot fungus C. *versicolor.* The degradation of the mixture of congeners that constitute Aroclor 1254, demonstrate the non-specific nature of the lignin-degrading system of the white rot fungi. Hexa- and pentachlorinated biphenyls were more resistant to the biodegradation by the enzyme systems of the fungus. Tri- and tetrachlorinated biphenyls, that could be detected with the GC-MSD seemed to be more rapidly degraded. Thomas et al. (1992) also concluded that mineralisation of chlorinated biphenyls to CO_2 by *P. chrysosporium* was affected adversely by the degree of chlorination.

4.4. H_2O_2 production, pH, and biomass determination

The decrease of H_2O_2 production in the culture fluid of our study, after 8-10 days of incubation was attributed to the N/C starvation of glyoxyl oxidase. The increase in H_2O_2 production after day 17 may be due to the utilisation of the carbon made available by the mineralisation of the phenolic PCB compound. The glyoxyl oxidase may need to adapt itself to the new carbon source. Glyoxyl oxidase is active within a broad pH range, with a maximum at pH 6. Glyoxyl oxidase activity is approximately 50% of its maximum at pH 4.5 (Kersten and Kirk, 1987). This might be another possible explanation for the sudden increase in H_2O_2 production between day 13 and 17. The increase in pH might be responsible for this increase by bringing the medium pH nearer to the optimum pH for glyoxyl oxidase.

The fungus C. *versicolor* altered the pH of the culture medium. The pH optimum for MnP for *Phanerochaete sordida* is between 4.0 and 4.5 (Rüttimann-Johnson et al., 1994) and the pH of between 4.5 and 5.5 that was prevalent for the first 15 days of incubation is most probably the optimum pH for the MnP from C. *versicolor.* The cultures with no added manganese probably produced LiP that was responsible for the degradation of the Aroclor 1254. The pH of these cultures was, for most of the incubation period, between 4 and 4.5. This corresponded to optimum pH for lignin degradation which is 4.5 (Kirk et al., 1978; Rüttimann-Johnson et al., 1994). Comparison of C. *versicolor* mycelial biomass production in all the cultures indicated that Mn had no significant effect on growth. This was also shown by Brown et al. (1990).

5. Conclusions

These studies show that the wood-rotting fungus C. versicolor was able to remove Aroclor 1254 in nitrogen-deficient medium. Also shown was a positive relationship between the concentration of MnO_2, added on day 4 of incubation and the rate and extent of Aroclor degradation. Previous studies have shown that the addition of MnO_2 to cultures of *P. chrysosporium* had the ability to improve production, enzymatic activity,and stability of the lignin peroxidase enzyme produced by this fungus (Bonnarme and Jeffries, 1990; Kern, 1989, 1990). The effect of MnO_2 on the production and activity of Mn-dependent peroxidase has not yet been determined.

The addition of high concentrations of MnO_2 to cultures of *C. versicolor* had the ability to improve production, enzymatic activity and stability of the manganese(II)-peroxidase enzyme produced by this fungus (Kern, 1989). The results showed that there existed a relationship between MnO_2 concentration and the production of manganese(II)-peroxidase and H_2O_2 production. Both these enzymes are necessary for biodegradation of xenobiotics. The manganese(II)-peroxidase enzyme showed a similar rate of decrease of Aroclor as the lignin peroxidase enzyme. The data indicated that both enzymes degraded the less chlorinated biphenyls with greater ease than the highly chlorinated biphenyls. More work needs to be done on the mineralization of PCB to CO_2 and a proposed pathway for the degradation of PCB. The study clearly shows the regulatory

effect of Mn on the enzyme system of *C. versicolor,* and the potential of MnP to remove environmental persistent pollutants.

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