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Effect of polydimethylsiloxane oxygen carriers on the biological bleaching of hardwood kraft pulp by *Trametes versicolor**

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Summary. Improving the availability of oxygen by adding polydimethylsiloxanes (PDMS) oxygen carriers to *Trametes versicolor* cultures increased pulp brightening. The presence of the oxygen carriers in cultures of *T. versicolor* with hardwood kraft pulp increased the growth rate of the fungus, but not the ultimate biomass yield. The PDMS also stimulated brightening of hardwood kraft pulp by *T. versicolor* immobilized in polyurethane foam. A threefold increase in the oxygen uptake rate in *T. versicolor* cultures with PDMS was observed. This increase can be explained by elevated oxygen transfer rate and attributed to the surfactant properties of PDMS.

Introduction

Conventional bleaching of chemical pulps with Cl_2 produces polluting chlorinated organics. The pulp and paper industry needs a cleaner technology for pulp bleaching; biological bleaching is a promising alternative to chlorination (Reid and Paice 1991).

Incubation with the white-rot fungus *Trametes versicolor* results in brightening of hardwood kraft pulp and removal of residual lignin from the pulp, i.e. bleaching (Paice et al. 1989). The degradation of lignin by white-rot fungi is an oxidative process (Kirk and Farrell 1987), which in previous studies using *T. versicolor* has been shown to occur faster in an atmosphere of oxygen than in air (Reid and Seifert 1982). In preliminary experiments we found that the oxygen supply in foam-stoppered cultures of *T. versicolor* was apparently sufficient for growth, but limiting for bleaching since purging with O_2 increased brightening (Kirkpatrick et al. 1990b).

One of the limiting factors in the transfer of oxygen to microbial cultures is the poor solubility of oxygen in aqueous media (Prins and van't Riet 1987). Conventional methods for increasing oxygen solubility in liquid media involve increasing the dispersion of the gas by increased agitation, increasing the partial pressure of O_2 in the gas phase, or decreasing the temperature of the solution. Oxygen concentration in a culture medium can also be increased by adding substances called oxygen carriers, i.e. better solvents for oxygen than water (Rols et al. 1990). Two groups of compounds have been used as oxygen carriers: perfluorinated hydrocarbons (Adlercreutz and Mattiasson 1982; Mattiasson and Adlercreutz 1987) and polydimethylsiloxanes (Leonhardt et al. 1985), in both of which the solubility of oxygen is 30–40 times higher than in water. Previous studies have shown that both polymeric dimethylsiloxanes and, to a lesser extent, perfluorinated hydrocarbons do not have any major adverse effect on the growth and metabolism of microbial cells (Leonhardt et al. 1985).

In this work we have used co-polymers of dimethylsiloxanes, with ethylene and propylene oxides (PDMS) as oxygen carriers. As shown in Table 1, these polymers are also mild, non-ionic, high-molecular-mass surfactants. The amphiphilic nature of these compounds and their lack of toxic effects on fungi made them ideal candidates for this study. The aim of this work was to determine whether an increased oxygen supply using oxygen carriers would lead to a more rapid or extensive bleaching of hardwood kraft pulp (HWKP) by *T. versicolor*.

Materials and methods

Growth of T. versicolor cultures

The standard flask culture conditions used in this study were incubation of *T. versicolor* (strain PPRIC No. 52) at 25° C and 200 rpm (2.5 cm radius) in 200 ml of 10 g/l mycological broth (Difco, Detroit, Mich., USA; low pH product) containing 2.0% (w/v) HWKP in 500-ml erlenmeyer flasks stoppered with polyur-

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Table 1. Physicochemical properties of polydimethylsiloxanes

Oxygen carrier	Viscosity (cs)	Non-siloxane moiety (%)[a]	Oxygen solubility a[b]	Oxygen solubility b[c]	Surface tension (α) (dynes/cm)[d]
PDMS-130	130	82	0.1038	0.0817	52.3
PDMS-1800	1800	Unknown	0.1510	—	62.4
Water (control)			0.0283		86.0

[a] Provided by the manufacturer
[b] Oxygen solubility (alpha factor) is defined as the volume of gas (ml) dissolved in 1 ml of test solution at 25° C and was determined using concentrated oxygen carrier preparations with a Clark electrode
[c] Value determined for 0.5% PDMS solution in defined medium with gas chromatography (see Materials and methods)
[d] Surface tension values were measured at the critical micelle concentration (approx. 10 µg/ml for both PDMS-130 and PDMS-1800). The approximate molecular masses of PDMS-130 and PDMS-1800 are 8 kDa and 100 kDa, respectively

ethane foam plugs (Canlab, Mississauga, Ont., Canada; T 1385). All results are expressed as the mean obtained from triplicate cultures, with standard deviations not exceeding ±5%. The cultures of *T. versicolor* immobilized in polyurethane foam were prepared as described earlier (Kirkpatrick et al. 1990a). *T. versicolor* cultures were also grown in fermentors (Chemap, Volketswil, Switzerland) equipped with a 3.5-l fermentation tank and marine impeller. All baffles were removed from the tank to prevent the deposition of the pulp on the sides of the fermentor and excessive foaming. Cultures were grown at 25° C and 200 rpm in 2 l of 0.5% mycological broth containing 2% HWKP and sparged with air at 260 ml/min. pH and dissolved O_2 (pO_2) were continuously monitored.

Analytical techniques

Preparation of handsheets and determination of brightness. Handsheets were prepared using 20–30 ml of 2% pulp suspension as described previously (Kirkpatrick et al. 1989). The handsheets were allowed to dry between saturation pads (Mandel Scientific, Guelph, Ont., Canada) pressed with stacked books. Brightness (reflectance at 457 nm), relative to barium sulphate used as a 100% brightness standard, was measured using a Perkin Elmer (Norwalk, Conn., USA) lambda 3B spectrophotometer fitted with a reflectance accessory.

Determination of biomass. Biomass produced by *T. versicolor* cultures in the presence of wood pulp was measured by determination of glucosamine. Glucosamine is a cell wall component and therefore its concentration is proportional to accumulated biomass (Kirkpatrick et al. 1989). Samples (0.5 ml) of cultures were centrifuged at 16000 rpm for 5 min. The pellet was washed several times with water, resuspended with water up to 0.5 ml and homogenized with an Omni 1000 homogenizer (Omni International, Waterbury, Conn., USA). Next, the sample was mixed with 0.5 ml of 12 N HCl and hydrolysed at 110° C for 12 h under nitrogen. After hydrolysis, HCl was removed by evaporation and the glucosamine concentration was determined using standard amino acid analysis (Beckman 7300 High Performance Analyzer, Palo Alto, Calif., USA). The biomass was also determined by change in total dry weight of the pelleted pulp and mycelium (Kirkpatrick et al. 1989).

Measurement of dissolved O_2. The alpha factors, i.e. the volume of oxygen (ml) dissolved in 1 ml of test solution, were measured for the concentrated solutions of PDMS using Clark's electrode, and were similar to the literature data (Leonhardt et al. 1985). Therefore, the oxygen concentration in the culture medium containing 0.5% of oxygen carriers should be increased only by 2–3%. Due to the surfactant properties of dimethylsiloxane co-polymers it was very likely that these compounds could increase the oxygen concentration in the culture medium by reducing surface tension (Prins and van't Riet 1987). In order to determine how much oxygen was actually dissolved in the aqueous medium, 100 ml of 0.5% PDMS-130 solution in a defined medium was saturated with oxygen and 8 ml was transferred to a 15 ml serum-stoppered vial containing 470 mg NaCl (1 M final concentration of NaCl). The concentration of the gases released into the headspace were measured by gas chromatography. Samples of 2 ml were withdrawn with a gas-tight syringe and used to fill a 0.5-ml sample loop on a Perkin-Elmer Sigma 2000 gas chromatograph. The samples were analysed at an oven temperature of 70° C and a helium carrier flow-rate of 20 ml/min. The gases were quantified with a thermal conductivity detector and an LSI-100 integrator. Carbon dioxide was separated on a 10 × 0.6 cm molecular sieve column, and O_2 and N_2 were separated on a 30.5 × 0.3 cm Porapak N column. A three-fold increase in oxygen solubility (Table 1) for culture medium containing 0.5% PDMS was found.

The pO_2 probe reading in the fermentor was set to 100% after saturating media with or without PDMS-130, with air. The presence of PDMS-130 in the culture medium changed the ratio between the pO_2 read by the fermentor's oxygen (Clark) electrode and the concentration of dissolved oxygen in the medium.

Infrared spectroscopy. A 10% solution of PDMS in dichloromethane was placed in a flow-cell (d = 0.1 cm) equipped with Irtran windows, and scans (800–4000 cm^{-1}) were performed using a Pye Unicam (Philips Sci., Cambridge, UK) model PU 9512 IR spectrophotometer with solvent as a reference.

Oxygen carriers

Co-polymers of dimethylsiloxane and ethylene and propylene oxide (Petrarch Systems, Bristol, Pa., USA) were added to the culture medium at 0.5% final concentration, unless stated otherwise. Some of the physico-chemical properties of these compounds are presented in Table 1. These compounds are amphiphilic and behave like medium-strength surfactants. The surface tension of PDMS solution was measured with a Surface Tensiomat (Fisher, Ottawa, Ont., Canada; Model 21) and the critical micelle concentration calculated by extrapolation to zero surfactant concentration.

Results and discussion

Effect of PDMS on HWKP bleaching by free fungal mycelium

The addition of siloxanes to cultures before inoculation improved bleaching of HWKP by *T. versicolor* (Table 2). The PDMS alone caused only a small increase in brightness of the pulp – an effect which was accompanied by a corresponding decrease in pulp kappa number, i.e. lignin concentration. The brightness increase resulting from incubation with the fungus and oxygen carrier together was greater than the total brightness increase caused by the fungus or oxygen carrier separately, leading us to conclude that the fungus and oxygen carriers were acting synergistically. Both dimethylsiloxane co-polymers increased brightening of HWKP by *T.*

Table 2. Effect of PDMS on the biological bleaching of hardwood kraft pulp by *Trametes versicolor* in mycological broth

Treatment		Increase in brightness[a]
Fungus	PDMS[b]	
−	−	0.0
+	−	15.1±1.3
−	PDMS-130	1.8±0.1
+	PDMS-130	22.0±0.7
−	PDMS-1800	2.0±0.7
+	PDMS-1800	22.1±0.3

[a] Brightness was measured as described in Materials and methods, and expressed as a percentage of the brightness of barium sulphate (standard). Handsheets were made from 5-day-old cultures. Data are means of triplicates ±SE

[b] Final concentration in the culture medium was 0.5%

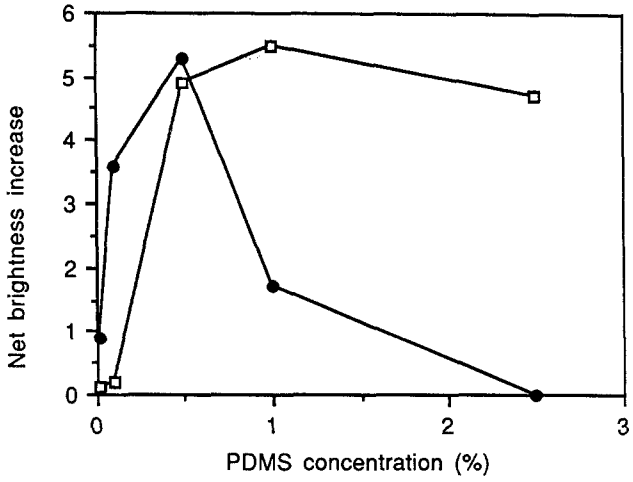

Fig. 1. Net effect of polydimethylsiloxanes (PDMS) on the biological bleaching of hardwood kraft pulp (HWKP). Net brightness was calculated as the difference between the brightness measured for HWKP treated with *Trametes versicolor* grown in medium containing PDMS and controls where pulp was treated with fungus alone for 5 days. The brightness of the control pulp was 44.4%: ●, PDMS-130; □, PDMS-1800

versicolor. In the case of PDMS-1800, this effect could only be seen when at least 0.5% siloxane was added to the cultures (Fig. 1). Much less PDMS-130 was required to cause a significant increase in HWKP brightening by *T. versicolor*. The effect of PDMS-130 was diminished when concentrations of this siloxane higher than 0.5% were used.

Unfortunately, the low solubility of PDMS-1800 in water made this polymer very difficult to separate from pulp fibres. To remove this siloxane, washing of the pulp samples with acetone and dichloromethane was required. This, however, did not prevent formation of glossy and oily handsheets. On the other hand, removal of PDMS-130 from pulp fibres did not present any problem and less than 1% of this polymer was found attached to the pulp after simply washing it with water.

To examine whether the oxygen carriers affected fungal growth as well as bleaching ability, cultures of *T. versicolor* containing HWKP were incubated in the presence and absence of 0.5% (v/v) PDMS-130. The rate of consumption of glucose and amino-nitrogen, as well as changes in culture pH and increase in total dry weight of cultures (an indication of fungal growth) were not significantly different for cultures incubated in the presence and absence of oxygen carriers. On the other hand, the rate of increase in HWKP brightness and the titre of extracellular laccase activity (Wolfenden and Willson 1982) were threefold greater for cultures incubated with PDMS.

Interestingly, surface tension measurements in culture filtrates showed an increase in surface tension after a 5-day treatment of HWKP with *T. versicolor*. For culture filtrates containing PDMS-130 and PDMS-1800 there were 75% and 25% increases in the surface tension, respectively. This observation can be explained in a number of ways – either the fungus metabolizes the polyalkyleneoxide component of the oxygen carrier, or there is free-radical mediated aggregation of PDMS, or PDMS forms complexes with culture metabolites that decrease its surfactant properties.

In order to test which of the above hypotheses applied to alteration of PDMS-130 properties, 5-day-old cultures of *T. versicolor* containing HWKP and 0.5% PDMS-130 were tested. The culture filtrate was separated and the pulp was washed with excess water. The filtrate was evaporated in vacuo and the solid residue was suspended in dichloromethane. The insoluble residue was filtered off and the solvent was evaporated under vacuum. Gravimetric analysis of the brown-coloured oily residue indicated that almost 100% of the siloxane added to the culture had been recovered. The oily residue was further tested by IR spectroscopy. The spectrum of PDMS-130 treated with *T. versicolor* was identical to that of non-treated siloxane. Quantitative assessment of the major bands typical for PDMS-130 (2850, 1720 and 1450 cm^{-1}) indicated that there was no decomposition or modification of the siloxane. Therefore the loss of surfactant properties by PDMS-130 exposed to *T. versicolor* cultures should be attributed to formation of PDMS-metabolite complexes.

HWKP bleaching by T. versicolor immobilized in polyurethane foam

As described previously (Kirkpatrick et al. 1990a) HWKP can also be bleached by treatment with *T. versicolor* immobilized in polyurethane foam, with no apparent presence of free fungus in the pulp samples. Limited contact between fungus and cellulose fibres gave an opportunity to test the extracellular factors involved in biological bleaching. As in the case of free mycelium, PDMS-130 increased the brightness of the pulp treated with immobilized, pre-grown *T. versicolor* by an average 10% brightness points. This observation implies that oxygen was important not only for the growth of the fungus but also for the extracellular bleaching process.

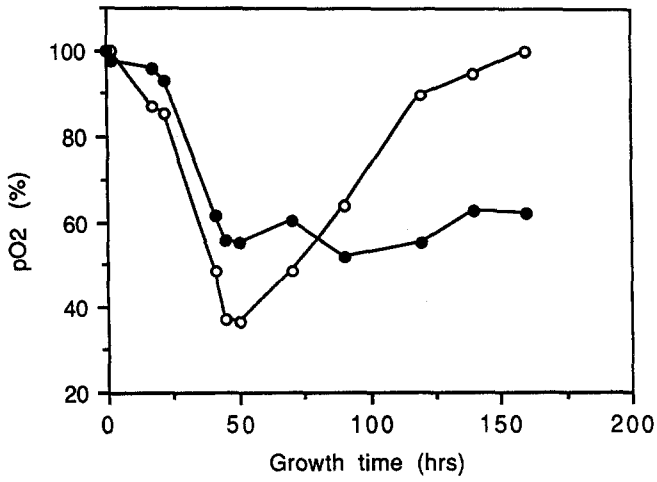

Fig. 2. Effect of PDMS-130 on dissolved oxygen in *T. versicolor* cultures grown in a fermentor. After sterilization, culture medium containing HWKP (with or without PDMS-130) was saturated with nitrogen to establish $pO_2=0$ and then with air to set the 100% saturation value. During calibration the temperature was kept at 25° C and stirring at 200 rpm: O, control; ●, 0.5% PDMS-130

Monitoring of the biological bleaching of HWKP in fermenters

In order to better assess the oxidative processes taking place during the biological bleaching of HWKP we have used a fermentor equipped with pO_2 and pH probes, allowing on-line monitoring of all the above parameters. Samples (30 ml) of the culture were taken daily for preparation of handsheets and for nutrients and glucosamine determination. It was also important to establish to what extent an increased solubility of oxygen caused by PDMS-130 affected the growth of *T. versicolor*. Indeed, the presence of PDMS-130 in the culture medium supported a higher apparent level of pO_2, especially during the most oxygen-demanding phase of fungal growth (Fig. 2) between 40 and 70 h. During that phase the biomass (measured as glucosamine concentration in the solid fraction of the culture) production rate in cultures with PDMS-130 was threefold faster than in control cultures. Nevertheless, after 7 days, the amount of biomass produced by cultures grown with or without siloxane in the medium was practically the same. In both cases it was 24 μg glucosamine/ml, equivalent to approx. 0.3 mg dry biomass/ml of *T. versicolor* culture.

A higher rate of fungal growth, implying a higher oxygen uptake rate (OUR), and at the same time smaller decrease in pO_2 than in the control must be attributed to increased oxygen transfer (OTR) caused by PDMS-130 (Eq. 1). It is possible that the increase in OTR in cultures with PDMS-130 was even higher than threefold when compared to the control cultures, since pO_2 values in cultures with PDMS-130 were higher during the most oxygen-demanding phase of growth (Fig. 2).

After 70 h of growth, the pO_2 began to rise, reaching 100% saturation in the control culture after 160 h. The pO_2 profile for the culture containing PDMS-130 showed a decrease to 50–55% saturation at its lowest point, but unlike the control it reached only 60% saturation after 70 h and stayed at this level for the rest of the fermentation. One possible explanation for this phenomenon is the decrease in surfactant properties of PDMS-130 from binding to fungal metabolites, as described above. As in flask experiments with free and immobilized fungus, the presence of PDMS-130 in the culture medium enhanced HWKP brightness by almost 20% brightness points. The maximal brightness of HWKP treated in the fermentor with *T. versicolor* and PDMS-130 was reached on day 7 of the treatment, i.e. 2 days after maximal brightness of the control. This may indicate that oxygen carriers can prolong the oxidative processes catalysed by this microorganism and thus be responsible for the failure of pO_2 to return to 100% in PDMS cultures. This phenomenon will be the subject of separate studies.

The OTR from gas phase to liquid phase is affected both by the solubility of oxygen in the liquid phase and the interfacial area (Prins and van't Riet 1987; Rols et al. 1990).

$$OTR=k_L a(C^*-C) \quad (1)$$

where a is the interfacial area, $k_L a$ is the volumetric oxygen transfer coefficient (h^{-1}), and C^*, C are the saturation and actual dissolved oxygen concentrations, respectively (Rols et al. 1990).

We suggest that PDMS-130 could increase the OTR by:

1. Increasing C^*, i.e. increasing the solubility of O_2 in the medium.
2. Increasing a, because of its surfactant effect.
3. Increasing k_L, by making the liquid-gas interface attractive to O_2 as shown in Fig. 3.

The decrease in surface tension by PDMS-130 may cause a better oxygen (air) dispersion in the medium and a larger interfacial area. An estimated threefold increase in OTR caused by PDMS-130 could also be related to the particular properties of this co-polymer. Due to its chemical composition and its amphiphilic nature this compound will occupy the interface facing the gas-phase with its hydrophobic, dimethylsiloxane portion, and the liquid-phase with its alkylene oxide,

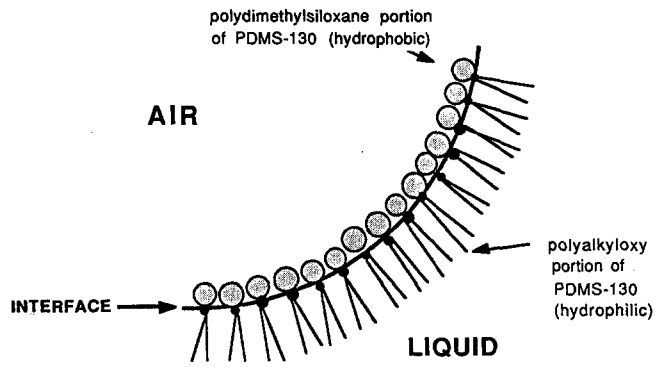

Fig. 3. Schematic illustration of oxygen transfer through air-liquid interface containing PDMS-130

hydrophilic part of the molecule (Fig. 3). Since the hydrophobic portion of the co-polymer is an exceptionally good medium for dissolving gases (Leonhardt et al. 1985), oxygen passage through the interface should be faster than in the case of other surfactants, e.g. Tween 80. Some surfactants actually decrease k_L, apparently by lowering surface mobility (Aiba et al. 1973; Prins and van't Riet 1987). The surfactant properties of PDMS-130 may also enhance the solubility and removal of lignin from HWKP (Table 2). Recently, the synthesis of perfluoro-polyethoxylated amphiphilic copolymers was described (Selve et al. 1990). These compounds, like PDMS, show both oxygen carrier and surfactant properties and could also be useful for biological bleaching.

In summary, we conclude that for the culture conditions described here, increased oxygen supply increased the ability of the fungus to bleach HWKP. This observation is consistent with the effect of increased oxygen supply on lignin biodegradation by other white-rot fungi (Reid and Seifert 1982). PDMS oxygen carrier can effectively enhance biological bleaching of kraft pulp, and probably other O_2-limited biological processes.

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