Removal of color and estrogenic substances by fungal reactor equipped with ultrafiltration unit

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Abstract Removal/degradation of color components and endocrine disruptors (EDs) by a bioreactor employing a white rot fungus *Trametes hirsuta* IFO4917 equipped with ultramembrane filtration (UF) unit was proposed and experimentally investigated. Among 20 white-rot fungal strains, *T. hirsuta* IFO4917 was screened as a most effective white-rot fungus for removal/degradation of color and EDs. This strain could effectively decolorize humic acid and degrade a wide range of Eds: bisphenol A (BPA), nonylphenol (NP), 17 beta-estradiol, estrone and estriol, although di-(2-ethylhexyl) phthalate (DEHP) could not be degraded. A bench scale (10 L), sequencing batch reactor using this fungus was developed and applied to decolorization of a melanoidin containing synthetic wastewater (4,200 color unit). The fungus was immobilized onto polyurethane foam cubes to stably maintain the biomass, and UF was applied to achieve a complete solid/liquid separation. The wastewater was decolorized in a main bioreactor and the resultant biologically treated wastewater was subjected to UF to obtain permeate as the effluent. The concentrate containing the remaining colored components with higher molecular weights was returned to the fungal bioreactor for further decolorization. In this fungal/UF system, 70% of the decolorization was constantly achieved at HRT of 2 days. The fungal/UF system was scaled up to a pilot-scale plant (200 L), and applied to the treatment of the secondary effluent from a night soil treatment process containing color components (1,000 color unit) and some EDs, NP, 4-*t*-octylphenol (OP), DEHP and benzophenone. 65–70% of decolorization efficiency was achieved at a 1.5 day cycle sequencing batch operation. NP, OP and benzophenone were removed efficiently with removal of 94%, 89% and 81%, respectively. However, the removal of DEHP was not so effective (45%).

Keywords Decolorization; degradation of endocrine disruptors; immobilization onto polyurethane foam; *Trametes hirsuta* IFO4917; ultrafiltration; white-rot fungi

Introduction

Problems of recalcitrant organic compounds including natural/synthetic color components and endocrine disruptors (EDs) have received substantial attention recently. For their resistance to biological degradation, conventional wastewater treatment, such as activated sludge processes, cannot effectively degrade or remove those contained in industrial wastewaters, night soil and/or landfill leachates. Therefore, wastewaters containing recalcitrant compounds are generally treated by physico–chemical processes, such as ozonation, photocatalytic degradation and/or activated carbon adsorption at present. However, these have the common problem of the high cost. Since employing biological means can be a cost-effective way for treating organic wastewaters in general, novel biological processes applicable to efficiently degrade or remove recalcitrant compounds are required to be developed.

White-rot fungi are the most promising candidate microbes which may be utilized for developing such novel biological wastewater treatment processes. White-rot fungi possess extracellular lignin-degrading enzymes which have high oxidoreductive potential with very relaxed- or none-substrate specificity in addition to H_2O_2-generating systems, and this allows them to degrade a wide variety of high-molecular complex compounds and

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xenobiotic compounds. The compounds which have been reported to be degraded by white-rot fungi include synthetic dyes (Cripps *et al*., 1990), natural color components like humic acids (Blondeau, 1989), chlorinated organic compounds like PCBs, DDT and dioxines (Arisoy, 1998; Bumpus *et al*., 1985), and polyaromatic hydrocarbons (Novotny *et al*., 1999).

In spite of the great potential of white-rot fungi for degradation of recalcitrant organic compounds, wastewater treatment processes employing white-rot fungi have not been fully investigated and have never been practiced to date. There has been no or very little information on the fungal treatment of synthetic and natural EDs which are frequently found in wastewater treatment plants, such as bisphenol A (BPA), nonylphenol (NP), 4-*t*-octylphenol (OP), di-(2-ethylhexyl)phthalate (DEHP), 17 beta-estradiol (E2), estrone (E1) and estriol (E3). In this study, color removal and EDs degradation by a bioreactor employing a white rot fungus *Trametes hirsuta* IFO4917 equipped with ultramembrane filtration (UF) unit was proposed (fungal/UF system), and its performance was experimentally investigated.

Screening of white-rot fungi for color removal and ED degradation

Several fungal strains collected from a type culture collection (IFO, Osaka, Japan) and gifted from Dr. T. Hattori, Forestry and Forest Products Research Institute, were examined for their potential to decolorize humic acids and to degrade Eds, in order to select the most suitable strain for the fungal/UF system.

The fungal strains were cultivated in the *N*-limiting medium containing 10 g glucose, 0.1 g NH_4NO_3, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$, 0.001 g $MnSO_4 \cdot 5H_2O$ and 0.001 g $CuSO_4 \cdot 5H_2O$ per litre (pH 4.5) at 28°C on a rotary shaker for about 2–3 days. The mycelial pellet and cell-free supernatant (extracellular enzyme solution) of the resultant culture obtained by centrifugation were used for the decolorization tests and ED degradation tests, respectively. For the decolorization test, washed mycelial pellet (collected from 30 mL of the medium) was inoculated to 100 mL of the N-limiting medium supplemented with 0.5 g/L humic acid (sodium salt) as a typical color component, and further cultivated in a 300 mL flask. The color unit of the medium was monitored at absorbance at 390 nm and the decolorization efficiency after 2 days was determined. For the ED degradation tests, BPA, NP, DEHP, E1, E2 and E3 were used as EDs frequently detected from the wastewater. 0.6 mL of the extracellular enzyme solution was added to 10 mM sodium malonate buffer (pH 4.5) containing 20 mg/L ED to be the total reaction volume of 3 mL in a test tube. The reaction was carried out at 28°C with reciprocal shaking for 24 hours, and the ED removal during the reaction was determined by HPLC assay.

The results of the screening tests of the 20 white-rot fungal strains are summarized in Table 1. Although none of the fungal strains showed significant ability to degrade DEHP, humic acid decolorization and degradation of the other EDs could be effectively performed by some strains. *T. hirsuta* IFO4917, *T. hirsuta* 1567 and *Irpex lacteus* 18, especially, showed relatively high potentials to both humic acid decolorization and degradation of a wide range of EDs. Although *Trametes versicolor* IFO30340 and *Phellinus gilvus* 110 could also effectively degrade a wide range of EDs, their decolorization potential was much lower than the previously mentioned strains (the latter strain produced a soluble pigment and decolorization could not be determined). From the results, *T. hirsuta* IFO4917 was selected as the most suitable strain for developing the fungal/UF system and was used for further studies, because its physiological properties including lignin-degrading enzymatic system have been well characterized before (Miyata *et al*., 1998). *T. hirsuta* 1567 and *I. lacteus* 18 also seem to be promising candidates, however, further characterization is necessary for their practical applications.

Table 1 EDs and humic acid degradation by white-rot fungi

White-rot fungi	Degradation (control %)						Humic acid decolorization
	E1	E2	E3	BPA	NP	DEHP	(%)
Trametes hirsuta 133	35.0	47.2	12.4	17.2	27.2	3.7	20.2
Trametes hirsuta 1567	63.1	>99.9	>99.9	>99.9	94.8	2.8	43.2
Trametes hirsuta 1674	7.5	37.8	48.8	41.9	94.4	1.4	50.6
Trametes hirsuta IFO 4917	24.4	51.6	60.9	36.5	92.9	7.6	57.2
Trametes orientalis 1070	20.7	23.4	40.5	0.0	0.0	11.1	10.2
Trametes orientalis 1071	0.0	0.0	7.0	3.7	0.0	9.6	17.1
Trametes versicolor 1670	0.0	21.2	61.4	29.1	42.0	0.0	50.8
Trametes versicolor IFO 30340	0.0	60.4	91.2	42.5	51.6	12.2	19.1
Trametes versicolor IFO 30388	30.1	37.8	3.9	5.9	0.0	3.5	14.3
Lenzites betulina 130	0.0	11.3	90.7	17.1	5.4	12.0	39.1
Lenzites betulina IFO 6266	14.1	16.6	21.7	0.0	0.0	0.9	23.6
Daedaleopsis tricolor 164	12.1	17.3	25.2	0.0	3.1	0.0	25.4
Fomes fomentarius 150	0.0	0.0	23.5	0.0	22.9	17.0	–
Pycnoporus coccineus 866	11.6	50.4	1.6	94.1	82.5	9.3	32.7
Irpex lacteus 18	0.0	57.4	49.8	23.7	76.1	0.0	59.7
Phanerochaete chrysosporium IFO 31249	7.9	16.3	17.1	0.0	0.0	1.5	7.1
Ganoderma applanatum 136	10.9	21.8	35.8	15.3	81.6	0.0	49.4
Phellinus gilvus 110	45.9	88.6	>99.9	40.6	91.1	8.5	–
Pleurotus ostreatus IFO 30879	0.0	0.0	15.4	0.0	21.1	0.0	38.0
Perenniporia fraxinea 1509	19.7	5.5	0.0	0.0	0.0	0.0	40.9

–, not determined

Color removal by bench-scale fungal/UF system

The experimental set-up used for the lab-scale decolorization study of the fungal/UF system is illustrated in Figure 1. 1,000 pieces of polyurethane foam cubes (PUF; $12 \times 12 \times 15$ mm) immobilizing the *T. hirsuta* IFO4917 cells were prepared in a 12 L glass bottle as the fungal bioreactor. As the membrane filtration unit of this system, a tangential flow UF system provided by Takuma Co. using the polysulfone ultramembrane 1″HF-43-PM30-PB [RomiPro: hollow fiber diameter, 1.1 mm; membrane area, 930 cm^2; molecular weight cut-off (MWCO), 30,000 Da] was utilized. The lab-scale fungal/UF system was operated in a sequencing batch mode. The bioreactor was fed with 10 L of the model colored wastewater supplemented with 1,000 mg/L ethanol as the substrate for fungal decolorization. The model wastewater contained peptone and meat extract as the main organic components and synthetic melanoidins (Miyata *et al.*, 1998) as the typical color components (4,200 color units; color unit was determined according to the combination method of the platinum cobalt method and measurement of the absorbance at 465 nm). The fungal bioreactor was aerated at 5 L/minute from the bottom for 3 days and decolorization was performed at 30°C under non-sterilized condition (the 1st cycle). The fungally decolorized wastewater was sent to the stock tank and subjected to the UF unit at a pressure of 1.5 kgf/cm^2 until 5 L of the permeate was obtained. The concentrate remaining in the stock tank (5 L) was heated with a heater at the defined temperature for 10 minutes for killing microbes which contaminated the fungal reactor. Then 5 L of the fresh model wastewater as influent was fed to the fungal bioreactor with returning 5 L of the heat-treated concentrate (the 2nd cycle). We propose to utilize UF for complete separation of excess fungal cells and removal of higher molecular weight colored components as a post-filter. UF may be effective not only for the above-mentioned purposes but also for concentrating the extracellular decolorizing enzymes. This may allow more extensive and efficient decolorization in the proposed fungal/UF system, because colored components with higher molecular weights are retained longer and the higher enzymatic activities are maintained in the fungal bioreactor. From the

2nd cycle, the fungal bioreactor was aerated for 1 day, and these operations were repeated daily (HRT = 2 days). The membrane was washed with 0.3% sodium hypochlorite solution for 1 hour between the cycles, and the same membrane was used.

The lab-scale fungal/UF system was first operated under the non-sterilized condition. Figure 2 shows the result of sequencing batch decolorization by the system without heating the concentrate. Although temperature and pH were maintained at the optimal condition for the fungus, the air/water-born microbes contaminated the fungal bioreactor (more than 10^6 cfu/ml). As a result the fungal decolorization efficiency was drastically lowered as the operation proceeded, although the color unit of the UF permeate was maintained below 1,000, leading to the accumulation of colored components in the fungal bioreactor. Boman *et al*. (1991) also reported that a fungal bioreactor was less effective for purifying a bleach plant effluent than expected, because the white-rot fungi could not adapt long to the non-sterile experimental condition.

In order to reduce the effects of microbial contamination, the UF concentrate was heated at 50°C for 10 minutes before being returned to the fungal bioreactor. This heat inactivation could lower the air/water-born microbes as cfu by approximately 80%, while both MIP and MnP activities in the retentate showed no significant loss. When the heat inactivation was done at 60 or 70°C for 10 min, more than 99% of the air/water-born microbes were killed. However, the denaturation of organic components in the retentate including the decolorizing enzymes was observed, and this seemed to have adverse effects on the fungal bioreactor and UF treatment, e.g. extraordinary foaming, decrease in the permeate flux, etc. With the heat inactivation at 50°C for 10 minutes, the fungal/UF system showed a relatively high and stable decolorization performance as shown in Figure 3. At a nearly steady state, the fungal bioreactor showed an average decolorization rate of ca. 1,300 color units/day and the UF unit an average color unit rejection efficiency of ca. 60%. The whole fungal/UF system (fungal bioreactor + UF) constantly achieved about 70% of the decolorization, therefore, the contribution of the fungal bioreactor alone to the total decolorization was approximately 45%. As we assumed, it was experimentally confirmed that the UF was effective not only for physically removing higher-molecular-weight colored components, which could not be decolorized by the fungus, but also for concentrating the extracellular decolorizing enzymes, manganese peroxidase (MnP), manganese independent peroxidase (MIP) and laccase, which were produced by the fungus (data not shown).

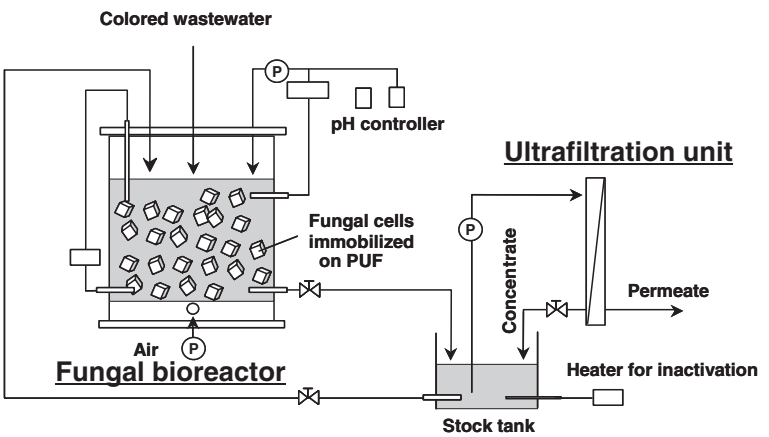

Figure 1 Schematic diagram of sequencing batch fungal/UF system for decolorization of colored wastewater

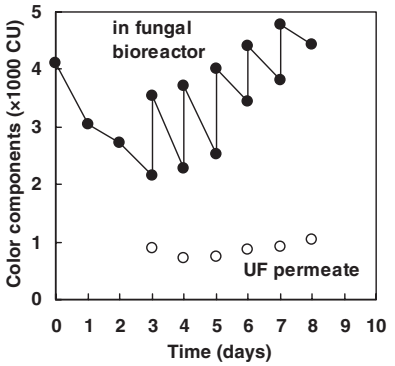

Figure 2 Decolorization of artificial model colored wastewater by the fungal/UF system without heat inactivation in 1 day-cycle sequencing batch mode (HRT = 2 days). Changes in color components in the fungal reactor (●) and permeate (○) are shown. The first 3 days were the start-up period

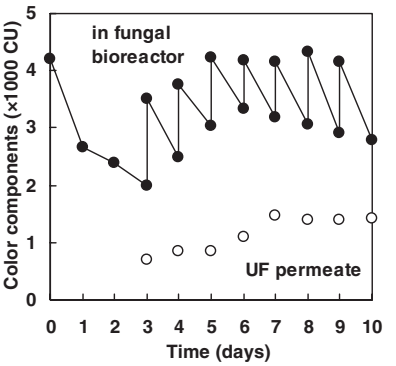

Figure 3 Decolorization of model colored wastewater by the fungal/UF system with heat-inactivation in 1 day-cycle sequencing batch mode (HRT = 2 days). Changes in color components in the fungal reactor (●) and permeate (○) are shown. The first 3 days were the start-up period

Color and ED removal by pilot-scale fungal/UF system

The fungal/UF system was scaled up to a pilot-scale plant (working volume, 200 L), and applied to the treatment of the secondary effluent from a biological treatment process of night soil in order to evaluate the possibility of the practical use. The schematic diagram of the pilot-scale fungal/UF system is shown in Figure 4. The secondary effluent, that is the influent to the pilot-scale fungal/UF system, contained a considerable concentration of color components (approximately 1,000 color units). NP, OP, DEHP and benzophenone which are recognized or doubted as EDs, were also detected in the influent, although natural estrogens like E1–E3 were not found at the significant levels. Operations were done in a trial-and-error mode to optimize the decolorization and ED removal efficiencies under non-sterilized conditions.

Using the pilot-scale fungal/UF system, 65–70% of decolorization efficiency was achieved, when the fungal/UF system was operated at a 1.5 day cycle sequencing batch mode with addition of ethanol at 1.3 g/L of the system influent (an optimized condition). The fungal decolorization accounted for approximately 60% of the decolorization by the whole system. A long-term treatment led to the accumulation of acetic acid which tended to inhibit the fungal decolorization. Transformation of ethanol, which as added to the fungal

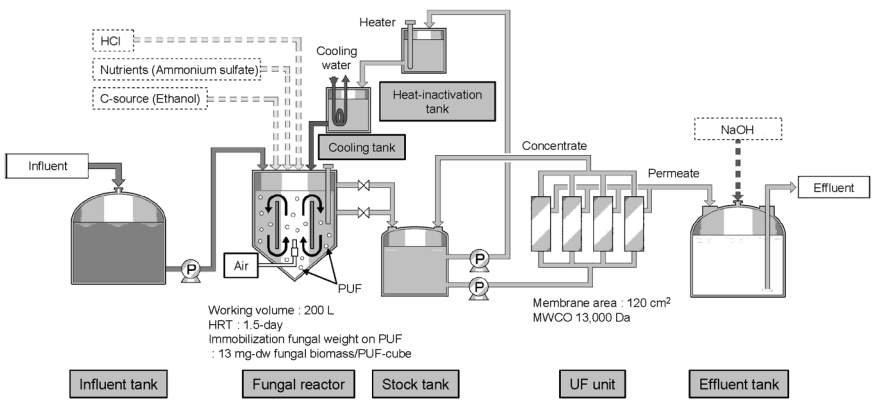

Figure 4 The schematic diagram of the pilot-scale fungal/UF system for decolorization of colored wastewater

Table 2 Removal efficiency of color components and EDs by fungal/UF system

Color and EDs		Influent	Effluent	Removal (%)
Color (CU)		1260	400	68
EDs (μg/L)	NP	1.6	<0.1	>94
	OP	2.9	0.32	89
	DEHP	1.1	0.6	45
	Benzophenone	0.13	<0.025	>81

bioreactor as the carbon source, into acetic acid seemed to be caused by the metabolism by bacteria or yeast contaminated the system. NP, OP and benzophenone were also degraded efficiently with removal of 94%, 89% and 81%, respectively. However, the removal of DEHP was not so effective (45%). A summary of the performance of the pilot-scale fungal/UF system under this condition is given in Table 2.

Conclusion

The experimental results strongly suggested that the fungal/UF system proposed here has a great potential for application to the treatment of a variety of wastes/wastewaters containing colored components and EDs. In addition to the application to the secondary effluent from the night soil treatment processes demonstrated here, treatment of leachate containing EDs, binding to lignin and humic substances, from waste disposal sites by this system would be of interest in future studies. Design and operational parameters of the fungal/UF system, such as the volume fraction of PUF in the fungal bioreactor, timing and concentration of the ethanol addition, HRT of the fungal bioreactor, and MWCO of the UF unit, should be further optimized for its practical use.

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