African Journal of Biotechnology Vol. 7 (3), pp. 286-289, 5 February, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

*Full Length Research Paper*

# Fungal treatment of hemp-based pulp and paper mill wastes

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Accepted 4 January, 2008

**This paper examined the ability of *Penicillium camemberti* to degrade hemp-based pulp and paper plant bleachery effluents in batch and up-flow column reactor studies. In batch tests, the highest removals for acid-line effluents (67% AOX (adsorbable organic halogens), 44% TOC 8 total organic carbon), 97% color) were obtained with 2 g/l acetate concentration in 10 days. Acid-line and alkali-line composite effluent was also fed to a column reactor with of 17 mg/l. AOX concentration. In column studies, the highest removals (57% AOX, 67% TOC, and 74% color) and (57% AOX, 48% TOC and 73% color) were obtained with 0.5 and 0.2 g/l feed acetate concentration in 20 days, respectively. Gas chromatography analysis indicated drastic reductions at low molecular weight adsorbable organic halogen compounds.**

**Key words:** Hemp, bleaching, adsorbable organic halogens, pulping, *Penicillium camemberti*, molecular weight, up-flow reactor.

## INTRODUCTION

Traditionally, spruce and pine are used for chemical softwood pulp to produce strong thick paper. Chemically treated hardwood pulp was introduced when smoother and thinner printable paper was required. The mills of hemp, flax, cotton, sugarcane bagasse, esparto, wheat straw, reeds, sisal, abaca produce so called specialty papers such as cigarette paper, filter paper, coffee filters, tea bags (Van Roekel, 1994).

Despite its low lignin content, hemp bast fiber is somewhat difficult to pulp and bleach. The fibers are cooked for several hours at elevated temperature and pressure until, all fibers are separated from each other. After cooking, the cooking chemicals and extracted binding components are separated from the fibers by washing with excess water. This is where most of the polluting waste emerges from the process. Bleaching treatments often use chlorine compounds, which are also discharged into the environment (Van Roekel, 1994).

Pulping effluents are normally treated biologically for standard parameters such as BOD and COD; but biological treatment is usually not complete. Parameters unique to these wastes along with the classical ones are color and AOX and are virtually persistent thought the treatment cycle. The color originates from pulping and pulp bleaching processes while AOX originates exclusively from chlorine bleaching. Bergbauer and Eggert (1992) states that organic halides are known to have toxic, mutagenic and carcinogenic repercussions in the environment and tend to persist in the ecosystem. In the recent decade, certain wood-rotting fungi have been identified for the ability to degrade lignin and were implied in the treatment of halogenated organics in the pulping and bleaching effluents.

The primary objective of the study was to analyze the ability *Penicillium camemberti* which was found to be very effective in treating softwood pulping and bleaching effluents (Taşeli et al., 2004) and chlorinated model compounds like PCP, 2-chlorophenol and trichloroacetic acid (Taşeli and Gökçay, 2005), to degrade hemp-based pulping and bleaching effluents in batch and in up-flow column reactor studies.

## MATERIALS AND METHODS

### Wastewater

Wastewater samples obtained from Turkish State Paper Industries' (SEKA) Kastamonu Pulping and Paper Plant were used for the batch

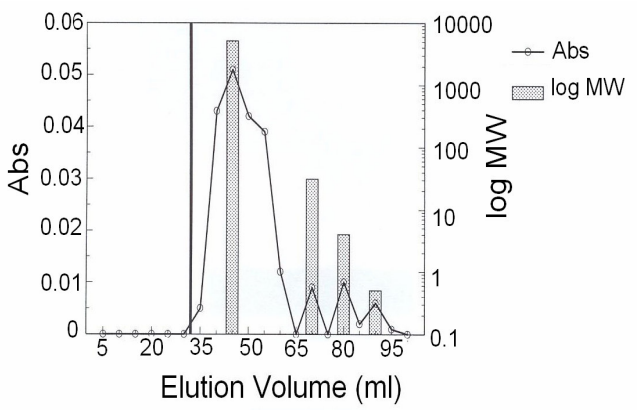


**Figure 1.** Molecular weight distribution of acid-line effluents (Note: Those with elution volumes less than 32 ml have molecular weight greater than 30 000).

and continuous column experiments. Kastamonu pulping plant uses mainly hemp fibers and stalks for raw material to produce cigarette paper. A 3 stage bleachery process is applied to the cooked pulp in the following sequence: Chlorination (C), Alkali extraction with caustic soda (E) and two Hypochlorite (H) stages. In this plant effluents are collected in 3 discharge channels consisting of alkali-line originating from pulping of hemp, paper machine-line originating from paper machine and acid-line. Acid-line wastewaters include solely chlorination stage wash waters whereas alkali-line mainly discharges wash waters from caustic extraction and the two-hypochlorite stages.

#### Biological tests

The *P. camemberti* used in this study has been isolated from chlorination-stage acidic effluents of SEKA-Kastamonu Pulp and Paper Plant in Turkey. The isolated fungus was identified through elaborated biochemical tests (Pitt, 1993). During experiments, hemp based pulping effluents were supplemented with 2 g/l, 0.5 mg/l or 0.2 g/l of acetate and basal salts medium having the following composition: 2 g/l $KH_2PO_4$, 0.5 g/l $MgSO_4$, 0.1 g/l $CaCl_2$, and 0.12 g/l $NH_4Cl$ and 0.001 g/l thiamine. The pH was adjusted to 4.5 - 5.0, and temperature to 25 ± 2°C. Batch culturing was carried out in 200 ml hemp based bleachery effluent samples placed in 500 ml conical flasks that were incubated on a rotary shaker at 80 rpm.

#### Chemical analysis

Adsorbable organic halogens (AOX) analyses were carried out according to German DIN 38409 Norm. The soluble organics were first adsorbed onto pure activated carbon particles and then filtered off on polycarbonate filters, washed with a nitrate solution and combusted in the furnace of the Euroglass 500 AOX analyzer. The chloride release was detected and recorded by the instrument as mg/l AOX.

Gas chromatography analyses were performed on a Perkin Elmer Autosystem 1020 Plus Gas Chromatograph. Firstly, gas chromatograph was calibrated with standard mix solution including target compounds. The calibration procedure was repeated prior every 5 samples. Secondly, effluent samples were first preconditioned with methanol and then were passed through C18 solid phase extraction columns. Organics retained on the C18 column were eluted with freshly distilled chloroform. The collected chloroform phase was dried by passing through anhydrous $Na_2SO_4$ and further concentrated down to 0.1 ml in a micro Kuderna Danish concentrator. The concentrated samples were then injected into a gas chromatograph with electron capture detector and CP Sil-5 capillary column. (30 m, 0.25 mmID, 0.25 micron film thickness).

Total organic carbon (TOC) content of the effluents was determined using the total organic carbon analyzer, model 1555B, Ionics. It was calibrated upon start-up at the laboratory and the calibration was checked at least once a week using a standard solution of known concentration. It can be calibrated for any range from 0 - 2 ppm to 0 - 2000 ppm. In the experiments, a wastewater sample of either 20, 100 or 200 µl (volume depends on the range) was injected into a reaction chamber, packed with a catalyst and held at a fixed temperature. In the TOC reaction chamber a copper oxide or platinum catalyst was used and the chamber controlled at 850°C.

The carbon or inorganic carbon is converted to $CO_2$, which is directly proportional to the concentration of total or inorganic carbon by the action of the catalyst and the elevated temperature. Samples were analyzed for total carbon (TC) and total inorganic carbon (TIC). TOC content of the original sample was found by subtracting TIC from TC.

Relative color at absorbance of 465 nm was measured using the Pharmacia Biotech Spectrophotometer. The UV scans of effluents were obtained by scanning between 200 - 400 nm using a Secomam UV- VIS spectrophotometer.

#### Molecular weight (MW) distribution analyses

Molecular weight (MW) distribution analyses were carried out in a 1 m by 1.5 cm Sephadex G-50 column using 0.1 N LiOH-NaCl solutions as eluent. Fractions were collected and ring structures were followed with absorbance at 280 nm by using an UV Spectrophotometer. The column was suitably calibrated using three reference materials with known molecular weights (Taşeli and Gökçay, 2006).

#### Up-flow column reactor

The biological treatability experiments were conducted in a bench-scale up-flow tubular column reactor with a 6.7 cm inner diameter and 55.7 cm height. The column reactor consisted of a feed tank, a feed pump and the column itself, having an inlet, an outlet, and 4 sampling outlets (Taşeli et al., 2004).

## RESULTS AND DISCUSSION

Before molecular weight (MW) distribution analysis it was expected that low molecular weight compounds and high molecular weight compounds dominate in acid-line and alkali-line effluents, respectively. In order to confirm this, molecular weight distribution analysis of both lines were conducted and results are given in Figures 1 and 2. When these figures are examined it is clear that low MW compounds (MW < 1000) dominate in acid-line effluents whereas high MW compounds (MW between 10 000 and 30 000) dominate in alkali-line effluents.

The ability of *P. camemberti* to dechlorinate and decolorize hemp-based pulping and bleaching effluents was examined in both shaking and non-shaking batch cultures at 25°C, pH 5 and using acetate as primary car-

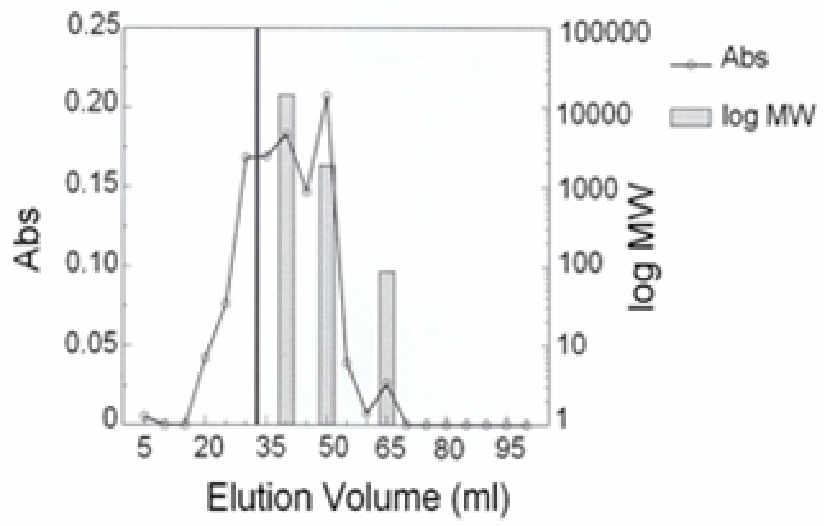


**Figure 2.** Molecular weight distribution of alkali-line effluents (Note: Those with elution volumes less than 32 ml have molecular weight greater than 30 000).

**Table 1**. Results of batch experiments.

| Conditions | Non-shaking[(1)] | | | Shaking[(2)] | | |
|---|---|---|---|---|---|---|
| Effluent | AOX removal (%) | TOC removal (%) | Color removal (%) | AOX removal (%) | TOC removal (%) | Color removal (%) |
| Acid-line effluent | 34 | 82 | 95 | 67 | 44 | 97 |
| Alkali-line effluent | 34 | 66 | 52 | 35 | 21 | 65 |
| Acid-line and alkali-line effluent | 40 | 73 | 52 | 49 | 28 | 68 |

[(1)]Hemp-based bleachery effluent + mineral salts + 2 g/l acetate, pH 5, 25°C.
[(2)]Hemp based bleachery effluent + mineral salts + 2 g/l acetate + 0.05% Tween 80, pH 5, 25°C.

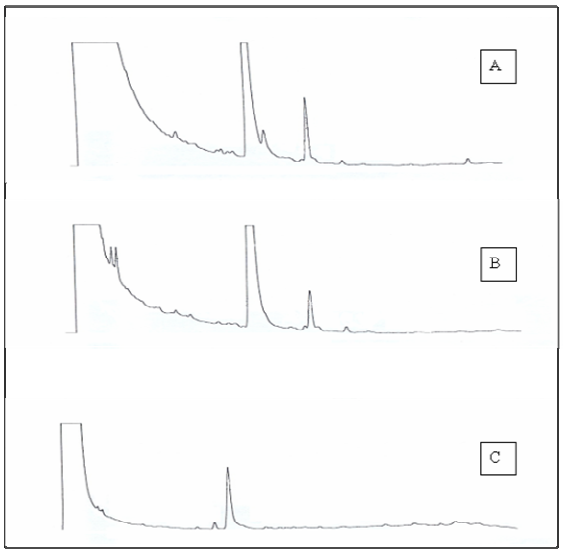


**Figure 3.** GC analysis of acid-line and alkali-line composite effluent before fungal treatment (A), after fungal treatment under non-shaking conditions (B) and under shaking conditions (C).

bon source. The flasks were incubated for 10 days and the solution contained in the flasks were measured on first day and on 10 day of incubation in terms of AOX, TOC and color. 10 day of incubation was chosen since it was proved by the earlier study that 10 day was the optima for the fungus in batch studies (Taşeli et al., 2004). As can be seen from Table 1, batch experiments conducted in shaking flasks resulted in 67% AOX, 44% TOC and 97% color removal for acid-line effluent. It was found that 35% AOX, 21% TOC and 65% color were removed from the alkali-line effluent. Finally removals of 49% AOX, 28% TOC and 68% color were achieved with acid-line and alkali-line composite effluents. It is clear that, small molecular size of the organics presumably present in acid-line effluents is effectively treated by the fungus. Figure 3 showing the gas chromatograms (GC) of acid-line and alkali-line composite effluent before and after column treatment under non-shaking and shaking conditions confirm that *P. camemberti* is very effective in removing low molecular weight compounds especially under shaking conditions. This result is also in accord with the earlier study in which it was proved that the fungus

**Table 2.** Results of up-flow column experiments.

| Effluent | Acetate conc. (g/l) | AOX removal % | TOC removal % | Color removal % |
|---|---|---|---|---|
| Alkali-line effluent | 0.5 | 53 | 36 | 46 |
| | 0.2 | 47 | 38 | 56 |
| Acid-line and alkali-line effluent | 0.5 | 57 | 67 | 74 |
| | 0.2 | 57 | 48 | 73 |

Condition: Hemp based bleachery effluent + mineral salts + 0.2 or 0.5 g/l acetate, pH 5, 25°C.

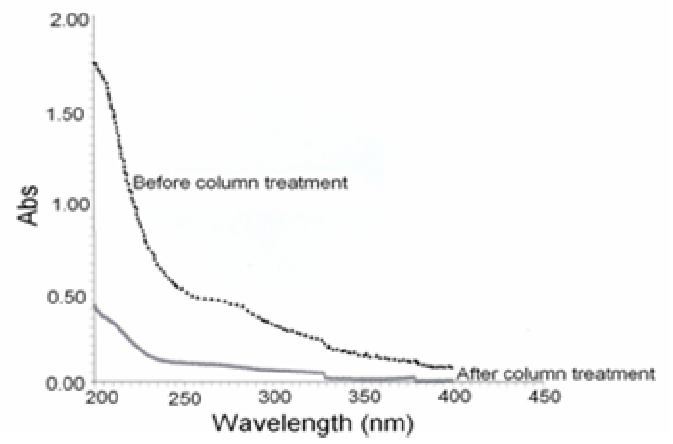


**Figure 4.** UV scans of acid-line and alkali-line bleachery effluent sample before and after column treatment.

effectively removed small sized phenolics (MW < 1000) presents in the softwood bleachery effluents implying toxicity reduction in the effluents (Taşeli and Gökçay, 2006).

The continuous up-flow column reactor used earlier for the treatment of softwood bleachery effluents (Taşeli et al., 2004) and chlorinated model compounds like PCP, 2-chlorophenol and trichloroacetic acid (Taşeli and Gökçay, 2005) was further operated in the laboratory by feeding it with alkali-line effluents and a sample of combined 1:1 acid-line and alkali-line composite effluents without any dilution since optimum initial AOX concentration of 19 mg/l (Taşeli and Gökçay, 1999) was not exceeded. The results are tabulated in Table 2. As can be seen from table 57% AOX, 67% TOC and 74% color removals with 0.5 g/l feed acetate concentration and 57% AOX, 48 % TOC and 73% color removals with 0.2 g/l feed acetate concentration were obtained in 20 days. There was no significant effect of feed acetate concentration on removal efficiencies.

UV scans of acid-line and alkali-line composite sample before and after column treatment are given in Figure 4. When spectrographs are examined it is clear that there is an obvious difference between before and after fungal treatment at critical absorbance bands of 280 nm and 254 nm corresponding to phenol and aromatic ring resonances, respectively. This indicates that the fungus effectively opens aromatic rings of the chlorolignins during treatment. Moreover, the peak observed at 280 nm before fungal treatment is found to have completely removed after fungal treatment.

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