Upflow column reactor design for dechlorination of chlorinated pulping wastes by *Penicillium camemberti*

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A *Penicillium camemberti* strain isolated in our laboratory has been studied for its ability to degrade chlorinated pulping wastes, presumably containing a variety of chlorinated polyphenols. In batch tests, the highest removals (76% AOX, 61% color and 65% TOC) were obtained with 0.2 g/l feed acetate concentration. The tendency of the fungus to dechlorinate bleachery effluents better under non-shaking conditions and to attach onto surfaces suggested the use of immobilized cells rather than freely suspended ones in further exploitation of the process. An upflow glass wool packed column reactor established with this fungus could be operated for nearly two years in the laboratory. At best around 70% AOX could be removed from chlorinated pulping wastes in 7.3 h of contact with no aeration and with a minimal amount of carbon supplement (0.2 g/l). Finally, an asymptotic mathematical formula for determining Michaelis-Menten kinetic rates has been derived. The kinetic rates $K_m$ (the Michaelis constant or saturation constant for the substrate) and $V_m$ (the product of maximum rate for the enzymatic reaction and biomass concentration) were then calculated as 126.386 mg/l and 2.83017 mg/l h, respectively.

1. Introduction

The main constraints of effluent discharges from the pulp and paper mill industry are adsorbable organic halids and the brown or black color due to dissolved lignin-based compounds. The biological treatment of pulp bleachery effluents has been under investigation for some years now. These compounds are usually biologically persistent and in some cases are also toxic. Conventional wastewater treatment, such as activated sludge or aerated lagoons, is known to be not completely effective in treating chlorolignins (Bergbauer and Eggert, 1992). Evidently, biological systems, which currently seem most promising in the treatment of bleachery effluents, are those supporting wood-degrading fungi. In an earlier study it has been reported that AOX (adsorbable organic halogens) removals of around 20% were possible by *Penicillium sp.36, 24% by Penicillium sp.48, 19% by Aspergilus japonicus and 23% by Aspergilus fumigatus* (Milstein et al., 1988).

A fungus, which is able to remove close to 76% AOX, 61% color and 65% total organic carbon (TOC) from chlorinated pulping wastes in batch tests has been isolated and tentatively identified as *Penicillium camemberti* (Taseli and Gokcay, 1999). This was the first time that this species was used for the degradation of chlorinated organics. A continuous upflow packed bed reactor was designed and operated successfully in our laboratory for nearly two years with somewhat higher performances than those obtained in batch tests.

Research efforts in our laboratory have been focused on the development of novel fungal bioreactor technology that permits continuous and efficient treatment of chlorinated effluents from the paper industry and from other sources. Use of immobilized *P. camemberti* appears to be a promising technology to achieve this goal.

The primary objective of the present article was to analyze the dechlorination kinetics in a continuous upflow...
packed bed bioreactor system using \( P. \) \textit{camemberti}. An understanding of the kinetics of color, \textit{TOC} and \textit{AOX} degradation should provide a basis for process analysis and design of pilot scale studies as well as determining conditions for optimum removal with this fungus. The hydraulic residence time (HRT) within the column was used as the primary determinant parameter in the studies. The parameters and conditions that enhance bioreactor performance in terms of decolorization and dechlorination were also investigated.

2. Materials and methods

\textit{Wastewater.} Wastewater samples obtained from Turkish State Paper Industries’ (SEKA) Dalaman Pulping and Paper Plant were used for the continuous column experiments. The Dalaman pulping plant uses mainly pine softwood for raw material. The Kraft process is employed for pulp production in the works and cellulose is cooked in a vertical Camyr pressurized cooker. A six stage bleachery process is applied to the cooked pulp in the following sequence: Chlorination (C), Alkali extraction with caustic soda (E), Hypochlorite (H), Chlorine dioxide (D), Alkali extraction (E) and Chlorine dioxide (D) stages. The abbreviation CEHDED is used in this paper to mean chlorinated pulping wastes. The average \textit{AOX} concentration observed in 24-h composite samples taken from the end of bleachery effluent line varied between 29.6 and 66.46 mg/l. This corresponds to 2.18 and 11.48 kg \textit{AOX}/ton pulp produced, respectively.

\textit{Organism and culture conditions.} The \( P. \) \textit{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 elaborate biochemical tests (Pitt, 1993). During the experiments chlorinated pulping effluents were supplemented with 2 or 0.2 g/l of acetate and basal salts medium having the following composition: 2 g/l \( \text{KH}_2\text{PO}_4 \), 0.5 g/l \( \text{MgSO}_4 \), 0.1 g/l \( \text{CaCl}_2 \), 0.12 g/l \( \text{NH}_4\text{Cl} \) and 0.001 g/l thiamine. The pH was adjusted to 4.5–5.0, and temperature to 25 \( ^\circ \text{C} \). Batch culturing was carried out in 200 ml bleachery effluent samples placed in 500 ml conical flasks that were incubated on a rotary shaker at 80 rpm.

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

\textit{Color Measurements.} Relative color at absorbance of 380 and 465 nm was measured using a Pharmacia Biotech Spectrophotometer.

\textit{Total Organic Carbon Analysis (TOC).} The organic content of the effluents was determined using a total organic carbon analyzer, model 1555B, Ionics.

\textit{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 shown in Fig. 1 consisted of a feed tank, a feed pump and the column itself, having an inlet, an outlet, and four sampling outlets. The bioreactor was operated in a continuous manner; the feed solution was either a concentrate or its diluted form as required for the experiments and was continuously supplied to the reactor from its bottom and the products were continuously withdrawn from its top.

The up-flow fungal column reactor used in this study was prepared by filling a PVC column with glass wool, which was previously seeded with the isolated fungus. The seeding of glass wool was carried out in shaking flasks using the culture medium described above, in order to homogeneously distribute fungal cells within the glass wool support matrix. The flasks were incubated for 10 days at 20 \( ^\circ \text{C} \) with acetate. At the end of this period infected glass wool was transferred into the empty column with a packing density of 43.62 g/l. The feed solution was pumped into the column reactor at a constant hydraulic loading rate of 2.12 l/l.d. At the end of six months of operation with 2 g/l acetate, the VSS (volatile suspended solids) concentration of the immobilized fungal biomass reached 21,500 mg/l (Taseli, 1997).

3. Results and discussion

3.1. Performance of the column reactor

The performance of the bioreactor was affected by a number of operating variables including HRT, color, AOX and co-substrate concentrations. The effect of HRT was studied by changing the volumetric hydraulic loading to
the column and by measuring the corresponding AOX, TOC and color removals with time.

The kinetic behavior of the bioreactor was first investigated by determining the effect of influent AOX concentration on the bioreactor performance. The bioreactor was operated with influent AOX concentrations of 9, 19 and 36 mg/l at a constant hydraulic loading rate of 2.12 l/l.d. Samples taken from the reactor inlet and outlet were analyzed for AOX. The purpose of this experiment was also to investigate the effect of AOX levels on bioreactor performance. A 36 mg/l initial AOX concentration had a marked inhibitory effect on the system. The highest AOX removal was obtained when the feed was 19 mg/l AOX.

In a similar experiment the effect of acetate concentration as co-substrate on the kinetic behavior of the bioreactor was examined. The AOX removal efficiency was studied by using 2, 0.5 and 0.2 mg/l acetate concentrations. At best 61% of color, 65% of TOC and 76% of AOX removals were achieved in the bioreactor with 0.2 g/l feed acetate concentration.

### 3.2. Kinetics of dehalogenation

The reaction rate \( r_c \), was described in terms of Michaelis-Menten kinetics in the reactor. By assuming plug flow regime inside the reactor it is possible to write a mass-balance equation around the packed-bed reactor. Fig 2 exhibits the flow behavior in the column reactor with 1% NaCl as feed.

The so-called Michaelis-Menten kinetics is recognized as a suitable model (Grady Leslie and Lim) for the degradation of AOX. The model consists of the equation

\[
 r_c = \frac{V_m S}{K_m + S}
\]

where \( r_c \), \( V_m \), \( K_m \) and \( S \) are the reaction rate, the maximum rate for the enzymatic reaction, the Michaelis constant or saturation constant for the substrate and AOX concentration, respectively. The mass balance can then be written as

\[
\frac{K_m}{V_m} \ln \frac{S_{\text{ini}}}{S_{\text{out}}} + \frac{1}{V_m} (S_{\text{ini}} - S_{\text{out}}) = \frac{V}{Q}
\]

where \( Q, S_{\text{ini}}, S_{\text{out}} \) and \( V \) are the volumetric flow rate, AOX concentration before treatment, AOX concentration after treatment and reactor volume, respectively. The important kinetic coefficients \( V_m \) and \( K_m \) are known as Michaelis-Menten constants.

Eq. (2) is equivalent to

\[
y e^{S_{\text{out}} (K_m) Y} = e^{S_{\text{ini}} (K_m) - (V_m/K_m) (V/Q)}
\]

where we have defined the ratio

\[
y = \frac{S_{\text{out}}}{S_{\text{ini}}}
\]

The series expansion of the exponential function (Abramowitz and Stegun) on the left-hand side is

\[
e^{S_{\text{out}} (K_m) Y} = 1 + \frac{S_{\text{ini}}}{K_m} Y + \frac{S_{\text{ini}}^2}{2 K_m} Y^2 + \ldots
\]

in which higher powers of \( Y \) can be neglected provided that the ratio \( Y \) is sufficiently small, i.e., \( Y \ll 1 \). Therefore, an asymptotic form of Eq. (3) is expressible as

\[
y + \frac{S_{\text{ini}}}{K_m} Y^2 = e^{S_{\text{ini}} (K_m) - (V_m/K_m) (V/Q)}
\]

which is a quadratic equation in \( Y \). It is an easy matter to find the two solutions of this equation, which are

\[
y_1 = -\frac{1}{2} \frac{K_m}{S_{\text{ini}}} + \frac{1}{2} \sqrt{\frac{K_m^2}{S_{\text{ini}}^2} + 4 \frac{K_m}{S_{\text{ini}}} e^{S_{\text{ini}} (K_m) - (V_m/K_m) (V/Q)}}
\]

and

\[
y_2 = -\frac{1}{2} \frac{K_m}{S_{\text{ini}}} - \frac{1}{2} \sqrt{\frac{K_m^2}{S_{\text{ini}}^2} + 4 \frac{K_m}{S_{\text{ini}}} e^{S_{\text{ini}} (K_m) - (V_m/K_m) (V/Q)}}
\]

However, the second solution \( Y_2 \) should be rejected since it is strictly negative, and, hence, cannot describe a physical phenomenon. Thus solving \( S_{\text{out}} \) from \( Y_1 \) we obtain

\[
2S_{\text{out}} = -K_m + K_m \sqrt{1 + 4 \frac{S_{\text{ini}}}{K_m} e^{S_{\text{ini}} (K_m) - (V_m/K_m) (V/Q)}}
\]

Furthermore, the square root factor on the right hand-side can be approximated by

\[
\sqrt{1 + 4 \frac{S_{\text{ini}}}{K_m} e^{S_{\text{ini}} (K_m) - (V_m/K_m) (V/Q)}}
\]

\[
\approx 1 + 2 \frac{S_{\text{ini}}}{K_m} e^{S_{\text{ini}} (K_m) - (V_m/K_m) (V/Q)}
\]
from which we arrive at the relation
\[ S_{\text{out}} = S_{\text{ini}} e^{(S_{\text{ini}}/K_m) - (V_m/K_m) V/Q} \]  
(11)
suitable for the regression analysis of the experimental data.

Actually, if we deal with the exponential model
\[ y = e^{a+bx} \]  
(12)
the regression analysis can be performed with the variables
\[ y = Y = \frac{S_{\text{out}}}{S_{\text{ini}}}, \quad x = \frac{1}{Q} \]  
(13)
and parameters
\[ a = \frac{S_{\text{ini}}}{K_m}, \quad b = -\frac{V_m V}{K_m} \]  
(14)
corresponding to the intercept and the slope of the logarithm of the best fit curve.

The data used for the regression analysis and the results are presented in Tables 1 and 2, respectively. The data were collected at different periods of the experiment upon when the system reached steady state conditions. The data presented in Table 2 indicate that the model statistically fits to the actual data at over 95% confidence.

It is obvious that \( K_m \) is calculated from the intercept \( a \) in Eq. (14) for a given \( S_{\text{ini}} \). Then \( V_m \) is obtained from \( b \) in Eq. (14) for a specified value of the void volume \( V \). For example, we found that \( K_m = 126.386 \text{ mg/l} \) when \( S_{\text{ini}} = 20.626 \text{ mg/l} \). Similarly, \( V_m = 2.83017 \text{ mg/l h} \) when \( V = 532.589 \text{ ml} \). Note that the void volume, here, is equal to (total volume) \(-\) (bed volume).

In order to find the actual \( V_m \) of the Michaelis-Menten equation we should divide the computed \( V_m \) by the fungal biomass, which was measured as 21,424 mg/l. Therefore we have \( V_m = 0.000132 \text{ h}^{-1} \).

In an earlier study using the white-rot fungus \( P. \ chrysosporium \), which was immobilized in a packed bed reactor employing silica-based porous support, biodegradation of 2-chlorophenol was studied. The Michaelis-Menten constants for \( K_m \) and \( V_m \) were reported as 132 and 45 ppm/h, respectively. The \( K_m \) value obtained for the reactor configuration of this study was comparable to the value reported in the literature for 2-chlorophenol (Lewandowski et al., 1990), but a large difference between the \( V_m \) values was observed. The difference could be due to a variety of reasons, such as temperature, type of organism employed, type of co-substrate etc. The experiments were conducted at 25 °C in this study, whereas it was 39 °C in the latter one. Moreover, 0.2 g/l acetate was used as co-substrate in this study, it was 2 g/l glucose in the latter one. However it is highly possible that the difference is primarily due to the temperature effect, since \( V_m \) is a rate constant whereas \( K_m \) is merely a concentration.

4. Conclusions

An appropriate asymptotic form of the usual Michaelis-Menten kinetics in the reactor has been introduced to model the AOX degradation. The approach is based on the assumption that the ratio \( Y \) defined in Eq. (4) is sufficiently small. The exponential model \( y = Y = e^{a+bx} \) tends toward zero as \( x \) approaches infinity, due to the fact that the slope \( b \) is always negative. This means that as the variable \( x \) in Eq. (13) increases, \( Q \) decreases and hence biological treatment vanishes. Thus, it is clear that there would be no treatment if \( Q \) were zero in the limit.

The experimental results of this study showed that the continuous upflow packed bed reactor containing glass wool-immobilized fungus \( P. \ camemberti \) removed 61% of the color, 65% of TOC and 76% of the chlorinated organics from chlorinated pulping effluents.

The upflow fungal column of \( P. \ camemberti \) was found to be robust enough to be employed successfully in the treatment of pulping effluents, as it retained activity and stability through the course of 2 years of continuous operation.

Acknowledgements

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References


