PROJECT REPORT
2000-8

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Colour Removal from Pulp Mill Effluents Using Immobilized Horseradish Peroxidase

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ISBN 1-55261-066-7

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Colour Removal from Pulp Mill Effluents Using Immobilized Horseradish Peroxidase

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June 2000

ABSTRACT

Previous studies have reported that the Horseradish peroxidase enzyme (HRP) catalyzes colour removal from pulp mill effluents. Immobilized enzyme is generally more resistant to inactivation and is more conveniently retained within a reactor, so a straightforward technique to immobilize HRP using basic ion exchange resin was demonstrated. A reliable assay to determine the activity of immobilized enzyme was developed.

The ability of four ion exchange resins to immobilize enzyme and retain the enzyme in the presence of liquid shear was tested. All of the strongly basic resins was able to immobilize the enzyme. However, Amberlite IRA 400 resin was shown to retain the most active enzyme after being subjected to liquid shear.

Colour removal tests using the immobilized enzyme in the presence of hydrogen peroxide (needed to activate the enzyme) showed that the enzyme was not able to remove colour. This suggested that the colour forming compounds are not substrates of HRP, which is able to catalyze the oxidation and subsequent precipitation of aromatic compounds. This was in disagreement with previous published reports, in which HRP was used successfully to decolorize effluent from bleach processes that used elemental chlorine in at least one step of the bleach process. These results suggest that the colour forming compounds in effluent from the elemental chlorine free bleach processes used in the present study are fundamentally different from those in the previous studies. Specifically it may be inferred that these compounds are not aromatic.

Although the enzyme was unable to remove colour, the Amberlite IRA 400 ion exchange resin removed colour very effectively. This was also contrary to results reported from previous studies which showed the ion exchange resin to remove only minimal amounts of colour from pulp mill wastewater.

Further research is warranted to characterize the major classes of compounds responsible for colour in effluent from elemental chlorine free bleach processes, in order to design colour control technologies on a rational basis. Studying the application of ion exchange technology to the removal of colour and other ionic material from pulp mill wastewaters is also warranted. This technology has potential applications in progressive plant closure.

ACKNOWLEDGEMENT

The authors wish to thank Ms. Rhiannon Johnson of Weyerhaeuser Grande Prairie and Ms. JoAnne Volk of Weldwood of Canada Lt. Hinton Division for providing samples used in this study.

Funding for this study was provided by grants from the Sustainable Forest Management Network and the Natural Sciences and Engineering Research Council of Canada.

INTRODUCTION

The manufacture of kraft pulp is a multistage process. The object of pulping is to separate the cellulose fibers from the lignin which binds the fibers together. The resulting pulp is used in papermaking and the manufacture of a variety of other paper products. Kraft pulp is specifically manufactured for its high tensile strength, brightness and capacity to absorb water (Walden et al. 1985). It is most often manufactured using an alkaline chemical process. The two Alberta pulp mills which contributed samples used in this investigation, employ this alkaline chemical process to produce bleached kraft softwood pulp.

Kraft pulp mill final effluent is a combination of several process wastewaters. These processes include digesting, brownstock washing, bleaching and drying of pulp. The effluent contains various components such as lignin derivatives and spent pulping liquors. This wastewater has a high organic load and must be treated prior to discharge into natural receiving waters. Concern has arisen over the many potential environmental effects including: abnormal pH, high suspended solids concentration, oxygen demand, toxicity, bioaccumulable organics, mutagenics, and colour (Walden et al. 1985). Treatment of pulp mill effluent is usually accomplished by first removing solids by settling and then applying biotreatment. Biotreatment of effluent prior to discharge greatly reduces the concentration of most of the component organic chemicals, which reduces their potential environmental impacts. However, biotreatment does not remove colour.

Pulp mill effluents typically have a dark brown colour. Coloured effluents may inhibit the transmission of light in natural waters, thus causing potential damage to aquatic plants and other organisms. Also, colour causes an aesthetic problem in natural areas. The colour itself is attributed to solubilized lignin residues from the pulping and bleaching processes, with the majority coming from the bleaching steps. (Walden et al. 1985). The colour causing compounds have been described as chromophoric lignin (Royer et al. 1990). It is believed that the residues which cause colour are comprised of coniferaldehyde units, aryl ketones, quinone methides, and quinones (Walden et al. 1985). These quinones are derived from the original lignin, as well as from the degradation of cellulose and sugars, the auto-oxidation of catechols, and the demethylation of guaiacyl and syringyl groups in the lignin. The structure of the lignin and its residues will vary with the type of wood being pulped (Walden et al. 1985). It is also believed that phenols and chlorophenols are major building blocks in pulp mill effluent colour. Ferrer and co-workers (1991) reported that phenolic residues released in the alkaline extraction stages of wood pulp bleaching account for over 50% of the colour load. The task of identifying the precise structures which form colour has not been undertaken.

Several strategies are currently available to remove colour from pulp mill effluent. These include both physical processes such as membrane technologies and chemical processes such as ozonation. Despite the availability of these options colour removal remains a problem for many kraft mills. Oxygen delignification, which is incorporated into the pulping process, has been adopted at several mills. An advantage of this process is that it is able to remove a considerable amount of colour causing compounds which are then captured and burnt during chemical recovery.

The available methods involve considerable startup costs, and can not meet increasingly stringent effluent colour standards in under all circumstances. Therefore, research is needed to develop alternative colour removal methods that can complement or replace existing processes. Enzymatic treatment has been reported to effectively remove colour from pulp mill effluents (Ferrer et al., 1991; Peralta Zamora et al., 1998).

Enzymatic Treatment

The development of enzyme-catalyzed treatment processes to selectively remove specific compounds from industrial wastewaters has been the focus of a considerable amount of research since the 1970's (Aitken 1993). Pure enzymes isolated from their parent organisms are often commercially available and offer several advantages over both chemical and biological treatment. Among the enzymes studies, peroxidase enzymes have been shown to be particularly effective at removing aromatic compounds from wastewaters. When activated by hydrogen peroxide, these enzymes oxidize aromatic compounds to produce free radicals which spontaneously form polymers. As reactions continue, these polymers increase in size until they precipitate from solution (Nicell et al. 1995). Horseradish peroxidase (HRP) has been used to remove phenols and chlorophenols (Davis and Burns 1990), as well as colour from pulp mill effluent (Ferrer et al. 1991; Peralta Zamora et al. 1998). HRP processes have been optimized and enzyme inactivation reduced by the use of protective additives such as polyethylene glycol (Nakamoto and Machida, 1987). While these applications are encouraging, further research is required to assess the applicability of this technology to the kraft pulp industry in Alberta.

Research Requirements

Effluent components can vary greatly from mill to mill. Lignin, the material which holds wood fibers together is broken apart in the pulping process, and varies greatly with climate, wood type and the geology of the area (Walden et al. 1985). Thus, it can not be predicted that because HRP removed colour from effluent in a kraft pulp mill in Brazil (Peralta Zamora et al. 1998) or in Chile (Ferrer et al. 1991) that it would definitely be effective on kraft pulp mill effluent in northern Alberta.

In designing a reactor, soluble enzyme would be difficult to retain within the reactor. It is known that immobilizing the enzyme (attaching it to a solid support medium) can extend its useful life. Peralta-Zamora (1998) effectively immobilized HRP on ion exchange resins, and used the enzyme to remove kraft pulp mill effluent colour. The resins retained a large amount of active enzyme. The ion exchange resins used to immobilize HRP in previous studies are not available in North America. Therefore, research to identify a suitable resin is required. In this investigation, various resins will be tested for their ability to retain active HRP. Reliable methods of measuring immobilized enzyme activity are currently lacking. Therefore a new method should be developed.

Study Goals

The goals of this study are as follows:

- To immobilize HRP on various ion exchange resins;
- To develop a reliable assay to measure the activity of immobilized enzyme;
- To determine which of the exchange resins tested retains the most active HRP;
- To evaluate the ability of the resins to retain active enzyme in the presence of liquid shear;
- To investigate the ability of the immobilized enzyme to remove colour from kraft mill effluents;
- To determine the optimum point within a mill to apply the treatment; and
- To determine the relative contributions of the HRP, peroxide and resin to colour removal.

It should also be noted that the methods developed in this investigation are to be mindful of cost. Enzymatic treatment of pulp mill effluent must be designed to be low cost and reliable.

MATERIALS AND METHODS

Established methods were used to quantify the activity of soluble enzyme and to determine colour concentrations. For determination of immobilized enzyme activity, a new method was developed and is described below. A detailed account of the materials and methods used in the study may be found in Grant (2000).

Immobilized HRP Assay

In order to determine the activity of the enzymes immobilized on the different media used in this investigation, a reliable method needed to be developed. The method used by (Peralta Zamora et al. 1998) of taking the soluble HRP activity of the filtered fractions after immobilization was found to be unusable, as there was no significant difference between the activity of the original solutions and the activity of the filtered fractions after immobilization. However, this was not convincing evidence that no enzyme was being immobilized, due to the large amount of enzyme in the supernatant solution and the possibility that a small amount relative to the supernatant was immobilized. Thus, a new method was required that would be quick, reliable, and reproducible.

A colorimetric assay was developed to function in a manner similar to the soluble HRP assay. That is, the rate of colour formation in a reaction mixture of 10 mM phenol, 2.4 mM AAP and 0.2 mM H_2O_2 and an immobilized enzyme sample was measured at 510 nm. Measured amounts of medium (with enzyme attached) were added to 100 mL batches of this mixture and stirred at a constant rate. At regular intervals, 1 mL was removed from the reaction vessel and put into a semi-micro cuvette Added to the cuvette was 100 μL of catalase solution. The catalase quenches the reaction by converting very quickly any remaining peroxide to oxygen and water and thus stops the reaction and prevents any more colour from forming in case the removed sample contains active enzyme sheared by the mixing of the reaction vessel. The colour intensity was then measured over time to calculate the amount of active enzyme immobilized on the medium per gram medium added. The intervals and amounts of media added were chosen based on achieving a linear trace of colour production versus time, during which the assay is accurate; that is, when the formation of colour is directly proportional to the amount of peroxide being used up by the enzyme. The peroxide and other reactants are in excess throughout the assay and the rate of colour development is proportional to the enzyme activity.

RESULTS

The first experimental portion of this study involved testing the soluble assay to determine activity. This portion was to establish that the enzyme was indeed active and its activity could be measured reliably. The next step was to immobilize the enzyme. Once immobilized, its activity was determined. The best medium was found to be IRA-400, based on activity retained and resistance to the shearing force test. The IRA-400 was then used to test colour removal from kraft mill final effluent in a factorial design experiment which tested the effects of enzyme dose, peroxide level and PEG level. Once optimum conditions were established, colour removal tests were performed on a weaker colour kraft effluent, as well as a combined bleach plant effluent from each of the two mills.

Enzyme Immobilization and Retention

The amount of enzyme immobilized per unit mass of resin is not the only important criterion in finding a suitable resin for immobilizing HRP. The resin must be able to retain HRP in an active form under the turbulent conditions that are likely to exist within a reactor. Thus, HRP was immobilized on each of four ion exchange resins, and the immobilized enzyme was then subjected to the liquid shear forces caused by rapid mixing. The results are indicated in Table 1.

Table 1. Summary of immobilization and fluid shear test results

Medium	Initial Immobilized Enzyme Activity (units/gram)	Activity Retained after Shear Test (units/gram)	Activity Released (%)
IRA 67	0.08	0.047	41.1
IRA 400	1.0	0.98	1.63
IRA 410	0.25	0.23	10.64
IRA 900	1.25	0	116.8

IRA 900

IRA 900 is a macroreticular type 1 polystyrene strongly basic anion exchanger which contains quaternary ammonium groups. It contains large pore sizes, forming a sponge-like matrix which is capable of removing large size soluble organic particles. It was used in the Cl^- form. The average activity retained on the IRA 900 resin was 1.25 units/gram of resin with a standard deviation of 0.14 units/gram over 6 trials.

The IRA 900 resin had shown the highest capacity for immobilizing active HRP, as shown in Table 1. However, once subjected to the fluid shear, the supernatant liquid showed that an average of 117% of the active enzyme had been sheared off by the stirred liquid. It is possible to have a number greater than 100% here if some of the HRP had been immobilized in an orientation that blocked access to the enzyme’s active site. When subjected to shear forces, apparently the all of the enzyme was released and some, or all, of the inactive enzyme was re-activated. Based on these results, IRA 900 was eliminated from further consideration.

IRA 67

IRA 67 is a weakly basic anion exchanger. It has a crosslinked acrylic divinylbenzene copolymer matrix. The functional ion in IRA 67 is a tertiary amine. It was used in the free base form. This resin did not immobilize much active enzyme, averaging only 0.08 units/gram of resin tested with a standard deviation of 0.02 units/gram over 6 trials.

The fluid shear test resulted in an average of 41% release of active immobilized enzyme. It should be noted that the total amount of active HRP immobilized was only 0.08 units/gram before the shear test. Thus, there was a very small amount of enzyme to deal with in the first place. Due to the very small amount of immobilization, coupled with a relatively high removal by shear, the IRA 67 resin was also eliminated from further consideration.

IRA 400

IRA 400 is a strongly basic gel anion exchange resin. The functional group is a quaternary ammonium group and the matrix is a polystyrene divinylbenzene copolymer. This resin retained an average of 1.0 unit/gram of resin. The standard deviation over the 6 trials was 0.4 units/gram, indicating a larger range of values.

IRA 400 resin immobilized the second largest amount of active enzyme. The resin also demonstrated the greatest ability to withstand shear. When subjected to the fluid shear test, an average of only 1.63% of the active enzyme was released from IRA 400. This was the best combination of immobilization and shear resistance. Therefore, IRA 400 was selected for use in the subsequent colour removal tests.

IRA 410

IRA 410 is another strongly basic anion exchanger that has type 2 quaternary amine as its functional group in a crosslinked styrene divinylbenzene copolymer matrix. The IRA 410 resin did not yield results as repeatable as IRA 400 or 900. The average activity retained on the IRA 410 was 0.23 units/gram of resin with a standard deviation of 0.12 units/gram.

The IRA 410 resin also performed relatively well in the fluid shear test, averaging 10.64% release of the active HRP. However, since the average retention of active enzyme was only 0.25 units/gram, it was eliminated from further consideration.

Colour Removal Trials

The ability of HRP immobilized on IRA 400 resin to remove colour from pulp mill wastewaters was tested. A factorial experimental design was followed to test the main effects and interactions of the three independent variables: polyethylene glycol, hydrogen peroxide and immobilized enzyme dose. These factors were tested at three levels yielding a 3^3 factorial design. Polyethylene glycol (PEG) is an additive used to protect the enzyme from rapid inactivation, and hydrogen peroxide is needed to initiate the enzyme's catalytic cycle. The dose of immobilized enzyme is expressed in terms of combined mass enzyme and resin, and indicated as *enzyme/resin*. Keeping in mind that approximately 1 unit of enzyme activity is immobilized on each gram of IRA 400, the masses cited are essentially equivalent to the units of active enzyme present. The main results are summarized below. A full account of all results is available in the Grant (2000).

Each of the experimental trials was carried out in duplicate. The colour removals observed in each pair of replicates differed by 5% or less. The results of these tests are shown in Table 2.

Table 2. Colour removal achieved during the factorial experiment.
Initial colour of all samples was 1065 platinum cobalt colour units (pccu).

Trial	PEG (mg/L)	H_2O_2 (mM)	Enzyme/Resin Dose (g)	Colour Remaining (pccu)	Colour Removed (pccu)
1	0	0	5	490	575
2	200	0	5	460	605
3	400	0	5	465	600
4	0	5.8	5	330	735
5	200	5.8	5	415	650
6	400	5.8	5	365	700
7	0	11.6	5	300	765
8	200	11.6	5	310	755
9	400	11.6	5	315	750
10	0	0	15	290	775
11	200	0	15	320	745
12	400	0	15	330	735
13	0	5.8	15	250	815
14	200	5.8	15	255	810
15	400	5.8	15	280	785
16	0	11.6	15	195	870
17	200	11.6	15	220	845
18	400	11.6	15	220	845
19	0	0	30	180	885
20	200	0	30	180	885
21	400	0	30	180	885
22	0	5.8	30	180	885
23	200	5.8	30	155	910
24	400	5.8	30	150	915
25	0	11.6	30	130	935
26	200	11.6	30	145	920
27	400	11.6	30	145	920

As can be seen in Table 2, the colour removal is highest for highest values of the factors tested, suggesting that additional colour removal could be achieved at higher doses. Nevertheless, the high HRP/resin dose trials yielded up to approximately 85% colour removal from the kraft mill final effluent sample.

Effects and Interactions

The main effects and interactions of the factors tested were evaluated using Yates' Algorithm (Box and Draper 1987). The ANOVA method was applied subsequently to determine the significance of these values and hence of the factors and interactions. This procedure indicated that only the enzyme/resin and the hydrogen peroxide doses were significant at a 95% confidence level. These results led to the following empirical linear model to indicate the relative effect of each independent variable on colour removal from kraft mill effluent:

Colour removed (pccu) = 111.4(Enzyme/Resin dose in g) + 50.8(H_2O_2 conc. in mM) [1]

As indicated in equation [1], the effect of the enzyme/resin dose is greater than that of the peroxide. The colour removals achieved in each of the trials is indicated in Figure 1. It is evident that the dependence of colour removal on the peroxide and enzyme/resin doses is not linear. Therefore, while equation [1] may be used to indicate the relative importance of the peroxide and enzyme/resin doses, it is not an acceptable model to describe colour removal. A residuals plot of the fit of equation [1] to observed data as well as a complete account of the application of Yates' Algorithm and the ANOVA procedure may be found in Grant (2000).

Because the effect of interaction between the two independent variables was found to be insignificant (at the 95% confidence level), the effect on colour removal of peroxide and enzyme/resin dose is additive. The relationship between substrate removal and hydrogen peroxide or enzyme concentration has generally been found to be linear. The non-linear nature of the colour removal relationship observed in the present study suggests that the colour removal may not be attributed solely to the action of peroxide and enzyme. A non-linear empirical model to describe the dependence of colour removal on peroxide concentration and enzyme/resin dose was found to be:

$$\text{Colour Removed} = 8.80[\mathrm{H_2O_2}] + 478.80[\text{Enzyme/Resin}]^{0.17} \qquad [2]$$

Where colour removal is expressed in pccu; peroxide concentration is in mM; and enzyme/resin dose is in g. The coefficient of determination (r^2) of the fit of equation [2] to the data shown in Figure 1 was calculated to be 0.93, indicating that much of the variation of colour removal with the two independent variables is described by equation [2]. The fit of this model to experimental results is shown in Figure 1.

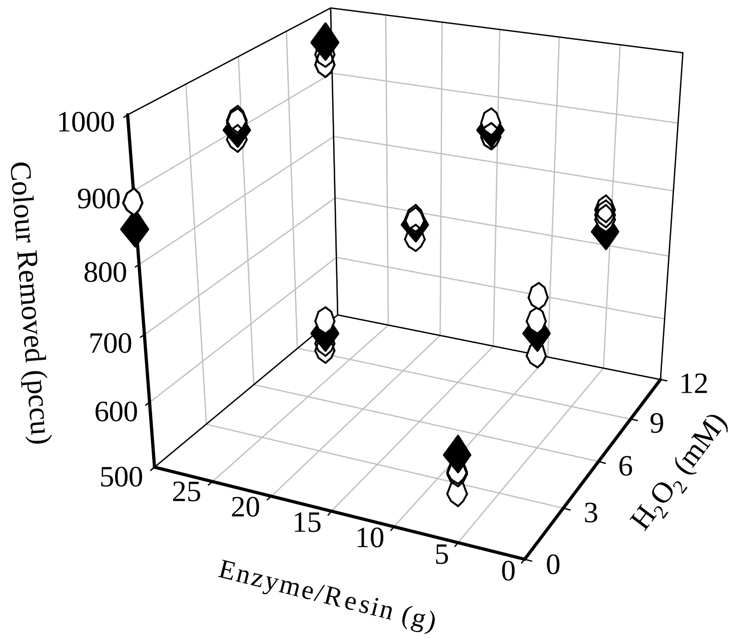

Figure 1. Measured and modeled colour removal from pulp mill final effluent having a 1065 pccu initial colour (! measured removal; ♦ modeled removal).

Determination of Optimal Point of Application

The optimal conditions from the factorial experiment on the final kraft effluent were determined in order to test the colour removing potential on other waste samples. As both peroxide and enzyme/resin effects are positive in equation [1], and the highest doses provided the best results, the high dose levels were used for the other effluent samples. That is, 11.6 mM H_2O_2 and 30g of HRP/resin. To determine at which point in the bleach process the treatment is most effective, colour removal from three other kraft effluents was tested These included: a low colour final effluent from a the Weldwood of Canada pulp mill in Hinton; a mixture of acidic and caustic effluent from the Weldwood bleach plant adjusted to pH 6.8; and a mixture of Weldwood D_0 (acidic effluent from first chlorine dioxide bleaching step) and E_{op} (caustic effluent from oxygen/peroxide alkaline extraction step) adjusted to pH 6.8. All tests were performed in triplicate and the average result reported. The results are listed below.

Weldwood Final Effluent

Final effluent from the Weldwood of Canada kraft pulp mill in Hinton, Alberta was tested under the conditions described above. Initial pH of the effluent was 8.8. The characteristics of this final effluent differ from those of the Weyerhaeuser effluent. For example, the initial colour of the Weldwood final effluent was only 420 pccu, as opposed to the 1065 pccu for the Weyerhaeuser final effluent. The main reason for the difference is the presence of an oxygen delignification step prior to bleaching in the Weldwood mill. This step is known to greatly reduce colour. After 20h at 25°C with 11.6 mM H_2O_2 and 30g HRP/IRA-400, the final colour of the Weldwood effluent was 110 pccu, a 73% or 310 pccu removal.

Weldwood Acid and Caustic Sewers

The combined wastes from the acidic and caustic sewers from the Weldwood bleach plant have higher colour levels than the Weldwood final effluent. This is because the bleaching effluents are the major contributors to pulp mill effluent colour. Initial colour of this stream was 590 pccu. After treatment, the 30 pccu of colour remained in the sample. This corresponds to 95% removal of the colour initially present.

Weyerhaeuser Acid and Caustic Sewers

The combined acidic and caustic bleach plant effluent from the Weyerhaeuser mill contained the highest colour of all effluents tested. This mill did not use oxygen delignification at the time samples were collected. The initial colour of this effluent was 1365 pccu. The colour remaining after treatment was 370 pccu, which represents a 73% colour removal.

Immobilized Enzyme Assay to Test Residual Activity After Use

After the enzyme/resin was used to remove colour from the Weyerhaeuser effluent a portion was saved and tested again for any remaining HRP activity. It was found that a small amount of enzyme activity remained, 0.03 units/gram of resin. Data from this trial and calculations can be found in Grant (2000).

Colour Removals by Soluble Enzyme vs. IRA 400 Resin

It is known that some ion exchange resins are capable of adsorbing large organic molecules. Thus, it is possible that a significant portion of the colour removed in the trials was removed by the resin itself, and not specifically by the action of the enzyme. In order to determine this, control trials were performed: these consisted of using soluble HRP without resin, and resin alone. Results from these trials are shown in Table 3. It is evident from these results that colour removal by enzyme is insignificant, and that enzyme may in fact increase the amount of colour in a sample. However, the IRA 400 ion exchange resin is very effective at removing colour from the Weyerhaeuser final effluent.

Table 3. Results of control tests (1065 pccu initial colour).

Resin Dose (g)	Colour Removed (pccu)	HRP Dose (units of activity)	Colour Removed (pccu)
5	590	15.3	-104
15	780	153	-232
30	840	800	-169

Figure 2 contains a comparison of the results of colour removal by the ion exchange resin alone to removals predicted by equation [2] (with a peroxide concentration of 0). The close agreement between measured and predicted removals indicates that equation [2] may be used to predict colour removal by Amberlite IRA 400 resin.

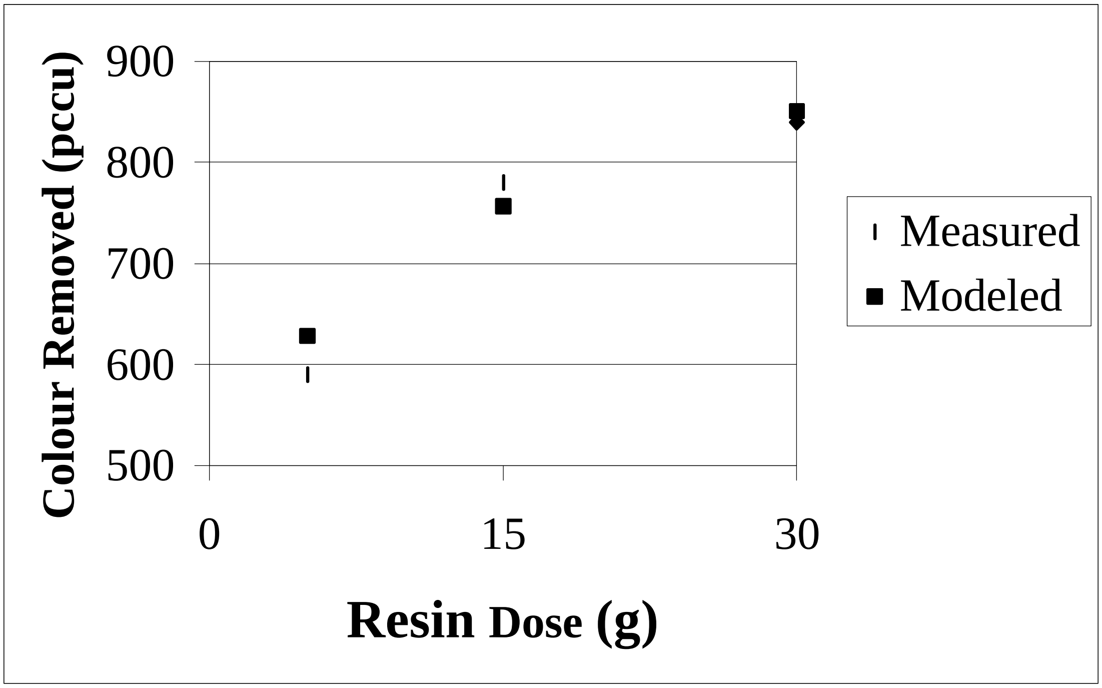

Figure 2. Comparison of measured and modeled colour removal by resin alone.

DISCUSSION

Comparison of the Resins

All four resins were basic anion exchangers. IRA 67 is a weakly basic exchanger. It has a tertiary amine as its functional group in a crosslinked acrylic divinylbenzene copolymer matrix. The pore spaces are smaller in the IRA 67 resin than in the others. It is the only weakly basic resin tested. It retained the least amount of active HRP, much less than the others. The most obvious difference is that the exchanger is weakly basic, rather than strongly basic with smaller pore sizes. As these are the main physical differences between the resins, it is likely that these are the main contributing factors to the lack of retention of active enzyme.

IRA 410 is a strongly basic exchanger, which, like IRA 400, has quaternary amines as functional groups. Like IRA 400 and IRA 900, it has a crosslinked polystyrene divinylbenzene copolymer matrix as its structure. However, IRA 410 retained much less active enzyme than IRA 400 and IRA 900. It is most likely that the weaker affinity for the HRP is due to a smaller pore size which is unsuitable for HRP adsorption and ion exchange. The enzyme molecules may not fit the pore sizes well such that the majority of the active sites are not able to function properly.

IRA 400 resin is also a strongly basic anion exchanger. It also has a quaternary ammonium as the functional group. The greater affinity for the HRP is most likely due to larger pore sizes which allow the HRP to remain securely immobilized while keeping the enzyme's active sites available for reaction.

IRA 900 resin has the same structure and functional group as IRA 400. It was able to retain more active HRP, but lost all of the retained activity during the shear test. It is most likely that the pore sizes are large enough to allow diffusion of the enzyme to exchange sites, yet offer no protection to the shearing force of the moving fluid, causing rapid desorption. The results of the shear test suggested that not only was all of the active enzyme removed, but also a substantial amount of enzyme that had been immobilized in an inactive configuration was re-activated upon desorption.

The best of the resins tested was IRA 400 for retaining the most immobilized active HRP under turbulent conditions. IRA 400 apparently has an appropriate pore size for enzyme diffusion while offering protection from shear forces. The quaternary amine functional groups are apparently suitable for enzyme immobilization.

Colour Removal

Previous studies, such as that of Peralta Zamora (1998), used immobilized HRP to remove colour from pulp mill effluent. This colour removal was achieved by the removal of aromatics which HRP acts on. These chromophoric bodies are polymerized until they precipitate from the solution. In the previous studies, both soluble and immobilized HRP were effective in removing colour forming bodies from the effluents tested. In this investigation it was found that neither soluble nor immobilized enzyme was responsible for any of the colour removed in the trials. Instead, the colour was removed by the ion exchange resin itself.

Hydrogen peroxide also contributed to colour removal, but to a lesser extent. Hydrogen peroxide is a strong oxidant that is known to break down colour bodies, and is sometimes added to pulp mill effluent to reduce colour before discharge to the receiving water. Therefore, some contribution to colour removal by hydrogen peroxide had been expected.

The IRA 400 resin removed an average of 27 pccu from final kraft effluent per gram of resin provided. Rohm and Haas, who manufacture the resin, state that the large pores, sponge-like matrix and strong basicity of IRA 900 and IRA 400 allow larger soluble organics to diffuse from solution into the resins. An article by Fitch (1980) describes cases where ion exchange technology was used to remove pollutants and colour from bleach plant effluent. Weakly basic ion exchange resin was reported to remove 70% of colour in highly coloured (12000-14000 colour units) bleach plant effluent. Fitch (1980) stated that soluble organics with strongly ionized molecular groups will be strongly attracted to ion exchange resin and will bond very tightly. These stronger organics will have such an affinity for the resin that they will even displace weaker organics.

Colour Structure

The fact that HRP did not remove colour suggests that the colour causing compounds in the samples employed in the present study are fundamentally different from those of the previous studies. Colour forming bodies are thought to be largely made up of chlorophenols, quinones, and aromatic ketones, high molecular weight compounds with many aromatic rings. However, because no colour was removed by the HRP, it may be inferred that the colour forming bodies in the effluents tested do not contain significant amounts of aromatic compounds.

The work of Peralta Zamora and co-workers (1998) showed that a small portion of the decolorization of final effluent could be attributed to adsorption to the resin support. However, no colour was removed from the E1 effluent and the combined bleach plant effluents by the resin. This is very different from the results of the current study, where colour was removed from each type of wastewater sample by the resin. This difference is further indication that it is likely that the colour forming species in the Brazilian study are very different from the colour forming species in the Alberta kraft mill effluent tested in this investigation.

A possible explanation for the difference in colour body structure in the Alberta mills and previous studies is in the bleaching sequence. Both of the mills tested in this investigation use an elemental chlorine free bleaching sequence. Elemental chlorine free bleaching is a relatively new process which uses chlorine dioxide bleaching steps rather than elemental chlorine. Alberta mills were converted to elemental chlorine free bleaching in the early 1990’s in order to prevent the release and formation of dioxins and furans such as 2,3,7,8-tetrachlorodibenzodioxin to the receiving waters. Each of the previous studies which used HRP to remove pulp mill effluent colour was performed on effluent from mills which still used at least one elemental chlorine bleaching step. There is very little information available which compares colour structure from elemental chlorine bleaching processes with colour structure from elemental chlorine free bleaching processes. However, Kemeny (Kemeny and Banerjee 1997) wrote that substituting chlorine dioxide for chlorine increases the population of lower chlorophenol congeners. Moreover, these lower congeners are much more biodegradable than more highly substituted chlorophenols. If these chlorophenols made up the colour, and were in fact highly biodegradable, colour would be lowered across the effluent treatment system. This is not the case at either one of the mills that contributed wastewater samples. Thus, the colour is not made up of chlorophenols, at least not lower congeners, in any large part.

It is also possible that the reason for the difference in the effluent components may be due to differences in climate, geology and the makeup of lignin in different types of wood. None of the previous studies used northern boreal forest softwood. It is possible that the structure of lignin in other climates with differing soil geology varies so greatly that the compounds solubilized during the pulping process are different. If this is the case, it could explain why the colour can be removed by HRP from effluent in Brazil by Peralta-Zamora (1998) but not in northern Alberta.

Comparing the Effluents

Analysis of the test results shows that the largest amount of colour removed per gram of resin was for the high colour bleach plant effluent from the mill which did not use oxygen delignification (Weyerhaeuser). For this effluent, an average removal of 33 pccu/gram resin was observed. The next highest amount was for Weyerhaeuser final effluent at 30 pccu/gram resin, which was also the next highest colour waste tested. The Weldwood bleach plant effluent and final effluent, respectively had removals of 18 and 10 pccu/gram resin. The resin removes the most colour from the high colour wastes. Thus, the highest colour stream makes the best candidate for this treatment. The resin did not remove all the colour present in the sample. It is possible that the colour bodies removed in the oxygen delignification process are the same colour bodies removed by the resin IRA 400. If this is in fact the case, then perhaps ion exchange technology could be investigated as a low cost alternative to oxygen delignification.

Because the resin removes the most colour from the high strength colour streams, it would be most prudent to use this technology on the bleach plant effluent.

CONCLUSIONS

This investigation has lead to the conclusions outlined below.

Method for Determining Activity of Immobilized Enzyme

A method was developed to determine the amount of active enzyme immobilized on a solid support. This method involved a small batch assay. At known time intervals, small samples were removed, the reaction was stopped and the colour developed at 510 nm was measured. The linear increase of colour with time at this wavelength is proportional to enzyme activity. This method proved to be reproducible and reliable.

Comparison of Ion Exchange Resins

Four basic anion exchange resins were compared for their capacity to immobilize active enzyme and retain it when exposed to shearing forces. It was found that the strongly basic resin Amberlite IRA 400 performed the best of those considered, retaining 1.0 unit of activity /gram and showing excellent resistance to fluid shear.

Colour Removal

Both soluble and immobilized enzyme were shown to be incapable of removing colour from pulp mill final effluent. However, strongly basic IRA 400 ion exchange resin proved very effective at removing colour from the wastewater samples.

Colour Structure

Traditionally, the structures of colour forming bodies were thought to be largely phenols, and other aromatics. These compounds are substrates of HRP and so have been successfully removed in the past by the enzyme. However, in this investigation the HRP was unable to remove any colour. An obvious difference between wastewaters on which HRP was effective and those on which HRP was ineffective is the bleach process. HRP has been shown to catalyze colour removal from wastewaters from bleaching processes that employ elemental chlorine. The samples used in the current study were from mills that operate elemental chlorine free bleach processes. This suggests that the elimination of elemental chlorine from the bleach process leads to the formation of colour forming compounds that are fundamentally different from those which are formed when elemental chlorine is used in the bleach process. Specifically, this study provides evidence that elemental free bleaching of pulp yields colour causing compounds that are not aromatic.

RECOMMENDATIONS

Further research is required before any of the technologies and ideas developed in this study can be used in industrial applications. Such research should include investigating the application of ion exchange technology to the removal of colour and other ionic material, and an assessment of the structure of the specific components of pulp mill effluent colour.

Colour Forming Compounds

The inability of HRP to remove any colour from the effluent led to the conclusion that the colour forming compounds were not phenolic or other aromatic structures. In order to design colour removal technology on a rational basis, it is important that future study includes the characterization of the major classes of colour causing compounds.

Colour Removal by Ion Exchange Resins

Amberlite IRA 400 resin was shown to remove colour effectively, with up to 85% of a sample's colour being removed. Further studies into the use of ion exchange resins to remove colour is warranted. This research should include investigation of various ion exchange resins from the perspective of colour removal ability and the extent of regeneration possible. It is likely that ionic material in addition to colour forming compounds will be removed by the resins. Therefore, ion exchange technology may be a useful tool toward progressive plant closure.

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