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ABSTRACT

A new method has been elaborated for the removal of phenols and aromatic amines from industrial wastewaters. It involves the treatment of solutions containing the pollutants with horseradish peroxidase and hydrogen peroxide. Such treatment results in precipitation of phenols and aromatic amines from water due to their enzymatic crosslinking. This approach was used to remove over 40 different phenols and aromatic amines (including known human carcinogens and mutagens) from water. For many pollutants, the efficiencies of the enzymatic removal are very high (exceeding 99%), whereas for others they are significantly lower. It was discovered that easily removed phenols and aromatic amines greatly enhance the enzymatic precipitation of those that have relatively low removal efficiencies. This finding is of great importance for the treatment of "real" industrial wastewaters, which always contain mixtures of pollutants. It was found that a crude preparation of peroxidase is as efficient in the removal of pollutants as the highly purified enzyme. The peroxidase treatment has been successfully used to dephenolize different samples of real industrial aqueous effluents.

INTRODUCTION

Various aromatic compounds, in particular phenols and aromatic amines, are present in wastewaters of numerous industries such as coal conversion, petroleum refining, organic chemicals and dyes, resins and plastics, iron and steel, and ore mining and dressing. For instance, phenol, cresols (methylphenols), and xylenols (dimethylphenols) are produced by the high-temperature carbonization of coal. In the United States, which possesses about one-half of the world's estimated coal reserves, these processes will increase in importance with time as alternative supplies of fossil fuels diminish.

Most phenols and aromatic amines are toxic. Some (e.g. benzidine and its derivatives, naphthylamines, aminoazobenzenes, and o-toluidine) have been determined to be human carcinogens (Searle, 1976; Slein and Sansone, 1980; Fishbein, 1979). Others (e.g., aniline and its derivatives, 2,4,6-trichlorophenol, phenylenediamines, and 4-amino-2-nitrophenol) are on the OSHA list of suspected carcinogens (Anonymous, 1980). Many more are referred to as "priority pollutants" by the U.S. Natural Resources Defense Council decree (e.g., 2-chlorophenol, 2,4-dimethylphenol, nitrophenols, phenol, and pentachlorophenol). Therefore, removal of such chemicals from industrial aqueous effluents is of great practical significance.

Current methods for the removal of phenols and aromatic amines from wastewaters include microbial degradation, adsorption on activated carbon, chemical oxidation, solvent extraction, membrane processes, and irradiation (Slein and Sansone, 1980; Fair and Geyer, 1965; Sittig, 1977). All of these methods, although feasible and useful, suffer from some serious shortcomings such as high cost, low efficiency, incompleteness of purification, or formation of hazardous by-products. Hence, novel alternative methods are very much needed. This project has dealt with development of such a method.

RESULTS AND DISCUSSION

Peroxidase-Catalyzed Precipitation of Phenols and Aromatic Amines from Water

We began this study by examining the effect of horseradish peroxidase and hydrogen peroxide on several phenols that have been determined by the EPA to be hazardous pollutants (Environmental Protection Agency, 1979). These include phenol itself, o-chlorophenol, cresols, and resorcinol. In all cases, such treatment resulted in the gradual formation of a precipitate which could be separated easily from the solution by sedimentation or filtration. That is, such treatment can result in at least some water purification.

To obtain more detailed information on the peroxidase-assisted removal of toxic phenols from water, we have investigated in greater detail the reaction of horseradish peroxidase and H_2O_2 with one of them, o-chlorophenol. To describe quantitatively the degree of water purification achieved by removal of dissolved phenols we shall use the parameter "removal efficiency" (Lanouette, 1977) which is defined as the percentage of the pollutant removed from solution under given conditions.

Upon addition of H_2O_2 (1 mM) and horseradish peroxidase (1 unit/ml) to a solution of o-chlorophenol (0.1 g/liter) in 10 mM phosphate buffer (pH 6.0), the solution immediately turns dark brown, followed by gradual separation of a brown precipitate. After removal of this precipitate by centrifugation, a colorless solution is obtained. To determine the removal efficiency caused by this treatment, a solution of o-chlorophenol is analysed before and after addition of the peroxidase system. Under the aforementioned conditions, treatment for 3 h results in 99.9% removal efficiency. Reduction of the treatment time causes a decrease in the removal efficiency.

We have purposely shown that the treatment of o-chlorophenol solutions with either 1 unit/ml peroxidase or hydrogen peroxide (up to 5 mM) alone for up to 24 h results in the absence of any precipitate and in no appreciable decrease in the phenol concentration. Hence, the removal of the pollutant described above should be attributed to the combined action of the enzyme and H_2O_2.

It was of obvious significance to study how the removal efficiency depends on reaction conditions. The efficiency of the enzymatic removal of o-chlorophenol from solution has a broad maximum between pH 6 and 8. Upon reducing the pH to 4.0 to removal efficiency decreases and then increases again in the range of pH 4.0 to 3.0.

The efficiency of peroxidase-assisted precipitation of 0.1 g/liter o-chlorophenol (0.8 mM) was investigated as a funciton of hydrogen peroxide concentration. The removal efficiency gradually increases upon increasing the concentration of H_2O_2 from 0 to 1 mM to reach the level of 99.8% which then remains constant within experimental error at higher concentrations of H_2O_2.

The removal efficiency for o-chlorophenol also increases with increase in peroxidase concentration. After a 3-h treatment (1 mM H_2O_2, pH 6.0) it reaches the maximum level at an enzyme concentration of 1

unit/ml. With longer treatment periods, the concentration of peroxidase needed to reach 99.8% removal efficiency is lower. Thus, with a 24-h treatment, it is less than 0.25 unit/ml. That is, an increase in the time of treatment can be used to offset the reduction in removal efficiency at low enzyme concentration.

An important question was whether the enzymatic removal of o-chlorophenol would be as effective at low concentrations of the pollutant as it is at 0.1 g/liter concentration. We studied the dependence of the removal efficiency on the concentration of o-chlorophenol (1 mM H_2O_2, 1 unit/ml peroxidase, 3-h treatment, pH 6.0) in the range 0.001 to 0.15 g/liter. It was found that within experimental error the removal efficiency is constant in this range.

Thus, it is apparent that peroxidase treatment results in effective removal of o-chlorophenol from water under various conditions. We therefore extended the enzymatic treatment to a wide range of other phenols and also aromatic amines. For each of them, the removal efficiency was measured as a function of pH, and the maximal efficiency is reported herein at the corresponding pH.

Table I shows the efficiencies of enzymatic removal from water of

TABLE I

Removal of Various Mononuclear Phenols from Water by Horseradish Peroxidase and Hydrogen Peroxide[a]

	Optimal pH	Removal efficiency[b] (%)
Phenol	3.5	85.3
Guaiacol (o-methoxyphenol)	5.5	98.0
m-Methoxyphenol	5.5	98.6
p-Methoxyphenol	7.0	89.1
o-Cresol	4.0	86.2
m-Cresol	4.0	95.3
p-Cresol	5.5	85.0
o-Chlorophenol	7.0	99.8
m-Chlorophenol	7.0	66.9
p-Chlorophenol	5.5	98.7
o-Aminophenol	3.5	53.5
m-Aminophenol	5.5	85.3
Resorcinol	3.5	84.1
5-Methylresorcinol	3.5	90.8
2,3-Dimethylphenol	4.0	99.7
2,6-Dimethylphenol	5.5	82.3

[a]Conditions: 0.1 g/liter aqueous solution of a phenol, 3-h treatment at room temperature, 1 unit/ml peroxidase, 1 mM H_2O_2.

[b]At the optimal pH.

16 different phenols. In some cases removal efficiencies are very high (e.g., for o- and p-chlorophenols, m-methoxyphenol, and 2,3-dimethylphenol), whereas in other they are much lower, in the range of 70 to 95%.

Table II shows removal efficiencies for seven naphthol derivatives and other binuclear phenols. For these chemicals enzymatic precipitation is much more effective than for mononuclear phenols; in most cases removal efficiencies exceed 99%.

TABLE II

Removal of Various Binuclear Phenols from Water by Horseradish Peroxidase and Hydrogen Peroxide[a]

	Optimal pH	Removal efficiency[b] (%)
1-Naphthol	4.0	99.6
1,3-Naphthalenediol	4.0	92.1
2-Nitroso-1-naphthol	4.0	98.9
2,7-Naphthalenediol	3.5	99.1
p-Phenylphenol	4.0	99.9
5-Indanol	7.0	96.3
8-Hydroxyquinoline	7.0	99.8

[a]Conditions: 0.1 g/liter aqueous solution of phenol, 3-h treatment at room temperature, 1 unit/ml peroxidase, 1 mM H_2O_2.

[b]At the optimal pH.

Efficiencies for the peroxidase-assisted removal of nine anilines and other aromatic amines are presented in Table III. Removal efficiencies of aromatic amines are similar to those for phenols (Table I).

The data in Tables I-III allow some conclusions to be reached regarding the relationship between the structure of a pollutant and the efficiency of its enzymatic precipitation. It seems that electron-donating substituents (e.g., methyl or methoxy groups) at the m-position

TABLE III

Removal of Aromatic Amines from Water by Horseradish Peroxidase and Hydrogen Peroxide[a]

	Optimal pH	Removal efficiency[b] (%)
Aniline	7.0	72.9
4-Chloroaniline	5.5	62.5
4-Bromoaniline	5.5	84.5
4-Fluoroaniline	7.0	86.4
4-Bromo-2-methylaniline	7.0	84.5
m-Phenylenediamine	7.0	98.6
4,4'-Methylenedianiline	7.0	88.9
N-(1-Naphthyl)-ethylenediamine	7.0	93.3
4'-Amino-2,3'-dimethylazobenzene	5.5	95.5

[a]Conditions: 0.1 g/liter aqueous solution of aromatic amine, 3-h treatment at room temperature, 1 unit/ml peroxidase, 2 mM H_2O_2.

[b]At the optimal pH.

favor the removal to a greater extent than those in o- or p-positions. The situation is exactly opposite for electron-withdrawing groups (such as Cl). An increase in hydrophobicity markedly improves the removal efficiency (see Tables I and II).

It appears that there are at least two independent factors affecting the overall removal efficiency of a phenol or an aniline from water. The first one is its reactivity toward peroxidase. The second one is the solubility in water of the product of peroxidase oxidation. Apparently naphthols have much higher enzymatic removal efficiencies than phenols because polymeric products of the former are more hydrophobic and hence more water insoluble than those of the latter.

Due to the adverse effect of one (or both) of the two factors discussed above some compounds listed in Tables I-III have relatively low enzymatic removal efficiencies (e.g., phenol, o-aminophenol, aniline, etc.). Moreover, some phenols such as o-, m- and p-nitrophenol, p-cyanophenol, and pyrogallol, failed to precipitate as a result of peroxidase treatment. Therefore, it might seem that the proposed enzymatic removal of pollutants would have a rather limited application. However, we have discovered a phenomenon which apparently overcomes this limitation.

We have found that easily removed pollutants (i.e., those that have high removal efficiencies) aid in the precipitation of other phenols and anilines. This phenomenon is illustrated by Table IV. One can see that the efficiency of the enzymatic removal of o-aminophenol is

enhanced markedly in the presence of easily removable phenols (Table IV).

TABLE IV

Efficiency of the Enzymatic Removal of o-Aminophenol in the Absence and in the Presence of Other Compounds[a]

Pollutant	Added compound	Removal efficiency (%)
o-Aminophenol	None	48.6
o-Aminophenol	2,3'-Dimethylphenol	95.1
o-Aminophenol	p-Phenylphenol	92.0
o-Aminophenol	1-Naphthol	84.9
o-Aminophenol	2,7-Naphthalenediol	95.3

[a]Conditions: 0.1 g/liter aqueous solutions of o-aminophenol and other compounds, 1 unit/ml peroxidase, 2.5 mM H_2O_2, pH 4.0, 3-h treatment at room temperature.

There are at least two alternative explanations for the effect described above. Supposing phenol itself is poorly removed because it has a low reactivity toward peroxidase, addition of more reactive compounds would increase the overall yield of free radicals in the system and therefore would result in a higher rate of formation of the polymeric products. An alternative explanation is that phenol may be sufficiently reactive toward peroxidase but the products of its enzymatic oxidation may have a low molecular weight and hence be fairly soluble in water. Addition of easily removable compounds (the products from which are obviously water insoluble) might result in formation of mixed polymers which are apparently nearly insoluble in water.

It occurred to us that the free radicals enzymatically produced from easily removed phenols and anilines could be used to remove even nonphenolic and nonaromatic amine compounds (which normally are not substrates for peroxidase). This, indeed, turns out to be the case. For instance, naphthalene does not react with peroxidase and H_2O_2 at all. However, in the presence of 100 mg/l 2,3-dimethylphenol, more than 60% of the naphthalene precipitates. The results are even more spectacular with azobenzene, a common intermediate in the chemical industry and a suspected carcinogen. Azobenzene alone does not react with horseradish peroxidase and H_2O_2. However, in the presence of 2,7-naphthalenediol and 1-naphthol, 99.9 and 99.3% of azobenzene, respectively, can be precipitated upon peroxidase treatment (20 mg/l azobenzene, 100 mg/l naphthalenediol or naphthol, 4mM H_2O_2, 300 units/l peroxidase, pH 4.0, 48-h treatment).

The discovery of the enhanced enzymatic removal of poorly removed

or nonremoved compounds in mixtures of pollutants has an important practical implication. Real industrial wastewaters contain many different pollutants. Hence, even if only a few of them can be easily precipitated by peroxidase, the removal of the others by the enzyme and hydrogen peroxide will be facilitated.

Enzymatic Dephenolization of Water

Phenol itself (C_6H_5OH) represents an important target for the removal from water because it is a hazardous pollutant and the major organic component of coal conversion and many other wastewaters.

Upon addition of horseradish peroxidase and H_2O_2, a solution containing phenol immediately turns brown, and a precipitate gradually forms -- a response not brought on by either regent alone. When 0.5 unit/ml peroxidase and 2 mM hydrogen peroxide were added to a 0.1 g/l (100 ppm or 1.1 mM) solution of phenol at pH 9, within 10 min the concentration of phenol in solution was reduced by half; no further reduction occurred during a subsequent incubation. Addition of more H_2O_2 did not increase the removal efficiency. However, addition of more enzyme did: another 0.5 unit/ml brought the removal efficiency to about 85%, and yet another 1 unit/ml brought it to about 99% (Fig. 1).

A plausible explanation of the incomplete removal of phenol by low concentrations of peroxidase was inactivation of the enzyme during the reaction. We confirmed that hypothesis by direct assay (Putter, 1974) of the peroxidase activity in samples withdrawn before and after the reaction: in all cases the enzymatic removal of phenol stopped the moment the peroxidase activity disappeared. Neither phenol nor hydrogen peroxide alone inactivated the enzyme under the same conditions. Hence, inactivation of peroxidase takes place during the enzymatic reaction, most likely due to interactions of the phenoxy radicals with the enzyme's active center. Calculations based on our experimental data indicate that, during its lifetime, one molecule of peroxidase can remove approximately 10,000 molecules of phenol (Fig. 1, pH 9).

Since the pH of industrial aqueous effluents may vary, it was important to examine the pH-dependence of the enzymatic removal of phenol. We found that horseradish peroxidase can precipitate phenol in a remarkably wide range -- from pH 3 to 12; the optimum is at pH 9 (Fig. 1), which fortunately is close to the typical pH of coal conversion, in particular coal gasification, wastewater (Singer et al., 1978). The removal efficiency was independent of whether the mixture was stirred, shaken or left undisturbed; however, it was higher when the enzyme was added incrementally rather than all at once.

One of the major drawbacks of conventional dephenolization methods is that they are generally effective only when phenol concentrations are either relatively low (e.g., adsorption on activated carbon and microbial degradation (Luthy and Tallon, 1980; Cavanaugh et al., 1977) or relatively high (e.g., solvent extraction (Cavanaugh et al., 1977)). Therefore, we examined our enzymatic treatment in the practically significant range of phenol concentrations: from as low as 0.01 g/l to as high as 5 g/l (pH 9). We selected concentrations of peroxidase and H_2O_2 based on three facts: (i) at 0.1 g/l phenol, no less than 1 unit/ml peroxidase and 2 mM H_2O_2 are required for a 99% removal efficiency (Fig.

1); (ii) H_2O_2 reacts stoichiometrically with phenol in peroxidase-catalyzed oxidation (Saunders et al., 1964); and (iii) the fraction of the enzyme inactivated by the phenoxy radicals is probably directly proportional to the initial phenol concentration. Thus, we varied the concentrations of the enzyme and H_2O_2 so that the ratios [peroxidase]/[phenol] and [H_2O_2]/[phenol] remained the same as for 0.1 g/l phenol. The removal efficiencies obtained were 98% for both 0.01 g/l and 5 g/l phenol, that is, peroxidase-catalyzed dephenolization is equally effective at low and high phenol concentrations provided that the concentrations of the enzyme and H_2O_2 are altered proportionally.

In addition to phenol, coal conversion wastewaters also contain ammonia, chloride, cyanide, thiocyanate and other constituents which adversely affect bacterial and other methods of dephenolization (Singer et al., 1978). We therefore investigated the effect of such contaminants on the peroxidase treatment. A typical coal gasification wastewater has the following characteristics: 2 g/l phenol, 5 g/l ammonia, 19 g/l chloride, 0.1 g/l cyanide, 1 g/l thiocyanate, pH 9.0. We examined the possibility of enzymatic dephenolization in this system. As was mentioned earlier, 2 units/ml peroxidase and 2 mM H_2O_2 remove 99% of phenol from a 0.1 g/l solution. When we added to that solution ammonia, chloride, cyanide, and thiocyanate in the concentrations indicated above, the same treatment resulted in only 10% removal efficiency. However, when we increased the concentration of phenol to 2 g/l and the concentrations of peroxidase and H_2O_2 proportionally, the removal efficiency in such a "more realistic" mixture jumped to 97% (as compared to 98% for 2 g/l phenol in the absence of other contaminants). Thus it appears that while components of coal gasification wastewaters inhibit peroxidase, this inhibition is insignificant at high, practically important concentrations of phenol, probably because the latter displace the inhibitors from the complexes with the enzyme.

Peroxidase Treatment of Real Industrial Wastewaters

In order to test the practicability of our enzymatic method, we have used it for the treatment of "real" industrial aqueous effluents. The wastewater sample was obtained from a chemical plant that produces triarylphosphates (used as flame retardants). This sample contained more than 150 different chemicals including various phenols, cresols, xylenols, isopropylphenol, and tert-butylphenol, as well as some nonphenolic compounds such as triarylphosphates and inorganic phosphate. The total concentration of phenols in the system was 105 ppm (105 mg/l).

The way these wastewaters are usually treated is as follows. First they are subjected to a bacterial degradation. This procedure takes several days and results in an 85-90% removal of phenols. The remaining phenols are then removed by adsorption on activated carbon. The bacterial treatment is carried out in open ponds. This makes it impossible to employ such treatment in the winter, since the bacteria do not degrade phenols at low temperatures.

Therefore, it was of particular interest to carry out our enzymatic treatment in the cold. Also, from the practical standpoint, it was highly desirable to treat water at pH 6.5-8.0, because lower pHs tend to cause the corrosion of steel columns with activated carbon and other

metal containers.

One liter of the wastewater was adjusted to pH 7 and placed in a refrigerator. Then 100 units of horseradish peroxidase and 2.5 mmole of H_2O_2 were added to the cold solution. The system was left in the refrigerator for 40 hours and then assayed for phenols. We found that as a result of this treatment, the concentration of phenols decreased from 105 to 3.7 ppm, that is, our treatment afforded a removal efficiency of 96.5%. Even at 25 units/l peroxidase and 2 mM H_2O_2 the removal efficiency was in the range of 85-90%. The crude peroxidase (see below) and the purified enzyme were equally effective. The treatment at room temperature leads to about the same removal efficiency as that at 4°C. (It is worth mentioning here that the precipitate enzymatically produced from pollutants, in principle, can be burned and the energy obtained can be used for heating.)

Our enzymatic treatment has also been successfully applied to real coal conversion wastewaters. We obtained a gallon of a flushing liquor from a coke plant. The sample had pH 8.6 and contained 0.4 g/l phenols. To the sample we added 8 unit/ml peroxidase (the minimal concentration for this phenolic strength, see above) and 14 mM H_2O_2. After 1 hr at room temperature the removal efficiency obtained was 97%. Hence, the peroxidase treatment was as effective with real wastewater as with model mixtures.

Removal of Pollutants with a Crude Preparation of Horseradish Peroxidase and with Other Peroxidases

Since this enzymatic treatment of industrial aqueous effluents is designed as a practical process, its cost should not be prohibitive. In all the experiments described above, a partially purified horseradish peroxidase obtained from a commercial supplier (Sigma, type II) was used. In order to reduce the potential cost of the treatment, the possibility of using a crude preparation of the enzyme was examined. To this end, whole horseradish roots were purchased in a local supermarket. The roots were minced with a blender. Then water was added and the suspension was stirred for a few minutes. After that is was pressed through cheesecloth to separate the juice which was later used as a source of peroxidase. The concentration of the enzyme in the juice was determined using the standard guaiacol assay (Putter, 1974).

Table V shows the results of the enzymatic precipitation of several different phenols with the commercial, purified peroxidase and with the crude enzyme preparation. As one can see, the removal efficiencies for this two types of treatment are comparable. Hence the purity of the enzyme is not essential for the treatment.

TABLE V

Removal of Phenols from Water by Purified and Crude Peroxidases[a]

Phenol	Removal Efficiency[b], %		pH
	"Sigma" enzyme	Crude enzyme	
Phenol	87.6	89.8	3.5
3-Methylphenol	85.3	99.6	4.0
4-Methylphenol	98.5	95.6	5.5
2-Chlorophenol	99.7	99.7	7.0
2,3-Dimethylphenol	99.6	99.3	4.0
1-Naphthol	97.4	99.6	4.0
2-Nitroso-1-naphthol	96.0	95.0	4.0
8-Hydroxyquinoline	99.9	99.7	7.0

[a]Conditions: 100 g/l phenols, 1000 units/l peroxidase, 2.5 mM H_2O_2, 21-h treatment at room temperature.

[b]The phenols were assayed by the method of Emerson (1943) (except for 4-methylphenol which was assayed in accordance with Arnow (1937)).

It was of obvious interest to study if the ability to precipitate phenols and aromatic amines is a unique feature of the horseradish peroxidase or whether peroxidases from other sources would be equally effective. To this end, we examined two commercially available peroxidases (from Sigma), lactoperoxidase from cows' milk and chloroperoxidase from Caldariomyces fumago. Upon the action of lactoperoxidase and H_2O_2 and H_2O_2 (800 units/mL enzyme, 2 mM H_2O_2, pH 5.5, 24-h treatment) on 100 mg/l benzidine, 1-naphthylamine, phenol, aniline, 2-methoxyphenol, 1-naphthol, 4-phenylphenol, 3-methylphenol, and 2-chlorophenol, removal efficiencies of 90.8, 35.1, 0, 0, 31.6, 32.0, 38.3, 15.4, and 13.7%, respectively, were obtained. With chloroperoxidase, the only compound from the aforementioned list that can be enzymatically precipitated is benzidine. Therefore it appears that although both lactoperoxidase and chloroperoxidase are capable of precipitating certain phenols and aromatic amines, the efficiency of their removal is low and not of a practical value.

We have also examined the feasibility of replacement of horseradish peroxidases with some other microbial peroxidases. The following peroxidases kindly provided by Cetus Corp. (Berkeley, CA) have been tested: 1) from Acetobacter, 2) from Bacillus, 3) from CMCC 256, 4) from CMCC 755, and 5) CMCC 834. (CMCC stands for Cetus Master Culture Collection).

First, we attempted to remove phenol at a concentration of 100 mg/l by treating with 2 mM H_2O_2 and 0.2 ml microbial peroxidase (total reaction volume of 10 ml). After more than 3 hours, at pH 4 - 9, we found

no evidence of precipitate; further our assay showed negligible removal efficiencies (due probably to inaccuracies in the assay). Horseradish peroxidase, on the other hand, at a concentration of 2 units/ml, removes approximately 95% of the phenol at pH 9 (under the same conditions).

Secondly, we attempted to remove guaiacol (o-methoxyphenol) since it is easily removed by horseradish peroxidase: 2 units/ml of the enzyme can remove nearly 100% of a 5 g/l solution of guaiacol. At pH 9, 100 mg/l guaiacol was treated with 2 mM H_2O_2 and 0.2 ml microbial peroxidase (total reaction volume of 10 ml); there was no precipitate in any of the cases; further, negligible removal efficiencies were measured for all five microbial peroxidases. At the same time, 2 units/ml horseradish peroxidase removed 97.7% of the guaiacol (according to our assay).

Next, we treated industrial wastewater (pH 4.65) from North Pond, Stauffer Chemical Company, with 2 mM H_2O_2 and 0.2 ml microbial peroxidase (10 ml total volume). Again, no significant removal was found, and no precipitate formed. 2 Units/ml horseradish peroxidase was able to remove 87.9% of the phenols from the wastewater.

To determine if any of the microbial peroxidases had any activity whatsoever, we used the peroxidase activity assay described by Putter (1974). Only peroxidase #3 (CMCC 256) had a significant enough activity to be measured by the assay. All the other microbial peroxidases had zero activity according to the assay.

Finally, we attempted to remove the carcinogen benzidine using the microbial peroxidases. Benzidine is very readily removed by horseradish peroxidase; 100 mg/ml benzidine·dHCl can be removed with as little as 1 mM H_2O_2 and 0.1 unit/ml horseradish peroxidase at pH 5.5. Several of the microbial peroxidases were able to remove significant amounts of benzidine. Data is tabulated in Table VI.

TABLE VI

Benzidine Removal by Microbial Peroxidase

(for conditions see text)
Removal efficiencies of benzidine were determined as described by Klibanov and Morris (1981)

Peroxidase Source	pH	3	4	5	6
Acetobacter		no ppt.	no ppt.	no ppt.	no ppt.
Bacillus		no ppt.	no ppt.	no ppt.	no ppt.
CMCC 256		94.3	95.0	97.7	96.7
CMCC 755		92.8	96.8	96.3	96.4
CMCC 834		65.5	61.9	53.5	52.5

Further, we treated 10 ml North Pond wastewater with just 0.2 ml microbial peroxidase (no hydrogen peroxide) to check for phenol oxidase

activities. Again, there was no precipitate formed and negligible removals were recorded.

In summary, none of the microbial peroxidases seems to have any significant potential in comparison to horseradish peroxidase.

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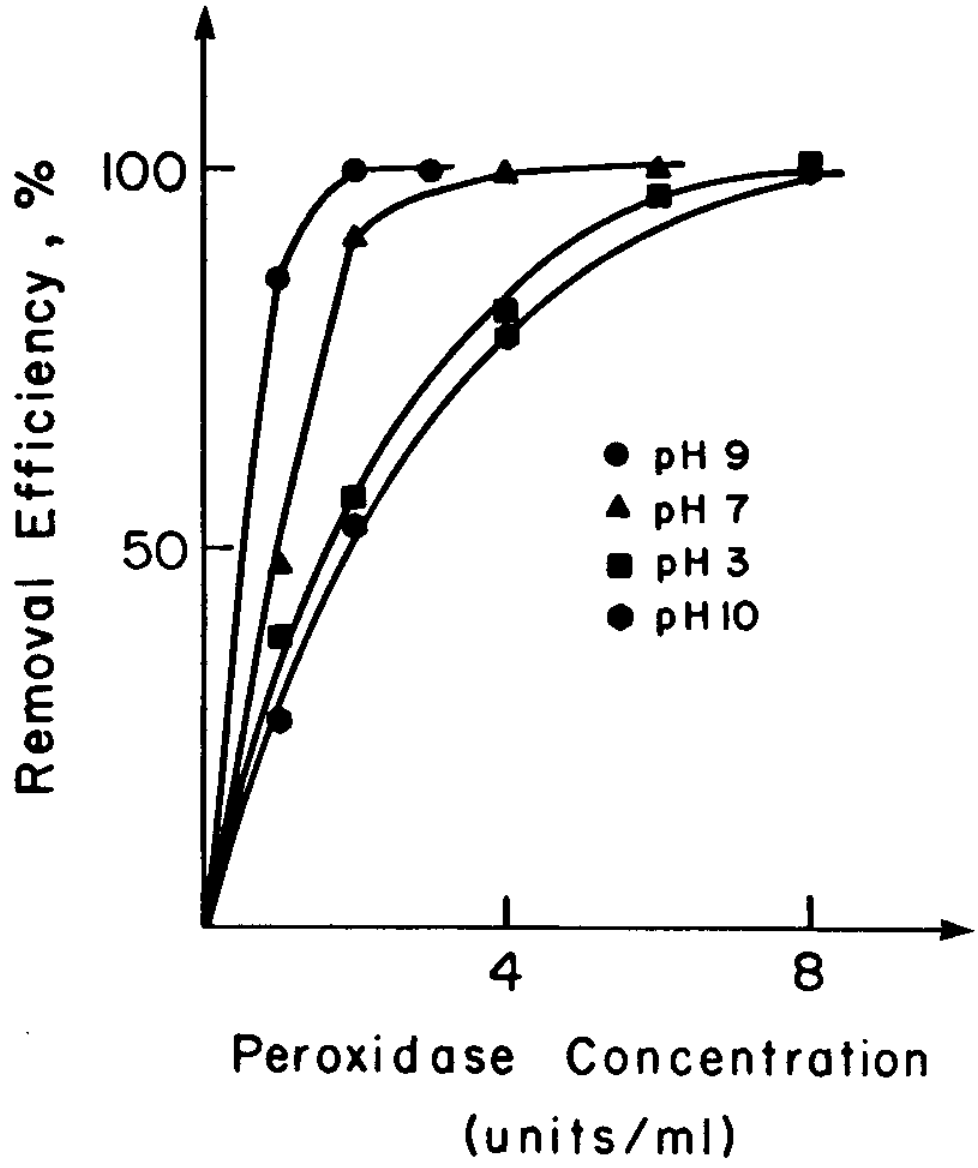

Figure 1. The dependence of peroxidase-catalyzed precipitation of phenol on the concentration of the enzyme at different pH. Conditions: 0.1 g/l phenol, 2 mM H_2O_2, room temperature, the corresponding portions of peroxidase added every half hour; the buffers used -- 0.1 M acetate for pH 3, 0.1 M phosphate for pH 7, and 0.1 M borate for pH 9 and 10.