Notice to Contributors

Science is taking drastic steps to reduce its backlog of accepted reports and thus to attain faster publication in future. This can only be achieved by a sharp temporary reduction in the rate of acceptance of manuscripts. The problem will be overcome by 1 October. Financial limitations prevent diminishing the backlog by substantially increasing the pages devoted to reports. Because of our large domestic and international circulation, the cost of delivering a two-page report to all our readers is about $1600. We need to reduce the backlog by 100 reports.

Until 1 October, the acceptance rate for reports will be about 10 percent. Unfortunately, many excellent manuscripts will be rejected. To save authors' time and to avoid asking conscientious reviewers to devote efforts to examining manuscripts that must ultimately be declined, some reports will be returned to authors without review. We regret any inconvenience that these temporary measures may entail.

Reports

Peroxidase-Catalyzed Removal of Phenols from Coal-Conversion Waste Waters

Abstract. *A novel, enzymatic approach has been developed for the removal of phenols from coal-conversion aqueous effluents. Treatment with horseradish peroxidase and hydrogen peroxide precipitates 97 to 99 percent of the phenol in a wide range of* p*H and phenol concentrations; both model mixtures and real industrial waste-water samples have been treated successfully. Other pollutants, such as polychlorinated biphenyls, can be enzymatically coprecipitated with the phenols.*

Phenols are the major organic pollutants in aqueous effluents from coal-conversion processes (*1*). Since most phenols are toxic (*2, 3*), they must be removed from waste waters if such processes are to be environmentally acceptable. Current methods for removing phenols from industrial waste waters include solvent extraction, microbial degradation, adsorption on activated carbon, and chemical oxidation (*3, 4*). Although these methods are effective, they suffer from such shortcomings as high cost, incompleteness of purification, formation of hazardous by-products, and applicability to only a limited concentration range (*3, 4*). Therefore, better technologies are needed.

We propose a novel approach to dephenolization of coal-conversion waste waters based on the use of peroxidase, the enzyme that polymerizes phenolic compounds to lignin in plants (*5*). This enzyme oxidizes numerous phenols with H_2O_2 (*6*), generating phenoxy radicals which diffuse from the active center of the enzyme into solution; there they react with phenol molecules, forming polyaromatic products (*6*). These insoluble polymers then precipitate out of solution and can be separated by simple filtration. We describe here how the peroxidase treatment may be used to remove phenols from both model and "real" samples of coal-processing waste waters.

Upon addition of horseradish peroxidase (*7*) and H_2O_2, a solution containing phenol immediately turns brown and a precipitate forms gradually, a response not brought on by the use of either reagent alone. To quantify the degree of water purification achieved, one may define "removal efficiency" (*3*) as the percentage of phenol removed from solution under a given set of conditions. When 0.5 unit of peroxidase per milliliter and 2 m*M* H_2O_2 were added to a phenol solution having a concentration of 0.1 g/liter [100 parts per million (ppm) or 1.1 m*M*] at *p*H 9, within 10 minutes the phenol concentration (*8*) 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 enzyme (another 0.5 unit/ml) brought the removal efficiency to about 85 percent, and addition of yet another 1 unit/ml increased it to about 99 percent (Fig. 1).

A plausible explanation for the incomplete removal of phenol by low concentrations of peroxidase is inactivation of the enzyme. We confirmed this hypothesis by direct assay (*9*) 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 H_2O_2 alone inactivated the enzyme under the same conditions. Hence, inactivation of peroxidase takes place during the enzymatic reaction, most likely as a result of the 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, *p*H 9).

Since the *p*H of industrial aqueous effluents may vary, we examined the *p*H dependence of the enzymatic removal of phenol. We found that horseradish peroxidase can precipitate phenol in a remarkably wide range—from *p*H 3 to 12. The optimum is at *p*H 9 (Fig. 1), which fortunately is close to the typical *p*H of coal-conversion, in particular coal-gasification, waste waters (*10*). 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 [for example, adsorption on activated carbon and microbial degradation (*11, 12*)] or relatively high [for example, solvent extraction (*12*)]. Therefore, we examined our enzymatic treatment in the practically significant range of phenol concentrations from 0.01 to 5 g/liter (*p*H 9). We selected concentrations of peroxidase and H_2O_2 based on three facts: (i) at a phenol concentration of 0.1 g/liter, 1 unit of peroxidase per milliliter and 2 m*M* H_2O_2 are required for a 99 percent removal efficiency (Fig. 1); (ii) H_2O_2 reacts stoichiometrically with phenol in peroxidase-catalyzed oxidation (*6*); 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 a phenol concentration of 0.1 g/liter. The removal efficiencies obtained were 98 percent for phenol concentrations of 0.01 and 5 g/liter; 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.

Coal-conversion waste waters contain, in addition to phenol, ammonia, chloride, cyanide, thiocyanate, and other constituents that adversely affect bacterial and other methods of dephenolization (*10*). We therefore investigated a typical coal-gasification waste water with the following composition (*10*): phenol, 2 g/liter; ammonia, 5 g/liter; chloride, 19 g/liter; cyanide, 0.1 g/liter; thiocyanate, 1 g/liter; *p*H 9.0. When we added 2 units of peroxidase per milliliter and 2 m*M* H_2O_2 to a 0.1 g/liter phenol solution containing ammonia, chloride, cyanide, and thiocyanate in the concentrations indicated above, this treatment (which in the absence of the contaminants afforded a 99 percent removal of phenol) resulted in a removal efficiency of only 10 percent. However, when we increased the concentration of phenol to 2 g/liter and the concentrations of peroxidase and H_2O_2 proportionally, the removal efficiency jumped to 97 percent (as compared to 98 percent for a phenol concentration of 2 g/liter in the absence of other contaminants). Thus it appears that, although components of coal-gasification waste waters 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.

The aforementioned experiments were performed with reconstituted aqueous effluents (*10*). To test our enzymatic method with "real" coal-conversion waste waters, we obtained a gallon of a flushing liquor from a coke plant (*13*). The sample had *p*H 8.6 and a phenol concentration of 0.4 g/liter (*8*). To the sample we added 8 units of peroxidase per milliliter (the minimal concentration for this phenolic strength) and 14 m*M* H_2O_2 (*14*). After 1 hour at room temperature, the removal efficiency was 97 percent.

Easily removed phenols (that is, those having high removal efficiencies) aid in the enzymatic precipitation of hard-to-remove phenols and other aromatics (*15*). This phenomenon is probably due to the fact that the phenoxy radicals enzymatically produced from easy-to-remove phenols can react with and coprecipitate hard-to-remove ones, or that hard-to-remove pollutants can adsorb on the precipitating polymers of easy-to-remove phenols. We endeavored to determine whether peroxidase can coprecipitate pollutants other than phenols from the coke plant waste-water sample. Polychlorinated biphenyls (PCB's) are toxic and persistent organic pollutants whose removal from water poses a difficult environmental problem (*16*). 4,4′-Dichlorobiphenyl and 2,4,5-trichlorobiphenyl are representative PCB's that are prevalent in commercial products (*17*). Addition of horseradish peroxidase and H_2O_2 to a 10-ppm solution of 4,4′-dichlorobiphenyl and a 3-ppm solution of 2,4,5-trichlorobiphenyl (*p*H 9) resulted in no measurable removal of the pollutants (*18*). But, when the same concentrations of the PCB's were dissolved in the coal-conversion waste-water sample, the enzymatic treatment (60 units of peroxidase per milliliter, 100 m*M* H_2O_2) removed 91 and 86 percent of the pollutants, respectively. These findings have two important implications. (i) Even pollutants that are unreactive toward peroxidase might be enzymatically precipitated if the waste water also contains other pollutants that are readily removed by the enzyme. (ii) In dealing with two waste-water streams, one responsive to the peroxidase treatment and the other not, it might be beneficial to combine them prior to the enzymatic treatment; the enzyme could then coprecipitate pollutants from both streams.

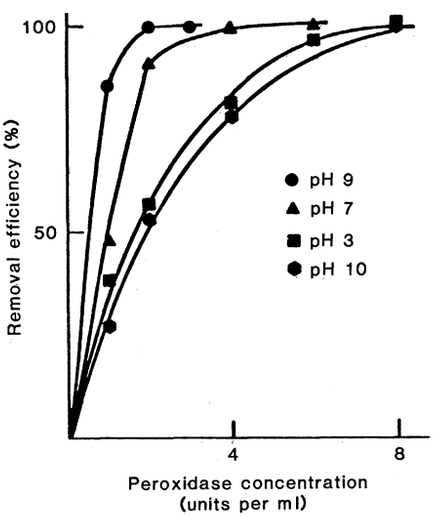

Fig. 1. The dependence of peroxidase-catalyzed precipitation of phenol on the concentration of the enzyme at different *p*H. The conditions were as follows: phenol concentration, 0.1 g/liter; H_2O_2 concentration, 2 m*M*; room temperature; corresponding portions of peroxidase added every half hour. The following buffers were used: 0.1*M* acetate for *p*H 3, 0.1*M* phosphate for *p*H 7, and 0.1*M* borate for *p*H 9 and 10.

One possible use of the proposed dephenolization method would be to replace conventional bacterial degradation of phenols in open ponds. The peroxidase treatment is much less sensitive than bacterial degradation to variations in *p*H, phenol concentration, other toxic pollutants, and temperature. Moreover, the enzymatically produced precipitate might be recovered for use as a fuel. To make the proposed method more economically feasible (*19*), one could either replace the horseradish peroxidase with a microbial counterpart possessing a similarly broad substrate specificity or clone the horseradish peroxidase genes into efficient enzyme-producing microorganisms.

ALEXANDER M. KLIBANOV
TSU-MAN TU
KEVIN P. SCOTT
Laboratory of Applied Biochemistry, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. C. E. Jahnig and R. R. Bertrand, *Chem. Eng. Prog.* **72** (No. 8), 51 (1976); N. S. Dailey, in *Environmental, Health, and Control Aspects of Coal Conversion*, H. M. Braunstein, E. D. Copenhaver, H. A. Pfuderer, Eds. (Oak Ridge National Laboratory, Oak Ridge, Tenn., 1977), vol. 1, chap. 4.
2. E. L. Baker *et al.*, *Arch. Environ. Health* **33**, 89 (1978); *U.S. Environmental Protection Agency Catalog* (Environmental Protection Agency, Washington, D.C., 1979).
3. K. H. Lanouette, *Chem. Eng. (N.Y.)* **84** (No. 22), 99 (1977); W. M. Throop, *J. Hazardous Mater.* **1**, 319 (1977).
4. J. A. Klein and D. D. Lee, *Biotechnol. Bioeng. Symp.* **8**, 379 (1978).
5. K. Freudenberg and A. C. Neish, *Constitution and Biosynthesis of Lignin* (Springer, New York, 1968); J. M. Harkin and J. R. Obst, *Science* **180**, 296 (1973).
6. B. C. Saunders, A. G. Holmes-Siedle, B. P. Stark, *Peroxidase* (Butterworth, London, 1964).
7. The horseradish peroxidase (E.C. 1.11.1.7) (Sigma Chemical Company) had a specific activity of 175 purpurogallin units per milligram of solid. The molecular weight is about 40,000 (*6*).
8. The concentration of phenol was determined by the classical method of E. Emerson [*J. Org. Chem.* **8**, 417 (1943)]. In the case of peroxidase-treated phenol solutions, the assay was done after precipitate removal by centrifugation.
9. J. Putter, in *Methods of Enzymatic Analysis*, H. U. Bergmeyer, Ed. (Academic Press, New York, 1974), vol. 2, p. 685.
10. P. C. Singer, F. K. Pfaender, J. Chinchilli, J. C. Lamb, in *Environmental Aspects of Fuel Conversion Technology*, F. A. Ayer and M. F. Massoglia, Eds. (Publication EPA-600/7-78-063, Environmental Protection Agency, Washington, D.C., 1978), p. 461.
11. R. G. Luthy and J. T. Tallon, *Water Res.* **14**, 1269 (1980).
12. E. C. Cavanaugh *et al.*, *Environmental Assessment Data Base for Low/Medium BTU Gasification Technology* (Publication EPA-600/7-77-1256, Environmental Protection Agency, Washington, D.C., 1977), vol. 2, p. D-18.
13. The sample was provided by D. C. Lang and T. Barnett, Inland Steel Company, East Chicago, Ind.
14. Peroxidase and H_2O_2 were added as four equal portions (2 units per milliliter and 3.5 m*M*, respectively) every 15 minutes. The mixture was not buffered, and no stirring or shaking was used.
15. A. M. Klibanov, B. N. Alberti, E. D. Morris, L. M. Felshin, *J. Appl. Biochem.* **2**, 414 (1980); A.

M. Klibanov and E. D. Morris, *Enzyme Microb. Technol.* **3**, 119 (1981).
16. K. Kay, *Environ. Res.* **6**, 202 (1973); "Polychlorinated biphenyls, a report by the Committee on Assessment of Polychlorinated Biphenyls in the Environment" (National Academy of Sciences, Washington, D.C. 1979).
17. D. Sissons and D. Welti, *J. Chromatogr.* **60**, 15 (1971); O. Hutzinger *et al.*, *Chemistry of PCB's* (CRC Press, Boca Raton, Fla., 1982).
18. 4,4′-Dichlorobiphenyl and 2,4,5-trichlorobiphenyl were determined by gas chromatography (180-cm glass column packed with Analab's Super Pak 20M; oven temperature, 160°C; carrier gas, N_2; flow rate, 20 ml/min), following centrifugation of the aqueous solution (after the peroxidase treatment) and chloroform extraction.
19. B. N. Alberti and A. M. Klibanov, *Biotechnol. Bioeng. Symp.* **11**, 373 (1981).
20. Supported by grants from the Environmental Protection Agency (R808619) and the M.I.T. Sea Grant. The views expressed herein do not necessarily reflect the opinion of the Environmental Protection Agency and no endorsement of that agency should be inferred.

1 February 1983; revised 4 April 1983

Holocene Timberline Fluctuations in Jasper National Park, Alberta

Abstract. *Pollen, fossil logs, and macrofossils from three high-elevation sites in the Maligne Range, Jasper National Park, Alberta, provide the first detailed record of timberline fluctuations in the Canadian Rockies during the last 8700 years. Timberlines were much higher than at present between 8700 to 5200 years ago but oscillated significantly in elevation, with a major episode of timberline recession punctuating two periods of high timberline between about 6700 to 5900 and about 8700 to 7000 years ago. Since 5200 years ago, regional timberlines have generally receded with perhaps brief reversals, reaching their lowest recorded positions sometime after 500 years ago.*

Past fluctuations in the alpine timberline have proved to be sensitive indices of Holocene climatic changes in several areas of North America (*1*). We report the results of what we believe to be the first detailed investigation of Holocene timberline changes in the Canadian Rockies based on pollen, fossil logs, and macrofossils recovered from three sites above the present timberline in the Maligne Range, Jasper National Park, Alberta. These results provide a clearer definition of the Holocene climatic history of this area, particularly the Hypsithermal, than has been possible before now and are compared with data for other sites in the North American Cordillera.

The Maligne Range forms the interfluve between the Athabasca and Maligne rivers (Fig. 1) and consists of clastic Cambrian and Precambrian rocks rising to 2500 to 3000 m above sea level. Climatic data are sparse (*2*). Rainfall increases with elevation and ranges from 500 to 1000 mm per year (1300 to 5000 mm of snowfall). The mean annual temperature for Jasper (elevation, 1061 m) is 2.8°C, with July and January means of 15.2°C and −12.2°C, respectively. The Maligne and Athabasca valleys lie primarily within the subalpine forest zone and are dominated by varying proportions of *Picea engelmanni* Parry (Engelmann spruce), *Abies lasiocarpa* (Hook.) Nutt. (subalpine fir), and *Picea glauca* (Moench) Voss (white spruce). The summits and upper reaches of valleys in the Maligne Range are mantled by alpine tundra. All the sites reported here lie above present timberlines (Fig. 1) (*3*) and are surrounded by heath (*Cassiope* spp.) tundra communities intermixed with stands of *Salix arctica* Pall (arctic willow). Occasional dwarf subalpine fir (*Abies lasiocarpa* krummholz) occurs in the vicinity of the Watchtower Basin site.

Pollen cores recovered from the Watchtower Basin and Excelsior Basin sites (*4*) provide the most complete and continuous records of past timberline oscillations in this area. We reconstructed the direction and magnitude of past timberline changes from ratios of selected pollen taxa, using regression equations derived from the relation between altitude and modern surface samples (Fig. 2) (*5*). When plotted against eleva-

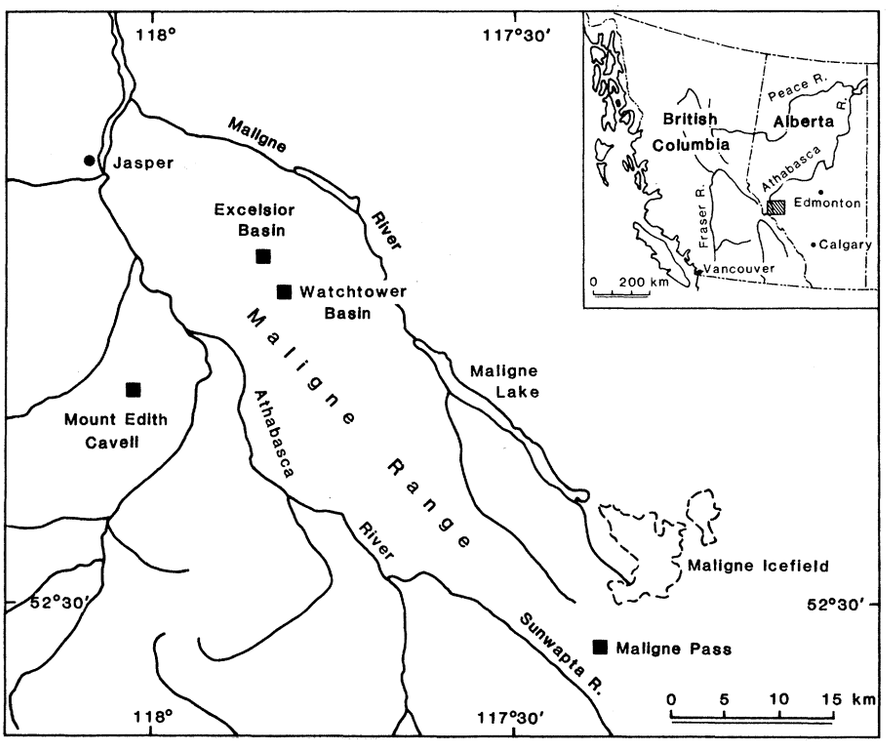

Fig. 1. Sketch map of a part of Jasper National Park, showing the site locations.

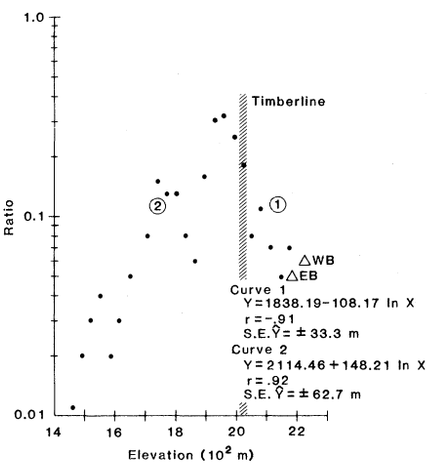

Fig. 2. Elevational changes in *Abies/Pinus* pollen ratios (dots) from modern surface samples collected on Mount Edith Cavell, approximately 10 km south of Jasper. The regression line defined by curve 1 includes all samples above the timberline; curve 2 is based on all samples below the timberline; r = correlation coefficient; S.E.$\hat{y}$ = standard error of the estimated value of y. *Abies/Pinus* ratios (triangles) for surface samples of the Watchtower Basin (*WB*) and Excelsior Basin (*EB*) cores are shown for comparison.