REMOVING OF PHENOL FROM WASTE WATERS BY OXIDATION TO MELANIN WITH MUSHROOM POLYPHENOLOXIDASE

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Phenol can be removed from cokery effluents by oxidation to melanin on the action of mushroom polyphenoloxidase. Under optimum conditions — pH 8·8, temperature 30°C — 98% phenol can be removed in this manner within 48 h.

The problem of removing phenol from phenolic effluents from cokeries cannot be regarded completely settled in technical practice. The technique currently used in plants is biological treatment with activated sludge after dilution with sewage water[1]; other procedures[2], such as spraying over dumps, are being abandoned. The reported oxidation of phenol in waste waters with air[3] is of theoretical value only.

Since, however, pure phenol solutions are known to be oxidized to melanin on the catalytic action of polyphenoloxidase[4] (diphenol–O_2:oxido–reductase, E.C.1.14.18.1.), we have studied in the present work the possibility to make use of this enzyme reaction for the removing of phenol from waste waters.

EXPERIMENTAL

Materials

Phenolic effluent was obtained from the cokery of Plant 1, Vítkovice — Klement Gottwald Iron and Steel Works, Ostrava-Vítkovice. The phenol content was appr. 1·5 g/l. In addition to phenol, resorcinol was present in amounts about 0·2 g/l. Phenol reagent grade purity (POCH, Poland) used as the standard was double distilled.

Enzyme preparation: Mushrooms, fresh or refrigerated to —20°C, were homogenized in a mixer with distilled water in 1 : 1 ratio. The supernatant obtained on centrifugation (5 min at 2500*g*) served as the enzyme preparation. Its activity for phenolic water as the substrate (phenol concentration 0·01 mol l^{-1}) was in the range 112—200 ncat/ml at pH 8·8 and temperature 30°C. If 0·01M pyrocatechol was used as the substrate, the activity of the enzyme preparation was ten times higher under the same conditions. The enzyme preparation was not purified, in order to approach industrial conditions.

Methods

Determination of phenol (modification according to[5] without distillation): To 1 ml of the reaction mixture was added 0·1 ml of 1·2M-HCl. After shaking mixture, the precipitated melanin was centrifuged and the supernatant was twenty times diluted. For the determination, 1 ml of the diluted sample was used and 4 ml of water and 0·2 ml of diazotated *p*-nitroaniline solution were added, the mixture was shaken and allowed to stand for 15 min. The absorbance was measured at 490 nm against distilled water on a spectrophotometer Unicam SP 800.

Photometric determination of melanin[6]: 2 ml of formamide was added, to 0·2 ml of the reaction mixture and the absorbance was measured at 440 nm.

Enzyme oxidation of phenolic waste water: Unless stated otherwise, a mixture of 5 ml of the phenolic waste with 1 ml of the enzyme preparation was agitated and allowed to incubate in an closed test tube at preselected pH and temperature for a time simultaneously with the corresponding blanks. After the incubation, the melanin increment and/or the phenol decrement were determined. The results reported are averages always from five replicates.

RESULTS

In the first series of experiments, the suitable enzyme-to-substrate ratio was sought. In the ratios 1 : 50 and 1 : 30 the reaction almost did not proceed, and its rate was low even at the ratio 1 : 10. The ratio 1 : 5 emerged as the lowest, still satisfactory, and was therefore applied throughout the subsequent experiments.

Preliminary investigations proved that the loss of phenol corresponded well with the formation of melanin. This allowed us to monitor the course of most reactions by determination of melanin, which is methodically much simpler; the content of phenol was determined only before and after the incubation.

Time course and kinetics of the reaction: The time dependences revealed that the reaction order varies during the reaction course, the rate constant could not be therefore evaluated. The curves of the logarithms of concentration *versus* time indicate that two reactions may be involved, the first faster and the second slower, with the change near 5 h (Fig. 1).

Effect of temperature: The effect of temperature on the course of the reaction was examined in the region between 25–41°C, in the time from 2 to 48 h The analytical values were corrected for the auto-oxidation of phenol, the phenols present in the enzyme preparation, and colouration of the phenolic water. The pH value applied, pH 8·8, was adjusted by mixing the phenolic water and borate–phosphate buffer in the 1 : 1 ratio. It can be seen from Fig. 2 that the optimum temperature is 30°C and the differences between the temperatures given are very small. Fig. 1 demonstrates that in this manner phenol can be removed nearly quantitatively: as little as 2% from the initial quantity remains in the solution after 48 h.

Effect of pH: For the investigation of the time dependences at different pH values, the phenolic water was mixed with buffers of various pH or with distilled water in the

ratio 1 : 1. The reference phenol sample was diluted with distilled water. It follows from Figs 3 and 4 that the optimum pH for the reaction is 8·8, the reaction proceeds faster with pure phenol than with phenolic waste water (although the initial phenol concentration was somewhat lower in the former case), and the reaction is evidently promoted by a mere addition of the buffer.

DISCUSSION

Originally we supposed that the phenolic water might contain inhibitors to the enzyme reaction – too high a concentration of cyanides for instance – which would inhibit the reaction altogether. However, the kinetic dependences demonstrate that the reaction does proceed, its rate being quite satisfactory for technical practice. The efficiency of the phenol removal was considerably higher than expected. Within the incubation period of 28 h, 85% phenol was removed; this is a sufficient quantity with regard to the fact that this process is only intended as a prepurification step such that subsequent routine biological treatment of the phenolic effluent with activated sludge could be applied without additional dilution.

The little dependence of the reaction on the temperature is also of value for technical practice: the process is not susceptible to temperature variations within the region

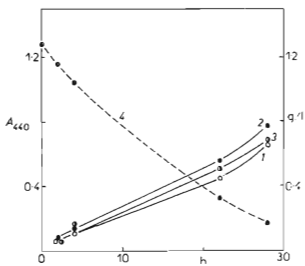

FIG. 1

Time Dependence of the Melanin Increment and Phenol Decrement at Different Temperatures

Mixtures of 2·5 ml of the phenolic water, 2·5 ml of borate-phosphate buffer and 1 ml of the enzyme solution were incubated; *1*—*3* melanin, *4* phenol, *1* 25°C, *2* 30°C, *3* 35°C, *4* 30°C.

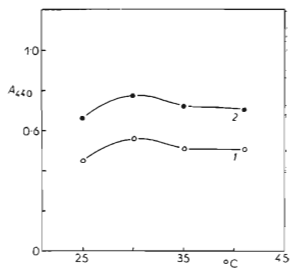

FIG. 2

Dependence of Melanization on the Temperature

The incubation mixture contained 2·5 ml of the phenolic water, 2·5 ml of borate–phosphate buffer and 1 ml of the enzyme solution; melanization after *1* 22 h, *2* 28 h incubation.

examined (25–41°C), and it can be employed even at 40°C, it being not necessary to cool the phenolic water down so intensively.

Owing to the content of ammonia, the phenolic water is usually slightly basic, its pH thus approaching the optimum value appropriate for the reaction.

Should the phenolic water be subsequently subjected to biological treatment, phosphorus would have to be supplied[2]; this could be accomplished by adding phosphate, pH 8·8.

Under the experimental conditions applied, the polymeric melanin remained in the solution, not flocculating as occurring frequently during this reaction[4]. It has therefore to be either precipitated by acidifying or removed by adsorption on bentonite. Both procedures appear to be technically tractable.

Significant from the technical point of view is the fact that the stoichiometry of the reaction is preserved for the phenolic waste water, as proved by employing polyphenoloxidase from another source[7]. The ratio of 2 mol of oxygen per one mol of phenol required for the oxidation of the latter to melanin, as against the 7 mol of oxygen necessary for complete oxidation to carbon dioxide and water, represent an almost three-fold economy.

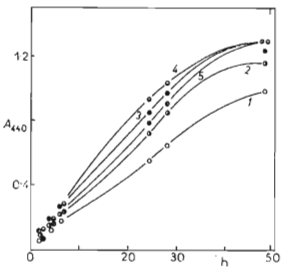

FIG. 3

Time Dependence of the Melanin Increment at Different pH Values

The incubation mixture contained 2·5 ml of the phenolic water, 2·5 ml of borate–phosphate buffer and 1 ml of the enzyme solution; pH: *1* 7·3, *2* 8·1, *3* 8·4, *4* 8·8, *5* 9·1.

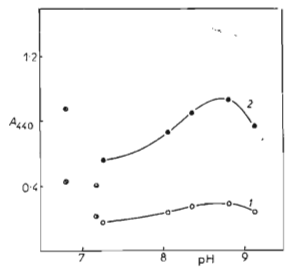

FIG. 4

Dependence of Melanization on pH

The incubation mixture contained 2·5 ml of the phenolic water, 2·5 ml of borate–phosphate buffer and 1 ml enzyme solution; melanization after *1* 6 h, *2* 24 h incubation. For a comparison: ⊕, ⊗ phenol diluted with distilled water, ◑, ◒ phenolic waste water diluted with distilled water, after 6 and 24 h, respectively.

There is, however, also a substantial drawback in this process, the enzyme preparation-to-substrate ratio in unreasonably high. To tackle this problem, further experiments would be called for, *e.g.*, to seek for an inexpensive source of equally active polyphenoloxidase. Conclusions from the parallel study[7] using polyphenoloxidase from potatoes was in this respect also negative – the requisite enzyme-to-substrate ratio was still higher. A solution to this problem might be the application of polyphenoloxidase bound to a support such as diethylaminocellulose[8] or polyacrylamide gel[9], which would permit the enzyme to be used repeatedly.

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