Treatment of Bleach Baths for Reuse in Dyeing with Immobilized Thermo-Alkali-Stable Catalases

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Currently in textile finishing, more than 100 L of water are consumed during the processing of 1 kg of textiles.[1] Catalase enzymes can be used in bleaching effluents to convert H_2O_2 to water and oxygen allowing reuse of the bleach bath; e.g., in dyeing. Even though catalase is one of the most effective biocatalysts in terms of turn-over numbers,[2] cost of enzyme for the degradation of H_2O_2 of bleaching effluents could be reduced by immobilization of the enzyme or the whole organisms.[3-5] Immobilization of whole cells is used for biological textile effluent treatment and thus it was interesting to determine whether a mixed population of alkalithermophilic bacteria would also be able to eliminate H_2O_2.

In this study, we have immobilized a mixed population of thermo-alkalophilic *Bacilli* and compared the degradation capacities of the whole cells to those of free catalase.

MATERIAL AND METHODS

Mixed Population

A 6-l-standard bioreactor (DIN 38412, 1994) was used for continuous cultivation of the mixed population enriched from the wastewater obtained from a textile finishing company and for its immobilization on light expanded clay. The culture medium consisted of 5 g/L yeast extract, 5 g/L peptone from casein, and 1 g/L KH_2PO_4 at 60C and pH 9.0, buffered with 50 mM $NaHCO_3/Na_2CO_3$. A trace element solution (1% w/w) containing 2500 mg/L Na_2EDTA, 100 mg/L $ZnSO_4 \cdot 7H_2O$, 30 mg/L $MnCl_2 \cdot 4H_2O$, 300 mg/L H_3BO_3, 200 mg/L $CaCl_2 \cdot 6H_2O$, 10 mg/L $CuCl_2 \cdot 2H_2O$, 20 mg/L $NiCl_2 \cdot 6H_2O$, 900 mg/L $Na_2MoO_4 \cdot 2H_2O$, 30 mg/L $Na_2SO_3 \cdot 5H_2O$, and 1000 mg/L $FeSO_4 \cdot 7\ H_2O$ was added. A bacterium identified as a *Bacillus* sp (*Bacillus* SF) was isolated from the mixed population and cultivated under the same conditions.

Induction of Catalases

Catalase production by both the mixed populations and by *Bacillus* SF was induced by the addition of various compounds such as 150 µM hydrogen peroxide (30 %), 3.9 µM Paraquat, 1000 µM L(+)-ascorbic acid, and 1000 µM pyrogallol. Cultivation was carried out using 100 mL buffeld Erlenmeyer flasks in a rotary shaker at 60C using the same medium as described for the bioreactor.

Assay and Enzyme Stability

Cells were harvested and centrifuged at 3000 g and the pellet was suspended in 50 mM NaH_2PO_4 buffer (pH 7.0). Cell disruption was carried out using a sonification unit (Bandelin Sonoplus HD 70, Berlin, Germany). Cell debris was removed by centrifugation at 6500 g and the remaining supernatant was stored at 4C. Catalase activity was determined by monitoring the degradation of H_2O_2 spectrophotometrically at 240 nm as described previously by Aebi.[2] The assay mixture contained 0.1 mL of enzyme preparation, 1 mL of a 26 mM H_2O_2 stock solution and 0.9 mL of buffer. The enzyme preparation (1 mL) was diluted with 9 mL buffer in eprouvettes (50 mM $NaHCO_3/Na_2CO_3$ for pH 9 and 10, 50 mM NaH_2PO_4 for pH 7 and 8) that were shaken at 50 rpm in a water bath at 50C and 60C. Samples were withdrawn at specified time intervals.

Degradation with Immobilized Cells

A 10-g sample of wet, immobilized organisms from the reactor were transferred into a column, which was kept at 50, 60, and 65C. A solution of 300 mg/L H_2O_2 in 50 mM Tris HCl buffer (pH 9.0) was pumped through the column (2.5 mL/min) and the decrease of the H_2O_2 concentration in the effluent was monitored spectrophotometrically at 240 nm.

RESULTS AND DISCUSSION

Mixed populations of bacteria were enriched in a continuous reactor at 65C and pH 9.5. The predominate species growing under these extreme conditions were identified as three new representatives of the *Bacillus* genus. Only few thermoalkalophilic *Bacilli* have been described previously such as *Bacillus* sp. TAR-1,[6] *Bacillus thermocatenulatus*,[7] *Bacillus thermoalcaliphilus*,[8] or an anaerobic strain LBS3.[9] Anaerobic alkalithermophiles seem to be more abundant than aerobics.[10]

Two mixed populations (MP1 and MP2) and the isolated *Bacillus* sp. SF were treated with known catalase in-

TABLE I.

Half-Life [$t_{1/2}$] of Isolated Catalases from MP1 in [h]

pH	20C	40C	50C	60C	70C
7	weeks	15	9	4	0.25
8	weeks	24	12	22	0.33
9	weeks	36	30	20	0.17
10	weeks	23	22	5	0.08

TABLE II.

H_2O_2 Degradation with Immobilized Mixed Population (MP1) in the Column Reactor

No.	temp. [C]	clay [g]	flow [mL/min]	H_2O_2 [mg/L]	$v_{deactivation}$ [s^{-1}]	half-life [h]
1	50	30	1.5	979	$35 \cdot 10^{-5}$	-
2	50	10	2.5	150	$3.6 \cdot 10^{-5}$	5.50
3	50	10	2.5	300	$7.0 \cdot 10^{-5}$	2.74
4	60	10	2.5	300	$8.5 \cdot 10^{-5}$	2.25
5	65	10	2.5	300	$8.7 \cdot 10^{-5}$	2.20

ducers as described and compared with a blank. Pyrogallol and H_2O_2 seemed to be poor inducers compared to ascorbic acid and Paraquat. In general, none of the inducers showed substantial increase in catalase activity. Obviously the different strains composing the mixed population showed different responses to the inducers.[11]

Catalases isolated from a mixed population MP1 showed remarkable stabilities at high temperatures and pH values with a half-life of 20 hours at pH 9 and 60C (Table I). In contrast, at the same conditions a half-life of only of 2.2 hours was measured for the whole cells (Table II). However, using a treatment time of about one hour for bleaching effluents, the mixed population could be regenerated by incubation with nutrients (data not shown) between two subsequent treatment steps. Trial No. 2 shows highest degradation capacity. The first trial shows the lowest catalase activity of the immobilized mixed population due to the high concentration of H_2O_2, which seemed to be toxic to the organisms. The increasing temperature obviously had a significant effect on the half-life. The initial H_2O_2 decomposition rate in trial No. 2 was 18.5 mg/L•min giving a specific decomposition rate of 631 mg/L•min•g dry weight and 1.85 mg/L•min•g expanded clay, respectively. Considering the flow rate of 2.5 mL/min, 3.4 kg of wet Leca with immobilized microorganisms would be enough to degrade all the peroxide in 1 m^3 of bleaching effluent within one hour.

CONCLUSIONS

Catalases seem to be attractive for the degradation of H_2O_2 in bleaching effluents. Although whole cell systems were less stable, this process could be advantageous when simultaneously used for the degradation of other effluent components.

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