J. Food Sci. Nutr.
Vol. 7, No. 1, p.33~36 (2002)

Biodegradation of Hydrogen Peroxide in Semiconductor Industrial Wastewater with Catalase from *Micrococcus* sp.

Sung-Hoon Oh, Hee-Jong Yu*, Moo-Sung Kim*, Sung So* and Hyung-Joo Suh†**

Department of Food and Biotechnology, Ansan College of Technology, Gyonggi-do 425-792, Korea
**Biotech. Division, Pacific Corporation, Gyonggi-do 425-120, Korea*
***Department of Food and Nutrition, College of Health Sciences, Korea University, Seoul 136-703, Korea*

Abstract

A catalase from *Micrococcus* sp. isolated from soil was applied to degrade hydrogen peroxide in wastewater from a semiconductor industry. The degradation rates of hydrogen peroxide increased with increasing reaction time and catalase concentrations in the reaction mixture. However, in the presence of aluminum chloride or chloride oxide used in detergent compounds, the degradation rate of hydrogen peroxide was not affected. Enzyme stabilizers and antifoam did not affect the degradation rates of hydrogen peroxide.

Key words: catalase, *Micrococcus* sp., semiconductor, wastewater

INTRODUCTION

Hydrogen peroxide (H_2O_2) is a powerful oxidant that is used as a bleaching agent or microbicide in paper, food, textile, and semiconductor industries. Although hydrogen peroxide itself is a toxic substance, when decomposed, it becomes oxygen and water. In light of the growing concern over the Earth's environmental problems, hydrogen peroxide is considered as a very promising candidate to replace chlorine as a bleaching or sterilizing agent (1). However, because hydrogen peroxide is a very toxic substance, it should be decomposed before disposal. If a catalase that effectively decomposes hydrogen peroxide can be applied, it can be utilized to decompose hydrogen peroxide contained in industrial waste. Catalase (EC 1. 11.1.6) is an enzyme well known for its ability to decompose hydrogen peroxide to water and dioxygen (2). Soluble catalases (e.g. from bovine liver or *Aspergillus niger*) are commercially available for use as catalysis for the decomposition of hydrogen peroxide. In recent years, the environmental impact of the paper, food, textile, and semiconductor industries has been reduced through the improvement of productivity and waste treatment technologies (3,4). In particular, a large amount of a mixture of sulfuric acid and hydrogen peroxide is required to clean the surface of silicon wafers, for example to strip photo-resist from the wafer. This mixture accounts for about 40% of total amount of chemicals required to clean semiconductor wafers. The waste mixture is first diluted with considerable water to lower the heat of hydration. Next, the waste mixture is neutralized with a base such as calcium hydroxide (5,6). The waste mixture is then coagulated with an inorganic flocculant such as $Al_2(SO_4)_3$. Following coagulation, the waste mixture is flocculated by a polymer flocculant to discharge wastewater and sludge (7). Although there are few examples of applications of catalase in the conductor industry, there may be great potential for such applications. In wastewater treatment in such industries, hydrogen peroxide should be removed before an activated-sludge treatment, because hydrogen peroxide damages microorganisms present in the treatment system.

The objective of this study was to evaluate the application of a catalase from *Micrococcus* sp. to decompose hydrogen peroxide effectively in reaction mixture system and wastewater. Therefore, in this study, we discussed the possibility of industrial applications in conductor wastewater using catalase degradation systems.

MATERIALS AND METHODS

A bacterial strain and medium

Micrococcus sp. producing catalase was isolated from soil. The preliminary morphological and biological characteristics of the strain coincide with those of *Micrococcus* sp. The strain was cultured in the basal medium containing 2% glucose, 2% peptone, 4% yeast extract, and 0.5% NaCl (8). The incubation was carried out at 30°C for 70 hr. Bacterial growth was monitored at OD_{600} (DU-65

†Corresponding author. E-mail: suh1960@unitel.co.kr
Phone: 82-2-940-2853, Fax: 82-2-941-7825

Spectrophotometer, Beckman, Fullerton, USA).

Assay of catalase

This procedure is used to determine the catalase activity, expressed as Baker Units (9). For a standard assay, an aliquot of no more than 1.0 mL of the sample, previously diluted to contain approximately 3.5 BU (Baker Units) of catalase. A hounded mL of 1.5% hydrogen peroxide in a 50 mM phosphate buffer (pH 7.0) previously adjusted to 25°C was placed in a 200 mL beaker then catalase was added to the 200 mL beaker, which was immediately stirred for 5 to 10 s. The mixture was incubated at 25°C until the reaction was completed. After stirring vigorously for 5 sec, 4.0 mL from the reaction mixture was added into a 50 mL Erlenmeyer flask. Five mL of 2 N sulfuric acid was added into the flask. Then 5.0 mL of 40% potassium iodide solution, freshly prepared, was added. One drop of 1% ammonium molybdate solution was also added and mixed. While continuing to mix, titration with 0.25 N sodium thiosulfate was rapidly carried out to a colorless endpoint. A blank determination with 4.0 mL of peroxide substrate solution was performed. One BU is defined as the amount of catalase that decomposes 264 mg of hydrogen peroxide under the conditions of the assay.

Degradation of hydrogen peroxide using the reaction mixture and wastewater

The degradation of hydrogen peroxide was initiated by adding various concentrations of the catalase. Approximately 3.5 BU of catalase was added into a 200 mL beaker. One hundred mL of 100 ppm hydrogen peroxide in 50 mM phosphate buffer (pH 7.0) previously adjusted to 25°C was added into a 200 mL beaker and stirred immediately for 5 to 10 sec. The mixture was reacted at 25°C until the reaction was completed.

The wastewater containing hydrogen peroxide was obtained from G Semiconductor Co. and stored at 4°C before use. The pH of wastewater (pH 1.71) was adjusted to pH 7.0 with $CaCO_3$. The degradation of 100 mL of waste water containing 57 ppm hydrogen peroxide was initiated by adding various concentrations of the catalase. After the reaction, residual hydrogen peroxide was measured.

RESULTS AND DISCUSSION

Degradation of hydrogen peroxide in catalase reaction mixture

The strain *Micrococcus* sp. was cultivated to produce catalase in the medium containing 2% glucose, 2% peptone, 4% yeast extract, and 0.5% NaCl. After 46 hr of cultivation, the lysozyme (360 mg, 23,900 units/mg) was added to the culture broth (1.8 L). The lysis was passed through a filter press. The filtrate was concentrated in an UF 5/4040 (Prochem Tech Internation Inc., New York, USA) using an ultrafilter (G-50, 1,5000 cut off) to remove any substances smaller than about 15 kDa. The final concentrate was used for the catalase.

Fig. 1 shows the degradation of hydrogen peroxide with the catalase reaction mixtures. The decomposition rate of hydrogen peroxide increased with increasing time and catalase concentrations. After 60 min, above 90% of hydrogen peroxide was decomposed in the mixture added with 15 ppm catalase. The decomposition kinetics of hydrogen peroxide for 20 min showed the first order reaction kinetics. The specific rate constants (k_1) of catalase with various enzyme concentrations [5 ppm (0.06 BU), 10 ppm (0.12 BU) and 15 ppm (0.18 BU)] were 0.02510^{-2}, 0.03510^{-2}, and 0.04710^{-2}, respectively. The k_1 values, which were calculated from equation ($d[H_2O_2]/dt = k_1 [H_2O_2]$), were increased with the enzyme concentrations of catalase.

Effect of aluminum chloride and chloride oxide on catalase activity

The inhibitors may come into contact with stained clothes during a soaking or prewashing stage prior to contact with the peroxide or persulfate bleaching agent or, alternatively, during the washing step. Of particular importance are compositions, which comprise a mixture of detergent compounds, aluminum chloride and chloride oxide. Therefore, it is very important that the compounds in wastewater using hydrogen peroxide as washing with bleaching agents should not affect the catalase activity. Fig. 2 shows the degradation of hydrogen peroxide using the catalase reaction mixture with detergent compounds. In the presence of aluminum chloride or chloride oxide used in detergent

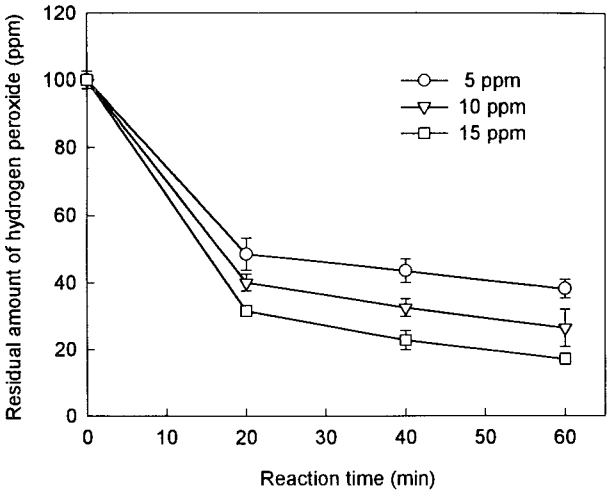

Fig. 1. Residual amounts of hydrogen peroxide treated with catalase in reaction mixture. Catalase has activity of 120 BU/mL. All measurements were performed at pH 7.0, 30°C in reaction mixture.

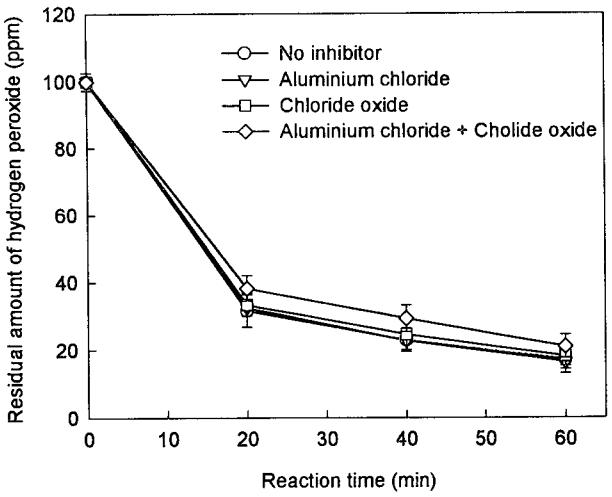

Fig. 2. Residual amounts of hydrogen peroxide treated with catalase under the additions of inhibitors in reaction mixture. Aluminum chloride (400 ppm), chloride oxide (50 ppm) and a mixture of aluminum chloride and chloride oxide were added to the reaction mixture.

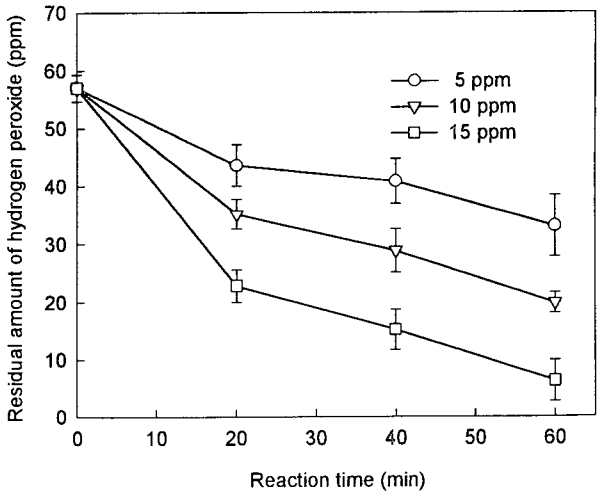

Fig. 3. Residual amounts of hydrogen peroxide treated with catalase in wastewater. Before treatment with catalase (120 BU/mL), pH of wastewater (pH 1.71) was adjusted to pH 7.0 with calcium chloride.

compounds, degradation of hydrogen peroxide was not affected. However, in the presence of a mixture of aluminum chloride and chloride oxide, degradation of hydrogen peroxide was slightly reduced, but not different significantly ($p<0.05$). These results indicated that the application of catalase for the treatment of wastewater in a semiconductor industry was not affected by aluminum chloride or chloride oxide.

Degradation of hydrogen peroxide in wastewater and effect of additives on catalase activity

Hydrogen peroxide residuals in the wastewater can be 57 ppm or higher during wafer washing. A catalase produced from *Micrococcus* sp. was applied to degrade hydrogen peroxide in the wastewater (Fig. 3). About 90% of hydrogen peroxide was decomposed after 6 min of reaction with the addition of 15 ppm catalase.

To prevent the loss of a catalase activity during degradation of hydrogen peroxide in wastewater, glycerol, ferrous sulfate, magnesium sulfate and NaCl were added to the reaction mixtures. Generally, the catalase of microorganisms such as yeast is located in organelles such as peroxisomes (10), which have membrane structures composed of hydrophobic materials surrounded by hydrophilic components. This suggests that catalase might be stable in a hydrophobic state (11). Therefore, the addition of NaCl or glycerol can be increased to hydrophobicity of catalase.

An antifoam agent is added for the removal of foams during the treatment of wastewater. The reaction products from the reaction of these compounds with unknown compounds in wastewater might affect the treatment of hydrogen peroxide with catalase. Fig. 4 shows the effect of several agents on the degradation rate for hydrogen peroxide in wastewater. After 20 min, sodium chloride, glycerol, ferrous sulfate, and magnesium sulfate slightly increased the degradation of hydrogen peroxide, but there were not different significantly ($p<0.05$). However, antifoam slightly reduced the degradation of hydrogen peroxide, but it did not show any significant difference ($p<0.05$) compared to the control. The agents for the stabilization of catalase and removing of foam did not affect the wastewater treatment.

Therefore, these results have a possibility of biodegradation of hydrogen peroxide in wastewater of semiconductor industry. However, for the industrial applications of catalase, immobilization of catalase will be further studied and carried out.

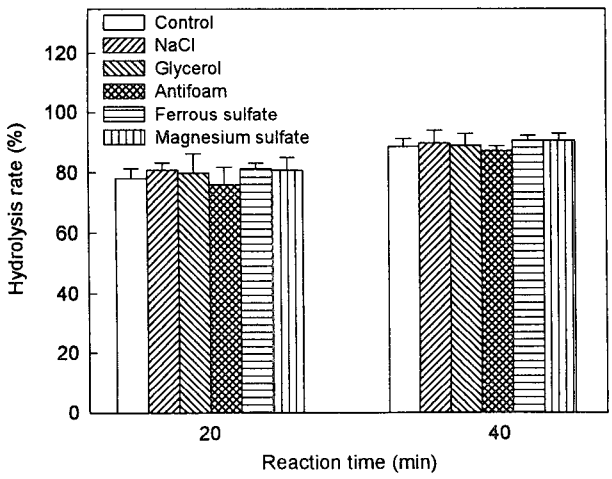

Fig. 4. Effect of stabilizer and antifoam on hydrolysis of hydrogen peroxide in wastewater. The hydrolysis was carried out in wastewater reactions containing with the agents (100 ppm) and catalase (15 ppm).

REFERENCES

1. Yumoto I, Yamazaki K, Kawasaki K, Ichise N, Morita N, Hoshino T, Okuyama H. 1998. Isolation of *Vibrio* sp. S-1 exhibiting extraordinarily high catalase activity. *J Fermen Bioeng* 85: 113-116.
2. Seip JE, Cosimo RD. 1992. Optimization of accessible catalase activity in polyacrylamide gel-immobilized *Saccharomyces cerevisiae*. *Biotechnol Bioeng* 40: 638-642.
3. Akertek E, Tarhan L. 1995. Characterization of immobilized catalase and their application in pasteurization of milk with H_2O_2. *Appl Biochem Biotechnol* 50: 291-303.
4. Larisch BC, Duff SJB. 1997. Effect of H_2O_2 on characteristics and biological treatment of TCF bleached pulp mill effluent. *Wat Res* 31: 1694-1700.
5. Goto A, Yamasaki K. 1999. A new wastewater treatment technology for mixed acid drainage containing fluorine. *IEEE T Semiconduct M* 12: 295-301.
6. Yamamoto K, Nakumura A, Hase U. 1999. Control of cleaning performance of an ammonia and hydrogen peroxide mixture (APM) on the basis of an kinetic reaction model. *IEEE T Semiconduct M* 12: 288-294.
7. Inagaki Y, Nishizaki M. 1999. Reclamation of waste mixture of sulfuric acid hydrogen peroxide used to clean semiconductor wafers. *Proc IEEE Int Symp* 267-270.
8. Rafii F, Lunsford P, Hehman G, Cerniglia CE. 1999. Detection and purification of a catalase-peroxidase from *Mycobacterium* sp. Pyr-1. *FEMS Microbiol* 173: 285-290.
9. Committee on Food Institute of Medicine. 1996. *Food chemical codex*. 4th ed. National Academic Press, Washignton DC, U.S.A.
10. Tanaka A, Yasuhara S, Osumi M, Fukui S. 1977. Immobilization of yeast microbodies by inclusion with photocrosslinkable resins. *Eur J Biochem* 80: 193-197.
11. Lida T, Maruyama D, Fukunaga K. 2000. Stabilization of entrapped catalase using photo-crosslinked resin gel for use in wastewater containing hydrogen peroxide. *J Chem Technol Biotechnol* 75: 1026-1030.

(Received January 2, 2002; Accepted February 16, 2002)