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BASIC STUDIES FOR CONTINUOUS DEGRADATION OF HYDROGEN PEROXIDE BY THE HOLLOW FIBER CONTAINING MICROCOCCUS LYSODEIPLICUS IFO 3333 CELLS

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A hollow fiber containing bacterial cells having high catalase activity was used as a reactor for degradation of hydrogen peroxide. The relative activity of the hollow fiber microbial cells to the intact cells was 57 % at pH 7.0 in a batch-wise reaction. When the hollow fiber reactor was applied to industrial waste water such as bleached wheat bran containing 10 mM hydrogen peroxide, the degradation ratio was 75 % for the first 200 h.

Catalase have been widely used for the assay or degradation of hydrogen peroxide. Immobilized catalase has also been applied in the food industry.[1] However, catalase seems to be rather unstable after immobilization.[2,3] Recently, Kimura et al.,[4] reported the convenient technique to entrap the microbial cells by using photocrosslinkable material. Further, Tanaka et al.,[5,6] reported studies on the continuous degradation of hydrogen peroxide by using immobilized yeast microbodies. The hollow fiber mass transfer devices[7,8] have recently been noted as having great potential for carrying out enzyme reactions. In this work, we studied the possibility of utilizing the hollow fiber Micrococcus lysodeiplicus IFO 3333 cells for the continuous degradation of hydrogen peroxide. The results indicated that the hollow fiber microbial cells would be usefull in degradation of hydrogen peroxide of the waste water of bleached wheat bran.

The buffer used in the present study was 0.07 M(1 M = 1 mol dm^{-3}) sodium phosphate, pH 7.0. Hydrogen peroxide concentration was determined using a colorimetric method.[9] The hollow fiber reactor used was Asahikasei 15p(polyacrylonitrile) minimodule. The parameters are: internal fiber diameter, 0.8 mm, wall thickness, 0.6 mm. The nominal molecular weight cut off is 6000. The length of the hollow fiber was 5 m. Batch-wise reactions were carried out in a beaker(100 ml) anchored in a water bath at 20°C. The cell concentration was 0.1 mg(dry cells)/ml, and the initial substrate concentration was 100 mM.

The batch-wise activity of the hollow fiber microbial cells at pH 7.0, 20°C was 57 % of that of the intact cells. As shown in Fig. 1, the optimum pH of the hollow fiber microbial cells was shifted about 0.5 pH unit to the alkaline region than that of the intact cells. Further, the hollow fiber microbial cells have wider ranges of the optimal pH than the intact cells.

Fig. 2 shows that the optimum temperature of the hollow fiber microbial cells were about 5°C higher than that of the intact cells. The optimal temperature for the hollow fiber microbial cells was observed to be 25°C. The shift of the optimal temperature suggested the stabilizing of catalase in the hollow fiber.

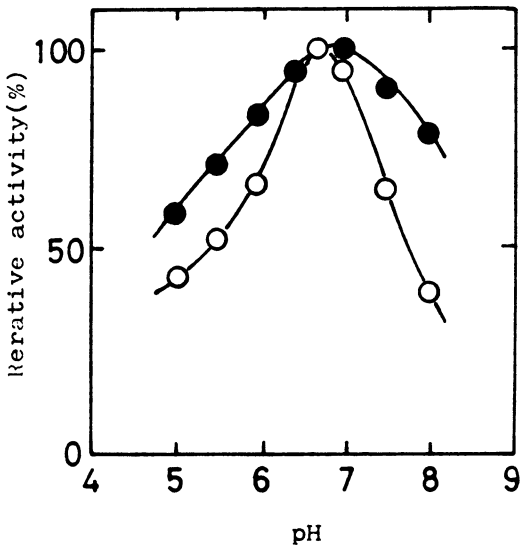

Fig. 1. Effect of pH on the catalase of microbial cells at 20°C in 0.07 M phosphate buffer.

A solution of 100 mM hydrogen peroxide was treated for 30 min with the hollow fiber reactor containing 0.1 mg(dry cells) /ml of the microbial cells.

○ : Intact cells

● : Hollow fiber microbial cells

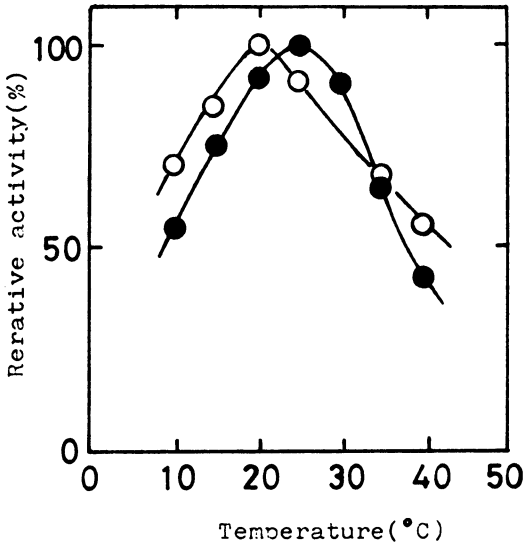

Fig. 2. Effect of temperature on the catalase of microbial cells in 0.07 M phosphate buffer, pH 7.0.

The conditions are the same as those in Fig. 1.

○ : Intact cells

● : Hollow fiber microbial cells

Lineweaver-Burk plots of the both of intact and hollow fiber microbial cells are shown in Fig. 3. Km and Vmax values calculated from the figure were 8.2×10^{-2}M and 2.4×10^{-3}M $min^{-1}$for the hollow fiber microbial cells, and 7.8×10^{-2}M and 2.7×10^{-3}M $min^{-1}$for the intact cells, respectively. Km and Vmax values of the hollow fiber microbial cells are similar to those of the intact cells. Inactivation of the enzyme does not observe by containing the intact cells in the hollow fiber.

Fig. 4 shows the continuous degradation of hydrogen peroxide using the industrial waste water of bleached wheat bran by the hollow fiber microbial cells. Continuous degradation system of hydrogen peroxide by the hollow fiber microbial cells is illustrated in Fig. 5. The hollow fiber reactor could treat continuously the waste water containing 10 mM hydrogen peroxide at a flow-rate of 50 ml/h. Degradation rate of hydrogen peroxide was 75 % for 200 h at pH 7.0, 25°C, as shown in Fig. 4.

Proliferation of the bacteria in the hollow fiber was observed after 8 days reaction with the waste water. The hollow fiber microbial cells would be

useful for the continuous degradation of hydrogen peroxide in industrial waste water such as bleached wheat bran.

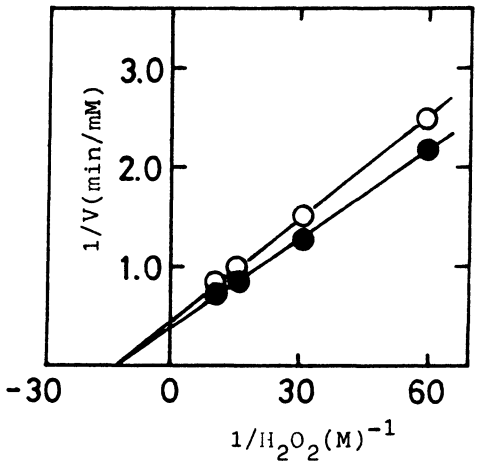

Fig. 3. Lineweaver-Burk plots of the intact cells and hollow fiber microbial cells.

All measurements were performed at pH 7.0, 25°C in batch-wise reactions. The activity was measured by a colorimetric method.[9)]

○ : Intact cells

● : Hollow fiber microbial cells

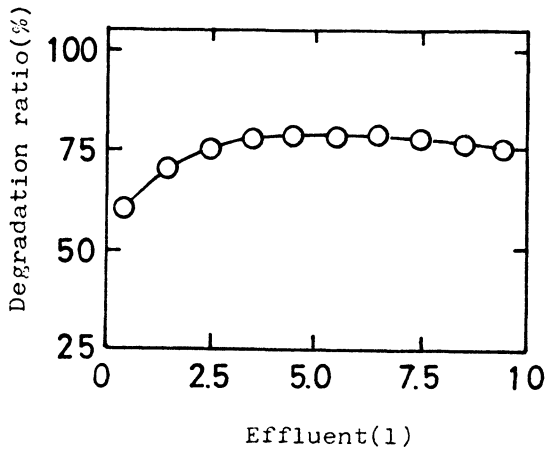

Fig. 4. Continuous degradation of hydrogen peroxide in the waste water of bleached wheat bran containing 10 mM hydrogen peroxide at a flow-rate of 50 ml/h, pH 7.0, 25°C.

The waste water was treated with hollow fiber reactor containing 1.8 mg(dry cells)/ml of the microbial cells.

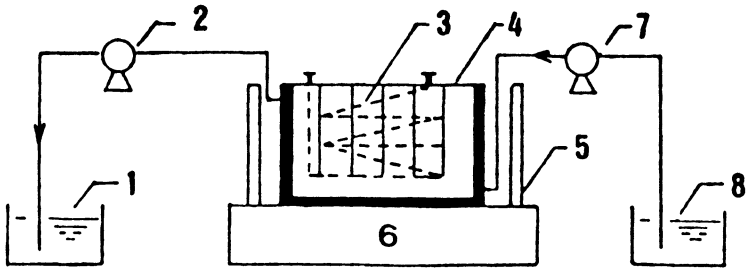

Fig. 5. Continuous degradation system of the hollow fiber microbial cells.

1: Reservoir, 2: Draw pump, 3: Hollow fiber microbial cells, 4: Asahikasei 15p minimodule, 5: Water bath, 6: Magnetic stirrer, 7: Feed pump, 8: Waste water of bleached wheat bran containing hydrogen peroxide.

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