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LYSIS OF BACTERIA IN AEROSOL BY IMMOBILIZED ENZYMES

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Bacteriolytic enzymes (protease and β-1,3-glucanase) produced by *Achromobacter lunatus* were immobilized in collagen membrane. Approximately 20% of intact *Bacillus subtilis* in aerosol was lyzed by the bacteriolytic enzyme–collagen membrane at the space velocity of 2×10^3.

INTRODUCTION

Recently many immobilized enzymes have been prepared and used for various practical purposes (1,2). The bacteriolytic enzymes have been immobilized in collagen membranes (3). Intact bacteria such as *P. aeruginosa*, *P. solanacearum*, *X. oryzae*, and *S. aureus* in aqueous solution have been lyzed by bacteriolytic enzyme–collagen membranes. It is well known that diseases are associated with airborne agents (4). Medical scientists have long concerned themselves with the study of bacteria aerosol. The disinfection of liquid and aerosol viral systems using immobilized enzymes has been reported by Kirwan et al. (5). Lysis of bacteria in aerosol by the bacteriolytic enzyme–collagen membrane was examined in this study. *Bacillus subtilis* was used for this experiment.

MATERIALS AND METHODS

Bacteriolytic enzymes (produced by *Achromobacter lunatus* Type YL 16,000 u/g) were obtained from the Amano Pharmaceutical Co. Collagen was purified by the method previously reported (6). Other solvents and reagents were commercially available analytical reagents or laboratory grade materials. Deionized water was used in all experiments.

Bacillus subtilis was cultured at 30°C for 48 h in 50 ml of tap water (pH 7.0) containing 1% glucose, 1% peptone, 1% beef extract, and 0.2%

sodium chloride. Eight-nine grams of 0.9% collagen fibril suspension (pH 3.0) and 0.8 g of bacteriolytic enzymes were mixed and cast on a plastic net (20 cm × 40 cm). The membrane was dried at room temperature for 24 h, and sprayed with 1% glutaraldehyde solution. The enzyme–collagen membrane was rolled and inserted into a biocatalytic reactor (7) (ϕ 2.2 cm × 50 cm, acrylic plastic). The enzyme activity of the reactor was 128 units (in the liquid phase experiment). A block diagram of the system for continuous lysis of bacteria is shown in Fig. 1. Bacteria suspension (0.2 mg/ml) containing 1.5 mM sodium laurylsulfate was atomized with a Lumina PS-1 atomizer from the Fuyo Seiki Co. at a flow rate of 5 ml/h. The atomizer produced droplets in the range from 10 to 100 μm when operating at the appropriate air pressure. The relative humidity within the reactor was not measured but would be near 100% because of the good contact between the air and droplets and the relatively large ratio of liquid to air. Lysis of bacteria was determined from the turbidity and ribonucleic acid content of the suspension (3).

RESULTS AND DISCUSSION

The bacteriolytic enzymes used in these experiments were protease and β–1,3-glucanase. Figure 2 shows the relationship between the space velocity and the lysis of *B. subtilis*. As shown, the lysis of *B. subtilis* occurred below

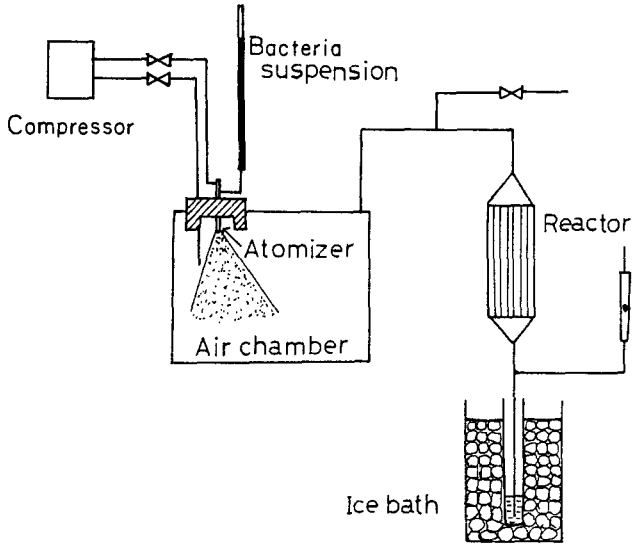

FIG. 1. A diagram of the system for continuous lysis of bacteria.

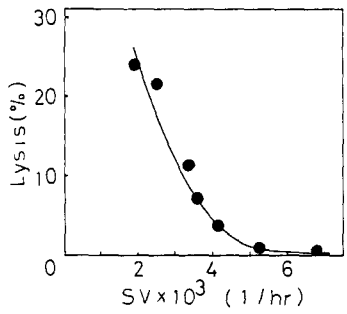

FIG. 2. Relationship between the space velocity and the lysis of *B. subtilis*. The reaction was performed at 15°C.

the space velocity of 4×10^3. Approximately 20% of *B. subtilis* was lyzed at the space velocity of 2×10^3. Control experiments were also performed with the enzyme–collagen membrane containing heat-denatured enzymes under identical conditions. In this case, no increase of RNA content was observed in condensed water (see Fig. 1). Therefore, no lysis of bacteria occurred on control experiments. *Bacillus subtilis* is a rod-shaped cell (0.7-0.8 × 2.0–3.0 μm). From diffusion experiments, the size of the collagen fibril network was found to be about 300 Å × 300 Å. Therefore, it was difficult for the bacteria to penetrate the collagen membrane. The bacteriolytic enzymes bound or entrapped on the surface of collagen membrane could react with *B. subtilis* cell walls. In this experiment, the detergent, sodium laurylsulfate, was added to the *B. subtilis* suspension. The lysis of bacteria also occurred without detergent. However, the velocity of lysis was one-fifth that under presence of detergent. It is well known that the outer layer of gram-negative bacteria (containing a complex mixture of lipid, protein, and lipopolysaccharide) is removed with detergent treatment (8). As *B. subtilis* is a gram-positive bacterium, the cell wall was mainly composed of peptidoglycan, a substance that contributes to the rigidity of bacterial cell walls. The structure of the *B. subtilis* cell wall may be changed with detergent treatment. Therefore, the hydrolysis of the *B. subtilis* cell wall was accelerated with detergent treatment.

Further developmental studies in this laboratory are being directed toward applying the enzyme–collagen membrane to sterilization of other bacteria in aerosol.

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