# CHAPTER III

# METHODOLOGY

## 3.1 Catalyst Preparation

Monolith honeycomb, cordierite, from Corning Glass was selected as support in this study. Two preparation techniques were used. The first technique is an ion-exchange. The other technique is similar to simple incipient wetness.

By ion-exchange technique, a catalyst was prepared by dipping a monolith into silver-amine complex solution which was formed by mixing $AgNO_3$ (Aldrich) and $NH_4OH$ in distilled water for a given period of time with continuous stirring. The soaked monolith was then immersed into formalin (37 % formaldehyde solution). The solution was allowed to diffuse and reduce the silver-amine complex species adsorbed on the washcoat surface. The final catalyst was air dried at 80 °C for 3 hours and was ready for use in killing experiment. To prepare catalysts with various silver contents, this may be done by either increasing silver concentration in solution or allowing longer dipping time.

The other group of catalysts was prepared by the way similar to incipient wetness method. A monolith was first dipped into a $AgNO_3$ aqueous solution for 10 minutes. The moist monolith was dried at 110 °C for 1 hour. The dipping and drying process was repeated several times. The final catalyst was then placed in a furnace. The temperature was ramped to 800 °C and

maintained for a given period. Silver-amine complex solution was sometimes used.

## 3.2 Catalyst Characterization

Average pore size and BET surface area of original monolith were determined by using $N_2$ as absorbent at 77 K. However, all samples were heated at 120 °C for 3 hours prior to test.

Silver content of catalysts was determined by measuring the silver concentration of the spent solution by atomic absorption spectroscopy. The silver loading of each catalyst was then calculated based on 20 % $Al_2O_3$ washcoat on monolith. Some properties such as the dispersion, the surface morphology and the variation of particle sizes were carried out by SEM, (JEOL model 5200). SEM pictures of original monolith were also taken.

## 3.3 Microorganisms and Cell Suspension Preparation

*E.coli* culture obtained from the Department of Microbiology, Chulalongkorn University, was used as a studied bacteria. *E.coli* was cultivated on a slant of nutrient agar and incubated at 37 °C for 20 hours. The resulting growth was harvested by washing off the growth by sterile buffered water. The suspended liquid was then centrifuged, discarded the supernatant liquid and resuspended. The cell suspension was shaken to disintegrate the bacteria and was ready for use in the killing study.

### 3.4 Sterile Buffered Water

To prepare stock phosphate buffered solution, 34.0 g potassium dihydrogen phosphate ($KH_2PO_4$) was dissolved in 500 ml distilled water. The pH was adjusted to 7.2 with 1 N sodium hydroxide (NaOH), and diluted to produce 1 liter of solution. Buffered water was prepared by adding 1.25 ml stock phosphate buffered solution and 5.0 ml magnesium chloride solution (81.1 g $MgCl_2 6H_2O$ per liter) to each liter of water. Sterilization was done by autoclaving at 121 °C for 20 minutes.

### 3.5 Sample Collecting Container

All sampling bottles were sterilized by autoclaving at 121 °C for 20 minutes. For each 100 ml of sample to be collected, 0.1 ml 10% sodium thiosulfate ($Na_2S_2O_3$) and 0.3 ml 15% disodium salt of ethylenediamine tetra-acetic acid (EDTA) were added prior to sterilizing the bottle as suggested in *Standard Method for the Examination of Water and Wastewater.*

### 3.6 Analytical Methodology for *E.coli* by Membrane Filtration

To prepare m-FC agar, the media was heated to dissolve completely and allowed to cool to about 50 °C. The media was then added to the bottom of 50 x12 mm sterile petri dish and allowed to solidify. The sterile filter holder was attached to a filter flask which was connected to a suction pump. A sterile membrane filter was placed with grid side up, on the platform of the filter unit by using sterile forceps. The liquid sample was poured into the funnel and draw the sample through the membrane filter by applying suction. After all the sample was passed through the filter, the membrane was rinsed with sterile buffered water. The membrane filter was transfered and placed on the m-FC

media. The membrane filter was placed on m-FC media with a rolling action in order to avoid trapping air bubbles between the filter and the agar media. After replacing the top of petri dish, the membrane filter and the media were incubated at 44.5 °C for 24 hours. *E.coli* colonies are blue visible colonies which were than counted.

### 3.7 Killing Study Procedure

The primarily used column for single-pass killing study was a down-flow fixed column of the diameter of which fitted to the diameter of monolith which was 3.5 cm diameter and 4.0 cm long.

Six liters of distilled water to be suspended with *E.coli* was first prepared in a 10-liter flat bottom flask. Silicone was used as inlet passage to connected between the 10-liter flask and the top of the test column. Similarly, water effluent passage was connected to the bottom of the column. The catalyst studied was placed in the column. The whole system had to be sealed and sterilized by autoclaving for 20 minutes at 121 °C. The whole system was then allowed to cool to room temperature. The system water was aerated to obtain the saturation level of dissolved oxygen by zero grade air which was prefiltered by air-vent bacterial filter to prevent undesired bacterial contamination. The experimental set up of the system is shown in Figure 3.1.

The following procedure was used for all single-pass killing study. An amount of *E.coli* culture was suspended into the sterile system water being tested just before beginning the experiment to produce an initial *E.coli* concentration of about $10^4$ CFU/ml (colony forming units/ml). The water sample was first collected and measured for pH, dissolved oxygen and system temperature. The peristaltic pump (Cole-Parmer, digital console drive pump)

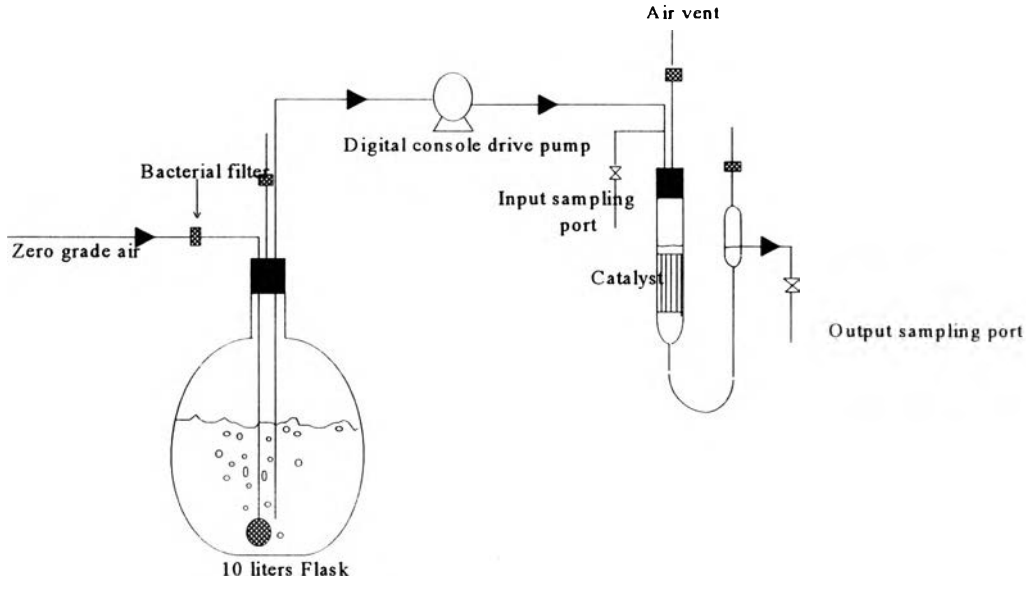


Figure 3.1 The experimental system for killing study.

was adjusted to a flow rate of 200 ml/min. The influent sample was collected. The output sample was then collected at 3 minutes after system water flowing through the column. The experiment was further conducted at the flow rates of 300 and 400 ml/min. The overall experiment was carried out within 20 minutes or less in order to minimize both stavation and multiplication effects. *E.coli* concentrations of all samples were tested by membrane filtration technique. The visible colonies observed on plates were counted and averaged by two replicates of each sample after 24 hours incubation at 44.5 °C. Silver content in each effluent sample was determined by atomic absorption spectroscopy.

### 3.8 Toxicity Test of Silver Ions

In order to see the effect of silver ion on survival of *E.coli*, silver ion prepared by the same method used for atomic absorption spectroscopy was distributed in distilled water to a desired concentration. *E.coli* were added to

distributed in distilled water to a desired concentration. *E.coli* were added to give initial *E.coli* concentration of about $10^4$ CFU/ml. Samples were removed at particular times and tested by membrane filtration technique. The final *E. coli* concentration of each sample was averaged by the counts of two replicates.