CHAPTER III
EXPERIMENTAL SECTION

3.1 Materials

3.1.1 Reactant Gases

The gases used in this experiment were research-grade oxygen (HP, 99.8 percent purity), nitrogen (UHP, 99.999 percent purity), and air (zero grade air) which were supplied by Thai Industial Gas (TIG) and PraxAir. All gases were used in order to control the dissolved oxygen level in the water .

3.1.2 Chemical

Silver nitrate (AgNO3) used for this catalyst preparation was AR grade of 99.9 percent purity obtained from Carlo Erba and BDH Laboratory.

3.1.3 Supports

Two types of alumina chosen as support for silver catalyst were alumina balls and alumina powder. Alumina balls with 90% purity and having 5 mm diameter were obtained from Cernic International Co., Ltd. Alumina powder prepared by sol-gel method has a relatively high specific-surface area. The two type of alumina was chosen as high and low specific-surface-area materials for this work.

3.2 Catalyst Preparation

In this study, the catalyst was prepared by impregnation technique. The alumina powder was firstly washed with hot distilled water and then heated to 300 °C in air for one hour in order remove any adsorbed materials. The alumina was cooled to room temperature in a dessicator. The purified dried alumina was impregnated with the silver precursor. The silver precursor used for this catalyst preparation was prepared by dissolving silver nitrate in distilled water. The ratio of the distilled water value to the amount of catalyst was 5 times the amount of silver nitrate were changed by following the percent loading of silver on alumina that used in the experiment. This precursor solution was slowly poured into the beaker containing alumina powder with stirring until it got wet evenly. Once the powder looked wet, addition of the silver nitrate solution was stopped. The alumina powder was then dried in air at 110 °C for 2 h.The alumina powder was cooled down in a desiccator. After drying , the alumina powder was soaked the silver nitrate solution again. This process was repeated until all of the silver nitrate was absorbed by the powder alumina. The alumina was dried in air at 110 °C for 2 h and then calcined at 200 °C for 1 h, 300 °C for 1 h, 400 °C for 1 h and 500 °C for 4 h with a constant heating rate of 10 °C/min. After that, the alumina was slowly cooled to room temperature and stored in a dessicator.

For α-alumina balls, the silver catalyst was also prepared by the impregnation method. The similar procedure as mentioned before was also used to deposit silver nitrate on the surface of the alumina balls. The different step was the calcination temperature which was 800 °C for 8 h. This high temperature for calcination was to ensure strong adhesion of the metalic silver to the alumina surface.

3.3 Catalyst Characterization

The silver catalysts prepared were characterized regarding to their physical and chemical properties using BET surface area analyser, X-ray Diffraction and Scaning Electron Microscope. The silver loading on alumina was determined by using Atomic Absorption Spectroscope.

3.3.1 Surface Area Measurement

Brunauer-Emmet-Teller (BET) method,AUTOSORB-1, was applied to determine the surface area of both types of alumina. The sample was heated in flowing He at 140 °C for 16 h. before nitrogen adsorption measurements. The characterization was based on the physical adsorption of nitrogen at the liquid nitrogen temperature (77 K). The specific surface area was calculated from five points of nitrogen adsorption isotherm. The average pore radius and average pore volume were also calculated at P/P_0 ratios close to unity.

3.3.2 X-ray Diffraction (XRD)

X-ray diffraction,(X'pert TW3710ATD Phillip) is usually used to identify the structure and composition of a crystalline material. The crystallite size can be found from the broadening of an X-ray diffraction peak, measured at one-half the height. An estimate of the dimension of the crystallites is calculated by the Scherrer formula :

$$d = k\lambda/(b \cos\theta)$$

where

d = the crystallite size (°A)

k = a constant (frequency nearly unity)

λ = a wavelength (°A)

θ = the corresponding Bragg angle (degree)

b = the peak width at the middle height (radian)

3.3.3 Scaning Electron Microscope(SEM)

The properties such as the dispersion, the surface morphology and the variation of particle sizes were determined using SEM,(JEOL model 5200). The sample was prepared by sticking on stub and coated with gold.

3.3.4 Atomic Absorption Spectrometer (AAS)

The AAS, VARIAN model 300/400, was employed to determine the content of silver on the alumina. Two hundred miligrams of the prepared catalyst was dissolved in HNO_3 solution with the acid to water ratio of 1 to 1 and then heated to 100 °C for 1 h. A standard solution of 1,000 ppm from Merck was used to establish a calibration curve. The amount of silver loaded on the alumina support was obtained by measuring the absorbance of the solution prepared.

3.4 Culture Media Preparation

8 g. of nutrient broth and 150 g of granulated agar, manufactured by Becton Dickinson, were dissolved in 1 litre of distilled water. The media solution was then sterilized by using an autoclave operated at 121°C for 15 min. This sterilized media solution was called slant.

For m-FC agar, 3.7 g of m-FC Broth Base and 15 g of Bacto-Agar were mixed well with 100 ml distilled or deionized water. 1 ml of a 1% solution of

Bacto Rosolic Acid in 0.2 N NaOH was added. If necessary, the solution was adjusted to pH 7.4 with 1 N HCl. The solution was then boiled.

3.5 Microorganisms and Cell Suspension Preparation

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

3.6 Sterile Buffered Water

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

3.7 Sterilization of Glassware and Materials

Before sterilization, all test-tubes, flasks and bottles were stoppered with non-absorbent cotton-wool so as to form a firmly fitting plug. Alternatively, loosely fitting metal caps were used. Pipettes were plugged with cotton-wool and the ends were singed. Pipettes and Petri-dishes to be sterilized in the hot-air oven were placed in metal containers or individually in paper.

Before the culture media and aqueous solutions were sterilized in an autoclave, they were covered with grease-proof paper to protect the cotton-wool plugs from excessive wetting by steam.

All glassware containing bacterial cultures or otherwise containated were sterilized by autoclaving for 20 min at 121 °C. In addition, this thermal treatment also made all solids to be removed easily. The sterilized glasswares were then rinsed well with tap water and, followed with soaking in a detergent solution and then cleaning with a brush. The final rinsing step was carried out by using hot water to wash for the first five times and then to be washed three times with the distilled water. All the clean glassware was finally dried in an oven before using.

All disposable pipettes which had to be sterilized by autoclaving for 20 min at 121 °C before discarding.

3.8 Sample Collecting Container

All sampling bottles were sterilized by autoclaving at 121 °C for 20 minutes. For each 100 ml of sample to be collect, 0.1 ml 10% sodiumthiosulfate ($Na_2S_2O_3$) and 0.3 ml 15% disodium salt of ethylenediamine tetraacetic acid (EDTA) were added prior to sterilizing the bottle as suggested in *Standard Method for the Examination of Water and Wastewater.(1985)*

3.9 Examination of *E.coli* by Membrane Filtration Method

Membrane filtration method was selected to determine number of *E. coli* in samples. This method was to filter a measured volume of sample through a filter membrane to trap all *E. coli* on the filter membrane. The filter membrane was then placed on a growth medium in a peri dish. Each bacterium retained on the filter grew to form a small colony. The number of *E. coli* present in the sample was directly determined by counting the number of colonies and it was expressed in terms of number per 100 ml of the original sample. The membrane filter technique has been widely adopted for use in water quality studies since it requires much less laboratory apparatus than the standard multiple-tube technique. Portable membrane filter apparatus has been developed for conducting coliform tests in the field. Special apparatus required to conduct membrane filter coliform tests includes filtration units, filter membranes, absorbent pads, forceps, and culture dishes.The apparatus of membrane filtration is shown in Figure 3.1. The filter-holding assembly, constructed of glass or porcelain, was sterilized by boiling or autoclaving. For filtration, the assembly was mounted on a side-arm filtering flask which is evacuated to draw the sample through the filter. Filter membranes used were 50 mm diameter disks with pore openings of 0.45 μm, small enough to retain microbial cells. Filters used in determining bacterial counts had a grid printed on the surface for ease in counting colonies.

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 x 12 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 of blue visible spots were than counted.

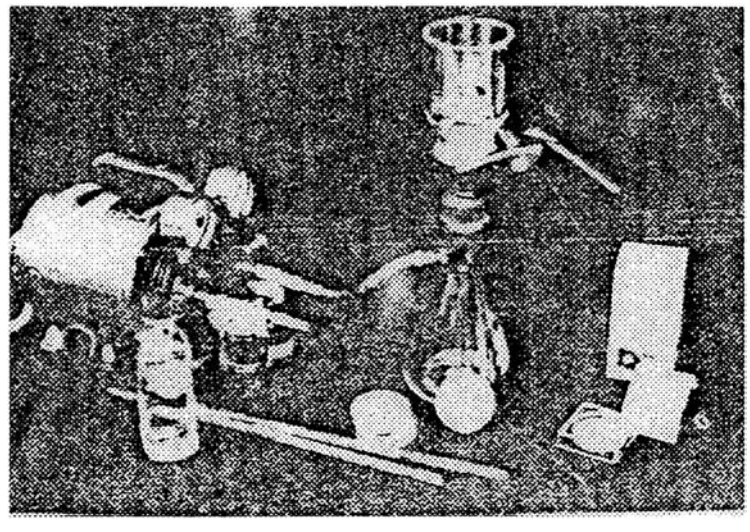

Figure 3.1 Laboratory apparatus for coliform testing by the membrane filter technique.

3.10 Procedure of Disinfection Study

An apparatus used for single-pass killing study was a down-flow fixed column having a diameter of 3.5 cm and 20 cm in the height. Six liters of distilled water to be suspended with *E.coli* was first prepared in a 10-liter flat

bottom flask. Silicone tube was used as inlet passage between the 10-liter flask and the top of the test column. 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 min at 121 °C. The whole system was then allowed to cool to room temperature before use. The studied 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 studied system is shown in Figure 3.2.

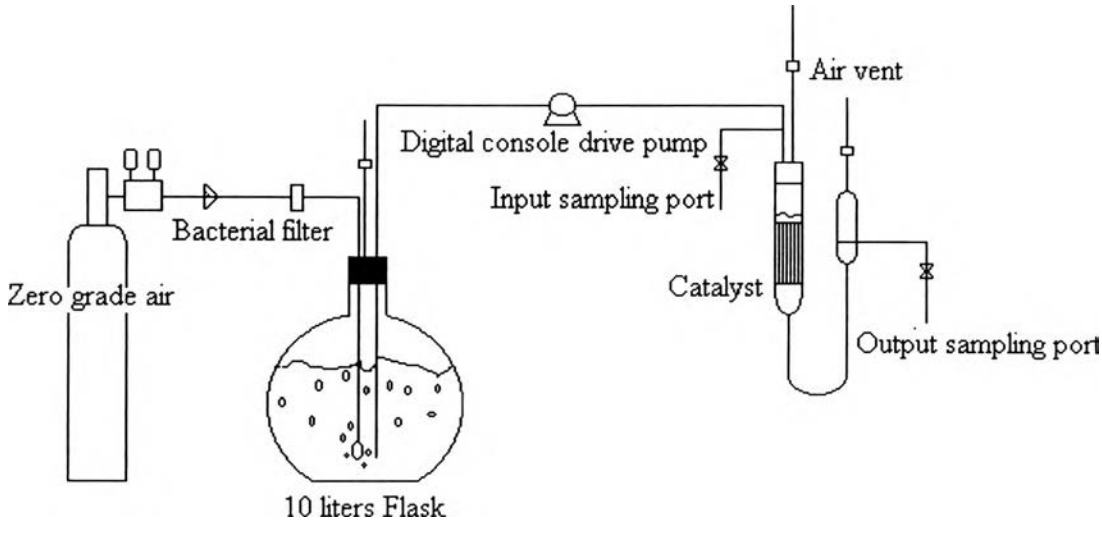

Figure 3.2 Experimental system for disinfection study.

The following procedure was used for all single-pass killing study. A desired 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 10^3 CFU/100 ml (colony forming units/100 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) was adjusted to a flow rate of 20 ml/min. The influent sample was collected. The output sample was then collected at 3 min after system water flowing through the column. The experiment was further conducted at the flow rates of 50, 100 and 150 ml/min for alumina powder system. The flowrate for the alumina balls system were 100, 200, and 300 ml/min, respectively. 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 determined 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 spectrometer.