

ANTIBACTERIAL TESTING METHOD FOR TiO₂ COATED PAPER

DEVELOPMENT OF ANTIBACTERIAL
EFFICACY TESTING METHOD
FOR TiO₂ COATED PAPER
IN THE PRESENCE OF LIGHT

BY

PARISA ZOGHI, B.ENG.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Master of Applied Science

McMaster University

© Copyright by Parisa Zoghi, August 2007

MASTER OF APPLIED SCIENCE (2007)
(Chemical Engineering)

McMaster University
Hamilton, Ontario

TITLE: Development of Antibacterial Efficacy Testing Method for TiO₂ Coated

Paper in the Presence of Light.

AUTHOR: Parisa Zoghi, B. Eng (Sharif University of Technology, Tehran, Iran)

SUPERVISOR: Professor Robert H. Pelton

NUMBER OF PAGES: x, 44

Abstract

Photocatalytic TiO_2 coated paper was prepared as antibacterial paper to investigate bactericidal activity of TiO_2 in the presence of UV light. When TiO_2 exposed to UV light, it produced hydroxyl radicals which were strong oxidative groups and could damage the cell wall causing death of bacteria. The goal of this work was to develop a promising method for microbiological examination of antibacterial paper and to test the disinfection properties of photocatalytic TiO_2 coated paper after UV illumination.

Three different methods were modified to test antibacterial effect of TiO_2 coated paper. The disk diffusion method, the washing method, and the membrane filter method. It was found that disk diffusion technique would not work because of insolubility of TiO_2 in water but it could be used for any other antibacterial paper that consisted water soluble agent. The results from membrane filter method agreed with those of washing method showing the reduction of *E.coli* colonies for TiO_2 coated paper after exposure to UV, comparing to blank paper.

With use of washing method, it was shown that by changing some functions such as: increasing TiO_2 content on the paper, increasing UV irradiation time or UV intensity, survival ratio of the bacteria decreased.

Acknowledgments

I would like to first express my sincere gratitude to my supervisor, Professor Robert H. Pelton, for supervising this master thesis and his excellent guidance and valuable comments on the work and giving me the opportunity to be part of SENTINEL bioactive paper network, and always understanding my personal issues and supporting me like my real father.

I also wish to thank my co-supervisors, Professor Carlos Filipe, for his helpful advices on microbiology parts of my work, and Professor Michael Brook for his supports to enhance the quality of this study. Special thanks to Dr. Lily Geng who contributed to this work with her new ideas and advice on experimental designs. Special acknowledgement and thanks go to SENTINEL Bioactive paper network and Natural Science and Engineering Research Council of Canada for financial support of this work.

My special thanks go to Professor Luba Brovko from Guelph University for discussions which helped to develop ideas put forward. I wish to acknowledge the help of Dr. Haifeng Wang also from Guelph University for his microbiology trainings and Professor Mansel Griffiths' group in Canadian Research Institute for Food Safety (CRIFS) from University of Guelph for providing *E.coli* ATCC 11229.

My thanks go to Professor Huining Xiao and Yong Guan from University of New Brunswick for permission to use their antibacterial handsheets. I would like to express my thanks to Xin Ge for all his supports on microbiology tests that were greatly enhanced by the assistance of him.

I am grateful to Steve Kupritch for the SEM training and measurements, to Marilyn Timleck for the CLSM training and measurements, to Frank Gibbs for the TGA measurements, and to summer student Scott Gow for his assistance on the TiO₂ coating experiments.

My special thanks go to my best friend Dr. Poupak Mehrani for her ongoing support throughout my study and new life in Canada. Many thanks go to all colleagues at McMaster Interfacial Technologies group for their help and friendship. I'm also grateful

to all my friends at McMaster, Dr. Hormoz Eslami, Maryam Emami, Hamzeh Hashemi, Geetha Mahadevan who made my life at McMaster such an enjoyable experience. I would like to thank Mr. Doug Keller (labarotory manager), Ms. Connie Barry, Ms. Lynn Falkiner, Ms. Kathy Goodram, Ms. Andrea Vickers and Ms. Justyna Derkach for their administrative and technical assistance.

Finally, I am grateful to my husband, Hooman Hojatie, for his love and encouragement, my son, the little bundle of joy, Parshawn Hojatie, who was born during my master study and grew up like a little man with understanding my situation by being the most intelligent boy I've ever seen. My deepest gratitude goes to my mother, Sakineh Rezakhani, and my father, Taghi Zoghi, for being an idol and source of inspiration to me throughout my life, to my sister Gita Zoghi and my brother Behrang Zoghi for believing in me, and their love.

Table of Contents

ABSTRACT	iii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	vi
LIST OF FIGURES	viii
LIST OF TABLES	x
1 INTRODUCTION.....	1
1.1 BACKGROUND INFORMATION: BRIEF REVIEW OF MICROBIOLOGY	1
1.1.1 Morphology of the cell.....	1
1.1.2 Shape and size of bacteria.....	2
1.1.3 Diagram and elements of bacteria.....	2
1.1.3.1 Cell wall and cell membrane.....	3
1.1.3.2 Cytoplasm	3
1.1.3.3 Ribosome	3
1.1.3.4 Flagella.....	3
1.1.3.5 Pili	3
1.1.4 Gram-positive, gram-negative	4
1.1.5 Growth	4
1.1.6 Environment.....	4
1.1.7 Nutrition.....	6
1.1.8 Culture media.....	6
1.1.9 Direct and indirect Measurements of microbial growth	6
1.1.10 Bacteria in the air	8
1.1.11 Bacteria in water	9
1.1.12 The death of bacteria.....	9
1.2 LITERATURE REVIEW	10
1.2.1 Photochemistry of TiO ₂	10
1.2.2 History of TiO ₂ photocatalyst as an antibacterial agent.....	11
1.2.3 Methods to evaluate disinfection property of antibacterial paper.....	13
2 EXPERIMENTAL PROCEDURE.....	18
2.1 OBJECTIVE	18
2.2 MATERIALS.....	18
2.3 PREPARATION AND CHARACTERIZATION OF TiO ₂ SUSPENSION.....	18
2.4 PREPARATION OF PAPER SAMPLES.....	19
2.4.1 TiO ₂ coated paper: Spray coating.....	19

2.4.2	Other antibacterial paper.....	20
2.5	CHARACTERIZATION OF TiO ₂ ON PAPER.....	20
2.6	MEASUREMENT OF UV INTENSITY OF LABORATORY UV LAMP.....	21
2.7	LURIA-BERTANI (LB) BROTH PREPARATION	22
2.8	E.COLI CULTURE PREPARATION	22
2.9	DISINFECTION TEST METHODS.....	23
2.9.1	Disk diffusion method.....	23
2.9.1.1	Introduction.....	23
2.9.1.2	Procedure	23
2.9.2	Membrane filter method using MacConkey agar	24
2.9.2.1	Introduction.....	24
2.9.2.2	Procedure	24
2.9.3	Washing method	25
2.9.3.1	Procedure	25
3	RESULTS AND DISCUSSIONS	27
3.1	PAPER SAMPLES	27
3.2	TiO ₂ SUSPENSION PROPERTIES	28
3.3	CHARACTERIZATION OF TiO ₂ ON PAPER.....	29
3.4	DISINFECTION TEST METHODS.....	29
3.4.1	Disk diffusion method.....	29
3.4.2	Membrane filter method using MacConkey agar	31
3.4.3	Washing method	34
3.4.3.1	Effect of TiO ₂ content on disinfection	37
3.4.3.2	Effect of UV illumination time on disinfection	38
3.4.3.3	Effect of UV intensity on disinfection	39
4	CONCLUSIONS	41
5	REFERENCES.....	43

List of Figures

<i>Figure 1.1 Diagram of cell: (a) Prokaryotic cell, (b) Eukaryotic cell</i> ¹	2
<i>Figure 1.2 (a) Gram-positive, (b) Gram-negative cell wall structure</i> ¹	4
<i>Figure 1.3 Bacterial growth cycle based on viable count and optical density</i> ¹	5
<i>Figure 1.4 3-D imaging with CLSM (copied from http://probes.invitrogen.com)</i>	7
<i>Figure 1.5 Live/Dead staining (copied from http://probes.invitrogen.com)</i>	8
<i>Figure 1.6 Photocatalysis mechanism of TiO₂ particle</i>	10
<i>Figure 1.7 E. coli colonies on selective media as red or magenta</i> ¹	16
<i>Figure 1.8 Disk diffusion method measurement (copied from http://ftp.cdc.gov)</i>	17
<i>Figure 2.1 Spray coating apparatus</i>	20
<i>Figure 3.1 Particle size(μm) vs. volume percentage for TiO₂ suspension with 1g/l concentration.</i>	28
<i>Figure 3.2 SEM image of TFP45, TiO₂ as white particles are seen on filter fibers.</i>	29
<i>Figure 3.3 Disk diffusion method for TiO₂ coated paper (TFP225), No inhibition ring.</i> ..	30
<i>Figure 3.4 Disk diffusion method for GSH, A) 19 mm disk examined At McMaster, B) 10 mm disks results sent from UNB, a & c: BHS. b & d: GHS</i>	31
<i>Figure 3.5 Membrane filter method for blank paper as control.</i>	32
<i>Figure 3.6 Membrane filter method for A) BFP and B) TFP225 with 30 min UV illumination with intensity of 23 W/m²</i>	33
<i>Figure 3.7 Membrane filter method for BFP and TFP225 without UV and with 30 minutes UV illumination with intensity of 23 W/m²</i>	33
<i>Figure 3.8 Membrane filter method for A) BHS and B) GHS.</i>	34

<i>Figure 3.9 Left side of plate: No colonies grow from washed TFP112 with present of UV light for 30 minutes with 23 W/m² intensity, Right side of plate: colonies grow from washed TFP112 without UV light irradiation.</i>	<i>35</i>
<i>Figure 3.10 Washing method: left plate, result for TFP112 with 30 minutes UV illumination with 23 W/m² intensity. Right plate, result for TFP112 without UV illumination.</i>	<i>36</i>
<i>Figure 3.11 Washing method: Bactericidal effect of TiO₂ coated paper with present of UV light for 30 minutes and 23 W/m² intensity.</i>	<i>37</i>
<i>Figure 3.12 Washing method: Effect of TiO₂ content on disinfection with 30 minutes UV illumination time and 23 W/m² intensity.</i>	<i>38</i>
<i>Figure 3.13 Washing method: Effect of UV exposure time on disinfection property of TFP112 with 23 W/m² UV intensity.</i>	<i>39</i>
<i>Figure 3.14 Washing method: Effect of UV intensity on disinfection for TFP112 with 30 minutes UV irradiation time.</i>	<i>40</i>

List of Tables

<i>Table 2.1 Classification of paper samples</i>	<i>21</i>
<i>Table 3.1 Paper sample labeling</i>	<i>27</i>

1 Introduction

Antibacterial effect of photocatalytic TiO_2 coated paper was studied in this work and three methods were proposed to test the disinfection effect of bactericidal paper. Basic information about microbiology was given as background information, and then literatures and standards that have been published on this purpose were reviewed in first chapter. Experimental procedures was performed in second chapter, then results and discussions in third chapter, and finally conclusions and references were presented in forth and fifth chapters respectively.

1.1 Background information: Brief Review of Microbiology

This chapter introduces very brief information about bacteriology such as morphology of the cell, growth and death of bacteria as background knowledge to this work.

1.1.1 Morphology of the cell

There are two structural types of cells as shown in Figure 1.1 described below:

- Eukaryotic cells include Algae, fungi and protozoa which are larger in size and have more complex structure than other cells. Nucleus, mitochondria and chloroplasts are membrane-enclosed structures that are specific internal structure of eukaryotes. Plants and animals are also made of eukaryotic cells.¹
- Prokaryotic cells include Bacteria and Archaea that have simpler structure than eukaryotic cells and lack membrane-enclosed structures.¹

In present work bacteria were used as a model microorganism, therefore properties and specifications of bacteria will be studied in here.

1.1.2 Shape and size of bacteria

Bacteria are small rod-shaped organisms that are not visible by naked eye. They can be observed by using different type of microscopes.² The average diameter of bacteria cell is about $1\text{ }\mu\text{m}$ and average length is 1 to $5\text{ }\mu\text{m}$.¹

1.1.3 Diagram and elements of bacteria

There are many different elements in each bacterium as shown in Figure 1.1 (a), which have different duties described below:

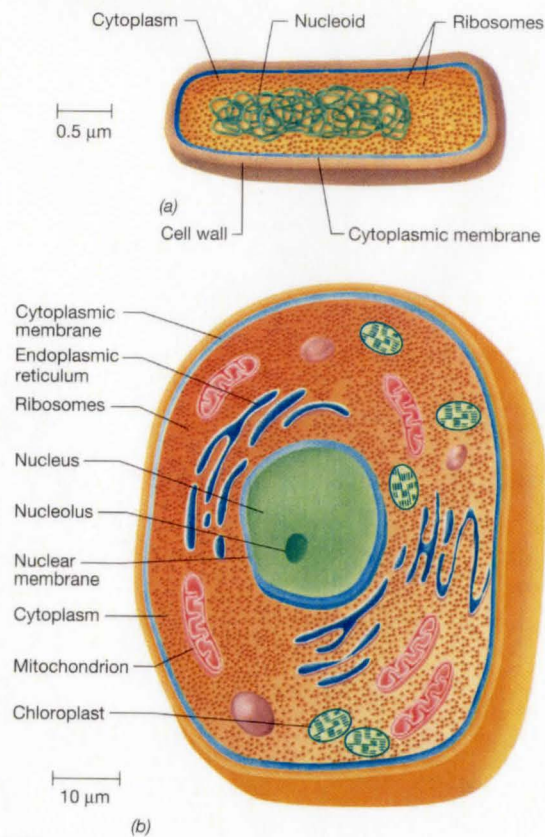


Figure 1.1 Diagram of cell: (a) Prokaryotic cell, (b) Eukaryotic cell¹

1.1.3.1 Cell wall and cell membrane

Nutrients and other substances enter the cell through cell membrane, also waste material and other products exit the cell through same membrane.¹ Cell wall is semi-permeable that controls substances like nutrients and waste product that exchange between the cell and its surroundings.^{2, 3} It is known as the much stronger layer that is placed outside of cell membrane.¹

1.1.3.2 Cytoplasm

Cytoplasm is jellylike material inside the cell that is responsible for absorption and metabolism of the food.³ Cytoplasm consist of water, macromolecules (proteins and nucleic acids) and ribosomes.¹

1.1.3.3 Ribosome

Ribosomes that synthesize proteins of the cell are made of ribonucleic acid (RNA) and proteins. They are small organic molecules within cytoplasm.¹

1.1.3.4 Flagella

Flagella are rod shape tails that attached to the cell wall and to the cytoplasmic membrane of the cell. They are made from proteins and each bacterium may have one or more of them that help bacteria to move.⁴

1.1.3.5 Pili

Pili are external structure of bacteria as thin, tube shape proteins that are involved in the adhesion of bacteria to other cells or surfaces.⁴

1.1.4 Gram-positive, gram-negative

Bacteria are divided to two different categories based on their cell wall structure: gram-positive (e.g. *Bacillus*, *Listeria*, and *Staphylococcus*) and gram-negative (e.g. *E.coli*, *Salmonella*, and *Pseudomonas*). Gram-positive cells have simpler but thicker cell wall and in contrast gram-negative cell walls have more complex multilayered structure as shown in Figure 1.2.¹

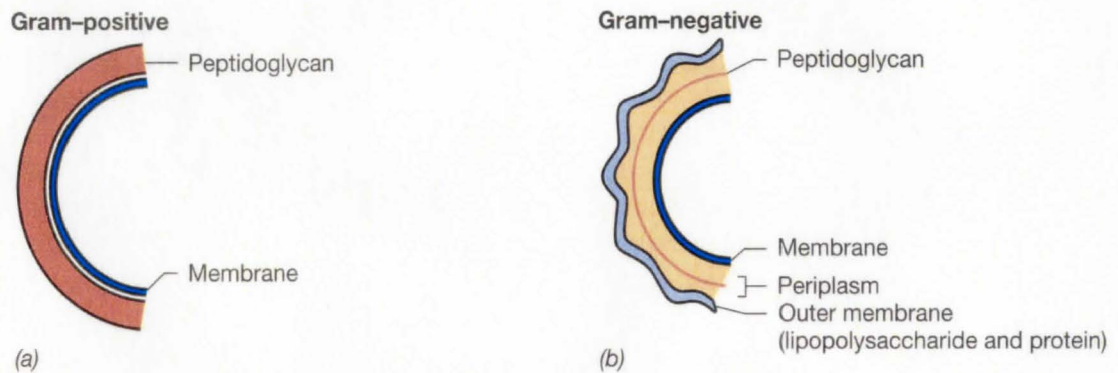


Figure 1.2 (a) Gram-positive, (b) Gram-negative cell wall structure¹

1.1.5 Growth

Bacterial growth involves an increase in the number of cells by a process called binary fission that each cell divides into two new cells. There is a typical growth cycle for bacteria that usually starts with lag phase and then exponential phase followed by stationary phase and finally death phase as shown in Figure 1.3.¹

1.1.6 Environment

There are four major environmental factors that influence bacterial growth: pH, temperature, oxygen, and water availability. Most microorganisms grow at pH values

between 5 and 9 that are common for most natural environments. Very few microorganisms can grow at pH values below 2 and above 10.¹

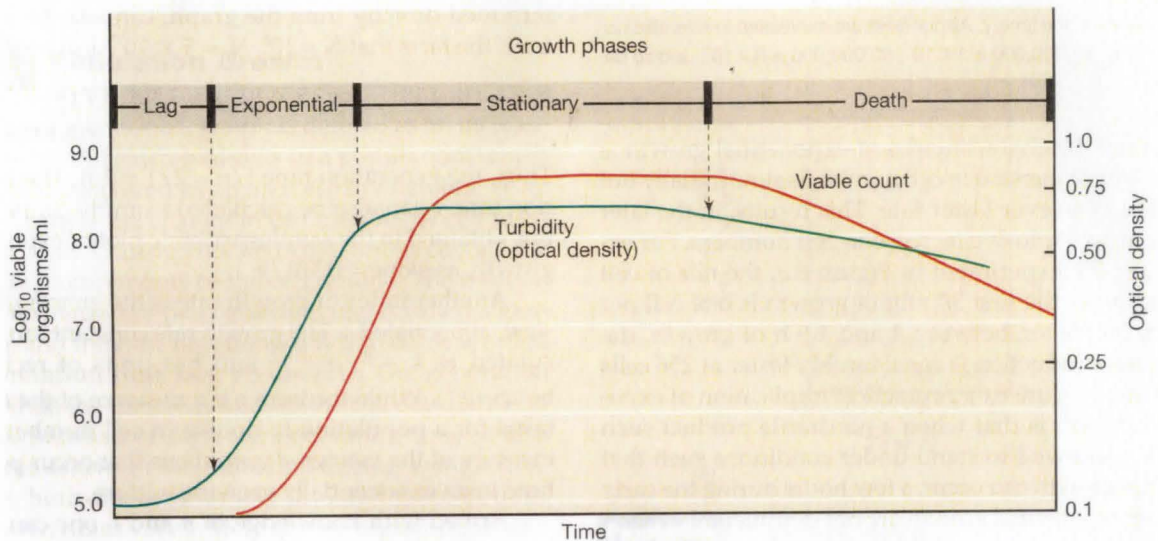


Figure 1.3 Bacterial growth cycle based on viable count and optical density¹

Each organism has different temperatures as minimum, optimum and maximum at which it can grow. For instance, optimum temperature of *E.coli* is 39°C, the minimum is 8°C and the maximum is 48°C.¹

All microorganisms need water for their growth because they absorb materials in solution.¹ Too low osmotic pressure of medium make water to pass through the cell and swelling of the cell will happen, also high osmotic pressure of solution cause shrinkage in cell cytoplasm and it pulls away from the cell wall.³

For oxygen requirement microorganisms can be divided into two main groups: aerobes and anaerobes. Oxygen can also occur in toxic form that may damage the

microorganism. Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$) are the major oxidants that can kill bacteria.¹

1.1.7 Nutrition

The chemical tool for a cell to make monomers of its own macromolecules is called nutrition. Carbon, nitrogen, phosphorous, sulfur, potassium, magnesium, calcium, sodium and iron are the major elements of macromolecules in the cell such as proteins, nucleic acids and others. Some cells also need growth factors that are organic compounds and include vitamins, amino acids, purines and pyrimidines.¹

1.1.8 Culture media

Liquid solution of different nutrients that are suitable for the laboratory growth of microorganisms is called culture media and it can be prepared by mixing certain amount of organic and inorganic chemicals to distilled water.¹

After preparing proper culture media for a specific microorganism then it is time to add organisms to the media; this process is called inoculation. For making a pure culture (culture with single kind of organism) it is needed to use aseptic techniques to prevent contamination with other microorganisms that exist everywhere. Gelling agent is used to solidify the liquid culture media and make solid culture media to immobilize cells and allow them to form visible masses called cell colonies.¹

1.1.9 Direct and indirect Measurements of microbial growth

Total and viable counts are two direct measurements of bacterial growth. Direct microscopic count method either on dry sample in slides or samples in liquid is done by

counting the number of cells per unit area, giving the number of cells per chamber volume and then gives the total number of living and dead cells in a suspension. If we are interested in number of live cells, viable cell counting method can be used that often called plate count or colony count. In this process each viable cell can produce one colony of the cells. Several 10-fold dilutions are usually done to have between 30 to 300 colonies on each plate that is a reasonable number from both statistical and microbiological point of view.¹

Confocal scanning laser microscopy (CSLM) is also a common 3-D imaging tool that is a computerized microscope with laser light source. It can store digital images from different layers of thick specimen and overlay them in a single image as three-dimensional image (Figure 1.4). Cells should be stained with fluorescent dye to be more visible.¹

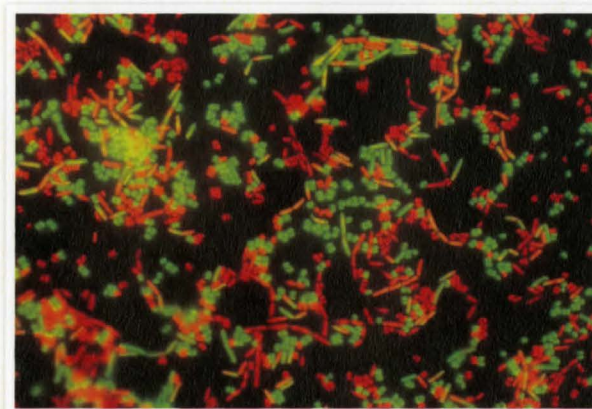


Figure 1.4 3-D imaging with CSLM (copied from <http://probes.invitrogen.com>)

Different staining can be done for various purposes, for instance Live/Dead staining is used to determine number of live or dead cells in a suspension. Live bacteria in green and dead bacteria in red are shown in Figure 1.5.¹

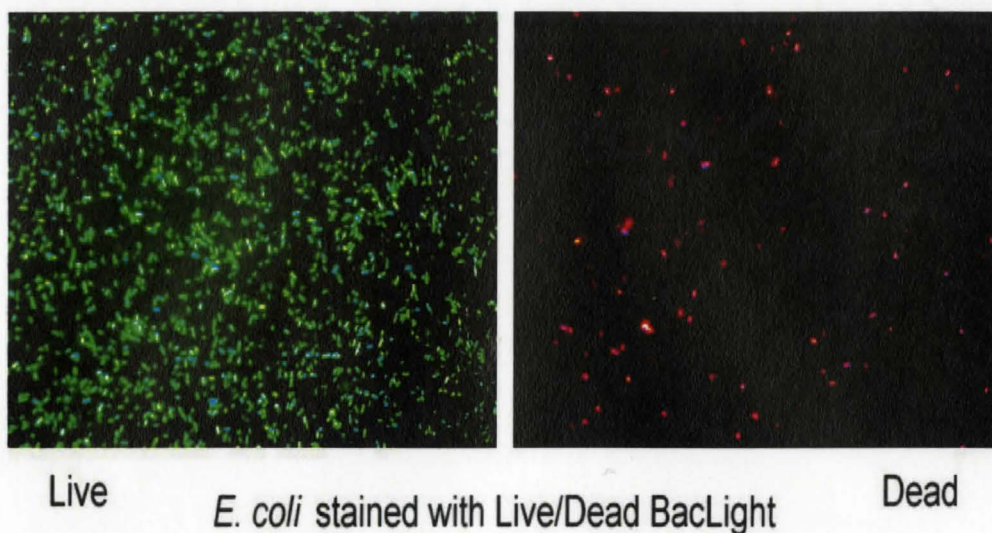


Figure 1.5 Live/Dead staining (copied from <http://probes.invitrogen.com>)

The only indirect method for measurement of bacterial growth is turbidity measurements using spectrophotometer that is very fast and easy. The more turbid the suspension the more number of cells present on it.¹

1.1.10 Bacteria in the air

Air is not considered a natural bacterial environment because there is less nutrients and moisture in the air compared with other natural environments and also presence of sunlight can prevent bacterial growth as well. However, many microorganisms live in the air and cause contamination in food and laboratories or cause infection. Microorganisms that live in the mouth can be carried into the air through coughing or sneezing that called droplet infection.⁵

There are two general methods for killing of bacteria in the air, one is irradiation with UV and the other is using bactericidal chemicals as sprays or aerosols.⁵ The other

suitable method for purification of air is using filtration to remove dust and microorganisms.²

1.1.11 Bacteria in water

There are specific standards for amount of bacteria in potable water. Bacteriological analysis is one of the most suitable analyses for water supply to determine presence of pollution and also prevent epidemics in water supplies.^{2, 5} Using chemicals such as hypochlorite and liquid chlorine, UV, and ozone are the general methods to kill bacteria in water.⁵

1.1.12 The death of bacteria

There are many factors that may kill or remove bacteria or inhibit the growth of bacteria. Two main methods include sterilization and disinfection. Sterilization which is removal and destruction of all living cells are done by physical agents such as drying, heating (e.g. autoclave), light and UV irradiation, or by filtration or by chemical agents. Disinfection is also done by using chemical substances that are toxic such as acids and alkalis, oxidizing agents (e.g. hydrogen peroxide), coagulants which coagulate proteins and inactivate enzymes to kill bacteria (e.g. silver nitrate), the dyes that mostly inhibit the bacterial growth, and surfactants (e.g. soaps).^{1, 3}

1.2 Literature review

In this section first photochemistry of TiO_2 will be introduced and then the history of TiO_2 photocatalyst as an antibacterial agent will be reviewed. Finally various methods and standards that have been used for antibacterial evaluation of bactericidal paper will be discussed.

1.2.1 Photochemistry of TiO_2

The basic concept of TiO_2 photocatalysis is that light with less than 380 nm wavelength has greater energy than the band gap energy of TiO_2 (3.2 eV), this energy can generate photoelectrons (e^-) in the conduction band and holes (h^+) in the valence band. The common fluorescent black light has the maximum output of about 365 nm, which is ideal for TiO_2 photo-activation.⁶ Figure 1.6 shows the mechanism of photocatalytic reaction of TiO_2 .

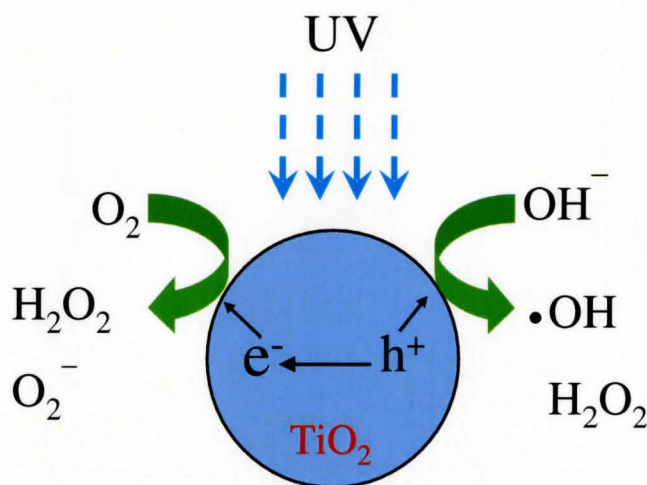
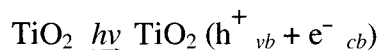
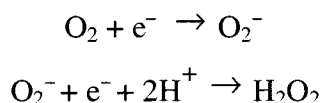


Figure 1.6 Photocatalysis mechanism of TiO_2 particle

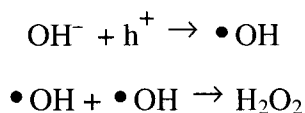
Oxidation and reduction reactions of TiO_2 with UV light are shown in following equations:⁷⁻⁹



Reductive reactions:



Oxidative reactions:



Superoxide (O_2^-) and hydroxyl radicals ($\bullet\text{OH}$) that are formed through photocatalytic reactions can attack organic compounds and decompose them.¹⁰ They can also attack the cell membrane and the cell wall causing disruption and death of the cell.¹¹

1.2.2 History of TiO_2 photocatalyst as an antibacterial agent

Fujishima and Honda were the first scientists who discovered photocatalytic activity of TiO_2 in 1972.¹² Since then researchers have been working in this field and discovered several functions for this chemical such as purification of water and wastewater, deodorization, disinfection, self cleaning, cancer therapy and so on.⁶ One of the important achievements was microbiocidal effect of TiO_2 that was found by Matsunaga et al. in 1985 for the first time.¹³ Then scientists started to use TiO_2 photocatalyst as an antibacterial agent on various substrates such as concrete, tile, glass, paint, fabric, and paper.⁶ For the application of bactericidal TiO_2 on the paper there have been only two publications and few patents.

TiO₂-containing paper have been prepared and tested in lab scale by Matsubara et al. in 1995.¹⁴ TiO₂ photocatalyst was successfully applied on paper in papermaking process. Deodorizing effect of TiO₂-containing paper was investigated by the photocatalytic decomposition of gaseous acetaldehyde. The antifouling function of TiO₂-containing paper was examined by the decomposition of adsorbed cigarette nicotine. Antibacterial effects were confirmed by using *Escherichia coli* on the TiO₂-containing paper and it was observed that *E.coli* was almost sterilized on TiO₂-containing paper in 90 minutes exposure to UV fluorescent light.¹⁵ Daoud et al. also studied bactericidal effect of TiO₂ coated cellulose fibers in 2005.¹⁰

Nakashima et al. in 1998 published a patent about photocatalyst-containing paper. Water soluble polymer (e.g. starch) and coagulant (e.g. ammonium sulfate) were used along with photocatalyst (e.g. TiO₂) to make photocatalyst-containing paper that can eliminate volatile substances and gas.¹⁶ In 2003 Kurogo et al. invented a medical face mask with TiO₂ photocatalyst coating and improved sterilization and deodorization properties of face mask in their work.¹⁷

Pelton et al. have recently reviewed the colloidal property and photochemistry of TiO₂ and also summarized fabrication and applications of photocatalytic paper.⁶ They have studied that to add TiO₂ photocatalyst to paper there were two methods in conventional papermaking. First method was called “wet-end addition” where the TiO₂ was deposited onto individual fibers before the formation of sheet and second method was called “size press treatment” where the dry paper sheet was coated with a photocatalyst by passing through a bath of chemicals.⁶

1.2.3 Methods to evaluate disinfection property of antibacterial paper

In the article that Matsubara et al. published in Japanese, it has not been mentioned that how antibacterial efficacy test of photoactive paper was conducted but it has been concluded that TiO_2 containing paper can sterilize *E.coli* in 90 min UV illumination.^{14, 15}

Daoud et al. examined antibacterial activities of fibers coated with TiO_2 dynamically and statically.¹⁰ Modified procedure of the shake flask method (ASTM E2149-01) was used to assess dynamic antibacterial activities of photocatalytic cellulose fiber. The main concept of this method was placing the known amount of sterile test specimens into the 250 ml flasks. Then the formed inoculum of bacteria in nutrient broth was diluted with PBS (phosphate buffer solution) to final concentration of $1.5 \sim 3 \times 10^5$ CFU/ml and added to the flasks. Then flasks were capped and shaken at 37°C and 250 rpm at certain periods of time. After predetermined time, 1 ml of solution was taken and diluted to 1:5 with sterile water and 0.1 ml of diluted solution was transferred to and spread on agar plates. After incubation of plates at 37°C for 24 hours, the number of viable cells was counted and CFU/ml was calculated.¹⁰ It has not been mentioned in the article that how and when the samples were exposed to UV light but it was understood that it should have been set during shaking period.

With using dynamic method different substrates were examined in bacteria solution introducing various contact time. It was concluded that TiO_2 coating is able to not only kill the bacteria but also protect substrates against the biofilms formation.¹⁰

The static method was suggested by Dauod et al. to assess static antibacterial

activities of photocatalytic cellulose fiber. In order to do so, four disks (5cm ϕ) of specimens were placed in separate jars and capped immediately after adding 1 ml of the bacteria inoculum to each jar. Then 100 ml PBS was added to each jar immediately for zero contact time test and they were shaken for 1 minute. For other contact times jars were incubated at 37°C and then the same procedure was repeated. The resulting solution was diluted to 1:5 ratios and 0.1 ml of it was transferred to and spread in agar plate. After incubation of plates at 37°C for 24 hours, the number of viable cells was counted and CFU/ml was calculated.¹⁰ Again in this method explanation about UV exposure procedure was missing but could be understood that UV was introduced when the jars were placed in incubator for certain contact time. Also it was not clear if the samples were soaked in or wetted with bacteria solution.

The reduction rate of bacteria growth in static method was less than that of dynamic method. It was suggested that shaking method enhances the contact and then reduction rate. In general same results were achieved for static and dynamic methods in case of comparing substrates and contact times.¹⁰

The International Organization for Standardization (ISO) released a standard for microbiological examination of pulp, paper and board (ISO 8784-1) that was basically total count of bacteria based on disintegration.¹⁸ The whole procedure was run under aseptic conditions. Paper samples were mixed in Ringer's solution (containing 2.5 g/L NaCl, 0.105 g/L KCl, 0.12 g/L CaCl₂, 0.05 g/L NaHCO₃) in a disintegrator jar to obtain 1% fiber suspension. The fibers were then disintegrated (in disintegrator or stomacher) until free from the fiber clumps. 10 ml of fiber suspension were transferred to each 150

mm Petri dishes immediately after disintegration. 30 ml of culture media were added to each plate. Plates were moved back and forth and side to side for better dispersion of fiber into the media, and then were rested to solidify. After incubation at 37°C for 48 hours plates were examined for the number of colony-forming units. Then the total colony number per gram dry mass of the sample was calculated.¹⁸

The above mentioned ISO standard and the methods that Daoud et al. have been used were considered in present work and modified to washing method that will be explained in experimental procedure section.

The United States environmental protection agency (EPA), submitted a method to evaluate amount of *E.coli* in water by using membrane filtration and selective culture media (EPA-821-R-02-023).¹⁹ The direct count of *E.coli* colonies was provided by this method. Sample water was passed through a membrane filter and bacteria were captured and retained on the surface of membrane. The membrane was then placed on a differential and selective medium and incubated at 35 °C for 2 hours and then 44°C for 22 hours. Red or magenta colonies were grown on the surface of membrane filter (Figure 1.7) and were counted by illuminated lens or stereoscopic microscope. Number of *E.coli* per 100 ml of water sample was calculated.^{19, 20} The EPA method was also modified in present study as membrane filter method using MacConkey agar that will be explained in experimental procedure chapter.

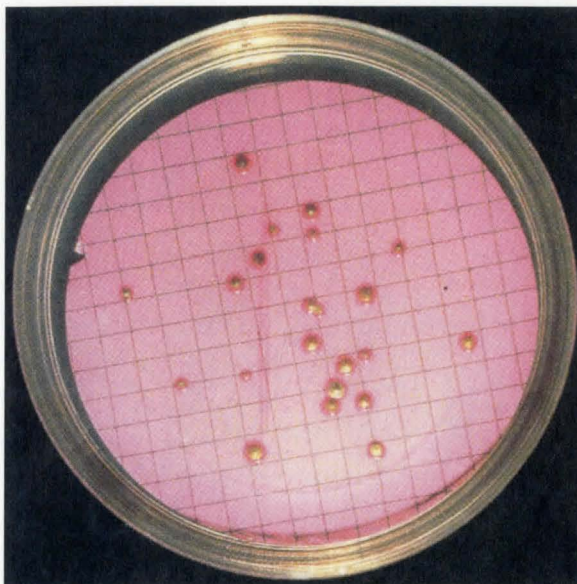


Figure 1.7 E. coli colonies on selective media as red or magenta ¹

The disk diffusion method was developed in 1940s by Heatley for testing antimicrobial susceptibility; introducing paper as an adsorbent to carry antimicrobial solutions.²¹ In 1966 Bauer, Kirby, Sherris, and Turck published their practical method for disk diffusion technique (commonly known as the Kirby-Bauer test)²² and in 1975 this method was modified and accepted as disk diffusion standard by standard National Committee for Clinical Laboratory Standards.²³ Medium inoculated with pure bacterial suspension and paper disks (6 mm ϕ) containing antimicrobial solutions were placed on the surface of bacterial lawn on Petri dishes. Plates were incubated at 37°C for 18 hours and after incubation, inhibition zones were measured as shown in Figure 1.8. The smaller the zone of inhibition, the more resistance bacteria to the antibiotic.²³ This method was also used in present thesis with the same name that will be introduced in experimental procedure part.

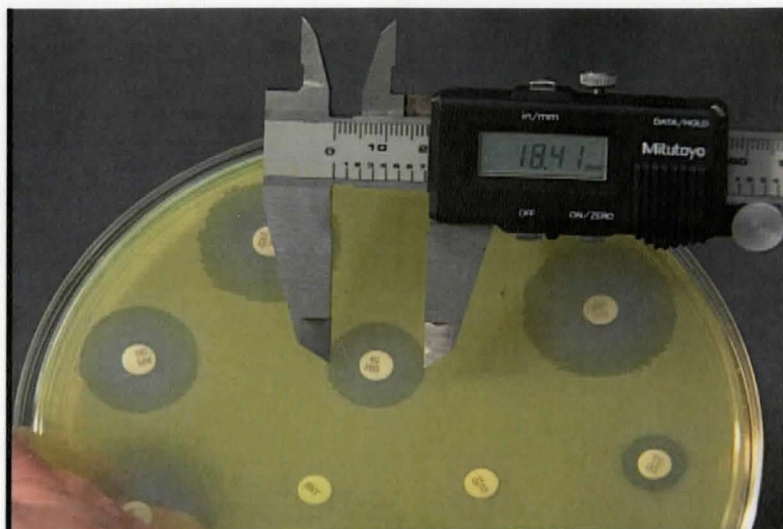


Figure 1.8 Disk diffusion method measurement (copied from <http://ftp.cdc.gov>)

In summary the dynamic method based on shaking flask standard that Daoud et al. used to evaluate antibacterial activity of their samples was taken in aqua environment. For the static method the fiber samples were soaked with bacteria suspension. These two methods can evaluate the disinfection property of antibacterial paper in solution that were modified to test antibacterial efficacy of TiO_2 coated papers when bacteria are deposited onto the paper surface in non-aqua environment. Standard disk diffusion method which was used to test susceptibility of antibiotics was modified in present work to observe diffusion ability and disinfection activity of antibacterial agent. Standard membrane filter method was also used to evaluate number of coliforms in water but it was modified for direct observation of bacteria colonies on the paper surface.

2 Experimental Procedure

2.1 Objective

The purpose of this project was to develop a method to evaluate the disinfection properties of photocatalytic TiO₂ coated paper. Three methods were evaluated - the disk diffusion method, the washing method and the membrane filter method.

2.2 Materials

Whatman filter paper # 4 was used in this study with 150mm diameter. TiO₂ powder P-25 from Degussa was used in all experiments. *Escherichia coli* ATCC 11229 strains were harvested and pre-cultured by Dr. Mansel Griffiths' group in Canadian Research Institute for Food Safety (CRIFS) from University of Guelph. Bacto™ Trypton, BBL™ Agar granulated and Difco™ MacConkey agar all from Becton, Dickinson and company, Yeast extract from EMD chemical Inc. and NaCl from Bioshop were used as received for culture media preparation.

2.3 Preparation and characterization of TiO₂ suspension

TiO₂ suspensions with concentrations of 1 g/l were prepared by dispersing 1g of TiO₂ powder Degussa P-25 into 1 liter of distilled water (MILLIPORE water from MILLI-Q Water System). The suspension was stirred for 10 minutes using magnetic stirrer (VWR 375 Hotplate/Stirrer) and 15 minutes in ultrasonic cleaner bath (BRANSONIC® 3510) to give a uniform dispersion. The TiO₂ suspension was constantly stirred while in use or storage to prevent aggregation and was used within 2 days. The suspension was stored in the dark to prevent any photocatalytic reaction with sunlight.

Important properties of TiO_2 suspension such as pH, particle size distribution and Zeta potential were measured. The pH value of suspension was tested by using a Beckman $\phi 390$ pH meter. pH meter probe was placed in suspension while it was stirred with magnetic stirrer. The pH value was read from the instrument and recorded. Particle size distribution was tested by Mastersizer 2000. TiO_2 suspension was poured on the sample tank and stirred, then machine was run and data were collected from the computer based on user manual. Mobility was tested by a BIC zeta plus Zeta Potential Analyzer. To make a sample solution for this purpose, 1 ml of TiO_2 suspension were diluted with 1 ml of NaCl solution and 18 ml of water in a 20 ml vial. 3 ml of final sample solution was poured to the special sample cell and placed in the machine, the program was run 8 times and data were collected from the computer based on user manual.

2.4 Preparation of paper samples

2.4.1 TiO_2 coated paper: *Spray coating*

A commercial paint spray (Wagner Power Painter PRO, 2400 PSI, Wagner spray tech corp., China) was used on high power to deposit TiO_2 particles onto paper (Figure 2.1). Filter paper was placed on a polished stainless steel plate (160 mm Φ) fixed in a plastic ring (160 mm Φ). Painter was fixed with stands and clamps 15 cm above the paper surface. TiO_2 suspension was sprayed onto the filter paper while stirred for 5 seconds for each sheet of paper and then air-dried over night in control room at 23 ± 0.2 °C and $50 \pm 1\%$ humidity. Spray coating apparatus is shown in Figure 2.1



Figure 2.1 Spray coating apparatus

2.4.2 Other antibacterial paper

GHS antibacterial paper that contains antibacterial guanidine-polymer-grafted starch that is water soluble polymer was prepared in University of New Brunswick in Dr. Xia's group and was used as received. All paper samples were labeled as shown in Table2.1.

2.5 Characterization of TiO_2 on paper

The ash contents were measured for TiO_2 coated paper to quantify the amount of TiO_2 on each paper sheet. Ash content was measured using TAPPI standard procedure.²⁴ About 3 ~ 3.5 g of TiO_2 coated paper was weighed and placed in oven-dried (at 900 °C for 30 ~ 60 minutes) crucibles and heated to 105 °C for 30 minutes to oven-dry then

weighed. This procedure was repeated until the weight of sample was constant to the nearest 0.1 mg. Then moisture content of paper was calculated. Crucibles were then gradually heated to 900 °C to completely combust the paper and recover the ash. Then crucibles were cooled to room temperature and weighed again to calculate ash content percentage ($\text{ash}\% = \text{weigh of ash (g)} / \text{weigh of test specimen (g, moisture free)} \times 100$). Scanning electron microscope pictures were also taken for a visual image of the TiO_2 treated filter paper.

Table 2.1 Classification of paper samples

Paper Sample Name	Description	TiO_2 suspension concentration for spray coating (g/l)	TiO_2 content (mg/m^2)
BFP	Blank Filter Paper, Whatman #4	0	0
TFP45	TiO_2 coated Filter Paper, Whatman #4	0.2	45 ± 10
TFP112	TiO_2 coated Filter Paper, Whatman #4	0.5	112 ± 20
TFP225	TiO_2 coated Filter Paper, Whatman #4	1	225 ± 50
GHS	Guanidine-polymer-grafted starch coated handsheet received from UNB	N/A	N/A
BHS	Blank handsheet received from UNB	N/A	N/A

2.6 Measurement of UV intensity of laboratory UV lamp

A Blak-Ray® XX-15BLB UV Bench Lamp (11.5V ~ 60Hz/0.68 amps) with 365 nm peak emission was used in all experiments to irradiate TiO_2 coated paper with UV.

The UV intensity was measured using a Traceable® Ultra Violet Light Meter (VWR). The distance between UV lamp and samples was about 18 cm and the intensity was varied from 2 to 23 W/m² depending on the place of sample.

2.7 *Luria-Bertani (LB) broth preparation*

A LB broth was prepared in the lab by dissolving 10 g Bacto™ Trypton, 5 g Yeast extract (EMD), and 5 g NaCl (Bioshop) in 1 liter distilled water and mix it with magnetic stirrer in a beaker. The media was poured in 250 ml flasks, 50 ml in each flask. Then flasks were closed and sealed with cotton balls and foil then sterilized in autoclave for 20 minutes at 121 °C and stored for future usage.

2.8 *E.coli culture preparation*

E.coli (ATCC 11229) was taken from the freezer and thawed in room temperature, then 100 µl of it was aseptically cultured in 50 ml of LB broth media using biosafety cabinet (Napflow Napco, class II, Type A/B3) and placed in rotary shaker (Lab-Line, Barnstead lab-line, 4633, USA) at 37°C for 18 hours with 200 rpm speed to reach concentration of 10⁸ colony forming units per milliliter (CFU/ml). The number of CFU/ml in the inoculum was determined and standardized by standard plate count method and turbidity measurement by spectrophotometer. To have 10⁸ CFU/ml bacterial concentration, *E.coli* was grown to optical density of 0.8~1 at 600 nm wavelength. For plate count method the required bacterial concentration was adjusted to 1:10⁶ folds by dilution with mili-Q water. It was diluted in a 10-fold series to 10⁶ and shaken for 10 seconds in mini vortexer (VWR). Then 100µl of the bacterial solution was pipetted and

spread onto the agar plates. The plates were incubated for 18 hours at 37°C (Shel lab incubator, 1575R, Sheldon manufacturing Inc., USA), and then the numbers of colonies were counted and CFU/ml was calculated.

2.9 Disinfection test methods

2.9.1 Disk diffusion method

2.9.1.1 Introduction

The disk diffusion method was developed for testing antimicrobial susceptibility of antibiotics. Medium was inoculated with pure bacterial suspension and paper disks with antimicrobial solutions were placed on the surface of bacterial lawn on Petri dishes. It was incubated for 18 hours at 37°C and after incubation, inhibition zones were measured.²³

2.9.1.2 Procedure

The strain of *E.coli* ATCC 11229 inoculum was prepared in LB broth with 10⁸ CFU/ml cell suspension concentrations as explained in section 2.8. LB agar plates were prepared in the lab (1.5 % agar in LB broth, autoclaved and poured in Petri dishes and solidified). The surface of the agar plate (LB agar Petri dish) was streaked with 100µl of pure *E.coli* suspension (10⁸ CFU/ml) which was spread with sterile plastic spreader. Plate was rotated in 90 degree and spread again to cover the whole agar surface with bacterial lawn. The plate was allowed to air-dry for 5 to 10 minutes. The paper sample was punched to 19 mm discs and placed on top of agar with sterile tweezers and tapped with the tweezers to make sure that the paper contacted the agar surface. For TiO₂ coated

papers, they were illuminated by UV light (Blak-Ray® XX-15BLB UV Bench Lamp, 11.5V ~ 60Hz/0.68 amps) for 30 minutes before incubation. Then plates were incubated for 18-24 hours at 37°C (Shel lab incubator, 1575R, Sheldon manufacturing Inc., USA). The zones of growth inhibition were measured by a ruler after the end of incubation period. This testing was repeated for BFP, TFPs, BHS, and GHS samples.

2.9.2 Membrane filter method using MacConkey agar

2.9.2.1 Introduction

Membrane filter method (MF) is used to determine existence of *E.coli* in water. In this method a water sample is passed through the membrane that captures the bacteria. The membrane containing the bacteria is placed on a selective medium and incubated. Red or magenta colonies grow on the surface of membrane filter that show the number of bacteria on the water sample.¹⁹

Difco™ MacConkey Agar (Becton, Dickinson and company, MD 21152, USA) is one of the selective and differential plating media that is mainly used for isolation and detection of gram negative bacteria such as *E.coli* from various sources such as food and water. *E.coli* grows as pink/red colonies on the surface of agar. The approximate amount of each ingredient per one liter of media is: peptone 17g, proteose peptone 3g, Lactose 10 g, Bile salts No.3 1.5 g, Sodium chloride 5g, agar 13.5 g, neutral red 0.03g, crystal violet 0.001g.

2.9.2.2 Procedure

50 g of the MacConkey agar powder was suspended in 1 liter of purified water. The powder was completely dissolved in water by boiling for 1 minute with heating and

frequent agitation, and then autoclaved at 121°C for 15 minutes. The solution was then cooled to 50 °C and aseptically poured to Petri dishes to solidified using biosafety cabinet (Napflow Napco, class II , Type A/B3).

The strain of *E.coli* ATCC 11229 was aseptically cultured and diluted in Mili-Q water to 1:2X10⁶ fold using biosafety cabinet (Napflow Napco, class II , Type A/B3). 100µl of diluted *E.coli* suspension was pipetted evenly to the paper (Whatman #4, 70 mm diameter, blank or coated with TiO₂). Then they were exposed to UV light (Blak-Ray® XX-15BLB UV Bench Lamp) for 30 minutes then were placed on to MacConkey agar plates and incubated for 24 hours at 37°C (Shel lab incubator, 1575R, Sheldon manufacturing Inc., USA). Plates were examined after incubation period and numbers of colonies were counted and recorded. This method was repeated for BHS and GSH with 19 mm diameter for comparison.

2.9.3 Washing method

2.9.3.1 Procedure

The strain of *E.coli* ATCC 11229 was aseptically cultured in LB broth and diluted in Mili-Q water to 0.2X10⁶ CFU/ml. In order to do so, 20µl of cell suspension was added to 10 ml of Mili-Q water using biosafety cabinet (Napflow Napco, class II , Type A/B3) and mixed in mini vortexer (VWR) for 8 seconds. BFP and TFP112 were cut to 2cm X 2cm squares, wrapped with aluminum foil and autoclaved at 120°C for 20 minutes. The paper samples were placed in the 20 ml glass vials and then 50µl of diluted cell suspension was pipetted onto each paper sample evenly. Paper samples were exposed to UV light (Blak-Ray® XX-15BLB UV Bench Lamp, 11.5V ~ 60Hz/0.68 amps). After UV

illumination, 10 ml Mili-Q water was added to each vial to achieve $1:10^5$ CFU/ml as final dilution. Vials were shaken for 15 minutes in the rotary shaker (Lab-Line, Barnstead lab-line, 4633, USA) at 200 rpm to wash the cells off the paper and suspend them in water. 100 μ l of result water was spread on agar plates and incubated at 37°C for 18 hours in incubator (Shel lab incubator, 1575R, Sheldon manufacturing Inc., USA) and the colony-forming units (CFUs) were then counted. $1:10^5$ CFU/ml dilutions of initial cell suspension was made and 100 μ l of solution was plated for viable count as control.

3 Results and discussions

All experiments were run in duplicates for statistical purposes. Error ranges were given in each test and averaged for plotting. Generally for plate count, the greater the number of viable cells, the more variation was seen in the counting. These variations in number of colonies were intrinsic errors in plate count that was about 10%.

3.1 Paper samples

Different TiO₂ coated paper samples were coated using various TiO₂ suspension concentrations, and the amount of TiO₂ on paper samples were measured by ash content method. Blank handsheets and antibacterial coated handsheets were received from university of New Brunswick to compare with TiO₂ coated papers. All samples were labeled as presented in Table 3.1.

Table 3.1 Paper sample labeling

Paper Sample Name	Description	TiO₂ suspension concentration for spray coating (g/l)	TiO₂ content (mg/m²)
BFP	Blank Filter Paper, Whatman #4	0	0
TFP45	TiO ₂ coated Filter Paper, Whatman #4	0.2	45±10
TFP112	TiO ₂ coated Filter Paper, Whatman #4	0.5	112±20
TFP225	TiO ₂ coated Filter Paper, Whatman #4	1	225±50
GHS	Guanidine-polymer-grafted starch coated handsheet received from UNB	N/A	N/A
BHS	Blank handsheet received from UNB	N/A	N/A

3.2 *TiO₂ suspension properties*

TiO₂ suspension with 1g/l concentration was prepared for characterization experiments by dissolving TiO₂ Degussa P-25 powder in distilled water. Then the suspension was characterized for pH using pH meter, particle size distribution using Mastersizer 2000, and electrophoretic mobility using Zeta Potential Analyzer. The electrophoretic mobility of the suspension was studied at pH 4.64. The mean electrophoretic mobility was measured as $1.4 \text{ m}^2/\text{Vs} \cdot 10^{-8}$ showed that TiO₂ was positively charged in this pH. Particle size distribution result is shown in Figure 3.1 showing that TiO₂ had various particle sizes in suspension from 0.3 μm to 10 μm .

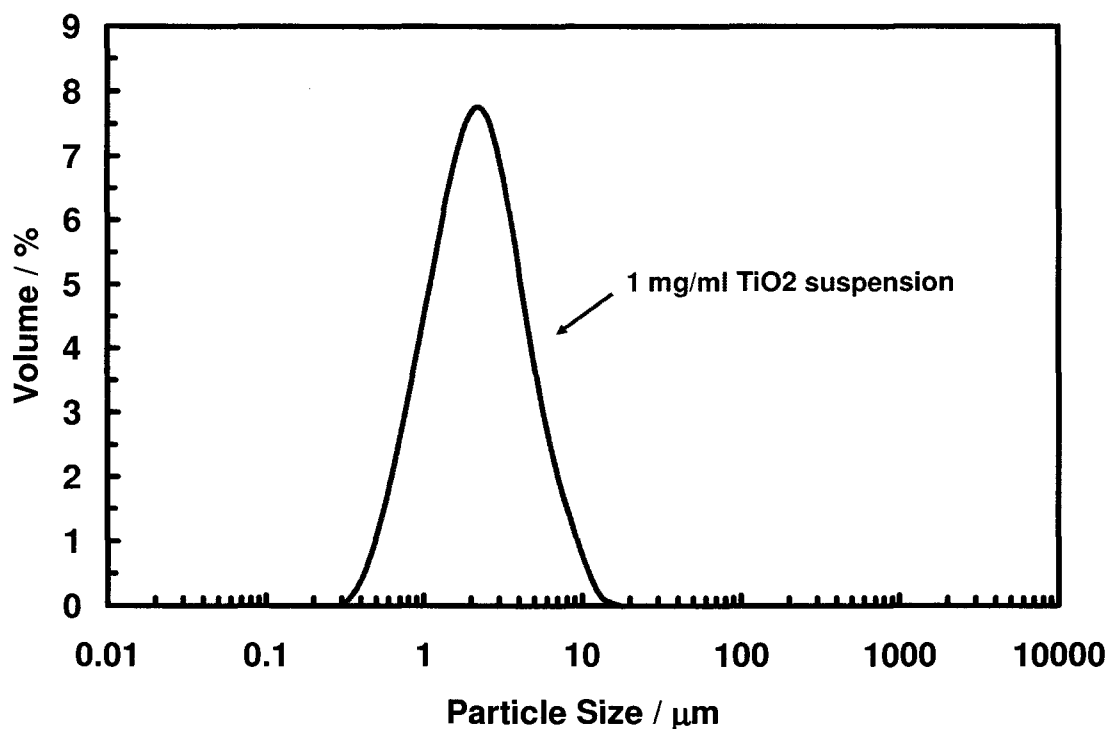


Figure 3.1 Particle size (μm) vs. volume percentage for TiO₂ suspension with 1g/l concentration.

3.3 Characterization of TiO_2 on paper

The Ash content of TFP225 sample was calculated to be 0.17 % which corresponds to a TiO_2 content of $225 \pm 50 \text{ mg/m}^2$. For TFP225 sample as a 19mm disk, TiO_2 content was calculated as 0.06mg and for 70mm disk as 0.86mg. Similarly this experiment was repeated for TFP45 and TFP112. Results are listed in Table 3.1.

Scanning electron microscopy was used to confirm the presence of TiO_2 particles on the paper fibers as shown in following picture (Figure 3.2). White particles on the paper fibers prove the presence of TiO_2 on the paper.

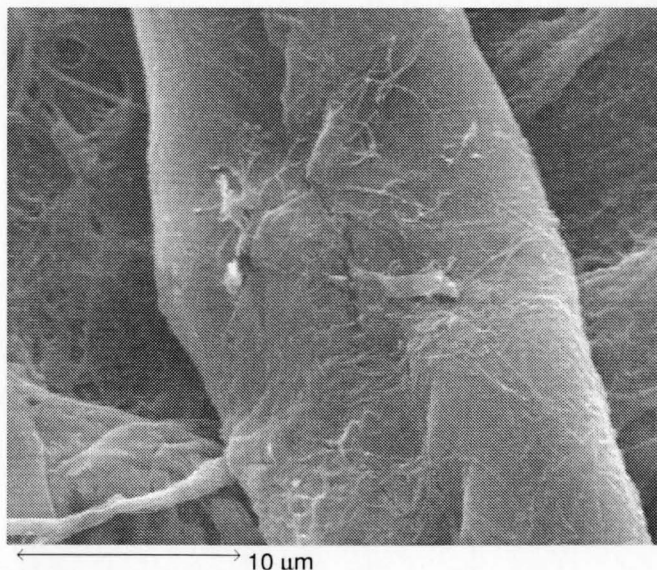


Figure 3.2 SEM image of TFP45, TiO_2 as white particles are seen on filter fibers.

3.4 Disinfection test methods

3.4.1 Disk diffusion method

TFP225 and BFP samples were punched to 19 mm disks. They were placed on agar plates containing *E.coli* ATCC 11229 bacterial lawn. Plates were exposed to UV

light and then incubated for 18 hours at 37°C. They were observed for existence of inhibition ring around the paper disk. The result for disk diffusion method showed no inhibition zone around TiO_2 coated paper on bacterial lawn as shown in Figure 3.3. It was found that the reason for this is because TiO_2 is insoluble in water and unable to diffuse to the media to kill bacteria and create the inhibition ring.

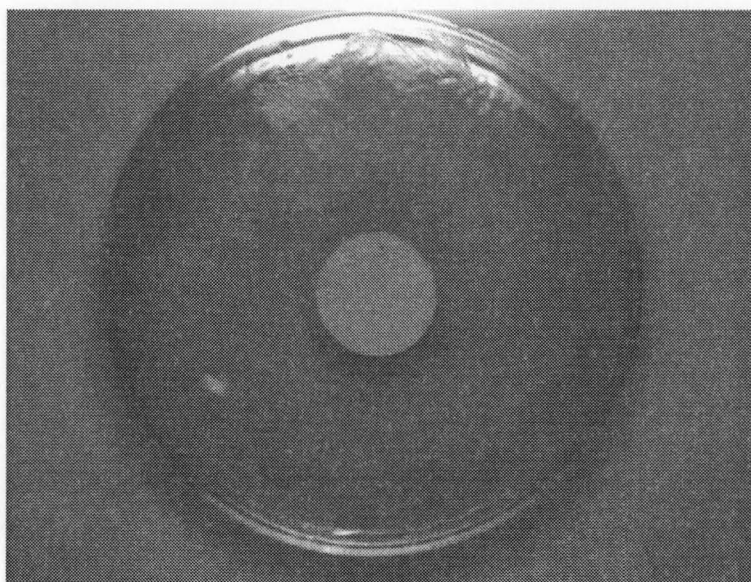


Figure 3.3 Disk diffusion method for TiO_2 coated paper (TFP225), No inhibition ring.

The disk diffusion method was repeated for GHS antibacterial paper that contains antibacterial guanidine-polymer-grafted starch that is water soluble polymer. Figures 3.4 A and B show an inhibition ring around the paper disk proven the ability of GHS for killing bacteria and diffusion of antibacterial agent to the media. All these results confirmed that disk diffusion method can be used for determination of antibacterial activity of water soluble chemicals

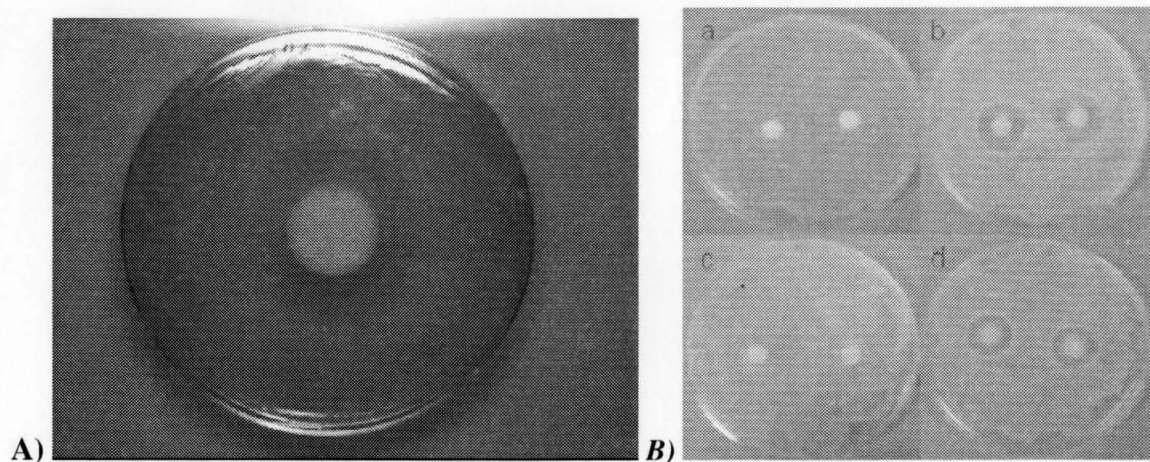


Figure 3.4 Disk diffusion method for GSH, A) 19 mm disk examined At McMaster, B) 10 mm disks results sent from UNB, a & c: BHS. b & d: GHS

3.4.2 Membrane filter method using MacConkey agar

TFP225 and BFP samples were cut to 70 mm disks then sterilized. 100 μ l of *E.coli* ATCC 11229 was pipetted to each disk. Papers were then placed on MacConkey agar plates. TFP225 samples were exposed to UV light prior to placing in agar plates. Cell colonies grew after incubation as pink dots directly on the paper. Figure 3.5 shows the colonies which have been grown on BFP sample. Equal amount of bacteria suspension (100 μ l) were streaked directly on the agar plate and incubated for 18 hours at 37 °C. Bacteria colonies were grown on plate and were counted as control to compare the growth of bacteria on agar (control) to those on paper samples. Growth of cells decreased from 50 ± 10 colonies on agar plate to 42 ± 6 colonies on blank paper. This can be an indication that paper act as a barrier to show bacterial growth.

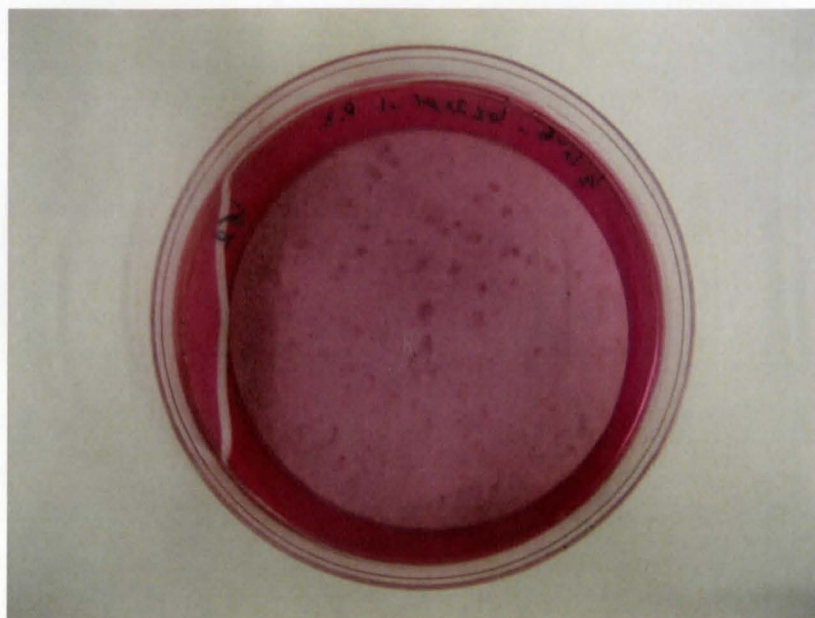


Figure 3.5 Membrane filter method for blank paper as control

BFP sample (70 mm ϕ), as control and TFP225 (70 mm ϕ) sample, each of them containing 100 μ l of *E.coli* ATCC 11229 were exposed to UV light for 30 minutes and incubated for 18 hours at 37 °C. Number of cell colonies decreased in BFP with 30 minutes UV illumination from 42 ± 6 to 11 ± 1 colonies. For TFP225 samples with 30 minutes UV irradiation almost all bacteria were killed. The results are presented in Figure 3.6 and 3.7.

This method was repeated for sterilized GHS and BHS paper samples in 19 mm disks as. Results were achieved qualitatively. Bacteria colonies were grown on BHS (control) and there has been no bacteria growth on GHS as can be seen in Figure 3.8.

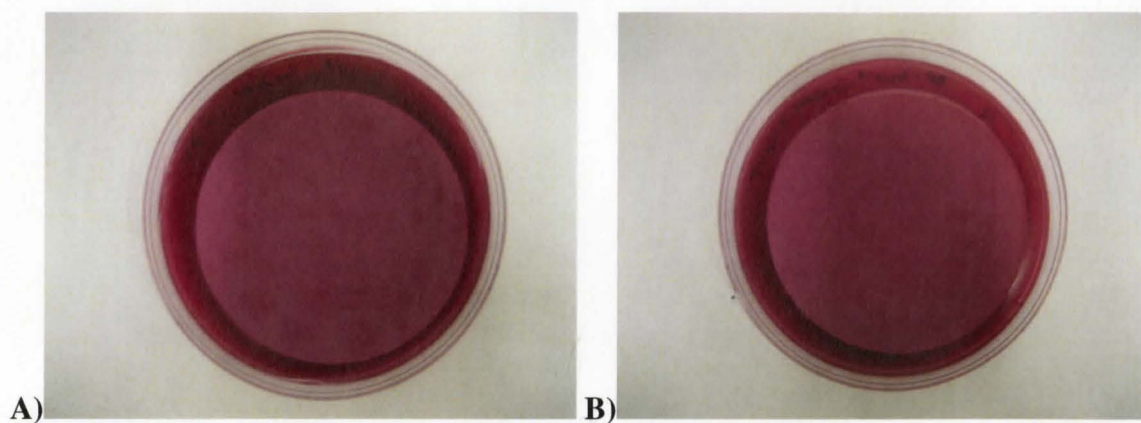


Figure 3.6 Membrane filter method for A) BFP and B) TFP225 with 30 min UV illumination with intensity of 23 W/m²

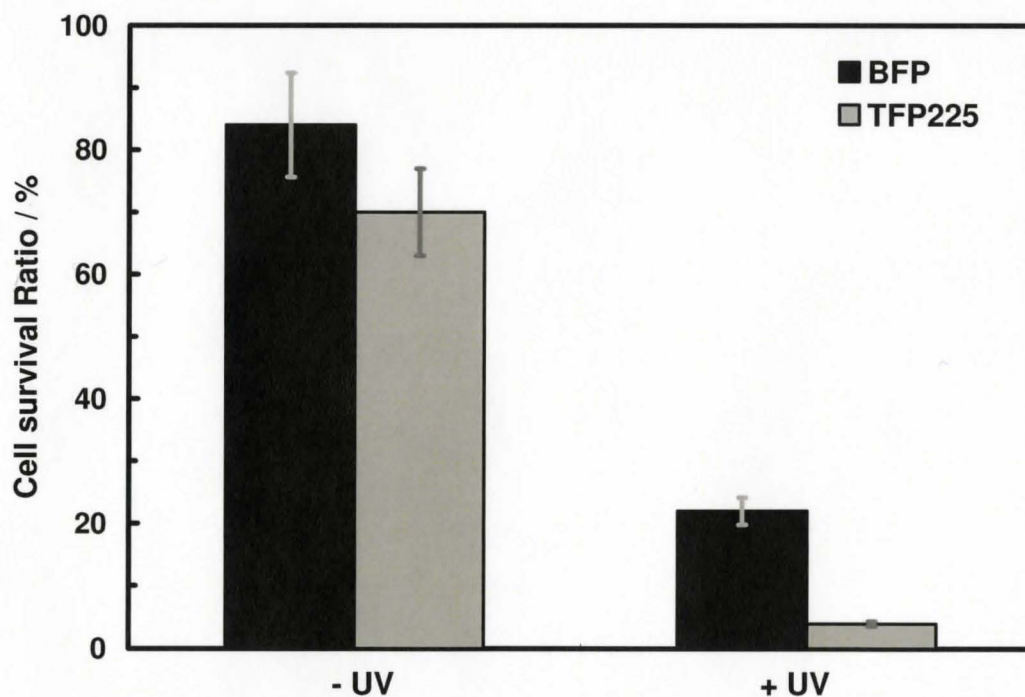


Figure 3.7 Membrane filter method for BFP and TFP225 without UV and with 30 minutes UV illumination with intensity of 23 W/m²

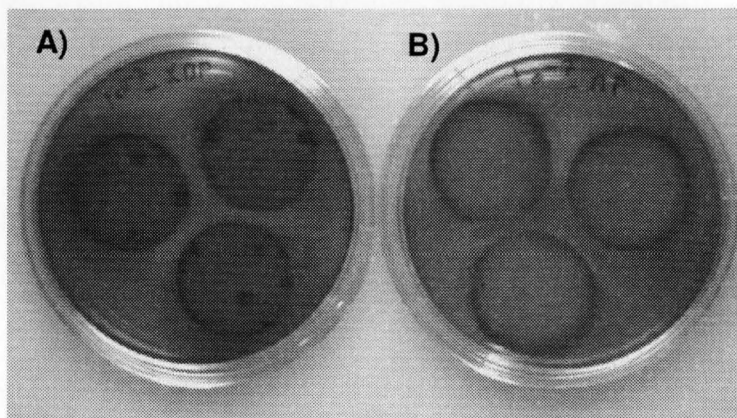


Figure 3.8 Membrane filter method for A) BHS and B) GHS.

This unique method seems quantitatively and qualitatively promising method to observe disinfection property of antibacterial paper directly on the paper surface. This method is less complicated, and involves shorter and easier procedure than other methods and unlike disk diffusion method it is independent to antibacterial agent's water solubility.

3.4.3 Washing method

TFP112 and BFP (for control) samples were cut to 20X20 mm squares and sterilized in autoclave for this experiment. *E.coli* ATCC 11229 was pipetted to the paper samples which were then treated with UV light. Paper samples were washed with sterile water in vials with shaking on shaker and then aliquots were streaked on agar plates. Bacteria colonies were counted after 18 hr incubation at 37 °C. Photographs shown in Figure 3.9 present the result of incubation of bacteria washed off from the paper samples. The left half of the plate was inoculated with aliquots from washed TFP112 containing bacteria in presence of UV light for 30 minutes. Right half of the plate is the inoculation

of aliquots from washed TFP112 containing bacteria without UV light. Left half of the plate shows no growth of cells which means that all bacteria have been killed by TiO_2 and UV light. Right half shows growth of bacteria colonies means that TFP112 without UV light does not have ability to kill bacteria.

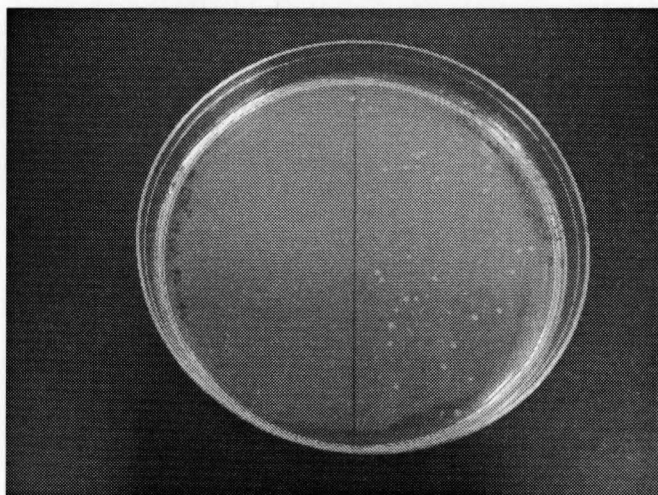


Figure 3.9 Left side of plate: No colonies grow from washed TFP112 with present of UV light for 30 minutes with 23 W/m² intensity, Right side of plate: colonies grow from washed TFP112 without UV light irradiation.

With applying more concentrated bacterial suspension on the paper samples in washing method, more colonies on the agar plates were grown. In Figure 3.10 the plate in the left is inoculation of aliquots from washed TFP112 containing bacteria after 30 minutes UV illumination and the plate in the right is inoculation of aliquots from washed TFP112 containing bacteria without UV illumination. The results show that fewer colonies on the left plate than on the right plate, indicating that bacteria were killed more with TFP112 in presence of UV light than without UV illumination.

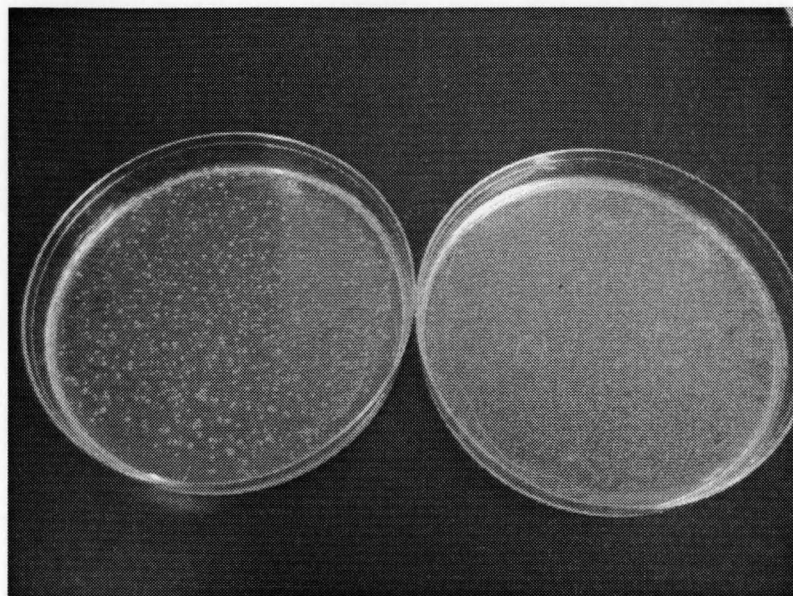


Figure 3.10 Washing method: left plate, result for TFP112 with 30 minutes UV illumination with 23 W/m² intensity. Right plate, result for TFP112 without UV illumination.

In each test 100µl of cell suspension with 1:10⁵ dilutions (as control suspension) was inoculated in agar plates and incubated for viable colony count considering as total number of colonies in initial cell suspension (CFU/ml). The other data were collected from plate counts, and then calculated as proportion to CFUs of control suspension by following equation:

Cell survival ratio = 100 X number of colonies in each plate/total number of colonies in initial cell suspension.

BFP and TFP112 without exposure to UV and also BFP with UV exposure were examined as control. Finally TFP112 was tested with 30 minutes UV irradiation. Results are shown in Figure 3.11. It was shown that for BFP samples almost 80% of the cells can be collected from the paper using washing method. Also with TFP112, the amount of

bacteria that remains on the paper is even more than BFP. After 30 minutes UV irradiation number of cell colonies decreased to 40 % and with effect of TiO_2 and UV, this amount decreased to almost 0%. *E. coli* is almost sterilized by TFP112 after 30 minutes UV irradiation.

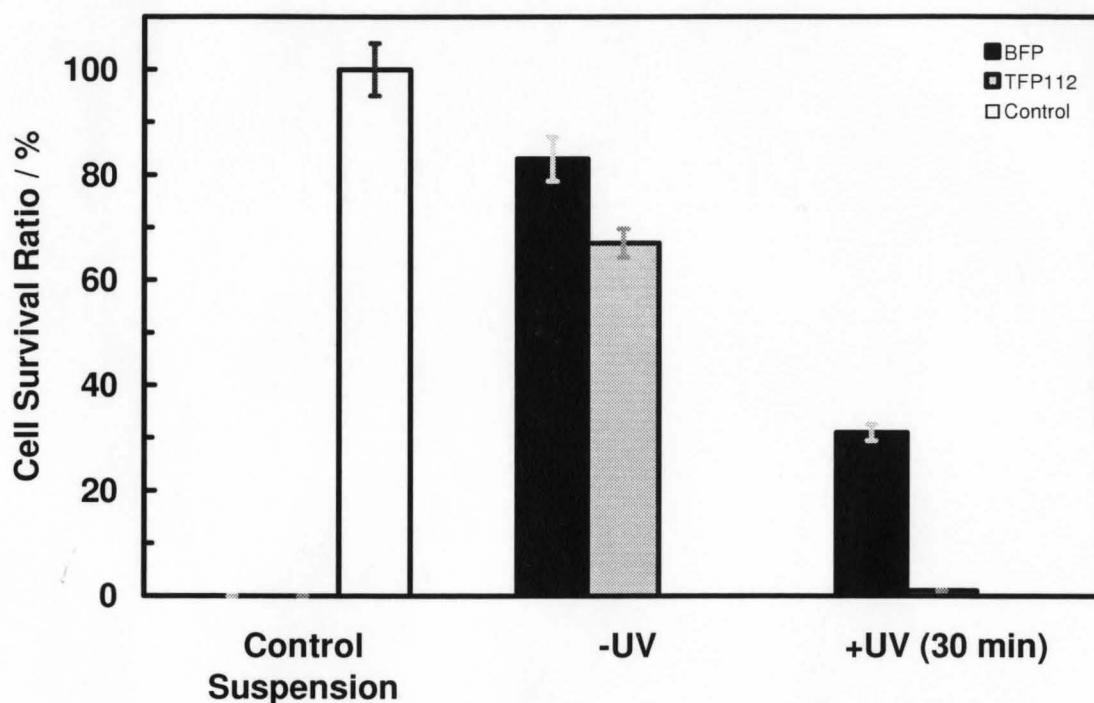


Figure 3.11 Washing method: Bactericidal effect of TiO_2 coated paper with present of UV light for 30 minutes and 23 W/m² intensity.

3.4.3.1 Effect of TiO_2 content on disinfection

Different concentrations of TiO_2 suspension (0.2, 0.5, and 1 g/l) were used in spray coating process and TiO_2 coated papers with different TiO_2 contents were prepared to study the effect of TiO_2 content in disinfection property of antibacterial paper. The amount of TiO_2 was measured by ash content test explained in experimental procedure

section and it was varied from 45 to 225 mg/m². The percentage of survived bacteria decreased from 80 % to 0% by increasing TiO₂ content as shown in Figure 3.12. It was found that with constant bacterial concentration, the more the amount of TiO₂ on the paper, the more hydroxyl radicals were produced, then the more bacteria were killed.

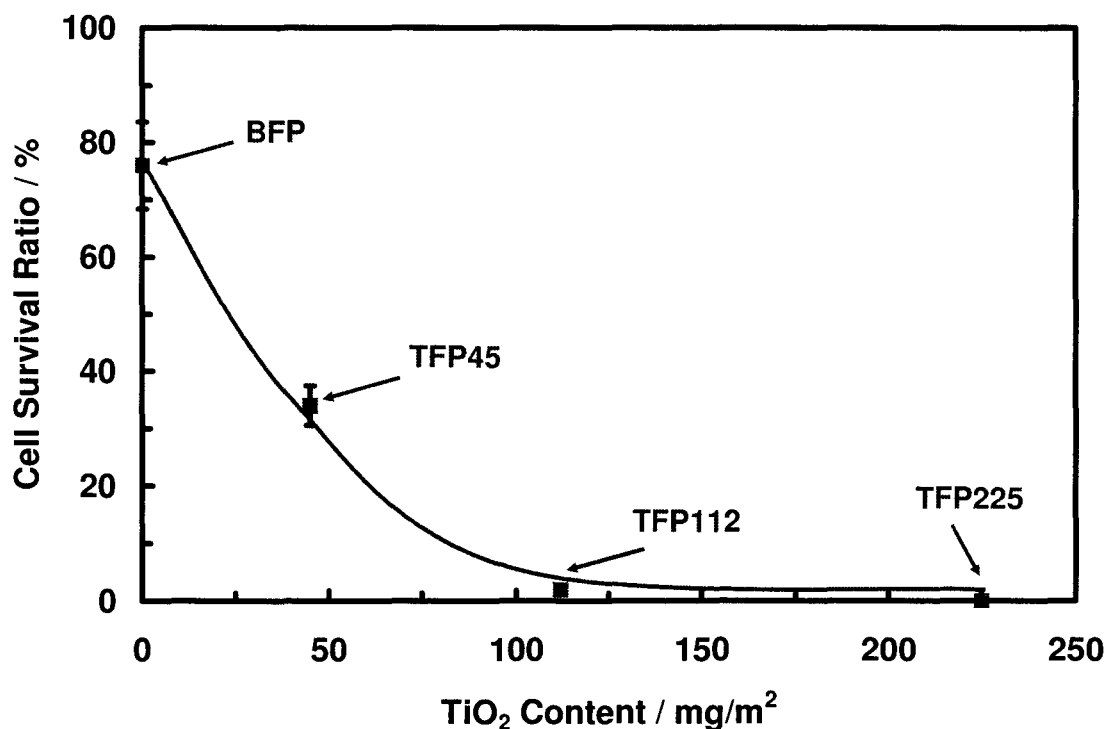


Figure 3.12 Washing method: Effect of TiO₂ content on disinfection with 30 minutes UV illumination time and 23 W/m² intensity.

3.4.3.2 Effect of UV illumination time on disinfection

TiO₂ coated paper(TFP112) was made by spray coating method with 0.5 g/l TiO₂ suspension. Washing method was used with various UV illumination time to determine the effect of UV exposure time on disinfection property of TiO₂ coated paper. By increasing the UV exposure time from 0 to 30 minutes, 10 minutes intervals, number of cell colonies decreased significantly from 70% to 0% as shown in Figure 3.13. This result

showed that the longer UV irradiation, the more hydroxyl groups were produced resulting more bacterial death than less UV exposure time.

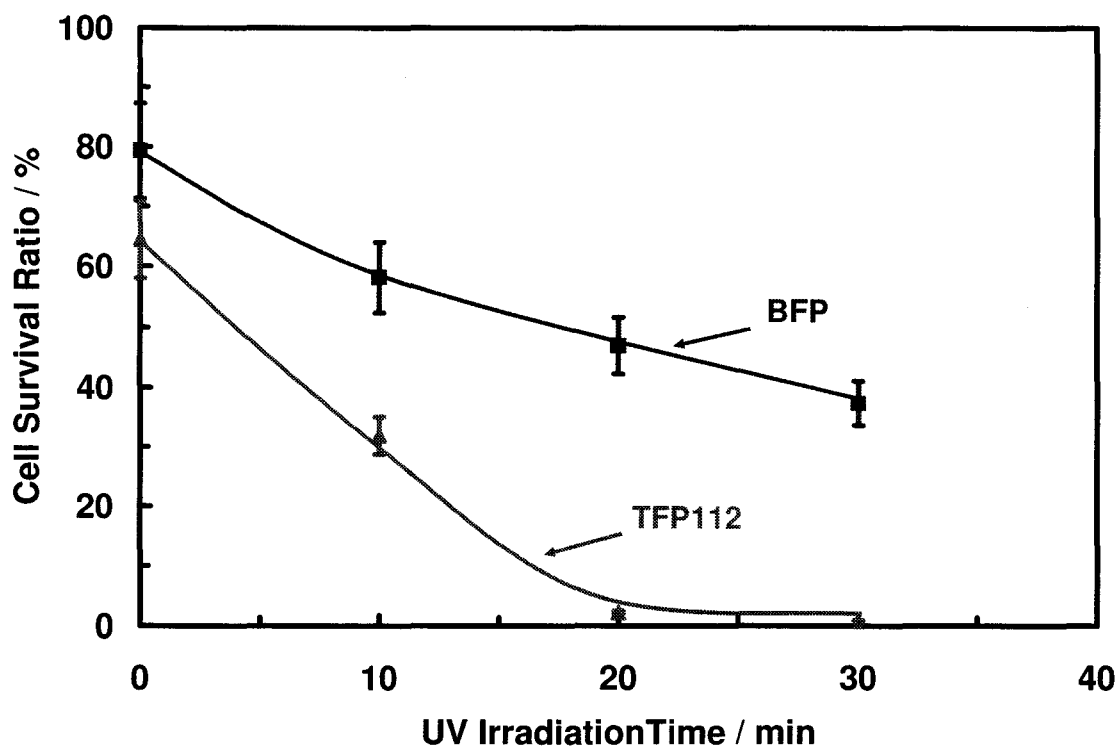


Figure 3.13 Washing method: Effect of UV exposure time on disinfection property of TFP112 with 23 W/m² UV intensity.

3.4.3.3 Effect of UV intensity on disinfection

TiO₂ coated paper (TFP112) was made by spray coating method with 0.5 g/l TiO₂ suspension. Washing method was used with various UV intensities to determine effect of UV intensity on disinfection property of TiO₂ coated paper. By increasing the UV intensity from 0 to 23 W/m², survival ratio of the cells decreased significantly from 70% to 0 % as shown in Figure 3.14. It was found that number of live cells started to decrease sharply after even very low increase in UV intensity. It showed that even with very low

UV intensity such as 5 W/m^2 , only 10% of the bacteria could survive after 30 minutes exposure.

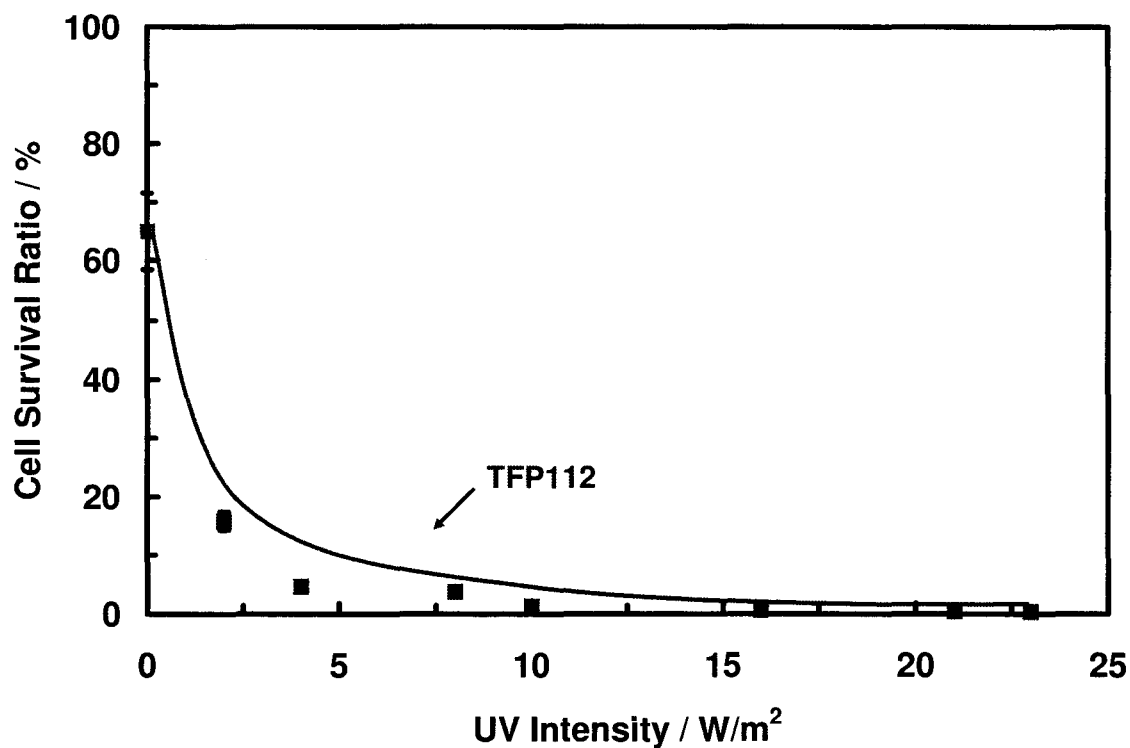


Figure 3.14 Washing method: Effect of UV intensity on disinfection for TFP112 with 30 minutes UV irradiation time.

4 Conclusions

The disk diffusion technique relies on two important functions that affect creation of inhibition zone: antimicrobial activity and mobility. The disk diffusion method is not suitable for the immobilized antimicrobial agents that are not soluble in water such as TiO_2 . In this study it was concluded that TiO_2 particles and subsequently hydroxyl radicals can not migrate from the paper far enough to diffuse into the agar and inhibit the growth of bacteria and create an inhibition zone around the paper disk. But this method can be used for other antibacterial agents that are water soluble and able to diffuse into the agar.

The modified membrane filter method can be used for qualitative and quantitative experiments as colonies are viable and countable directly onto the paper surface. For TiO_2 coated paper it was concluded that number of colonies were decreased after UV illumination. MacConkey agar has been designed for growth of gram-negative bacteria such as *E.coli.*. For gram-positive bacteria or any other kinds of microorganisms an appropriate media must be selected.

The results from washing method agreed with those from membrane filter method showing the decrease in number of bacteria after 30 minutes UV irradiation. Using washing method, numbers of tests were done to evaluate many factors influencing disinfection activity of TiO_2 coated paper, for instance TiO_2 content on the paper, UV irradiation time and UV intensity. It was shown that with increasing TiO_2 content on the paper, antibacterial activity of TiO_2 coated paper increases. Also it was concluded that with increasing the UV exposure time to 30 minutes for TFP112 almost all bacteria were

sterilized. With increasing UV intensity to 23 W/m^2 all bacteria were killed with TFP112 in half an hour.

Finally, it is suggested that washing method and modified membrane filter technique can be used together for testing antibacterial efficacy of TiO_2 coated paper.

5 References

1. Michael T. Madigan, J. M. M., Jack Parker, *Brock Biology of Microorganisms*. 10th ed.; **2003**.
2. Tanner, F. W., *Bacteriology, A textbook of microorganisms*. 4th ed.; **1948**.
3. Henrici, A. T., *The biology of bacteria : an introduction to general microbiology*. 3rd ed.; **1948**.
4. Walter, W. G., McBee, Richard H., Temple, Kenneth L, *Introduction to microbiology*. **1973**.
5. Bryan, A. H., Bryan, Charles A, Bryan, Charles G, *Bacteriology : principles and practice*. 6th ed.; **1968**.
6. Pelton, R.; Geng, X.; Brook, M., Photocatalytic paper from colloidal TiO₂-fact or fantasy. *Advances in Colloid and Interface Science* **2006**, 127, (1), 43-53.
7. Bolton, J. R.; Cater, S. R., Homogeneous photodegradation of pollutants in contaminated water: An introduction. *Aquatic and Surface Photochemistry* **1994**, 467-490.
8. Sunada, K.; Kikuchi, Y.; Hashimoto, K.; Fujishima, A., Bactericidal and Detoxification Effects of TiO₂ Thin Film Photocatalysts. *Environmental Science and Technology* **1998**, 32, (5), 726-728.
9. Hoffmann, M. R.; Martin, S. T.; Choi, W.; Bahnemann, D. W., Environmental Applications of Semiconductor Photocatalysis. *Chemical Reviews (Washington, D. C.)* **1995**, 95, (1), 69-96.
10. Daoud, W. A.; Xin, J. H.; Zhang, Y.-H., Surface functionalization of cellulose fibers with titanium dioxide nanoparticles and their combined bactericidal activities. *Surface Science* **2005**, 599, (1-3), 69-75.
11. Maness, P.-C.; Smolinski, S.; Blake, D. M.; Huang, Z.; Wolfrum, E. J.; Jacoby, W. A., Bactericidal activity of photocatalytic TiO₂ reaction: toward an understanding of its killing mechanism. *Applied and Environmental Microbiology* **1999**, 65, (9), 4094-4098.
12. Fujishima, A.; Honda, K., Electrochemical photolysis of water at a semiconductor electrode. *Nature (London, United Kingdom)* **1972**, 238, (5358), 37-8.

13. Matsunaga, T.; Tomoda, R.; Nakajima, T.; Wake, H., Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiology Letters* **1985**, 29, (1-2), 211-14.
14. Matsubara, H.; Takada, M.; Koyama, S.; Hashimoto, K.; Fujishima, A., Photoactive TiO₂ containing paper: preparation and its photocatalytic activity under weak UV light illumination. *Chemistry Letters* **1995**, (9), 767-8.
15. Matsubara, H.; Takada, M.; Koyama, S., Research on application of photoactive TiO₂ to paper. *Kinshi Kenkyu Kaishi* **1996**, 34, 36-9.
16. Nakashima, Y.; Tomotake, Y. Photocatalyst-containing paper. 97-40074 10226983, 19970207., **1998**.
17. Kurogo, S. Medical face mask with photolysis catalyst coating. 2002-101911 2003250920, 20020228., **2003**.
18. ISO-8784-1, International Organization of Standardization (ISO). Pulp and paper board microbiological examination. Part1: Total count of bacteria, yeast and mould based on disintegration.
19. EPA-method-1603, The United States environmental protection agency. *E.coli* in water by membrane filtration using modified mTEC.
20. ISO-9308-1, International Organization of Standardization (ISO). Water quality, Detection and enumeration of *Echerchia coli* and coliform bacteria-Part 1: membrane filtration method.
21. Heatley, N. G., A method for the assay of penicillin. *Biochemical Journal* **1944**, 38, 61-5.
22. Bauer, A. W., Kirby, W.M.M., Sherris, J.C. & Turck, M, Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* **1966**, 45, 493-6.
23. NCCLS-19:21, National committee for clinical laboratory standards. Performance standards for antimicrobial susceptibility testing, **1999**.
24. TAPPI, T413 om-02, Ash in wood, pulp, paper and paper board: combustion at 900°C, Test method, **2006**.