DEVELOPMENT OF NANOPARTICLE AND NANOTEXTILE BASED DECONTAMINATION SYSTEMS FOR BIOLOGICAL AGENTS

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ABSTRACT

DEVELOPMENT OF NANOPARTICLE AND NANOTEXTILE BASED DECONTAMINATION SYSTEMS FOR BIOLOGICAL AGENTS

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TiO_2 is used from sunscreen to wall paint and it found new application areas with the development of nanotechnology. An important utilization area of TiO_2 is decontamination and filtration. Similarly, the antimicrobial property of silver has been known from ancient times, but the effect mechanism is not fully understood.

The aim of this study was the determination of antimicrobial effects of TiO_2, its doped derivatives and silver loaded zeolite. The photodegradation effect of TiO_2 was investigated.

In this study; powder of TiO_2, TiO_2 coated textile, tungsten doped and tungsten-zinc doped TiO_2 coated textile were prepared. After preparation, antimicrobial properties of materials were investigated by applying the indicator microorganisms, namely *E.coli,* as Gr (-) bacteria, *S.aureus as* Gr (+) bacteria, *B. atrophaeus* as Gr (+) bacterial spores, *E.dermatitidis* as fungi and *A.niger* as fungi spore were used for experiments.

Photocatalytic effect of TiO_2 was better observed on *E.coli* at 2 hours of illumination, especially with tungsten zinc doped textile. The antimicrobial efficiency was as follows: *E.coli* > *S.aureus* > *A.niger* spores > *E.dermatitidis*> *B.atrophaeus* spores.

TiO_2 coated textile surfaces affected the enzymatic activity starting from 1 hour illumination. Same time was needed for observing photodegradation activity.

Antimicrobial effect of silver showed similar result with TiO_2. The effecting concentration of silver increased in following order; *E.coli*< *S.aureus*< *E.dermatitidis* *A.niger* spores< *B.atrophaeus* spores < *S.infantis*.

TiO_2 related part of this work was conducted in the frame of SAN-TEZ Project No: 00860.STZ.2011-1. Nanobiz Nanobiotechnological Systems R&D Ltd. Corporation funded silver loaded zeolite part of the study.

Keywords: Titanium dioxide, silver zeolite, photocatalysis, textile, antimicrobial surface

ÖZ

BİYOLOJİK AJANLAR İÇİN NANOPARTİKÜL VE NANOTEKSTİL TABANLI DEKONTAMİNASYON SİSTEMLERİNİN GELİŞTİRİLMESİ

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Günümüz teknolojisinde güneş kreminden duvar boyalarına kadar pek çok alanda kimysal süreçlerde sıklıkla kullanılan TiO2, nanoteknolojinin gelişmesiyle birlikte yeni kullanım alanları bulmuştur. TiO2 nin faydalanıldığı en önemli alanlar dekontaminasyon ve filtrasyondur. Benzer şekilde eski zamanlardan beri gümüşün antimikrobiyal özelliği bilinmektedir. Ancak etki mekanizması hala tam anlamıyla anlaşılabilmiş değildir.

Bu çalışmanın amacı TiO2, TiO_2 nin katkılı türevlerininve gümüş yüklü zeolitin antimikrobiyal etkinliklerinin belirlenmesidir. Ayrıca TiO_2 nin kumaş üzerinde kendi kendini temizleme özelliği de çalışılmıştır.

Bu çalışmada toz haldeki TiO_2, TiO_2 ile kaplanmış tekstil, tungsten metali ile katkılandırılmış ve tungsten, zinc metalleri bileşimi ile katkılandırılmış TiO_2 kaplı tekstiller hazırlanmıştır. Hazırlanan bu tekstillerin antimikrobiyal özellikleri indikatör mikroorganizmaların uygulanması ile araştırılmıştır. Çalışmada deneyler için Gr (-) bakteri olarak *E.coli,* Gr (+) bakteri olarak *S.aureus* Gr (+) bakteri sporu olarak

B.atrophaeus, fungi hücresi olarak *E.dermatitidis ve* fungi sporu olarak *A.niger* kullanılmıştır.

TiO_2 nin 2 saat ışıma ile, özellikle tungsten ve çinko bileşimi ile katkılandırılmış olan kumaş ile, en iyi *E.coli* üzerinde etki gösterdiği gözlemlenmiştir. TiO_2 nin antimikrobiyal etkinliği şu sırayı takip etmektedir; *E.coli> S.aureus> A.niger* sporu> *E.dermatitidis> B.atrophaeus* sporu.

Ayrıca TiO_2 1 saat ışımadan itibaren enzim aktivitesini etkilemiştir. Aynı sure kendi kendini temizleme özelliğinin gözlemlenmedi için de yeterlidir.

Gümüşün antimikrobiyal etkisi de TiO_2 ile benzer sonuçalr vermiştir. Etki için gereken konsantrasyonlar şu sıra ile artmaktadır.; *E.coli< S.aureus< E.dermatitidis< A.niger* sporu< *B.atrophaeus* sporu <*S.infantis*.

Bu çalışmanın TiO_2 ile sürdürülen kısmı SAN-TEZ No: 00860.STZ.2011-1. Projesi çerçevesinde gerçekleştrilmiştir. Gümüş yüklü zeolite ile sürdürülen kısmına ise Nanobiz Nanobiyoteknolojik Sistemler Ar&Ge Ltd. Şirketi tarafından kaynak sağlanmıştır

Anahtar Sözcükler: Titanyum dioksit, gümüş zeolite fotokataliz, tekstil, antimikrobiyal yüzey

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TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

NOMENCLATURE

Symbols

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ATSDR	Agency for Toxic Substances and Disease Registry
CoA	coenzyme A
C	speed of light, m/s^2
DNA	deoxyribonucleic acid
E	electron charge
eV	electron-volt
E	Photon energy
FDA	US Food and Drug Administration
H_2O_2	hydrogen peroxide
K	Potassium
M	Molarity
N	Negative
O.D.	Optical Density
P	Positive
RNA	ribonucleic acid
TiO_2	Titanium dioxide
UV-Vis	ultraviolet-visible region
UVA	ultraviolet A region
UVB	ultraviolet B region
W	tungsten
Zn	zinc

Greek Letters

λ	Wavelenght

Abbreviations

CVD	chemical vapor deposition
ROS	reactive oxygen species
SOV	surface oxygen vacancies

CHAPTER 1

INTRODUCTION

Due to the rising demand for healthy living, there is an intense curiosity to the materials that have the capacity of killing harmful microorganisms. With these types of materials, it is possible to coat the surfaces of common objects touched by people in daily life, to make them antiseptic and so incapable to transfer bacterial infections. [Tillller *et* at., 2001]. These types of disinfectants have a larger biotic spectrum when they are checked against antibiotics. [Wong *et* al., 2006].

In the microbiological areas such as laboratories and medical, for decreasing the bacterial number and preventing transmission, it is necessary to decontaminate the surfaces routinely and intensively. Common manual disinfection methods, used wiping, are not useful in long term, they are hard to make standardization, and they are needed time and employee [Kühn *et* al., 2003].

Because of the broad use of antibiotics and advent of microorganisms which are more resistant and have virulent strains, there is a rising necessity for new sterilization techniques, as another option.[Wong *et* al.,2006].

Because of the rising effects of infectious diseases and developing resistance of bacteria, researchers have been searching new antimicrobial agents. Nanosized materials started to be considered as new antimicrobial agents because they have high surface area to volume ratio, and they have unique properties in the means of physically and chemically [Rai *et* al., 2009].

From the perspective of energy reduction and protection of natural environment, production of low temperature antimicrobial materials became important recently, because normal materials do not have any antimicrobial specialty and need some changes. A possible alternative can be obtained from light guiding materials. They can be covered with a certain semiconductors and be provoked with indirect ultraviolet A light (320-400 nm) [Lin *et* al., 1998].

The rising apprehension of the public about environmental pollutants has evoked the necessity of new novel treatment methods, so an increasing attention about photo catalysts attained due to its effectiveness as pollutant degradation. [Beydoun., 2000]. The photocatalytic process has some advantages over other types of process such as

biological and classical chemical processes. Firstly, because the photocatalytic process is not characteristic it has a destructive capability in a wide spectrum. Secondly, the reaction is so mighty that it can cause fully mineralization of organic compounds. Thirdly, the photocatalytic process is not susceptible to organic toxicity. Therefore, the process can be appealing for the degradation of recalcitrant and toxic compounds. As another advantage, the process has equal effect on the liquid and gaseous streams. Lastly, there is chance of using sunlight as UV source rather than man made light for the process, which decreases the energy cost. In the heterogeneous photocatalytic systems semiconductor particles are used, these particles are in contact with the medium of the reaction. With exposed to catalyst light exited states are occurred. These states can start subsequent process resembling redox reactions and molecular transformation [Chang *et* al.,2000].

Because semiconductors have convenient electronic structure, light absorption trait, properties of charge transport, exited state lifetimes they are beneficial photocatalysts. In a semiconductor, there is a band gap between the upper side of the filled valence band and the lower side of the vacant conduction band. The electron transportation between these bands can appear only with energy change. Thus, excitation of an electron between bands can occur with absorption of a photon of energy, which is more than the band gap energy. For the photocatalytic processes interceded by semiconductors, the light actuated formation of an electron hole pair is essential [Kondarides *et* al., 2010].

Recently, it has been conducted that semiconductor assisted photocatalyst, because of its high capacity of degradation of recalcitrant chemicals in both gaseous and aqueous systems, also it is a cost effective process. Especially, titanium dioxide is the most interested semiconductor; because of its high photochemical reactivity, low-cost, stability and toxicity [Gouvera *et* al., 2000]. Semiconductor materials have high effect against microorganisms. Even though, most of the research shows the results of semiconductor photocatalytic reaction, there are not many industrial applications. In the research, mostly titanium dioxide powder is used. But due to usage of powder need stirring is examined, applying semiconductor coatings to the materials is developed. As a result, this gave them a self-cleaning specialty [Mills *et* al., 2003].

However, there are some disadvantages of TiO_2 .In visible light, it can not be activated, UV light is necessary for activation because its activation energy is 3.2 eV [Maxwan *et* al., 2011]. Also, its photocatalytic activity is not so high [Trapalis *et* al., 2003].

There are three different crystalline structures of TiO2 that change with the temperature; these are anatase, rutile and brookite. Due to different structures, these have different physical and chemical properties. The most stable ones are anatase and rutile structures. The rutile structure is the most stable; anatase form is more active ones. [Tang *et* al., 1995].

With light, under 400 nm, there happens a giving of electron from valence band to conduction band in a semiconductor photocatalysts. There is a positive potential of valence band, so creation of hydroxyl radicals at the surface is possible, in contrast there is a negative potential of conduction band that can cause the reduction of molecular O_2.

This hydroxyl radical is an oxidizing agent, so it attack the organic materials and causes oxidation [Chang *et* al., 2000].

As different from semiconductors, silver nanoparticles have broad bacterial spectrum and developing of resistance possibility is not high. One of the advantages of silver is that it has almost no side effect. Therefore, using silver in dressing or other medical materials can generate a new perspective of antibiotic resistance [Ip *et* al., 2005].

Recently, silver-based polymers are represented in industrial and academic area. These composites have the specialty of the high temperature processibility coming from thermoplastics and antimicrobial property of silver. The silver-based antimicrobial materials are not toxic for human, they are stable at high temperature and they are long lasting biocides [Kumar & Münestedt, 2005].

In this study, the aim is to improve nano based decontamination systems against microorganisms. These systems become usable in daily life, such as in the textile, door handle, machine etc and they are not toxic for human. The effect of the system should be observed on different types of organisms.

For studying, we choose TiO_2 as semiconductor photocatalyst. In the all photocatalytic semiconductors TiO_2 is predominant due to it is chemically and biologically inert, it has low cost, stable, production is easy, catalyze reactions efficiently and it is non toxic [Maxwan *et* al.,2011].There are some reported incidences of TiO_2 such as photocatalytic water splitting and light induced hydrophilic surface. With the help of hydrophilic property of surface, water can spread across the surface as a result making surface anti-fogging [Popielarski *et* al., 1998].

In this study photocatalytic, semiconductor TiO_2 nanoparticles were prepared with conventional methods. Then, for detection of antimicrobial properties, different microbial cells were used. In addition, enzyme inhibition activity and photodegradation was tested.

Moreover, we studied with silver zeolite as antimicrobial agent. Because silver ions can inhibit metabolic enzymes and have good antimicrobial property, it was chosen. The zeolite was chosen because silver atoms can be changed with the zeolites surface atoms. In aqueous solution, zeolite releases the silver ions [Quintavalla & Licini, 2002].

The silver zeolite was prepared with chemical methods and antimicrobial property was checked on different organisms.

CHAPTER 2

LITERATURE REVIEW

2.1. Antimicrobial Agents

There are found different microbial agents having different usage areas. From old times, the antiseptic properties of aromatic and medicinal plants have been known. For example, plant volatile oils are used as antimicrobial agents. They are obtained from non-woody plants. The antimicrobial properties of these volatile oils are investigated for usage in different areas such as cosmetic, food and pharmaceutical industries [Dorman & Dean, 2000].

From plants, useful antimicrobial photochemical can be obtained. These are simple phenol and phenolic acids, quinones, flavones, flavanoids, flavonols, tannins, terpenoids and essential oils, alkaloids, lectines and polypeptides [Cowan, 1999].

Another example of antimicrobial agent, fatty acids can be considered. Fatty acids have antifungal and bactericidal effects. They generally act as anionic surface, when a surface is anionic; it has low potential for physiological pH value [Kabara *et* al., 1972].

In wine, bacteria and yeasts could not survive because wine has high alcohol concentration and low pH. But sometimes these organisms can spoil wine, so to prevent this, preservatives such as sulfur dioxide, an antimicrobial agent, sorbic acid/potassium sorbate, a fungistatic agent can be added [Dharmadhikari, 2013].

Different from these antimicrobials, for adding antimicrobial specialty in a polymer matrix, there are different ways. Direct incorporation of volatile and non-volatile agents into polymers, adsorption of antimicrobial agents or coating, by using ion or covalent linkages immobilize the antimicrobial to the surfaces [Radheshkumar & Münstedt, 2006].

Recently there is a development of nanosized material. This type of materials can prevent the growth of biological species. There are antimicrobial materials for coating surfaces such as silver, copper, iodine or polypropylene. Also, hybrid type and organic nanosized antimicrobials are investigated. These materials can be used in different areas

such as food packaging, anti-fouling coatings, antimicrobial textiles etc.[Cioffi and Rai, 2012].Polyethylene (PE) and polypropylene (PP) are plastics that used generally in engineering area and for biomedical applications. With proper surface treatments, they can be biocompatible and antimicrobial [Abdou *et* al., 2008].

Metals can change enzyme structure and function, they can accelerate hydrolysis also or nucleophilic displacements [Thurman *et.* al, 2009].

Copper metal is used as antimicrobial material in health care science. By contacting with microorganisms, it can kill them effectively. Nowadays copper and copper alloys were used in touch surfaces like door handles or bed rails [Grass *et* al., 2011]

Silver has high toxicity against microorganisms as in the form of ion or as in the compounds. Its biocidal specialty can affect different species of bacteria.

In ion exchange fibers, dental resin composites and medical device coatings, silver ions are been using as antimicrobial agents [Sondi *et* al., 2010].

2.2. TiO_2 as a Photocatalyst

Powder of TiO_2 has been used for white pigment at old times. Nevertheless, TiO_2 is stable only at the dark. With UV light, it becomes active, and this specialty of TiO_2 has been known since 20^{th} century. For instance, its photobleaching effect on dye was reported at 1938. It was firstly called as photocatalyst by Mashio *et* al. They reported autooxidation of solvent and formation of H_2O_2.

At the first 1970s, the possibility of solar photoelectrolysis has been reported, so it can be understood that water can be split into hydrogen and oxygen under UV light. At late 1970s, researchers studied with TiO_2 for the destruction of the pollutants and at early 1980s, it was demonstrated that the destruction of harmful materials in the water and air was possible. Also at these times study for immobilization of TiO_2 was started [Hashimoto *et* al., 2005]. When TiO_2 powder was used in water or air, separation process and stirring during application was necessary, so film coating of TiO_2 occurred as an option [Lim *et* al., 2003].

When came to 1990s it was questioned that utilization of TiO_2 coated material, so the coated surface be kept clean. After this, the antibacterial effect of TiO_2 was discovered. It was understood that even under weak UV radiation, TiO_2 has antibacterial effect and it is harmless for human [Hashimoto *et* al., 2005].

2.3. Environmental Applications of Semiconductor Photocatalyst

The primary advantageous of heterogeneous photocatalyst against accepted methods is the possibility of mineralizing of toxic substrate completely, although there is

no added reagent. To a photocatalysts become ideal, it must have high reaction rate under light, it must become photostable, chemically and biologically inactive, moreover it is easy to find and cost effective. TiO_2 photocatalysts provides these features [Augugliaro *et* al, 2010].

Processes, which are carried out by semiconductor photocatalyst, are accepted as feasible solutions for environmental processes, especially for purification of water and air [Gamage *et* al., 2010]. Also, it was proven that they can be used against microorganisms and viruses, in the cancer cell inactivation studies, for cleaning oil, for water photosplitting and odor control, for nitrogen fixation, especially TiO_2. [Hoffman *et* al., 1995]

As mention above, in the early researches of semiconductor photocatalysts, it was centered that removal of organic pollutants from water. Yet, recently it was understood that they could remove organic pollutants from gaseous environment, also. Because volatile organic compounds can cause malodorous air, and usage of semiconductor photocatalyst for removal of them make photocatalyst photodeodorisation. Furthermore as other significant applications, they can use titanium for prevention of stains and can be used for disinfection under ambient situations [Serpone *et* al., 1988].

In a worldwide human have been facing with concerns about safe drinking water. Contaminated water in subtropical areas, toxicity of water due to inactivation technology, limited amount of groundwater has been always problems for humanity [Castillo-Ledezma *et* al., 2011].

Recently, researches shows that titanium can be used for gas sensing, photochemical hydrogen production, self-cleaning photocatalysts, photocatalytic reduction of CO_2, biomedical implants, drug delivery [Shankar *et* al,2009]. Also it has TiO_2 can have antifouling [Minabe *et* al., 2000] and antibacterial functions [Kikuchi *et* al., 1997].

Semiconductor photocatalysts are utilized solar energy. Normally, in surface cleaning materials high amount of energy is used for this process, also high amount of chemical releases to air or water occur, and it brings high costs together. For the elimination of these problems, researchers have been trying to find biochemical and analytical solutions and using semiconductor photocatalyst is a solution for destruction or transformation of these dangerous chemicals [Hoffman *et* al.1995].

TiO_2¨have some advantageous when it is used in construction and building materials, such as, superhydrophilicity, self-cleaning. Also in urban area, the pollutants have a compatible characteristic for degradation photocatalytic [Augugliaro *et* al, 2010].

2.4. Semiconductors

There are two energy bands of non-metallic materials, these are filled lower valance band and absolutely empty upper energy conduction band. If there are any electrons are or are not in the conduction band, determines the conductivity of a material. The band gap energy is known as the energy gap between conduction and valance band [Abrams *et* al., 2005].

Semiconductors are mainly light absorbers. Due to they have appropriate electron combination structure, exited –state lifetimes and charge carrying capacity they are used as photocatalyst. With an illumination with an equal or larger energy than band gap energy, the photocatalytic surface excites the electrons in the valance band to the conduction band. It causes the occurring of a positive hole in the valance band and an electron in the conduction band, as shown in Figure 2.1 [Thiruvenkatachari *et* al.,2008].

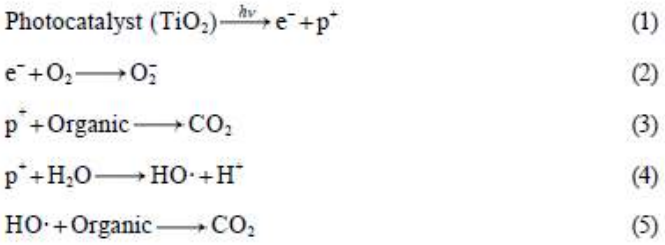


Figure 2.1 The principle mechanism of a semiconductor photocatalytic reaction

In semiconductors, the band gap is not so broad, so they do not have high conductivity. With the help of impurities, conductivity of the semiconductor can be improved. Above the temperature of absolute zero the conduction band is not empty, there are electrons. When the impurities are added into the semiconductors, it creates holes by donating extra electrons into the lattice or removing electrons from lattice. As another way, it can be said that impurity atoms creates new energy states in the structure of semiconductor. While impurities donating electrons, an extra level near to the conduction band appears, that donates electrons to the conducted band. As a result, by rising the charge carrier density, it increases also conductivity. When impurities accept electrons, near to the valance band a new level is created by the holes. Therefore, semiconductor absorb generally UV spectrum, the conduction band is filled with excited electrons [Fujishima *et* al., 2000].

There are two types of semiconductor in the means of electrical conduction. While electrons are the main carriers in n-type such as TiO_2, and while holes are the main carriers in p-type.

As mention before, holes are added to the semiconductors with the addition of impurity. When this donor gives extra electron it will be got by n-type semiconductor, when the donor absorbs the free electrons, it will be got by p-type. As a result of both cases this donor gives to the semiconductor extra current carriers [Walters *et* al., 2000]. In Figure 2.2, the structures of n type and p type semiconductors can be seen.

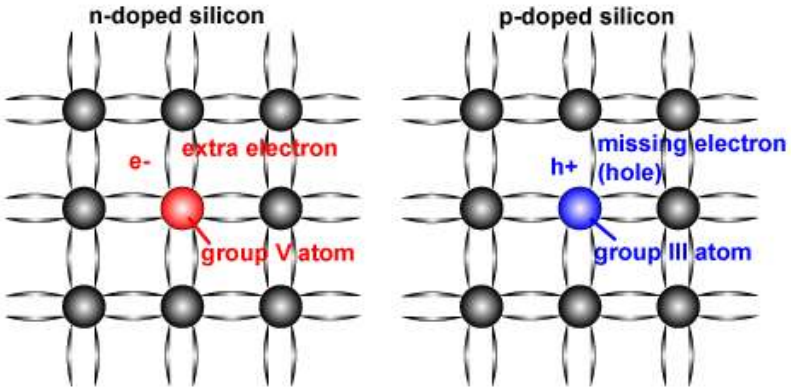


Figure 2.2 n-type and p-type silicon semiconductors [URL 1]

2.5. Optical Absorption Edge Energy of the Nanoparticles

By using its absorbance spectrum in the UV-Visible range, it can be understood that the magnitude of the band gap energy of the semiconductor. Absorbed light in the infrared region means lower energy level and it can give data about vibrations of lattice, on the other hand in the UV-Vis range means valance and conduction bands' energy and it gives information about crystallite size, surface electronic properties and oxidation states. UV-Vis range is important because when the crystallite size of the material become close to nanometer range, it can turn into semiconductor.

Some microscale sized metals can display semiconductor properties due to high amount electrons at the surface, it is a thermodynamically favored process. This behavior creates maintainable semiconductor properties and gives opportunity to create high technology products [Marzan *et* al., 2003].

Terminology and the cluster size of the material are important for characterization of the surface electronic structure of the semiconductor. In insulation materials, there is a huge gap between the valence and conduction bands known as forbidden band gap. In micro scale sized materials, this band is defined as the energy that is necessary for removing electrons from valence band to conduction band. On the contrast for nano scale materials this gap can be defined as the minimum photon energy which is necessary for

excitation of an electron from highest occupied molecular orbital to the lowest occupied molecular orbital. With the change of the crystallite size of the semiconductor, it should be detected the band gap for characterization of the features. The UV-Vis spectroscopy may be used for measuring the optical absorption edge energy or band gap [Kato *et* al., 1995].

2.6. Semiconductor Photocatalysts

Recently there is a rising interest to the semiconductor photocatalyst especially due to the reaction of the semiconductor photosensitizes are thermodynamically feasible [Mills *et* al., 2002]. They present useful way for water and air purification and self-cleaning. The photocatalytic reaction of semiconductors is stimulation of the photoreaction with a catalyst [Mills *et* al. 1993]. Generally, this reaction is the oxidation of an organic compound with oxygen. This reaction can be shown as follows; in Figure 2.3.

$$\text{Organic} + O_2 \xrightarrow[\text{semiconductor}]{h\nu \geq \text{bandgap energy}} CO_2 + H_2O + \text{mineral acids}$$

Figure 2.3 The principle of reaction mechanism of semiconductor photocatalyst

If there are hetero atoms in the main organic compound mineral acids are formed [Mills *et* al., 2002].

There are factors that affect photocatalytic activity. These are nanomaterials size, form such as particle and film, dopant species [Liqiang *et* al., 2006], semiconductors crystal structure, surface area and hydroxyl group density and pores at the surface. All these have effects on the occurrence of electron hole pairs at the surface, so adsorption and desorption redox processes [Minabe *et* al., 2000].

2.7. Precursors

Precursors are necessary for the production of TiO_2 containing materials such as films or nanopowders. Different types of precursors can be used for reaction.

Alcohols can produce metal salts which are known as alkoxide M $(OR)_x$. M is metal and R is alkyl group. In the reaction, alkoxide reagents hydrolysis and condensate

as a result metal hydroxide forms [Shwartz, 1997]. These precursors control the hydrolysis and condensation reaction during process. [Shwartz *et* al., 2004].

Hydrolysis and condensation rates could be controlled with the help of organic chelating agent. For example, acetyl acetone, diols and alkanolamines. They give stability to the reaction. [Nolan *et* al., 2009].

Mixed ligand precursor can be used for chemical deposition. For instance, titanium isopropoxide was used as precursor for the TiO_2 nanostructure preparation [Lakshmi *et* al., 1997]. Also tin tetrachloride pentahydrate can be used for conversion of a better precursor. Below, in Figure 2.4, reaction mechanism of titanium isopropoxide is shown during sol gel method [Sonowane *et* al, 2002].

$$Ti(OR)_4 + 4\ H_2O \rightarrow Ti(OH)_4 + 4ROH \uparrow$$
$$Ti(OH)_4 \rightarrow TiO_2 + 2H_2O \uparrow$$

Figure 2.4 Reaction mechanism of titanium isopropoxide in the sol gel procedure

2.8. Doped TiO2

Although TiO_2 is a good photocatalyst by itself, its efficiency is limited because of photoinduced electron hole pairs are occurred during the process rapidly. Some studies conducted to develop photocatalytic activity of TiO_2, advise the deposition of TiO_2 with noble metals. These metals can capture the electron-hole pairs, causes reduction of these pairs during process and so rise the absorption capability of TiO_2 under the UV-vis. But some of the noble metals are expensive, so using them reset the cost effectiveness of using TiO_2. As a result, researchers have been trying to find more practical elements [Xin *et* al., 2005].

When it is considered that TiO_2 is only activated by UV, but solar light contains 5% UV light and TiO_2 has low quantum efficiency for improving the catalytic performance of TiO_2 different dopants can be used [Li *et* al., 2006].

There are researches that using metal ions for doping quantum sized TiO_2. As a result they showed that doping with different ions such as Fe^{3+}, Ru^{3+}, Mo^{5+}, V^{4+} and Re^{5+},rise the photoreactivity, however doping with Co^{3+} and Al^3 decrease the photoreactivity. Doping of TiO_2 is a complex function due to importance of dopant concentration, the dopants energy level in TiO_2 lattice, the distribution, electron donor concentration, electronic configuration and light intensity [Yu *et* al., 2002].

2.8.1. Tungsten Doped TiO2

Doping TiO_2 with WO3, bad gap energy 2.8 eV, increase the photoreactivity. There are different ways for doping TiO_2 with WO_3. It can be by using wetness, ultrasonic nebulization and after flame hydrolysis technique, conducting multi-step grafting procedure with using ethanol as precursor or with sol gel method. By using sol gel methods researchers proved that 3% (M) doping of WO_3 into TiO_2 is provided the highest activity in the destruction of methylene blue. Also doped TiO_2 showed much hydrophilic surface after irradiation with ultra band gap light. It decreases the contact angle between water droplets and the surface. With this specialty doped TiO_2 materials can be used as commercial as self-cleaning window [Rampaul *et* al., 2003]

Similarly there are other researches about tungsten doped TiO_2. It was claimed that WO_3 can enhance the photocatalytic activity of TiO_2 in different ways. It can prevent the electron hole pair recombination, it can alter the light absorption band from UVA to UV-Vis, and it increases the acidity of the surface by absorbing more hydroxyl group, also. [Tobaldi *et* al., 2013].

2.8.2. Nitrogen Doped TiO2

It is a powerful way to use N dopant for modification of TiO_2. It extends the adsorption light from UV to visible area by narrowing the band with substitution of the lattice oxygen with nitrogen. Generally, this type of dope is prepared by NH_3 treatment of TiO_2 from the atmosphere at so high temperature. But it causes low surface area due to agglomeration and break down of pore structure. Recently new ways for doping with N is trying to find [Li *et* al., 2006].

2.8.3. Zinc Doped TiO2

Another modification method for improving TiO_2 is using Zn or ZnO. It boosts the photocatalytic and photoelectric performance of TiO_2, also it can increase photoinduced charges' separation rate. Recently there are few studies mention the effect of zinc on the surface oxygen vacancies (SOVs). SOVs can attribute the creation of the surface photovoltage spectrum sub-band response and it is suggested that SOVs have an important role in the photovoltage and photocatalytic reaction [Tobaldi *et* al., 2013]

In another research it was shown that Zn doped TiO_2 has greater photoactivity than pure TiO_2 because zinc ions on the surface facilitate the charge separation. Also they provide larger surface area and pore size, it can be helpful for the degradation reaction at a time that reaction with organic molecule [Xu *et* al., 2005].

2.9. Process for Preparing Solution and Chemical Routes

For the coating solution, precursors are used. Depending on the compounds reactivity and the conditions the chemical interaction occurs, between the starting reagents.

There are different ways for fabrication of TiO_2 films and nanoparticles. First one is sol-gel method, it uses alkoxide as precursor and contained hydrolysis and condensation reaction. The second one is metal organic decomposition, it uses carboxyl ate as precursor and it do not contain any condensation reaction. Lastly there is hybrid route for the process, it has few condensation reactions and it is used generally for the processes which has more than one precursors [Djaoued *et* al., 2001].

2.10. Production Techniques

There are some common ways for the production of TiO_2 particle and films; these are reactive sputtering technique, chemical vapor deposition (CVD) and sol-gel method [Sunada *et* al., 1998].

Reactive sputtering technique can be used for high packing density and strong adhesion. It has low cost. Yet, it is a very non-linear process, so deposition rate can change with an increase in the flow rate of oxygen gas in the system [Ohno *et* al., 2003].

Chemical vapor deposition is used generally in the literature for synthesizing functional film For the particle formation, there is an interaction between the reactions on the heterogeneous surface and the reactions in the homogenous gas phase, this interaction affects the growth rate and the structure of the films [Seifried *et* al., 2000].

Sol-gel process is an essential method using to develop the functional coatings. Because with the different advantages with the sol-gel method it is possible to produce films which have wanted features. This method is also known as wet process due to formation of the solution is occurred in an organic solvent. [Brinker *et* al., 1990].

Sol-gel method is a general process and different types of precursors can be used for the process. The main idea of the process is using inorganic salts or alkoxides. However alkoxides are not dissolved in water mostly, so primary hydrolysis can be necessary [Legrand-Buscema *et* al., 2002]

There are three approaches to prepare sol-gel monolith. The first one is the gelation of colloidal powders, the second one is hydrolysis and alkoxide precursor polycondensation with a drying process and the last one is hydrolysis and polycondensation of alkoxide precursors followed by drying under ambient atmospheres [Hench *et* al., 1990].

Generally, there are highly reactive alkoxide titanium precursors in the method, so they are hydrolyzed and condensed quickly. However, this can cause the precipitation of amorphous particles. To deal with these types of problems water is used as hydrolysis

agent and some methods such as addition of ionic liquids and organic additives can be used [Choi *et* al., 2006].

2.11. Nanoparticle Formation

By using methods above powders or films, congaing solutions are produced, and then the formation of the product come to think. Some conditions are necessary for the formation. Substrates should be wetted by the solution, solution should stay as stable through aging and it should have a leaning toward crystalinization.

For wetting the substrate, the contact angle is important. The contact angle between the substrate and the solution should not be higher than 90˚. For adjusting the wetting controlling the tension of the surface is most suitable way, so using of having low surface tension materials as precursor is a good choice [Yusuf *et* al.2001].

2.12. Contact Angle

The interfacial tensions' relation determines the wetting of a solid. The ratio of these tensions is important for the contact angle between the surface of the solid and the water droplet. If this angle is 0˚, the solid will be completely wetted and the surface energy is high, if this angle is 180˚, the solid will not be wetted and the energy of the surface is low.

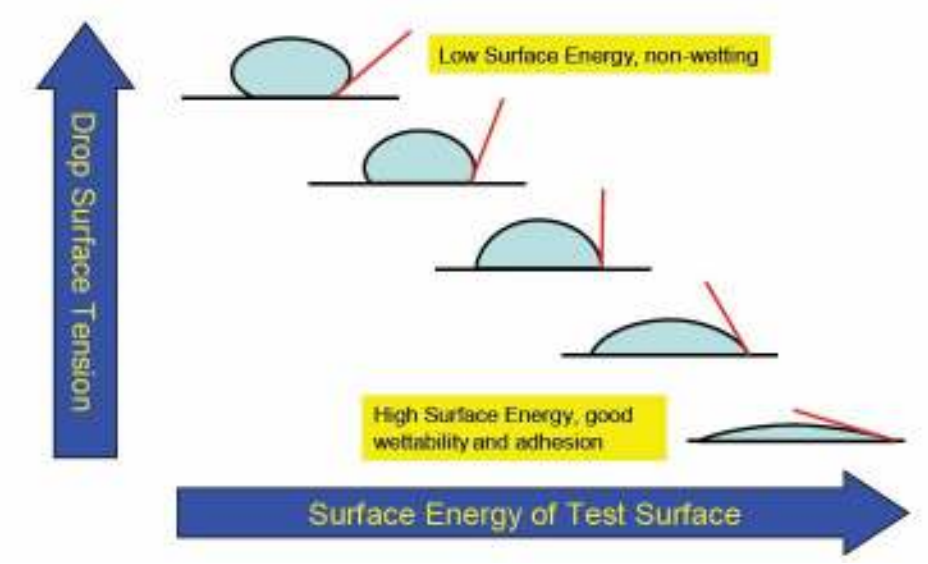


Figure 2.5 Relationship between the contact angle and wetting [URL 2]

There are some researches show that the contact angle goes up and is restored in dark places. It can be observed that the contact angle increases slowly and stays low for a long period in dark places because it is not radiated constantly with a UV source. [Machida *et* al., 1999].

Another research which supports this suggestion claims that UV illumination creates a highly hydrophilic and oleophilic surface. The wettability changes independent of the photocatalytic activity of different TiO_2 particles such as anatase or rutile forms. Moreover, with a few days storages in a dark place the high amphiphilicity of the particles surface is maintained [Wang *et* al.,1998].

2.13. Hydrophilicity

Hydrophilicity is a specialty of a material corresponds to the affinity of water. This interaction between material and the water occurs due to the surface chemistry of the material. So it can be said that hydrophilicity is depend on the absence or presence of certain chemical groups, this is OH^- groups, act as absorption site for water molecules [Bäcklund *et* al., 1991]

When it is considered that the photoinduced hydrophilic conversion of TiO_2 samples show different crystallinization, it was understood that, the structure of crystal is dependent to the photoinduced hydrophilic conversion [Watanabe *et* al., 1999].

2.14. Hydrophobicity

Hydrophobicity is known as the tendency of nonpolar species to accumulate in water solution, as a result decrease the hydrocarbon-water interfacial area [Breslaw, 1991]. Hydrophobic materials have not high surface tension and active groups on the surface. So they do not form hydrogen bond with water [Erkan,MS Thesis, 2005].

Due to contact angle of hydrophobic surfaces greater than 90^o, they do not have good wettability. With the increase in the degree of contact angle, the adhesion ability decreases [Benedix *et* al., 2000].

There are researches show that hydrophobicity can be amplified by surface roughness. When hydrophobicity is enhanced greatly, it creates superhydrophobicity. This has some application area. It can create an option for surface tension generated drop motion in microfluidic devices [Patankar, 2004].

It is claimed that TiO_2 photocatalysts, was made on teflon sheets with porous by using ion assisted deposition method, have super hydrophobic surfaces as coating materials. With UV light, organic pollutants are degraded as photocatalytically, that are weaken the water repelling specialty of the surface. TiO_2 loaded surface can help to keep super-hydrophobic properties of teflon surface [Yamashita *et* al., 2003].

2.15. Coating Process

There are some benefits of coating textile with inorganic sol. Firstly 50 nm particle diameter sol based metal oxides make well adherent transparent layers. Moreover, they are stable against light, heat chemical and microbial attack and they can develop the mechanical properties of the textile. They can help embedded functional additives to carry. Also, it is easy to produce them at room temperature and normal pressure [Mahltig *et* al., 2005].

There are different techniques for deposition TiO_2 solution. The most common finishing techniques are padding and coating for textile material. Padding is the contacting material with TiO_2 consisting material, generally by immersing and squeezing it. Coating method is based on knife coater. This method is the continuous spreads of TiO_2 containing solution on the textile [Dong *et* al., 2007].

Dip coating is a useful way for depositing sol-gel produced particle or films on a surface. Batch dip coating process has different process these are immersion, start up, deposition, drainage, evaporation. During deposition process there are different forces and these have effect on the thickness of the TiO_2 product. These forces are the surface tension gradient, gravity, viscous drag upward on the liquid by the moving substrate, inertial force of boundary liquid layer, pressure of disjoining or conjoining.

Spin coating technique can be used for the application of a thin uniform film to flat surface. It has a large usage area such as color television screens and magnetic storage discs [Lawrence, 1988]. The basic mechanisms of spin coatings are fluid mechanics, solvent transport and film formation [Bornside *et* al., 1989].

At the first step of this technique, coating fluid is deposited on the substrate. It can be done by using nozzle, spraying etc. It is important wetting the surface of the substrate fully. At the second step, acceleration of substrate reach final. Because the fluid should be thin enough for co-rotating with wafer, so there will be no differences in the fluid thickness. As a result of this step, at the third step the thickness of the fluid start to be thinning. At the final wafer, around the rim, there could be little bead of coating thickness differences due to surface tension, rotation rate and viscosity. At the other stage solvent evaporation starts. Due to solvents removes the viscosity of the solution and freezes the coating, coating starts to turn into gel. After this point, some post treatments can be needed such as heating [Erkan, MS Thesis, 2005].

At the last part of the coating, there is a drying step for vaporization of alcohol, combustion and carbonization of organic compound [Colgan *et* al., 2004].

After final drying and heating for removing the free particles, the sample was washed with water under sonication [Bozzi *et* al., 2005].

2.16. Mechanism of Photocatalytic Process

When TiO_2 is irradiated with UV light, it creates reactive species. These reactive species play role in the mineralization of organic compounds. These events start with absorption of a photon and creation of electron-hole pairs. These electron-hole pairs can take a role in redox reaction or recombine. The reaction mechanism of these events is shown in Figure 2.7. High amount of photon-generated electron-hole pairs can recombine with dissipation of heat. Some electron-hole pairs can go to surface and can react with adsorbed electron donors and acceptors. TiO_2 particles can trap these electrons, so reduce the recombination rate. To become degradable for an organic compound, it must be adsorbed on the surface of the photocatalyst [Krishna *et* al., 2005].

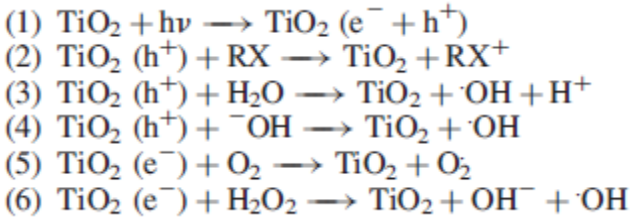


Figure 2.6 Reaction mechanism of UV light irradiated TiO_2

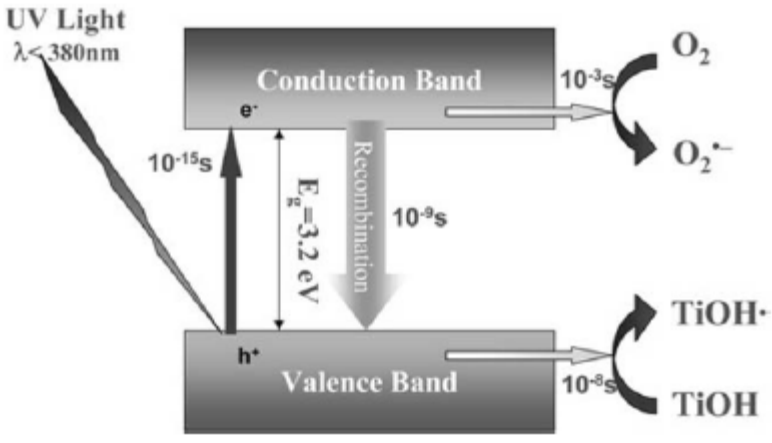


Figure 2.7 Steps in photoelectrochemical mechanism [Krishna *et* al.,2005]

In another way the mechanism can be explained like this. With coming UV light, the catalyst is excited and electron pairs or holes are created. These electrons give reaction with oxygen and superoxide radical anions are generated as products. Also the generated holes give reaction with water and hydroxyl radicals are produced. With these two radicals and species adsorbed on the titanium dioxide surface, redox reaction occurs [Augugliaro *et* al, 2010].

2.17. Mechanism of Microbial Inactivation

When TiO_2 is irradiated, while there is a direct contact with microorganism, the oxidative mechanism affects firstly microbial surface. There are polyunsaturated phospholipids in the cell membrane of bacteria, these are very prone to ROS attack, produced from irradiation of TiO_2 [Augugliaro *et* al, 2010].

It was stated that because of photocatalytic activity, oxidation of intracellular coenzyme A was observed, which brought together cell death with decrease in respiratory reaction. Firstly, the cell wall and membrane differences were not considered this report. However, this inactivation process was proportional to the complexity and the thickness of the cell wall. The photocatalytic inactivation time can be decreased with an increase in reactive species [Krishna *et* al., 2005]. The difference of sensitivity according to biological systems was ordered as virus, bacterial cell, bacterial spore [Huang *et* al., 2000].

In another study, it was claimed that because of cell membrane disruption causes leakage intracellular K^+ ion, cell death occurs. They showed that treatment with TiO_2 broke the cell wall of *Streptococcus sobrinus* at first 60 minutes and cell disruption occurred after 120 minutes [Saito *et* al., 1992].

There are other reports about the effects of TiO_2 photocatalytic activity. It was found that endotoxin was degraded by this activity and this caused membrane damage [Sunada *et* al.,1998], disruption of normal activities of cell associated with cell membrane due to lipid peroxidation [Maness *et* al., 1999], decomposition of the cell wall and membrane concluded with leakage caused cell death [Lu *et* al.,2003].

The usage of TiO_2 for inactivation of marine bacteria was stated. The result of this study creates the idea of using TiO_2 to prevent bacterial cell accumulation and biofilm formation [Lim *et* al., 2003].

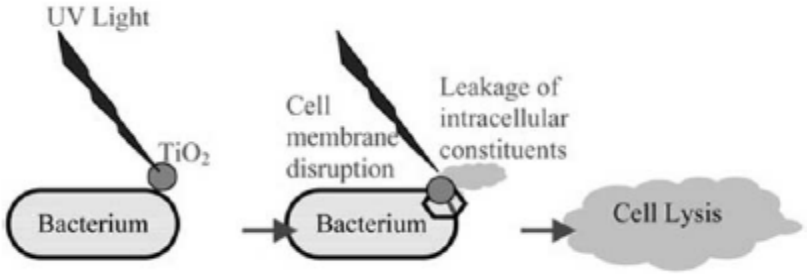


Figure 2.8 Photocatalytic killing of bacterium [Krishna *et* al., 2005]

It is suggested that there is a potential of inactivation of bacteria over whole area by using light guidance. With water and oxygen, reactive OH-radicals are created by TiO_2 and UV-A [Kühn *et* al., 2003].

By using transparent TiO_2 film with illumination, it was shown that there was a decrease in the number of *E.coli* even bacteria was separated from surface [Kikuchi *et* al., 1997].

2.18. Increasing the Photocatalytic Activity of Semiconductor

The photocatalytic activity depends on the excited electrons. These electrons move, so they can initiate reactions, also they are not stable and they can recombine fast. For a semiconductor the photocatalytic effect depends on the ratio between the surface charge carrier transfer rate and electron recombination rate. If electron-hole recombination rate is high, no time is left for other reactions. For decreasing, the recombination rate and extends the light absorption of titania some transition metal cations can be doped into titania. Pores on the surface of titania inhibit the mobility of electron. When dopants are added, these pores are filled so the mobility of electron rise, as a result surface conduction increases, this means the increasing of photocatalytic activity [Shah *et* al., 2002].

2.19. Silver as an Antimicrobial Agent

Silver have been using as biocide since ancient times, but still its mechanism could not be understood fully. It is claimed that silver has different mechanisms against different organisms. One of them is interaction between silver and amino acid, silver and DNA, creation of reactive oxygen species and cell membrane damage [Cioffi *et* al., 2012].

Silver can interact between thiol groups of proteins. This cytotoxic effect inhibits the biological functions of organisms. For example in respiration, it is necessary t transfer of electrons. However, silver causes cell membrane damage and membrane become permeable to protons, so the gradient is lost. With loss of gradient, respiration is accelerated, trying to expel more protons for restoration of losing gradient. During this process superoxides or hydroxyl radicals are created, these are toxic for organisms [Holt et al., 2005].

It was stated that silver ions could inhibit the phosphate ion intake and stimulate the phosphate ion efflux. Also, it was observed that the accumulation of glutamine, proline and mannitol [Rosenberg *et* al., 1974].

In another study, it was observed that silver treated bacteria cells have large electron light region in DNA. Also, there were granules near these regions. It was thought that these granules were occurred because of cell self defense mechanism. When this mechanism could not protect DNA effectively, because of high number of silver ions the cell filled with these granules [Feng *et* al., 2000].

It is thought that metals can cause the generation of ROS in solution with dissolved oxygen ions [Stohs *et* al, 1995]. When this situation is considered, silver nanomaterials not ions can kill bacteria. It was proven that antimicrobial property of silver can be observed with the presence of oxygen [Yoon *et* al., 2008]. In addition, silver particles can create excess ROS [Park *et* al., 2006].

Another mechanism of silver is direct damage to cell membrane. It was observed that silver nanoparticles could penetrate into membrane [Feng *et* al, 2000]. This mechanism is still not known fully. However, it was thought that this situation can be observed with the electrostatic attraction of positive charged silver ions and negative charged bacterial cell surface [Raffi *et* al., 2008]. Another hypothesis is that a process starts with the interaction of silver and thiol groups in the cell surface [Amro *et* al., 2000].

It was stated that silver nanoparticles could degrade lipopolysaccharides, causes accumulation of them in the membrane. Inside the membrane, they create pits and this causes the membrane permeability to increase [Li *et* al., 2008].

2.20. Application of Silver

Silver metal was acknowledged with its catalytic specialty. It can oxidize methanol to formaldehyde and ethylene. Silver is a good conductor, it is chemically stable and it has catalytic and antimicrobial activity [Sharma *et* al., 2009].

It has several advantages in surface application. Firstly, it has large spectrum of against microorganisms. It is easily tolerated by human tissue. It can be used with different materials due to it is so compatible. There is not much resistance against silver. Lastly, it can be both applied to the surface or submatrix easily [Gibbins & Warner, 2005].

Due to silver has a good antimicrobial property it can find large application area such as biomedical applications, purification of water and air, cosmetics and clothing areas, production of food and it can be used in lots of household materials. While nanotechnology developing, using silver in consumer products gain interest. These can be respirators, household water filters, antimicrobial sprays, detergents, shoes, dietary supplement, toys, cell phone, laptop etc. [Marambio-Jones and Hoek, 2010].

At the beginning of 1000 B.C. for making water, potable silver was used. In 1700s for treating people with venereal diseases silver nitrate was used. In 19th century by using silver nitrate, granulation tissues were moved and for newly burns treatment it was used again. Nowadays clinicians started to use silver wound dressing because of bacteria developing resistance [Rai *et* al., 2009].

There are some other examples of silver, using in health area due to silver shows affect in a broad range of microorganisms and it has low toxicity for human. It was reported that with using solution of silver salts, infections of newborn eye was treated. Recently in clinical wound dressing, silver is using, also coated biomedical materials such as catheters, contain silver. Catheters are important as main source of nosocomial infections in hospitals. Because silver usage, prevent colonization of bacteria, it is very important in medical area [Dallas *et* al.2011].

2.21. Silver Zeolite

Zeolite is a type of hydrated sodium aluminosilicate crystalline material and it has a porous. Its main building block is TO_4 (tetrahedron). Sometimes phosphorus or other metals can be found in structure. Zeolite can be used in industrial applications such as molecular sieves, ion exchangers and catalysts [Falcioni & Deem, 1998].

It has a strong affinity for Ag^+, zeolite can bind silver ions and they can show antimicrobial activity together [Kawahara *et* al., 2000]. During this process, free cations contact with the microorganisms in the environment. They inactivate enzymes, interrupt RNA replication and with an oxidative process prevent respiration. It was shown that the antimicrobial effect of silver zeolite against different type of bacteria and fungi [Casemiro *et* al., 2008].

Silver zeolites release silver ions constantly (10 ppb), and this create a long-term activity in the means of antimicrobial [Vermeiren *et* al., 2002]. In Figure 2.9 it can be seen the ion exchange mechanism of zeolite.

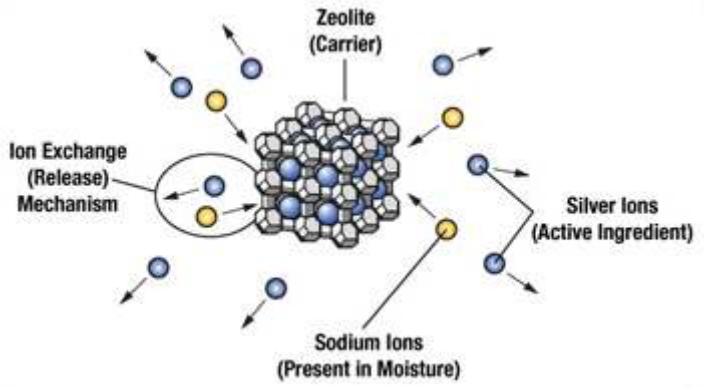


Figure 2.9 Ion exchange mechanism of zeolite [URL 3]

In the ion exchange mechanism, solid phase has charge, and there is a balance between opposite charge ions. In aqueous solution, diffusion process occurs. Ions released from solid phase and ions from environment diffuse into solid. For solid phase, active charge is important; it determines the ion exchange capacity. Zeolite has high ion exchange capacity as solid structure [Inglezakis, 2005]

There are different reasons for choosing silver zeolites. Ag^{+} is known as the only noble monopositive cation. It is stable in water and there is no hydrolysis during process. When compared to other noble metals, silver is the only one, which could be exchanged into zeolite in a liquid. Moreover, the process provides a good observation opportunity with silver's reversible oxidation-reduction [Sun & Seff, 1994].

2.22. Synthesis of Silver Based Nanoparticles

Silver nanoparticles can be obtained by using physical methods. These methods can be laser ablation in liquids, inert gas condensation process, ultrasonic assisted reduction, and photoinduced synthesis or irradiation reduction.

There are also found chemical approaches for production. These are chemical reduction, the template method, electrochemical reduction and microemulsion method.

The most common method is chemical reduction method. In this process, Ag^{+} reduces various complexes, and silver atom occurs. Agglomeration of clusters is followed after this. These clusters cause the formation of silver particles. Generally, borohydride or citrate is used as reluctant [Cioffi *et* al., 2012].

2.23. Safe Usage of Nanoparticles

Organizations are carried out researches for determining the non-toxic usage amount of nanoparticles.

The Occupational Safety and Health Administration (OSHA) regulate occupational exposure to TiO2. The permissible exposure limit (PEL) is 15 mg/m^3 as total dust (8-hr time-weighted average [TWA] concentration) [Department of Health and Human Services, 2011].

Also there are different standards for titanium dioxide. In the USA it is approved for using in food coloring as 1 % up by weight of the finished food. For coloring drugs, it can be used 358 mg per dosage with the standards listed in FDA.

FDA approved titanium dioxide in 21 CFR with the code of 73.575. The EU Scientific Committee for Food (SCF) evaluated TiO2 did not pronounce any daily intake for food. JECFA concluded that there is no significant absorption or tissue storage for TiO_2 and there is no necessity for determining a daily intake [Schoneker and DeMerlis, 2010].

For silver, EPA proposed 0.1 mg/L usage due to skin discoloration. Also EPA has showed that silver is not a carcinogenic agent.

The Occupational Safety and Health Administration (OSHA) determine the cubic amount of silver as 0.01 mg/m^3 in air for a workplace, 8 hr/day. The National Instıtue of Occupational Safety nd Health agreed this data. The American Conference of Governmental Industrial Hygienists recommended 0.1 mg/m^3 as limit for silver metal and 0.01 mg/m^3 for soluble silver compounds [ATSDR, 1999].

2.24. Main Cell Types of Microorganisms

Different microorganisms have different type of cell structure and wall. The effect of photocatalytic activity is related with these differences. In the Figure 2.10types of the microbial cells are presented [Shuler *et* al., 1992].

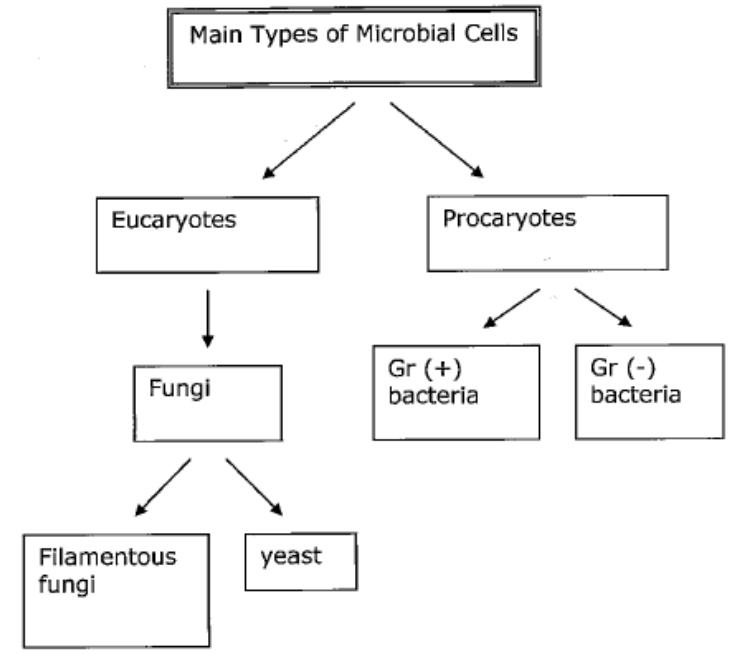


Figure 2.10 Main cell types of microorganisms

2.24.1. Prokaryotic Cells

The main difference between prokaryotic and eukaryotic cells is that the prokaryotic cell do not have membrane enclosed nucleus.. Another important difference between these two type cells is that prokaryotes' DNA is organized in multiple chromosomes as closed loop. [Weeks *et* al., 2008].

Prokaryotic cells have cell wall, surrounded the cell. With this wall the structural strength of cell increases and the cell can conserve its integrity at different external conditions. Under the wall, the cells have membrane which controls the transferring of materials between external environment and cell [Raven *et* al., 1996].

Generally, cell wall contains a polysaccharide molecule known as peptidoglycan. In some bacteria, peptidoglycan is thick and create a network around the outer surface of the cell.. In other type of bacteria there is a thin peptidoglycan is found between two plasma membranes. These two different cell wall types can be distinguish using a specific process called as gram staining [Raven *et* al.,2008].

By using gram staining bacteria can be classified as gram positive and gram negative. Under the microscope when these different cells are dyed, they are screened as different color due to differences in their cell wall structure [Raven *et* al.,2008].

2.24.1.1. Gram-negative Bacteria

The Gram-negative bacteria have a thin layer of peptidoglycan containing cell wall and the outside of this layer, the cell has a second membrane, which is quite different from plasma membrane. [Purves *et* al., 2003]. The cell envelop assists to maintain of cellular compounds and transport undesirable compounds to the outside [Laskin *et* al., 1973].

The mostly known Gram-negative bacterium is *E.coli.* The common structure of Gr(-) type bacteria is given Figure 2.11.

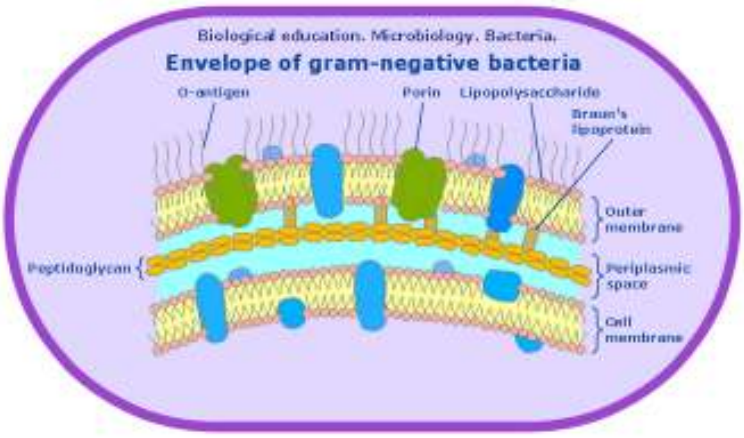


Figure 2.11 Gram-negative bacteria cell wall structure [URL 4]

2.24.1.2. Gram-positive Bacteria

Gram-positive bacteria have several sheets of peptidoglycan stacked one upon another [Madigan *et* al., 2005]. They do not have any outer membrane [Todar, 2000].

Peptidoglycan can be destroyed with some agents such as lysozyme, it is a protein that breaks the glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine [Madigan *et* al., 2005].

The mostly known Gr (+) bacteria are *B.subtilis* and *S.aureus.* The general structure of Gr (+) bacteria cell is represented in Figure.

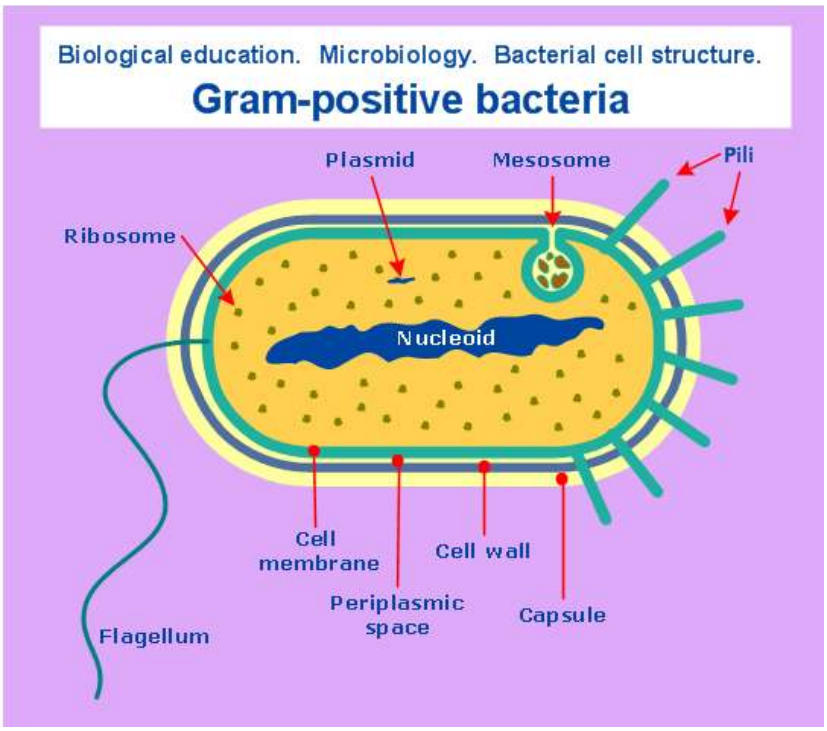


Figure 2.12 Gram-positive bacteria cell wall structure [URL 5]

2.24.2. Eukaryotic Cells

Eukaryotic cells are far more complex than prokaryotic cells. They have plasma membrane around the cell. Outer of the membrane there could be a cell wall or cell coat depending on the organism. DNA is stored at nucleus, which is surrounded by a membrane. [Purves *et* al., 2003]. Animals, plants and some microbial cells belong to eukaryotes. Yeasts (under fungi) and fungus are the microbial species of eukaryotes. In

2.24.2.1. Fungi

Fungi are classified as lower eukaryotes. They produce chitin, which is a polysaacaride, modified form of cellulose, used in the cell wall structure. It provides more resistance. Usually fungi grow as tubular filaments. These filaments are known as hyphae and they can be made stronger by chitin [Erkan, MS Thesis, 2005].

2.24.2.2. Yeasts and Molds

Yeasts and molds are the subgroups belong to fungal phyla. Yeasts are the unicellular form of fungi. [Purves *et* al.,2003].

Yeast has a cell envelop which includes the plasma membrane, the preiplasmic space and the cell wall.. The cell wall is thick and contains little amount of chitin. The cell wall has glucans in the structure; contribute the strength of the wall.

Molds are higher fungi. The cell wall usually do not have a fibrillar structure and can contain melanin. The mostly known species are *Aspergillus* and *Penicillium.*

2.25. Aim of the study

This study aims to research the antimicrobial effect of TiO2 nano particles, silver loaded zeolite particles and TiO2 coated textiles on different microorganisms namely, *E.coli, S.aureus, A.niger* spores, *E.dermatitidis* and *B.atrophaeus* spores.

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1. Preparation of TiO_2 Containing Materials

In this study titanium, isopropoxide and acetic acid were used as starting material. Distilled water was used for hydrolysis. Titanium isopropoxide (0.2 mol) and acetic acid (0,2 mol) were mixed at room temperature for 15 minutes. This mixture was added 290 ml distilled water and stirred for 1 hour at 700 rpm. After 1 hour 4 ml 65 % of nitric acid was added to solution and temperature was risen from room temperature to 80 ˚C. This solution was stirred for 40 minutes. Another 75 minutes was needed for peptization. For cooling the solution 370 ml distilled water was added. Then the solution was autoclaved at 250 ˚C for 12 hours. After autoclave, 2.4 ml 65 % nitric acid was added and sonication was done. Sonication was applied with 15 pulse/second. Lastly, rotary-evaporator was used and 13 % TiO_2 was obtained. (Appendix B)

After TiO_2 particles were obtained with sol-gel method, they were checked for structure. The obtained particles had 100 % anatase structure and they were 20-30 nm.

After synthesizing of nanoparticles, 10 % acrylic dispersion was added to particles. Then dip coating process was started. The solution was placed into a container. Then it was highly pressed with cylinder. Cylinders had 10 cm diameter, and made from rubber. The pressures of cylinders were 3 bars. The passing speeds of the textiles were 5 m/min. After textiles were dried in a ram type drier at 150 ˚C for 15 minutes. Lastly, another type of drier, which had fans, was used for completing of drying process. The speeds of fans were 2000 rpm, and the drier had 5-6% moisture.

The textiles are supreme weaving type, 30/1 cotton fiber. The textiles were processed with peroxidase bleachery.

3.2. Analysis of Particle and Textiles

3.2.1. SEM analysis

SEM analysis were done with Field Emission Scanning Electron Microscope (FESEM: FEI Nova NanoSEM 430) in the laboratory of Prof. Dr: Macit Özenbaş, ODTÜ.

3.2.1.1. Spectroscopic analysis

Spectroscopic analysis of powder was done with UV-Vis spectrophotometer (Shimadzu-UV 1201) in the wavelength (λ) between 290-500 nm ranges.

3.3. Preparation of Silver Zeolite

0,450 gram of silver nitrate and 1 gram of zeolite were added. Into 100 ml distilled water. The half of this solution was mixed with stirrer (CORNING PC-420 5) during overnight in 100 ml of distilled water**.** Other day the mix was filtered and left for drying at 100-150 ˚C in an oven. After it was dried, it was weighted and same amount of sodium borohydride was added into mix. 20 ml of ethylene diamine was added this mix and again stirred during overnight. The next day with 6 steps of centrifuged with distilled water the mix was washed. The centrifuge speed was 6000 rpm at 4 minutes. After washing it was left for drying at 37 ˚C. (Appendix D)

Controls: For understanding the effect of process, same process was done with only zeolite.

3.4. Antimicrobial Tests

3.4.1. Microorganisms and their growth conditions

E.coli (ATCC25922] was cultured aerobically in Luria-Bertani (LB) broth (Appendix A) at 37 ˚C with using a rotary shaker (130 rpm) for 16 hours. *E.coli* was maintained on LB agar (Appendix A). The LB agar containing plates were incubated at 37 ˚C overnight and for future use stored at 4 ˚C.

S.aureus (ATCC 14028) was cultured aerobically in Tryptic Soy (TS) broth (Appendix A) at 37 ˚C with using a rotary shaker (130 rpm) for 18 hours. Then maintained on TS agar (Appendix A). Plates were incubated at 37 ˚C overnight and for future use stored at 4 ˚C.

S.infantis was cultured aerobically in Tryptic Soy (TS) broth (Appendix A) at 37 ˚C with using a rotary shaker (130 rpm) for 18 hours, same as *S.aureus*. Then, maintained on TS agar (Appendix A). Plates were incubated at 37 ˚C overnight and for future use stored at 4 ˚C.

For determination of cell concentration viable count procedure on agar plate with serial dilution with 0.9 5 NaCl was used. Before all photocatalytic reactions, determination step was done.

B.atrophaeus was cultured aerobically in LB broth at 37 ˚C with using a rotary shaker (130 rpm) for 16 hours. Cells were maintained on NYSM agar (Appendix A). After sporulation, 1 week, with several washing steps and centrifuges spores were collected. 0,05 % Tween-20 was used at one washing step. Collected spores were stored at 4 ˚C and used directly as spore solution.

E.dermatitidis was cultured aerobically in Yeast Peptone Dextrose (YPD) broth (Appendix A) at 30 ˚C on a rotary shaker (130 rpm) for 36 hours. *E.dermatitidis* were maintained on PD agar (Appendix A). Plates were incubated at 30 ˚C for 36 hours and stored at 4 ˚C for future use.

A.niger (ATCC 16404) was cultured aerobically in Potato Dextrose (PD) broth (Appendix A) at 30 ˚C on a rotary shaker (130 rpm) for 40 hours. Cells were maintained on PD agar (Appendix A). Plates were incubated at 30 ˚C for 5days.After vegetative cells turned into spores, they were collected with 0.9 % NaCl solution. After 4 washing steps with centrifuged (8000 rpm, 5 min.), they were collected. At the fourth step of the washing procedure 0, 05 % Tween-20 was used. Spores were stored at 4 ˚C for future use and directly used as spore suspension for the tests.

3.4.2. Illumination for TiO_2

For photocatalytic process in the experimental system, a solar light stimulator lamp, Ultra-Vitalux light (Osram) was used for illumination for elimination of external sun light intensity alterations.

Solar light stimulator should mimic natural sun light well. For example, their spectral distribution should be compared. In Figure 3.1, there is a comparison graph for sun and solar stimulator.

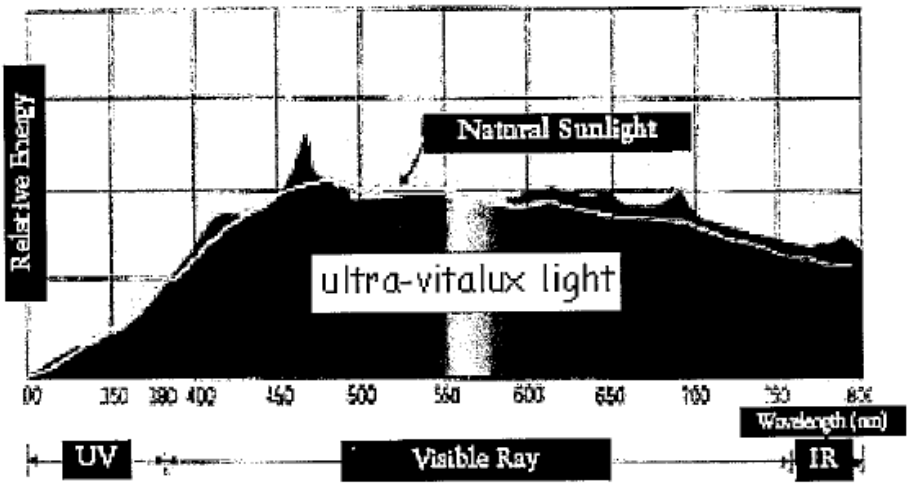


Figure 3.1 Comparison graph of spectral distribution of natural sunlight and solar stimulator

3.4.3. Irradiation and antimicrobial effect test of TiO_2

For testing powder and textiles, we used some parts of different methods and by adapting them into our experiments we created our experimental procedure. These methods were AATCC Test Method 100-2004 and JIS Z 2801. Appropriate parts of these methods were used in our experiments by combining.

After using viable count procedure, cell numbers were calculated and used bacterial cell count was adjusted with 0.9 % NaCl dilutions. Firstly, grown bacterial cells were centrifuged at 4500 rpm for 4 minutes. After collecting pellets, the optical density value of the suspensions was adjusted to 0.1 at 595 nm, with spectrophotometer (Shimadzu UV-1201). This 0.1 value was taken as 10^0 and accepted as stock suspension. For testing powders, 0.1 % TiO_2 powder was added into 27 ml % 0.9 NaCl solution and 1/10 diluted of stock suspension of for *E.coli, S.aureus, E.dermatitidis* were prepared. For *B.atrophaeus* spores and *A.niger* spores the beginning amounts were adjust to 10^{-2} with serial dilutions from stock spore solution. From these dilutions, 3 ml was added into TiO_2 containing solution. For mixing, magnets were used, and microorganism containing solutions were mixed at 300 rpm for 2, 4, and 6 hours depending on the organism. During mixing samples were illuminated from above with a solar light stimulator (Osram, ultra-vitalux, 300 W), 3 meters away from tested materials. There was no changes in the temperature during procedure. During illumination, cell suspensions were removed for taking sample at various time intervals. So time effect on different organisms was observed. 100 µl of remove suspensions were spread onto LB agar and incubated at 37˚C for *E.coli* and *B.atrophaeus* for overnight, were spread onto TS agar for *S.aureus* and incubated at 37˚C, were spread onto PD agar for *E.dermatitidis* and *A.niger* and were

incubated at 30 ˚C for 36 hours to determine the survival of microorganisms but counting the colony forming units (CFUs). Each evaluation was carried as duplicate. In our experiments there were 3 biological replicates, which contained 3 technical replicates.

Controls: On the microorganisms, the effect of UVA light alone were observed with another sample that did not contain TiO_2. The stability of the microorganisms and effect of powder alone on the microorganisms were measured in powder containing sample in the dark. For determining the initial number of microbial cells, which were applied in the liquid sample 100µl of suspension was spread onto agar plates and incubated according to necessary condition for growing microorganism. Used NaCl (Appendix 2) solution was also spread onto agar for checking if there was any contamination.

For coated material testing, grown bacterial cells were centrifuged (Labnet Spectrafuge 24D) at 4500 rpm for 4 minutes. And after collecting pellets the optical density value of the suspension adjust to 0.1 with spectrophotometer. This 0.1 value was taken as 10^0 and accepted as stock suspension and 1/10 diluted of stock suspension of for *E.coli, S.aureus, E.dermatitidis* were prepared. For *B.atrophaeus* spores and *A.niger* spores the beginning amounts were adjust to 10^5 with serial dilutions. 1 ml of the microbial suspension was spread onto the (25 cm^2) coated and uncoated textiles. For preventing vaporization of suspension from textiles, humidifying chambers were prepared. Samples were illuminated from above with a solar light stimulator (Osram, ultra-vitalux, 300 W). There were no changes in the temperature during procedure. Times for maximum activity were adjusted with several experiments according to microorganism. After the time finished textiles were placed into 99 ml 0.9 % NaCl containing jars and mix with shaker (Wiseshake SHO-1D) for 5 minutes at 100 rpm. 100 µl of this suspensions were spread onto LB agar and were incubated at 37˚C for *E.coli* and *B.atrophaeus* for overnight, were spread onto TS agar for *S.aureus* and were incubated at 37˚C, were spread onto PD agar for *E.dermatitidis* and *A.niger* and were incubated at 30 ˚C for 36 hours to determine the survived of microorganisms by counting the colony forming units(CFUs). Each evaluation was carried as duplicate.

Controls: On the microorganisms, the effect of UVA light alone were observed with uncoated textile. The stability of the microorganisms and effect of coating alone on the microorganisms were measured coated textile in the dark. For determining the initial number of microbial cells, which were applied in the liquid sample 100µl of suspension was spread onto agar plates and incubated according to necessary condition for growing microorganism. Used NaCl solution was also spread onto agar for checking if there was any contamination.

3.4.1. Antimicrobial Tests for Silver Zeolite

Same organisms and mediums were used with TiO_2 tests.

The different amounts of silver zeolites were speeded on the plates with beads. After left for drying at 37 ˚C.

Cells were prepared as mentioned before with centrifugation and O.D. adjustment procedure. From suspension with 0.1 O.D. value at 595 nm , 100 µl of diluted to$10^{-4}$with 0.9% NaCl suspension was inoculated on the agar plates. For spore solution, directly used 10^{-4} dilution for inoculation. After plates were incubated for different organisms at different condition as mentioned before. After growth of microorganisms counted method was used. Each evaluation was carried as duplicate

Controls: As control, only zeolite containing plates were used with same condition as silver zeolite.

The schematic representation of titanium dioxide was shown in Figure 3.2 and for silver zeolite experiments was shown in Figure 3.3.

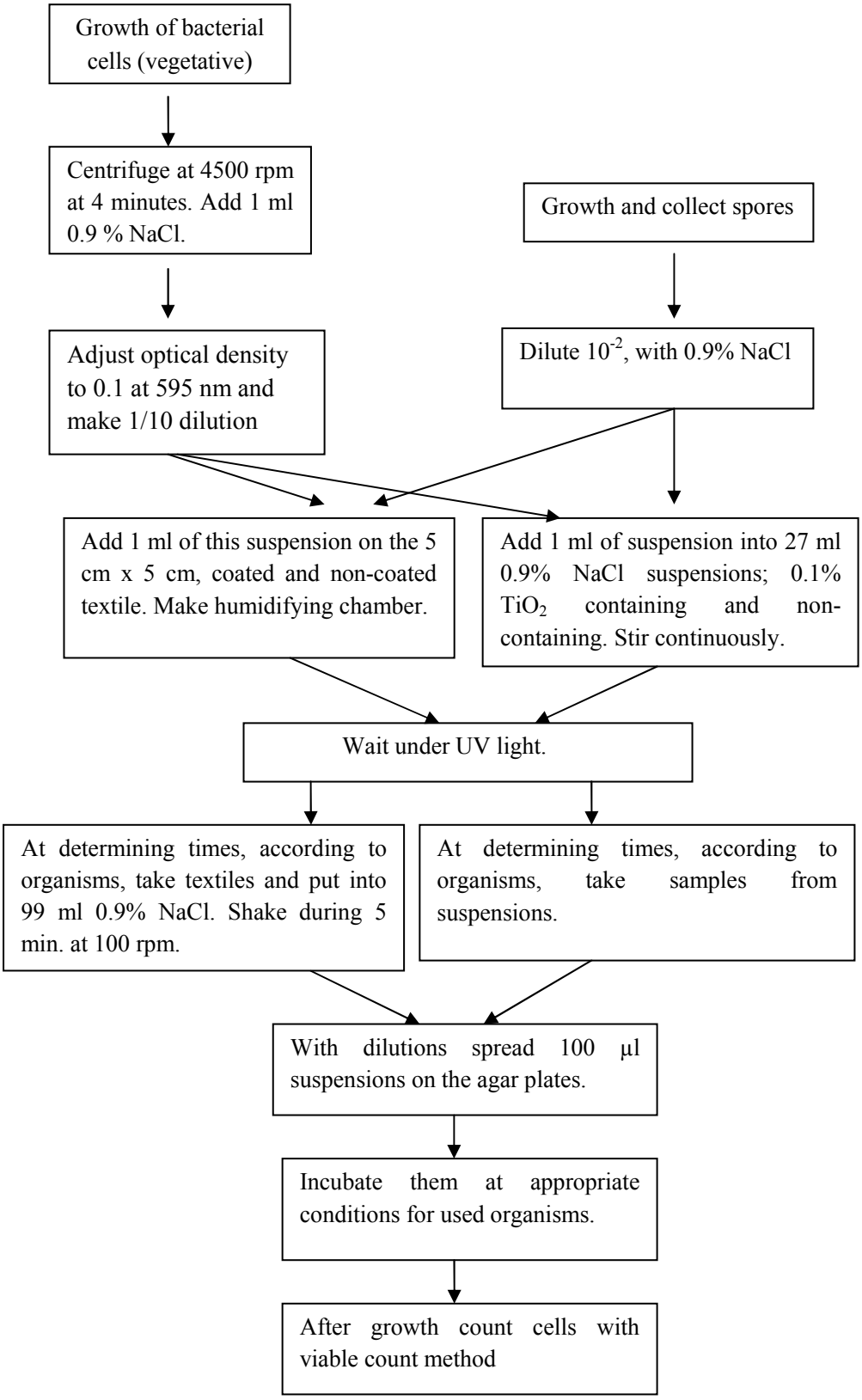


Figure 3.2 Schematic representation of titanium dioxide experiments

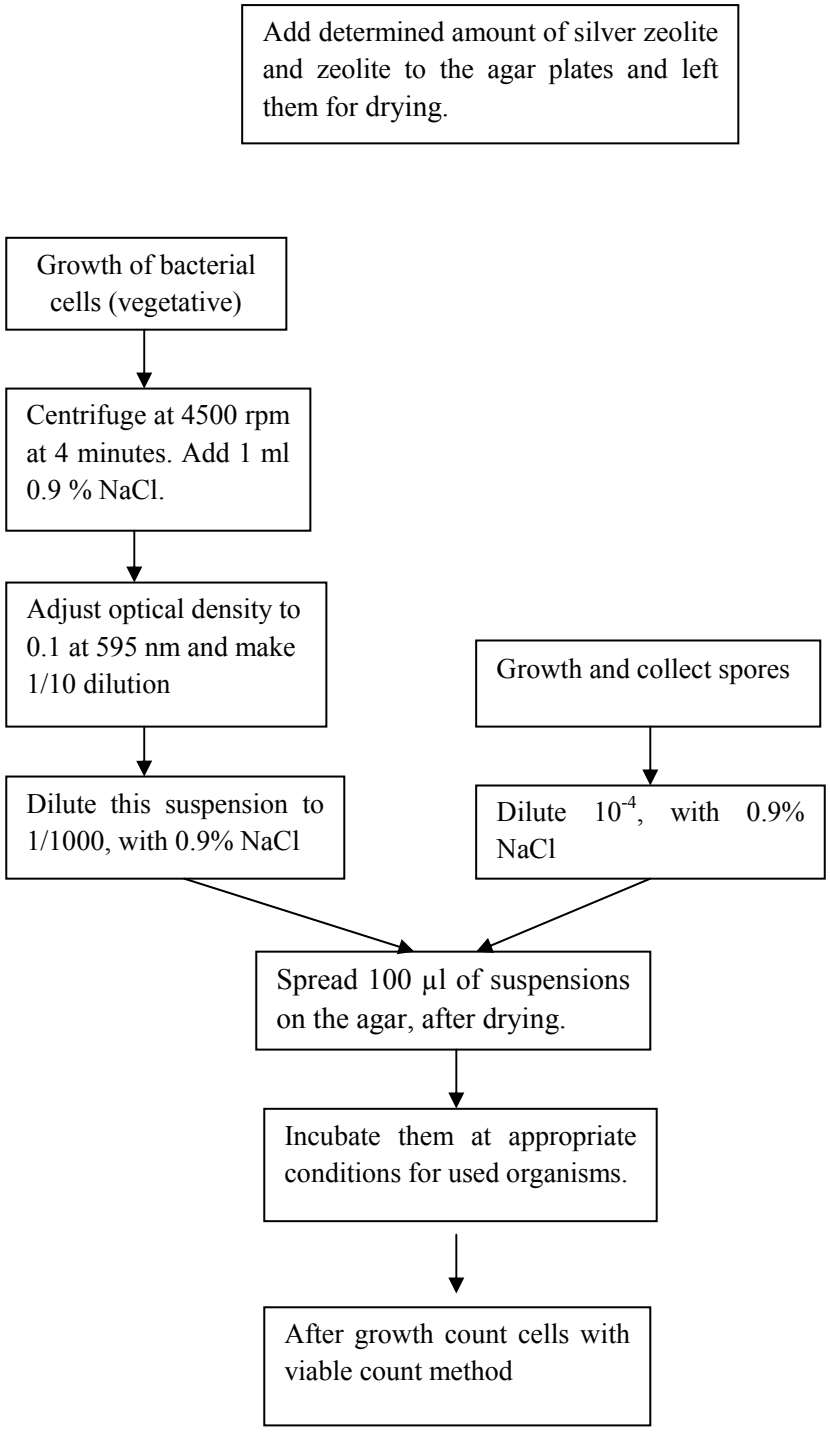


Figure 3.3 Schematic representation of silver zeolite experiments

3.5. Photodegradation effect test for TiO_2

Coated and uncoated textiles (4 cm^2) were prepared. 150 µl of 2 x 10^{-3} M methylene blue (Appendix C) was added to textiles. Textiles were placed under solar light stimulator, 3 meters away from light (Osram, ultra-vitalux, 300 W) for illumination. Waited for 1 hour, 2 hours, 4 hours and 6 hours. The color change was observed.

Controls: The effect of UVA light alone was observed with using uncoated textile. The effect of coating alone was observed with using methylene blue added textile in the dark.

3.6. Enzyme inhibition test for TiO_2

For the test, coated and uncoated textiles (4 cm^2) were prepared. 150µl of peroxidase enzyme (Appendix C) was added on the textile. Textiles were placed under solar light stimulator, 3 meters away from it (Osram, ultra-vitalux, 300 W). At 1 hour, 2 hours, 4 hours and 6 hours one textile was taken from under light and 50µl of ABTS (B) was added onto textiles as substrate. After 5 minutes waiting for emission, 50µl of H_2O_2 (Appendix C) was added on the textiles as catalyst. The color change was observed.

Controls: The effect of UVA light alone was observed with using uncoated textile. The effect of coating alone was observed with using enzyme added textile in the dark.

3.7. Antimicrobial Tests for Silver Zeolite

Same organisms and mediums were used with TiO_2 tests.

The different amounts of silver zeolite were spread on the plates with beads. After left for drying at 37 ˚C..

Cells were prepared as mentioned before with centrifugation and O.D. adjustment procedure. From suspension with 0.1 O.D. 100 µl of diluted to$10^{-4}$with 0.9% NaCl suspension was inoculated on the agar plates. For spore solution, 10^{-4} dilution was directly used for inoculation. After plates were incubated for different organisms at different condition as mentioned before. After growth of microorganisms, the survival of microorganisms was determined by counting the colony forming units (CFUs). Each evaluation was carried as duplicate. Experiments contained 3 biological replicates, whizh consisted 3 technical replicates.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Determination of Optical Absorption Edge Energy of the Particles Depending on the Spectra

By using UV-Vis absorbance important information about semiconductor can be obtained such as surface electronic properties or species oxidation states. Spectroscopic analyses of the powder was done with using UV-Vis spectrophotometer (Shimadzu UV-1201) in the wavelength range of 290-500.

In the Figure 4.1, the UV-Vis spectrum for TiO_2 powder can be seen. For analyses, TiO_2 powders were added into distilled water and distilled water used as blank.

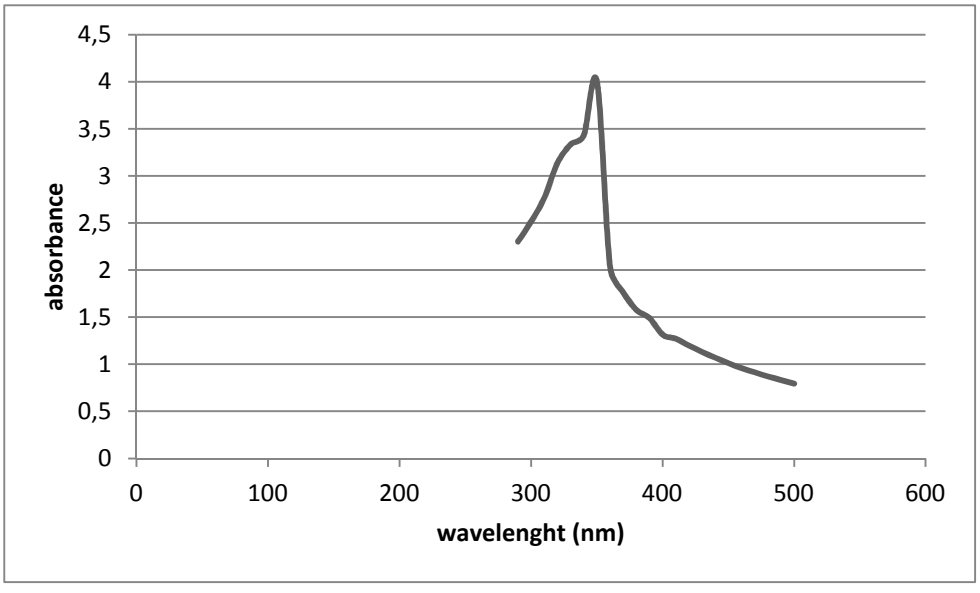


Figure 4.1 UV-Vis spectra of TO_2 powder

As seen in the figure between 300-370 obtained maximum absorbance from powder. UVB range is between 280-315, UVA range between 315-400 nm [Gruijl, 2000]. The powders absorbed light mostly in UVA range.

4.2. SEM Results of TiO_2 Textiles

In Figure 4.2 and Figure 4.3 SEM results of TiO_2 coating and non coating textile can be seen.

Non –coated textile

Coated textile

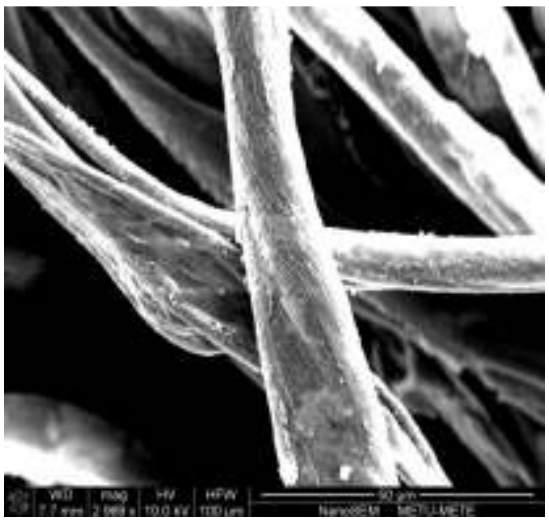
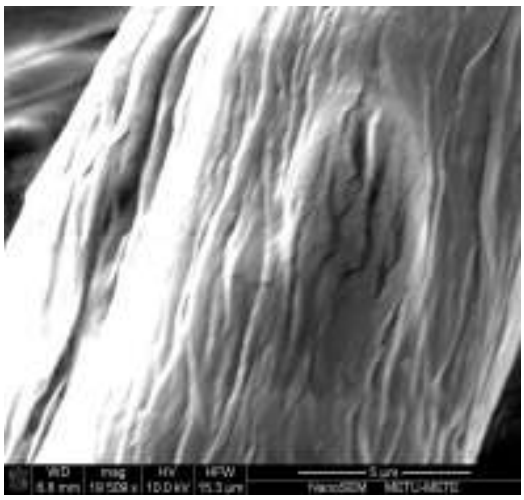


Figure 4.2 SEM results of coated and non-coated textiles at 3000 x magnification

Non –coated textile



Coated textile

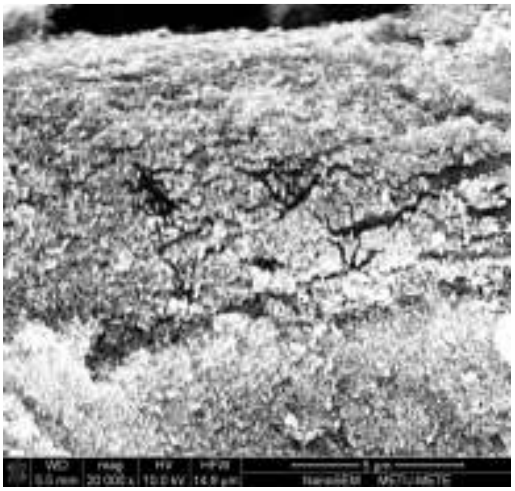


Figure 4.3 SEM results of coated and non-coated textiles at 20 000 x magnification

4.3. Antimicrobial Properties of the Nanoparticles and Textiles

4.3.1. TiO_2 Nanoparticles and Textiles

For understanding the antimicrobial properties of nanoparticles and their coatings, their effects on different organisms were tested. Due to the photocatalytic effect of used nanoparticles was first observed on the cell wall [Huang *et* al.,2000], *E.coli* and *S.aureus* were chosen as gram (-) and gram (+) prokaryotic cells and *B.atrophaeus* spore as bacterial spores, as eukaryotic cell *E.dermatitidis* and *A.niger* spores were used.

For each organism, cell suspensions were prepared with 0.9% NaCl solution after centrifugation of overnight growing cells. The optical density of this suspension was adjusted to 0.1 at OD 600 nm. From this suspension, 100 µl with 10^{-4} dilution was applied on the agar plate, so during each experiment, the number of the cells in the microbial suspension was determined with viable count method. For observing the effect of UVA, microbial suspension was incubated on the coated textile surface and for powder in another suspension with nanoparticle. Also for understanding if there is any effect of coating only, microbial suspension was incubated on the coated surface in the dark and suspension with nanoparticle in the dark for understanding the effect of preparation process on powder.

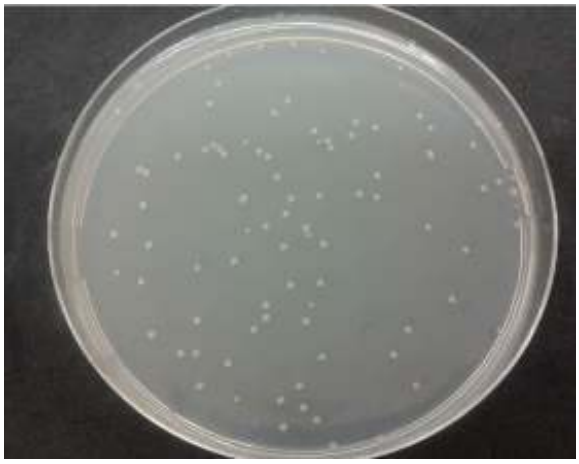


Figure 4.4 Number of *E.coli* cells in the 100 µl microbial suspension on the agar plate, with 10^{-4} dilution that have 0.1 optical density value (221±10 CFU/100 µl were counted)

Figure 4.4 shows the number of *E.coli* cells in the microbial suspension that applied on the agar plate, used during the photocatalytic experiments testing of the antimicrobial effects of nanoparticles. Almost in all experiments, number of the cells survived after applied to the uncoated material under the light (Figure 4.5) was equal to the number of the cells survived after applied to the coated material in the dark (Figure 4.6). Hence, it can be understood that, there is no effect of illumination or coating alone. Similar situation was observed for powder also. Number of cells survived after applied no powder containing solution under the light was equal to the number of cells survived after applied to powder containing solution in the dark.

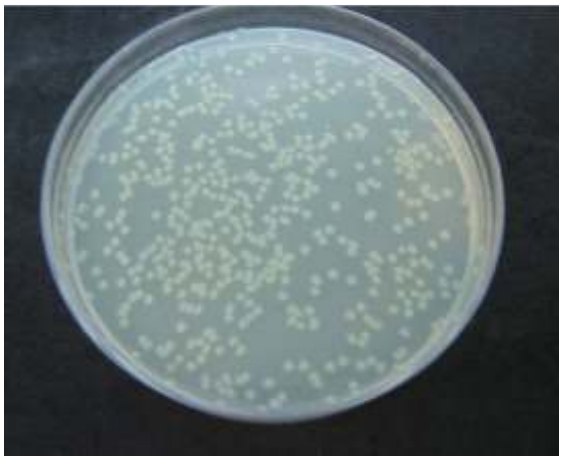


Figure 4.5 Number of *E.coli* cells in the microbial suspension of 1 ml applied on the uncoated surface/suspension and illuminated with 10^{-4} dilution that have 0.1 optical density value [240±8 CFU/100 µl]

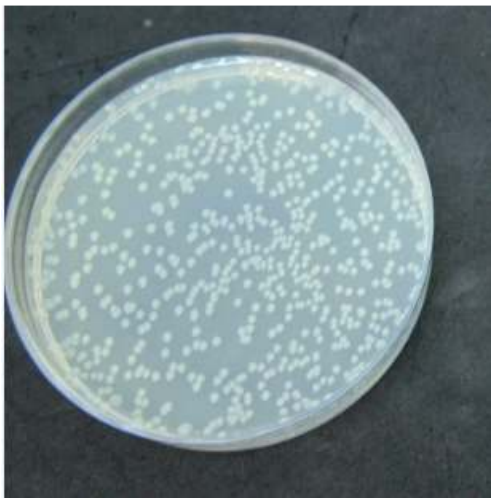


Figure 4.6 Number of *E.coli* cells in the microbial suspension of 1 ml applied on the coated surface/suspension and incubated in the dark with 10^{-4} dilution that have 0.1 optical density value [258±7 CFU/100 µl]

As shown in the Figure 4.5 and Figure 4.6, numbers of *E.coli* cells in the 100 µl suspension treated with the TiO_2 then kept in the dark and in the suspension without any treatment under illumination are counted as 240 and 258 CFUs, respectively. Same experiments were also done with *S.aureus, E.dermatitidis* and *A.niger* spores for control. The experiment results of all the microorganisms are given in Table 4.1. So, there is no

effect of only coating and only UVA against *E.coli, S.aureus, B.atrophaeus, E.dermatitidis* and *A.niger*.

Table 4.1 Results of coating and illumination control experiments on textiles

	Number of cells/ 100 µl		
	Original microbial suspension applied on the agar- with 10^{-4} dilution from stock	On the illuminated uncoated surfaces- with 10^{-4} dilution	On the coated surface kept in the dark- with 10^{-4} dilution
E.coli	261±10 CFU	275±5 CFU	258±7 CFU
S.aureus	713±4 CFU	734±3 CFU	717±4 CFU
B.atrophaeus	605±7 CFU	611±8 CFU	613±6 CFU
E.dermatitidis	130±6 CFU	134±6 CFU	139±6 CFU
A.niger	406± 4CFU	421±8 CFU	431±3 CFU

Table 4.2 Results of preparation process effect and illumination control experiment on nanoparticles.

	Number of cells/ 100 µl		
	Original microbial cell suspensions applied on the agar- with 10^{-4} dilution from stock	The illuminated no powder containing cell suspensions with 10^{-4} dilution	The powder containing cell suspensions kept in the dark- with 10^{-4} dilution
E.coli	232±6 CFU	281±7 CFU	271±5 CFU
S.aureus	817±6 CFU	795±7 CFU	821±7 CFU
B.atrophaeus	600±8 CFU	580±9 CFU	617±6 CFU
E.dermatitidis	320±8 CFU	317±9 CFU	301±4 CFU
A.niger	396± 4CFU	456±11 CFU	481±6 CFU

4.3.2. Silver Zeolite Nanoparticles

For understanding the antimicrobial properties of nanoparticles and the process mechanism, their effects on different organisms were tested. *E.coli* and *S.aureus* were chosen as gram (-) and gram (+) prokaryotic cells and *B.atrophaeus* spore as bacterial spores, as eukaryotic cell *E.dermatitidis* and *A.niger* spores were used.

For each organism, cell suspensions were prepared with 0.9% NaCl solution after centrifuged of overnight growing cells. The optical density of this suspension was adjusted to 0.1. From this suspension, 100 µl with 10^{-5} dilution was applied on the agar plate, so during each experiment, the number of the cells in the microbial suspension was determined with viable count method. For observing the effect of silver containing nanoparticles, microbial suspension was incubated on the plate, which contained silver. Also for understanding if there is any effect of process only, microbial suspension was incubated on the plate, which contains only zeolite.

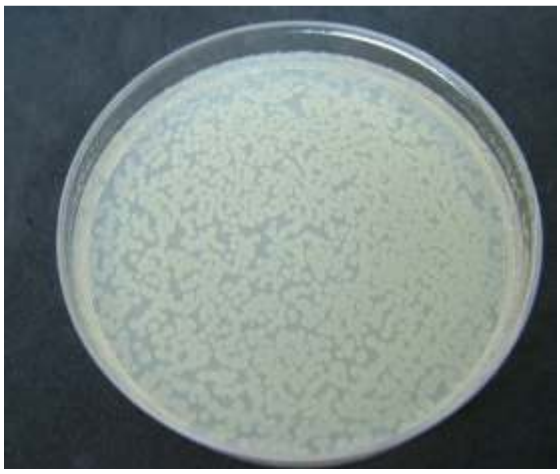


Figure 4.7 Number of *E.coli* cells in the 100 µl suspension on the agar plate, with 10^{-4} dilution that have 0.1 optical density value (700±4 CFU/100 µl were counted)

Figure 4.7 shows the number of *E.coli* cells in the microbial suspension that applied on the agar plate, used during antimicrobial effect of silver zeolite testing. Almost in all experiments, number of the cells survived after applied to the only zeolite containing plates (Figure 4.8) was equal to the number of the cells survived after applied to the empty plate (Figure 4.9). Hence, it can be understood there is no effect of only zeolite

Figure 4.8 Number of *E.coli* cells in 100 µl suspension on the zeolite containing plate with 10^{-5} dilution that have 0.1 optical density value [740±6 CFU/100 µl]

As shown in the Figure 4.5 and Figure 4.66, numbers of *E.coli* cells in the 100 µl suspension treated on the empty plates and on the zeolite containing plates were 700 and 740 CFUs, respectively. Same experiments were also done with *S.aureus, E.dermatitidis* and *A.niger* spores for control. The experiment results of all the microorganisms are given in Table 4.3. So, there is no effect of only zeolite against *E.coli, S.aureus, B.atrophaeus, E.dermatitidis* and *A.niger*.

Table 4.3 Results of zeolite effect control experiments

	Number of cells/ 100 µl	
	Original microbial suspension applied on the agar- with 10^{-4} dilution from stock	On the zeolite containing plate- with 10^{-4} dilution
E.coli	700±4 CFU	740±6 CFU
S.infantis	985± 9 CFU	1050± 11 CFU
S.aureus	799±3 CFU	820±7 CFU
B.atrophaeus	817±5 CFU	833±6 CFU
E.dermatitidis	558±5 CFU	554±3CFU
A.niger	348± 3 CFU	355±4 CFU

4.4. Photocatalytic Effect on the Antimicrobial Properties of TiO_2 Nanoparticles

4.4.1. Antimicrobial Effect Against *Escherichia coli*

4.4.1.1. Powder of TiO_2 Nanoparticles

After the 0.1 % nanoparticle containing and non-containing suspension illuminated 2 hours against *E.coli*, the photocatalytic effect's results were given Figure 4.9 and Figure 4.10. After 2 hours under the light, the number of cells in the suspension without nanoparticle was 281 and the number of cells in the suspension with 0.1 % nanoparticle was 0 CFUs. This was 100% decrease in the survival ratio of *E.coli* due to photocatalytic effect.

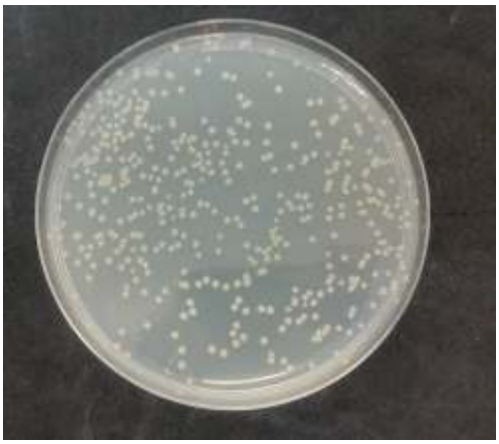


Figure 4.9 Number of *E.coli* cells survived in the suspension without nanoparticle (281 ± 7CFU/100 µl were counted)

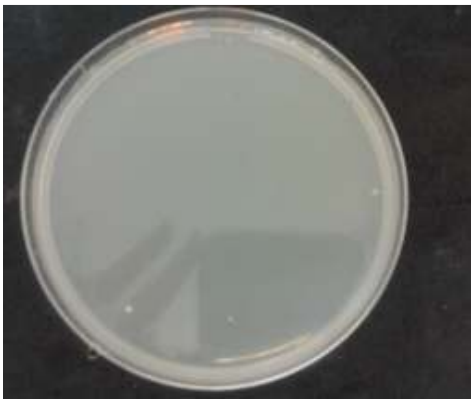


Figure 4.10 Number of *E.coli* cells survived in the suspension containing 0.1 % nanoparticle (0 ± 0 CFU/100 µl were counted)

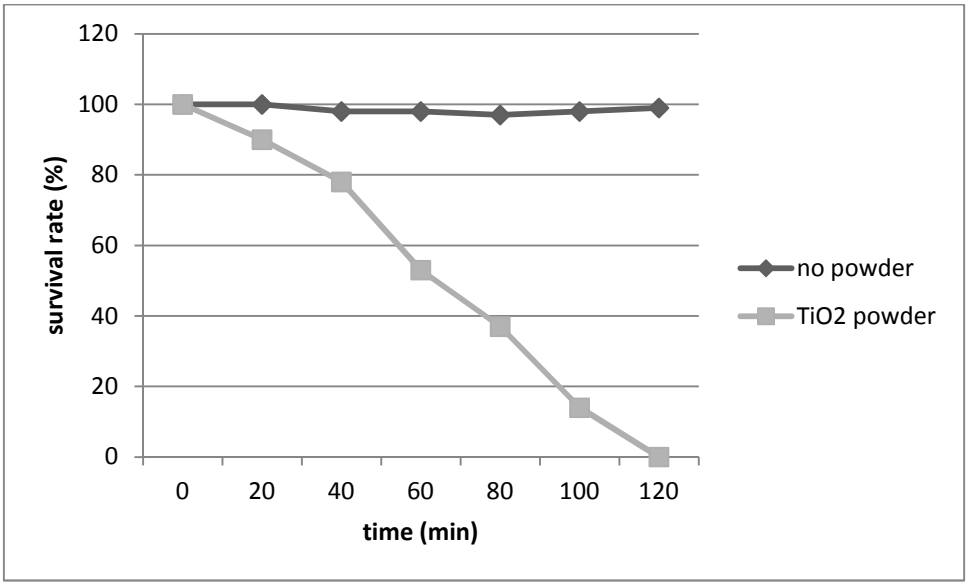


Figure 4.11 Survival ratio of *E.coli* under UV-Vis with TiO_2 powder containing and non -containing suspensions (initial number of *E.coli* was 281±7 CFUs/100 µl)

Huang *et* al. [1999] reported 96% of *E.coli* cells were inactivated after 60 minutes illumination with the use of 0.1 % TiO_2 nanoparticle. Also they claimed that a better activity can be achieved with increasing the surface area of TiO_2 and making smaller particle size by sonification.

4.4.1.2. TiO_2 Coated Textiles

After 2 hours of illumination of TiO_2 coated and uncoated textile against *E.coli*, the results of photocatalytic effect were given Figure 4.12 and Figure 4.13. After 2 hours of illumination, the number of cells on the uncoated textile was 275 and the number of cells on the coated textile was 58 CFUs. This was 79% decrease in the survival ratio of *E.coli* due to photocatalytic activity.

Figure 4.12 Number of *E.coli* cells survived on the uncoated textile (275 ±5 CFU/100 µl were counted)

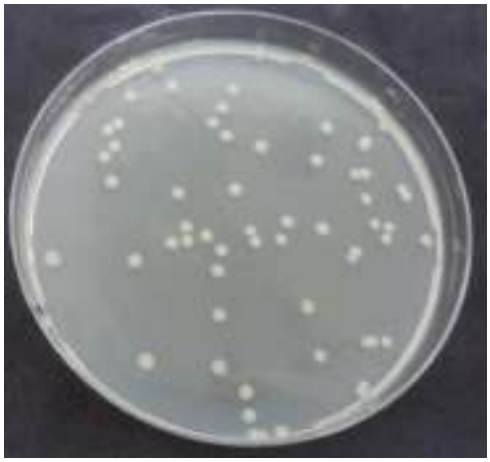


Figure 4.13 Number of *E.coli* cells survived on the coated textile (58 ±6 CFU/100 µl were counted)

4.4.1.3. Tungsten Doped TiO_2 Coated Textile

For increasing the conductivity of a semiconductor, using metal doping process is used in the literature [Zaharescu *et* al., 1993]. Tungsten can improve photocatalytic activity by preventing the recombination of electron-hole pairs. Also the chemical change can enhance acidity at the surface [Couselo *et* al., 2008].

After 2 hours of illumination of tungsten doped TiO_2 coated and uncoated textile against *E.coli*, the results of photocatalytic effect were given Figure 4.14 and Figure 4.15. After 2 hours of illumination, the number of cells on the uncoated textile was 298 and the number of cells on the coated textile was 38 CFUs. This was 88% decrease in the survival ratio of *E.coli* due to photocatalytic activity.

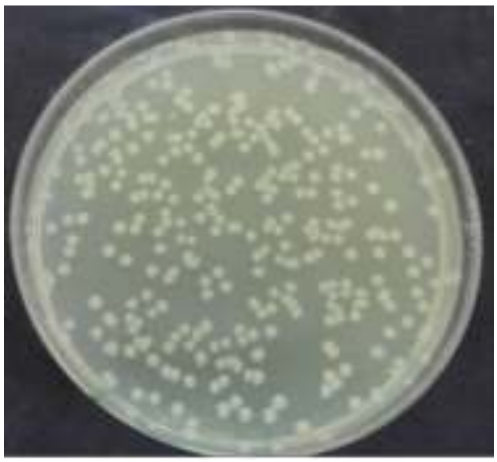


Figure 4.14 Number of *E.coli* cells survived on the textile that uncoated (298 ±5 CFU/100 µl were counted)

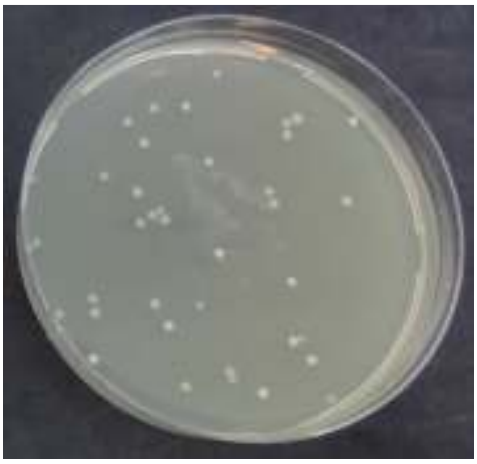


Figure 4.15 Number of *E.coli* cells survived on the textile that was coated and doped with tungsten (38 ±4 CFU/100 µl were counted)

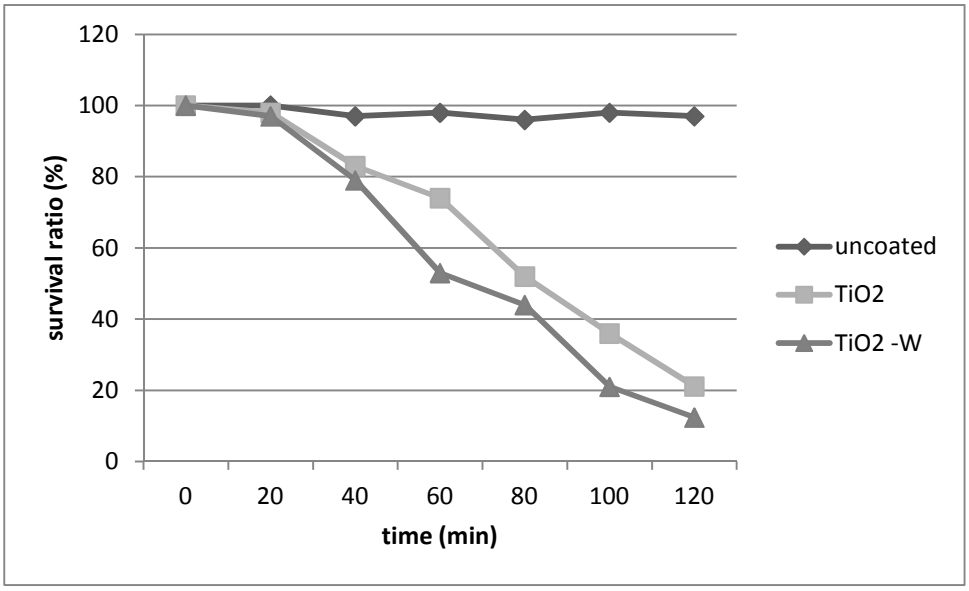


Figure 4.16 Survival ratio of *E.coli* under UV-Vis on the TiO_2 and TiO_2-W coated textiles (initial number of *E.coli* was 313±5 CFUs/100 µl)

The effect of tungsten doping is seen in Figure 4.16. Tungsten doped TiO_2 coated textiles showed higher photocatalytic activity when comparison is done with only TiO_2 coated textile at the end of 2 hours of illumination. After 60 minutes illumination the decrease of the survival ratio can be seen significantly.

4.4.1.4. Zinc and Tungsten Doped TiO_2 Coated Textile

Doping with zinc oxide nanocrystals changes the photoluminescent, photocatalytic, and magnetic properties of the nanocrystalline semiconductor. Yet, there is not much research about this dopant [Bohle *et* al.2010]. Combine effect of tungsten and zinc together has not been studied in the literature yet.

After 2 hours of illumination of zinc-tungsten doped TiO_2 coated and uncoated textile against *E.coli*, the results of photocatalytic effect were given Figure 4.17 and Figure 4.18. After 2 hours of illumination, the number of cells on the uncoated textile was 304 and the number of cells on the coated textile was 16 CFUs. This was 94.5% decrease in the survival ratio of *E.coli* due to photocatalytic activity.

Figure 4.17 Number of *E.coli* cells survived on the textile that was uncoated (304 ±6 CFU/100 µl were counted)

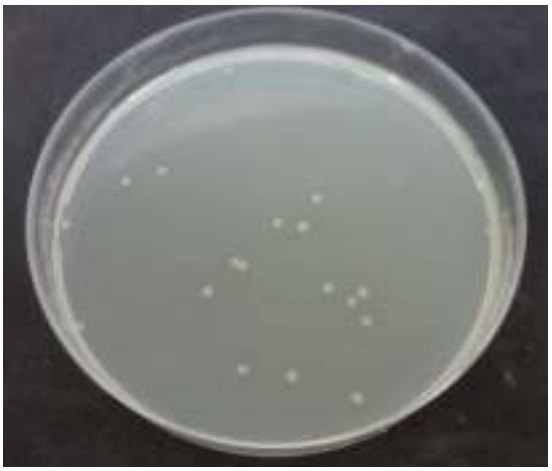


Figure 4.18 Number of *E.coli* cells survived on the textile that was coated with W-Zn doped TiO_2 (304 ±6 CFU/100 µl were counted)

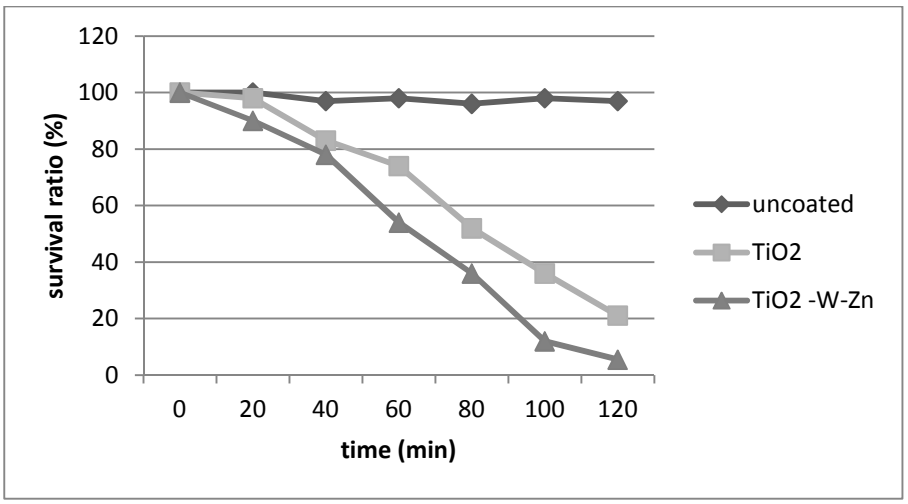


Figure 4.19 Survival ratio of *E.coli* under UV-Vis on the TiO_2 and TiO_2-W-Zn coated textiles (initial number of *E.coli* was 313±5 CFUs/100 µl)

The effect of tungsten doping is seen in Figure 4.19. Tungsten and zinc doped TiO_2 coated textiles showed higher photocatalytic activity when comparison is done with only TiO_2 coated textile at the end of 2 hours of illumination. After 50 minutes illumination the decrease of the survival ratio can be seen significantly. Tungsten and zing together showed more photocatalytic effect on *E.coli* than only tungsten doped titanium dioxide coated textile.

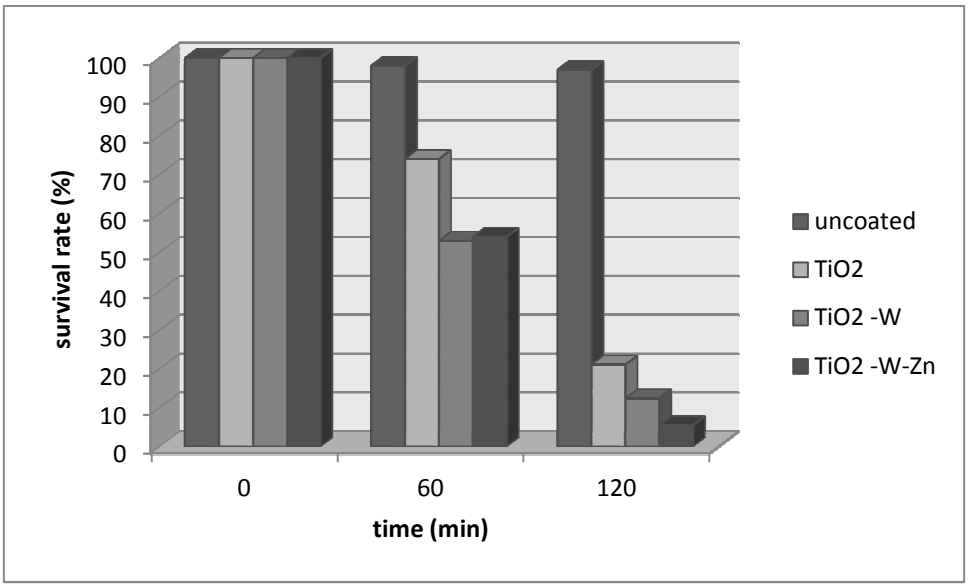


Figure 4.20 Survival ratio of *E.coli* with 2 hours of illumination on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles

In Figure 4.21, efficiency of all tested materials during 2 hours can be seen. The most effected material was titanium dioxide powder.

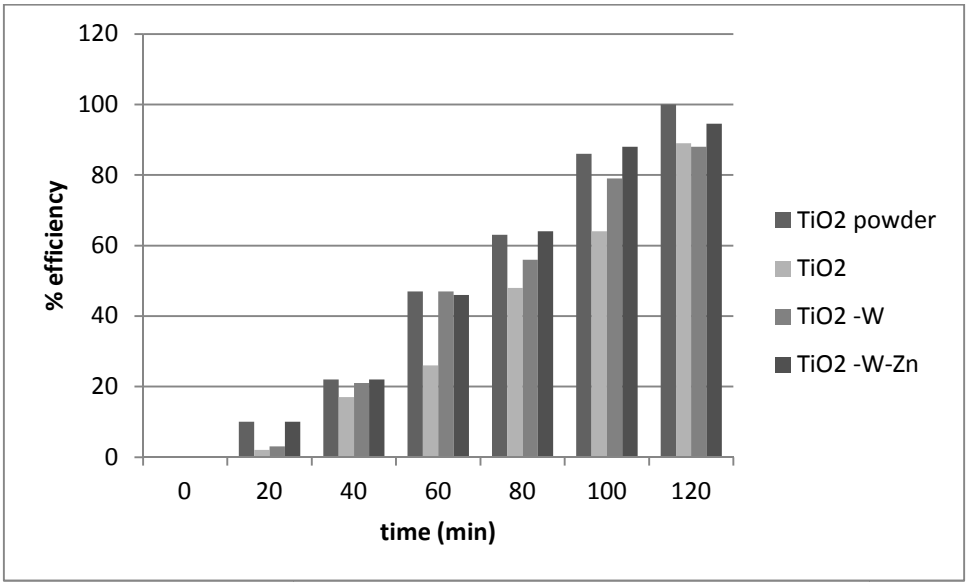


Figure 4.21Efficiency of all tested TiO_2 coated materials against *E.coli*

4.4.2. Antimicrobial Effect Against of *Staphylococcus aureus*

4.4.2.1. Powder of TiO_2 Nanoparticles

In the literature it was shown that inactivation photocatalytic efficiency of Gr (-) bacteria was higher than that of Gr (+) bacteria. Gr (+) bacteria have a thick cell wall made of peptidoglycan, but Gr (-) bacteria have a thin cell wall made of a layer of peptidoglycan. Also, they have an outer membrane, which contains lipids, and is separated from the cell wall by the periplasmic space. Although cell wall structure of Gr (-) bacteria is relatively more complex than that of Gr (+) cells, Gr (-) bacteria responded better for photocatalytic inactivation indicating cell wall destruction is probably not necessary for inactivation. Cell membrane damage is measured as loss of potassium ions, proteins and RNA from bacterial cells or as an increase in intracellular calcium ions. On the other hand, free radicals can react with the nucleic acids of the cell. It is also possible that reactive radicals are absorbed by the peptidoglycan layer of the Gr (+) bacteria without doing any fatal damage, while the opposite might be true for the Gr (-) bacteria [Pal *et* al., 2007].

So a higher antimicrobial effect on *S.aureus* cells was expected in our experimental system.

After the 0.1 % nanoparticle containing and non-containing suspension illuminated 4 hours against *S.aureus*, the photocatalytic effect's results were given Figure 4.22 and Figure 4.23 After 4 hours under the light, the number of cells in the suspension without nanoparticle was 795 and the number of cells in the suspension with 0.1 % nanoparticle was 2 CFUs. This was almost 100% decrease in the survival ratio of *S.aureus* due to photocatalytic effect.

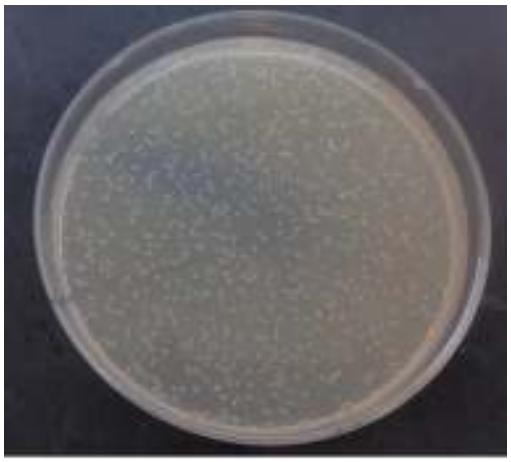


Figure 4.22 Number of *S.aureus* cells survived in the suspension which did not contain TiO_2 powder (795 ±7 CFU/100 µl were counted)

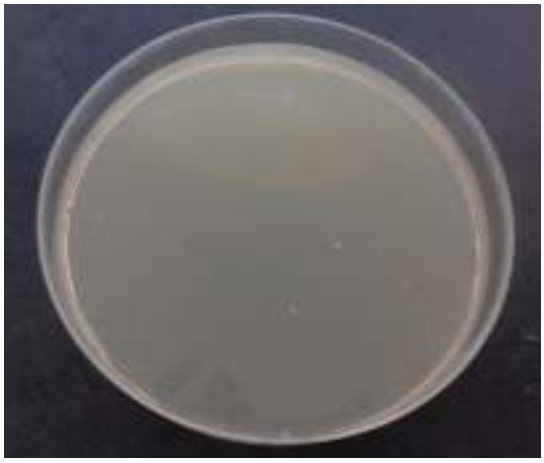


Figure 4.23 Number of *S.aureus* cells survived in the suspension of TO_2 powder containing (2 ±0,5 CFU/100 µl were counted)

Antimicrobial effect against *S.aureus* after two hours of illumination was higher when compared to *E.coli* cells. As explained before, this expected difference was due to cell wall differences.

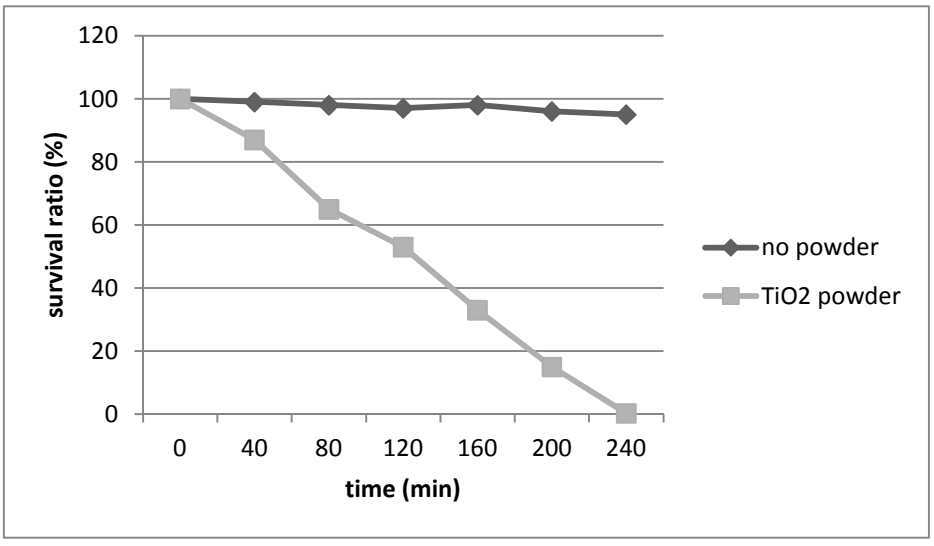


Figure 4.24 Survival ratio of *S.aureus* under UV-Vis with TiO_2 powder containing and non -containing suspensions (initial number of *S.aureus* was 795±7 CFUs/100 µl)

4.4.2.2. TiO_2 Coated Textiles

After 4 hours of illumination of TiO_2 coated and uncoated textile against *S.aureus*, the results of photocatalytic effect were given Figure 4.25 and Figure 4.26. After 4 hours of illumination, the number of cells on the uncoated textile was 734 and the number of cells on the coated textile was 8 CFUs. This was 99% decrease in the survival ratio of *S.aureus* due to photocatalytic activity.

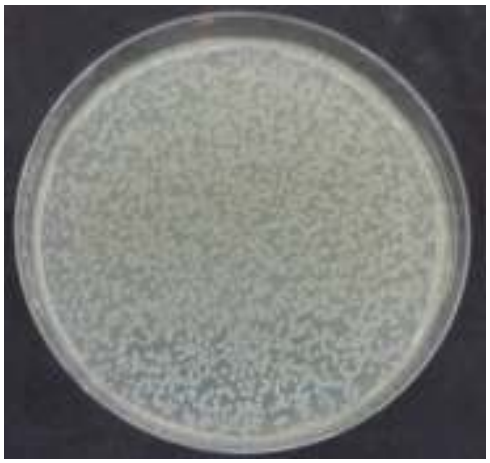


Figure 4.25 Number of *S.aureus* cells survived on the uncoated textile (734 ±3 CFU/100 µl were counted)

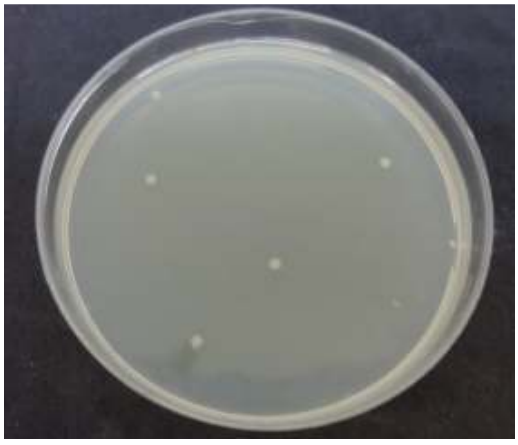


Figure 4.26 Number of *S.aureus* cells survived on the TiO_2 coated textile (8 ±2 CFU/100 µl were counted)

4.4.2.3. Tungsten Doped TiO_2 coated Textile

It was expected to doping semiconductor with tungsten increase the activity by rising the conductivity of the surface.

As it can be seen in Figure 4.27 and Figure 4.28, the tungsten coated textile exhibited an efficient effect on *S.auereus.* After 4 hours of illumination, the number of cells on the uncoated textile was 715 and the number of cells on the coated textile was 6 CFUs. This was 99% decrease in the survival ratio of *S.aureus* due to photocatalytic activity. Already TiO_2 coated textile showed high effect against *S.aureus*, there was not much increase with the addition of tungsten in 4 hours of illumination.

Figure 4.27 Number of *S.aureus* cells survived on the textile that was coated with W doped TiO_2 (715±3 CFU/100 µl were counted)

Figure 4.28 Number of *S.aureus* cells survived on the textile that was coated with W doped TiO_2 (6 ±1 CFU/100 µl were counted)

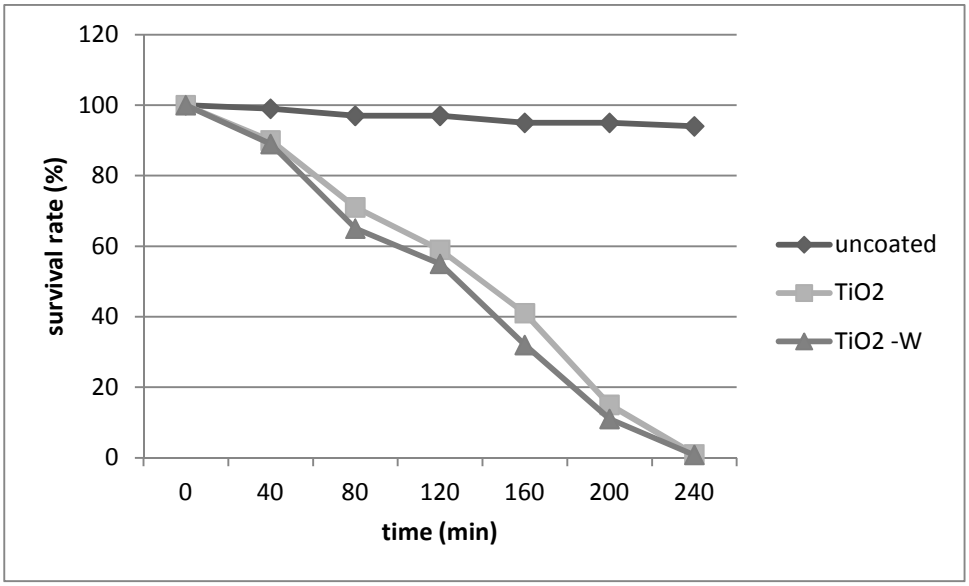


Figure 4.29 Survival ratio of *S.aureus* under UV-Vis on the TiO_2 and TiO_2-W coated textiles (initial number of *S.aureus* was 715±3 CFUs/100 µl)

4.4.2.4. Zinc and Tungsten Doped TiO_2 coated Textile

After 4 hours of illumination of zinc-tungsten doped TiO_2 coated and uncoated textile against *S.aureus*, the results of photocatalytic effect were given in Figure 4.30 and Figure 4.31. With 4 hours of illumination, the number of cells on the uncoated textile was 783 and the number of cells on the coated textile was 6 CFUs. This was 99% decrease in the survival ratio of *S.aureus* due to photocatalytic activity.

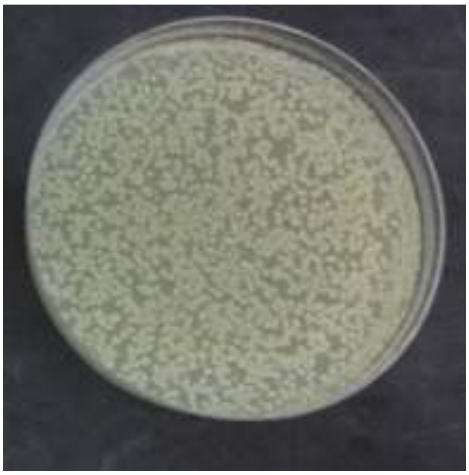


Figure 4.30 Number of *S.aureus* cells survived on the textile that was uncoated (783 ±6 CFU/100 µl were counted)

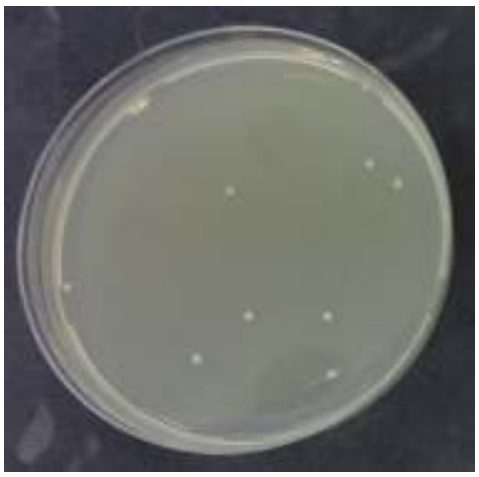


Figure 4.31 Number of *S.aureus* cells survived on the textile that was coated and doped with tungsten and zinc (6 ±0,5 CFU/100 µl were counted)

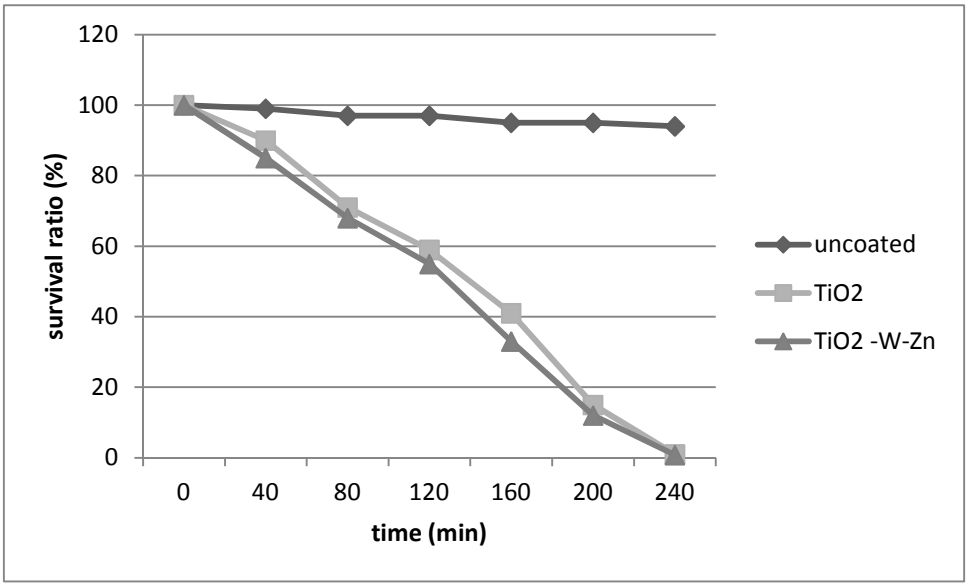


Figure 4.32 Survival ratio of *S.aureus* under UV-Vis on the TiO_2 and TiO_2-W-Zn coated textiles (initial number of *S.aureus* was 715±5 CFUs/100 µl)

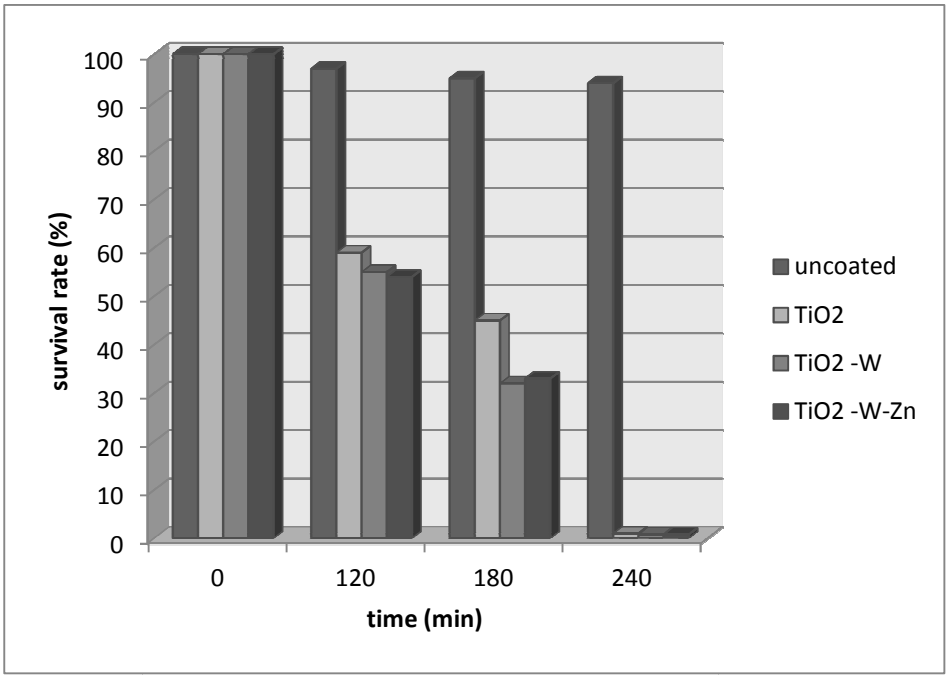


Figure 4.33 Survival ratio of *S. aureus* with 4 hours of illumination on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles

In Figure 4.32 it can be seen that, compared to bare TiO2 particles, the doped textiles have higher efficiency after 3 hours of illumination. However, all coated textiles have high effect against *S.aureus* under UV-Vis light at 4 hours as shown in Figure 4.33.

In Figure 4.34 effect of all tested materials were shown. Almost all of the materials have shown same amount of effect.

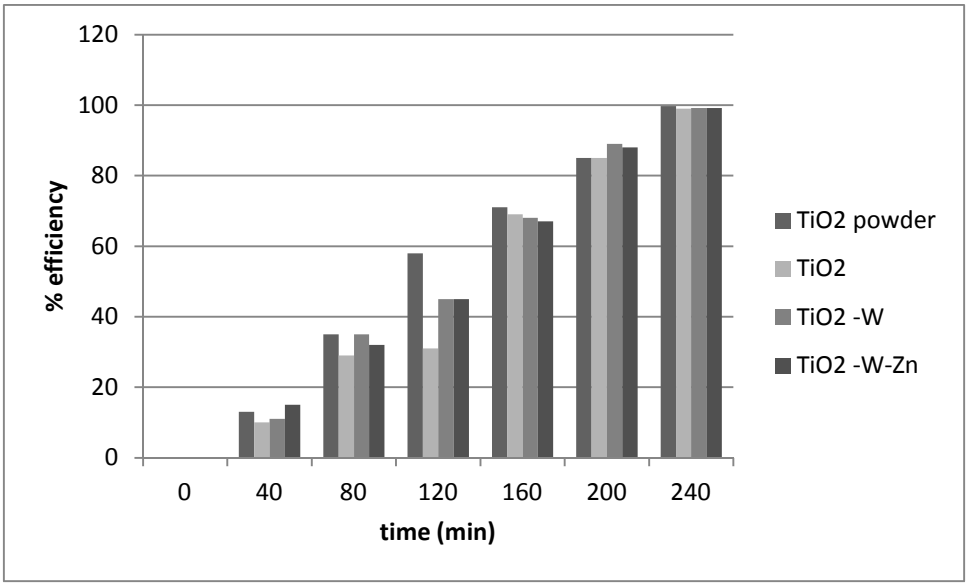


Figure 4.34 Efficiency of all tested TiO_2 coated materials against *S.aureus*

4.4.3. Antimicrobial Effect Against of *Bacillus atrophaeus*

4.4.3.1. Powder of TiO_2 Nanoparticles

Bacillus species are Gr (+) bacteria and they are spore forming. Spores show 5 to 50 times more resistance to UV radiation than vegetative cells. Also these two life forms have different handling mechanism for damage (Nhung *et* al., 2012). So it is hard to inactive them with same illumination time as for Gr (-) bacteria.

For observing the effect of 0.1 % TiO_2 powder against *B. atrophaeus* cells were stirred for 10 hours. The results of TiO_2 powder's photocatalytic effect were given in Figure 4.35 and Figure 4.36. The number of cells in the no powder containing suspension was 580 and the number of cells in the powder containing suspension was 65 CFUs. This was 89% decrease in the survival ratio of *B.atrophaeus* due to photocatalytic activity.

Figure 4.35 Number of *B.atrophaeus* cells survived in the no powder containing suspension (580 ±9 CFU/100 µl were counted)

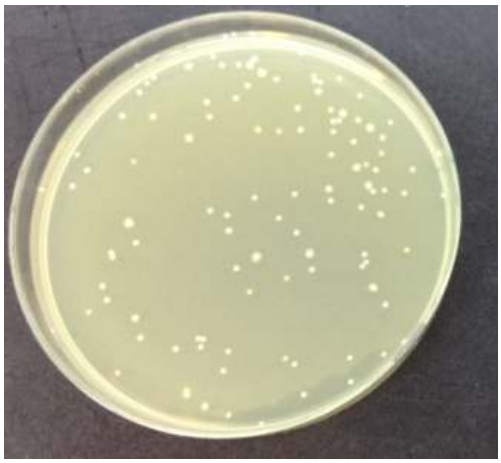


Figure 4.36 Number of *B.atrophaeus* cells survived in the TiO_2 powder containing suspension (65 ±7 CFU/100 µl were counted)

As shown in figures it needed more time for inactivation of Gr (+) bacteria spores when same amount of photocatalyst was used with Gr (-). When it was compared to the 2 hours data of *E.coli* and *B.atrophaeus* it was observed that there was almost no effect against *B.atrophaeus*. Similarly, when 6 hours results of *B.atrophaeus* was considered it was seen that almost only half of the spores were inactivated. The different time results are represented in Figure 4.37.

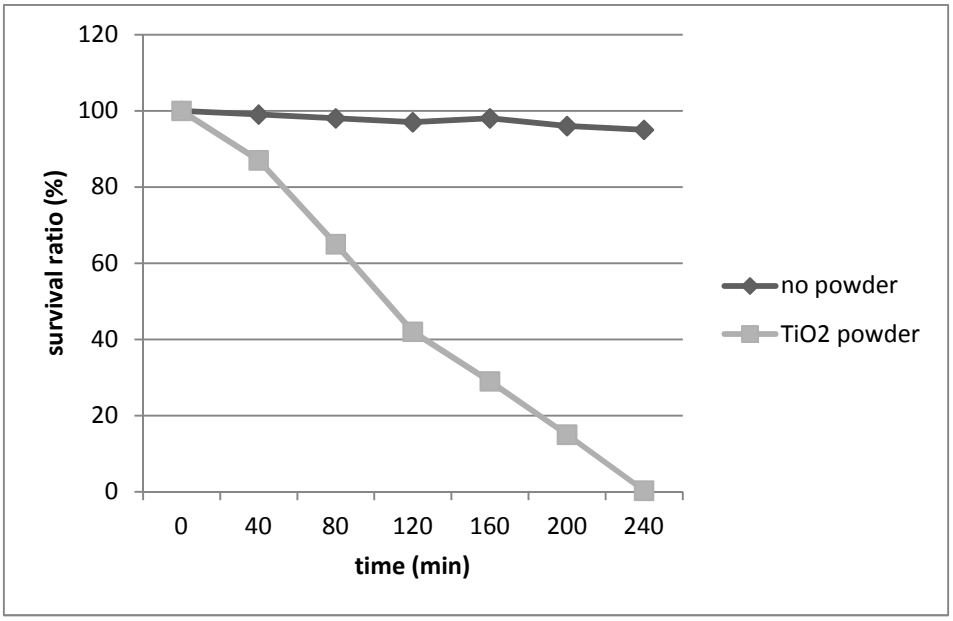


Figure 4.37. Survival ratio of *B.atrophaeus* under UV-Vis with TiO_2 powder containing and non -containing suspensions (initial number of *B.atrophaeus* was 600±8 CFUs/100 µl)

4.4.3.2. TiO_2 Coated Textiles

After 6 hours of illumination of TiO_2 coated and uncoated textile against *B.atrophaeus*, the results of photocatalytic effect were given Figure 4.38 and Figure 4.39. After 6 hours of illumination, the number of cells on the uncoated textile was 611 CFUs and the number of cells on the coated textile was 15 CFUs. This was 98% decrease in the survival ratio of *B.atrophaeus* due to photocatalytic activity.

Coated textile showed effect quicker than powders. This can be due to powders were aggregated into suspension during 10 hours stirring, so by decreasing surface area could not have contact with spores properly.

Figure 4.38 Number of *B.atrophaeus* cells survived on the uncoated textile (611 ±8 CFU/100 µl were counted)

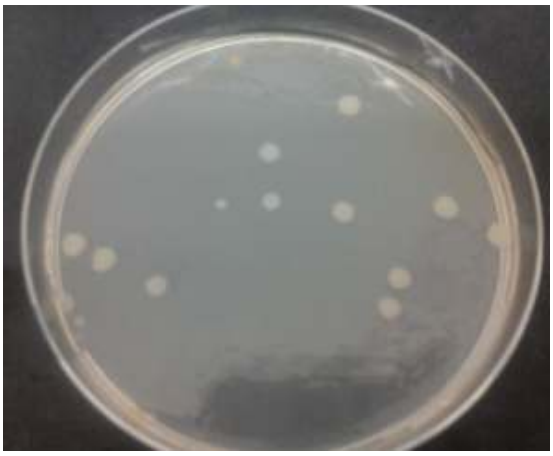


Figure 4.39 Number of *B.atrophaeus* cells survived on the TiO_2 coated textile (11 ±2 CFU/100 µl were counted)

4.4.3.3. Tungsten Doped TiO_2 coated Textile

Differently from other results doped textile show low efficiency against *B.atrophaeus* spores. It can be seen in Figure 4.40 and Figure 4.41 that the effect of tungsten coated textile after 6 hours of illumination, the number of cells on the uncoated textile was 718 and the number of cells on the coated textile was 582 CFUs. This was 19% decrease in the survival ratio of *B.atrophaeus*.

According to researches sometimes doping material such as silver, can combine together at high temperature during doping process. This situation can decrease antibacterial efficiency of photocatalytic material (Le *et* al., 2011). During preparation

process at the some parts of the textile, tungsten particles can be aggregate and decreasing surface areas could not be enough for inactivation of bacterial spores.

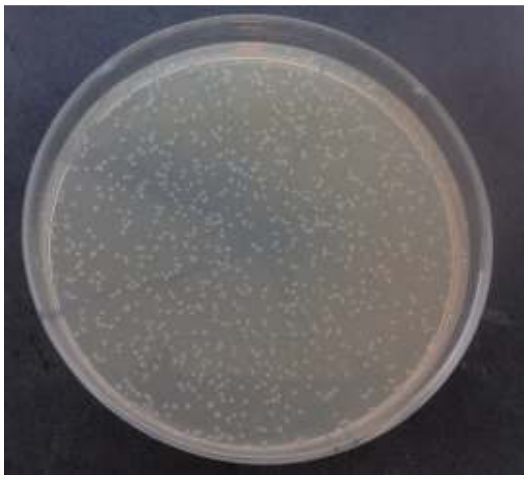


Figure 4.40 Number of *S.aureus* cells survived on the textile that was coated with W doped TiO_2 (715±4 CFU/100 µl were counted)

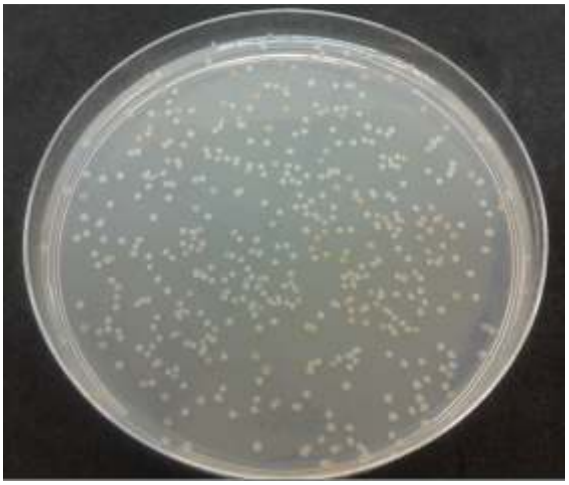


Figure 4.41 Number of *B.atrophaeus* cells survived on the textile that was coated with W doped TiO_2 (582 ±5 CFU/100 µl were counted)

In Figure 4.42 survival ratio of spores was shown.

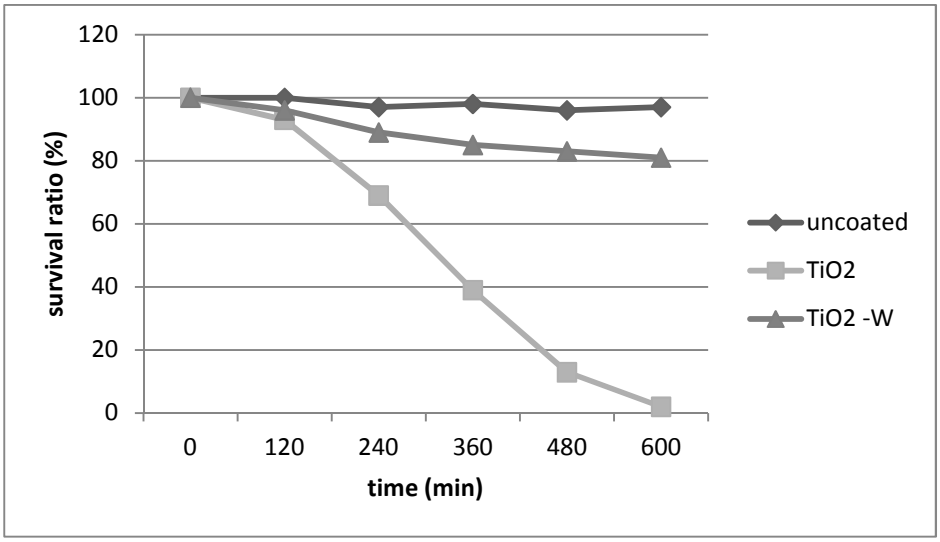


Figure 4.42 Survival ratio of *B.atrophaeus* under UV-Vis on the TiO_2 and TiO_2-W coated textiles (initial number of *B.atrophaeus* was 611±8 CFUs/100 µl)

4.4.3.4. Zinc and Tungsten Doped TiO_2 coated Textile

After 6 hours of illumination of zinc-tungsten doped TiO_2 coated and uncoated textile against *B.atrophaeus*, the results of photocatalytic effects were given Figure 4.43 and Figure 4.44. With 6 hours of illumination, the number of cells on the uncoated textile was 780 CFUs and the number of cells on the coated textile was 586 CFUs. This was 25% decrease in the survival ratio of *B.atrophaeus* due to photocatalytic activity. The results were similar with tungsten doped textiles.

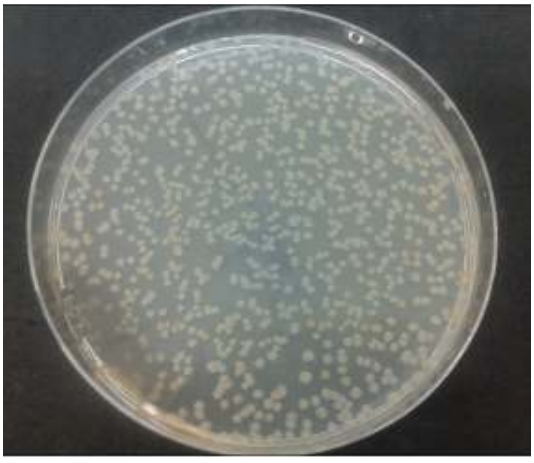


Figure 4.43 Number of *B.atrophaeus* cells survived on the textile that was uncoated (780 ±5 CFU/100 µl were counted)

Figure 4.44 Number of *B.atrophaeus* cells survived on the textile that was coated and doped with tungsten and zinc (586 ±4 CFU/100 µl were counted)

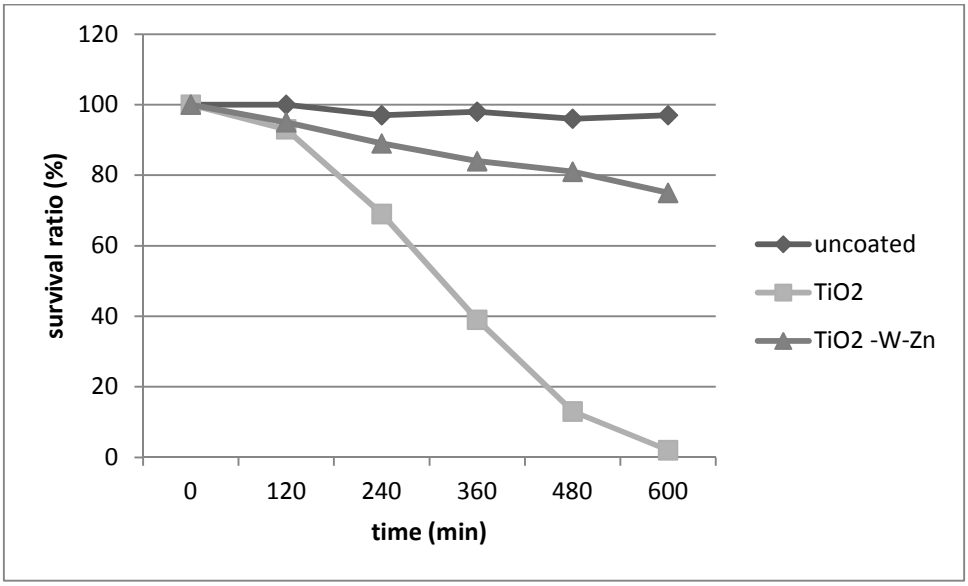


Figure 4.45 Survival ratio of *B.atrophaeus* under UV-Vis on the TiO_2 and TiO_2-W-Zn coated textiles (initial number of *B.atrophaeus* was 611±8 CFUs/100 µl)

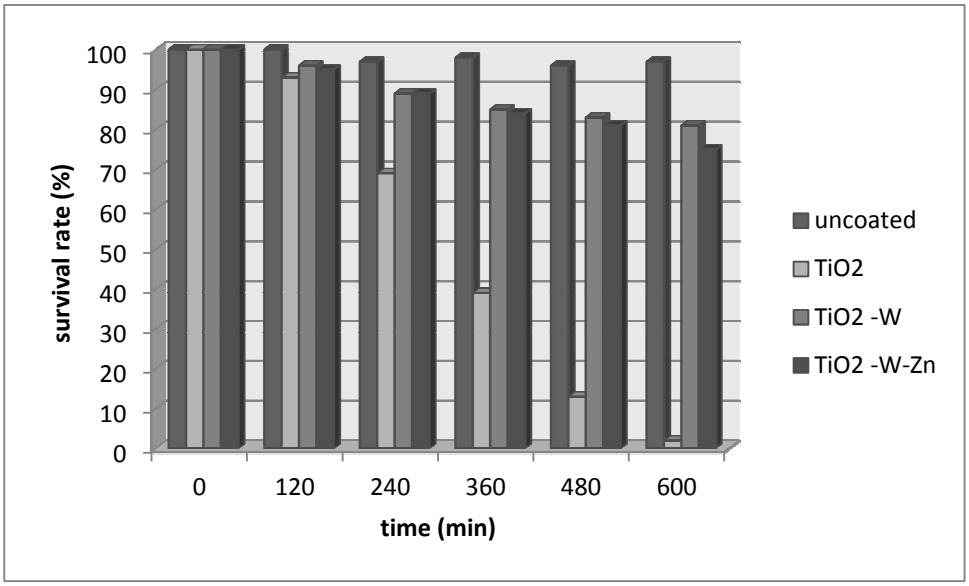


Figure 4.46 Survival rate of *B.atrophaeus* with 6 hours of illumination on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles.

In Figure 4.45, it can be shown that the doped textiles have not high efficiency at 6 hours. However, only TiO_2 coated textiles affected *B.atrophaeus* highly under UV-Vis light at 6 hours as shown in Figure 4.46Figure 4.46.

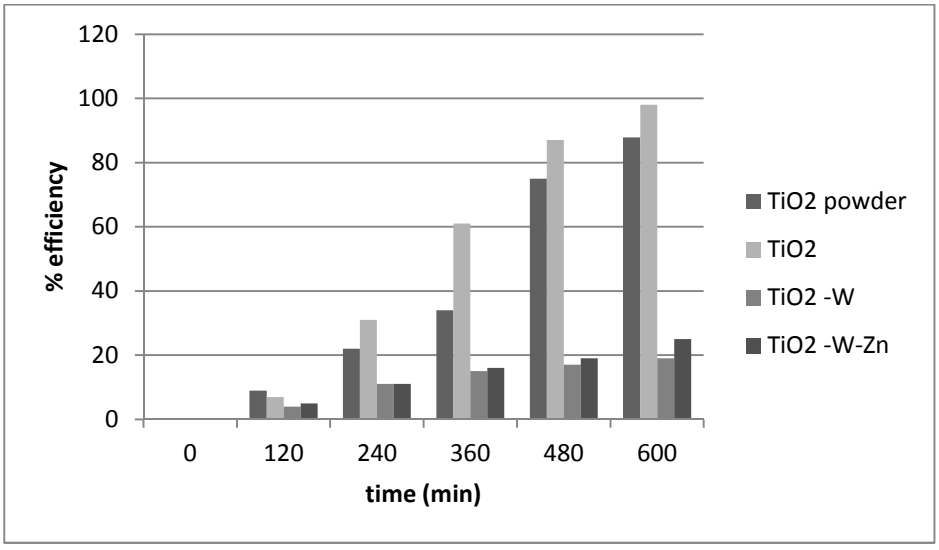


Figure 4.47 Efficiency of all tested TiO_2 coated materials against *B.atrophaeus* spore.

It can be seen in the Figure 4.47, the effect of all tested materials. When coated textiles were compared, doped textiles did not show high effect as non-doped.

4.4.4. Antimicrobial Effect Against of *Exophiala dermatitidis*

4.4.4.1. Powder of TiO_2 Nanoparticles

As yeast organism, *E.dermatitidis* was chosen. In the literature, effect of photocatalyst was not studied in this organism before.

In literature different yeast cells such as *C. albicans* was studied to evaluate the antimicrobial effect of photocatalytic semiconductor. It was shown that the yeast cells that interact with the photocatalytically active surfaces, can be destroyed after irradiation [Kühn *et* al., 2003].

For observing the effect of 0.1 % TiO_2 powder against *E.dermatitidis* cells were stirred for 6 hours. The results of TiO_2 powder's photocatalytic effects were given Figure 4.48 and Figure 4.49. The number of cells in the no powder containing suspension was 317 CFUs and the number of cells in the powder containing suspension was 47 CFUs. This was 85 % decrease in the survival ratio of *E.dermatitidis* due to photocatalytic activity.

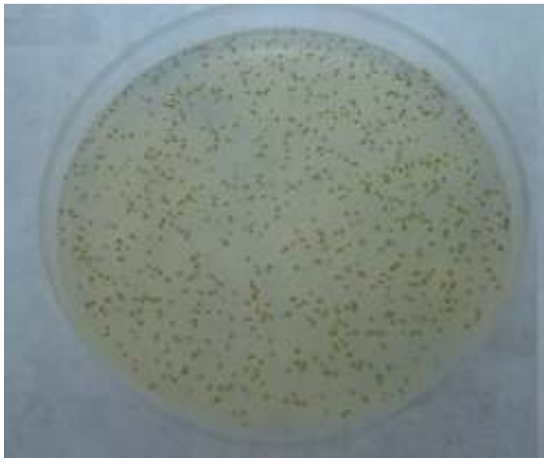


Figure 4.48 Number of *E.dermatitidis* cells survived in the no powder containing suspension (317 ±9 CFU/100 µl were counted)

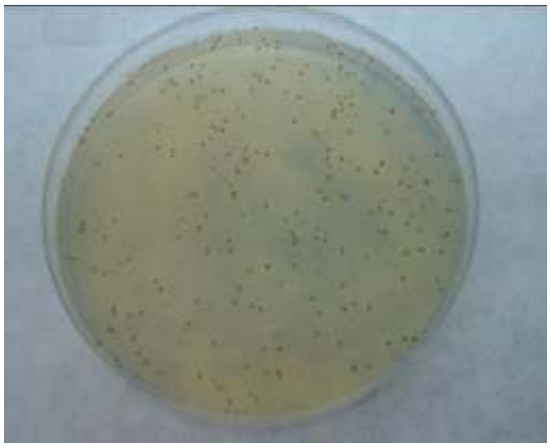


Figure 4.49 Number of *E.dermatitidis* cells survived in the TiO_2 powder containing suspension (47 ±3 CFU/100 µl were counted)

As shown in figures, yeast needed more time than Gr (-) bacteria due to its cell wall structure. When 2 hours data of *E.coli* and *E.dermatitidis* was compared, it was observed that the effect of photocatalyst on *E.dermatitidis* was not high enough. Similarly when 4 hours results of *S.aureus* and *E.dermatitidis* were compared it was seen that inactivation of Gr (+) bacteria was easier than yeast.

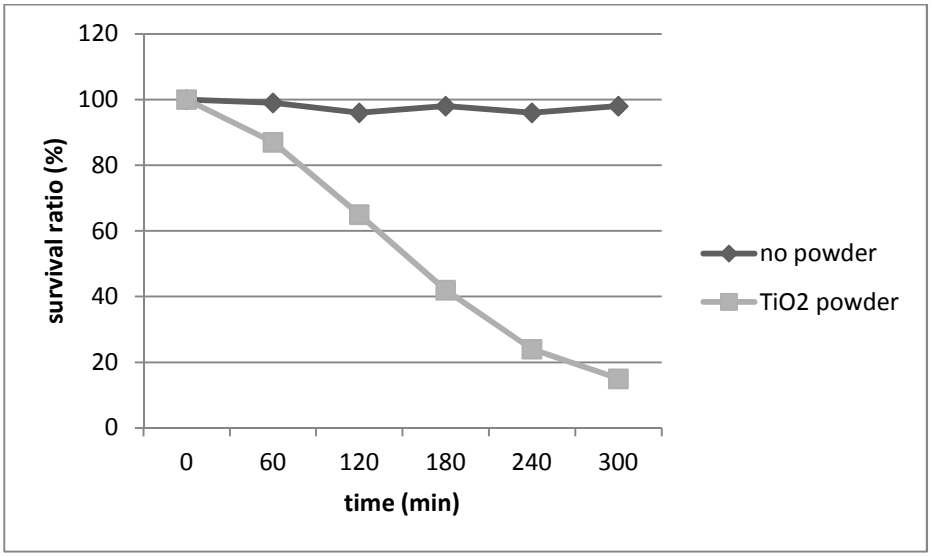


Figure 4.50 Survival ratio of *E.dermatitidis* under UV-Vis with TiO_2 powder containing and non -containing suspensions (initial number of *E.dermatitidis* was 317±9 CFUs/100 µl)

It was seen in Figure 4.50 especially after 4 hours the effect of TiO_2 powders was significant.

4.4.4.2. TiO_2 Coated Textiles

After 6 hours of illumination of TiO_2 coated and uncoated textile against *E.dermatitidis,* the results of photocatalytic effect were given Figure 4.51 and Figure 4.52. After 6 hours of illumination, the number of cells on the uncoated textile was 134 CFUs and the number of cells on the coated textile was 34 CFUs. This was given 75% decrease in the survival ratio of *E.dermatitidis* with the photocatalytic activity.

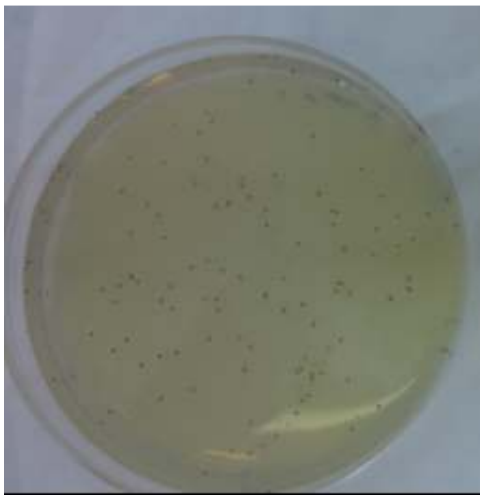


Figure 4.51 Number of *E.dermatitidis* cells survived on the uncoated textile (134 ±6 CFU/100 µl were counted)

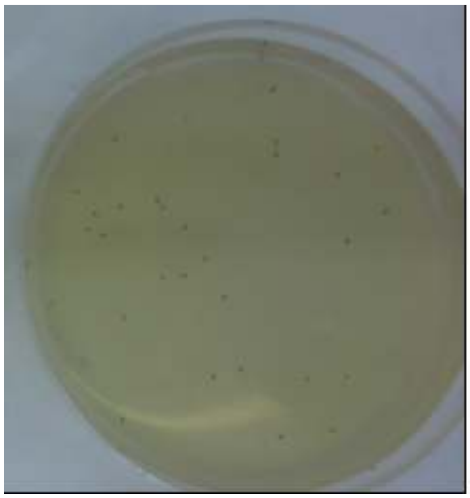


Figure 4.52 Number of *E.dermatitidis* cells survived on the TiO_2 coated textile (34 ±3 CFU/100 µl were counted)

4.4.4.3. Tungsten Doped TiO_2 coated Textile

Doped textile show efficiency against *E.dermatitidis* but with little difference from non doped textiles. It can be seen Figure 4.53 and Figure 4.54 the effect of tungsten coated textile after 6 hours of illumination, the number of cells on the uncoated textile was 136 CFUs and the number of cells on the coated textile was 33 CFUs. This was 76% decrease in the survival ratio of *E.dermatitidis*.

The reason of observing little difference by doping may be the higher repair rate of cell wall on the agar [Davis *et* al., 1973].

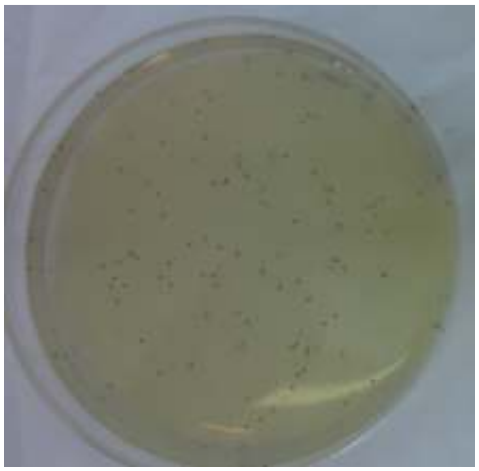


Figure 4.53 Number of *E.dermatitidis* cells survived on the textile that was coated with W doped TiO_2 (136±3 CFU/100 µl were counted)

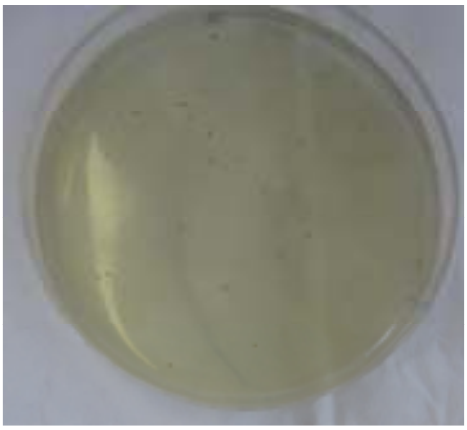


Figure 4.54 Number of *E.dermatitidis* cells survived on the textile that was coated with W doped TiO_2 (33 ±1 CFU/100 µl were counted)

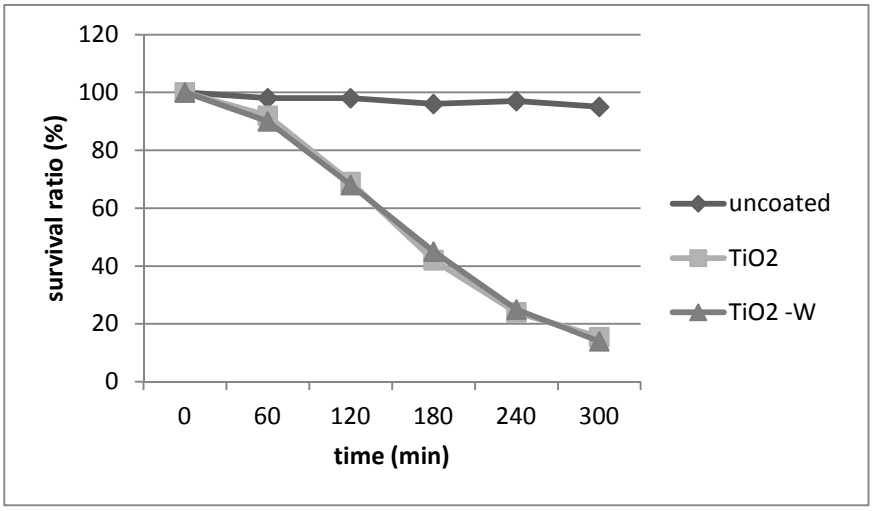


Figure 4.55 Survival ratio of *E.dermatitidis* under UV-Vis on the TiO_2 and TiO_2-W coated textiles (initial number of *E.dermatitidis* was 134 ±6 CFUs/100 µl)

4.4.4.4. Zinc and Tungsten Doped TiO_2 coated Textile

After 6 hours of illumination of zinc-tungsten doped TiO_2 coated and uncoated textile against *E.dermatitidis*, the results of photocatalytic effect were given Figure 4.56 and Figure 4.57. With 6 hours of illumination, the number of cells on the uncoated textile was 140 CFUs and the number of cells on the coated textile was 34 CFUs. This was 76% decrease in the survival ratio of *E.dermatitidis* due to photocatalytic activity. The results were similar with tungsten doped textiles. There was no significant difference form he non-doped textile.

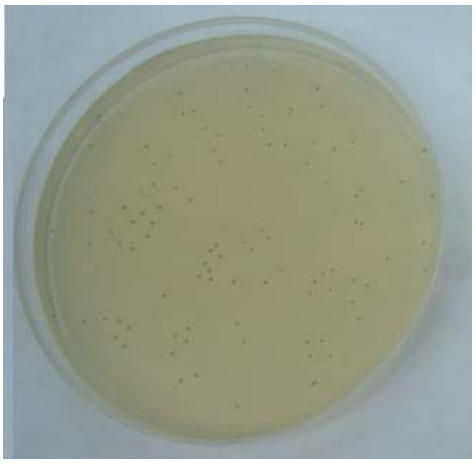


Figure 4.56 Number of *E.dermatitidis* cells survived on the textile that was uncoated (140 ±4 CFU/100 µl were counted)

Figure 4.57 Number of *E.dermatitidis* cells survived on the textile that was coated and doped with tungsten and zinc (34 ±2 CFU/100 µl were counted)

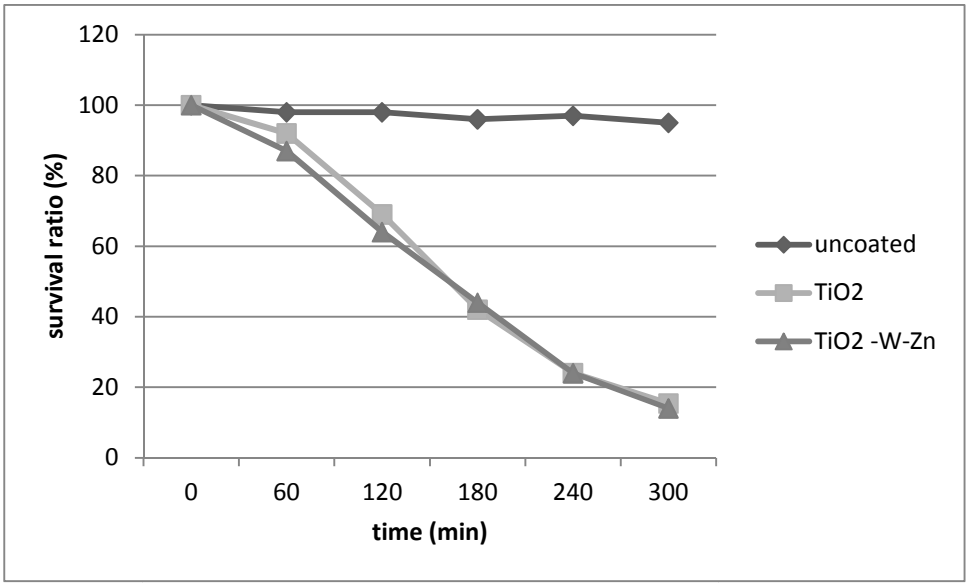


Figure 4.58 Survival ratio of *E.dermatitidis* under UV-Vis on the TiO_2 and TiO_2-W-Zn coated textiles (initial number of *E.dermatitidis* was 134±6 CFUs/100 µl)

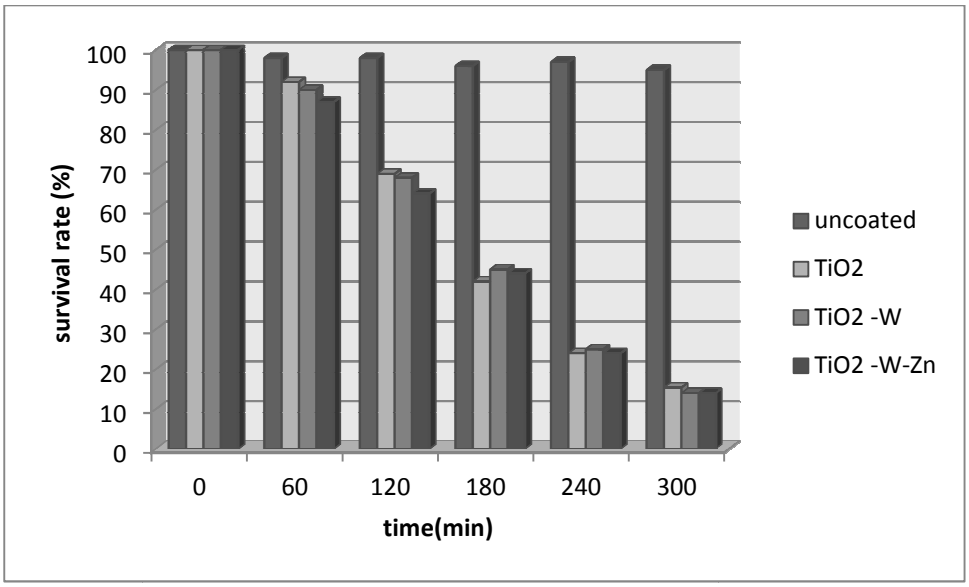


Figure 4.59 Survival rate of *E.dermatitidis* with 6 hours of illumination on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles.

In Figure 4.59, it can be seen that the doped textiles have not higher efficiency than the textile coated only with TiO_2 at 6 hours. But coated textile gave high inactivation for yeast.

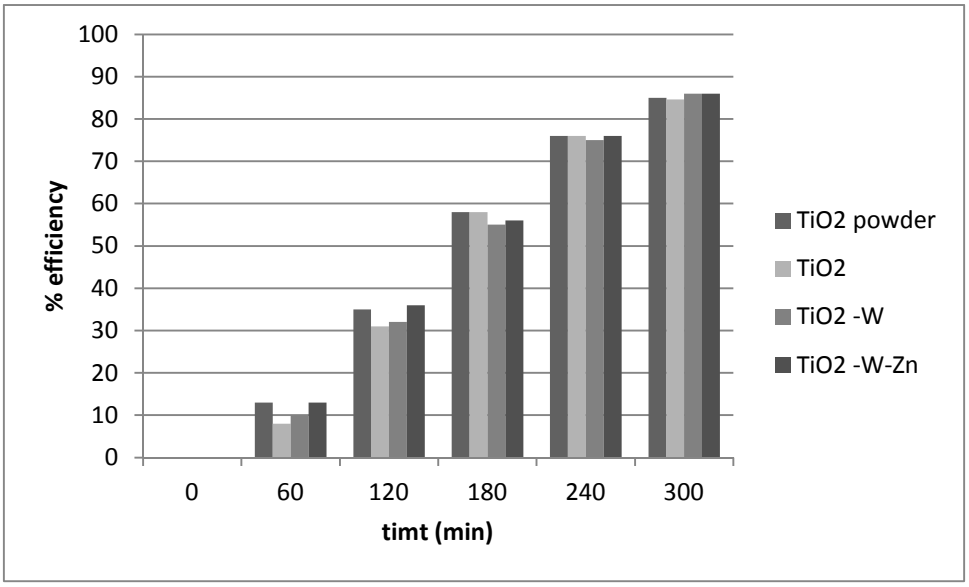


Figure 4.60 Efficiency of all tested TiO_2 coated materials against *E.dermatitidis*

Figure 4.60 summarized the results of all tested materials. Al materials showed close effects to each other. Addition of zinc to the tungsten doped textile did not change the efficiency.

4.4.5. Antimicrobial Effect Against of *Aspergillus niger*

4.4.5.1. Powder of TiO_2 Nanoparticles

For observing the effect of 0.1 % TiO_2 powder against *A.niger* spores were stirred at 4 hours. The results of TiO_2 powders photocatalytic effects were given Figure 4.61 and Figure 4.62. The number of cells in the no powder containing suspension was 456 CFUs and the number of cells in the powder containing suspension was 35 CFUs. This was 92% decrease in the survival ratio of *A.niger* spores due to photocatalytic activity.

Figure 4.61 Number of *A.niger* cells survived in the no powder containing suspension (456 ±11CFU/100 µl were counted)

Figure 4.62 Number of *A.niger* cells survived in the TiO_2 powder containing suspension (47 ±3 CFU/100 µl were counted)

When results was compared to 2 hours data of *E.coli,* it was observed that inactivation of *A.niger* spores take more time. However when 4 hours results of *S.aureus* and *A.niger* spores were compared it was seen that inactivation of Gr (+) bacteria was slightly easier than fungi. Nevertheless, when spores were considered it was harder to inactivate bacterial spores than fungal.

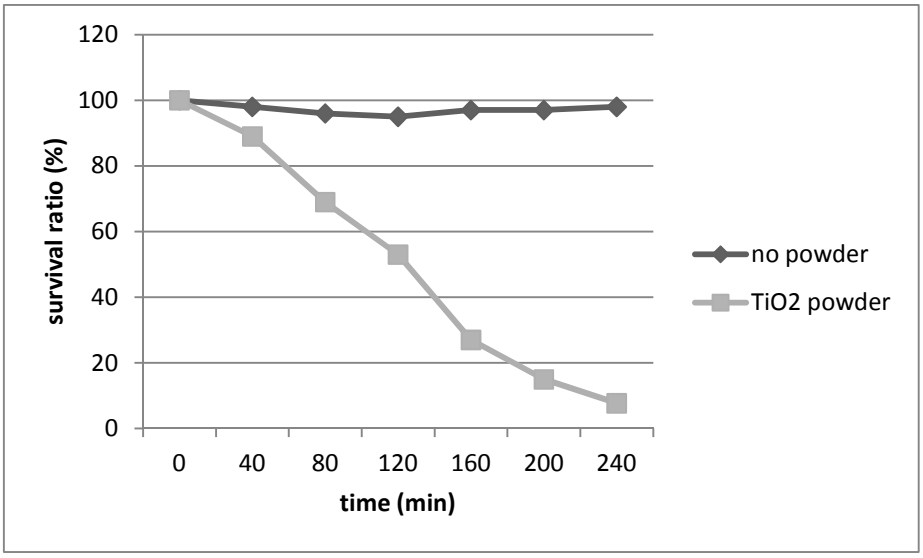


Figure 4.63 Survival ratio of *A.niger under* UV-Vis with TiO_2 powder containing and non -containing suspensions (initial number of *A.niger* was 456±11 CFUs/100 µl)

As seen in Figure 4.63, especially after 2 hours the effect of TiO_2 powders was significant. Nevertheless, high efficiency can be observed after 4 hours.

4.4.5.2. TiO_2 Coated Textiles

After 4 hours of illumination of TiO_2 coated and uncoated textile against *E.dermatitidis,* the results of photocatalytic effect were given Figure 4.64 and Figure 4.65. After 4 hours of illumination, the number of cells on the uncoated textile was 421 CFUs and the number of cells on the coated textile was 51 CFUs. This was given 88% decrease in the survival ratio of *A.niger* with the photocatalytic activity.

Figure 4.64 Number of *A.niger* cells survived on the uncoated textile (421 ±8 CFU/100 µl were counted)

Figure 4.65 Number of *A.niger* cells survived on the TiO_2 coated textile (51 ±3 CFU/100 µl were counted)

4.4.5.3. Tungsten Doped TiO_2 coated Textile

Doped textile show efficiency against *A.niger* but the difference from non doped textiles is not so much. However the ratio of inactivation of TiO2 coated textile was already high. It can be seen in the Figure 4.66 and Figure 4.67, the effect of tungsten coated textile after 4 hours of illumination, the number of cells on the uncoated textile was 432 CFUs and the number of cells on the coated textile was 38 CFUs. This was 90 % decrease in the survival ratio of *A.niger*.

The reason of observing little difference by doping may be the higher repair rate of cell wall on the agar [Davis *et* al.,1973].

Figure 4.66 Number of *A.niger* cells survived on the textile that was coated with W doped TiO_2 (432±4 CFU/100 µl were counted)

Figure 4.67 Number of *A.niger* cells survived on the textile that was coated with W doped TiO_2 (35±2 CFU/100 µl were counted)

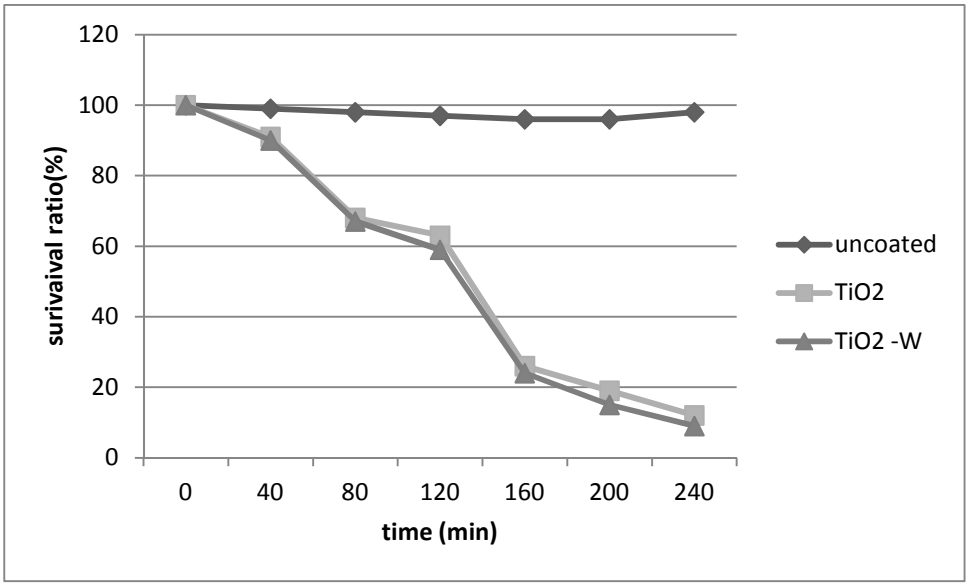


Figure 4.68 Survival ratio of *A.niger* under UV-Vis on the TiO_2 and TiO_2-W coated textiles (initial number of *A.niger* was 421 ±4 CFUs/100 µl)

4.4.5.4. Zinc and Tungsten Doped TiO_2 coated Textile

After 4 hours of illumination of zinc-tungsten doped TiO_2 coated and uncoated textile against *A.niger* the results of photocatalytic effects were given Figure 4.69 and Figure 4.70. With 4 hours of illumination, the number of cells on the uncoated textile was 402 CFUs and the number of cells on the coated textile was 40 CFUs. This was 90% decrease in the survival ratio of *A.niger* due to photocatalytic activity. The results were similar with tungsten doped textiles. There was not any significant differences form non doped textile.

Figure 4.69 Number of *A.niger* cells survived on the textile that was uncoated (402 ±5 CFU/100 µl were counted)

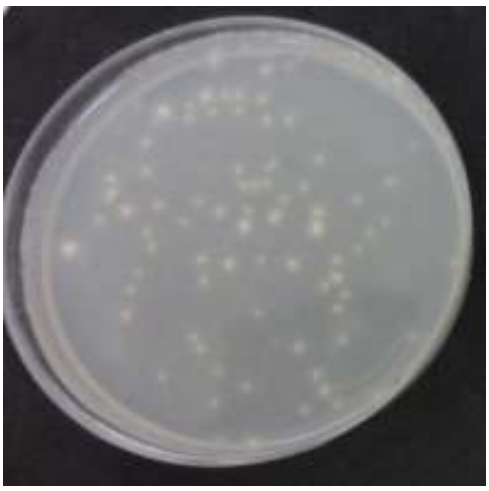


Figure 4.70 Number of *A.niger* cells survived on the textile that was coated and doped with tungsten and zinc (40 ±2 CFU/100 µl were counted)

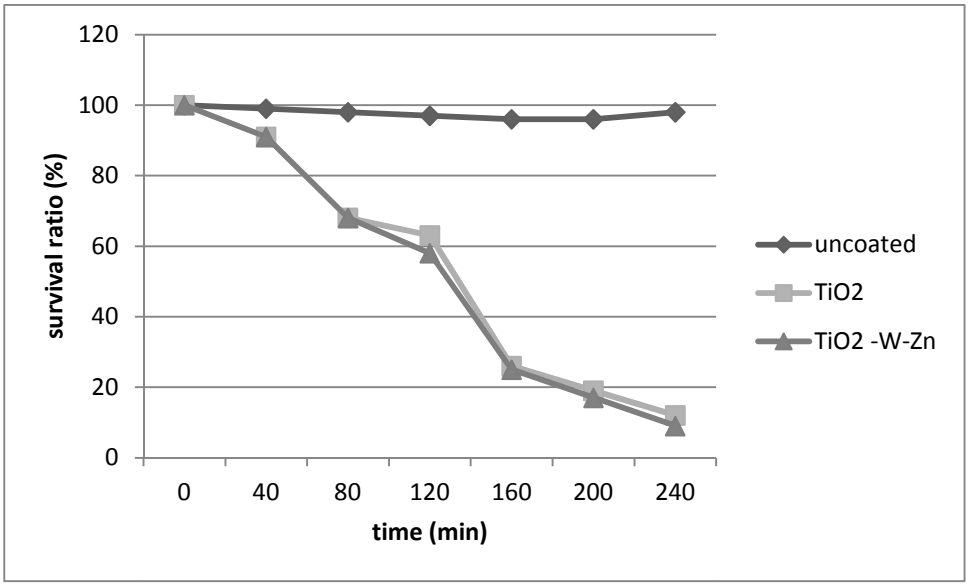


Figure 4.71 Survival ratio of *A.niger* under UV-Vis on the TiO_2 and TiO_2-W-Zn coated textiles (initial number of *A.niger* was 421±4 CFUs/100 µl)

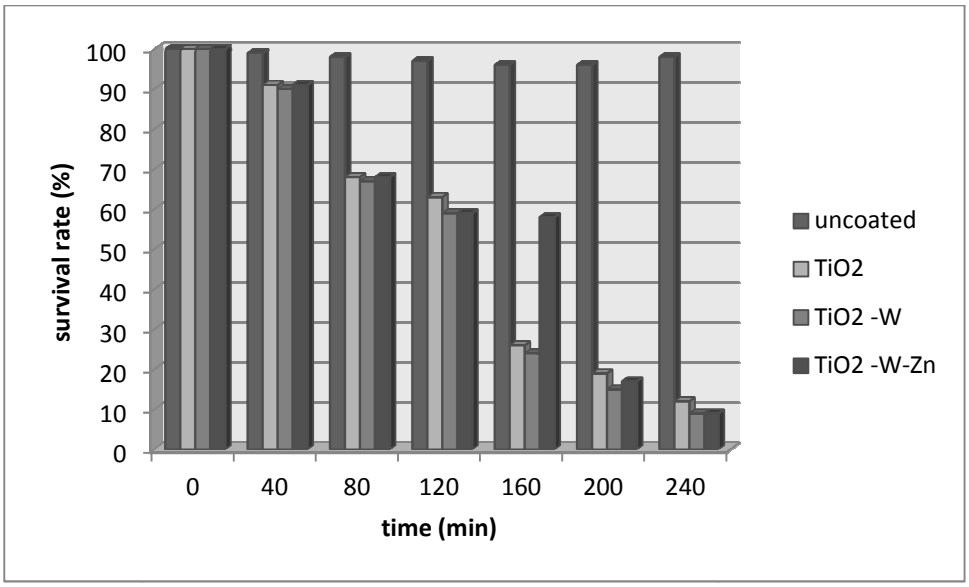


Figure 4.72 Survival rate of *A.niger* with 4 hours of illumination on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles.

In Figure 4.71 and Figure 4.72, it can be seen that the doped textiles have not higher efficiency than the textile coated only with TiO_2 at 6 hours. But coated textile gave high inactivation for fungi.

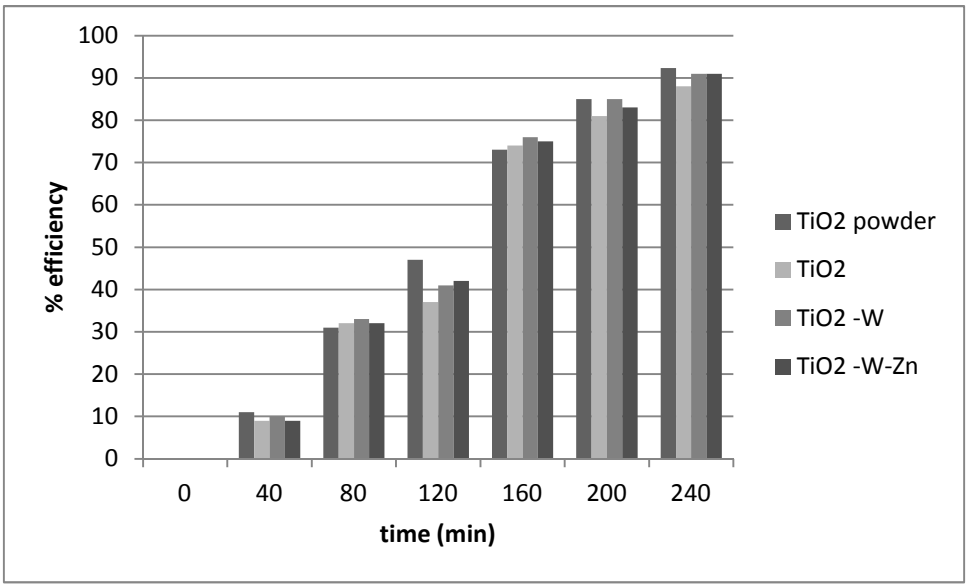


Figure 4.73 Efficiency of all tested TiO_2 coated materials against *A.niger* spore

In Figure 4.73 it can be seen powder showed the highest effect against *A.niger* spore and doping of textile caused increase in the efficiency.

In summary *E.coli, S.aureus, B.atrophaeus* spores, *E.dermatitidis* and *A.niger* spores were illuminated with TiO_2 powder, TiO_2 coated textiles and doped TiO_2 coated textiles.

It was observed that the antimicrobial efficiency of TiO_2 powder and coated textiles against different organisms is as follows: *E.coli* > *S.aureus*> *A.niger* spores > *E.dermatitidis* > *B.atrophaeus* spores.

Since *E.coli* is a Gr (-) bacteria it has a thin cell wall and it has periplasmic space, *S.aureus* is a Gr (+) bacteria so its cell wall is thicker, so it has more strength. As seen in the results for inactivation of *S.aureus* more time was needed than *E.coli* for both powder and textile applications. When it is considered that the first step of inactivation is decomposition of cell wall, it can be claimed that gram positives has more time for inactivation than gram negatives [Kühn *et* al., 2003]. However powder and textiles were not shown significant effects on *B. atrophaeus* spores. It was stated that bacterial spores

can handle with environmental stress conditions since they do not have an active metabolism [Lee *et* al., 2005]. Similar information is stated at different studies such as the reason of high resistance of bacterial spores is slow kinetic of inactivation [Vohra *et* al., 2005].

For *A.*niger spores, the results showed that 4 hours was enough to kill spores. It has a cell wall also, so it was harder to kill *A.niger* spores than *E.coli.* The response of fungi spores against irradiation is different from the response of bacterial spores due to difference between morphology, cytology, reproductive cycle and growth mechanism. However, bacterial spores show more resistance to irradiation than fungal spores [Wellheaiser *et* al., 1992]. A similar situation was observed in our experiments.

For *E. dermatitidis* 6 hours was needed for both powder and textiles. It is a fungi type, so it has a cell wall. However yeasts can protect themselves by producing capsule [Marshall Cavendish Corporation, 2008].

As seen in the results the higher photocatalytic effect of doped textiles at 2 hours was observed against *E.coli.* It was due to its simple cell wall structure. W doped textile causes 8.5% increase and W-Zn doped textile showed a 15% increase during 2 hours against *E.coli* when compared to non-doped coated textile. However, W doped textile alone did not change the effect of photocatalytic activity for other microorganisms. The effect of doping against cell wall containing organisms was almost same. W doped textile did not affect so much *E.dermatitidis, S.aureus,* and *A.niger* spore. There was an increase about 1-2% for cell wall containing organisms and addition of zinc to the tungsten coated textile did not change the increasing amount. However, there was no change for *B.atrophaeus* spore.

In all photocatalytic experiments, the higher effects were obtained against *E.coli*. Formation of uniformly distributed W and Zn ions on the coated surface, increase the conductivity so as antimicrobial efficiency. The effect of 2 hour illumination for all tested textiles can be seen in Figure 4.74.

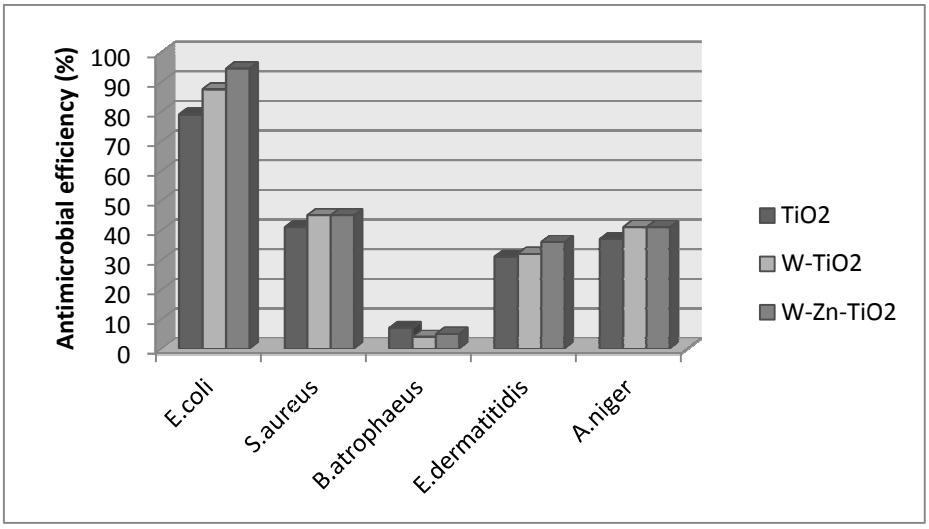


Figure 4.74 Comparison of antimicrobial efficiencies of TiO_2 coated doped and non-doped textiles against different microorganisms for 2 hours under illumination

The results of powders antimicrobial efficiency against all organisms for 1 hour and 2 hours can be seen in Figure 4.75.

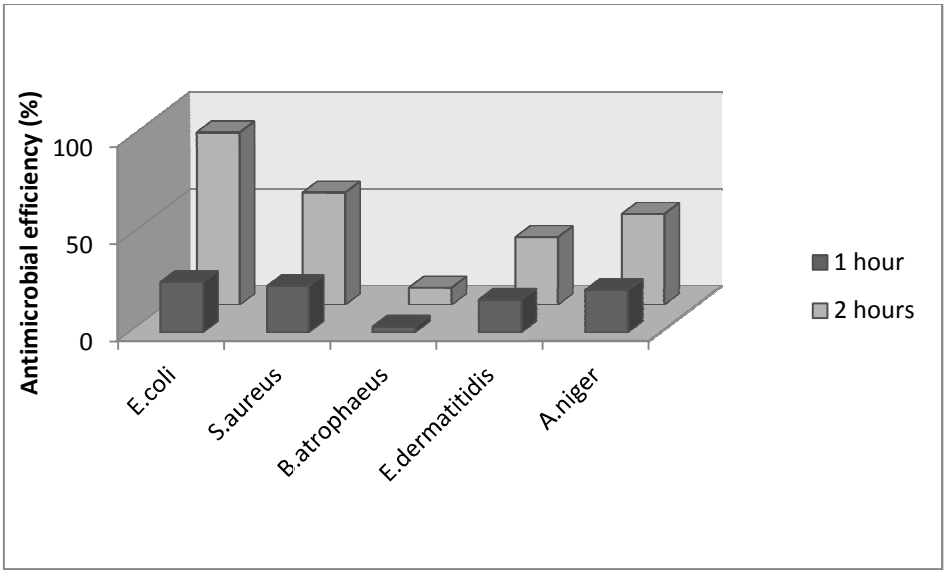


Figure 4.75 Comparison of antimicrobial efficiencies of TiO_2 powder against different microorganisms for 1 hour and 2 hours under illumination

4.5. Antimicrobial Effect of the Silver Zeolite Particles

4.5.1. Antimicrobial Effect Against *Escherichia coli*

After the 100 μg/ml silver zeolite containing and 100 μg/ml zeolite containing plates were incubated with *E.coli*, the antimicrobial effect's results were given Figure 4.76 and Figure 4.77. After overnight growth on the zeolite containing plates the cell number was 740 and the number of cells on the silver zeolite containing plates were 22 CFUs. This was 97% decrease in the survival ratio of *E.coli*, due to antimicrobial effect.

Figure 4.76 Number of *E.coli* cells survived on the 100 μg/ml zeolite containing plate [740±6 CFU/100 μl]

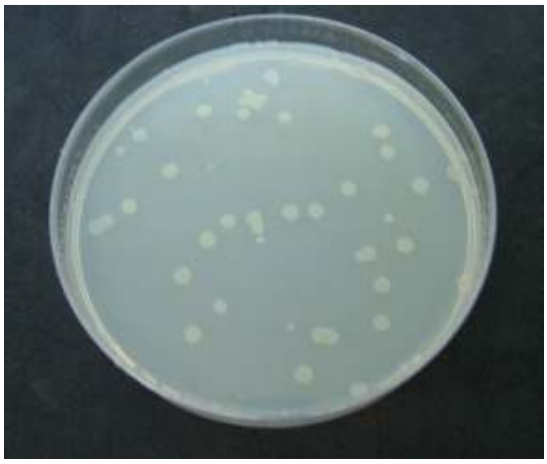


Figure 4.77 Number of *E.coli* cells survived on the 100 µg/ml silver zeolite containing plate [22±4 CFU/100 µl]

4.5.2. Antimicrobial Effect against *Salmonella Infantis*

For comparing effect of silver zeolite, it was tested on same type of organism. *S.infantis* is a Gr (-) type bacteria, however, higher amount of silver zeolite was required for obtaining high effect on *S.infantis.* By using 1000 µg/ml of silver zeolite 97 % decreasing effect was observed. After overnight growth on the zeolite, containing plates was 1050 and the number of cells on the silver zeolite containing plates were 32 CFUs. The results are shown in Figure 4.78and Figure 4.79.

Figure 4.78 Number of *S.infantis* cells survived on the 1000 µg/ml zeolite containing plate [1050±11 CFU/100 µl]

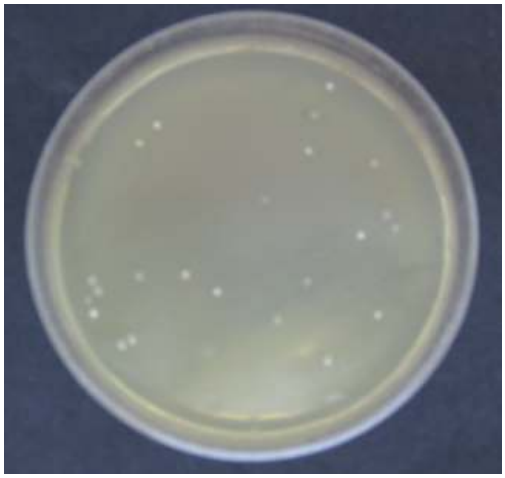


Figure 4.79Number of *S.infantis* cells survived on the 1000 μg/ml silver zeolite containing plate [32± 3 CFU/100 μl]

When results were compared with *E.*coli, it was seen that *Salmonelle* species needed more silver nanoparticles. In some studies it was reported that *Salmonella* species can show resistance against silver ions. The first known example of this situation was reported from Massachusetts General Hospital [Gupta *et* al., 2001].

Some bacteria, belong to *Salmonella* species can contain plasmid; generally it was pMG101, that have silver resistance. This plasmid has siiE gene, which has a code for a Ag(I) binding protein and an Ag efflux pump system; SilCBA and SilP, these are chemiosmotic efflux pump and ATPase efflux pump [Silver, 2006].

4.5.3. Antimicrobial Effect against *Staphylococcus aureus*

After the 200 μg/ml, silver zeolite containing and 200 μg/ml zeolite containing plates were incubated with *S.aureus* the antimicrobial effect's results were given in Figure 4.80 and Figure 4.81. After overnight growth on the zeolite, containing plates was 820 and the number of cells on the silver zeolite containing plates was 266 CFUs. This was 67, 5% decrease in the survival ratio of *S.aureus*, due to antimicrobial effect.

Figure 4.80 Number *S.aureus* of cells survived on the 200 μg/ml zeolite containing plate [820± 7 CFU/100 μl]

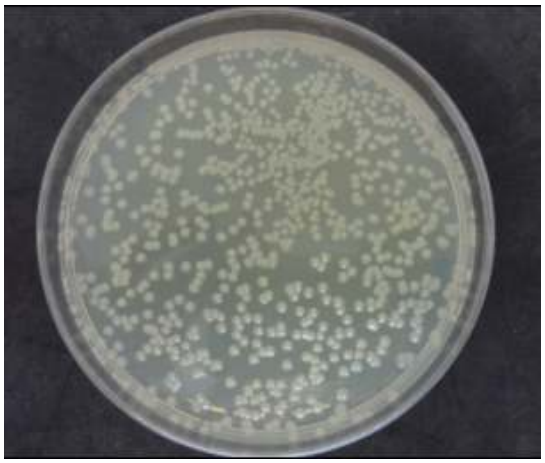


Figure 4.81 Number of *S.aureus* cells survived on the 200 μg/ml silver zeolite containing plate [266±5 CFU/100 μl]

As mentioned before gram positive bacteria contain cell wall. The cell wall contains peptidoglycan. These peptidoglycan have negative charge, so they can bind positive silver ions. As a result, Gr (+) bacteria show more resistance against silver than Gr (-) ones [Monteiro *et* al., 2009].

4.5.4. Antimicrobial Effect against *Bacillus atrophaeus* spores

After the 500 μg/ml silver zeolite containing and 500 μg/ml zeolite containing plates were incubated with *B.atrophaeus* spores, the antimicrobial effect's results were

given in Figure 4.82 and Figure 4.83. After overnight growth on the zeolite, containing plates was 740 and the number of cells on the silver zeolite containing plates was 83 CFUs. This was 90% decrease in the survival ratio of *B.atrophaeus* spores, due to antimicrobial effect.

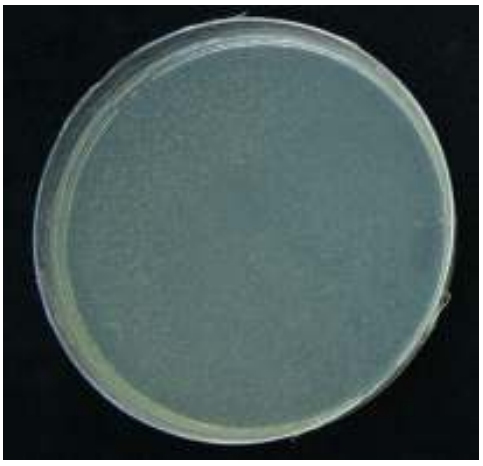


Figure 4.82 Number *B.atrophaeus* of spores survived on the 500 µg/ml zeolite containing plate [833±6 CFU/100 µl]

Figure 4.83 Number of *B.atrophaeus* spores survived on the 500 µg/ml zeolite containing plate [83±4 CFU/100 µl]

In *B.atrophaeus* spores different from killing, also bacteriostatic effect was observed. The cells are smaller than normal cells.

4.5.5. Antimicrobial Effect against *Exophiala dermatitidis*

After the 500 µg/ml silver zeolite containing and 300 µg/ml zeolite-containing plates were incubated with *E.dermatitidis,* the antimicrobial effect's results were given Figure 4.84 and Figure 4.85. After overnight growth on the zeolite, containing plates was 554 and the number of cells on the silver zeolite containing plates was 0 CFUs. This was 100% decrease in the survival ratio of *E.dermatitidis*, due to antimicrobial effect.

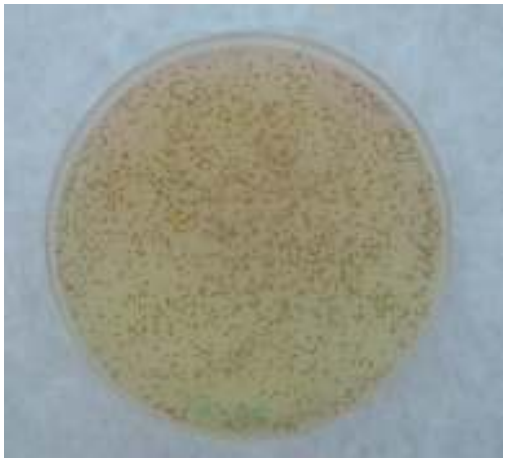


Figure 4.84 Number *E.dermatitidis* cells survived on the 300 µg/ml zeolite containing plate [544± 3 CFU/100 µl]

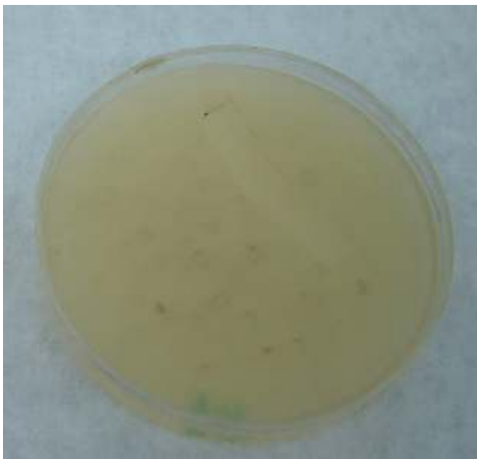


Figure 4.85 Number of *E.dermatitidis* cells survived on the 300 µg/ml silver zeolite containing [0±0 CFU/100 µl]

4.5.6. Antimicrobial Effect Against *Aspergillus niger* spores

After the 400 µg/ml silver zeolite containing and 400 µg/ml zeolite-containing plates were incubated with *A.niger* spores, the antimicrobial effect's results were given Figure 4.86 and Figure 4.87. After overnight growth on the zeolite, containing plates was 355 and the numbers of cells on the silver zeolite containing plates were CFUs. This was 100% decrease in the survival ratio of *A.niger* spores, due to antimicrobial effect.

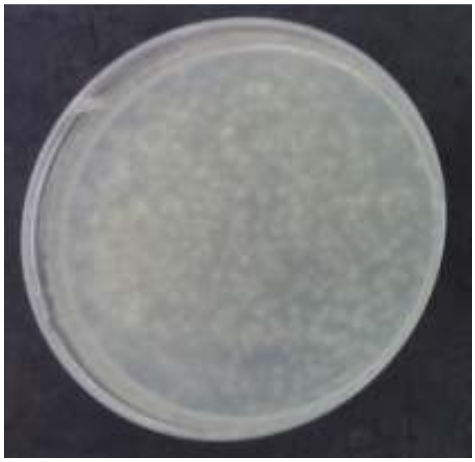


Figure 4.86 Number of *A.niger* spores survived on the 400 µg/ml silver zeolite containing [355±4 CFU/100 µl]

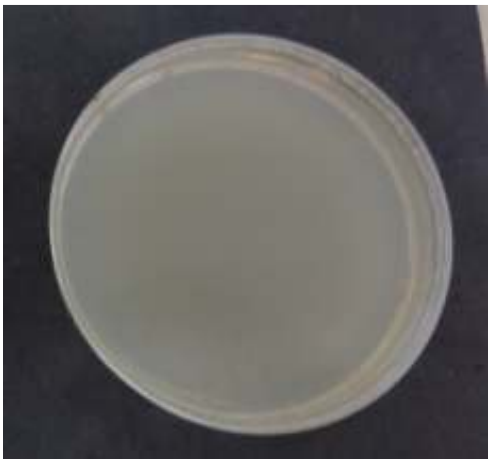


Figure 4.87 Number of *A.niger* spores survived on the 400 µg/ml silver zeolite containing [0±0 CFU/100 µl]

The result of silver zeolite was similar withTiO_2. Different concentrations showed effect against organisms. The minimum amount was affected against *E.coli* due to it is a Gr (-) microorganism. 100 µg/ml silver zeolite exhibited 97% antimicrobial efficiency. 200 µg/ml of silver zeolite killed 67.5% of *S.aureu.*cells. According to our experimental resulte the antimicrobial efficiency is as follows: *E.dermatitidis* >*A.niger* spores> > *B.atrophaeus* spores.

Similar reasons as mentioned before, could have affected the results. Spores needed more particles to be killed and cell wall containing microorganisms needs more silver zeolite than non-containing one. Different from titanium, silver showed more effect on *E.dermatitidis*. 300 µg/ ml Ag zeolite was enough for obtaining 100 % effect, but this effect was observed with 400 µg/ml Ag zeolite against *A.niger*. For titanium, it was said that yeast can capsule sometimes under stress conditions. However if it did not produce capsule, it was easier to kill yeast than killing fungi spores.

In Figure 4.88 it the sensitivity of different organisms to different silver zeolite concentrations are given.

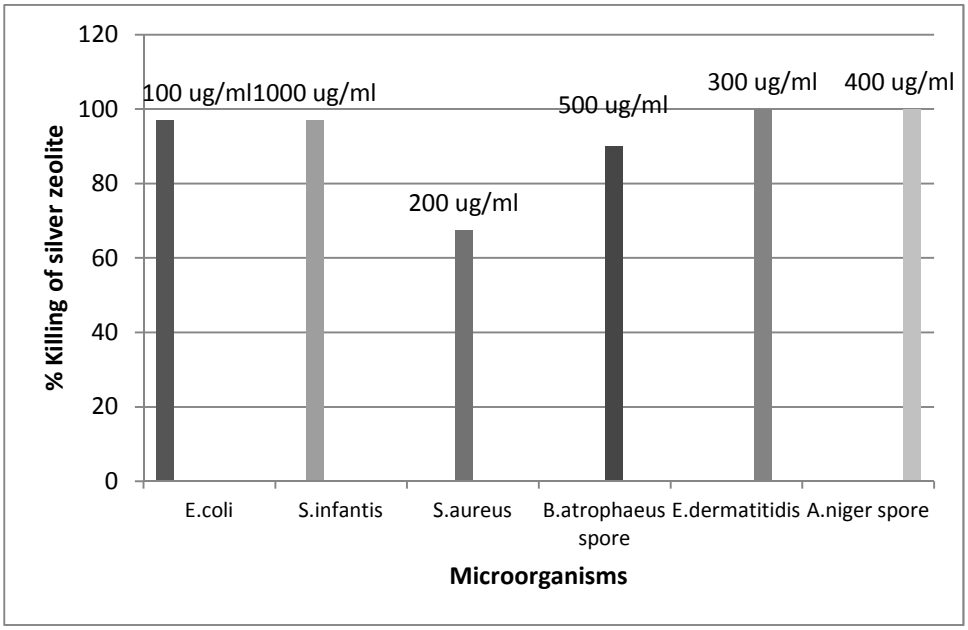


Figure 4.88 The necessary amount of silver zeolite for killing different microorganisms.

4.6. Photodegradation Effect Test

For observing photodegradation, effect of TiO_2 methylene blue dye was used. The results of the experiments were given in Figure 4.89, Figure 4.90, Figure 4.91, Figure 4.92 and Figure 4.93 from 0 minutes to 360 minutes.

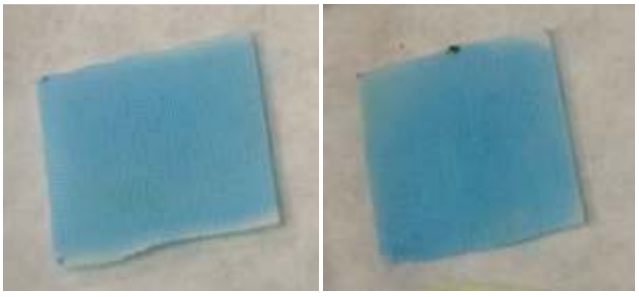


Figure 4.89 The result of methylene blue dyed uncoated and coated textile after 0 minute illumination

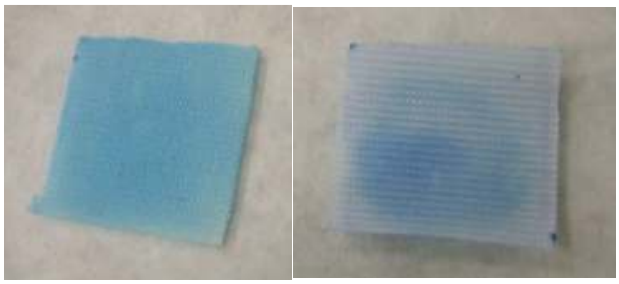


Figure 4.90 The result of methylene blue dyed uncoated and coated textile after 60 minutes illumination

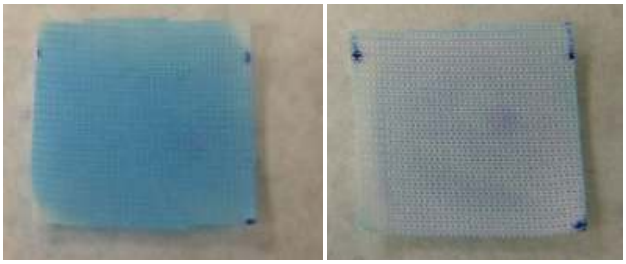


Figure 4.91 The result of methylene blue dyed uncoated and coated textile after 120 minutes illumination

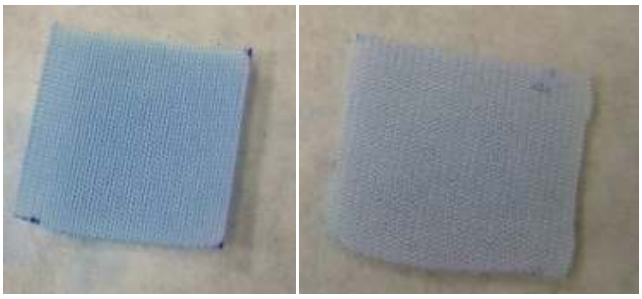


Figure 4.92 The result of methylene blue dyed uncoated and coated textile after 240 minutes illumination

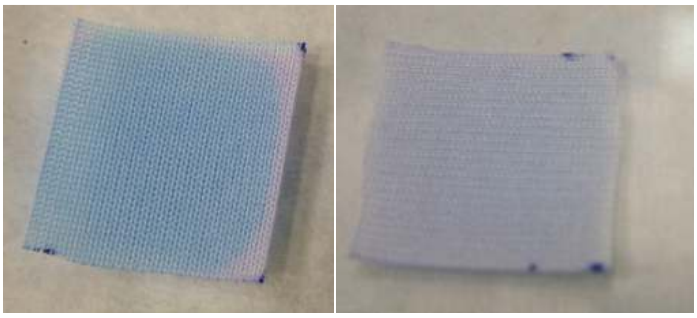


Figure 4.93 The result of methylene blue dyed uncoated and coated textile after 360 minutes illumination

As seen in the figures the color start bleaching at 60 minutes. However, after 4 hours the color of uncoated textile showed bleaching due to evaporation of dye.

Bleaching of dye can be occurred due to active oxygen species that are created at the titanium dioxide surface and transferred in the gas phase [Tatsuma *et* al., 2009]. In the Figure 4.94, the degradation mechanism of TiO_2 was shown.

$$\text{dye} + h\upsilon \rightarrow \text{dye}^* \quad (1)$$

$$\text{dye}^* + TiO_2 \rightarrow \text{dye}^{+\bullet} + TiO_2(e) \quad (2)$$

$$TiO_2(e) + O_2 \rightarrow TiO_2 + O_2^{-\bullet} \quad (3)$$

$$O_2^{-\bullet} + TiO_2(e) + 2H^+ \rightarrow H_2O_2 + TiO_2 \quad (4)$$

$$2O_2^{-\bullet} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (5)$$

$$H_2O_2 + TiO_2(e) \rightarrow {}^{\bullet}OH + OH^- + TiO_2 \quad (6)$$

$$\text{dye}^{+\bullet} + (O_2^{-\bullet} \text{ or } {}^{\bullet}OH) \rightarrow \text{the degraded products} \quad (7)$$

Figure 4.94 Photoreaction mechanism of degradation of dye under visible irradiation [Chen *et* al., 2001]

In aqueous solutions, methylene blue can be totally mineralized with circulation over TiO_2 films under illumination. The destruction rate obeys the first order kinetics, but rate constant decreases with the solutes initial concentration [Matthews, 1989].

Different studies showed that TiO_2 shows photocatalytic activity under UVA light and can degrade organic dye methylene blue. Also, if the amount of TiO_2 is high, that could decrease the degradation of methylene blue. In same study the results claimed that degradation of methylene blue was maximum after 20 minutes under light [Wang *et* al., 2013].

In the experiments when uncoated and coated textiles were compared the great difference between colors can be seen after 60 minutes. Between 4 hours and 6 hours, there was not much difference in the color of coated textiles

4.7. Enzyme Inhibition Test

Peroxidase enzyme catalyzes the reaction of oxidation of different substrates by help of hydrogen peroxide. This enzyme can play important roles in the cell growth, cell differentiation, cell wall component polymerization and secondary metabolites oxidation [Dequaire *et* al., 2002].

For understanding if TiO_2 coated textiles can degrade proteins thus inhibit the enzyme activity, peroxidase enzyme was used at 2 units concentration (Appendix C). The results of the study were represented in Figure 4.95, Figure 4.96, Figure 4.97, Figure 4.98 and Figure 4.99.

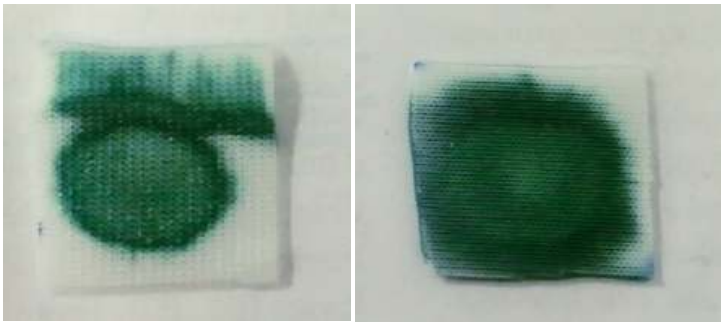


Figure 4.95 The effect of illumination of uncoated and TiO_2 coated textile on enzyme activity at 0 minutes.

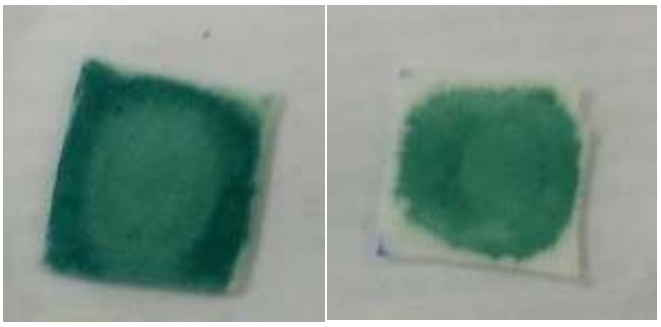


Figure 4.96 Effect of illumination of uncoated and TiO_2 coated textile on enzyme activity at 60 minutes.

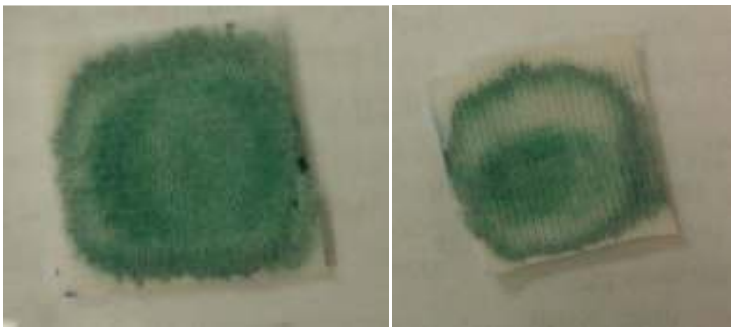


Figure 4.97 Effect of illumination of uncoated and TiO_2 coated textile on enzyme activity at 120 minutes.

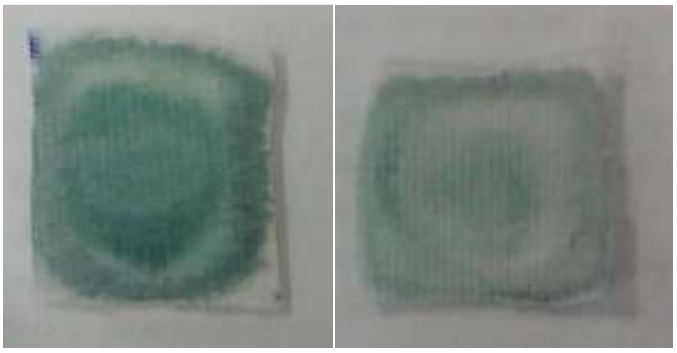


Figure 4.98 Effect of illumination of uncoated and TiO_2 coated textile on enzyme activity at 240 minutes.

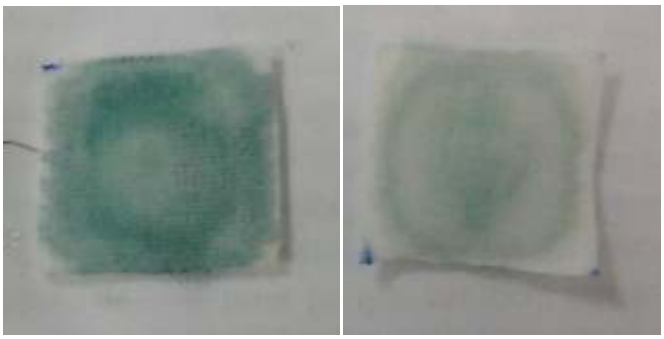


Figure 4.99 Effect of illumination of uncoated and TiO_2 coated textile on enzyme activity at 360 minutes.

TiO_2 can inhibit the enzyme activity by showing photocatalytic effect. In the study after illumination we added substrate to the enzyme, if enzyme was working correctly; it was waited for color change as a result of enzyme activity. Starting from 1 hour, the color turned lighter tones because the decrease of enzyme activity. Especially after 6 hours there was a distinct difference between coated and uncoated textiles. These results clearly show that the coated textiles can degrade the proteins. This result is an indication that, the coated textiles would exhibit antiviral activity.

Effect of TiO2 on enzymatic activity has been studied in the literature. It was stated that soil enzyme activities such as protease, peroxides was lower in the TiO_2 containing soils, than control soils [Du *et* al.,2011]. It was studied that the effect of TiO_2 nanoparticles on pepsin enzyme. The results showed that nanoparticles were affected the polarity of the enzyme not to change in the sequence integrity. Also α- helix and β-turns were disappeared and there was a decreasing in the fraction of β sheets [Zhu *et* al., 2010]. Similarly, TiO_2 has decreasing activity against the peroxidase enzyme belongs to the fish species by causing oxidative stress and creating antioxidant depletion [Kartigarani *et* al., 2012].

As a brief conclusion, TiO_2 nanoparticles and coated textiles affected all tested organisms the results can be shown in Table 4.4 and Table 4.5.

Table 4.4 Effect of TiO_2 nanoparticles against all tested organisms

Microorganisms	**Time**	**Decreasing**
E.coli	2 hours	100 %
S.aureus	4 hours	100 %
B.atrophaeus spore	10 hours	89 %
E.dermatitidis	6 hours	85 %
A.niger spore	4 hours	92%

Table 4.5 Effect of TiO_2 coated textiles against all tested organisms

Microorganisms	**Time**	**Decreasing**		
		TiO2 coated textile	W doped TiO2 coated textile	W-Zn doped TiO2 coated textile
E.coli	2 hours	79 %	87,5 %	94,5 %
S.aureus	4 hours	99 %	99 %	99 %
B.atrophaeus spore	10 hours	98 %	19%	25 %
E.dermatitidis	6 hours	75 %	76 %	76 %
A.niger spore	4 hours	88 %	90%	90 %

Silver zeolite has shown antimicrobial effect against all tested organisms also, the results were shown in Table 4.6.

Table 4.6 Effect of silver zeolite

Microorganisms	**Amount**	**Decreasing**
E.coli	100 µg/ml	97 %
S.infantis	1000 µg/ml	97 %
S.aureus	200 µg/ml	67,5 %
B.atrophaeus spore	500 µg/ml	90 %
E.dermatitidis	300 µg/ml	*100* %
A.niger spore	400 µg/ml	*100* %

In self cleaning experiment, we determined the time as 60 minutes, needed for observing photodegradation. For inhibition of enzyme inactivation, 60 minutes was enough, also. After 60 minutes the color started bleaching due to decreasing interaction between enzyme and substrate.

CHAPTER 5

CONCLUSION

This study was focused on the preparation of antimicrobial particles (TiO_2 powder and silver loaded zeolite), TiO_2 coated textiles, tungsten doped TiO2 coated textile, tungsten and zinc doped TiO_2 coated textiles. Photocatalytic effect of the above mentioned material was studied by various methods, such as: antimicrobial effect against microorganisms, enzyme inhibition effect and photodegradation effect. The conclusions were as follows:

- Powder TiO_2 had better antimicrobial effect against organisms than textiles.
- Coated textiles showed better antimicrobial effect against microorganisms than uncoated textiles, except *B.atrophaeus* spores.
- The antimicrobial efficiency against different microorganisms was as follows: *E.coli (*gram negative) >*S.aureus* (gram positive)> *A.niger* spores (fungi; mold) > *E.dermatitidis (fungi; yeast)s*> *B.atrophaeus* spores (gram positive).
- All tested materials exhibited the most efficient effect against *E.coli*.
- Coated textile with tungsten and zinc doped TiO2 showed the most efficient antimicrobial activity against *E.coli*, 95%, *S.aureus* 45 %, *E.dermatitidis* 36 %, *A.niger* 41 % and *B.atrophaeus* spores 9 % during 2 hours of illumination.
- The self cleaning, photodegradation, activity for methylene blue dye, 1 hour was enough for observing the degradation activity.
- The inhibition of enzyme peroxides was achieved with starting from 1 hour illumination with coated textile.
- Silver loaded zeolite particles exhibit a very efficient antimicrobial activity against different species of microorganism thus the antimicrobial effect was observed at different silver zeolite particle concentrations.
- For silver zeolite nanoparticles, same type of organisms (gram negative bacteria) can be affected with very different concentrations.

CHAPTER 6

RECOMMENDATION

In this study it was shown that sterilization of surfaces is possible by using a light-guiding material which consists of a semiconductor, so stimulated with direct UVA.

- As future study for understanding, the effect of photocatalysts better highly organized cells should be studied such as algae.
- The clear effect of doping with metal (W and Zn), it can be studied with other metals as dopant.
- Different surfaces such as glasses or ceramics can be coated with semiconductor for investigation of effect on different surfaces and developing antimicrobial specialty on different surfaces.
- The effect of microbial cell concentration should be studied for understanding if it is possible to inactivate cells at high concentration.
- The effect of bleaching effect on other dyes can be studied
- Silver can be combined with different materials for improving activity.
- Antiviral efficiency of the similar particles can be studied.

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APPENDIX A

MEDIUM AND AGAR BASES

1. **Luria Bertani (LB) Br**oth

   Basis: 1 liter (in distilled water)

   Luria Bertani Broth (Merck), 25 g

   pH: 7.0

2. **Luria Bertani (LB) Agar**

   Basis: 1 liter (in distilled water)

   Luria Bertani Broth (Merck), 25 g

   Agar-Agar (Applichem), 15 g

   pH:7.0

3. **Tryptic Soy (TS) Broth**

   Basis: 1 liter (in distilled water)

   Tryptic Soy Broth (Merck), 30 g

   pH: 7.3

4. **Tryptic Soy (TS) Agar**

   Basis: 1 liter (in distilled water)

   Tryptic Soy Broth (Merck), 30 g

   Agar-Agar (Applichem), 15 g

   pH:7.3

5. Nutrient Agar Yeast Salt Mixture (NYSM) Agar

Basis: 1 liter (in distilled water)

Nutrient Agar (Merck), 23 g

Yeast Extract (Merck) 0,500 g

Agar-Agar (Applichem), 5 g

Salt mixture, 5 ml

Contains;

Basis: 500 ml (in distilled water)

- $CaCl_2$ x $2H_2O$ (Applichem), 10 gram
- $MgCl_2$ x $6H_2O$ (Applichem), 20,35 gram
- $MnCl_2$ x $4H_2O$ (Applichem), 1 gram

pH:7.5

6. Yeast Extract Peptone Dextrose (YPD) Broth

Basis: 1 liter (in distilled water)

Yeast Extract (Merck), 10 g

Peptone from casein (Merck), 20 g

D-Glucose (Merck) 20 g

pH:6.0

7. Potato Dextrose (PD) Broth

Basis: 1 liter (in distilled water)

Potato dextrose broth (Sigma-Aldrich), 24 g

pH: 5.6

8. Potato Dextrose (PD) Agar

Basis: 1 liter (in distilled water)

Potato dextrose agar (Merck),

pH: 5.6

APPENDIX B

SAMPLE CALCULATIONS

B.1.Calculations for TiO_2 powder.

The molecular weights of species necessary for the calculations are shown in Table B.1.

Table B.1 Molecular Weights of Reactants

Specie	Molecular Weight (g/mol)
Titanium iso-porpoxide [$Ti[OCH(CH_3)_2]_4$] (Sigma-Aldrich)	284.22
Acetic Acid [$C_2H_4O_2$] (Norateks Kimya)	60.05
Nitric Acid [HNO_3] (Tekkim)	63,01
Water [H_2O] (Arıteks)	18,02

Basis: 0.2 mol $[Ti[OCH(CH_3)_2]_4]$

n of $Ti[OCH(CH_3)_2]_4$ = m of $Ti[OCH(CH_3)_2]_4$/ Mwt of $Ti[OCH(CH_3)_2]_4$

0,2 =m of $Ti[OCH(CH_3)_2]_4$/ 284,22

m of $Ti[OCH(CH_3)_2]_4$ =56,8 g

n of $C_2H_4O_2$ = m of $C_2H_4O_2$/ Mwt of $C_2H_4O_2$

0.2= m of $C_2H_4O_2$/ 60,5

m of $C_2H_4O_2$ = 12,1 g

APPENDIX C

ENZYME AND PHOTOCATALYTIC TEST CHEMICALS

1. **ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)**

   Basis: 100 ml (in distilled water)

   ABTS (AppliChem), 35 g

2. **Hydrogen Peroxide (H_2O_2), 35 %**

   Basis: 100 ml (in distilled water)

   H_2O_2 ,35% (Sigma- Aldrich), 0,05 µl

3. **Peroxidase**

   Basis: 1 ml (in distilled water)

   Peroxidase (Sigma), 1 mg- 266 units

   For 2 units from 200 units stock made 1/100 dilution with distilled water

   Preparation of 200 units 0,75 mg/ ml.

4. **Methylene Blue Dye**

   Basis: 100 ml (in distilled water)

   Methylene blue dye (Merck),0,075 g, 2×10^{-3} M

   M_w = 373,90 g/mole

APPENDIX D

SILVER ZEOLITE PREPARATION CHEMICALS

Basis in 100 ml (distilled water)

- $AgNO_3$, (Merck), 0,450 g
- Zeolite (GORDES, 30 micron), 1 gram
- Ethylenediamine (Merck), 200 ml
- Sodium Borohydrate (Sigma) 1:1 ratio with filtered silver zeolite

APPENDIX E

DATA OF GRAPHICS

Table E.1: Data of Figure 4.1, UV-Vis spectra of TiO_2 powder

TiO_2			
nm	ab		
289	0	440	1,068
290	2,301	450	1,01
300	2,515	460	0.957
310	2,767	470	0.913
320	3,135	480	0,87
330	3,334	490	0,831
340	3,43	500	0,793
350	4		
360	2,03		
370	1,762		
380	1,577		
390	1,489		
400	1,313		
410	1,27		
420	1,197		
430	1,129		

Table E.2 Data of Figure 4.11 Survival ratio of *E.coli* under UV-Vis with TiO_2 powder containing suspension

	TiO_2 powder
Time (min)	% survival
0	100
20	90
40	78
60	53
80	37
100	14
120	0

Table E.3 Data of Figure 4.16, Figure 4.19 and Figure 4.20 Survival ratio of *E.coli* under UV-Vis on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles

	TiO_2	TiO_2 -W	TiO_2-W-Zn
Time (min)	% survival		
0	100	100	100
20	98	97	90
40	83	79	78
60	74	53	54
80	52	44	36
100	36	21	12
120	21.1	12.4	5.5

Table E. 4 Data of Figure 4.24 Survival ratio of *S.aureus* under UV-Vis with TiO_2 powder containing suspension

	TiO_2 powder	
Time (min)	% survivor	
0	100	100
20	100	90
40	98	78
60	98	53
80	97	37
100	98	14
120	99	0

Table E.5 Data of Figure 4.29, Figure 4.32, Figure 4.33 Survival ratio of *S.aureus* under UV-Vis on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles

	TiO_2	TiO_2 -W	TiO_2-W-Zn
Time (min)	% survival		
0	100		100
40	90	89	85
80	71	65	68
120	59	55	55
160	41	32	33
200	15	11	12
240	1	0.8	0.8

Table E.6 Data of Figure 4.37 Survival ratio of *B.atrophaeus* spores under UV-Vis with TiO_2 powder containing suspension

	TiO_2 powder	
Time (min)	% survivor	
0	100	100
120	100	91
240	97	78
360	98	66
480	96	25
600	97	12.2

Table E.7 (cont'd) Data of Figure 4.42**,** Figure 4.45 and Figure 4.46 Survival ratio of *B.atrophaeus* spores under UV-Vis on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles

	TiO_2	TiO_2 -W	TiO_2-W-Zn
Time (min)	% survival		
0	100	100	100
120	93	96	95
240	69	89	89
360	39	85	84

	TiO_2	TiO_2 -W	TiO_2-W-Zn
Time (min)	% survival		
480	13	83	81
600	2	81	75

Table E.8 Data of Figure 4.50 Survival ratio of *E.dermatitidis* under UV-Vis with TiO_2 powder containing suspension

	TiO_2 powder	
Time (min)	% survivor	
0	100	100
60	99	87
120	96	65
180	98	42
240	96	24
300	98	15

Table E.9 Data of Figure 4.55, Figure 4.58 and Figure 4.59 Survival ratio of *E.dermatitidis* under UV-Vis on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles

	TiO_2	TiO_2 -W	TiO_2-W-Zn
Time (min)	% survival		
0	100	100	100
60	92	90	87
120	69	68	64
180	42	45	44
240	24	25	24
300	15,4	14	14

Table E.10 Data of Figure 4.63 Survival ratio of *A.niger* spores under UV-Vis with TiO_2 powder containing suspension

	TiO_2 powder	
Time (min)	% survivor	
0	100	100
40	98	89
80	96	69
120	95	53
160	97	27
200	97	15

Table E.11 Data of Figure 4.68, Figure 4.71 and Figure 4.72 Survival ratio of *A.niger* spores under UV-Vis on TiO_2 coated, W doped TiO_2 coated and W-Zn doped TiO_2 coated textiles.

	TiO_2	TiO_2 -W	TiO_2-W-Zn
Time (min)	% survival		
0	100	100	100
40	91	90	91
80	68	67	68
120	63	59	58
160	26	24	25
200	19	15	17

Table E.12 Data of Figure 4.88 The necessary amount of silver zeolite for killing different microorganisms.

	E.coli	*S.aureus*	*S.infantis*	*B.atrophaeus* spores	*E.dermatitidis*	*A.niger* spores
Ag-z/o(μg/ml)	100	200	1000	500	300	400
% Killing	98	70	97,4	92	100	100
	96	66	96	88	100	100
	97	66,5	97,3	90	100	100