



OPEN ACCESS INTERNATIONAL JOURNAL OF SCIENCE & ENGINEERING

STUDIES ON PHOTOCATALYTIC DEGRADATION OF *E. COLI* PRESENT IN DRINKING WATER USING COMMERCIAL, SYNTHESIZED AND DOPED TiO_2 AS CATALYST

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Abstract: Photocatalytic degradation employing semiconductors as photocatalyst is a promising and attractive method for the removal of pollutants in wastewater. TiO_2 has been identified as the most effective and useful photocatalyst. However, the applications of TiO_2 has been retarded by fast recombination of electron-hole pairs and their wide band gap which corresponds to the UV light. Therefore, the study with modifying TiO_2 in order to reduce the electron-hole recombination and sensitization towards visible light is one of the current hotspots in the photocatalyst research. Photocatalytic degradation experiments were carried out using *E. coli*, UV light was mainly used as photon source. The proposed study aims at investigating the above mentioned aspects by conducting an in-depth study of *E. coli* degradation using commercially available, sol-gel synthesized and doped TiO_2 . Experimental runs were carried out with varying inoculum levels of 1 ml/L, 10 ml/L, 20 ml/L and this was fixed at 20 ml/L and also with varying concentrations of commercially available TiO_2 i.e. 0.05 g/L, 0.1 g/L, 0.5 g/L, 0.7g/L, 1 g/L and 2 g/L in 100 ml and 1L total volume where 0.5 g/L was found to be optimum catalyst concentration for which the time taken was 2 h to degrade. Degradation studies were also conducted with sol-gel synthesized TiO_2 and Ag doped TiO_2 which took 9 and 6 min respectively for the optimum 0.5g/L catalyst concentration. Also Ag doped TiO_2 was tested under sunlight for which the degradation time was 30 min.

Keywords: Photocatalytic degradation, Photocatalyst, sol-gel, Ag doped, *E.coli*

I INTRODUCTION

Water pollution is a major problem in the global context, which is leading to worldwide cause of death and diseases that account for the death of more than 14,000 people daily [1,2]. Drinking Water contaminated with chemicals and pathogens like bacteria, viruses and fungi cause several water borne diseases like diarrhea, nausea, dysentery. In extreme cases some pathogens may infect the lungs, skin, eyes, nervous system, kidneys, or liver and the effects may be more severe, chronic, or even fatal. Of them diarrhea caused by *E.coli* is posing a heavy threat on adults as well as infants. It produces a toxin that damages the lining of the intestines resulting in hemorrhagic diseases [3,4]. The World Bank estimated that 21% of communicable diseases in India are related to unsafe water and diarrhoea alone causes more than 1,600 deaths daily in India [5]. Nearly 60% of infant

mortality can be linked to a water related infectious disease; globally, diarrhoea is the third largest cause of morbidity and the sixth largest cause of mortality, causing up to 2.2 million deaths per year. Morbidity and mortality are the greatest amongst children under five years of age [6,7]. Research has been done extensively to remove harmful disease causing pathogens and resulted in many conventional methods like chlorination, filtration, ozonation etc., which can effectively kill the pathogenic microorganisms, however the formation of carcinogenic undesirable disinfection by-products (DBPs) such as trihalomethanes (THMs), haloacetic acids and other dissolved organic halogens limits their usage [8,9]. Also the permissible level of *E.coli* in drinking water is zero colony forming units per 100ml which may not be achieved by these techniques.

Therefore there is an urgent requirement to investigate innovative processes which can overcome these

difficulties. Recent developments in this domain have led to some oxidation processes which are receiving increasing attention because of its potential to completely eradicate wide range of chemical as well as biological contaminants in water. Photo catalysis is an advanced oxidation process which involves the use of semiconductor photocatalyst (ZnO, TiO₂) and a photon source (UV lamp). It received worldwide attention due to its direct utilization of sunlight to achieve a variety of chemical reactions, renders to water splitting, organic pollutant degradation, and water microbial disinfection. TiO₂ is being extensively studied semiconductor catalyst for photocatalytic degradation of industrial effluents and microorganism. The mechanism of photocatalysis is based on interaction between photons emitted by a light source and catalyst particles (TiO₂) [10,11].

The main objective of the work is to synthesize catalyst (TiO₂) which can degrade microorganisms, directly in the presence of sunlight. This can be achieved by doping catalyst (TiO₂) with metals. The doped catalyst has smaller band gap than normal TiO₂ due to which less energy is required for photoexcitation, therefore the degradation process can be carried out under direct sun light. The present research work is focused on effect of commercial available, synthesized and doped TiO₂ on photocatalytic degradation process. Also the effect is tested under sunlight to know the activity of catalyst for the photocatalytic degradation.

II MATERIALS AND METHODS

2.1 Microorganism and growth media

Escherichia coli NCIM2065 a bacterial strain was used as target microorganism in photocatalytic inactivation studies. *E. coli* was obtained from the culture collections (NCIM, Pune, India) and was grown aerobically in nutrient broth (SRL, India) (13g in 1000ml distilled water) at 37°C. Solid medium of agar plates was prepared using nutrient agar media (SRL, India) (28g in 1000ml distilled water) and 2% agar powder (HiMedia, India) and were used in the viable count method for the analysis of inactivated samples.

2.2 Chemicals for photocatalytic degradation

The photocatalysts used in the study were commercially available TiO₂ (Fisher Scientific, India), Sol-gel synthesized TiO₂, Silver doped TiO₂ (Ag/TiO₂). Ag/TiO₂ synthesized by Sol-gel synthesis method using Titanium (IV) Isopropoxide as a titanium precursor (Avra Synthesis Pvt. Ltd) and 1N AgNO₃ as silver precursor.

2.3 Accessories for photocatalytic reactor

Photocatalytic inactivation was studied by using a high pressure mercury vapor lamp of 125 W (Philips) irradiating mainly in UV region (predominantly at 365 nm). Water was circulated to the reactor by using submersible pump (8W).

2.4 Viable count method

The inactivated samples were analyzed by standard plate count method (viable count method). The collected 1ml of inactivated samples were serially diluted, in sterile deionized water, such that the final count was not more than 200 CFU/mL (CFU = colony forming unit), and 100 µL of the diluted sample was spread on sterile agar plates. The plates were incubated overnight and fully grown colonies on plates were counted and recorded.

2.4.1 Colony forming unit and calculation

Colony-forming unit (CFU) is a measure of viable cells. In direct microscopic counts where all cells, dead and living, are counted, but viable count method measures only viable cells. Theoretically, one viable cell (viable defined as able to multiply via binary fission under the controlled conditions such as Nutrient medium, Temperature and Time) can give rise to a colony through multiplication. The CFU/ml can be calculated using the formula:

$$\text{CFU/ml} = (\text{number of colonies} \times \text{dilution factor}) / \text{volume taken on culture plate.}$$

2.5 Catalyst synthesis

Pure anatase phase TiO₂ was synthesized by Sol-gel synthesis method. The precursor of Titanium used for synthesis is Titanium (IV) isopropoxide (C₁₂H₂₈O₄Ti). 1ml of titanium (IV) isopropoxide was taken carefully by using a syringe. Dilute it by adding drop wise in to 20ml ethanol. It was mixed thoroughly for 1 h by using magnetic stirrer. Distilled water was added to the ethanol titanium precursor solution in the molar ratio of 1:4 (Ti: H₂O) under continuous mixing. The mixed solution was vigorously stirred for 1 h in order to form sols. After aging for a day at temperature of 75-80 °C in oven, the sols were transformed into gel form. The obtained gels were dried at 120 °C for 24 h to remove the water and organic materials. After that, the dried gel was then ground to a fine powder and calcinated at 450 °C for 2 h in muffle furnace. Finally the pure TiO₂ nanoparticles were obtained and the samples were send to PXRD analysis.

2.6 Doping of catalyst

Ag/TiO₂ was synthesized from the mixture containing TiO₂ precursor solution, gelatin solution and AgNO₃ solution. Precursor solution was prepared by mixing Titanium (IV) isopropoxide (TIPP), glacial acetic acid (AA) and distilled water (H₂O) in the same order with molar ratio of 1:10:200 (TIPP:AA:H₂O) under vigorous stirring. Three grams of gelatin was dissolved in 100 ml of deionized water, stirring at 60°C for 30 min and was added to TIPP precursor solution under vigorous stirring. To this solution 10ml of 0.1N silver nitrate (AgNO₃) solution was added drop wise. The solution was dried at 80 °C for 48 h in oven. The dried gel was then ground to a fine powder and calciated in a muffle furnace at 500 °C for 5 h.

2.7 Catalysts used in present study

The summary of the catalysts used in the present study along with sources is shown in Table 2.1.

Table 2.1: Summary of the catalysts used in the Present study

Catalyst	Source
Commercially Available TiO ₂	Fisher scientific company
Synthesized TiO ₂	Synthesized by Sol-gel Method
Ag/TiO ₂	Synthesized by Sol-gel Method

III RESULTS AND DISCUSSION

3.1 Photodegradation of E.coli without catalyst

Preliminary experiments were conducted to check the effect of UV radiation on E.coli degradation. Different inoculum levels of E.coli 100µl, 1ml and 2ml were taken and experiments were conducted without catalyst in 100ml sterile water for the effect of UV light on degradation studies. The times taken for degradation of inoculum under direct UV radiation were 15min, 30min and 150min with respective inoculum levels (Fig 3.1).

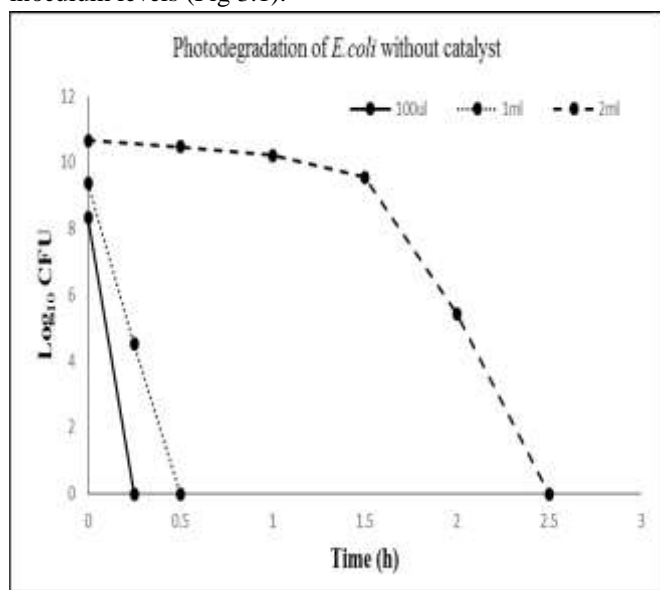


Figure 3.1: Photodegradation of E.coli without catalyst using different inoculum levels

Figure 3.1 infers degradation of inoculum with 100µl and 1ml takes place linearly whereas with 2ml it signifies degradation takes place exponentially. Comparing three inoculum levels, 2ml inoculum took 2.5h which is longer than the other two due to presence of high number of E.coli. As expected with increase in the number of E.coli there is increase in time of degradation therefore there is a need to minimize the time of degradation.

3.2 Photocatalytic degradation of E.coli with commercially available TiO₂

The effect of degradation of E.coli in the presence of TiO₂ catalyst was studied fixing the inoculum level to 2ml/100ml distilled water. Experimental runs were carried out with varying catalyst (commercially available TiO₂) concentrations of 0.05 g/L, 0.1 g/L, 0.5g/L and 1g/L in 100ml distilled water. It took 2.5h, 2.5h, 2h and 3h of time with respective catalyst concentrations to degrade E.coli (Fig 3.2). Catalyst concentrations of 0.05 and 0.1 g/L does not impose significant effect on degradation hence took 2.5h to degrade which is the same time as without catalyst. This is due to the presence of less number of active sites on catalyst surface to produce enough number of reactive oxygen species (ROS) to degrade E.coli. 0.5 g/L catalyst takes 2h to degrade which may be due to the presence of more active sites on catalyst surface to produce sufficient number of ROS to degrade E.coli.

Experiments with 1 g/L catalyst took 3h to degrade. The reason for taking more time may be due to self-recombination of excessive ROS resulting in the formation of an emulsion which blocks the radiation to reach catalyst particles (shadowing or screening effect. [12-14]). 0.5 g/L of catalyst is set as optimum catalyst loading concentration as it took less time compared to other concentrations of catalyst to degrade E.coli.

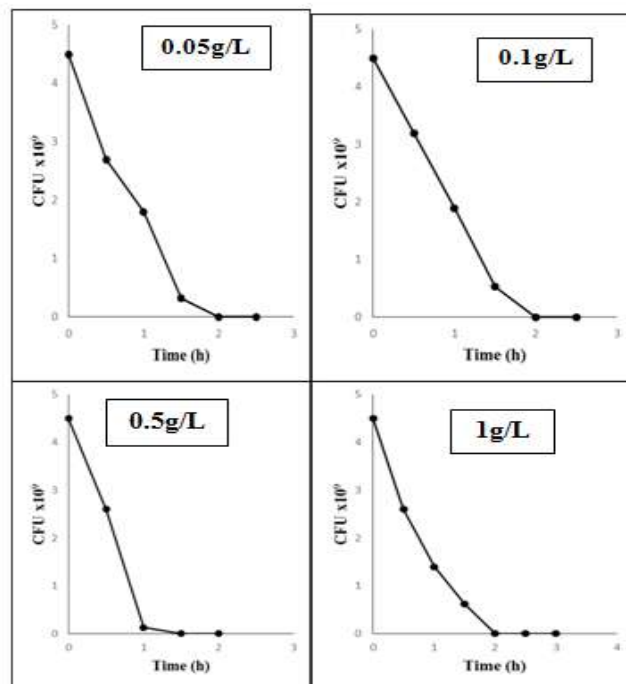


Figure 3.2: Photocatalytic degradation of E.coli with varying catalyst concentrations 0.05g/L, 0.1g/L, 0.5g/L and 1g/L

Fig 3.2 infers 0.05g/L of catalyst loading shows exponential degradation, 0.1g/L of catalyst loading shows

linear degradation till 1.5h followed by exponential degradation, 0.5g/L of catalyst loading shows linear degradation till 1h followed by exponential degradation and 1g/L of catalyst loading shows exponential degradation. The optimum catalyst concentration of 0.5g/L was in good agreement with Benabbou et al., (2007) [15], which is 0.35g/L and better than the optimum concentration 1g/L obtained by Manness et al., (1999),Nagaveni et al., (2004b), Wei et al., (1994) [15-17].

3.3 Scaling up of photocatalytic degradation of *E.coli* with commercially available TiO₂

The experimental volume was scaled-up to 1L and the experiments were carried out with varying catalyst concentrations 0.5g/L, 0.7g/L, 1g/L and 2g/L by keeping inoculum level 20 ml/L (2 ml/100 ml) constant. The effect of catalyst concentration on degradation of *E.coli* to purify 1L water was studied. The result signifies the degradation times as 2h, 3h, 3.5h and 3.5h respectively (Fig 3.3). With an increase in catalyst concentration beyond optimum point (0.5 g/L), there is increase in degradation time which may be due to the excessive ROS which undergo self-recombination resulting in increasing the turbidity which decreases the penetration depth of UV rays. Hence 0.5g/L is set as optimum catalyst loading level even for 1L. Fig 3.3 infers 0.5g/L of catalyst loading shows linear degradation till 1 h followed by exponential degradation, 0.7g/L of catalyst loading shows linear degradation till 1h followed by exponential degradation, 1g/L of catalyst loading shows exponential degradation and 2g/L of catalyst loading shows exponential degradation.

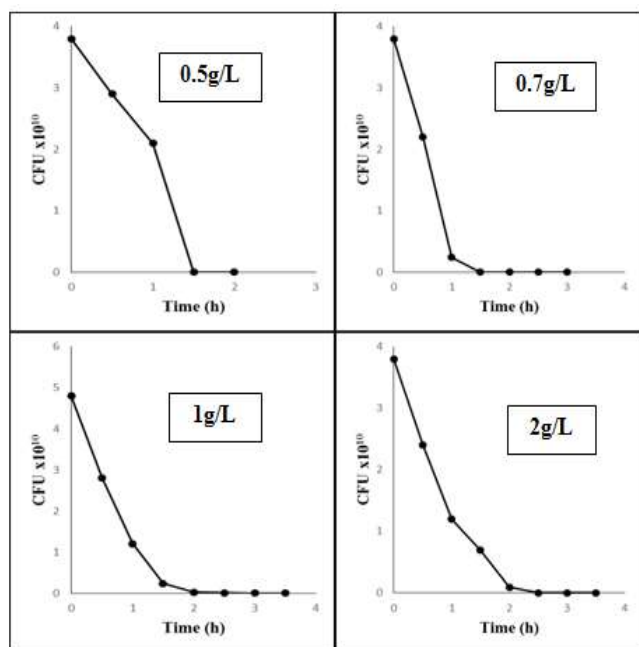


Figure3.3: Photocatalytic degradation of *E.coli* with varying catalyst concentrations 0.5 g/L,0.7 g/L, 1g/L and 2g/L

3.4 Synthesis of catalyst

The need for the use of synthesized catalyst is to decrease the degradation time by altering the catalyst structure to reduce band gap such that the degradation takes place faster. Also there is a need to alter the catalyst such that the photo excitation takes place under direct sunlight rather than under UV light. Photocatalytic degradation of *E.coli* with synthesized and doped TiO₂ is discussed in sections 3.4.1 and 3.4.2.

3.4.1 Photocatalytic degradation of *E.coli* with synthesized TiO₂

The commonly used method for synthesizing catalyst is sol-gel synthesis. Pure anatase phase TiO₂ was synthesized by using this method. The precursor solution used for synthesis is Titanium (IV) isopropoxide (C₁₂H₂₈O₄Ti). 1ml of titanium (IV) isopropoxide is taken carefully by using a syringe and diluted with 20ml of ethanol. It is mixed thoroughly for 1h by using magnetic stirrer. Distilled water is added to the ethanol titanium precursor solution in the molar ratio of 1:4 (Ti:H₂O) under continuous mixing. The mixed solution was vigorously stirred for 1h in order to form sols. After aging for a day at temperature of 75-80 °C in an oven, the sols were transformed into gel form. The obtained gels were dried at 120 °C for 24h to remove the water and organic materials. After that, the dried gel was then ground to a fine powder and calcinated at 450 °C for 2h in a muffle furnace. Finally the pure TiO₂ nanoparticles were obtained and the samples were send to PXRD analysis to check the purity and nature of the produced catalyst particles.

The PXRD analysis was done at IISc Bangalore and the obtained results were compared with standard JCPDS plots of TiO₂.The preferred orientation corresponding to the plane (101) at 25 2θ / degree was observed in the samples same as in the standard plot. All the diffraction peaks in the patterns can be indexed as anatase phase of TiO₂ nanoparticles and the diffraction pattern was in good agreement with JCPDS files # 21-1278 which is standard plot for anatase TiO₂.The surface area of the material 126 m²/g signifies that the catalyst particle is of 17-20 nm size. Fig 3.4 and 3.5 represent the peak patterns of standard plot and sample.

The synthesized TiO₂with altering concentrations of 0.2, 0.3, 0.4, 0.5and 0.6 g/L was taken and experiments were carried out, the resulting degradation times for the respective catalyst concentrations were 20min, 25min, 21min, 9min and 12min (Fig 3.6). Catalyst concentrations of 0.2, 0.3 and 0.4 g/L imposes effect on degradation as the produced catalyst was in nanometers with increased surface area, thus processes more active sites which on excitation increases activity of degradation. 0.5 g/L of catalyst concentration reduces the degradation time rapidly and slight increasing in the catalyst concentration to 0.6 g/L increases the degradation time. This

may be due to the fact that at optimum concentrations of catalyst, all the active sites are producing enough number of ROS to degrade *E.coli* thus increases the rate of degradation. Upon increasing catalyst concentration beyond optimum level the degradation time increases due to self-recombination of excessive ROS which results in the formation of an emulsion which blocks the radiation to reach catalyst particles due to increased turbidity (shadowing or screening effect, Nagaveni et al., 2007; Benabbou et al., 2007; Rincon and Pulgarin et al., 2003).

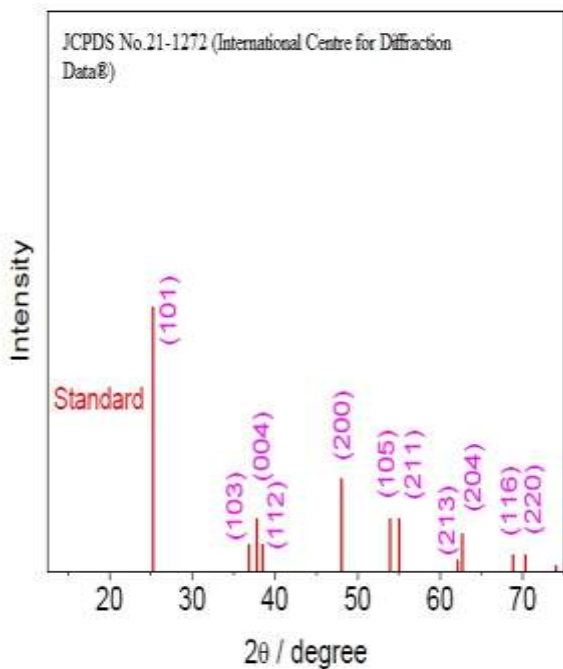


Figure 3.4: Peak pattern of standard plot JCPDS #21-1272

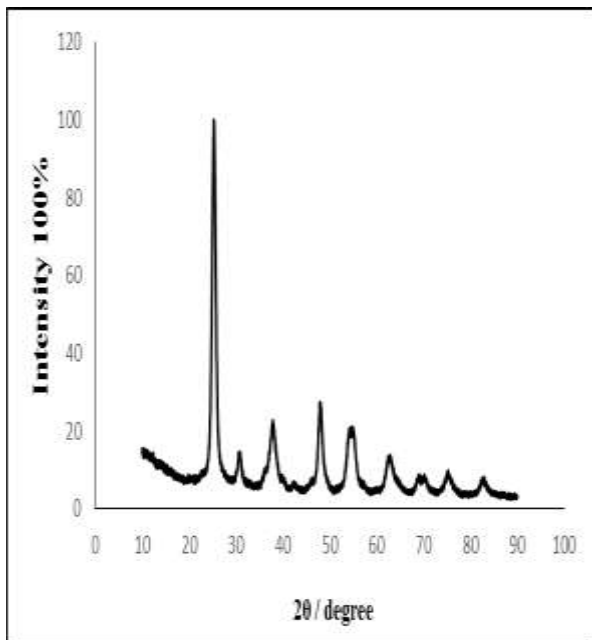


Figure 3.5: PXRD result of synthesized TiO₂ catalyst

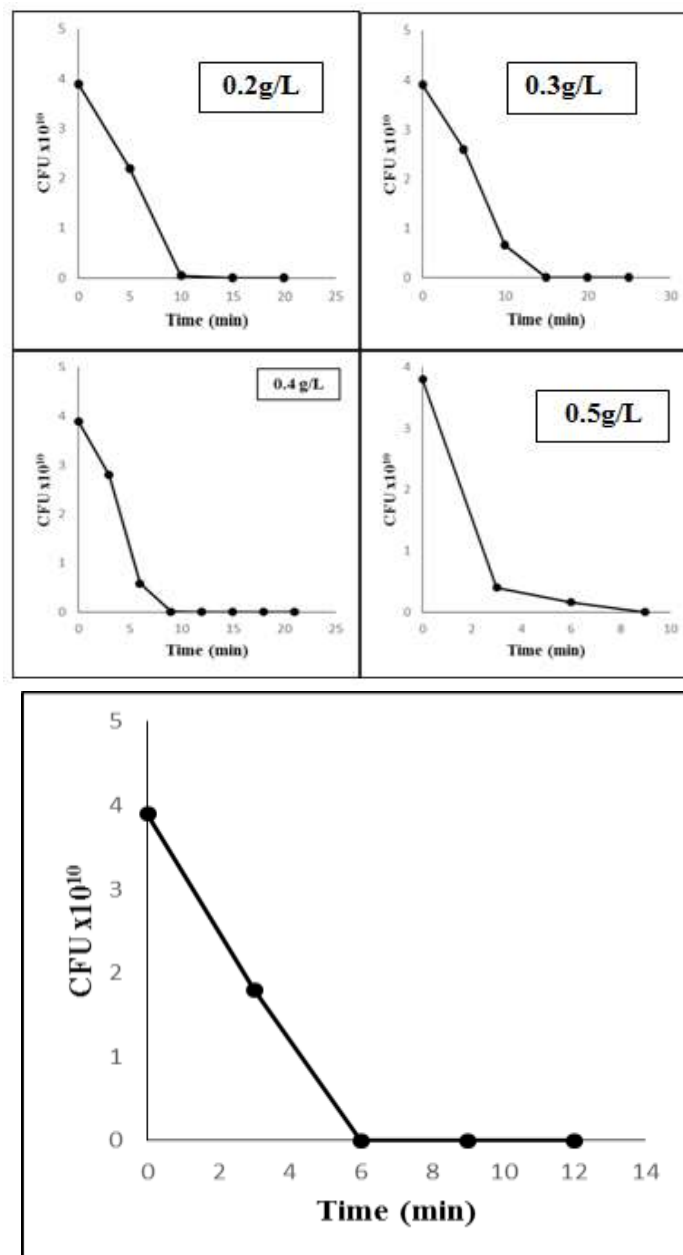


Figure 3.6: Photocatalytic degradation of *E.coli* with synthesized TiO₂ of different concentrations of catalyst.

3.5 Photocatalytic degradation of *E.coli* with Ag doped TiO₂

The synthesized catalyst can be further altered so that the degradation can be done under sunlight i.e., the photo excitation can be done with sunlight rather than UV to save energy or to make the process economical. Metal ion doping improves the electrocatalytic and photocatalytic activity of TiO₂. Transition metals doped on TiO₂ play the role of charge carrier trap, which allows for light absorption in the visible range and enhances surface electron excitation by visible-light excited Plasmon resonance. Silver doped-TiO₂ (Ag-TiO₂) in anatase form was prepared via sol-gel method. Ag/TiO₂ was synthesized from the mixture containing TiO₂ precursor solution, gelatin solution and 1N AgNO₃ solution.

Precursor solution was prepared by mixing Titanium (IV) isopropoxide (TIPP), glacial acetic acid (AA) and distilled water (H₂O) in the same order with molar ratio of 1:10:200 (TIPP:AA:H₂O) under vigorous stirring. 3 g of gelatin was dissolved in 100 ml of deionized water, stirring at 60°C for 30 min and was added to TIPP precursor solution under vigorous stirring. To this solution 10ml of 0.1N silver nitrate (AgNO₃) solution was added drop wise. The solution was dried at 80 °C for 48h in an oven. The dried gel was then ground to a fine powder and calcinated in a muffle furnace at 500°C for 5 h.

The synthesized Ag-TiO₂ was then investigated for enhanced catalytic activity and degradation studies were performed under UV and sunlight in 1L water with concentration of 0.5 g/L. The degradation of *E.coli* was done in 6min under UV and 30min under sunlight. Degradation with 0.5 g/L shows decrease in degradation time as the doped catalyst has Ag which results in decreasing of band gap thus increases efficiency. Also degradation under sunlight reduces the degradation time due to photoexcitation by light absorption in the visible range due to decreased band gap in the catalyst. The graph signifies the linear degradation of Ag doped TiO₂ under UV and sunlight (shown in Fig 3.7).

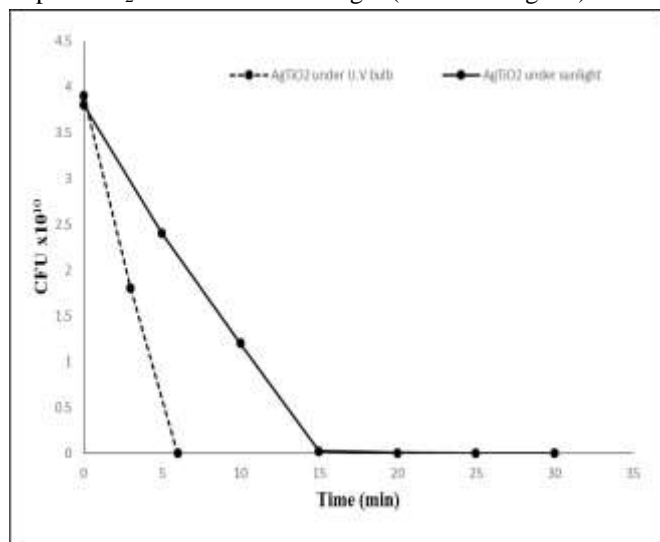


Figure 3.7: Photocatalytic degradation of *E.coli* with 0.5 g/L Ag doped TiO₂

IV CONCLUSION

The current methodology, Advanced Oxidation Processes (AOP) has huge potential to treat industrial and various organic pollutants including pesticides, microbes in waste water. The degradation of pollutants is carried out by employing semiconductors as photocatalyst. In this work, TiO₂ has been used as photocatalyst for degradation of *E. Coli* present in water. The present study focus, on the preparation, characterization and photocatalytic performance of commercial, sol-gel synthesized and Ag doped TiO₂ photocatalysts.

The degradation experiments were carried out using photocatalyst, UV light (UVA, 365 nm) which was mainly used as photon source. However, considering the economy of the process, photocatalytic degradation in presence of visible light was also studied. The catalyst used for photocatalysis was mainly employed in a suspended form. The photocatalytic degradation was confirmed by spread plate analysis.

Experimental runs were carried out with varying inoculum levels of 1 ml/L, 10 ml/L, 20 ml/L and the inoculum level was fixed at 20 ml/L. Experiments were carried out with varying concentrations of commercially available TiO₂ i.e. 0.05 g/L, 0.1 g/L, 0.5 g/L, 0.7g/L, 1 g/L and 2 g/L in 100 ml and 1L total volume and 0.5 g/L catalyst concentration was found to be optimum for which the time taken was 2 h to degrade. To study the effect of synthesized TiO₂ catalyst, TiO₂ nanoparticles were synthesized by sol-gel method and the samples were analyzed with PXRD. The surface area of the material is found to be 126 m²/g with a particle size of 17-20 nm. Degradation studies were also conducted with sol-gel synthesized TiO₂ and Ag doped TiO₂ which took 9 and 6 min respectively. Also Ag doped TiO₂ was tested under sunlight for which the degradation time was 30 min. The results emphasize that further experiments can be conducted in a large volume of contaminated water using solar energy.

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