

N-doped TiO₂ nano particles for ultra violet photocatalytic degradation of coliform and fecal coliform from hospital wastewater effluent

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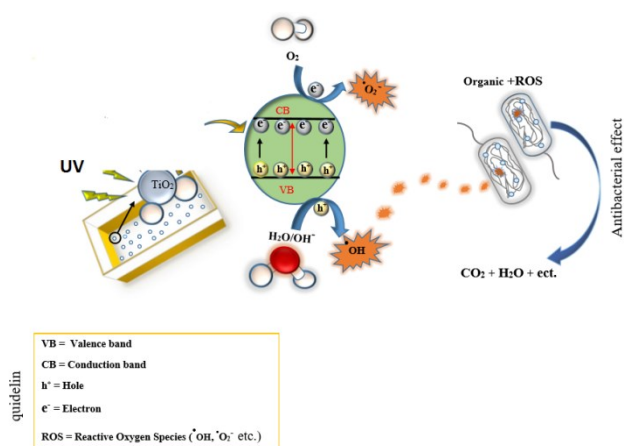
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Graphical abstract



Abstract

The photocatalytic process as an advanced oxidation process (AOP) has attracted worldwide attention in water and wastewater industry. The photocatalytic process can be used for water and wastewater disinfection. The purpose of this study was to remove coliform and fecal coliform from real hospital wastewater using UV and UV/N-doped TiO₂ photocatalytic process. A secondary effluent samples were taken from Ali-ebne Abitaleb Hospital in Zahedan. The real samples of effluent containing N-doped TiO₂ nanoparticles were exposed to UV light in a reactor. Multi-tube fermentation method (MPN/100ml) was used for counting of coliform and fecal coliform bacteria and evaluation of process efficiency. The results showed that the mechanism of inactivation of coliform and fecal coliform by N-doped TiO₂ nanoparticles and UV follows the first-order equation and simultaneous application of N-doped TiO₂ nanoparticles and UV (N-doped TiO₂/UV) increase the efficiency of photocatalytic process and deactivate completely the coliform and fecal coliform in 10 minutes. The results of this study also showed that with

increasing the contact time, the removal efficiency of coliform and fecal coliform increase in the presence of UV light. The highest efficiency of the nanoparticles in bacterial inactivation was achieved in dose 120 mg/l the presence of light UV and it was 100%. Nano-photocatalytic process can be used an efficient method for removal of coliform and fecal coliform from hospital real effluent, and N-doped TiO₂/UV process is a reliable antibacterial method for water and wastewater disinfection.

Keywords: Coliform, fecal coliform, N-doped TiO₂, disinfection, hospital effluent

1. Introduction

Hospitals are considered as high risk places because they located within city near human societies. Hospitals generate solid waste and wastewater. Hospital wastewaters has various pollutants that may generate environmental problems. This wastewater contain intestinal pathogens, including bacteria and viruses which may easily reach to water resources (Sponza and Alicanoglu, 2018). Increasing of microorganisms in discharged effluent increases concerns related to the health effects of human. Furthermore, presence of pathogens in the effluent limits or reduces the reusing of effluent.(Pecson *et al.*, 2015). Therefore, it is necessary to enforce stricter standards on the microbiological contamination of discharged effluent. Consequently, a good disinfection process is needed to be included in the treatment steps by considering the water and wastewater quality objectives in terms of technical reliability, economic and environmental criteria(Sun *et al.*, 2003). Traditionally, wastewater disinfection is done through chlorination or ultraviolet (UV) radiation. Chlorine and chlorine-based compounds cause the formation of mutagenic and carcinogenic compounds during water disinfection, and UV radiation requires expensive equipment such as UV lamps as well as increases energy consumption(Huang *et al.*, 2011). Recently, advanced oxidation processes (AOPs) have

been used to degradation of organic pollutants and disinfection of water and wastewater. AOPs as aqueous phase oxidation method based on the production of hydroxyl radicals, HO•, which results in the degradation of various compounds (Blanco *et al.*, 2012). Various AOPs have been used for treatment of wastewater, such as ozonation (O₃), photo-peroxidation (H₂O₂/UV-C), photo-catalysis (N-doped TiO₂/UV), and Fenton systems (Rosa *et al.*, 2015). The N-doped TiO₂ photocatalytic process represents a promising low-cost method for water and wastewater effluent (Kiwi *et al.*, 2014). In recent years, using of titanium dioxide (N-doped TiO₂) as a photocatalyst for inactivation of bacteria, viruses and protozoan parasites has been extensively reported (Wang *et al.*, 2015). Compared to other semiconductors, N-doped TiO₂ is suitable as a photocatalyst for water treatment, because of its characteristics such as highly photoreactive, cheap, nontoxic, chemically and biologically inert, and photostable. Studies have shown that pure anatase N-doped TiO₂ nanoparticles are more effective for inactivating bacteria than nanoparticles composed of anatase and rutile phase (Caratto *et al.*, 2013). When nano-N-doped TiO₂ is irradiated with UV light, valence band holes (h⁺) and conduction band electrons (e⁻) are generated by a chain of reactions. OH radicals, generated through water oxidation by photogenerated valence band and when in contact with microbial cells, cause oxidative stress, promote deleterious alterations in cellular structure and final inactivation (Xiao *et al.*, 2015). In this study, we investigated the efficacy of photocatalyzed N-doped TiO₂ and ultraviolet radiation to eliminate two major waterborne bacterial pathogens in humans, coliform and fecal coliform.

Table 1. Chemical characterization of the effluent

BOD ₅	90 mg/l
COD	270 mg/l
pH	7.3-7.8
TS	2850 mg/l
TSS	62 mg/l
EC	2.9- 4.7ms/cm ²
Turbidity	60-100 NTU
Temperature	20-25 °C

Table 2. Specifications of nano-TiO₂

Chemical formula	N-doped TiO ₂
Assay	99.7%
Behavior in water	Hydrophilic
Average particle size	<25 nm
BET surface area	50 ± 15m ² /g
Bulk density	4.26 g/L
Crystalline phase	Anatase

2. Material and methods

2.1. Materials

The wastewater used in this study was collected from Ali Ibn Abi Talib Hospital in Zahedan. It drains directly into the wastewater treatment plant (WWTP), where biologically active sludge treatment is used. Amber glass bottles were used for the collection of the samples. The samples were

transferred to the laboratory immediately after collection and Biochemical Oxygen Demand (BOD₅), Chemical Oxygen Demand (COD), pH, Total Solids (TS), Total Suspended Solids (TSS), EC and turbidity were measured for the raw samples. It was finally disinfected by the photocatalytic process. These parameters are considered as significant factors by Iranian Department of the Environment (DOE) for monitoring of hospital wastewater disposal. Sample analysis was done according to the standard methods (Association *et al.*, 1920) the mean of the influent BOD₅, COD, TSS, pH, EC and turbidity were reported in the Table 1 treated with photocatalysis. The commercially available titanium dioxide powder (N-doped TiO₂ P₂₅) was purchased from Sigma Aldrich Corp. physicochemical characteristics nano- N-doped TiO₂ were reported in the Table 2.

3. Reactor and light source

Photo-catalytic experiments were conducted in a laboratory-scale photoreactor. The outer surface photoreactor was surrounded by an aluminum layer to protect the samples studied. The interior glass container of the reactor has a total volume of 500 mL (10-cm diameter and 15-cm height) and in order to prevent the temperature increase of the solution (due to electricity consumption), the container was installed in a water reactor. Photo-catalytic experiments were conducted by using UV light, for which a mercury vapor lamp (4W) in length was installed 15 cm above the samples surface.

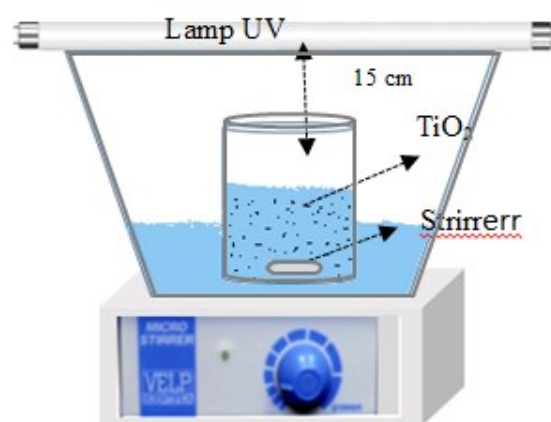


Figure 1. Schematic illustration of system setup used in the photocatalytic bacterial disinfection by N-doped TiO₂ under UV irradiation

4. Photocatalytic treatment

The study is based on two systems for wastewater sample disinfection. The first system of titanium dioxide nanoparticles without ultraviolet light and Second system titanium dioxide with ultraviolet light. In the examinations various parameters such contact time (3,5,7,10 min) and amount of N-doped TiO₂ (0,60,120 and 180 mg/l) were studied in terms of their effect on reaction process respectively. In any disinfection system, 350 mL of the sample were transferred in the reaction vessel and the appropriate amount of N-doped TiO₂ was added to achieve the desirable catalyst loading. In order to keep the catalyst

suspended in the sample body, the resulting mixture was magnetically stirred throughout the experiments. From the reaction vessel at sample various intervals of time was drawn and serially diluted up to 10⁵ times. The sample was grown on Liquid Broth Agar (LBA) and E. coli culture broth. All the experiments were repeated at least twice and mean values are quoted as results. Most Probable Number (MPN) of Coliform and Fecal Coliform in samples was measured as MPN/100 ml before and after examinations by using the fermentation technique in presumptive and confirmed tests. The disinfection efficiency, is calculated as:

$$E = \frac{C_i - C_f}{C_i} \times 100$$

C_i and C_f are the initial and final MPN/100ml respectively.

5. Results and discussion

5.1. FE-SEM

The FE-SEM was used to show the shape and morphology of un-doped and N-doped TiO₂ particles (Figure 2). The prepared nano-particles were found to be fine, irregular shape, slightly smooth surface and tend to agglomerate to form larger irregular grains. The diameter of particles was found to be 30-40 nm, which is in a good agreement with the crystal size obtained by XRD indicating that both un-doped and N-doped particle is nano-sized particles (Figure S1, Supplementary Material).

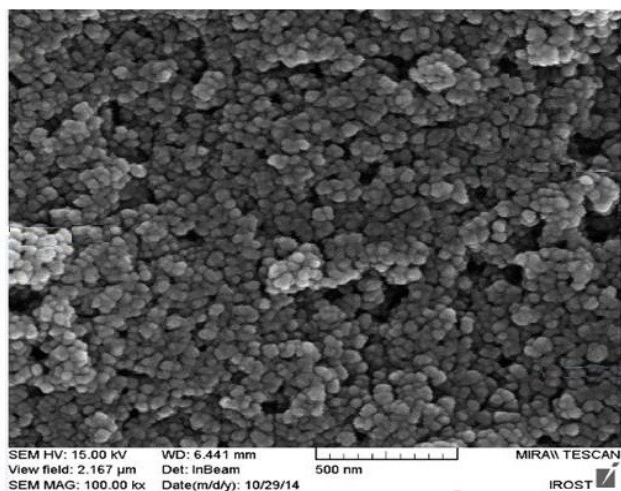


Figure 2. Image FE-SEM of Synthesis of N-doped TiO₂

An X-ray diffraction pattern was used to investigate the type of crystalline in material and also to know if any change was occurred after doping of N-doped TiO₂. Figure 3 a shows the XRD patterns of the un-doped and N-doped TiO₂ samples. As shown in the XRD pattern, all synthesized samples had a sharp diffraction peak indicating a good characteristic crystal. The distinctive peaks at 2θ = 25.49°, 37.14°, 37.99°, 38.76°, 48.35°, 54.12°, 55.33°, 62, 90° and 68.95°; correspond to the anatase (JCPDF Card No. 20-0387) were observed. The patterns also showed that the anatase was the main phase in un-doped and N-doped TiO₂ under all synthesis conditions.

These results revealed that the peak positions were nearly the same and no detectable dopant-related peaks were

observed, implying that the structure of N-doped TiO₂ has not been changed and also suggesting that nitrogen dopants do not react with TiO₂ to form new crystalline. It is noteworthy, that many documents have also reported that doping with the nitrogen ions have not exhibited additional phase except anatase. The pure anatase phase in N-doped TiO₂ could be due to the fact that the nitrogen dopants are so low and they have also moved into either the interstitial positions or into the substitution sites of the TiO₂ crystal structure. Compared to the n-doped TiO₂, the peak of N-doped TiO₂ samples exhibited a slight shift toward the lower angle corresponding to (1 0 1) plane of anatase (Figure 1b), indicating a lattice distortion of the N-doped TiO₂. These defects and disorderly state in the particles caused by nitrogen dopants are reported as key factor for absorption edge shift towards the visible-light region

The average crystallite size of un-doped TiO₂ and N-doped TiO₂ were calculated according to the Debye-Scherrer formula as the following:

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

where: D = the average crystallite size, k = a dimensionless shape factor (usually=0.9), λ = the wave length of the X-ray radiation (0.15418 nm for Cu Kα), β = the full width at half-maximum of the diffraction, and θ = the corresponding diffraction angle in degree [21].

The calculated results were 30, 30, 26 and 34 nm for un-doped TiO₂, NT1, NT2 and NT3 nano-particles, respectively.

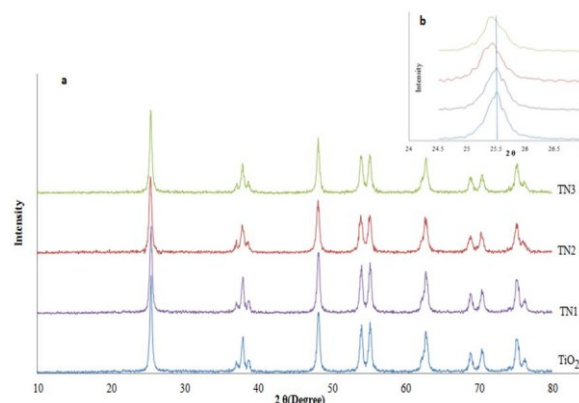


Figure 3. Image X-rd analyzes of Synthesis of N-doped TiO₂

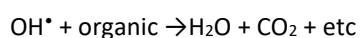
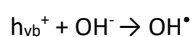
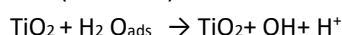
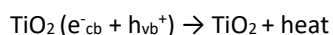
6. Effect of UV light irradiation and N-doped TiO₂

One of the major concerns in the water and wastewater industry is the removal of pathogens from water and wastewater effluents, which today the photocatalytic processes. In this context a significant role. Photocatalytic processes are capable of attacking organic compounds without producing byproducts. They produce free radicals such as hydroxide and superoxide and convert organic compounds to CO₂ and H₂O (Eslami *et al.*, 2018) Usually, the photocatalytic efficiency of N-doped TiO₂ in wastewater purification depends on the characteristics of the wastewater, photocatalytic loading, radiation field and light source (Iervolino *et al.*, 2018). First, bacteriological

experiment was performed on hospital wastewater sample and investigate The effect of nanoparticle and UV light. Results for the removal coliform and coliform from real hospital wastewater at a dose of 120 mg / l N-doped TiO₂ nanoparticles under conditions of dark, UV alone, N-doped TiO₂ alone and UV with titanium dioxide nanoparticles (UV\N-doped TiO₂) are shown in Figures 1 and 2 respectively. The number of coliforms and fecal coliforms in the presence of UV light and titanium dioxide decreased more than other conditions (UV light alone, N-doped TiO₂ nanoparticles alone, and darkness). So that after 10 minutes, the initial population of total coliform and fecal coliform in the wastewater sample has completely disappeared. The results of Chih-Yu Chen *et al.*, 2010 study showed that the removal efficiency of Escherichia coli and Staphylococcus aureus in the presence of UV-A light with N-doped TiO₂ (93.5% and 100%, respectively) was better than the absence of N-doped TiO₂ (76% and 78.3%, respectively) after 30 minutes of irradiation. They said in the system combined, more hydroxyl radicals are produced, resulting in higher bacterial inactivation (Chen *et al.*, 2010). Zazoli *et al.* (2015) showed that the death rate of bacteria in the presence of UV alone was higher than that of N-doped TiO₂ alone. However, the efficiency of the photocatalytic process (UV + nN-doped TiO₂) was more than UV alone or N-doped TiO₂ alone (Zazouli *et al.*, 2015).

7. The bacterial inactivation process

When N-doped TiO₂ is irradiated with ultraviolet or visible light with a wavelength shorter than 390 nm, it becomes a photocatalyst. If this light is absorbed by the semiconductor surface, it will have enough energy to overcome the energy barrier and excite an electron for electron transfer from the Valance Band (VB) to the conduction band (CB). In the valence band, when charge carriers are created in the band gap, they may move to the semiconductor surface and be absorbed by the reactants and results the formation of highly oxidizing hydroxyl and superoxide radicals (Klavarioti *et al.*, 2009) The ROSs that are produced invade the bacterial cell. Bacterial cells have two intracellular enzymes catalase (CAT) and superoxide dismutase (SOD) that protects them from ROSs attack. these two enzymes would be capable of converting respective ROSs into less or non-harmful substances. But the loss of activities of these two enzymes accelerated the accumulation of ROSs, leading to cell wall rupture , a rapid K leakage and release of protein and nucleic acids, leading ultimately to cell death (Naskar and Kim, 2019) TiO₂ +hv→ TiO₂ (e⁻cb + h⁺vb)



Also The results show that N-doped TiO₂ alone had the highest bacterial mortality rate compared to UV alone and was able to eliminate 0.99% of coliforms and 0.97% of fecal coliforms within 10 minute. However, the removal efficiency of coliform and fecal coliform in UV light

disinfection process alone was 93% and 88%, respectively. N-doped TiO₂ nanoparticles can inactivate bacteria due to their toxicity (Adams *et al.*, 2006). Mohammad Yousef Alikhani *et al.* (2013) stated that in the absence of UV irradiation, E. coli can be adsorbed on ZnO particles and ZnO removal efficiency is between 8 and 62% depending on the ZnO dose, pH of solution And the initial concentration of bacteria changed. The high inactivation of E.coli may have an inhibitory effect on the photocatalytic reaction, largely due to the reduction of ZnO uptake sites available (Alikhani *et al.*, 2013). Yi Li *et al.* (2013) stated that UV light has limited bacterial ability to kill gram-negative bacteria, while N-doped TiO₂ nanoparticles alone lead to greater bacterial inactivation (Li *et al.*, 2015). Ultraviolet radiation in the absence of N-doped TiO₂ may provide new sources of nutrients from existing biomass dead cells and organic compounds present in wastewater for active residual microorganisms, which increases their chances of survival On the other hand Dead bacteria and their excreted intracellular components form a screen against hydroxyl radicals that block light from penetrating, so "active residual bacteria" are protected from light and do not die (Chen *et al.*, 2010).

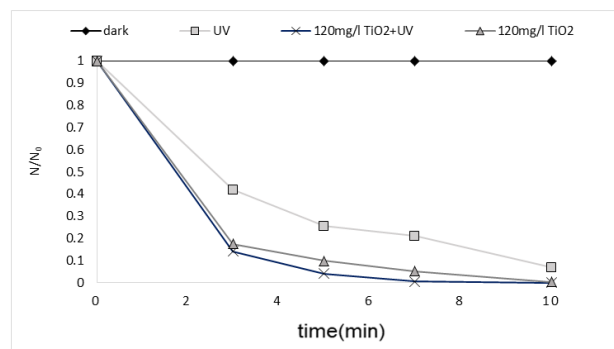


Figure 4. Influence of darkness, UV light, N-doped TiO₂ and UV \ N-doped TiO₂ nanoparticles on Total coliform removal; 120 mg /L titanium dioxide concentration

8. Effect of N-doped TiO₂ concentration

In another set of experiments, the effect of Degussa P25 N-doped TiO₂ loading on the survival time of coliform and fecal coliform during the photocatalytic disinfection of secondary treated hospital wastewater was investigated and is shown in Figures 4 and 5. In the presence of UV light by increasing the dose of titanium dioxide nanoparticles from zero to 120 mg / L the efficiency of removal of the coliform and fecal coliform increased with time. This increase in efficiency was for coliform from %93 to %100 and for fecal coliform from %88 to %100. But, as the dose of nanoparticles increased from 120 to 180 mg / l, the removal efficiency of both microorganisms decreased and reached to %99.8 and % 99.7 after 10 minutes, respectively. Increasing the dose of nanoparticles is an important parameter in increasing the efficiency of the photocatalytic process because it increases the probability of photocatalyst photon interaction. A study by Kai Ouyang *et al.* (2016) showed that low doses of nanoparticles do not completely absorb the light irradiated into the solution, resulting in the production of less active radicals in the

solution but increased dose of nanoparticles in solution the light absorption increased. As a result, active radicals higher are produced (Ouyang *et al.*, 2016). However, the greater the photocatalyst concentration, the lower the penetration of light into the slurry concentration. Romander P.S. Surrey *et al.* In 2012 stated that the optimum dose of catalyst for photocatalytic deactivation of *E. coli* was 0.1 g / L under artificial light, and the bacterial inactivation rate decreased with increasing catalyst dose (Suri *et al.*, 2012), while Radiman *et al.*, showed that low dose of N-doped TiO₂ (concentrations below 0.1 g / L) and concentrations above 2.5 g / l N-doped TiO₂ were not sufficient to inactivate *E. coli* and the optimal dose of N-doped TiO₂ to remove 10⁸ cfu / ml of *E. coli* was 1 g/ L. Increasing the dose of nanoparticles in the photocatalytic process results in the agglomeration of the nanoparticles, the reduction of the effective catalyst surface, the absorption and scattering of the irradiated light, thereby reducing the antibacterial activity (Rahim *et al.*, 2012).

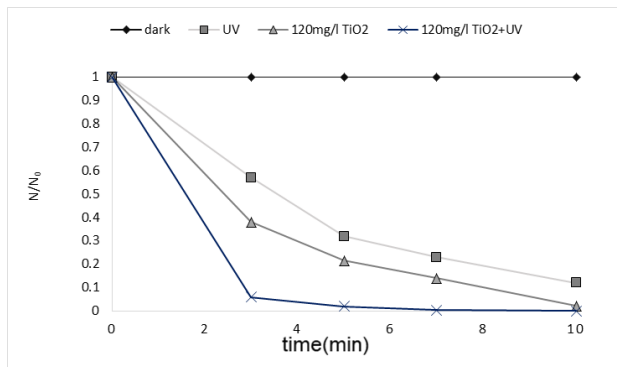


Figure 5. Influence of darkness, UV light, N-doped TiO₂ and UV \ N-doped TiO₂ nanoparticles on fecal coliform removal; 120 mg/L titanium dioxide concentration

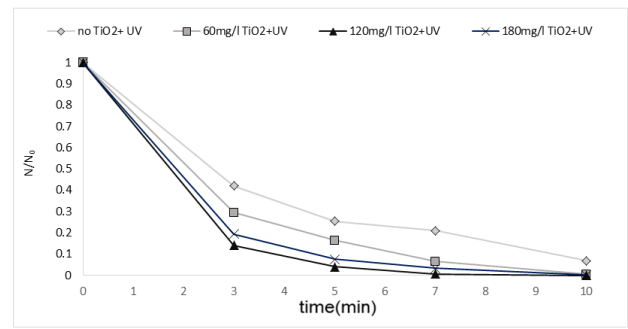


Figure 6. Influence of N-doped TiO₂ dose on coliform removal; 10 min contact time in the presence of UV light

9. Effect of contact time

The effect of changing contact time in the range 3-10 min on the bactericidal activity of 120mg/L of Degussa P25 N-doped TiO₂ was investigated and the results are shown in Figure 8. The results show that in the N-doped TiO₂ disinfection system with UV light, the removal efficiency also increases with increasing contact time. So that at a dose of 120 mg/l N-doped TiO₂ increasing the contact time from 3 min to 10 min increased the removal efficiency of the coliforms from 86% to 100% and the removal efficiency fecal coliform from 94% to 100% . Ruth Belinda *et al* in 2016 completely deactivated fecal coliform bacteria using a 1.5V cell potential and N-doped TiO₂/Ag the solution within 6 minutes(Domínguez-Espíndola *et al.*, 2017). the results of Nikos *et al.*'s study showed that even after prolonged UV irradiation (60 min) some cells in the natural waters were tested and the secondary purified wastewater sample remained alive (3% of all coliforms and 11% of Enterococci). They said in environmental samples, the types of bacteria and their growth modes vary with time. In addition, dead bacteria and the extracellular components excreted thereby protect the remaining active bacteria against light, thereby increasing the time required to further remove the bacteria and achieve higher efficiency (Lydakís-Simantiris *et al.*, 2010).

Table 3. Photocatalytic Disinfection Kinetic Coefficients of Coliform and Fecal Coliform

Type of microorg anism	Test condition	concentrati on(mg/l)	first-order		second -order		Pseudo-First		Pseudo-second	
			K	R ²	K	R ²	K	R ²	K	R ²
Coliform	N-doped TiO ₂	60	-0.2354	0.979	-0.000006	0.878	0.4527	0.5661	-0.00007	0.801
		120	-0.5172	0.9586	-0.0001	0.597	-0.4528	0.5514	-0.0013	0.5805
		180	-1.2873	0.9556	-0.0072	0.5392	-0.4521	0.5438	-0.0503	0.5355
	UV/N-doped TiO ₂	60	-0.4927	0.9561	-0.00009	0.6055	-0.4549	0.562	-0.0009	0.5876
		120	-0.7005	0.993	-0.0001	0.6552	-0.4531	0.5485	-0.0008	0.6194
		180	-0.596	0.9698	-0.0003	0.5844	-0.4536	0.5527	-0.0026	0.5708
Fecal Coliform	N-doped TiO ₂	60	-0.1279	0.9556	-0.000003	0.8817	-0.4309	0.6633	-0.00004	0.8775
		120	-0.3675	0.9554	-0.00005	0.507	-0.4268	0.571	-0.0005c	0.6247
		180	-0.5415	0.9568	-0.00008	0.6487	-0.4279	0.5647	-0.0006	0.6217
	UV/N-doped TiO ₂	60	-0.3645	0.9993	-0.00004	0.8012	-0.4269	0.5672	-0.0004	0.7301
		120	-0.8097	0.9916	-0.0005	0.6473	-0.4236	0.5431	-0.0037	0.6114
		180	-0.6085	0.9776	-0.0004	0.7195	-0.4267	0.5586	-0.0039	0.67

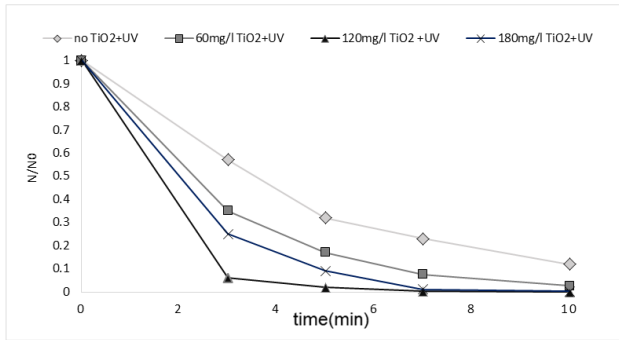


Figure 7. Influence of N-doped TiO₂ dose on Fecal coliform removal; 10 min contact time in the presence of UV light

In this study, in order to check the stability of the sample using a certain amount of nanoparticles, during several consecutive cycles of photocatalytic experiments, only 9% reduction in removal efficiency has appeared, as shown in Figures 9–11.

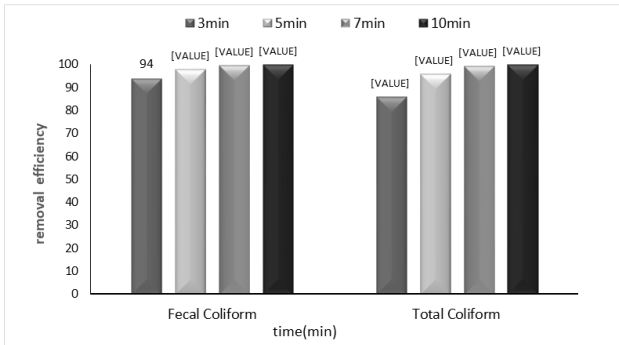


Figure 8. Influence of contact time on removal efficiency of total coliform and fecal coliform; 120mg/l N-doped TiO₂ dose and the presence of UV light

10. Kinetic modeling

Kinetic study can be useful for investigating and understanding the mechanism of contaminant removal. In this study, In this study investigated the Kinetic first-order, second -order, Pseudo-First and Pseudo- second equations. The values for the disinfection rate constant (k) under different conditions and different dose of N-doped TiO₂ together with their regression coefficients (R²) are presented in Table 3. By calculating the R² correlation coefficient for all four Kinetic models studied, it was concluded that the mechanism of inactivation of coliform and fecal coliform by N-doped TiO₂ nanoparticles and UV light follows the first-order equation.

$$\ln\left(\frac{N_0}{N_t}\right) = K_0 t$$

Where N₀ and N_t are the number of primary cells at the initial time and the number of living cells at the time of t (min) and k₀ the first-order reaction rate constants (min⁻¹), and t is the reaction time (min). The reaction rate constant (k₀) could be calculated from the slope of a plot of ln (N₀/N_t) versus (t). In this study, the effect of different disinfection systems the darkness, UV, N-doped TiO₂ and

UV\N-doped TiO₂ on the inactivation rate of coliform and fecal coliform were shown in diagrams 10 and 11.

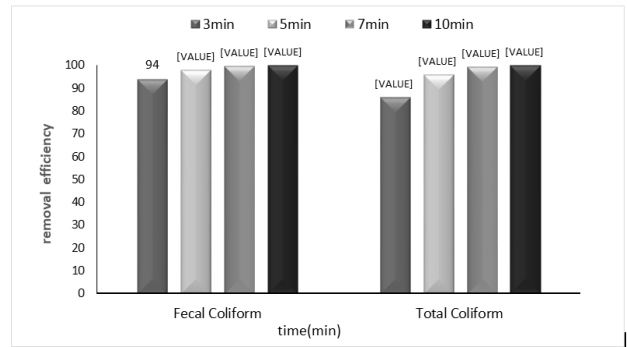


Figure 9. Stability of the sample using a certain amount of nanoparticles

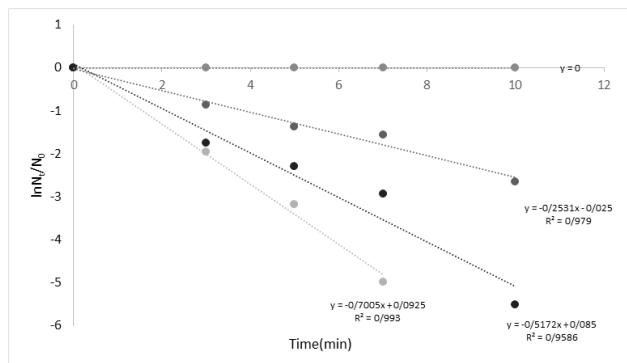


Figure 10. Kinetic Inactivation of Coliforms in Different Systems

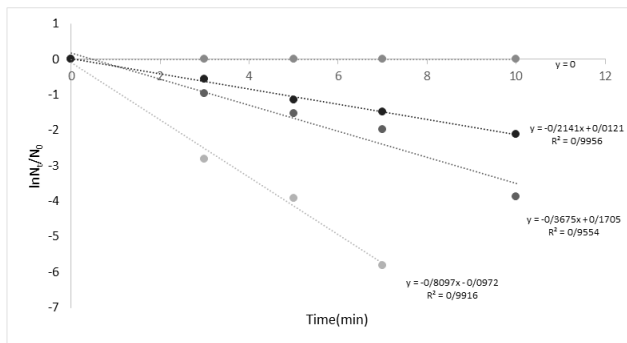


Figure 11. Kinetic Inactivation of Fecal Coliforms in Different Systems

Figures 6 and 7 show the highest constant of the removal reaction of coliform and fecal coliform for the UV \N-doped TiO₂ system, which is -0/7005 for coliform and -0/8097 for fecal coliform. In general, the results show that UV light and N-doped TiO₂ alone have poor disinfection ability in removing coliform and fecal coliform. Therefore, the reaction rate of the disinfection can be increased by a regression coefficient of 0.99 with the simultaneous use of N-doped TiO₂ and UV light (UV \N-doped TiO₂). Also Table 3 data show that in N-doped TiO₂ system alone with increasing dose N-doped TiO₂ from 60 mg/l to 180 mg/l constant rate of inactivation of coliform and fecal coliform during 10 min respectively from -0.2354 to -1. 2873 increased from -0/1279 to -0/5415. In fact, in this system there is a linear relationship between the dose of N-doped TiO₂ and the rate of bacterial inactivation (R² = 0.95). However, in the N-doped TiO₂ system with UV light, the

dose of 120 mg/l had the highest removal efficiency of fecal coliform and fecal coliform with regression coefficient of 0.99 and increased bacterial inactivation with increasing dose from 120mg/l to 180mg/l Speed has decreased.

11. Conclusion

In this study, photocatalytic removal of bacterial agents, especially microbial pullotion index bacteria by dioxide titanium nanoparticles with UV light was demonstrated and it was found that photocatalytic processes can be considered as a desirable method without producing byproducts for treatment and disinfection of hospital wastewater. This study showed that the disinfection process efficiency is influenced by the simultaneous use of N-doped TiO₂ and UV light, the dose of nanoparticles in solution and the contact time. In addition, the results showed that the efficiency of UV /N-doped TiO₂ process in removal of coliform and fecal coliform bacteria from effluent was more than UV light alone and N-doped TiO₂ alone and was able to inactivation all coliform and fecal coliforms in effluent within 10 minutes.

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