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A study on Inhibitory Effects of Titanium Dioxide Nanoparticles and its Photocatalytic Type on *Staphylococcus aureus, Escherichia coli* and *Aspergillus flavus*

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Abstract

Backgrounds and Objectives: Photocatalyst titanium dioxide nanoparticles can oxidize organic and inorganic compounds of microorganisms in aqueous solutions after exposure to UV light. In the present study, the inhibitory effect of titanium dioxide and its photocatalyst type on *Aspergillus flavus*, *Escherichia coli* and *Staphylococcus aureus* is investigated.

Materials and Methods: Toxicogenic strains of *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus* were cultured in their selective media and two groups of samples both included three different concentrations of nanoparticles (0.1, 0.5 and 1 g l^{-1}) and two control samples without any nanoparticles were considered. The first category of samples was placed on the shaker for 20 min, and the second category was irradiated by a UV lamp while shaking for 20, 40 and 60 min on a rotary shaker. Thereafter, they were cultured by using pour plate method in agar and after incubation the colonies were counted.

Results and Conclusion: Based on obtained results the photocatalyst titanium dioxide had an inhibitory effect at concentration of 1 g l^{-1} at the highest timeframe (60 min). In addition, the test variables *i.e.* the type of bacteria, concentration of nanoparticles and time had a significant effect on the growth inhibition of microorganisms. Regarding the economic aspects of contamination control and its importance in dairy products, application of photocatalystic nanoparticles of titanium dioxide is recommended.

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1. Introduction

Advances in manufacturing processes of nano-structures and nanomaterials with suitable properties led to increase in production of stable nanoparticles capable of applying in food and related industries. Nanotechnology has wide applications in food preservation and especially food packaging and in some cases it has been success-fully applied [1]. Drug-resistant infections will soon cause more than 10 million deaths in year and the death rate caused by super bugs will be even more than those of different types of cancer [2]. In fact, lack of food and population growth made it necessary to develop nanotechnology. The use of advanced technologies can be considered as a new approach in this sector of industry. Application of nanotechnology opens a new horizon for novel processing and production techniques in food industry.

Application of this field of science can also influence the economy significantly and improve life quality and standards [3]. Packaging perhaps is the most applicable industry to use the nanotechnology due to its crucial role in protecting the food products from physical damage and contamination [4]. In addition, utilization of these nanoplastics with high performance against microorganisms can increase the shelf life of food products. Shelf life of products packed with nano-materials showed to increase from 3 or 4 days to 2-3 weeks at room temperature [3,5].

Nanocomposite food packages consist of resins (either thermoset or thermoplastics *i.e.* polyethylene, polyamide, polystyrene) and nanofillers that enhance the dimensional stability, strength, heat resistance and barrier properties [6,7]. TiO_2 nanoparticles and nanoclay are used in nanocomposites in food packaging.

TiO_2 nanoparticles which are mainly used as plastic fillers are based on two categories of organic and inorganic (e.g. Fe, Si, Ti, Ca, etc.) materials. The latter are more used in food packaging [7].TiO_2 nanoparticles have been of interest to researchers in various disciplines thus far due to their physical, photocatalytic, low toxicity and allergic reaction properties[8].

On the other hand, the dairy products are well known for their role and value in human nutrition since long time ago [9]. Their rich and valuable nutritional characteristics make them to get easily exposed to various contaminations and thus spread pathogen agents. Therefore, milk borne diseases caused from contaminated dairy products can lead to drug resistant infections. Since the local dairy products contain the unpasteurized milk, the presence of milk borne pathogens in dairy products may be also significant [10]. The presence of *Escherchia coli* bacteria must be negative in both pasteurized and sterilized dairy products. Presence of coliforms in pasteurized milk indicates poor hygiene in pasteurization and packaging processes.

In addition, the poisoning caused by *Staphylo-coccus aureus* is known as one of the most common food poisoning. This Gram positive bacterium is an important pathogen in food hygiene and especially dairy products. This bacterium has a high tolerance against sodium chloride (in concentration up to 15%), and thus cause spoilage of dairy products containing high amount of salt [11]. The growth of molds and yeasts in dairy foods can occur fast and easily. An instance is *Aspergillus flavus* that is known as an important fungus due to producing aflatoxin as a carcinogenesis poison in human liver [12].

On the other hand, the increasing resistance of bacteria against antibiotics, human sensitivity towards antibiotic and occurrence of antibiotic-resistant pathogens in human body are the problems of using antibiotics as an antimicrobial agent to increase the shelf life of dairy products [13]. Thus, utilization of antimicrobial characteristics of titanium dioxide nanoparticles seems a necessity.

Nanoparticles may work through different mechanisms; they can directly react with microbial cell or indirectly cause DNA damage of the microorganism [14,15]. These nanoparticles are most functional in self-cleaning and disinfecting by coating surfaces in food industry [16]. Nowadays, TiO_2 Nanoparticles are used in transparent coating of foods as protection against the UV. According to European Food Safety Authority there is no safety concern for the consumer if the titanium nitride is used up to 20 mg kg^{-1} in only PET plastics intended for contact with all types of foodstuffs since there would be no migrations of these nanoparticles into food. Titanium dioxide is a semiconductor with a band gap of 3.2 eV that can become a photocatalyst for antibacterial reaction under the UV irradiation [17]. During a catalytic process, in the presence of TiO_2 water on the electrode surface is decomposed into hydrogen and oxygen.

Studies showed that many organic compounds decompose to mineral compounds during this process [18]. Hazardous organic compounds such as deodorants, pesticides, paint, etc. are the main pollutants that can affect the environment caused by means of pharmaceuticals, textiles, agricultures and food industries. Many of these toxins are capable of decomposing to harmless compounds (such as water and carbon dioxide) by photocatalytic reactions [19]. Spoilage of dairy products is a huge economic problem as each year almost one fourth of food worldwide lost their nutritional value due to microbial activities.

Nanoparticle of titanium dioxide is an example of nanotechnology products which can be used in packaging of dairy products [19]. It has been widely used as an antimicrobial agent in recent years due to its resistance to high temperatures, low solubility, high specific surface area, cost-effectiveness hydro-philic and strong oxidizing properties [20-22]. One of the noteworthy applications of this nanoparticle is eliminating the microorganisms causing food spoil-age in form of covered surfaces in the food package-ing industry [15,23].

Nowadays, access to healthy food for all the individuals is an indication of the level of economic development in a society. Thus, this project aimed to study the simultaneous influence of concentration and contact time of titanium dioxide nanoparticles and its photocatalyst type on some of milk-borne pathogens *i.e. A. flavus*, *E. coli* and *S. aureus*.

2. Materials and methods

2.1. Materials

Titanium dioxide nanoparticles in powder form with an average diameter of 30 nm and a purity of 99.98% was purchased from Pishgaman Nanomaterial Iranian Company (Mashhad, Iran). Lyophilized bacterial strains of Gram negative bacterium *E. coli* (ATCC8739), Gram positive

bacterium *S .aureus* (ATCC6538) and fungus *A. flavus* (PTCC5004) were provided from the Iranian Research Organization of Science and Technology.

Tests were conducted for all three groups of bacteria and fungus since antibacterial activity of TiO_2 nanoparticles may get influenced by cell wall of microorganisms. In the present study, Giolitti-Cantoni Broth for *S. aureus* and Lauryl Sulfate Broth for coliform were provided from Merck (Germany); Potato Dextrose Agar was purchased from Scharlau Company (Barcelona, Spain) for activation of *A. flavus*, and Mueller-Hinton agar was provided from Merck (Germany) for preparation of pure culture.

2.2. Analysis of the results of electron microscopy on titanium dioxide nanoparticles

Scanning Electron Microscopy (Carl Zeiss, Germany) was used for determination of diameter and also surface area to volume ratio of nanoparticles. As shown in Figure 1 nanoparticles of titanium dioxide are in a spherical shape with an average diameter of about 30 nm displaying recombinant proteins on the cell surface are those displaying single recombinant protein encoded by a gene expressed either from an episomal plasmid or integrated into the yeast genome.

2.3. Preparation of TiO_2 nanoparticles suspension

To follow the safety standards a 5% aqueous stock solution was prepared according to the special instruction. First 5 g of nanoparticle powder was weighted and transferred to an 250 ml Erlenmeyer, diluted to volume 100 ml and was autoclaved in 121°C for 15 min. Three different concentrations of 0.1, 0.5 and 1 g l^{-1} were prepared from the aqueous stock solution [19].

2.4. Activation of Strains

Microbial strains were activated based on the instructions provided as additional protocol of the manufacturer. First, inoculation was conducted by using a sterile loop and transferring lyophilized microorganisms into 10 ml sterile special liquid medium (GCB, LSB and PDA) which had been distributed in the test tubes. These inoculated medium were used as the source medium [23,24].

Microbial and fungal suspensions were prepared according to (0.5) McFarland Standard which is equivalent to 1.5×10^8 bacterium in each ml. For testing the turbidity of these suspensions, the absorbance was measured at 620 nm of wavelength by using a UV-VIS spectrophotometer (UNICO-2100, USA) and was ranged 0.1-0.8. For determination of CFU for each microorganism, the source stock was used and cultured by pour plate method in agar medium after preparation of different dilutions; thereafter the initial microbial population was obtained [25].

2.5. Antimicrobial effects of titanium dioxide nanoparticles

Two groups of samples were considered for evaluation of antimicrobial effect of nanoparticles of titanium dioxide and its photocatalyst type for each microorganism. The first group of samples (including four beakers) was considered for evaluating the effect of TiO_2 nanopatrticles and the second group of samples (including five beakers) was considered for evaluating the effect of photocatalyst type of nanoparticles in the presence of UV light. All samples were placed in the oven at 180°C for 1 h to get sterilized. Content of sample beakers for each microorganism included 20 ml of special liquid medium and 1 ml of the mixture suspension of microorganism or fungal with nano-particles in certain concentration. Three control beakers all containing medium and related suspension for each microorganism with no nanoparticles were prepared and one of them was exposed to irradiation by using a UV lamp (in order to use as control for group two), while the other two were covered by aluminum foil and kept in a dark place until use (in order to use as control for group one and two).

All samples were vortexed for 15 min before using nanoparticles in order to homogenize the suspensions. The 4 beakers of the first group of samples (which were prepared in order to evaluate the antimicrobial effects of different concentrations *i.e.* 0.1, 0.5 and 1 g l^{-1} of TiO_2 nanoparticles and one control) were placed on a shaker with a speed of 20 g for 20 min for 20 min. The 5 beakers of second group (which were prepared in order to evaluate the antimicrobial effects of different concentrations *i.e.* 0.1, 0.5 and 1 g l^{-1} of photocatalytic TiO_2 nanopart-icles with two control samples *i.e.* one exposed to UV irradiation and the other covered with aluminum foil) were placed on a shaker with a speed of 20 g for 20, 40 and 60 min while a UV lamp was placed at a distance of 8 cm from them. Thereafter, 1 ml of the processed suspension and MHA and PDA medium were added into plates by using pour plate method in agar.

Plates were placed upside down in incubator for certain time and temperature depending on the type of microorganism content *i.e. S. aureus* and *E. coli* bacteria incubated at 37°C for 24 h and *A. flavus* at 25°C for 72 h. Then, the colonies were counted. All the steps were replicated three times during the entire process at Biotechnology department, Agricultural Research Education and Extension Organization of East Azarbaijan [26,27].Then the number of colonies was calculated per milliliter, as follows [28].

$$N = \frac{\sum C}{n.d} \qquad \text{Eq 1.}$$

In this equation, N represents the number of colony forming units per milliliter, ΣC represents the set of colonies counted in triplicate, d represents dilution factor and n represents the number of plates counted. In this study, the simultaneous effect of both concentration and contact time of nanoparticles on microbial growth was performed using a factorial design. A Completely Randomized Design (CRD) was used with LSD value at the 5% level of significance. SAS software, version 9.1 ($P \leq 0.05$) was used to determine the relationship between the population of microorganisms and concentrations of nanoparticles. The average of three conducted tests was plotted in Microsoft office Excel, 2010.

3. Results and Discussion

3.3.1. Analysis of the results of electron microscopy on titanium dioxide nanoparticles

Scanning Electron Microscopy was used for determination of diameter and also surface to volume ratio of nanoparticles. As shown in Figure 1 nanoparticles of titanium dioxide are in a spherical shape with an average diameter of about 30 nm.

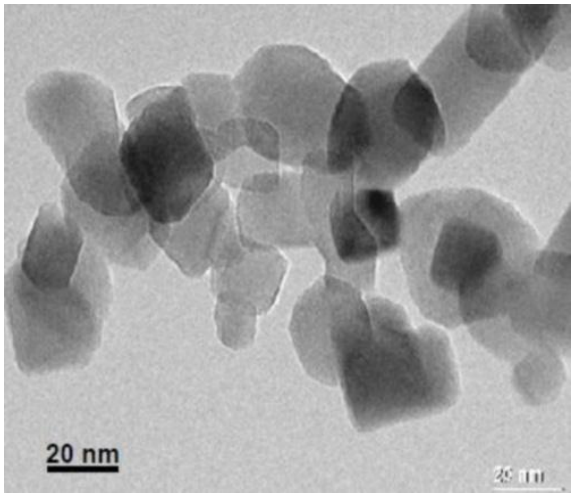

Figure 1. Scanning electron microscopy (SEM) of TiO_2 nanoparticles.

3.3.2. The effect of titanium dioxide nanoparticles and its photocatalytic type on *E. coli*

The effect of different concentrations of titanium dioxide nanoparticles on *E. coli* is given in Figure 2.

It shows by increasing concentrations of nanoparticles microbial population reduced compared to those of control sample.

Figure 3 illustrates changes in population of *E. coli* at varying time intervals under photocatalytic process and with presence of titanium dioxide nanoparticles. Microbial load significantly reduced by increasing the time of photocatalytic process. According to the obtained results, the efficiency of titanium dioxide nanoparticles undergone photocatalytic process was much higher in *E. coli* population removal compared to that of nanoparticles with no exposure to irradiation.

3.3.3. The effect of titanium dioxide and its photocatalytic type on *S. aureus*

Figure 4 shows the decrease in population of *S. aureus* in the presence of titanium dioxide nanoparticles. The simultaneous effect of contact time and concentration of titanium dioxide nanoparticles on microbial growth was investigated.

As shown in Figure 4 the average population of *S. aureus* bacteria in the control sample is 330 CFU ml^{-1}, indicating that this population reduced remarkably. In Figure 5 the effect of titanium dioxide irradiated by UV during certain time for photo-catalytic process, on *S. aureus* is shown. As seen in Figure 4 and Figure 5, by applying longer exposure time of irradiation and increasing the photocatalytic process, the average microbial population reduced, however, this impact is only slightly different with that of non-irradiated nanoparticles.

3.3.4. The effect of titanium dioxide and its photocatalytic type on *A. flavus*

The effect of titanium dioxide on population of *A. flavus* is shown in Figure 6. *A.flavus* population had no significant reduction compared to control sample and also in the presence of different concentrations of titanium dioxide. Nevertheless, as shown in Figure 7 by increase in concentration and time of irradiation, decrease in population of *A. flavus* was significant. Cell wall of *A. flavus* consists of long carbohydrate layers, long chain of polysaccharides along with glycoproteins and lipids which may be one of the reasons of its resistance. However this resistance can be overcome by generating oxidative agents during the photocatalytic process [29].

Antimicrobial impact of titanium dioxide on *S. aureus* and *E. coli* was more than that of *A. flavus*. These results are consistent with a report by Biebesheimer (2011) that studied the impact of antimicrobial of titanium dioxide and zinc oxide on the Gram positive enterococcus and Gram negative bacterium *E. coli* [30].

The results showed that the non-photocatalyticTiO_2 nanoparticles had less antimicrobial effect on *E. coli* cell wall compared to their effect on Enterococcus cell wall [23]. Another study conducted on controlling *E. coli* growth by titanium dioxide indicated high inhibitory effect against *E. coli*, which is in accordance to our results *i.e.* TiO_2 nanoparticles undergone photocatalytic process are much more effective against *E. coli* compared to those with no exposure to UV irradiation [23].

An earlier study by Jacoby et al., (1998) indicated that the mortality rate of bacteria depends not only on the toxic effect of photocatalystic TiO_2

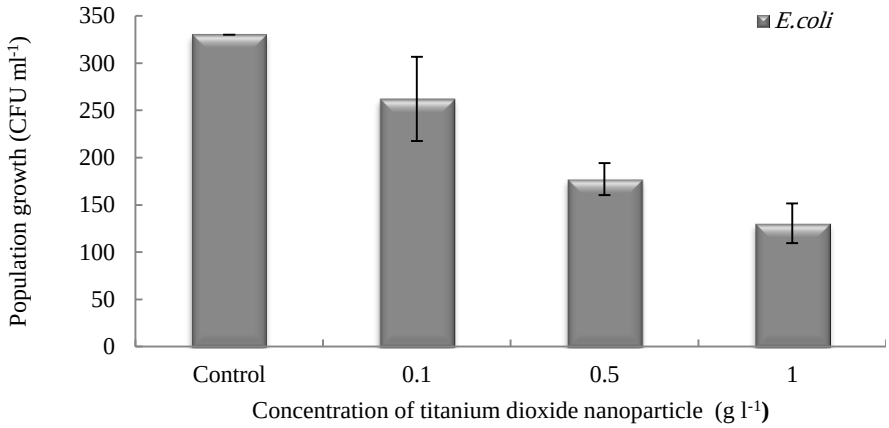

Figure 2. The effect of TiO_2 nanoparticles on *E. coli*. Error bars indicate mean±standard deviation of three replicates.

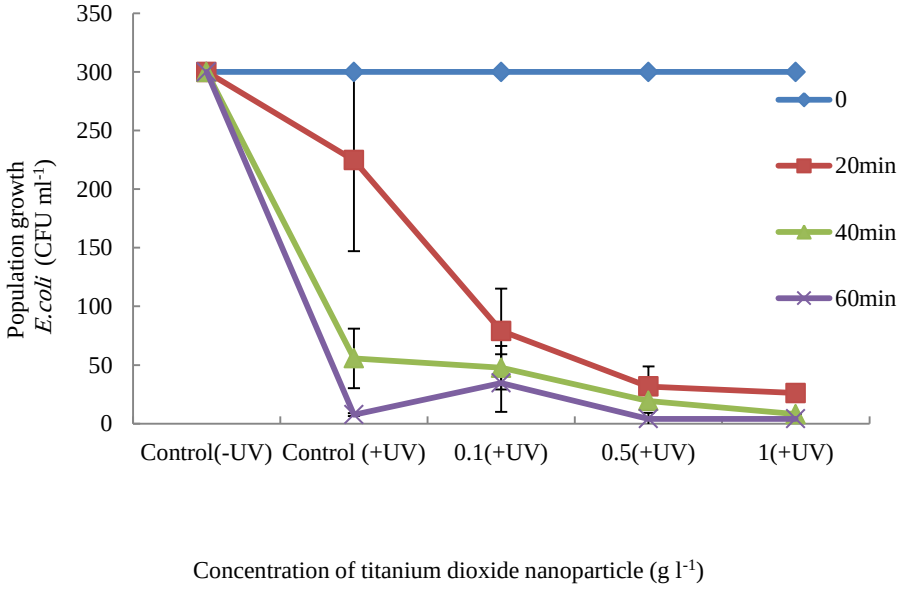

Figure 3. The effect of photocatalytic TiO_2 nanoparticles on *E. coli* bacteria (+UV: control samples irradiated with UV; -UV: non irradiated control samples; 0.1%+UV, 0.5%+UV,1%+UV: Irradiated samples with 0.1, 0.5 and 1 g l^{-1} of TiO_2 nanoparticles respectively). Error bars indicate mean±standard deviation of three replicates.

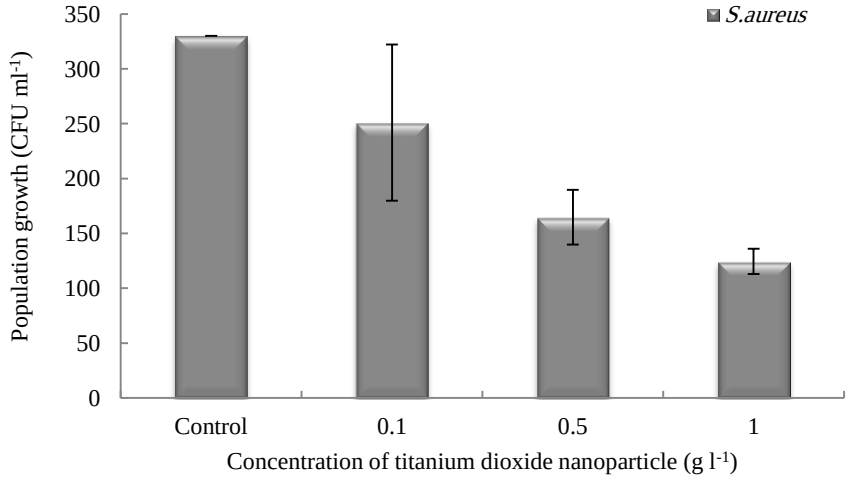

Figure 4. The effect of TiO_2 nanoparticles on *S. aureus*. Error bars indicate mean±standard deviation of three replicates.

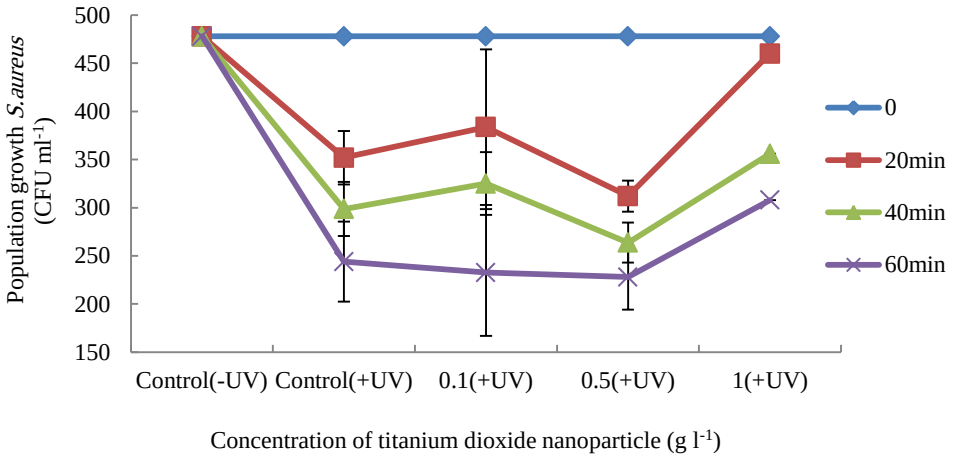

Figure 5. The effect of photocatalytic TiO_2 nanoparticles on *S. aureus* (+UV: sample irradiated with ultraviolet radiation, -UV: non-irradiated control samples, samples treated with 0.1, 0.5 and 1 g $l^{-1}$of TiO_2 nanoparticles). Error bars indicate mean±standard deviation of three replicates.

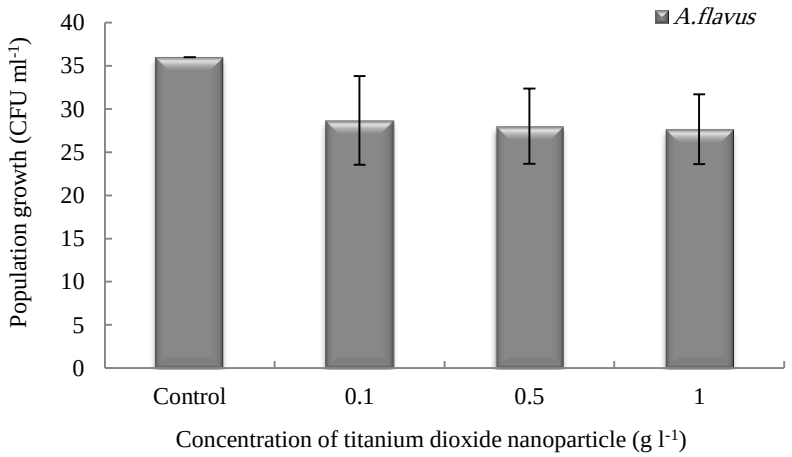

Figure 6.The effect of TiO_2 nanoparticles on *A. flavus.* Error bars indicate mean±standard deviation of three replicates.

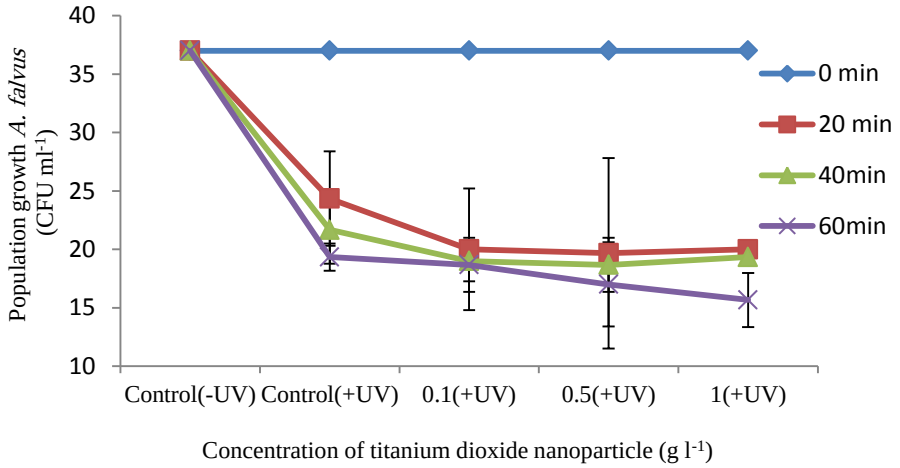

Figure 7. The effect of photocatalytic TiO_2 nanoparticles on *A. flavus* fungus (+UV: sample irradiated with ultraviolet radiation, -UV: non-irradiated control samples, samples treated with 0.1, 0.5 and 1 g $l^{-1}$of Tio_2 nanoparticles). Error bars indicate mean±standard deviation of three replicates.

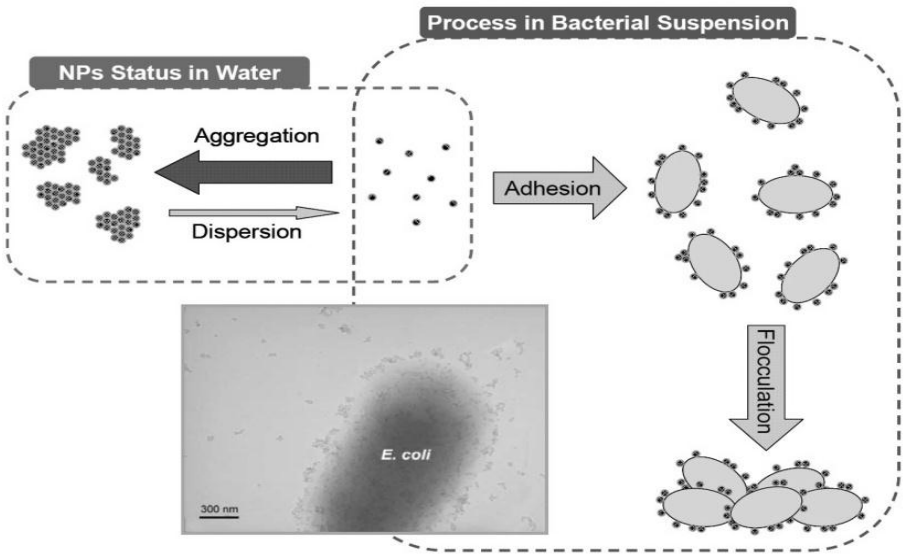

Figure 8. The mechanism of binding nanoparticles to *E. coli* cells [37].

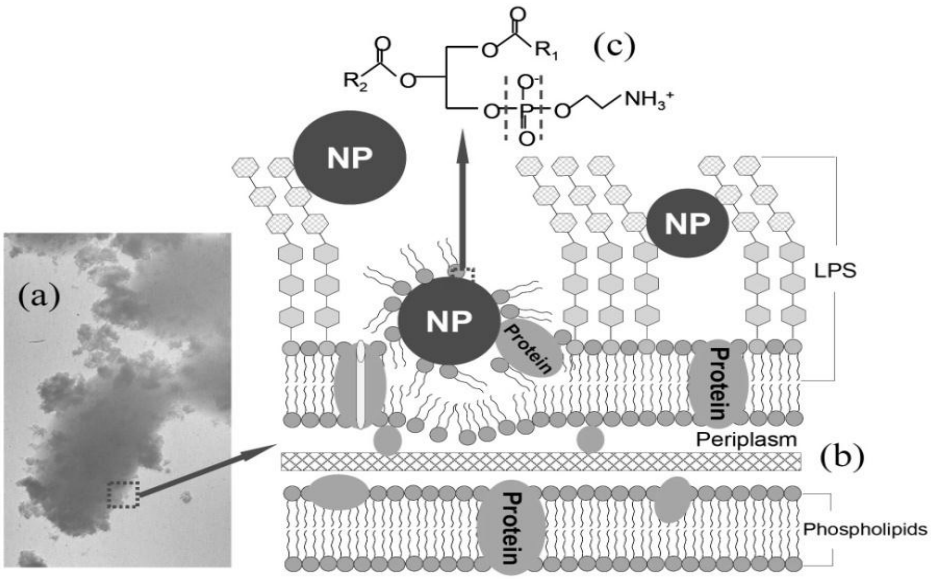

Figure 9. The mechanism of effects of TiO_2 nanoparticles on cell walls of *E.coli* [37].

but also on their cell structure and cell wall thickness. Thus, decomposition of bacterial components also plays role in bacterial death beside the toxic effect of photocatalytic TiO_2 nanoparticles.

According to this report, by irradiating the nanoparticles of TiO_2 in sufficient long time, organic matter in bacteria will completely turn into minerals. Our obtained results also tally with this finding. In another study by Cho et al., linear correlation was reported between concentration of hydroxyl free radicals and inactivation of *E. coli* in disinfection process by using photocatalytic TiO_2 nanoparticles and the present study achieved similar findings [33].

As a result, by increasing the time of UV exposure to the nanoparticles, concentration of hydroxyl radicals increases and many of cell structure components completely mineralized as the direct relationship between the concentration of hydroxyl free radicals and deactivation of *E. coli* were reported in previous studies [18,34,35].

In the present study, the relationship between the concentration of titanium dioxide and increase in time of photocatalytic process with elimination of *E. coli* population was proven.

According to the obtained results, Gram negative *E. coli* showed much higher sensitivity towards oxidative agents generated during the photocatalytic process. There are different results regarding the sensitivity of Gram negative and Gram positive bacteria. In some reports Gram positive types are known as the more sensitive species while there are studies which report the opposite [36]. This difference in results could be due to the fact that the effect of UV light and generation of oxidative agents in photocatalysis process can also take part in addition to the role of characteristics of bacterial strains. The results indicated that the toxic effects of titanium

dioxide are due to water solubility of metal ions of it. The negative charge throughout the bacterium cell wall cause lipids to react with free metal ions with positive charge in the solution and once enough amounts of nanoparticles bind to the bacterium cell wall, membrane fluidity would be affected and that leads to decomposition of membrane phospholipid structure, decrease in its hydrophobic properties and eventually bacterial death [37]. These strong bonds of nanoparticles to outer layer of *E. coli* and *S. aureus* bacteria make them being slippery in the culture medium. They also cause restraining the dehydration process, controlling enzymes in the periplasmic space and controlling synthesis of RNA, DNA and proteins [38]. Titanium dioxide nanoparticles cause peroxidation of polycyclic phospholipid compounds of microorganism's membrane and reduce the membrane monotony, membrane dependent respiratory function and at last cell death [39,40]. No exact mechanism is known to explain how inhibition of microbial growth takes place. Nevertheless, the difference of mortality rates between studied microorganisms might be due to their different resistance against damages caused by oxidative agents and that in turn depends on the type of microorganisms and cell wall structure [37].

4. Conclusions

Nowadays, there is a great competition in the food industry for application of novel technologies. Thus, utilization of the latest technologies is essential in order to maintain and expand the market of food industry. Nanoparticles today are widely used in food processing. One of their important applications is their ability in elimination of food borne pathogens as covered surfaces in food packaging industry. Thus, antimicrobial titanium dioxide nanoparticles can be a proper alternative to common antibiotics and antifungals since their use in packaging of food and dairy products would be more affordable. The present study indicated and proved the antimicrobial effects of titanium dioxide on some of milk borne pathogenic microorganisms. These antimicrobial properties can help to reduce microbial load of food and especially dairy products in the future. Nevertheless, as a suggested future study, it could be of high value to perform similar study in the frame of target dairy products, especially milk.

5. Acknowledgment

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6. Conflict of interest

The authors declare no conflict of interests.

References

1. Saraschandra N, Pavithra M, Sivakumar A. Antimicrobial application of TiO_2 coated modified polyethylene (HDPE) films. Arch Appl Sci Res. 2013; 5 (1): 189-194.
2. Lai C, Lee K, Xiao Y, Ahmad N, Veeraraghavan B, Thamlikitkul V. High burden of antimicrobial drug resistance in Asia. J Glob Antimicrob Resist. 2014; 2 (3): 141–147. doi:10.1016/j.jgar.2014.02.007.
3. Nakayama N, Hayashi T. Preparation and characterization of TiO_2 and polymer nanocomposite films with high refractive index. J Appl Polym Sci. 2007; 105 (6): 3662–3672. doi: 10.1002/app.26451.
4. Restuccia D, Spizzirri UG, Parisi OI, Cirrillo G, Curico M, Iemma F, Puoci F, Vinici G, Picci N. New EU regulation aspects and global market of active and intelligent packaging for food industry applications. Food Control. 2010; 21 (11): 1425-1435.
5. Fathi BC, Mohebbi M. Increased food safety with the use of nanotechnology. J. Nanotechnol. 2009; 4 (9): 16-18.
6. Ray SS, Okamoto M. Polymer/Layered Silicate Nanocomposites: A Review from Preparation to Processing. Prog. Polym. Sci. 2003; 28, 1539–1641.
7. Liyaghati L, Azizi M, Hussain B, Jokar M. The use of nanocomposites in packaging and food industry. Int. J. Nanotech. 2013; 10 (11): 14-18.
8. Chawengkijwanich C, Hayata Y. Development of TiO_2 powder-coated food packaging film and its ability to inactivate *Escherichia coli* in vitro and in actual tests. Int. J Food Microbiol. 2008; 123 (3): 288-292.
9. Rezaei M, Karimi F, Yahyaei M, Javdani H, Shahabi A, Farahi A. A survey of microbial total count and prevalence of *Escherichia coli* in raw milk in Markazi Province, Iran. Res Opin Anim Vet Sci. 2013; 3 (12): 474-477.
10. Vaziri S, Noroozi M. Evaluation of coliforms and *E. coli* contamination of Tabriz Lighvan local cheeses in Maragheh. Iran J Med Microbiol. 2012; 7: 23-28.
11. Spanu V, Spanu C, Virdis S. Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. Int J Food Microbiol. 2012; 153: 7-53.
12. Wogan GN. Aflatoxin as a human carcinogen, Hepatology, 1999; 3 (2): 573-575.
13. Brinsley K, Srinivasan A, Sinkowitz-Cochran R, Lawton R, McIntyre R, Kravitz G. Implementation of the Campaign to Prevent Antimicrobial Resistance in Healthcare Settings: 12 steps to prevent antimicrobial resistance among hospitalized adults-experiences from 3 institutions. Am J Infect Control. 2005; 33 (1): 53–54. doi: 10.1016/j.ajic.2004.12.003
14. Emamifar A. Applications of antimicrobial polymer nanocomposites in food packaging. Adv Nano Compos Technol. 2011; 2, 300-318.
15. Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, Alvarez PJJ. Antimicrobial nanomaterial for water disinfection and microbial control: potential applications and implications. Water Res. 2008; 42 (18): 4591-4602.
16. Haghi M, Hekmatafshar M, Janipour MB, Gholizadeh S, Faraz M, Sayyadifar F, Ghaedi M. Antibacterial effect of TiO_2 nanoparticles on pathogenic strain of *E.coli*. Int. J. Adv. Biotechnol. Res. 2012; l3: 621-624.

17. Gupta K, Singh RP, Pandey A, Pandey A. Photocatalytic antibacterial performance of TiO_2 and Ag-doped TiO_2 against *S. aureus. P. aeruginosa* and *E. coli*. Beilstein J Nanotechnol. 2013; 4: 345-351. doi: 10.3762/bjnano.4.40.

18. Hilmi A, Luong JHT. Utilization of TiO_2 deposited on glass plates for removal of metals from aqueous wastes. Chemosphere. 1999; 38 (4): 865-876. doi:10.1016/S0045-6535(98)00225-2.

19. Roy AS, Parveen A, Koppalkar A, Ambika Prasad MVN. Effect of nano titanium dioxide with different antibiotics against methicillin-resistant *staphylococcus aureus*. J Biomater Nanobiotechnol. 2010; 1 (1): 37-41. doi:10.4236/jbnb.2010.11005.

20. Xing Y, Li X, Zhang L, Xu Q, Che Z, Li W. Effect of TiO_2 nanoparticles on the antibacterial and physical properties of polyethylene-based film. Prog Org Coat. 2012; 73 (2-3): 219-224.

doi:10.1016/j.porgcoat.2011.11.005.

21. Tavassoli S, Mahdavi M, Hamzeh F, Rezvani MB, Ahmadian Babaki F, Saderi H, Kharazifard MJ. Effect of Nano Titanium Dioxide on Self-Cleaning and Antibacterial Properties of Dental Mirrors. J Dent Sch Shahid Beheshti Univ Med Sci. 2013; 31 (3): 177-185.

22. Han K, Yu M. Study of the preparation and properties of UV-blocking fabrics of a PET/TiO_2 nanocomposite prepared by in situ poly condensation. J Appl Polymer Sci. 2006; 100 (3): 1588-1593.

doi 10.1002/app.23312.

23. Jaderi Z, Maskooki A, Mortazavi A. The lethal and inhibition effects of titanium dioxide on harmful microorganisms in foods. J Res Innovat Food Technol. 2013; 1: 47-60.

24. Naddafi K, Zare M, Yunesian M, Mohammadi M, Rastkari N, Mousavi N. Bioassay for toxicity assessment of zinc oxide and titanium oxide to *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923 Bacteria. Iran J Health Environ. 2011; 4: 171-180.

25. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater. 2008; 4: 707-716. doi:10.1016/j.actbio.2007.11.006.

26. Rahim S, Radiman S, Hamzah A. Inactivation of *Escherichia coli* under fluorescent lamp using TiO_2 nanoparticles synthesized via sol gel method. Sains Malaysiana, 2012; 41: 219-224.

27. Akiba N, Hayakawa I, Keh ES, watanabe A. Antifungal effects of a tissue conditioner coating agent with TiO_2 photocatalyst. J Med Dent. Sci. 2005; 52 (4): 223-227.

28. Hoseinzadeh A, Samarghandi M, Alikhani, M, Godini H, Shams Khorramabadi Gh. The coefficient of sensitivity and lethal kinetic of *Escherichia coli* and *Staphylococcus aureus* compared to of zinc oxide and copper oxide. J Isfahan Med Sch. 2013; 20: 1153-1165.

29. Oppezzo OJ, Pizarro RA. Sublethal effects of ultraviolet A radiation on *Enterobacter cloacae*. J Photo-chem. Photobiol. B. 2001; 62: 158-165.

30. Bijan B, Zare M, Fatehizadeh M, Mohseni A, Toolabi A. Comparison of CuO and TiO_2 nanoparticles toxicity and antibacterial effects in the solid media. Int. J Environ Res Public Health. 2012; 6 (2): 167-176.

31. Biebesheimer OM. An investigation into metal oxide nanoparticle toxicity to bacteria in environmental systems using fluorescence based assays. PhD dissertation, The University of Toledo Digital Repository. Ohio, United States, 2011; 1-109.

32. Jacoby WA, Maness BC, Wolfrum EJ, Blake DM, Fennell JA. Mineralization of bacterial cell mass on a photocatalytic surface in air. Env Sci Technol. 1998; 37 (17): 2650-2653. doi: 10.1021/es980036f.

33. Cho M, Chung H, Choi W, Yoon J. Linear correlation between inactivation of *E.coli* and OH radical concentration in TiO_2 photocatalytic disinfection. Water Res. 2004; 38 (4): 1069-1077.

34. Othman SH, Abd Salam NR, Zainal N, Basha RK, Talib RA. Antimicrobial activity of TiO_2 nanoparticle-coated film for potential food packaging applications. Int J Photoenergy. 2014; 2014: 1-6.

doi: 10.1155/2014/945930.

35. Vijayaraghavan R. Zinc oxide based inorganic antimicrobial agents. Int. J. Sci. Res. 2012; 1: 35-46.

36. Rinco AA, Pularin C. Bactericidal action of illuminated TiO_2 on pure Escherichia coli and natural bacterial consortia: post-irradiation events in the dark and assessment of the effective disinfection time. Appl Catal B, 2004; 49: 99-112.

37. Jiang W. Bacterial Toxicity of Oxide Nanoparticles and Their Effects on Bacterial Surface Biomolecules. 2011. PhD dissertation, University of Massachusetts. Boston, United States.

38. Pin-ching M, Smolinski S, Blake DM, Huang Zh, Jacoby WA. Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. Appl Environ Microbiol. 1999; 65 (9): 4094-4098.

39. Wu TH, Tai YD, Shen LH. The novel methods for preparing antibacterial fabric composites containing nano-material. Solid State Phenom. 2007; 124: 1241-1244. doi:SSP.124-126.1241.

40. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. J Colloid Interface Sci. 2004; 275: 177-182. doi: 10.1016/j.jcis.2004.02.012.