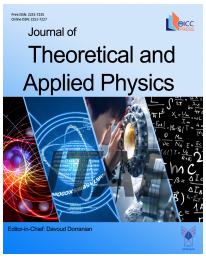

Volume 18, Issue 1, 182402 (1-10)

Journal of Theoretical and Applied Physics (JTAP)

https://dx.doi.org/10.57647/j.jtap.2024.1801.02

Photocatalytic properties of silica nanoparticles in water decontamination; adsorption and photocatalytic disinfection

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Original Research

Received:
17 July 2023
Revised:
12 October 2023
Accepted:
30 October 2023
Published online:
30 December 2023

Abstract:

In this experimental research the adsorption and photocatalytic properties of silica nanoparticles were used to disinfection water against the gram-negative *Escherichia coli* bacteria. Silica nanoparticles were synthesized by modified Stöber method and characterized. TEM image shows that the prepared nanoparticles were spherical with $20-30$ nanometers diameter and 4.25 eV bandgap energy. The disc diffusion test was employed to evaluate the antimicrobial properties of produced nanoparticles against the *Escherichia coli* bacteria. The optimal pH of water disinfection was determined for decontamination. And finally silica nanoparticles activity was assayed to disinfect the suspended *Escherichia coli* bacteria in water by adsorption and photocatalytic mechanisms. Results were compared with photolysis process. According to disc diffusion test, the antimicrobial effects of silica nanoparticles strongly depend of their concentration. Results confirm that silica nanoparticles are capable for cheap and fast decontamination of bacterial infected water.

Keywords: Silica nanoparticles; Antibacterial; Photocatalytic process; Disc diffusion test

1. Introduction

Concerns about environmental pollution have caused human beings to use new methods to eliminate them. Among environmental pollution, water contamination is a great threat for human health. Water pollution by microorganisms including bacteria, viruses and fungi are serious concern due to adverse effects on water quality [1, 2]. Different conventional physical and physicochemical methods including filtration, adsorption, ozonation, chlorination and flocculation have been examined for microbial disinfection of water [2]. Nevertheless, these methods have specified disadvantages including; formation of disinfection byproducts, high operation and maintenance cost, antibiotic drug resistance and Low disinfection efficiency for hazardous biological pollutants [3]. Nanotechnology acts an important role for conflict with the environmental pollution issues [4]. Brilliant physical and chemical properties of metal oxide nanoparticles have introduced a significant perspective for the elimination of environmental pollution, including bacterial pollution [5]. Nano sized oxides including AuO, AgO, Al_2O_3, ZnO, TiO_2, SiO_2, CuO are known as antimicrobial and photocatalytic factors. They are highly selective materials for water disinfection based on adsorption and photocatalytic disinfection [6–8]. Advances in technology of the preparation of metal oxide nanoparticles have led to improved performance of these nanoparticles in the field of antibacterial and photocatalytic activities [9–13].

These nanoparticles have antibacterial potential because of the fact that they can destruct the bacteria by interacting with the bacterial surface and penetrate to the cell membrane. The oxidative stress process destroys the lipids, carbohydrates, and proteins and finally the DNA of bacterial cell [14]. Furthermore their adsorptive performance and catalytic activities make them as an attractive candidate to microbial decontamination of water sources [15]. Their irradiation with photons having equal or more energy than the bandgap energy, leads to creation of electron-hole pairs in the conduction and valance band of the molecules on the surface of photocatalyst nanoparticle and capable them to react with water to generate free radicals for secondary reactions [16]. Reactive oxygen species (ROS) generated

by photocatalyst nanoparticles under illumination play a dominant role in the process of inactivating bacteria. They attack bacteria by creating damage to the cell membrane [17, 18].

Among the photocatalytic nanoparticles ZnO and TiO_2 have the most studies due to their qualified photocatalytic effect. Thus far, little literature is present for the use of silica nanoparticles for the photocatalytic disinfection. Silica nanoparticles are an attractive alternative for these two nanoparticles due to their biocompatibility nature and easy processing surface [19–21]. Silica nanoparticles due to the high surface area up to 1200 m^2/g, large pore diameter, regular pores, good mechanical stability, binding ability, hydrophilicity, water permeability, or fouling resistance, and excellent biocompatibility have been recommended for the removal of emerging pollutants from aqueous solutions. In this regard, it has been proven that silica nanoparticles are capable to improve catalytic performance due to enhanced contaminant adsorption on the catalyst surface [22–26]. On the other hand, high surface area will lead to better antibacterial activity at ambient temperature. Other features of these nanoparticles include easy surface modification, easy to store and the possibility of the recovering and reusing them from water or wastewater after the treatment process, have important economic and environmental consequences [27, 28].

Photocatalytic and adsorptive activities of silica nanostructures and nanocomposites were investigated in many researches. Qian et al. examined the photocatalytic activities of Mesoporous Silica [29]. Photocatalytic degradation of methyl red dye by silica nanoparticles were evaluated by Badr et al. [30]. Duhan et al. observed beneficent adsorption property of silica in photocatalytic dye degradation by enhancement of active sites on its surface [31]. Silica nanocomposite was employed as a highly efficient adsorbent for removal of several organophosphorus pesticides from the contaminated water by Liu et al. [32].

Rovani et al. reported the use of silica particles for the removal of acid orange 8 dyes from water. They were able to remove approximately all the dye pollution from the aqueous solution. The maximum adsorption capacity was 230 mg/g [33]. The efficiency of the silica nanoparticles as photocatalyst was tested to remove and degrade MO dye using an ultraviolet light illumination. Complete degradation was obtained in 90 s, using 10 g/L silica nanoparticles [34]. In a study, the silica nanoparticles are used to remove methylene blue dye from aqueous media. The nanoparticles achieved adsorption capacity of 679.9 mg/g with removal percentage of 80% [35]. In a study, a low cost mesoporous silica nanomaterial that can be used for the removal of methylene blue was developed. The maximum adsorption amount of methylene blue onto mesoporous silica obtained was 347.2 mg/g [36].

In this paper, we first investigated the antibacterial properties of silica nanoparticles synthesized by the modified Stöber method and then studied the photocatalytic disinfection and adsorption capability of this nanomaterial.

2. Materials and methods

2.1 Silica nanoparticles synthesis

Silica nanoparticles were synthesized using a modified Stöber method [37]. In this method silica nanoparticles were prepared by hydrolysis and condensation of (TEOS) Tetraethyl orthosilicate in ethanol. First ethanol (Merck, Germany) and deionized water were stirred for 5 minutes. Then a sufficient amount of TEOS (Merck, Germany), was added while sonicating, after 30 minutes a proper amount of Ammonia solution (25%, Merck, Germany) as a catalyst was slowly added to precursor solution, and the sonication of suspension was continued to become a white transparent homogeneous solution. Afterward the resulting product was kept for 12 hours at room temperature to perform the process of hydrolysis and condensation of TEOS. Then the products were centrifuged and washed with ethanol and deionized water several times to remove dust particles and then dried in oven at 60°C for overnight.

2.2 Characterization studies

For the characterization of the optical and structural properties of silica nanoparticles, UV–Vis spectroscopy (UV–Vis-NIR T80 spectrophotometer from PG instruments Ltd), X-ray diffraction (XRD) (with Cu-Kα radiation ($\lambda = 1.54060$ $\mathring{A}$), using a STOE–XRD diffractometer), scanning electron microscope ((SEM) (AIS2100) and transmission electron microscopy (Philips EM 208) were used. For XRD analysis, powder of silica nanoparticles was characterized in angular range of $10 - 80°$ with the step size of 0.05 for 2θ.

3. Antimicrobial activity studies

The antibacterial activities of the prepared nanoparticles have been investigated for *E. coli* by agar diffusion method [38].

First a stock suspension of *E. coli* was prepared with 0.9% NaCl solution. The concentration of the bacterial suspension was 1.5×10^8 colony forming units (CFU/mL). That was equivalent with 0.5 McFarland standards. 1 mL of the prepared bacterial suspension was swabbed over the surface of the nutrient agar (Merck, Germany) plates. Some Filter paper discs were made by a sterile Cork borer (6 mm diameter). Sufficient amount of silica nanoparticles were dissolved in 10 mL of dimethyl sulfoxide (DMSO) to prepare a stock solution. By diluting the stock of the Silica nanoparticles, final concentrations of the nanoparticles solution were obtained. 100 μL of each prepared nanoparticles solution was poured on each paper disc, while a paper disc was impregnated deionized water as a control. The paper discs were located in to the agar plates, and the plates were incubated for 24 h at 37°C in incubator. After that diameter of the inhibition zones formed around the discs were measured.

3.1 Photocatalytic tests

The Synthesized silica nanoparticles were evaluated for the photocatalytic inactivation of *E. coli* bacteria in water. To obtain a required concentration of *E. coli*, a stock suspension of *E. coli* was prepared with DI water. The density

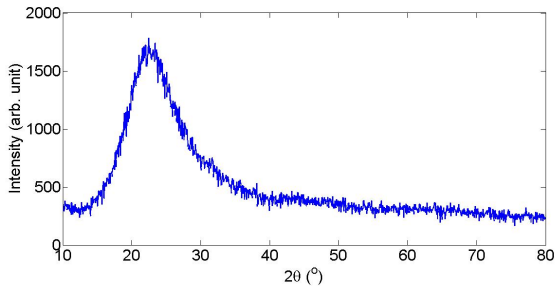

Figure 1. The X-ray diffraction pattern of silica nanoparticles.

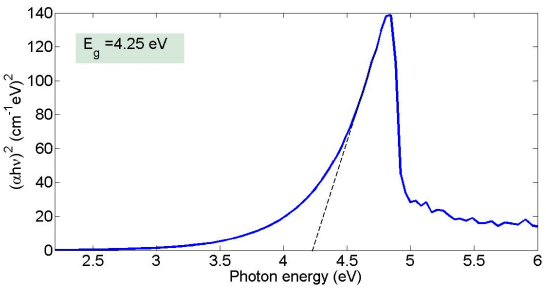

Figure 3. Bandgap energy of synthesized silica nanoparticles.

of the stock was 1.5×10^8 CFU/mL, In order to ensure the prepared turbidity; the suspension was adsorbed by UV-Vis spectrophotometer and the adsorption was adjusted between $0.08 - 0.1$. The stock was serially diluted in DI water and final concentration of *E. coli* was obtained. Therefore an initial *E. coli* concentration (1.5×10^4 CFU/mL) was used for all experiments. The photocatalytic effect of nanoparticles was studied in a batch system. The system was equipped with a UV-B lamp (Philips, TL 20W/01 RS SLV, $320 - 280$ nm). 50 mL DI water and the bacterial suspension were prepared in a glass beaker and the silica nanoparticles were dispersed in contaminated water at 1 g/L. This amount of silica nanoparticles was used in all experiments. The solution was irradiated for 60 minutes by UV-B lamp, while continuously stirred by a magnetic stirrer (440 rpm). Initially, to obtain the optimum pH in the photocatalytic process efficiency, three pHs in the acidic, neutral and alkaline ranges were evaluated. Three samples at different pH values were exposed to UV-B irradiation. Then Photocatalytic tests were carried out triplicates and 0.1 mL aliquots sampled after 30 minutes of irradiation. Eventually bacterial removal efficiency was determined by the spread plate (SP) method, with nutrient agar grown at 37°C during 24 h. after obtaining the optimum pH, the rest of the experiments were performed at this pH. To assess the inactivation time the volume of 0.1 mL aliquot of the solution was sampled every 15 minutes throughout the experiment with a total treatment time of 1 hour. Moreover, the colony counting of the untreated samples was also determined at t = 0 min. To ensure the results, the samples were plated in triplicate and incubated for 24 hours at 37°C and the colonies counted. In addition to Photocatalytic tests, photolysis (inactivation of *E. coli* in the presence of UV-B irradiation and in the absence of the photocatalyst) and adsorption (inactivation of *E. coli* in the presence of the photocatalyst in the absence of UV-B irradiation) tests were studied and the results were compared with the evaluations of the photocatalytic tests [39].

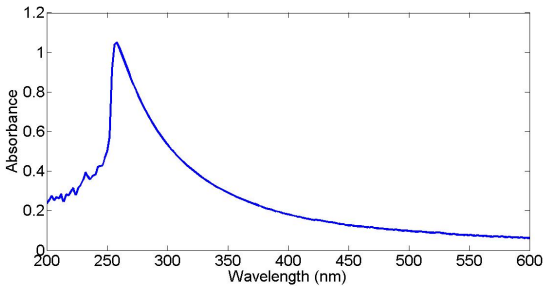

Figure 2. Absorption spectrum of synthesized silica nanoparticles.

4. Results and discussion

4.1 Characterization of silica nanoparticle

Using (Cu-Kα) powder X-ray diffraction (XRD) system crystalline structure of nanoparticles was characterized. The XRD pattern of produced silica nanoparticles is presented in Fig. 1, A single hump peak at $2\theta = 23°$ indicates the amorphous nature of silica nanoparticles.

Fig. 2 shows the absorption spectrum of silica nanoparticles. The spectrum consists of a single peak at $\lambda = 258$ nm. Similar result was observed by Rahman, Zhao, and Rice research groups [40–42]. The results indicated that silica nanoparticles have obvious absorption peak in the UV region due to excitonic absorption of silica nanoparticles as a dielectric nanomaterial. FWHM of absorption peak confirms that synthesized nanoparticles are almost uniform in size and spherical in shape. In the case of nanoparticles, plasmonic and excitonic absorption wavelength is sensitive to particle size.

The bandgap energy of produced nanoparticles was extracted by Tauc method. Result is presented in Fig. 3. Tauc method states that in the absorption edge region we have

$$(\alpha h\nu) = A(h\nu - E_g)^m \quad (1)$$

In this formula, α is the absorption coefficient and $h\nu$ is the photon energy, E_g is the optical bandgap energy, and A is the transition probability, which can be assumed to be a constant value in the considered optical frequency range. The value of m indicates the type of transition. $m = 1/2$, 2, 3/2 or 3 are correspond to allowed direct, allowed indirect, forbidden direct and forbidden indirect electronic transitions, respectively. To calculate the bandgap of the samples, we plotted the $(\alpha h\nu)^{(1/m)}$ versus the photon energy $h\nu$. This graph will be linear and the x-intercept of the line determines the amount of the optical bandgap energy of the material. Taking $m = 1/2$ corresponds to allowed direct transition of synthesized silica nanoparticles the bandgap energy of samples was found to be 4.25 eV. Bandgap energy of nanoparticles is very sensitive to their preparation

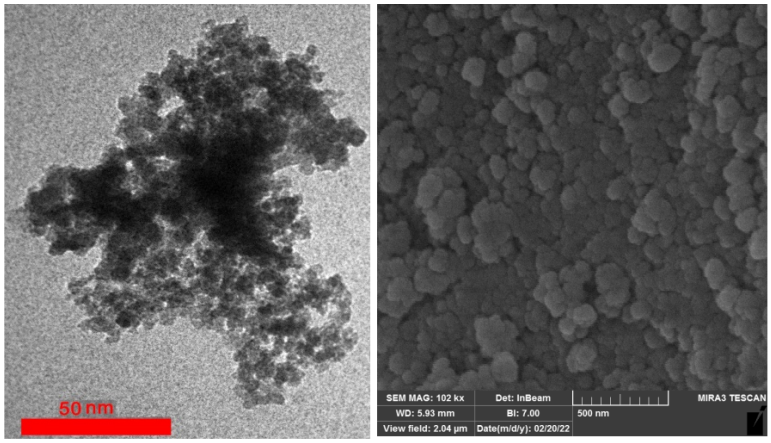

Figure 4. TEM image (a) and FESEM image (b) of synthesized silica nanoparticles by Stöber method.

method, size, morphology, and also it can be influenced by the existence of defects and impurities [43].

According to literature, bulk silicon is investigated by indirect bandgap absorption from 1.1 to 3.4 eV and two direct bandgap transitions starting around 3.2 eV and 4.2 eV [44]. In another report by Messih et al., the bandgap energy of $9-15$ nm composed SiO_2 nanoparticle was about 5 eV. The bandgap energy shows that mean size of silica nanoparticles should be in the range of $20-30$ nm [45].

For the case of our synthesized nanoparticles which are in the rage of $20-30$ nm the bandgap energy was increased to 4.25 eV which is the direct effect of the size of nanoparticles. The bandgap energy of nanoparticles increased inversely proportional to their size. The energy gap corresponds to the wavelength of 291.7 nm confirms that produced nanoparticles are capable to absorb UV- B and C photons.

TEM and FESEM images of synthesized silica nanoparticles by Stöber method are presented in Figs 4(a) and 4(b). TEM image reveals that nanoparticles are spherical in shape. Their average size is smaller than 30 nm. Image shows two regions. One of them at the center of the cluster is fully dark which is surrounded by lighter nanoparticles. Dark particles may be Si nanoparticles and light ones are particles which are combined with oxygen molecules. SEM image shows that the grains of nanoparticles are also spherical in shape. Their area and concentration is high enough to operate as an efficient photo absorber.

4.2 Antibacterial activity of silica nanoparticles

Diluted solutions of silica nanoparticles at concentrations of 80 μg/mL, 40 μg/mL and 20 μg/mL were used to investigate their antibacterial activity by agar diffusion method. Antibacterial activity was determined based on the zone of inhibition. Results for agar well diffusion assays are given in Fig. 5 and Table 1.

No inhibition zone was observed surrounding the soaked disc with 20 μg/mL concentrations. But Along with the increase of the concentration of the silica nanoparticles the growth of *E. coli* was inhibited more and more. Other studies confirm that increasing the concentration of nanoparticles has increased their antibacterial activity.

Halos observed around the discs may occur due to multiple modes including physical damage to cell membranes, ROS production and endo-lysosomal burden. The ability of nanoparticles to cross bacterial membranes, leading to physical damages on bacterial cell, which inhibit the growth of bacterial cells [46–48].

ROS production like H_2O_2 leads to inhibit the growth of bacterial cells. However the production of the active oxygen is just possible under UV irradiation but the antibacterial property of nanoparticles also could be detected without illumination. Silica nanoparticles may prompt as a conduit for electrons and promote photochemical reactions without exposing to any light source [28, 49, 50].

In studies that have been reported on the antibacterial properties of silica nanoparticles, Mosselhy et al. reported that silica nanoparticles is not efficient enough to inhibit the growth of bacteria [51]. In contrast, however Amna Sirelkhatim et al. reported that silica nanoparticles exhibit antimicrobial properties [52]. The effect of silica nanoparticles on the growth of P. aeruginosa gram-negative bacteria was studied by Kadhum. It was concluded that an inhibition zone was (20 mm) at a concentration of 10 μg/mL of silica nanoparticles in P. aeruginosa while the lower inhibition zone was (2 mm) at the concentration of 4 μg/mL. and no inhibition zone at the concentration of 2 μg/mL observed in P. aeruginosa [14]. Jiang et al. were investigated toxicity comparison between SiO_2, ZnO and TiO_2 nanoparticles in their study,

Table 1. Antibacterial activity of silica nanoparticles against *E. coli*.

concentration (μg/mL)	80	40	20
Zone of inhibition (mm)	8.72	7.77	0

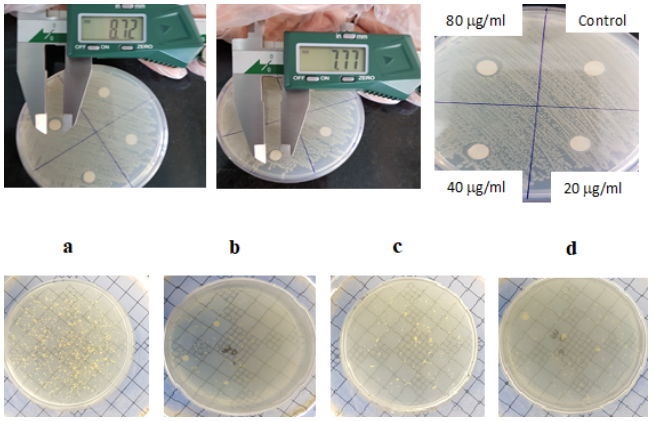

Figure 5. Antimicrobial activity of silica nanoparticles against E.coli. Petri dish images before (a) and after photocatalytic treatment at acidic pH (b), neutral pH (c) and basic pH (d).

and the study conducted in the absence of sunlight found a ranking of ZnO > SiO_2 > TiO_2. Accordingly the difference between the results of the various studies can be attributed experimental conditions such as presence or absence of light illumination, particle size, and nanoparticle dose. On the other hand however, some studies reported no significant size effects on the antibacterial activity of nanoparticles against *E. coli* cells, but the most studies have proven that the antibacterial ability increases with decreasing particle size [53–55].

4.3 Photocatalytic activity of silica nanoparticles

-Effect of initial pH on the photocatalytic inactivation

The effect of pH on photocatalytic efficacy was investigated at 4.5, 7 and 9 pHs. The effect of initial pH on the photocatalytic inactivation of *E. coli* in the presence of 1 g/L of Catalyst for 30 minutes illumination is shown in Fig. 6. No significant variation was observed in photocatalytic effect at different pHs. Negligible *E. coli* reaction to acid conditions was due to the presence of an acid tolerance response in the bacterium itself, which secreted the acid-induced proteins for bacterial acid-shock protection [56]. Bacterial removal efficiency at acidic, neutral and basic pHs was 68, 73 and 71 percentage, respectively. As respects, both *E. coli* and silica due to their isoelectric point (below 3.5) are negatively charged at pH = 4 – 9, Adjusting the initial pH of the water did not markedly affect the photocatalytic process [39, 57, 58]. Therefore, the neutral pH was selected for further studies. It is better to mention here that silica nanoparticles, despite their negative charge, to interact with the lipopolysaccharide layer of the bacteria outer membrane, probably through hydrogen bonding with the lipopolysaccharide hydroxyl groups. These interactions are likely stronger than cell-cell ones as they could break down the clustering state of *E. coli* cells [59].

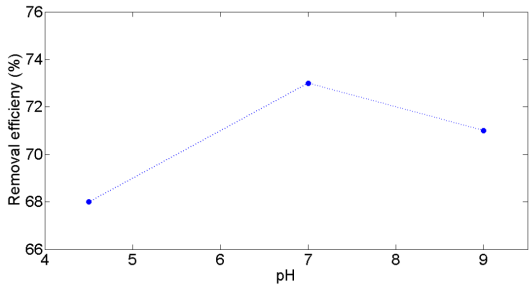

Figure 6. Effect of pH on photocatalytic removal efficiency.

-Comparison of different processes involved in the water contamination treatment

After determining the optimal pH, three different processes for the treatment of contaminated water were investigated. In the first study, the simultaneous effect of illumination source and photocatalyst (photocatalytic process) was investigated, and 0.1 mL aliquot of the contaminated water was sampled every 15 minutes for a total irradiation time of 60 minutes. In the second and third studies only illumination source (photolysis process) and photocatalyst (adsorption process) were investigated respectively. The removal efficiency at different time for all three studies is shown in Fig. 7. The best result was observed in the photocatalytic process, So that no infection was observed after 60 minutes of treatment. Actually the silica defects can be activated and produce hydroxyl or superoxide free radicals under the illumination source. In this regard, photo-generated holes and electrons are formed in the valence band (h^+_{VB}) and the conduction band (e^-_{CB}), respectively. Consequently, these photogenerated charge carriers then react with water or dissolved oxygen to produce reactive oxidizing species that decompose pollutants into smaller molecules as well as inactivate micro-organisms [18]. The photocatalytic interactions is depicted in Eqs. (2-6)

$$SiO_2 + h\nu \rightarrow e^-_{CB} + h^+_{VB} \quad (2)$$

$$h^+_{VB} + H_2O \rightarrow \cdot OH + H^+ \quad (3)$$

$$h^+_{VB} + OH^- \rightarrow \cdot OH \quad \text{and} \quad e^-_{CB} + O_2 \rightarrow \cdot O_2^- \quad (4)$$

$$\cdot O_2^- + H_2O \rightarrow \cdot OH + H^+ \quad (5)$$

$$\cdot OH + \text{microorganism} + O_2 \rightarrow \text{simpler products} \left(\text{salts}, CO_2, H_2O \text{etc.},\right) \quad (6)$$

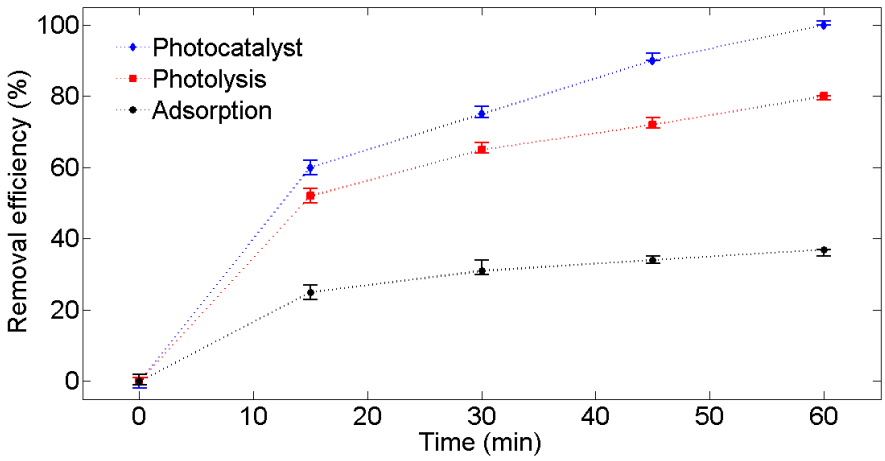

Figure 7. Removal efficiency of different processes including photocatalyst process, photolysis process and adsorption process.

Photocatalytic materials when come in contact with microorganisms via different energy such as electrostatic attraction, hydrophobic interactions and van der Waals, they exert their effect on the bacterial cell membrane and starts to influence the basic metabolism of the cells by different mechanisms. The cells could also experience various stresses such as oxidative stresses, membrane permeability imbalance, changes in cell shape, protein inhibition and alteration in metabolism and DNA damages [60].

Photolysis process is counted among the advanced oxidation processes (AOP) due to the production of free radicals. But the concentration of hydroxyl radicals produced in the hydrolysis process is less than the photocatalytic process. Photolysis process is mainly due to the absorption of UV by DNA and RNA which causes the formation of pyrimidin dimers [61]. About photocatalytic process silica nanoparticles are excited by photons of UV lamp and produce as electrons e^- and holes h^+ which could generate more free radicals. In this regard, the electrons interact with the adsorbed oxygen to form superoxide radicals, and the holes uptake by the surface hydroxyl group on the surface of the photocatalyst or adsorbed water molecules creates OH radicals. Among these free radicals hydroxyl radical (·OH) is the major and highly reactive oxidant which has an essential impact on the cell membrane. The produced free radicals penetrates cell through cell wall and cause cell death [2, 61].

Fig. 7 shows that adsorption of *E. coli* on to the silica nanoparticles can be responsible for 37% of removal efficiency after 1 hour of water treatment. Considering that, the both silica nanoparticles and the *E. coli* bacteria are negatively charged, It seems that trapping of *E. coli* into the surface of silica materials becomes a difficult process. However, silica nanoparticles have been reported to be a hopeful adsorbent for bacteria removal from wastewater due to pore volume, high surface area, identical pore size, porosity, controllable particle size and affinity for DNA [62, 63].

5. Conclusion

The efficacy of the photocatalytic process using silica nanoparticles on glass plates was evaluated to remove microbial contamination of water. The experiments were conducted on the removal of *E. coli* at different pH values by silica nanoparticles in the presence of UV-B radiation. The results showed that the effect of pH has not a significant role in the removal of E .coli bacteria. Other experiments were performed at neutral pH, and the efficacy of the photolysis and adsorption processes were compared with photocatalytic process at different time. Photocatalytic process showed the best bacterial removal efficiency due to the generation of free radicals by UV radiation to silica nanoparticles. In addition to the good performance of the photocatalytic process of silica nanoparticles, *E. coli* was significantly inhibited by the synthesized silica nanoparticles.

Ethical approval:
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval does not applicable.

Authors Contributions:
Mahsa Alizadeh, Conception; setup arrangement; data acquisition; analyzing the results; preparing the draft.
Davoud Dorranian, Supervisor; conception; analyzing the results; wrote the paper.
Amir Hossein Sari, Setup arrangement; analyzing the results; preparing the draft; checking the draft.

Availability of data and materials:
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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