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**Photocatalytic inactivation of marine *Vibrio fischeri* bacterium using visible light
driven nanoparticles for preventing seawater membrane biofouling**

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Abstract

Biofouling is a major problem in water membrane processes, especially in seawater reverse osmosis (SWRO) plants. Inactivation of *Vibrio fischeri* (a well-known marine bacterium forming biofilm) through photocatalysis *via* visible light was investigated in this work using active Fe₂O₃-TiO₂ nanoparticles. Five Fe₂O₃-TiO₂ photocatalysts with different weight percentage of Fe₂O₃ (0-5 wt.%) were synthesized using an ultrasonic-assisted co-precipitation method. The photocatalysts were characterized by powder X-ray diffraction (XRD), BET surface area, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and diffuse-reflectance spectroscopy (DRS). Based on the design of experiments, the synthesized photocatalysts were tested for inactivation of *V. fischeri* under visible light irradiation at different temperatures (25-35 °C) and different photocatalyst dosage (0.1-2 g/L). The photocatalytic microbial inactivation experiments were performed in artificial seawater appropriate for growth of the marine bacterium. The results revealed that the highest inactivation efficiency of *V. fischeri* was achieved when 1 g/L of 2.5 wt.% Fe₂O₃-TiO₂ were used, at 35°C. The antibacterial activity of photocatalysts was attributed to the formation of highly reactive oxygen species (ROS) during photocatalytic process. Photocatalytic inactivation of microorganisms using visible light-driven Fe₂O₃-TiO₂ photocatalysts, could introduce an innovative green method in pretreatment units of reverse osmosis plants to control the membrane biofouling.

Keywords

Seawater pre-treatment; Fe₂O₃-TiO₂ nano-photocatalyst; visible light; marine bacteria; membrane biofouling

48

49   **1. Introduction**

50   Providing of sufficient and clean water have been one of the intensive challenges in
51   the past decades, due to shortage of fresh water resources and growing population
52   (Cipollina et al., 2009; Fritzmann et al., 2007). Seawater is considered as a high
53   potential resource for drinking and industrial applications. Therefore, desalination
54   techniques have attracted much attention in water purification plants. Due to the high
55   energy and space efficiency about half of the industrial water desalination plants around
56   the world use reverse osmosis (RO) membranes (Greenlee et al., 2009). Membrane
57   fouling is a drawback of reverse osmosis and nano-filtration processes, resulting in an
58   increase in hydraulic resistance, pressure drop and subsequently decreases the
59   normalized flux (Matin et al., 2011; Nguyen et al., 2012).

60   Biofouling occurs as a result of the attachment and growth of microorganisms such
61   as bacteria and microalgae onto the membrane (Chiou et al., 2010; Komlenic, 2010). It
62   is one of the most commonly encountered fouling types in large and small scale RO
63   membranes. More specifically, biofouling causes a decline in membrane flux, increases
64   the feed pressure and pressure drop, increases salt passage and biodegradation of the
65   membrane (Flemming, 1997; Herzberg and Elimelech, 2007; Khan et al., 2010).
66   Therefore, the RO membrane lifetime decreases due to frequently cleaning stages. The
67   biofouling occurrence depends on different factors; including the concentration and type
68   of microorganisms contained in the water, pH level, concentration of dissolved oxygen,
69   concentration of nutrients and water temperature (Sutzkover-Gutman and Hasson,
70   2010). The goal of seawater pretreatment is to eliminate or reduce the potential of
71   scaling and biofouling on membranes and flow channels to minimize cleaning

frequency.

Biofouling can be controlled by using an effective pretreatment system integrated to other anti-scaling pretreatments. Disinfection is a common pretreatment in water desalination and purification plants. Chlorination, using organic biocides, UV-C irradiation, and ozonation are conventional disinfection methods used in the RO plants as pretreatment (Chong et al., 2010; Malato et al., 2009). These methods are often expensive and difficult in operation, for example chlorine, as a commonly used biocide, is very effective but membranes show a poor resistance to its highly oxidizing property. In order to protect membrane against chlorine, the feed water should be dechlorinated prior to the desalination step (Sutzkover-Gutman and Hasson, 2010). In addition, contact of chlorine with organic matters in seawater leads to the formation of carcinogenic substances such as trihalomethanes (Jamaly et al., 2014). Because of these problems, numerous works have been done to extend novel, less toxic and also effective techniques to prevent biofouling including ozone or ultraviolet (UV-C) treatment (Dobretsov et al., 2013; Harif et al., 2011; Laxma Reddy et al., 2017). Alternatively, advanced oxidation processes (AOPs) using photocatalysis have attracted much attention due to the *in-situ* formation of highly reactive oxidants (i.e. hydroxyl radicals ($\cdot\text{OH}$), superoxide ion ($\cdot\text{O}_2^-$) and hydrogen peroxide H_2O_2) capable of destroying microorganisms without further secondary effects (Chong et al., 2010; Pigeot-Rmy et al., 2011). Among different types of semiconductor materials, titanium dioxide (TiO_2) has been widely used due to its favorable catalytic properties, stability and low price. TiO_2 exists in three forms, namely, rutile, anatase and brookite. They differ in physical (e.g. crystal structure, stability, hardness, density) and optical (e.g. color, luster, brightness, refractive index) properties (Winkler, 2003). Both anatase and rutile phases

are commonly used as photocatalysts. Generally, the anatase phase has shown greater photocatalytic activity than the rutile phase (Coleman et al., 2005; Muggli and Ding, 2001). However, for reasons that are not still understood, mixtures of anatase and rutile have better photo-activity than either single phase (Rincón and Pulgarin, 2003; Tuzen et al., 2009). There are some reports, however, that the rutile phase presents the photocatalytic activity greater or comparable to the anatase phase (Ohno et al., 2002; Torimoto et al., 2002). This activity is attributed to the stronger absorption of rutile in the near ultraviolet (360–400 nm) than anatase (<370 nm) (Rincón and Pulgarin, 2003). However, because of some unfavorable properties of both rutile and anatase phases, (including high recombination rate and decreased visible light sensitivity) (Kuo et al., 2011), various modifications have been implemented to address these problems. In this regard, an intensive interest is to promote TiO₂ in order to increase its photocatalytic activity in exposure of visible light irradiation (Markowska-Szczupak et al., 2011). Doping TiO₂ with various metals (such as Fe, Cr, Sn, Pt, and V) is one of the modification methods that enhances the TiO₂ visible light absorption (Dvoranová et al., 2002; Klosek and Raftery, 2002). Among the various metals, iron has been considered as an efficient dopant for TiO₂ modification (Khan and Swati, 2016). Iron doping adds new states in the bandgap of TiO₂, and subsequently, increases the visible light absorption (Yamashita et al., 2003).

In this work, the activity of Fe₂O₃-TiO₂ nano-photocatalysts (with different amount of iron) was investigated as a pretreatment technique for the removal of the marine bacterium *Vibrio fischeri* from seawater. Specific objective of the study included the investigation of disinfection activity of the Fe₂O₃-TiO₂ photocatalysts in artificial nutrient seawater under continuous visible light irradiation. For this purpose, 0-5 wt.%

Fe₂O₃-doped TiO₂ photocatalysts were synthesized. A Response surface methodology (RSM) was applied to investigate the effect of temperature, iron percentage in photocatalyst and dosage of photocatalyst on inactivation of biofilm forming bacteria. Effective inactivation of marine bacteria in saline and nutrient water by visible light photocatalysis has been demonstrated. This gives promising prospects for preventing fouling in desalination units by pretreatment of membranes by this novel pretreatment method which can function at visible light wavelength.

2. Materials and methods

2.1. Materials and microorganism

All chemicals and reagents were reagent grade from Sigma-Aldrich and used without further purification and treatment. *V. fischeri*, a biofilm forming marine bacterium, was obtained from ABOATOX Co., Finland, and used as a bacterial strain.

2.2. Preparation of photocatalysts

The ultrasonic co-precipitation method was applied to synthesize Fe₂O₃-TiO₂ nanoparticles. Titanium tetrachloride (TiCl₄) and Iron (III) nitrate nonahydrate (Fe(NO₃)₃·9H₂O) were used as precursors for nanoparticle preparation. A solution of TiCl₄ (0.5 M) was made by slow addition of pure TiCl₄ to cold water under stirring. To synthesize nanoparticles, diluted NH₄OH solution was gradually added to TiCl₄ solution under ultrasound waves and stirring at 80 °C for 2 h, to reach a pH of 9. The synthesized TiO₂ nanoparticles were centrifuged and washed several times by distilled water to be neutralized, followed by drying at 100 °C overnight. In order to dope TiO₂ with different percentage of Fe₂O₃, the TiO₂ suspension at pH 7 was added to pre-

calculated amount of Na₂CO₃ aqueous solution under ultrasonication at 80 °C. Then the pre-calculated amount of iron salt was dissolved in distilled water and added to solution drop-wise under ultrasonication and stirred for 2 h. The Fe₂O₃-TiO₂ nanoparticles were centrifuged and washed several times by distilled water and dried in oven over night at 100 °C.

Finally, the samples were calcined at 250 °C at a rate of 5 °C/min for 3 h. TiO₂ nanoparticles with 0.00, 1.25, 2.50, 3.75, and 5.00 weight percent of Fe₂O₃ are denoted as T, IT1.25, IT2.5, IT3.75, and IT5, respectively.

2.3. Characterization of the photocatalysts

The X-ray diffraction (XRD) patterns of the synthesized photocatalysts were recorded using a Philips Analytical (X'pert Pro MPD) X-ray powder diffractometer with CuKα radiation in the 2θ range of 5–70° and at scanning rate of 1.5° (2θ)/min. The Debye-Scherrer equation was used to calculate the average crystallite size of the nanoparticles (Firooz et al., 2010) :

$$d_{\text{XRD}} = \frac{0.9\lambda}{\alpha \cos\theta} \quad (1)$$

Where d_{XRD} is the diameter of nanoparticle, $\lambda = 0.154060$ nm (in the case of CuKα), and α is the full width at half maximum intensity of peak.

Brunauer-Emmett-Teller (BET) specific surface area of synthesized photocatalysts was measured at the liquid nitrogen temperature by N₂ physical adsorption *via* the multi point method, using micrometrics Gemini surface area analyzer. Prior to BET measurements, samples were degassed at 200 °C for 1 h under N₂ flow. The approximate average particle diameter of samples from BET specific surface area were calculated using the following equation:

$$d_{BET} = \frac{6000}{\rho \times S_{BET}} \quad (2)$$

where S_{BET} is BET surface area (m^2/g) and ρ is the skeletal density (g/cm^3) (Banisharif et al., 2015; Maghsoodi et al., 2013).

The morphology and size of the synthesized photocatalysts were analyzed by scanning electron microscope (FEI Quanta 200 ESEM FEG). In addition, energy dispersive X-ray (EDX) spectra of the samples were obtained by scanning electron microscopy (FEI Quanta 200 ESEM FEG) and used for elemental analysis of the photocatalysts. In order to calculate the band gap of the synthesized photocatalysts, the diffuse reflectance spectra (DRS) of the samples were recorded at room temperature in the wavelength range of 200-800 nm using an Avaspec-2048-TEC spectrophotometer. Corresponding band gap of nanoparticles were calculated using Kubelka-Munk equation:

$$\alpha = \frac{k(h\nu - E_{bg})^{(1/n)}}{h\nu} \quad (3)$$

Where α is the absorption coefficient, k is constant, h is Planck constant, ν is frequency of light, E_{bg} is band gap energy, and n equals 0.5 for indirect semiconductors as TiO_2 (Cui et al., 2010).

2.4. Preparation of growth media and bacterial inoculant

Artificial seawater was prepared by dissolving, 28.13 g NaCl, 0.77 g KCl, 1.60 g $CaCl_2 \cdot 2H_2O$, 4.80 g $MgCl_2 \cdot 6H_2O$, 0.11 g $NaHCO_3$, and 3.50 g $MgSO_4 \cdot 7H_2O$ in 1000 mL distilled water. Liquid media (pH 7.8) was prepared by dissolving 10.0 g beef extract and 10.0 g peptone in 250 mL distilled water by heating. Artificial seawater and liquid media were autoclaved for 20 min at $121^\circ C$. After cooling to room temperature,

750 mL of artificial seawater was mixed with 250 mL of liquid media. This growth media was stored at 4°C and was used for bacteria cultivation and photocatalytic experiments. In order to make solid media, after dissolving beef extract and peptone, the pH readjusted to 7.3 and 20.0 g Agar was added to the liquid media and autoclaved at 121°C for 20 min. Directly after autoclaving, warm (55°C) sterile seawater was added to media. Then, the agar containing media was poured in petri dishes and stored at 4°C for viable colony counting tests.

To prepare bacterial inoculant, *V. fischeri* was inoculated into synthesized growth media and cultured overnight in shaking incubator with 110 rpm at 25°C under aerobic condition to final OD_{600nm} of 0.257 equivalent to 1 McFarland standard number (Matlock Brian and Beringer Richard, n.d.).

2.5. *Vibrio fischeri* inactivation assay

The activity of synthesized photocatalysts for inactivation of *V. fischeri*, as a seawater model bacterium, was investigated *via* a batch reactor shown in Fig. 1 using artificial seawater as growth medium. The system consists of a 400 mL Pyrex glass beaker (9.5 cm in diameter and 5.5 cm in height) with quartz lid, stirrer, water recirculation and temperature controlling system, air blower, light collimator, and light source located in enclosed box equipped with air conditioner to avoid lamps over temperature. For all the photocatalytic experiments, 1 mL of fresh *V. fischeri* with concentration equal to 1 Mcfarland, in exponential phase, was inoculated to 100 mL of growth media to achieve 3×10⁶ CFU/mL as initial concentration of *V. fischeri*. At the same time, the specific amount of sterile photocatalyst (in the range 0.1-2.0 g/L) was added to the reaction media. The mixture was stirred in exposure of light source at

constant temperature (in the range 25-35 °C) for 4 h. Two 9W fluorescent white lamp (Osram, G23, 2pin, 220V) and two 9W fluorescent UV-A lamp (Philips, PL-S, 2pin, 220V) were used as visible light and UV-A light source, respectively, providing a light intensity of 55 W/m² at the surface of reaction media. The UV-A lamps have wavelengths in the range of 350–400 nm at maximum intensity at 365 nm. The intensity of the visible lamp and UV-A lamp was measured by the LI-250A visible light meter and YK-35UV UV ultra violet light meter, respectively. The distance between light source and surface of the mixture was fixed at 10 cm. During the experiment, air was continuously bubbled to keep the dissolved oxygen concentration at a constant level. The temperature of the reactor was kept constant at desired temperature with recirculation of water around the reactor. For each run of experiments, the three selected variables (reaction temperature, catalyst concentration and catalyst composition) were adjusted based on the design of experiment. Liquid samples were taken in time intervals (0, 15, 30, 45, 60, 120, 240 min), during each experiment and inactivation of *V. fischeri* was investigated by measuring cell concentration (CFU/mL) through viable colony counting. Viable colony counting on agar-growth media was performed after standard serial dilutions using spread plate method (Buck and Cleverdon, 1960). The number of colonies was counted after incubating at 25°C for 48 h using electronic colony counter.

In order to investigate the effectiveness of photocatalytic process for inactivation of *V. fischeri*, three parallel control experiments were conducted: 1) in the absence of light source and presence of photocatalyst (i.e. dark control), 2) in the absence of photocatalyst and presence of light (i.e. light control) and 3) in absence of both light and photocatalyst (i.e. blank control). The control experiments were conducted using the optimum catalyst concentration and embedded Fe₂O₃ percentage at 30 °C.

2.6. Design of experiments and statistical analysis

Three independent parameters were selected as process variables including reaction temperature (A, °C), photocatalyst dosage (B, mg/L) and the percent of Fe₂O₃ in photocatalyst (C, wt.%). Full Central Composite Design (CCD), a design type under response surface method (RSM), with alpha value 2 was used to investigate the effects of three parameters on the response and to optimize the variables with twenty experiments. Final normalized cell concentration of *V. fischeri* was considered as response variable and was recorded in time intervals (0, 15, 30, 45, 60, 120, 240 min) during each experiment. The three variables (temperature, catalyst dosage, and catalyst composition) were considered as numerical factors, the levels were coded as $-\alpha$, -1, 0, +1, and $+\alpha$. The levels of numeric parameters are illustrated in Table 1. Analysis of variance (ANOVA) and optimization using RSM method were carried out by Design-Expert® software trial version 7.0.0. (Stat-Ease, Inc. Minneapolis).

3. Results and Discussion

3.1. Characterization of synthesized photocatalysts

The X-ray diffraction patterns of the synthesized Fe₂O₃-TiO₂ photocatalysts (with 0 to 5 wt.% Fe₂O₃), calcined at 250 °C for 3 h, are presented in Fig. 2. The XRD patterns of all samples were indexed with anatase TiO₂ (ICDD file no. 03-065-5714) and rutile TiO₂ (ICDD file no. 03-065-1118). Diffraction patterns (Fig. 2) represents peaks at $2\theta = 25.32^\circ$, 38.6° , 48.06° , and 62.7° that are related to (1 0 1), (0 0 4), (2 0 0), and (2 0 4) crystal planes of TiO₂ anatase structure. The other peaks at $2\theta = 27.47^\circ$, 36.12° , 41.31° , 44.58° , 54.33° , and 56.63° are related to (1 1 0), (1 0 1), (1 1 1), (2 1 0), (2 1 1), and (2 2

0) crystal planes of TiO₂ rutile structure. The ratio of anatase to rutile was calculated by Eq. 4 (Hp, 1966; Jones and Hockey, 1971) and presented in Table 2.

$$C_A = \frac{100}{(1+1.265 \frac{I_A}{I_R})} \quad (4)$$

I_A/I_R is the ratio of the intensities of peaks at 2θ=25.32 ° and 27.47°, that belong to anatase and rutile phase, respectively. The existence of clear and developed peaks indicates that all the photocatalysts are well-prepared crystalline nanoparticles. There is no diffraction peak related to any phase of iron species in XRD patterns of all samples (Hamadani et al., 2011; Zhu et al., 2004). This indicates that part of Fe probably entered into the structure of TiO₂ crystal without the formation of Fe₂O₃ and the rest of Fe was highly dispersed (small crystallites) as iron oxide on the surface of TiO₂ (Ganesh et al., 2012). The average crystallite size (d_{XRD}) of the photocatalysts, were estimated using Scherrer's equation, are reported in Table 2.

The BET specific surface area (S_{BET}) of photocatalysts are listed in Table 2. The nanoparticles surface area is in the range of 110-160 m²/g. Addition of Fe₂O₃ within different concentration range may lead to the larger or smaller surface areas (Adán et al., 2007; Asiltürk et al., 2009; Zhu et al., 2004). Based on the results, the BET surface area and pore volume of photocatalysts decreases with increasing the loading of Fe₂O₃ from 0 to 3.75 wt%. This is probably because of pore blockage due to highly dispersed iron oxide. Further addition of iron oxide beyond the 3.75 wt% leads to increased BET surface area and pore volume, because of the generation of some iron aggregates and grains at the TiO₂ surface (Adán et al., 2007).

The SEM micrographs of the pure and Fe₂O₃ doped TiO₂ photocatalysts are presented in Fig. 3a and Fig. 3b. It can be seen from the figures that the size of photocatalyst powder chunks is 0.2 μm. The compositional analysis of the nanoparticles

was performed by the EDX technique, and the pattern is displayed in Fig. 3c and Fig. 3d. The EDX pattern shows the separate peak of Ti, Fe and O, which confirms the successful doping of Fe in the TiO₂ host structure that leads to the oxygen deficiencies.

Understanding the absorption of UV-vis light by photocatalyst is an important factor in order to investigate the photocatalytic properties. The light absorption of photocatalysts in the range of 200-800 nm is plotted in Fig. 4. Band gap of the nanoparticles were calculated using Kubelka-Munk equation. The results are presented in Table 2. As shown in Fig. 4 and Table 2, the absorption edge of nanoparticles shifted to a longer wavelength region by increasing the Fe₂O₃, subsequently the band gap energy of nanoparticles decreases with increasing the iron content. This means that the visible light absorption of photocatalyst increases with increasing the iron content.

3.2. Model fitting and statistical analysis

Based on the experimental design presented in Table 3, twenty experiments were conducted in the exposure of visible light. An empirical quadratic polynomial equation was fitted for the response variable (*V. fischeri* concentration) as a function of numeric independent variables. The final model for *V. fischeri* normalized concentration in the exposure of visible light *versus* experimental variables calculated by Design-Expert software are presented below:

$$Y = 0.249 - 0.001517A + 0.09188B - 0.13748C - 0.012526AB + 0.00084AC + 0.038526BC + 0.000089A^2 + 0.13488B^2 + 0.014356C^2 \quad (5)$$

Where Y represents the normalized concentration of *V. fischeri* ($\frac{C}{C_0}$) in the exposure of visible light, and A, B and C represents temperature (°C), photocatalyst dosage (g/L) and Fe₂O₃ percent in catalyst (wt.%). The ANOVA of the quadratic polynomial

indicates that the models are highly significant for the normalized concentration of *V. fischeri*, as the F-value of the model is 30.76 and the corresponding P-value is <0.0001. This means that there is only a 0.01% chance that such model F-value would be as a result of noise. The model had R^2 value equal to 0.965, adjusted- R^2 value equal to 0.934, and predicted- R^2 value equal to 0.767. The high value of R^2 indicates that the second-order polynomial can be used to predict the *V. fischeri* inactivation in the experimental range (Fathinia et al., 2010). Generally, the lower the P-value and the higher the F-value, the larger the effectiveness of parameter on the response variable in corresponding model (Sahoo and Gupta, 2012). Therefore, according to Table 4, the results of ANOVA indicated that the catalyst dosage is the most effective variable among the three operating variables. Temperature and iron percentage have less effectiveness on response variable, respectively in experimental range and levels.

3.3. Analysis of response surface plots and effect of parameters

In order to investigate the effect of parameters (i.e. Temperature, photocatalyst dosage and Fe_2O_3 percentage) and find the optimum range for *Vibrio fischeri* inactivation, the response surface analysis was used. The response surface plots of normalized *V. fischeri* concentration are illustrated in Fig. 5 for the three pairs of parameters.

According to the RSM plots illustrated in Fig. 5a and Fig. 5b, generally higher temperatures favor the *V. fischeri* inactivation efficiency. Fig. 6a shows normalized *V. fischeri* concentration during photocatalytic process at three different temperatures. Higher temperatures accelerate the inactivation rate of bacterium. Oxidizing free radicals react with cell microorganism oxidizing their biomass organic matter according

to Arrhenius reaction rate (McKay et al., 2013), so increasing temperature in the range of 25-35 °C cause increased *V. fischeri* inactivation. It is notable that generally the efficiency of bacteria inactivation decreases at temperatures more than 40 °C, due to the recombination rate of photo-generated electron-hole pairs (Cui et al., 2010; Libera et al., 2010). In addition, the exothermic adsorption of bacteria to the photocatalyst surface decreases at higher temperatures (Malato et al., 2009; Wang and Ku, 2007).

In the case of photocatalyst dosage, lower concentration of photocatalyst is insufficient to generate required amount of oxidants to inactivate microorganisms (Das et al., 2015). *V. fischeri* inactivation efficiency first increased with increasing catalyst dosage from 0.1 g/L up to an optimum point ~1.0 g/L and then the inactivation efficiency decreased with increasing catalyst dosage from ~1.0 g/L to 2.0 g/L (Fig. 5a and c). The reason for this phenomenon is that when the catalyst dosage is increased, the number of active sites increases (Sakkas et al., 2007), however, increasing catalyst dosage results in increased turbidity, and subsequently decreased transmittance of light through the aqueous solution. So, there is an optimum value for catalyst dosage. The effect of photocatalyst dosage at three different values of catalyst concentration is shown in Fig. 6b. As illustrated, the inactivation of *V. fischeri* with photocatalyst concentration equal to 1.05 g/L is more effective than 0.1 and 2.0 g/L.

As presented in Table 2, the band gap values were decreased with increasing Fe₂O₃ content. This means that the amount of energy needed for electron excitation from valence band to conduction band decreases, so the light in the range of visible light can activate the photocatalytic mechanism of nanoparticles. Experimental results revealed that increasing the iron content from 0 to ~ 2.5 wt.% (Fig. 5b and c) results in an improvement in photocatalytic activity of nanoparticles. Addition of Fe³⁺ impurity to

TiO₂ nanoparticles induces local energy levels under the conduction band (Eq. 6) and on top of the valence band (Eq. 7) (Su et al., 2011):

According to Eq. 6 and Eq. 7, Fe³⁺ can act as photo-generated electrons and holes trapping centers changing the electron-hole recombination rate (Guo et al., 2013). Since the Fe²⁺ and Fe⁴⁺ are unstable species, interphase charge transfer to molecular oxygen and hydroxyl groups take place and •O₂ and •OH radicals, the most reactive oxidant species, are produced as well as Fe³⁺ is regenerated (Zhu et al., 2006). These oxidants significantly improve photocatalytic activity of iron doped TiO₂ (Li et al., 2012). Furthermore, the unstable Fe²⁺ ions can also be converted to Fe³⁺ by transferring electron to surface Ti⁴⁺ producing Ti³⁺ (Chen et al., 2015). Ti³⁺ species are considered as a reactive agent for H₂O and O₂ adsorbates producing oxidizing radicals. Oxygen vacancies and Ti³⁺ centers inhibit the photo-generated electrons/holes recombination and improve the visible light absorbance in the wavelength range of 400-520 nm (Pan et al., 2013). Increasing iron content from ~ 2.5 to 5.0 wt.%, cause a decrease of the photocatalytic activity of nanoparticles. Probably, exceeding the optimum level of iron results in over saturation of TiO₂ crystal lattice with Fe³⁺ ions. Afterwards, the excess iron combines with TiO₂ creating separate phases, and iron grains that lessen the photocatalytic activity. Khan et al. (Khan and Swati, 2016) reported that (Fe:Ti = 1:50) is the optimum molar ratio of iron to TiO₂. Furthermore, higher concentration of dopant can decrease the distance between trapping centers and subsequently, increasing the

recombination rate of electron/hole pairs (Cui et al., 2010; Libera et al., 2010). Fig. 6c shows the inactivation of *V. fischeri* during photocatalytic process using Fe₂O₃-TiO₂ with three different amounts of dopant percent. It is clear that the inactivation rate of *V. fischeri* using IT2.5 (2.5 wt.%) photocatalyst is significantly more than those for T (0.0 wt.%) and IT5 (5.0 wt.%).

3.4. Optimization and verification

The numerical optimization of photocatalytic process of *V. fischeri* inactivation was performed using Design-Expert 7.0.0 software. Minimizing the normalized *V. fischeri* concentration (C/C_0) after 4 h irradiation of visible light was set to be the goal of optimization criterion. Based on optimization results, the most effective domain for temperature, catalyst dosage and Fe₂O₃ percent was proposed in the range of 34.8-35.0 °C, 0.92-1.00 g/L and 2.47-2.64 wt.%, respectively. In order to verify the results from the proposed model, a subsequent experiment was carried out under optimal conditions (T=35 °C, catalyst concentration=1 g/L and Fe₂O₃ percent 2.5 wt.%). The final normalized concentration of *V. fischeri* was 0.0055 that is in good agreement with predicted value of 0.0061 at the same condition using Eq. 5.

To validate that the photocatalytic process is the major agent of *V. fischeri* inactivation, three control experiments were performed at 30 °C (i.e. dark, light and blank control). In addition, the efficiency of process was investigated under UV-A irradiation. Photocatalytic process using UV-A light is more effective in comparison with visible light and inactivate bacteria in less time, due to shorter wavelength. As shown in Fig. 12 light and photocatalyst are not effective individually. As illustrated in Fig. 12, in control experiments the concentration of *V. fischeri* increases significantly,

due to nutrient powerful growth media. Photocatalytic inactivation process not only slows down the growth and metabolic activity of bacterium but also kills the organisms effectively.

4. Conclusions

Five Fe₂O₃-TiO₂ nanoparticles with different weight percentage of Fe₂O₃ (0-5 wt.%) were synthesized via ultrasonic-assisted co-precipitation method for inactivation of *Vibrio fischeri* as a marine model bacteria causing biofouling. In order to investigate the inactivation effect of Fe₂O₃-TiO₂ photocatalysts on *V. fischeri* under visible light irradiation a set of experiments were performed at different temperatures and different photocatalyst dosage based on the central composite design of experiments. Photocatalytic experiments were conducted in nutrients containing seawater media containing *V. fischeri* marine bacteria. Using the obtained response surface plots, the effects of the temperature, Fe₂O₃ content in photocatalyst, and photocatalyst dosage on the photocatalytic inactivation of *V. fischeri* were investigated. The results show that using 1 g/L of 2.5 wt.% Fe₂O₃ doped TiO₂ has the highest efficiency of *V. fischeri* inactivation at 35°C of reaction temperature. Under these optimized conditions, the 2.5 wt.% Fe₂O₃ doped TiO₂ photocatalyst achieved a bacterial inactivation efficiency of 99.4% after 4 hours of visible light irradiation in nutrient media. Photocatalytic inactivation of microorganisms using visible light-driven Fe₂O₃-TiO₂ nano-photocatalysts could be a green method in pretreatment units of reverse osmosis plants to control membrane biofouling.

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583

584

Figure captions

Fig.1 – Schematic diagram of the experimental setup used for photocatalytic inactivation of microorganisms

Fig. 2 – X-ray diffraction patterns of calcined TiO₂ based photocatalysts with various percentage of Fe₂O₃ (0, 1.5, 2.5, 3.75, 5%)

Fig. 3 – SEM micrographs of pure TiO₂ (a), 5wt.%Fe₂O₃ doped TiO₂ (b), and EDX spectrum of pure TiO₂ NPs (c), 5wt.%Fe₂O₃-TiO₂ (d)

Fig. 4 – UV–vis diffuse reflectance spectra of photocatalysts, inset: the plot of Kubelka-Munk function of $[F(R). hv]^{1/2}$ vs. hv .

Fig. 5 – The response surface of the final normalized concentration of *Vibrio fischeri* as a function of: process temperature and photocatalyst dosage for IT2.5 photocatalyst (a), process temperature and Fe₂O₃ percentage at 1.05 g/L of photocatalyst dosage (b), photocatalyst dosage and Fe₂O₃ percentage at 30 °C (c).

Fig. 6 – Time variation of normalized concentration of *V. fischeri*: using 1.05 g/L of IT2.5 at different temperatures (a), using different dosage of IT2.5 at 30 °C (b), using 1.05 g/L of photocatalyst with different percentage of Fe₂O₃ at 30 °C (c), for control experiments (dark, light, blank) and photocatalytic experiments using 1.05 g/L of IT2.5 at 30 °C (d).

609 **Tables**

610 **Table 1 – Experimental parameters and levels**

Independent varriables	factors	Levels and values				
		$-\alpha$	-1	0	+1	α
Temperature (°C)	A	25	27.5	30	32.5	35
Photocatalyst dosage (g/L)	B	0.1	0.575	1.05	1.575	2
Fe ₂ O ₃ percentage (wt.%)	C	0	1.25	2.5	3.75	5

611

612 **Table 2 – Textural, structural, and band gap energies of synthesized photocatalysts**

Sample	BET surface area (m ² /g)	Pore volume (cc/g)	Average particle size (nm)	Crystallite size (dXRD, nm)	Anatase to rutile ratio (CA)	Band edge (nm)	Band gap (eV)
T	161	0.0138	8.8	9.1	29.2	380	3.25
IT1.25	133	0.0094	10.4	8.2	32.2	410	3.12
IT2.5	128	0.0086	11.0	10.23	33.4	430	2.9
IT3.75	111	0.0062	12.7	10.23	33.6	455	2.8
IT5	126	0.0070	11.1	10.23	32.8	480	2.65

613

614

615 **Table 3 – Design of experiments and actual and predicted values of response**

Run	Parameters			Response (Final normalized concentration of <i>Vibrio fischeri</i>)	
	Temperature (°C)	Photocatalyst dosage (g/L)	Fe ₂ O ₃ percent (%)	Actual	Predicted*
1	30.0	1.050	2.50	0.048	0.041256
2	30.0	1.050	2.50	0.052	0.041256
3	32.5	0.575	3.75	0.03	0.025728
4	32.5	1.525	1.25	0.06	0.081728
5	30.0	1.050	2.50	0.029	0.041256
6	32.5	1.525	3.75	0.14	0.132608
7	27.5	1.525	3.75	0.18	0.193254
8	30.0	1.050	2.50	0.05	0.041256
9	27.5	0.575	3.75	0.05	0.026875
10	25.0	1.050	2.50	0.08	0.079627
11	30.0	2.000	2.50	0.27	0.253865
12	30.0	1.050	0.00	0.141	0.1311
13	30.0	1.050	2.50	0.037	0.041256
14	27.5	1.525	1.25	0.15	0.152873
15	32.5	0.575	1.25	0.081	0.066347
16	35.0	1.050	2.50	0.006	0.007334
17	30.0	0.100	2.50	0.055	0.072105
18	27.5	0.575	1.25	0.072	0.077994
19	30.0	1.050	5.00	0.121	0.130862
2	30.0	1.050	2.50	0.031	0.041256

616 $*Y = 0.249 - 0.001517A + 0.09188B - 0.13748C - 0.012526AB + 0.00084AC + 0.038526BC +$

617 $0.000089A^2 + 0.13488B^2 + 0.014356C^2$

618

619 **Table 4 – ANOVA for the second order model**

Source	Sum of squares	F-value	P-value
Model	0.076	30.76	<0.0001
A	5.22E-03	18.94	0.0014
B	0.033	119.85	<0.0001
C	6.25E-08	2.268E-04	0.988
AB	1.77E-03	6.42	0.0297
AC	5.51E-05	0.2	0.664
BC	4.18E-03	15.19	0.003
A ²	7.79E-6	0.028	0.869
B ²	0.023	84.48	<0.0001
C ²	0.013	45.9	<0.0001
Residual	2.756E-03	-	-
Lack of fit	2.245E-03	4.4	0.0650
Pure error	5.108E-04	-	-

620