Anti-algal activity of Fe$_2$O$_3$–TiO$_2$ photocatalyst on Chlorella vulgaris species under visible light irradiation

Baniamerian, Hamed; Tsapekos, Panagiotis; Alvarado-Morales, Merlin; Shokrollahzadeh, Soheila; Safavi, Maliheh; Angelidaki, Irini

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Anti-algal activity of Fe2O3-TiO2 photocatalyst on *Chlorella vulgaris* species under visible light irradiation

Hamed Baniamerian\textsuperscript{a,b}, Panagiotis Tsapekos\textsuperscript{a}, Merlin Alvarado-Morales\textsuperscript{a}, Soheila Shokrollahzadeh\textsuperscript{b}, Malihe Safavi\textsuperscript{c,*}, Irini Angelidaki\textsuperscript{a,*}

\textsuperscript{a} Department of Environmental Engineering, Technical University of Denmark, Kgs. Lyngby, DK-2800, Denmark

\textsuperscript{b} Department of Chemical Technologies, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran

\textsuperscript{c} Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran

*Corresponding authors and addresses:

Malihe Safavi; Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), P.O.Box 15815-3538, Tehran, Iran

E-mail: m.safavi@irost.ir

Tel: +982156276637; Fax: +982156276265

Irini Angelidaki, Department of Environmental Engineering, Technical University of Denmark, Bld 115, 2800 Lyngby Denmark

E-mail: iria@env.dtu.dk, Tel.: +4545251429; Fax: +4545932850
Abstract

Many industries located in coastal areas use a large amount of seawater. Algal biofouling can be a major problem that hinders the efficiency of these industrial facilities. In most cases, seawater requires algal removal pre-treatment to avoid or mitigate biofilm formation. To remediate green microalgae, Fe₂O₃-TiO₂ nanoparticles with 2.5 % w/w Fe₂O₃ were applied as a visible light driven photocatalyst. The anti-algal activity of the photocatalytic pre-treatment using green microalgae, Chlorella vulgaris was tested. The experiments were carried out in freshwater, artificial seawater, and real seawater. Effect of photocatalyst dosage, visible light intensity, and water salinity on the removal of microalgae was investigated. The highest inactivation efficiency of Chlorella vulgaris was achieved under 55W/m² visible light irradiation when 0.25 g/L of Fe₂O₃-TiO₂ photocatalyst was used. The photocatalytic removal kinetics of Chlorella vulgaris followed the pseudo first order Langmuir-Hinshelwood model. The results revealed that the efficiency of photocatalytic removal of algae decreased with increasing of seawater salinity. The anti-algal activity of Fe₂O₃-TiO₂ nanoparticles was attributed to the generation of reactive oxygen species (ROS) through the photocatalytic process. H⁺ radical was shown to be the most important ROS that nanoparticles produced in the aqueous media. Using Fe₂O₃-TiO₂ nanoparticles in photocatalytic pre-treatment could be an efficient environmental-friendly method for micro-algal remediation in seawater under visible light.

Keywords
Seawater pre-treatment; photocatalysis; visible light; biofouling; microalgae; Chlorella vulgaris
1. Introduction

Industries located in coastal areas use a large amount of seawater in their processes. Biofilm formation in interior parts of industrial cooling systems, pipelines, and seawater desalination facilities, e.g. reverse osmosis membranes, is a major problem that occurs mainly due to the attachment and subsequent growth of algae, protozoa, and bacteria (Chiou et al., 2010). Biofilm formation can reduce lifetime, capacity, and heat transfer efficiency of cooling tower systems and also increase pressure drop that will subsequently increase operation and maintenance costs (Chede et al., 2019; Meesters et al., 2003). Among various microorganisms, algal cells are difficult to eliminate with conventional pre-treatment methods (Sathe et al., 2016). Therefore, it is necessary to apply an efficient and environmental friendly pre-treatment method to deactivate microalgae to protect coastal facilities. Algal cells have tendency to deposit on the membrane surface which can reduce membrane lifetime. Removal of micro-algal cells is crucial to improve the performance of water desalination plants and cooling systems. Several marine micro-algal species produce blooms that cause severe biofouling and can produce dangerous toxins. Most reports in the literatures are related to the treatment of freshwater microalgae, *Microcystis aeruginosa*, (Gavand et al., 2007), while other biofilm forming species such as *Chlorella* or *Dunaliella* has been less investigated. Among *Chlorella* species, *Chlorella vulgaris* showed signs of adaptation to high content of salinity. Moreover, the response shown by *Chlorella vulgaris* to rise in salinity is stronger than that of *Chlorella Salina*, which is presumably a salt-water resistant species (Talebi et al., 2013). The microalgae *Chlorella vulgaris*, is a microorganism widely found in lakes and marine environments and it can tolerate...
different levels of salinity (Liu et al., 2008). Thus, in this research *Chlorella vulgaris* was chosen as algal microorganism model.

Common seawater pre-treatment methods involve usage of chemical based disinfectants (such as chlorine, chlorine dioxide, chloramines, and ozone), which can effectively control the growth of microorganisms including bacteria, algae, fungi, and yeast (Li et al., 2014; Pichel et al., 2018). Formation of harmful disinfectant by-products is one of challenges of these methods that pose adverse health effects and cause environmental risks. Furthermore, desalination membranes can be degraded by these disinfectants, i.e. chlorine, ozone, which are strong oxidants (Marconnet et al., 2011).

Using ultraviolet (UV) irradiation can be an alternative way to inactivate microorganisms to avoid biofilm formation with less side effects. A drawback of UV irradiation is that it may be more expensive and energy consuming compared to chemical oxidation (Chi Zhang et al., 2018). Heterogeneous photocatalysis employing semiconductors activated by visible light continue to be a subject of research interest for developing water/seawater treatment technologies, aiming to prevent biofilm formation at low cost and with minimum side effects. Photocatalytic process is one of the main advanced oxidation processes (AOPs) that produce highly reactive oxidants (ROS), e.g. hydroxyl radicals (\(^{\text{\scriptsize \bullet}}\text{OH}\)), superoxide ion (\(\text{O}_2^-\)) and hydrogen peroxide \(\text{H}_2\text{O}_2\), through light incidence of a semiconductor. This process conducts degradation of the microorganisms without addition of chemicals and secondary effects such as membrane damage (W. Wang et al., 2015).

Titanium dioxide (TiO\(_2\)) is a well-known photocatalyst extensively used for disinfection applications due to its low price and stability (Geng et al., 2017). Roy et al. (2016) investigated the effect of commercial P25 TiO\(_2\) nanoparticles (NPs) on the
Chlorella and Scenedesmus microalgae species in freshwater media. TiO\textsubscript{2} NPs exerted oxidative stress on micro-algal cells under visible light and UVA irradiation through producing ROS species. In another research, Kim et al. (2016) used crystalline/amorphous reduced TiO\textsubscript{2} in the disinfection of the green microalgae Chlamydomonas in freshwater media. Similarly, ROS generated by the TiO\textsubscript{2} was responsible for inactivation of this green microalgal species. However, the large band gap energy of TiO\textsubscript{2}, 3.2 eV, restrict its application to UV light irradiation (Cuiqing Zhang et al., 2018). High recombination rate of photo-induced electron-hole pairs, which reduce life-time of charge carriers, and low visible light sensitivity of TiO\textsubscript{2} photocatalyst are drawbacks of TiO\textsubscript{2} photocatalysis (Chen et al., 2011). Due to these disadvantages, many research studies have been dedicated to develop visible light active TiO\textsubscript{2} photocatalyst, while maintaining a large active surface area. One of the most effective methods is doping TiO\textsubscript{2} with different materials such as metals, metal oxides, and non-metals (Baniamerian and Shokrollahzadeh, 2016). Iron is an environment-friendly, stable and low cost material with low band gap (2.2 eV) and high visible light adsorption capacity which makes it a promising candidate to modify TiO\textsubscript{2} photocatalyst (Liŭ et al., 2016; Zhang and Lei, 2008). Adding Fe\textsubscript{2}O\textsubscript{3} to TiO\textsubscript{2} structure introduces new levels in the band gap of TiO\textsubscript{2} and increases light absorption wavelength to make the use of visible light, i.e. solar irradiation, more efficiently for practical applications (Uyguner-Demirel et al., 2018). However, despite this interest, no one to the best of authors knowledge has investigated the effect of visible-light driven Fe\textsubscript{2}O\textsubscript{3}-doped TiO\textsubscript{2} against the growth of marine micro-algal cells as a biofouling preventive treatment. Furthermore, most researches are limited to disinfection of microorganisms in freshwater media which have completely different conductive properties than salt water.
environments.

In the current study, the activity of visible-light driven Fe$_2$O$_3$-TiO$_2$ photocatalyst for the removal of biofilm forming microalgae, *Chlorella vulgaris*, under visible light irradiation was investigated as a water pre-treatment technology. Light intensity and photocatalyst concentration are two important parameters on the point of economic and operating efficiency view. Salinity varies in different seas and oceans and can affect the photocatalytic process due to presence of ions. Therefore, the effect of operating parameters, e.g. photocatalyst concentration, visible light intensity, and seawater salinity on photocatalytic removal of microalgae was evaluated.

2. Materials and methods

2.1 Reagents and materials

Fe$_2$O$_3$-TiO$_2$ NPs with optimized 2.5 % w/w Fe$_2$O$_3$ content was synthesized via an ultrasonic-assisted co-precipitation method, as it has the highest efficiency of microorganisms inactivation among other Fe$_2$O$_3$ percentage based on our previous research (Baniamerian et al., 2018). The NPs were fully characterized and the results of characterization including crystallinity, size distribution, BET surface area, and band gap energy have been previously reported (Baniamerian et al., 2018). All chemicals and reagents were reagent grade from Sigma-Aldrich and used without further purification and treatment. Baltic sea water was taken from the coastal area in Klampenborg, Denmark. The seawater was filtered using 2.5 μm Whatman filter paper, then autoclaved and stored at 4 °C prior to using. The characterization of seawater is presented in Table 1.

Table 1: characterization of Klampenborg coastal area seawater
<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Ca$^{2+}$</th>
<th>K$^+$</th>
<th>Mg$^{2+}$</th>
<th>Na$^+$</th>
<th>Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>84.60±1.27</td>
<td>140.48±3.12</td>
<td>505.62±13.66</td>
<td>4387.56±208.49</td>
<td>15940.85±230.70</td>
<td></td>
</tr>
</tbody>
</table>

### 2.2 Tracing the ROS by photo-degradation of methylene blue

The ROS generated by 2.5wt% Fe$_2$O$_3$/TiO$_2$ NPs in the photocatalytic inactivation process was measured through the photocatalytic degradation of methylene blue (MB) dye using radical capturing techniques (Wang et al., 2018). Isopropanol (IPA), triethanolamine (TEA), and benzoquinone (BQ) were used to scavenge •OH, H$^+$, and •$O_2^-$, respectively. In each experiment, 0.1 g of photocatalyst was added to the 200 mL of MB solution with an initial concentration of 20 mg/L, which are the optimum amounts of photocatalyst and dye concentrations typically used in organic dye photo-degradation experiments (Li et al., 2016). An amount of 0.5 g of each scavenger was added to the solution in the assays to evaluate the quenching effect of scavenger. In order to prepare oxygen saturated, homogenous solution, air was blown into the solution using air pump and magnetic stirrer. Simultaneously, to reach the adsorption-desorption equilibrium the solution was kept in dark for one hour prior to the photocatalytic experiment. Photocatalytic degradation of MB dye was performed at 20 °C using the experimental setup explained in previous research (Baniamerian et al., 2018). Liquid samples were taken from the solution at time intervals during the experiment. The samples were centrifuged immediately after retrieval to remove photocatalysts. The MB concentration in the solution was measured by DR-3900 Spectrophotometer. The absorption peak at 665 nm was related to MB (Park et al., 2018). The concentration of dye in each sample was calculated using the peak intensity and calibration curve.
2.3 Algal cells and culture conditions

Batch cultures of *Chlorella vulgaris* were grown in several media i.e. freshwater (Van Wagenen et al., 2014), artificial seawater with different salinity (15000, 30000, and 45000 mg/L), and Baltic seawater (15940 mg/L salinity). Salinity was measured based on a method described by Boulton et al. (2014). The artificial seawater was made as described previously by Baniamerian et al. (2018) and enriched with nutrients (Guillard and Lorenzen, 1972). Salinity in artificial seawater was increased by dissolving more salts in deionized water. The cultures were grown in Erlenmeyer flasks above the fixed fluorescent bulbs with 136 μmol photons m\(^{-2}\) s\(^{-1}\) irradiation in a controlled temperature room of 20 °C as described by Van Wagenen et al (2014). Cultures were diluted with the related fresh medium to reach the optical density, OD\(_{750}\), of 0.1 prior to the photocatalytic experiments.

2.4 Photocatalytic inactivation of algae

The activity of Fe\(_2\)O\(_3\)/TiO\(_2\) NPs for removal of marine *Chlorella vulgaris* microalgae was investigated in a batch system equipped with a temperature controller. The range of 0-1 g/L was selected for testing photocatalyst dosage, as higher catalyst levels resulted in increased turbidity, and subsequently decreased transmittance of light through the aqueous solution (Baniamerian et al., 2018). In a 400 mL Pyrex glass beaker (9.5 cm in diameter and 5.5 cm in height), up to 1g/L of sterile photocatalyst was suspended in 200 mL of microalgae containing growth media. The initial optical density (OD\(_{750}\)) of *Chlorella vulgaris* for all experiments was selected equal to 0.1 which is a very high concentration of algal cells that have been reported in algal blooms (Masojidek et al., 2011; Sathe et al., 2016). The relationship between OD\(_{750}\) and *Chlorella vulgaris* cell number is expressed by (*Chlorella vulgaris* cell...
numbers = 3.04 × 10^{10} \text{OD}_{750}) equation reported by Masojídek et al. (2011). The suspensions were stirred in exposure of two 9 W fluorescent white lamp (Osram, G23, 2pin, 220 V) at 20 °C constant temperature. Three neutral density filters were made by printing different intensity shades of black ink on a transparent film. The transparent films were used in order to provide different light intensities (in range of 25-55 W/m²). The intensity of the visible light was measured by a handheld Li-COR quantum sensor (LI-250A). The distance between the light source and surface of the mixture was fixed at 10 cm. For each experiment, the variables, i.e. catalyst concentration, light intensity, and water salinity, were fixed based on experimental plan. At given irradiation time intervals (0, 4, 8, 12, 16, 20, 24 h) 1 mL of the suspension was collected and inactivation of \textit{Chlorella vulgaris} microalgae was investigated by measuring the chlorophylls content. Effect of three parameters, i.e. photocatalyst concentration (0-1 g/L), light intensity (25-55 W/m²), and water salinity (15000-45000 mg/L) on photocatalytic destruction of \textit{Chlorella vulgaris} was investigated as presented in Table 2.

**Table 2: experimental ranges and levels of independent variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Range and levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photocatalyst concentration (g/L)</td>
<td>0</td>
</tr>
<tr>
<td>Light intensity (W/m²)</td>
<td>25</td>
</tr>
<tr>
<td>Water salinity (mg/L)</td>
<td>0</td>
</tr>
</tbody>
</table>

2.5 Monitoring algal cell re-growth after photocatalytic treatment

After 24 h of photocatalytic treatment, algal cell suspension was monitored for algal
cell re-growth for up to 7 days. The contents of the experimental vessel (400 mL beaker) were transferred to aerated Erlenmeyer flasks and maintained under conditions ideal for *Chlorella vulgaris* growth as described in section 2.3. Re-growth of *Chlorella vulgaris* microalgae was investigated by measuring the chlorophylls content daily up to 7 days.

### 2.6 Chlorophyll measurement

The chlorophylls content of the algal cells is considered as an index for determining the growth of algae and is an important parameter in indicating the viability of cells during inactivation processes such as photocatalysis (Bhuvaneshwari et al., 2018). 2 mL of suspension were taken from the reaction media during the process and placed in microtubes. The samples were centrifuged at 12000 rpm for 10 min and the supernatant was removed (1.7 mL). In order to extract chlorophylls, 1 mL methanol was added to each sample and the tubes were shaken for 1 hour using a vortex mixer. The samples were centrifuged again at 12000 rpm for 10 min. The supernatant containing extracted chlorophylls was transferred into a 96-well microplate and absorbance was measured at 665 nm with BIOTEK Synergy microplate reader (Suzuki et al., 2017).

### 2.7 Statistical analysis

All analyses were performed in triplicate and mean±SD values are reported. The analysis indicates that the results are similar and no significant difference was observed in obtained data for each set of experiments. In order to reveal the statistically significant differences between the experiments and compare the results, one-way ANOVA was applied, following Tukey post hoc test (p < 0.05).
3. Results and Discussion

3.1 Analysis of reactive oxygen species (ROS) generated from photocatalytic activity

The ROS generated in the photocatalytic process were identified using radical scavenging technique. In this research, a number of different scavengers, i.e. IPA, TEA, and BQ, were selected to quench *OH, H+, and *O2–, respectively, if formed during the photocatalytic destruction of the algal cells. Fig. 1 shows the normalized concentration of MB dye (ratio of dye concentration/initial dye concentration) during photocatalytic experiment in presence of different scavengers (IPA, TEA, and BQ) as well as control experiments including dark control (absence of light and scavenger and presence of photocatalyst) and blank control (absence of scavenger and presence of photocatalyst and light). Adsorption and photocatalysis are the two main processes that work cooperatively to achieve the degradation of organic matters. Before photocatalysis functions, the catalyst should complete the capture of organic molecules, which is decided by the adsorption property of the catalyst. Prior to visible light irradiation, the suspension was stirred in the dark for 60 min to reach the adsorption/desorption equilibrium on the Fe2O3-TiO2 nanoparticles surfaces under experimental conditions. As shown in Figure 1, the presence of each radical scavenging agent in the photocatalytic process caused reduction in MB dye decomposition. The scavenging agents react with ROS during process and limit the photocatalytic oxidation efficiency. As an example addition of H+ scavenger (TEA) had the highest suppressing effect on photocatalytic decomposition of MB dye, while the addition of IPA and BQ inhibited the progress of photocatalytic destruction in lower extent. Therefore, the contribution of
the different ROS in the process is, $H^+ > OH^+ > O_2^-$, demonstrating that $H^+$ radical is the most important species produced by our synthesized NPs in the aqueous media. The observed algal destruction was attributed to the ROS generated during the photocatalytic process which can oxidize the organics matters (Mahmood et al., 2012). Free radicals can affect the fatty acid chain and deform the cell lipids causing permanent rupture of the cell membrane and subsequently DNA damage (Nel et al., 2006). Furthermore, ROS can degrade polysaccharides, cell walls, and extracellular polymeric substances of cells resulting cell inactivation (Gladis et al., 2010).

Fig. 1: Adsorption behavior of the photocatalyst in dark condition and photo-decolorization results during photocatalytic treatment in presence of different scavengers.

3.2 Effect of photocatalyst concentration on destruction of algal cells

Four different concentrations of $Fe_2O_3$-TiO$_2$ NPs were tested in freshwater media to investigate the effect of photocatalyst dosage and choose the most effective
concentration for inactivation of *Chlorella vulgaris*. Fig. 2a shows normalized chlorophylls concentration (as an index of algal viability) during photocatalytic treatment in presence of different dosages (0-1 g/L) of NPs under visible light irradiation (55 W/m²). The results demonstrated a dose dependent effect of photocatalyst on *Chlorella vulgaris* removal. The chlorophyll content would be oxidized during photocatalytic treatment, resulting in the destruction of photosynthetic system. These effects would finally lead to the inactivation of algal cells (Wang et al., 2018). As can be seen in Fig. 2a the algal concentration was higher in absence of NPs during irradiation of visible light (control). Adding 0.25 g/L of NPs showed the most intensive effect on inactivation of algal cells. Photocatalyst concentrations higher than 0.25 g/L resulted in decreasing the algae deactivation efficiency. Higher NPs concentrations increase water turbidity and thereby higher light scattering resulting in lower light penetration depth into the aqueous media (Kim et al., 2003).

Photocatalytic reactions take place on the surface of NPs and it is important to investigate possible mechanism for photocatalytic degradation of micro-algal cells. There are five fundamental steps which occur during all heterogeneous catalytic processes based on adsorption-type mechanism. These steps are including transfer of aqueous reactants, i.e. microorganisms, to the solid NPs, the adsorption of the reactants on the surface of NPs, reaction takes place and by-products generate, desorption of products, and transfer of products from the solid surface to the bulk liquid phase (Dalrymple et al., 2010). In order to destruct microorganisms, micro-algal cells like molecular reactants are required to be in contact with or in very close to the NPs surface. In photocatalytic researches, adsorption models have usually been applied for the elimination of chemical pollutants (Gusain et al., 2019; Tungudomwongsa et al.,
and not microorganisms. However, Marugán et al. (2008) developed their disinfection model for TiO₂ NPs based on Langmuir–Hinshelwood-type kinetics. In this research, the photocatalytic removal kinetics of *Chlorella vulgaris* with Fe₂O₃-TiO₂ NPs were described by fitting the experimental data to pseudo first order Langmuir–Hinshelwood model. The kinetic equation was described as follows (X. Wang et al., 2015):

\[
\ln\left(\frac{C_t}{C_0}\right) = -k_{\text{app}} t
\]

Eqn. 1

where \(C_0\) and \(C_t\) are the chlorophylls concentration at \(t=0\) and \(t\), respectively, while \(k_{\text{app}} (\text{h}^{-1})\) is the apparent pseudo first order reaction rate constant obtained by plotting -ln(\(C/C_0\)) vs. the reaction time (\(t\)).

The whole photocatalytic destruction process of micro-algal cells is divided into three steps (Wang et al., 2017): (a) generated ROS cause irreversible damage to the membrane protein, which subsequently results in electrolyte leakage and increase in conductivity of solution; (b) photosynthetic system is disturbed, because cell organelles would be exposed to oxidative environment produced by ROS. The accumulated ROS would attack the pigments and active proteins, which would accelerate the dying progress of the micro-algal cells; (c) oxidation products and metabolites, i.e. extracellular dissolved organic materials, react with ROS and strongly compete with micro-algal cells and its organelles.

Cell walls of microalgae consist of a polysaccharide and glycoprotein matrix providing the cells with a strong defence against its environment (Gerken et al., 2013), thus the photocatalytic process was performed during relatively long time, i.e. 24 h, which is longer than normal time for photocatalytic bacterial removal (Uyguner-Demirel et al., 2018). Fig. 2b shows the rate constant (\(k_{\text{app}}\)-value) for photocatalytic removal of
Chlorella vulgaris cells in terms of the concentration of photocatalyst in the reaction media. The increase in the concentration of photocatalyst more than 0.5 g/L decreased the photocatalytic removal of algae cells and subsequently the algae removal rate constant was decreased. The negative $k_{app}$-value in the case of control experiment indicates the net positive growth of algal cells under 55W/m$^2$ visible light at 20 °C. As it noted before, photocatalysis is a catalyst dosage-dependent process. A larger NPs concentration can produce more oxidative radicals by absorbing more light. Nevertheless, high NPs concentration reduces the transparency of suspension, hindering the energy from light radiation, and subsequently decreasing the degradation efficiency (Parra et al., 2002). In Fig. 2b, a continuous decrease in $k_{app}$-value is observed as the Fe$_2$O$_3$-TiO$_2$ NPs concentration exceeding 0.5 g/L, reaching a minimum value of 0.0198 for 1 g/L of Fe$_2$O$_3$-TiO$_2$ NPs. Among tested dosages of NPs, 0.25 g/L showed the maximum $k_{app}$-value, and consequently the best microorganism destruction efficiency. However, it seems there is an optimum dosage of NPs, between 0-0.5 g/L, in which the maximum destruction of microorganisms occurs.
Fig. 2: Time course of normalized concentration of algal chlorophylls (C/C₀) in the presence of different concentration of Fe₂O₃-TiO₂ NPs and 55 W/m² of visible light (a), and corresponding cell removal rate constant (k_{app}-value) based on Langmuir–Hinshelwood model (b).

A more careful attention to Fig. 2a revealed that the photocatalytic destruction of *Chlorella vulgaris* cells in this study can be divided into two phases in the point of reaction rate view. Phase I started from 0 to 4 h has the maximum destruction rate of micro-algal cells and could be attributed to a rapid adsorption of micro-algal cells onto
the NPs surface and subsequently rapid destruction of cells by generated ROS in vicinity of photocatalyst. During this phase the micro-algal cells were captured rapidly by the porous surface of Fe$_2$O$_3$-TiO$_2$ NPs. Phase II start from 4 to 24 h. Compared to phase I, destruction rate of micro-algal cells decreased. When the adsorption capacity of the NPs is gradually saturated, a cell layer will cover the surface of NPs. This may cause inhibition photocatalytic active sites from reacting with micro-algal cells. Furthermore, lysis and oxidation products strongly compete for ROS. Thus, slower destruction rate of *Chlorella vulgaris* cells was observed as moving towards the end of process. This finding is in agreement with other researches revealed that destruction rate of microorganisms slowed down gradually during photocatalytic process (Dalrymple et al., 2010; Marugán et al., 2008; Wang et al., 2017; Wu et al., 2018). The results revealed that efficient photocatalytic destruction of high concentration of *Chlorella vulgaris* in exposure of relatively low visible light intensity, 55 W/m$^2$, required long time, i.e. 24 h.

### 3.3 Effect of light intensity on algal destruction

The effect of light intensity on efficiency of photocatalytic algal removal in freshwater media at 20 °C is summarized in Fig. 3. Two control experiments were conducted in dark (dark control), in the absence and presence of Fe$_2$O$_3$-TiO$_2$ NPs to understand the effect of NPs on *Chlorella vulgaris*. No algal growth was observed during incubation of microalgae in the absence of light and NPs. No algal death was observed in the absence of light and presence of 0.25 g/L of NPs during 24 h indicating zero cytotoxic effect of Fe$_2$O$_3$-TiO$_2$ NPs on *Chlorella vulgaris*. Furthermore, according to the results inactivation rate of *Chlorella vulgaris* increased with increasing average light intensity from 25 to 55 W/m$^2$ in the presence of 0.25 g/L photocatalyst. These results revealed
that, when photocatalyst concentration is constant, the removal rate of algal cells depend
on the average light intensity. The visible light intensity of 55 W/m² in the presence of
0.25 g/L photocatalyst effectively reduced the algal concentration to a negligible level
in the freshwater after 24 hours treatment. When 25, 35, and 45 W/m² of visible light
was applied to the system, the algal concentration of media was reduced to 43%, 31%,
and 9.6% of the initial levels, respectively. The results show that more rapid algal
removal is occurred at higher light intensity. At higher light intensities, higher flow of
photons cause generation of more ROS on the surface of TiO₂-based photocatalyst that
attacks microalgae and increases the removal rate (Rincón and Pulgarin, 2003).
However, there is no linear relation between the light intensity and algal removal
rate. The electrical conductivity of algal suspension in freshwater was measured during
photocatalytic process. Fig. 4 shows the photocatalytic process in the presence of 0.25
g/L photocatalyst under 55 W/m² caused increase in electrical conductivity over
irradiation time whereas, the electrical conductivity of control experiment changed
slightly. This observation indicates the leakage of electrolyte (K⁺ ion) from damaged
algal cells and confirms the inactivation of Chlorella vulgaris during the photocatalytic
treatment (Wang et al., 2018). The salt content of saline media and seawater is high in
comparison with K⁺ ion released from algal cells. The salts exist in saline water
interfere with k⁺ released from dead algal cells, therefore the measurement of electrical
conductivity changes was measured only in freshwater media during photocatalytic
process.
Fig. 3: Normalized concentration of algal chlorophylls (C/C0) during time under different light intensities using 0.25 g/L Fe$_2$O$_3$ NPs at 20°C.

Fig. 4: Electrical conductivity of microalgae suspension in freshwater during photocatalytic process

3.4 Effect of salinity on process efficiency

The effect of different saline conditions on photocatalytic inactivation of *Chlorella*
*vulgaris* was investigated during visible light irradiation. Artificial seawater with different salinities 15000 mg/L (ASW1), 30000 mg/L (ASW2), and 45000 mg/L (ASW3), and Baltic seawater with 15940 mg/L (RSW) salinity were used as growth media for micro-algal cells. Photocatalysis can be affected by seawater composition and level of salinity (Rincon and Pulgarin, 2004). The final normalized concentration of chlorophylls after 24 hours of photocatalytic process in different saline mediums are shown in Fig.5. It was found that increasing the salt content resulted in lower efficiency of the inactivation process. The final normalized concentration of chlorophylls after 24 hours of the photocatalytic process was 0.127, 0.261, 0.317, and 0.112 in ASW1, ASW2, ASW3, and RSW media, respectively. The growth of *Chlorella vulgaris* was also investigated in all types of media (including artificial seawater with different levels of salinity and real seawater) under irradiation of 55 W/m² visible light and absence of NPs as control experiments. The control experiments suggested that the pattern of algal growth was not affected by salinity level within the tested salinity range. Chlorophylls measurements in control experiments showed that ratio of the chlorophylls concentration (after 24 hours irradiation) to initial concentration of chlorophylls reached to about 1.6 in all tested media. As noted before, photocatalysis by TiO₂ based NPs, produces ROS which causes damage to microorganisms membrane, proteins, DNA and subsequently inactivate microorganisms (Moncayo-Lasso et al., 2008). The results showed that the efficiency of photocatalytic inactivation of algal cells is affected by the composition of water, so that the presence of salt in water decreased the process efficiency. There are two possible reasons for the adverse effect of salts on photocatalysis (Rubio et al., 2013). The first possibility is the scavenging effect by halide ions such as Cl⁻, Br⁻, CO₃²⁻, and HCO₃⁻. Sodium chloride is the most abundant
inorganic salt in seawater (Rincon and Pulgarin, 2004), as it can be seen also in Table 1, the Na$^+$ ion and subsequently the counter ion, Cl$^-$, is the most abundant ions. Photo-induced $^*\text{OH}$ radicals can be scavenged by Cl$^-$ ions (Eqn. 2) (Takeda et al., 1998).

\[
^*\text{OH} + \text{Cl}^- \rightarrow \text{Cl}^* + \text{OH}^- \quad \text{Eqn. 2}
\]

Produced Cl$^*$ radicals also have oxidizing capability of organic substances, but at a lower rate than $^*\text{OH}$, as they have lower oxidation power (Kiwi et al., 2000). Another possible explanation for the decreased photocatalytic efficiency by the presence of salts, is that Cl$^-$ ions are adsorbed on the surface of TiO$_2$ NPs, and therefore, block the active sites, subsequently decrease the ROS generation (Barka et al., 2008). Other ions like HCO$_3^-$ probably acts as scavengers of H$^+$ formed on the TiO$_2$ surface reducing the inactivation rate of microorganisms. Furthermore, HCO$_3^-$ partially inhibits the photocatalytic reactions by generation of negatively charged layer on the TiO$_2$ surface; therefore the TiO$_2$ surface charge becomes less positive, leading to the decrease in the adsorption of microorganism (Rincon and Pulgarin, 2004). This confirms pervious findings stating that addition of NaCl, NaHCO$_3$, Na$_2$CO$_3$, CaCl$_2$, and KCl salts in presence of TiO$_2$ NPs, significantly reduces adsorption of organic matters and subsequently reduces the photo-degradation efficiency of organic substances (Bouanimba et al., 2015; Guillard et al., 2005).
In addition to the effect of salinity on photocatalytic efficiency of TiO₂-based NPs on microalgae inactivation, possible algal re-growth after photocatalytic treatment with Fe₂O₃-TiO₂ photocatalyst was monitored for 7 days. Results from previous sections revealed that photocatalytic treatment using 0.25 g/L of photocatalyst under 55 W/m² visible light in zero salinity media could effectively inactivate Chlorella vulgaris species. Long term anti-algal properties of freshwater (containing Chlorella vulgaris) treated with 0.25 g/L of Fe₂O₃-TiO₂ photocatalyst was shown by monitoring chlorophylls content of treated culture suspension over a period of 7 days. Complete re-growth inhibition of algal cells, in fresh media, treated with visible light driven NPs was confirmed, as no significant changes in chlorophylls content were observed after 7 days of incubation of the treated culture, at ideal growth condition.

4. Conclusions

TiO₂ nanoparticles with 2.5 % w/w Fe₂O₃ were found to be effective in reducing the
viability of green microalgae *Chlorella vulgaris* in both freshwater and seawater in
exposure of visible light irradiation. Up to 99% of algal removal was observed under 55
W/m² visible light irradiation after 24 hours in the presence of 0.25 g/L of the
photocatalyst. Among the different oxidizing ROS, H⁺ radical was concluded to be the
most important species that are produced by our synthesized NPs in the aqueous media
and decreased the viability of algal cells. Salinity had a negative effect on process and
efficiency of the photocatalytic destruction of algal cells decreased with increasing
water salinity due to the presence of ionic scavengers in seawater and salty waters.

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