Aquatic toxicity evaluation of TiO_2 nanoparticle produced from sludge of $TiCl_4$ flocculation of wastewater and seawater: *D. magna* and *V. fischeri*

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Abstract

Flocculation using titanium tetrachloride ($TiCl_4$) as a coagulant is an efficient and economical application because the flocculated sludge can be recycled to produce a valuable byproduct, namely titanium dioxide (TiO_2) nanoparticles. However, toxicity of $TiCl_4$ has not yet been assessed while it is used in water treatment. The aquatic toxicity of $TiCl_4$ flocculation process

was investigated to assess the environmental safety of the coagulant. *D. magna* and *V. fischeri* bioassays were carried out to evaluate the supernatant toxicity after $TiCl_4$ flocculation. Artificial wastewater, biologically treated sewage effluent and seawater were used to study the toxicity of $TiCl_4$ flocculation. Results showed that supernatant toxicity was very low when $TiCl_4$ flocculation was conducted (no observed effect concentration = 100 mg/L and lowest observed effect concentration = 150 mg/L exposed to *D. magna* and *V. fischeri*, respectively). Similarly, TiO_2 nanoparticles recovered from wastewater and seawater flocculated sludge were also found to have low toxicity. The regenerated TiO_2 nanoparticles indicated low toxicity values when compared to the commercial-TiO_2 nanoparticle, P-25.

Keywords: Aquatic toxicity, *D. Magna*, *V. fischeri*, flocculation, TiO_2 nanoparticle

Introduction

Chemical treatments of wastewater, surface water and seawater involve the removal or the conversion of contaminants either by the addition of chemicals or through other chemical reactions. Flocculation is one of the most commonly used chemical treatments of water. Flocculation can be applied to remove organic matter from contaminated water, which may cause trihalomethane formation during disinfection in waste or drinking water treatment plants. Aluminium sulfate, iron salt and polyaluminum chloride are mainly used as coagulants (DeWolfe et al., 2003). The flocculation processes using these salts produce a large amount of sludge that is disposed of either into a landfill and/or dumped into the ocean. However, the disposal of chemical sludge into landfills is no longer acceptable as it could cause secondary contamination

due to landfill leachate. In addition, landfills are being exhausted at a fast rate, therefore they are not considered to be a viable and/or sustainable option for the disposal of the large amount of sludge produced. On the other hand, serious coastal environmental consequences are making ocean disposal of sludge a limited option. Besides, sludge dumping into the ocean will be prohibited by the year 2010 according to the international law of London Dumping treaty.

Upton and Buswell (1937) were the first to investigate Ti-salt coagulant. They reported that Ti-salt coagulant had no advantage over aluminum sulfate in terms of fluoride removal but it was better in color removal. Recently, Lokshin and Belikov (2003) reported the lowest F^- concentration achievement after Ti-salt flocculation. The Ti-salt can be used in a wide range of p H for producing high quality of water and fast settling floc. Shon et al. (2007) investigated the possibility of sludge recovery using the Ti-salt as a coagulant. Sludge produced during $TiCl_4$ flocculation processes of wastewater can be incinerated to produce a valuable nanoparticle, namely TiO_2. An amount as high as 40 mg-TiO_2/L-wastewater of TiO_2 nanoparticle was produced from wastewater sludge generated by the $TiCl_4$ flocculation (Shon et al., 2007). The significant increase of TiO_2 demand due to its wide range of applications such as environmental technology, cosmetics, paints, paper and solar cells can be partially overcome through this TiO_2 recovery process. However, it is also important to investigate the toxic effect of the remaining Ti ions in the supernatant after $TiCl_4$ flocculation and in TiO_2 produced from sludge before its use in water treatment.

Titanium tetrachloride ($TiCl_4$) has been widely used in the industrial process as an intermediate in the production of titanium rutile, titanium oxide and titanium pigments. The cost of $TiCl_4$ is

comparable to iron chloride or aluminium sulphate so the flocculation processes followed by sludge incineration offer an efficient and economical method for the removal of organic matte r and sludge recovery. Protocols for the safe handling and use of bulk $TiCl_4$ are well docum ented due to its existing industrial uses (Kapias and Griffiths, 2005). Risk levels of $TiCl_4$ were mostly through inhalation exposure (Lee et al., 1986; Lewis, 1996). The minimum risk levels for $TiCl_4$ were 0.01 mg/m^3 for intermediate inhalation exposure and 0.0001 mg/m^3 for chronic inhalation exposure. However, no references have been found on the toxic effect of Ti ion in an aqueous phase, because it was not widely used in water treatment processes.

The toxicity of TiO_2 has been investigated by several bioassay procedures using *D. magna* and *V. fischeri* (Bozzi et al., 2004; Lovern and Klaper, 2006; Schaefer and Scott-Fordsmand, 2006). *D. magna* is one of the widely used bio-indicators as it is a critical medium in the food chain between the algae and fish (Lovern and Klaper, 2006). Lovern and Klaper (2006) investigated the toxicity of aggregated and 0.22-μm filtered TiO_2 (average size of 10 – 20 nm) in terms of Median Lethal Concentration (LC50) and 100% mortality using *D. magna*. They found that the aggregated TiO_2 is not toxic, while the LC50 and the 100% mortality of the filtered TiO_2 were 5.5 mg/L and 10 mg/L, respectively. Here, LC50 stands for the lethal concentration of the chemical that kills 50% of *D. magna* in a given time (48 hrs). Microtox® test depends on the attenuation of bioluminescence of *V. fischeri*. This test is a rapid and inexpensive technique for screening of contaminants (Jennings et al., 2001). The bioluminescence of *V. fischeri* decreases in the presence of toxic chemicals and experimental values of relative light intensity (light intensity normalized with respect to initial light intensity) at various concentrations of toxic chemicals are calculated in terms of EC50 values at which a 50% loss of bioluminescence is

obtained after 15 min exposure. Gellert (2000) reported that the decrease of bioluminescence intensity may be due to i) biomechanisms, which are responsible for the toxic chemical uptake by *V. fischeri* (diffusion across membrane, transport process and composition of the cell wall), ii) interactions of toxic chemicals with enzymes or allosteric effectors at critical steps of metabolic pathways which supply cell energy, and/or iii) interactions with the luciferase complex, which is responsible for the luminescence.

In this study, $TiCl_4$ flocculation was conducted with Artificial Wastewater (AW), Biologically Treated Sewage Effluent (BTSE) and Seawater (SW). TiO_2 produced from $TiCl_4$ flocculation of different water sources was characterized in terms of X-ray diffraction, surface area, photocatalytic activity and scanning electron microscopy. To secure the efficiency of the Ti-salt flocculation process, the toxicity of Ti ion in the supernatant after $TiCl_4$ flocculation and in TiO_2 recovered from the sludge was evaluated in terms of LC50 and mortality of *D. magna* and EC50 of Microtox® test. No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values for *D. magna* and *V. fischeri* on exposure of $TiCl_4$ and TiO_2 produced from different water sources were also investigated.

Experimental

Water sources

Three water samples representing various water sources (i.e., AW, BTSE and SW) were used in

this study. The composition of AW can be found elsewhere (Seo et al., 1997). The AW represents effluent organic matter generally found in domestic wastewater after the biological treatment. Tannic acid, peptone, sodium lignin sulfornate, sodium lauryle sulfate and arabic acid represent the larger molecular weight portion, while peptone, beef extract and humic acid comprise the organic matters of lower molecular weight (Shon et al., 2005). BTSE was drawn from Gaya sewage treatment plant in South Korea. It had the following characteristics: concentration of dissolved organic matter = 7.8 mg/L; chemical oxygen demand = 20 mg/L; pH = 7.3; total nitrogen = 23.8 mg/L; total phosphorus = 2.4 mg/L; and conductivity = 430 μS/cm. Seawater was drawn from south-western Korea and it was found to have the following characteristics: pH = 8.10; conductivity = 48.9 mS/cm; total dissolved solid = 32827 mg/L; dissolved organic matter concentration = 1.56 mg/L; and alkalinity = 78 mg/L as $CaCO_3$.

Flocculation with $TiCl_4$ coagulant

Flocculation was carried out with $TiCl_4$ as a coagulant for the three different water samples. Water samples were stirred rapidly for 1 minute at 100 rpm, followed by 20 minutes of slow mixing at 30 rpm, and finally 30 minutes of settling. The optimum concentration of $TiCl_4$ was determined based on the organic removal results obtained.

Preparation of TiO_2

Sludge produced from different water samples was dried and the dried sludge was placed in

furnace at 600°C for 24 hrs (Shon et al., 2007). After incineration, the color of powder changed from black to white indicating TiO_2 formation.

Characterization of TiO_2

Aggregated particle size of produced TiO_2 was studied by Scanning Electron Microscopy (SEM, Rigaku, Japan). Nitrogen adsorption–desorption isotherms were recorded using an ASAP 2020 model (Micromeritics Ins., U.S.A.) and the specific surface area was determined by the Brunauer–Emmett–Teller (BET) method. X-Ray Diffraction (XRD) images of anatase and rutile TiO_2 photocatalysts were analyzed to identify the particle structure using MDI Jade 5.0 (Materials Data Inc., USA). The crystallite size of powders was determined from the broadening of the corresponding XRD peaks by using Scherrer's formula. The photocatalytic activity test of TiO_2 was investigated under UV irradiation (Sankyo, F10T8BLB, three 10 W lamps) and visible light (Kumbo, FL10D, three 10 W lamps) using the method of photodecomposition of gaseous acetaldehyde. The concentration of acetaldehyde was measured by gas chromatography (Youngin, M600D, Korea).

***D. magna* toxicity test**

D. magna obtained from the Korea Institute of Toxicology were cultured and experimentally maintained according to the method described in the US Environmental Protection Agency (USEPA) manual (USEPA, 1993). Culture water for *D. magna* was reconstituted with moderately hard water (120 mg/L of $CaSO_4 \cdot H_2O$, 192 mg/L of $NaHCO_3$, 120 mg/L of $MgSO_4$

and 8 mg/L of KCl) at pH 7.8±0.2. *D. magna* were fed with a mixture of *Selenastrum capricornutum* (algae), yeast, trout chow and cerophyll. Acute 48 hrs toxicity tests using *D. magna* were conducted under static conditions (T = 25±1^{o}C under 16 hrs light and 8 hrs dark photocycle. The fed *D. magna* were transferred into test cups. Four replicates holding five neonates of less than 24 hrs of age, were used to test different $TiCl_4$ and TiO_2 concentrations in water. Each set of test comprised of different concentrations and a control. The mortality was defined as the number of dead organisms after 48 hrs of exposure. The mortality was analyzed using a probit method analysis (USEPA Probit Analysis Program). This allowed LC50 to be calculated. NOEC and LOEC values for *D. magna* exposured to different concentrations of $TiCl_4$ and TiO_2 produced from different water sources were also calculated using Dunnett's test using TOXSTAT software.

Microtox® test

The Microtox® test was performed according to the protocol described by Azur Environmental. This has been adopted as a standard method by ASTM International (2002). *V. fischeri* colonies were seeded onto samples with an adjusted osmotic pressure using an osmotic adjusting solution composed of sodium chloride. The luminescence inhibition after 15 min of exposure was considered as the endpoint, and it was measured using a Microtox® Model 500 Analyzer (AZUR Environment). USEPA probit analysis was used to calculate EC50 (the concentration that reduced light production by 50%). NOEC and LOEC values for the Microtox® test were also measured using Dunnett's test using TOXSTAT software.

Results and discussion

TiO_2 characterization

Jar test was conducted to identify the optimum dose of $TiCl_4$ in terms of organic removal in AW, BTSE and SW. The optimum doses of Ti-salt with AW, BTSE and SW were found to be 9.79 Ti-mg/L, 6.57 Ti-mg/L and 3.23 Ti-mg/L, respectively. In order to obtain TiO_2 nanoparticles, the settled floc produced by $TiCl_4$ flocculation of AW, BTSE and SW was incinerated at 600 ºC. After incineration, the nanoparticles produced were characterized in terms of XRD, BET surface area, photocatalytic activity and SEM.

XRD images were made to identify the particle structure of the settled floc after incineration at 600 ºC (Figure 1). The TiO_2 produced from sludge was compared with the commercially available P-25. The P-25 showed both antase (65%) and rutile (25%) structures. On the other hand, TiO_2 nanoparticles from AW, BTSE and SW were found to have only anatase structures after incineration at 600 °C. Shon et al., (2007) reported that the anatase TiO_2 produced from wastewater sludge changed to rutile when the incineration temperature was increased more than 1000 °C. It was due to the presence of C and P atoms in the incinerated sludge. At temperatures lower than 600 °C, amorphous structures were observed due to the remaining organic matter.

Table 1 shows the characteristics of TiO_2 produced from AW, BTSE and SW in terms of

crystallite size and BET surface area. The crystallite size was calculated by the Scherrer's formula (Suryanarayana, 1995). The crystallite size of TiO_2 from AW, BTSE, SW and P-25 was 6, 15, 40 and 25 nm, respectively. The difference in crystallite sizes among different water sources are probably due to impurities in the flocculated sludge after $TiCl_4$ flocculation and incineration. The BET surface area of TiO_2 nanoparticles from AW, BTSE, SW and P-25 were 76.3, 103.5, 68.1 and 42.3 m^2/g, respectively. The BET specific surface areas of TiO_2 nanoparticles produced from different waters were higher than that of the P-25.

The photocatalytic properties of TiO_2 nanoparticles produced from different water sources were investigated in terms of UV photodecomposition of gaseous acetaldehyde (Figure 2). The concentration of acetaldehyde was measured by gas chromatography. The photodecomposition rate of acetaldehyde by TiO_2-SW was compared to that by the P-25 photocatalyst. During the first 15 minutes, TiO_2-SW showed the highest adsorption rate among tested TiO_2.It was sequentially followed by TiO_2–AW, TiO_2-BTSE and TiO_2-P-25. The removal of acetaldehyde differed among tested TiO_2. Results showed that TiO_2–SW was the best photocatalysis followed by TiO_2-AW, then TiO_2-P-25 and finally TiO_2-BTSE. On the other hand the photocatalytic activity of all tested TiO_2 under visible light irradiation was nil.

Figure 3 shows SEM images of the TiO_2 powder obtained from different water sources. TiO_2 produced from AW and BTSE indicated an irregular crystal structure. The P-25 powder and the TiO_2 obtained from SW relatively consisted of a regular grain size. The irregular crystal structure may be due to the larger amount of impurities (in terms of organic compounds) found in AW and BTSE compared to that in SW. Shon et al. (2007) reported that the impurity ratio of TiO_2 nanoparticles produced from wastewater was about 20%. The TiO_2 obtained from BTSE was

found to be doped with carbon and phosphorus.

D. magna mortality of $TiCl_4$

Aquatic toxicity of $TiCl_4$ coagulant was investigated using *D. magna* mortality with different $TiCl_4$ concentrations (Figure 4). *D. magna* was exposed to $TiCl_4$ concentrations ranging from 0 mg/L to 200 mg/L. The exposure of *D. magna* to $TiCl_4$ concentrations of less than 80 mg/L showed no fatality. However, $TiCl_4$ concentrations of 100 mg/L and 200 mg/L exhibited 15% and 25% mortality, respectively.

Median lethal concentration of $TiCl_4$ was investigated in terms of LC5, LC10, LC15 and LC50. The LC5, LC10, LC15 and LC50 of $TiCl_4$ were 68.1 mg/L, 98.4 mg/L, 126.1 mg/L and 359.9 mg/L, respectively. LOEC and NOEC of $TiCl_4$ to *D. magna* were 100 mg/L and 150 mg/L, respectively. This means that $TiCl_4$ coagulant has low toxicity in water.

D. magna mortality of TiO_2 produced from different water sources

Figure 5 shows the aquatic toxicity of TiO_2 produced from different water sources based on *D. magna* mortality. TiO_2 concentrations varied from 0 mg/L to 200 mg/L. The maximum mortality recorded for TiO_2 produced from AW, BTSE and SW was less than 15%, while that of P-25 TiO_2 was 25% for a concentration of 100 mg/L. As all the TiO_2 particles indicated low mortality, the LC50 was not determined. Therefore, LC5, LC10 and LC15 were compared using USEPA Probit analysis program (Figure 6). The commercially available P-25 TiO_2 showed lower values of LC5

(5.9 mg/L), LC10 (28.4 mg/L) and LC15 (81.3 mg/L) compared to TiO_2 produced from AW, BTSE and SW.

The LOEC and NOEC values of TiO_2 produced from AW, BTSE, SW and P-25 to *D. magna* were calculated using Dunnett's test. LOEC and NOEC of P-25 were 80 mg/L and 100 mg/L, and those of TiO_2 from AW, BTSE and SW were not obtained due to low mortality of *D. magna*. This result clearly indicates that TiO_2 nanoparticles produced from AW, BTSE and SW have low toxicity in aqueous condition. This agrees with previous studies on TiO_2 toxicity in aqueous media (Johnson et al., 1986; Lovern and Klaper 2006). Johnson et al. (1986) reported that mean concentration values of TiO_2 required to immobilize 50% of *D. magna* was more than 1 g/L. On the other hand, Lovern and Klaper (2006) found that mortality with sonicated TiO_2 was less than 9% and the LC50 was zero for the sonicated TiO_2 solution. However, when exposed to the filtered TiO_2 with 0.22 μm microfilter, the LC50 was calculated to be 5.5 mg/L with the NOEC (1 mg/L) and LOEC (2.0 mg/L). Thus, when the filtered TiO_2 was used, more attention has to be paid when assessing the acute toxicity.

Microtox® bioassay of $TiCl_4$

Figure 7 shows the dose-response curve of *V. fischeri* exposed to different $TiCl_4$ concentrations. The results are based on Whole Effluent Toxicity (WET) test (15 min exposure). Light emission rapidly decreased with the increase of $TiCl_4$ concentration. The inhibition percentages at 62.5 mg/L and 1000 mg/L of $TiCl_4$ concentration were 5.84% and 72.3%, respectively. Based on USEPA probit analysis program, the inhibition of bioluminescence of *V. fischeri* (EC50) was at

739.3 mg/L of $TiCl_4$ concentration.

Microtox® bioassay of TiO_2 produced from different water sources

Figure 8 shows inhibition of bioluminescence on TiO_2 produced from different water sources. Here, the toxicity values (expressed in terms of percentage inhibition of bioluminescence) represent loss of relative bioluminescence emission. The percentage of inhibition significantly increased with the increase in concentration of TiO_2 produced from AW, BTSE and P-25. The percentage of inhibition of TiO_2 produced from SW at 125 mg/L, 500 mg/L and 1000 mg/L was 5.6%, 11.9% and 13.7%, respectively. Microtox® result suggests that the TiO_2 produced from SW had the lowest toxicity.

Figure 9 presents the threshold of TiO_2 concentration values (EC50) obtained after 15 min exposure. For the TiO_2 produced from SW, the EC50 value was not found. The maximum loss of bioluminescence was 14%. However, the EC50 values of TiO_2 produced from AW, BTSE and P-25 were 650.6 mg/L, 940.6 mg/L and 830.8 mg/L, respectively. The EC50 values showed the following decreasing trend for TiO_2 obtained from different water sources: TiO_2-BTSE > P-25 > TiO_2-AW > TiO_2-SW.

Table 2 shows the NOEC and LOEC values of the Microtox® test for the exposure to TiO_2 produced from AW, BTSE, SW and P-25. The NOEC and LOEC values of TiO_2 from AW, BTSE and SW were similar or higher than those of P-25. The relatively high EC50, NOEC and LOEC values indicate that there is no significant toxic effect on TiO_2. Schaefer and Scott-Fordsmand

(2006) reported similar results on TiO_2 exposure to *V. fischeri*. They found no toxic effect of TiO_2 (< 100 nm size) on *V. fischeri* even at a TiO_2 concentration of 1000 mg/L.

Conclusions

TiO_2 produced from flocculated sludge of AW, BTSE and SW were characterized in terms of X-ray diffraction, surface area, photocatalytic activity and scanning electron microscopy. TiO_2 from AW, BTSE and SW consisted of anatase structure after incineration at 600 °C. The size of TiO_2 from AW, BTSE and SW was 6 nm, 15 nm and 40 nm, respectively. The surface area of TiO_2 from AW, BTSE and SW was 76.3 m^2/g, 103.5 m^2/g and 68.1 m^2/g, respectively. The photocatalytic removal of acetaldehyde under UV irradiation showed the following decreasing sequence: TiO_2-SW > TiO_2-AW > TiO_2-BTSE.

Aquatic toxic effect of Ti ion in the supernatant after $TiCl_4$ flocculation and TiO_2 recovered from the sludge was examined in terms of LC50 and mortality of *D. magna* and EC50 of Microtox® test. The NOEC and LOEC of $TiCl_4$ using *D. magna* were 100 mg/L and 150 mg/L, respectively. The NOEC and LOEC values of *D. magna* exposed to TiO_2 produced from different water sources were not found. Regarding the Microtox® test, the inhibition of bioluminescence of *V. fischeri* (EC50) was at 739.3 mg/L of $TiCl_4$ concentration. The maximum loss of bioluminescence of *V. fischeri* exposed to TiO_2 produced from SW was 14%. The EC50 of TiO_2 produced from AW and BTSE was 650.6 mg/L and 940.6 mg/L, respectively. Based on the two toxicity tools, it can be concluded that $TiCl_4$ coagulant and TiO_2 produced from AW, BTSE and

SW have very low toxicity in aqueous condition.

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Table 1. Crystallite size and BET surface area of TiO_2 produced from different water sources

	Crystallite size (nm)	Surface area (m^2/g)
TiO_2 from AW	6±0.3	76.3±1.1
TiO_2 from BTSE	15±0.6	103.5±0.9
TiO_2 from SW	40±0.8	68.1±1.0
P-25 TiO_2	25±0.4	42.3±0.9

Table 2. NOEC and LOEC values for the Microtox® test on exposure of TiO_2 produced from AW, BTSE, SW and P-25.

	NOEC (mg/L)	LOEC (mg/L)
TiO_2 from AW	250	500
TiO_2 from BTSE	250	500
TiO_2 from SW	500	1000
P-25	250	500

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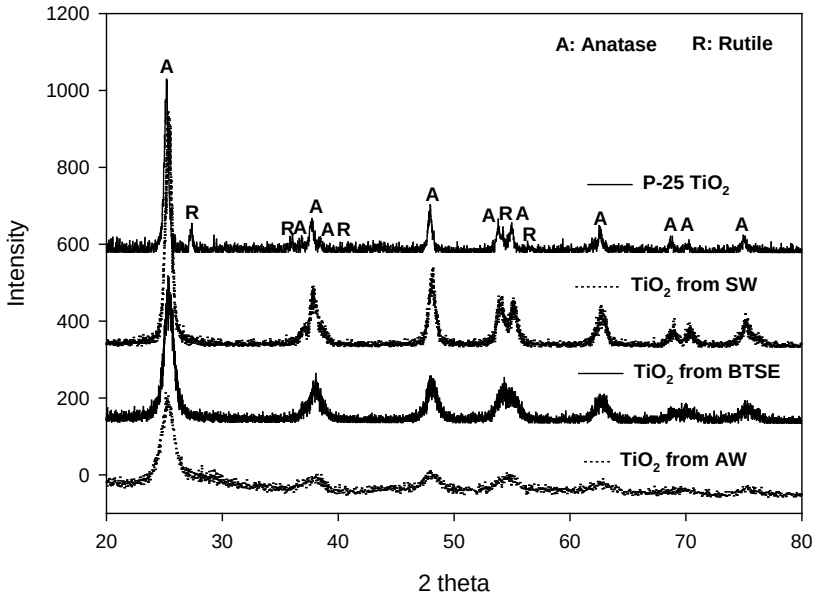

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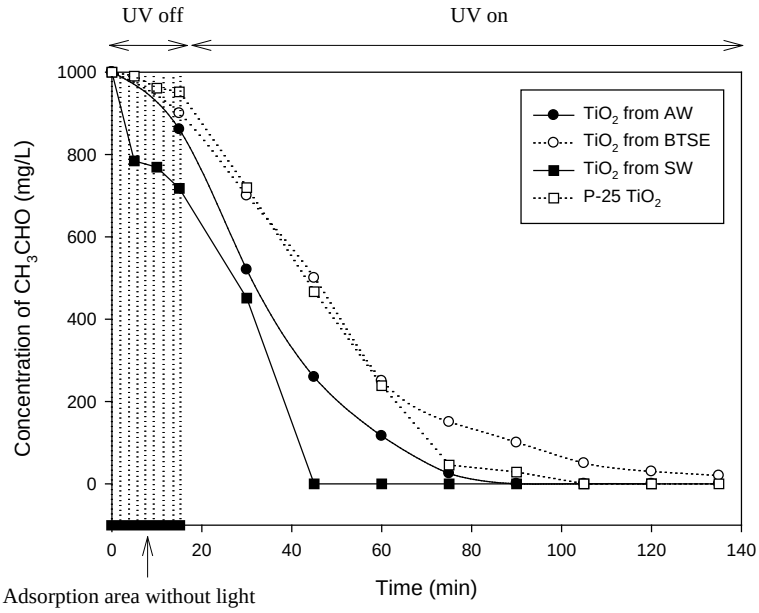
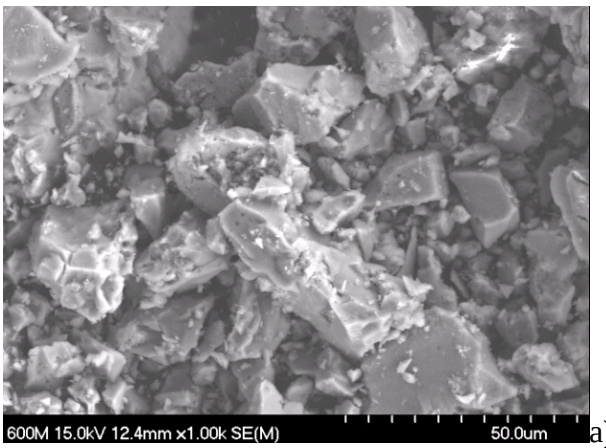
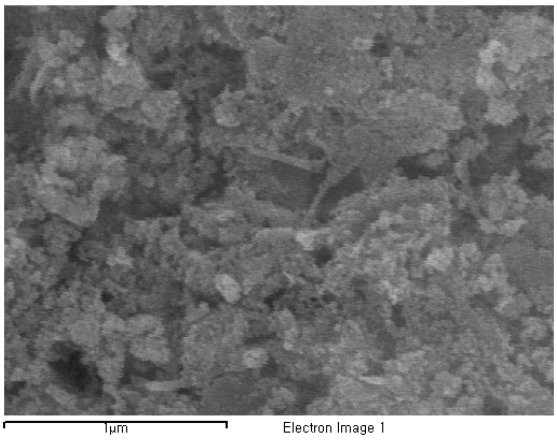


Figure 2. Adsorption and photodegradation of acetaldehyde under UV light using different TiO_2 (initial concentration of acetaldehyde = 1000 mg/L; UV irradiation = black light three 10 W lamps with a light power of 0.9 mW/cm^2)



a)



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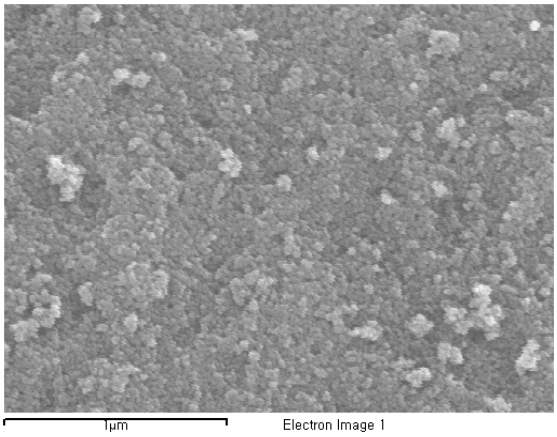
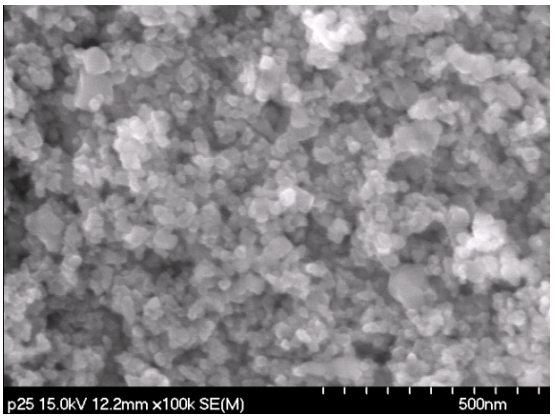


c)



d)

Figure 3. SEM images of the TiO_2 powders obtained from (a) AW, (b) BTSE and (c) SW as compared to (d) P-25

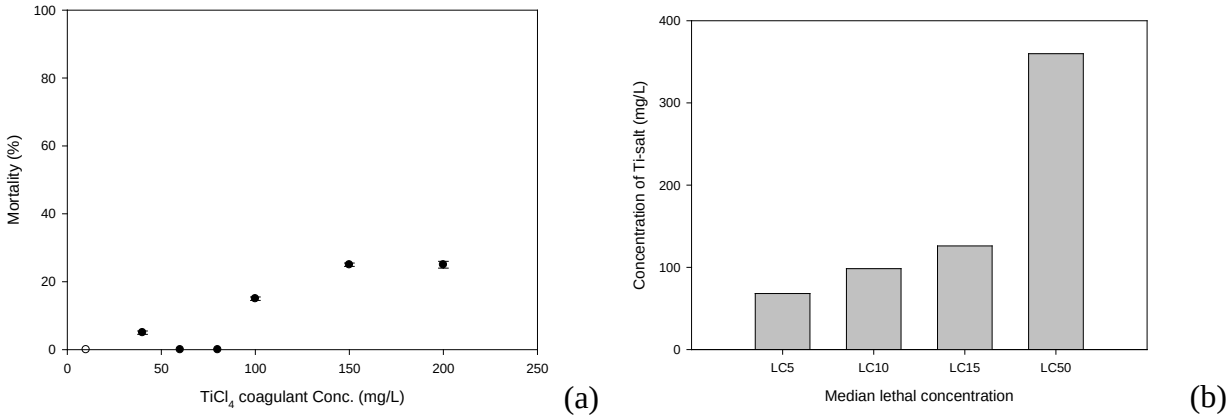


Figure 4. (a) Mortality of *D. magna* on exposure to $TiCl_4$ coagulant and (b) median lethal concentration of $TiCl_4$

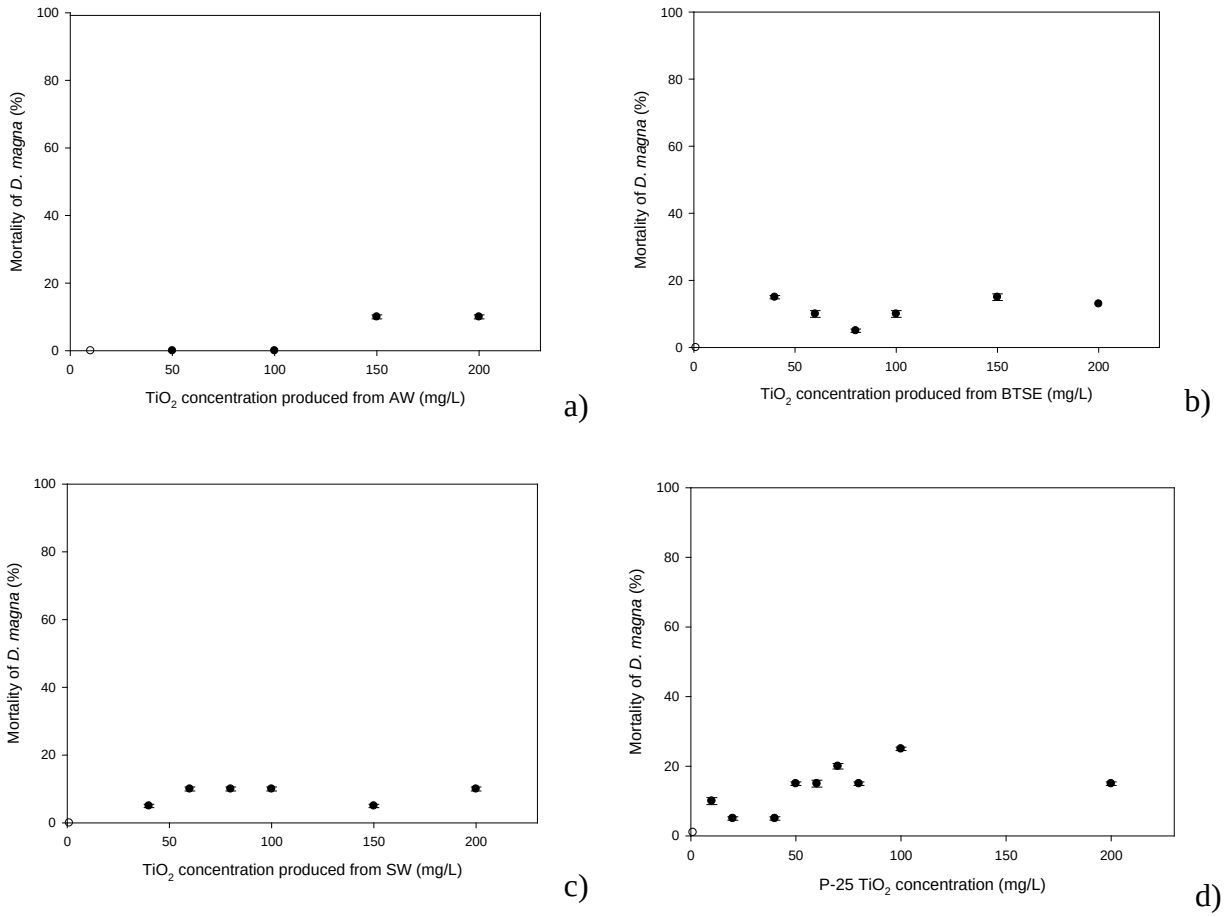


Figure 5. Mortality of *D. magna* exposed to TiO_2 produced from (a) AW, (b) BTSE, (c) SW and (d) P-25.

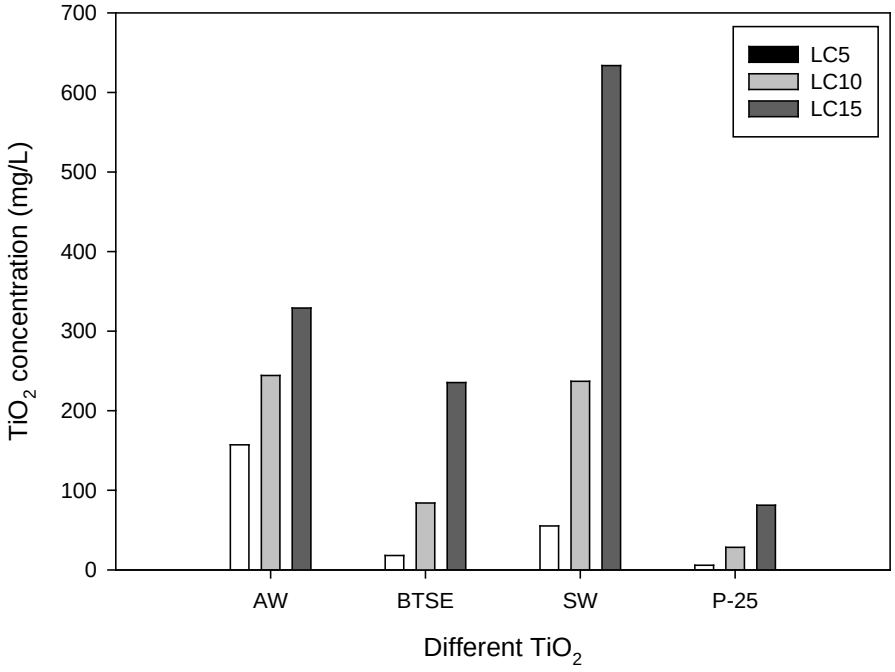


Figure 6. Concentration values of LC5, LC10 and LC15 for TiO_2 produced from AW, BTSE and SW

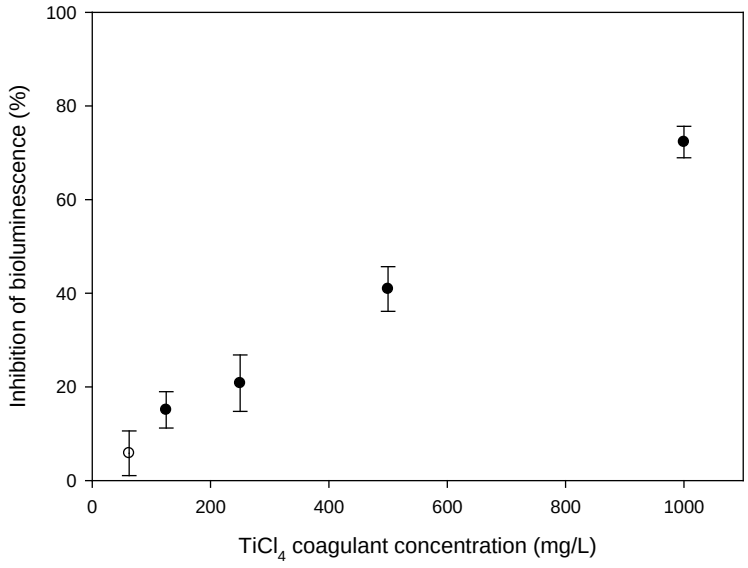


Figure 7. Dose-response curves of $TiCl_4$ coagulant in terms of light emission of *V. fischeri* exhibited by the whole effluent toxicity test (15 min exposure)

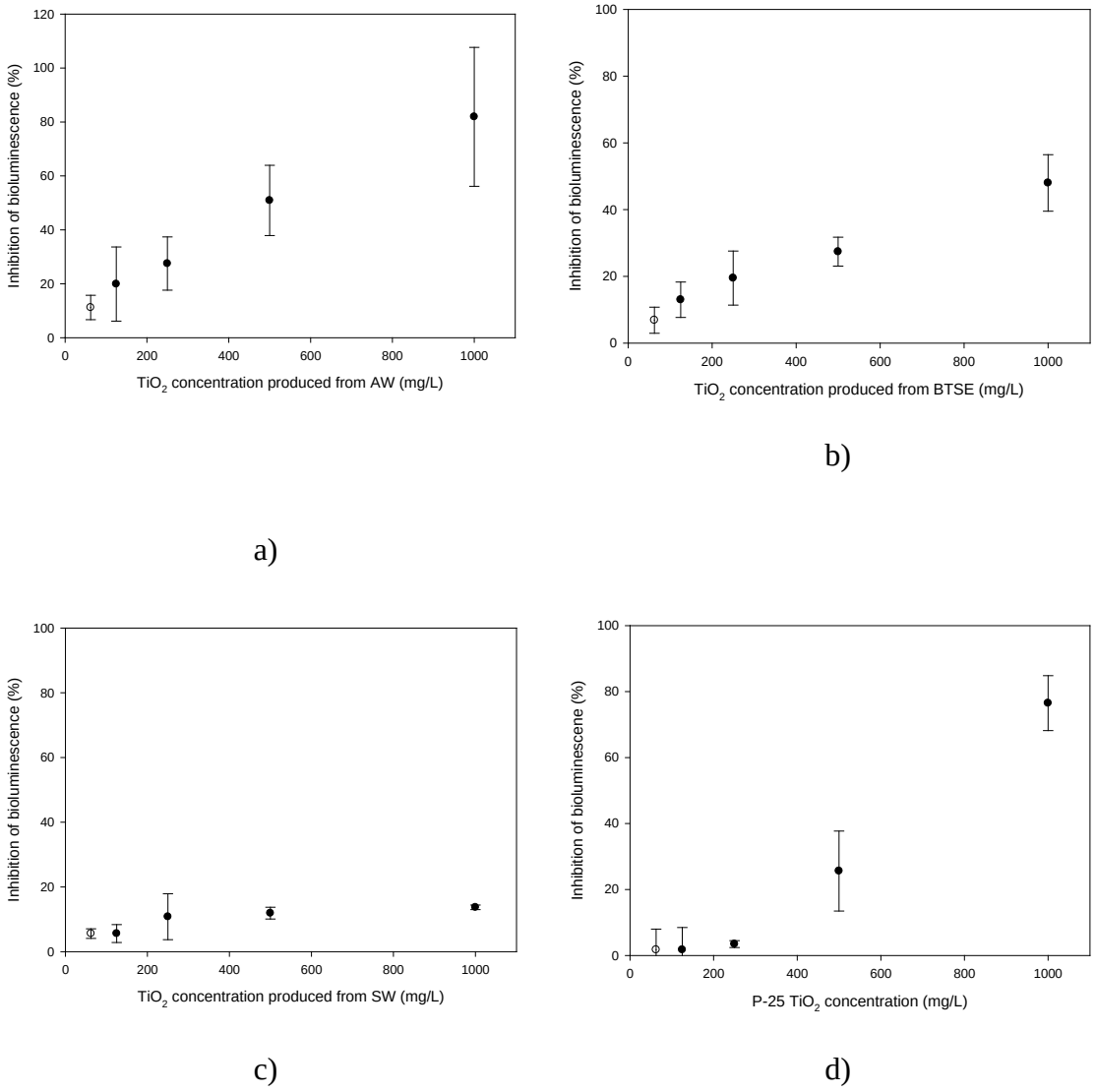


Figure 8. Inhibition of bioluminescence using *V. fischeri* exposed to TiO_2 produced from (a) AW, (b) BTSE, (c) SW and (d) P-25

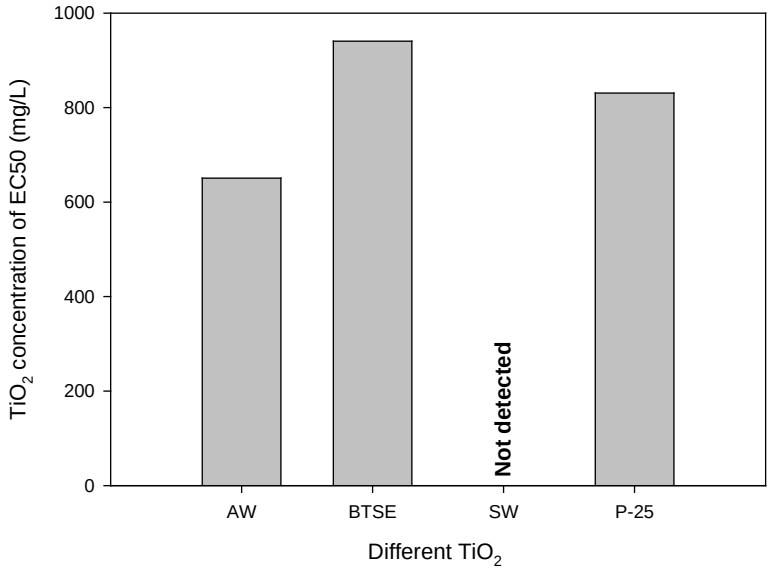


Figure 9. Threshold concentration values (EC50) after 15 min exposure of *V. fischeri* to different TiO_2