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# Degradation of Antibiotics (Trimethoprim and Sulphamethoxazole) Pollutants Using UV and TiO<sub>2</sub> in Aqueous Medium

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# Abstract

Experiment was carried out in two parts to investigate the degradation profile of trimethoprim and sulphamethoxazole, antibacterial compounds in aqueous phase. In the first part of study, the effects of UV and  $TiO_2$  on two antibiotics using the UV-water flow system (UVWFS) were examined. Obtained results revealed that trimethoprim and sulphamethoxazole pronounced 44 to 45% and 6 to 314% elevated rate of degradation in two TiO<sub>2</sub> doses, 0.05 and 0.1 g/l treatments with UV than only UV treatments which affords to draw a substantial conclusion that though both UV and  $TiO_2$  have a crucial impact but the synergistic photocatalytic effects of the  $TiO_2$  with UV attributes a rapid and higher degree of degradation of antibiotics compared to that of only UV at the same water flow rate. In this context, it may also be observed that effect of the dose of  $TiO_2$  was very little in trimethoprim degradation but a significantly greater effects was revealed in sulphamethoxazole that indicating same number of TiO<sub>2</sub> molecules acted more favourably on higher number of sulphamethoxazole molecules than trimethoprim by photocatalytic activity which enhancing the rate of degradation reaction resulting in the rapid decrease of sulphamethoxazole concentration level in water. In second part of the study, three (15, 42 and 80 cc/min) water flow rates were employed using UVWFS maintaining same UV and TiO<sub>2</sub> dose 0.1 g/l to ascertain the effect of water flow rate in the antibiotic transformation process. The degradation efficiency in 80 cc/min water flow treatment was 113 and 61% higher in trimethoprim and 103 and 51% higher in sulphamethoxazole compared to that of 15 and 42 cc/min water flow treatments, respectively which clearly indicated that the rate of water flow is a paramount important, largely influencing the photocatalysis reaction process of TiO<sub>2</sub> and UV with antibiotics in water medium possibly enhancing movement velocity of their molecules. Besides it, trimethoprim degraded rapidly over sulphamethoxazole which might be inferred that higher water flow substantially increases the rate of oxidation and photocatalytic transformation of trimethoprim over sulphamethoxazole in the presence of TiO<sub>2</sub> and UV.

Keywords: Antibiotics, UV, TiO<sub>2</sub>, Water flow system, Antibiotic degradation

# 1. Introduction

Recently, presence of antibiotic compounds in terrestrial and aquatic environment is of growing interest worldwide due to emergence as major threatened pollutants not only from environmental perspective but also from human health perspective. Antibiotics are used extensively in veterinary medicine for therapeutic purposes and as a growth promoter. In last decades, shrimp industry recognized as an important livelihood and developed rapidly because of attractive returns. A vast array of antibiotics are applied in the aquaculture especially in shrimp industry to enhance the production as well as to meet the global demand of such delicious and nutritious animal proteins in one hand and to strengthen and uplift the socioeconomic profile especially of the developing South East Asian countries. Use of antimicrobials in aquaculture essentially started with the work of Gutsell (1946), who recognized the potential usefulness of

sulphanamides for combating furunculosis. Following this, chloramphenicol, oxytetracycline, kanamycin, nifurprazine, oxolinic acid, sodium nifurstyrenate, flumequin, ciprofloxacin and others were introduced (Austin and Austin, 1993). Afterwards it appears as antibacterial, antiviral, antifungal, antiprotozoan and antimetazoan preparations, probiotics, immunostimulants, vaccines, bacterins, hormones, growth stimulants, anesthetics and bioremediatiors for both bioaugmentors and biostimulators.

Nowadays, antibiotic is the most common worrisome in the fish and shrimp industry that seepages through pond bottoms and discharges as wastes and effluents mixing into runoff water and crrying to downstrem covering tropical coastal mangroves destroying important natural habitat for shripm, fish, bird and people. Various substances in shrimp farm ponds can contaminate waters, including nutrients (nitrogen and phosphorus), metabolic wastes, antibiotics, or other medicines to protect shrimp, and suspended soil particles from erosion (Boyd and Green, 2002). Studies of fish farms have shown that the majority of antibiotics added in feed are not assimilated by fish but go into environment (Weston, 1996). Le and Munekage (2004) also reported that antibiotics residues may cause harmful effect on ecosystems in the coastal mangrove area. Once in the environment antibiotics can have a wide range of effects. Presently, indiscriminate application of antibiotics in aquaculture farms is resulting in suicidal affects due to developing mutagenic multi-antibiotic resistant viral, bacterial and fungal strains that causes boundless non preventive infectious diseases breakdown affecting aquatic organisms primarily - fishes and shrimps resulting in ultimate loss and devastation of farm. In addition to developing antibiotic resistant pathogens in surface water, it accumulates in the tissues of wild fish. According to Chua et al, (1989) if they accumulate in sediments, antibiotics may prevent natural bacterial decomposition and consequently alter the natural benthic environment. Antibiotic resistant bacteria was isolated from shrimp farm (Tendencia and de la Pena, 2001), from different shrimp farms of Viet Nam with 0.1 µg/ml antibiotic concentration (Le et al., 2005), from aquaculture sources (Akinbowale et al., 2006; Spanggaard et al., 1993) and luminous bacteria is also developing resistance to broad-spectrum antibiotics in certain shrimp culture systems of West Bengal (Sengupta et al., 2003).

On account of the above awful and trembling consequences of antibiotics, different countries have already taken up urgent steps regarding its application and removal from the environment imposing legislations and applying advanced degradation technology. Some methods have been developed concerning its degradation by means of biotic and abiotic agents that are retarded and incomplete process which further leading to contamination of environment. In this respect, some absorbents and natural zeolites have a wide variety of applications in the treatment of antibiotics using their unique adsorption and ion-exchange properties. Nowara et al. (1997) proposed that natural zeolite removes fluoroquinolone antibiotics by their high adsorptivity and sorption tendencies. According to Mansilla et al. (2007) and Herrmann (2005), photocatalytic reaction's of TiO<sub>2</sub> converts several classes of organic chemicals into less toxic and biodegradable compounds with the help of UV. Ozone is known to be an efficient oxidant for the degradation and removal of antibacterial agents in an aqueous state (Balcioglu and Otker, 2003; Huber et al., 2003; Ternes et al., 2004), as well as in the treatment of soils contaminated with conventional organic pollutants such as PAHs and PCBs (Nam and Kukor, 2000; Masten and Davies, 1997; Ohlenbusch et al., 1998). By considering these above mentioned facts, the goals of the present study are, to investigate the degradation capacity of widely used trimethoprim and sulphamethoxazole antibiotics employing UV-ray and TiO<sub>2</sub> in water phase and to determine the influence of water flow in its degradation process to reclaim aquatic environment from antibiotics troublesome situation.

# 2. Materials and methods

# 2.1 Synthesis of estuarine water

Low salinity (10 ppt) eschuarine water was prepared mixing sea water and ground water @ 1:3 ratio. According to Mark Flaherty et al. (2000) coastal shrimp farms generally, maintain pond salinity levels between 10 and 30 ppt, and commonly exchange 30 to 40% of the pond water volume each day to offset seepage or evaporation losses and maintain environmental conditions.

# 2.2 Antibiotic and titanium dioxide

Trimethoprim ( $C_{14}H_{18}N_4O_3$ ) (Figure 1) and sulphamethoxazole ( $C_{10}H_{11}N_3O_3S$ ) (Figure 2), bacteriostatic antibiotics and titanium dioxide (TiO<sub>2</sub>) of Sigma-Aldrich Chemical GmbH, Germany was obtained from local authorized agent. Aqueous solutions of antibiotics and TiO<sub>2</sub> were prepared at different concentrations using distilled deionized water.

# 2.3 Preparation of UV-water flow system

We developed an UV-water flow system (UVWFS) using a reservoir made up of stainless steel, ELEPON CR-I pump (Kitashiba Electric Co. Ltd., 50 Hz) having 10 to 100 cc/min water flow capacity and an UV lamp chamber of stainless steel (Aquapro, Model-UV 6GPM-H, ambient temperature 2 - 40°C) placed in a series and connected by pipes through which the water flows (Figure 3). Pump helps to flow the treated water of the reservoir through the UV-chamber and again recycled into the reservoir at a specific rate.

# 2.4 Experimental protocol

Two experiments were conducted in laboratory condition using UVWFC to determine the influence of UV and TiO<sub>2</sub> in the degradation process of antibiotics under different water flow rates. In experiment-I, used eight UWFCs were divided into four groups with two replicates (4x2) – one groups allotted for control (C) applied no UV and remaining three employed for only UV (UV), UV plus TiO<sub>2</sub> @ 0.05g/l (UV+T<sub>0.05</sub>) and UV plus TiO<sub>2</sub> @ 0.1g/l (UV+T<sub>0.1</sub>) treatments, respectively, whereas three groups of UWFCs with two replicates were used for 15, 42, 80 cc/min water flow, respectively maintaining same UV and TiO<sub>2</sub> concentration @ 0.1g/l (herein called as UVT<sub>15</sub>, UVT<sub>42</sub> and UVT<sub>80</sub>,). Reservoir of each UWFC was filled with 5 1 of trimethoprim and sulphamethoxazole dissolved distilled water (temperature 26°C and pH 7.4) and TiO<sub>2</sub> of specified doses were mixed gently to the antibiotic treated water of respected treatments in both experiments. Detailed experimental conditions of experiment-I and II are given in the Table 1.

#### 2.5 Antibiotic analysis

Water samples (100 ml) were collected from each reservoir at regular intervals using sterilized mechanical pipette and stored in neutral glass vial for the analysis of antibiotic concentration according to Samuelsen et al. (1994), Lunestad et al. (1995) and Borrego et al. (1996) with some modifications using high pressure liquid chromatography (HPLC) with Jasco liquid chromatography solvent delivery system (Tokyo, Japan, UV-2075) and Cosmosil packed column 5C18-AR-II (4.6 mm I.D x 250 mm). Sep-pak Plus PS-2 cartridge column for solid phase extraction were purchased from Waters Corporation Milford, Massachusetts USA. Used acetonitrile solvent system was of HPLC grade and obtained from Kanto Kagaku (Tokyo, Japan). Water samples (100 ml) were filtered through a 0.45 mm membrane filter and the water was applied to the sep-pack plus PS-2 solid phase extraction column at a flow rate of 1.0 ml/min and eluted with 1 ml of 60% acetonitrile solution to collect the desired fraction. Eluted 10  $\mu$ l aliquot was injected into the column and HPLC was run with a flow rate of 1.0 ml/min, column temperature 40°C and detection UV-265 nm. The mobile phase to analyze trimethoprim and sulphamethoxazole consisted of acetonitrile/25 mM ammonium acetate (25:75, v/v). Antibiotic content in the water was expressed  $\mu$ g/l comparing with known standard antibiotic concentrations

#### 2.6 Antibiotic degradation efficiency

The rate of reduction of antibiotic concentration in different treatments was calculated as degradation efficiency. The antibiotic degradation efficiency was written as  $\alpha$ .  $\delta$  is designated for antibiotic concentration, then initial and final concentration were represented as  $\delta_i$  and  $\delta_f$ , respectively. t was depicted as time, then final and initial time represented as  $t_f$  and  $t_i$ , respectively. The antibiotic degradation efficiency can also be calculated and expressed as  $\mu g/l/h$  using the following formula:

$$\alpha = \frac{\delta_i - \delta_f}{t_f - t_i}$$

#### 2.7 Statistical analysis

All results obtained from each treatment were statistically interpreted. To compare the mean reduced antibiotic concentration in different treatments, a one-way ANOVA was followed. Before analysis, the assumptions of normal distributions and homogeneity of the variance were checked using Kolmogrov–Smirnov. If the main effect was found to be significant, the ANOVA was followed by a LSD (least significance difference) test. All statistical tests were performed at 5% probability level using statistical package EASE and MSTAT.

#### 3. Results

#### 3.1 Experiment – I

The concentration of trimethoprim varied between 0.0 and 965.7  $\mu$ g/l in different treatments through out the period of experimentation. UV, UV+T<sub>0.05</sub> and UV+T<sub>0.1</sub> pronounced treatment dependent 73, 93 and 100% higher reduction response over the control. The lowest mean concentration 449.08  $\mu$ g/l in UV+T<sub>0.1</sub> showed 16 and 4% lower value than that of the UV and UV+T<sub>0.05</sub>, respectively (Figure 4a). There was a significant treatment difference in the degradation efficiency (2.768 – 26.15  $\mu$ g/l/h) in all treatments exhibiting 6.6 to 9.44 times higher over control (ANOVA, *P* < 0.05) (Figure 6a). Temporally, the concentration of trimethoprim exhibited a declining trend over time and reached zero level at time period 36<sup>th</sup> h in both UV+T<sub>0.05</sub> and UV+T<sub>0.1</sub> where as antibiotic existed in the water of UV treatments at the same time (Figure 4a).

A significant treatment dependence response was observed in the sulphamethoxazole concentration ranged from 0.0 to 887.87  $\mu$ g/l (ANOVA, P < 0.05). The mean concentration was minimum in UV+T<sub>0.1</sub>(168.58  $\mu$ g/l) and maximum in C (783.62  $\mu$ g/l) (Figure 4b). Though, no remarkable difference was found in the degradation efficiency (4.46 - 70.46

 $\mu g/l/h$ ) between UV (17.83  $\mu g/l/h$ ) and UV+T<sub>0.05</sub> (18.15  $\mu g/l/h$ ) but UV+T<sub>0.1</sub> exhibited significantly greater value (288 - 1500%) over the treatments and control (Figure 6a). As time progressed UV and UV+T<sub>0.05</sub> also showed a similar response in the sulphamethoxazole concentration reduction whereas a sharp concentration declining trend was found in case of UV+T<sub>0.1</sub> during the study period (Figure 4b).

Irrespective of treatment, the concentration reduction of sulphamethoxazole was more rapid compared to that of the trimethoprim in each respective treatment.

# 3.2 Experiment - II

The trimethoprim content of water varied (0.0–1020.8  $\mu$ g/l) significantly (ANOVA; P < 0.05) in all the treatments employed. The mean value was maximum (549.35  $\mu$ g/l) in the UVT<sub>15</sub> exhibiting the following order of variations: UVT<sub>15</sub> > UVT<sub>42</sub> > UVT<sub>80</sub> (Figure 5a). The degradation efficiency of trimethoprim in UVT<sub>80</sub> was 113 and 61% higher compared to that of UVT<sub>15</sub> and UVT<sub>42</sub>, respectively (Figure 6b). The concentration gradually decreased with time and reached at zero concentration level at the period of 36<sup>th</sup> and 48<sup>th</sup> h in UVT<sub>80</sub> and UVT<sub>42</sub>, respectively, whereas UVT<sub>15</sub> showed a substantial concentration until 48<sup>th</sup> h (Figure 5a).

The concentration of sulphamethoxazole registered a significant treatment dependent response throughout the period of study (ANOVA, p > 0.05). The mean concentration 168.03 µg/l in UVT<sub>80</sub> was lower (68 - 150%) compared to that of UVT<sub>15</sub> and UVT<sub>42</sub> (Figure 5b). A marked difference in degradation efficiency was found in three treatments showing the following order of variation: UVT<sub>15</sub> > UVT<sub>42</sub> > UVT<sub>80</sub> (Figure 6b). Though temporal responses were similar but a sharp declining trend in the concentration of sulphamethoxazole was encountered unlike trimethoprim in all treatments and obtained zero concentration at 36<sup>th</sup> and 48<sup>th</sup> h by UVT<sub>80</sub> and UVT<sub>42</sub>, respectively (Figure 5b).

Likewise, the concentration of sulphamethoxazole also showed a similar rapid reduction response compared to trimethoprim in three respective treatment in experiment - I.

# 4. Discussion

All findings obtained from the present study clearly demonstrated that UV,  $TiO_2$  and water flow rate played a key operating factors in the degradation pathway of antibiotics, since final trimethoprim and sulphamethoxazole concentrations of water varied from 0.0 to 33.93 µg/l and 0.0 to 22.26 µg/l in experiment – I and II, respectively, whereas initial concentration of trimethoprim (9.1104 – 1020.8) and sulphamethoxazole (845.6 – 887.87) was available in all treatments of both experiments. Inspite of that, antibiotic degradation efficiency was 6.6, 9.28 and 26.15 times in trimethoprim and 4.05, 4.2 and 1601 times in sulphamethoxazole greater values over control in experiment –I, which further strongly proved the effects of UV and TiO<sub>2</sub> on antibiotic degradation (Figure 6a).

Trimethoprim and sulphamethoxazole pronounced 44 to 45 % and 6 to 314% elevated rate of reduction in UV+T<sub>0.05</sub> and UV+T<sub>0.1</sub> over the only UV which indicating to draw a substantial conclusion that though both UV and TiO<sub>2</sub> have a crucial impact but the synergistic effects of the UV and TiO<sub>2</sub> attributes a rapid and higher degree of action on the degradation pathway of antibiotics compared to that of the only UV at the same water flow rate in experiment - I. Heterogeneous photocatalysis using TiO<sub>2</sub> is a procedure to promote the degradation of organic contaminants present in the aquatic environment (Pichat, 1994; Herrmann, 2005; Hoffmann et al., 1995; Mansilla et al., 2007). In trimethoprim, 34.8, 16.62 and 5.60 µg/l concentrations observed in UV, UV+T<sub>0.05</sub> and UV+T<sub>0.1</sub>, respectively at 36<sup>th</sup> h but complete degradation was occurred in UV+T<sub>0.05</sub> and UV+T<sub>0.1</sub> at 48<sup>th</sup> h, where as in sulphamethoxazole, the UV+T<sub>0.1</sub> attained complete degradation (i.e., no detection limit of concentration) at 24<sup>th</sup> h when 20.28 and 5.24 µg/l concentration existed in the UV, UV+T<sub>0.05</sub>. From this result it may be concluded that sulphamethoxazole degrade rapidly than trimethoprim under combined effect of UV and TiO<sub>2</sub> using UVWFM when time is concern. In this respect it may also be observed that influence of the dose of TiO<sub>2</sub> was very little in trimethoprim degradation but a significant effects was revealed in sulphamethoxazole implying that same number of TiO<sub>2</sub> molecules acted more favourably on higher number of sulphamethoxazole molecules than trimethoprim by photocatalytic activity which enhancing the rate of degradation reaction resulting in the rapid decrease of sulphamethoxazole concentration level in water.

In experiment – II, the degradation efficiency in UVT<sub>80</sub> was 113 and 61% higher in trimethoprim and 103 and 51% higher in sulphamethoxazole compared to that of UVT<sub>15</sub> and UVT<sub>42</sub>, respectively which obviously indicated that the rate of water flow is a paramount important, largely influencing the catalytic reaction process of UV and TiO<sub>2</sub> with antibiotics in water medium possibly enhancing movement velocity of their molecules (Figure 6b). In consideration of degradation period, we found that when complete degradation (i.e., no detection limit of concentration) occurred at 36<sup>th</sup> and 48<sup>th</sup> h in UVT<sub>80</sub> and UVT<sub>42</sub>, respectively in trimethoprim, only UVT<sub>80</sub> reached in complete degradation level at 48<sup>th</sup> h in sulphamethoxazole. Considering this result, it might be inferred that higher water flow substantially increases the rate of oxidation and photocatalytic transformation of trimethoprim over sulphamethoxazole with the help of TiO<sub>2</sub> and UV. Recently, advanced oxidation processes have been successfully employed on different antibiotic structures looking to transform pharmaceuticals into harmless substances (Reyes et al., 2006; Mansilla et al., 2007).

# 5. Conclusion and recommendations

Chemical aspects of photocatalytic reaction of  $TiO_2$  in the presence of UV was not considered in the present study for that reasons further study is going on to determine the stoichiometry of trimethoprim and sulphamethoxazole degradation reaction as well as their end products in aqueous phase. On account of these above discussion derived from the present study affords us to draw the following conclusions and suggestions:

1)  $TiO_2$  acts upon the antibiotics as a photocatalyst in the presence of UV and helps to degrade trimethoprim and sulphamethoxazole into simpler form that may be hydrocarbon of low molecular weight having no/less harmful impacts in the environment.

2) Synergistic and photocatalytic transformation effects of the  $TiO_2$  and UV are higher than that of the UV only in the antibiotic degradation pathway.

3) Sulphamethoxazole degrades rapidly than trimethoprim under combined effect of UV and  $TiO_2$  whereas; the effect of water flow is vice-versa in the degradation of trimethoprim and sulphamethoxazole.

4) Model of UV-water flow system may be a simple and low cost convenient tool can easily be used by the shrimp farmers as well as fish farmers not only in the antibiotic degradation process but also in other organic pollutants transformation to recover the ponds from suicidal effects of antibiotic troublesome situation. Under appropriate conditions mineralization of wide variety of compounds like chlorinated phenols, dyes, polyaromatic hydrocarbons and industrial effluents can be using  $TiO_2$  and UV (Vautier et al., 2001; Yeber et al., 2000).

5) After each production cycle, farmers should treat water with UV and  $TiO_2$  applying such model tools to save their precious and valuable shrimp crop and at the same time to improve the socioeconomic status by increasing profit instead of loss generally occurred by developing multi antibiotic resistant bacterial disease breakdown.

6) In environmental perspective, this UV-water flow treatment system imposes to the farmers to recycle the antibiotic contaminated water instead of drainage out in one hand and environment will be save from antibiotic pollutions and from rapidly development of antibiotic resistant bacterial strains on the other hand.

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Conditions		Treatments			
Experiment-I					
		С	UV	UV+T <sub>0.05</sub>	UV+T <sub>0.1</sub>
	Water volume (1)	5	5	5	5
	Water flow rate (cc/min)	42	42	42	42
	Concentration of	944.75	944.75	944.75	944.75
	Trimethoprim (µg/l)	$\pm 11.75$	$\pm 11.75$	$\pm 11.75$	$\pm 11.75$
	Concentration of Sulphamethoxazole	867.01	867.01	867.01	867.01
	(µg/l)	$\pm 17.76$	$\pm 17.76$	$\pm 17.76$	$\pm 17.76$
	Concentration of TiO <sub>2</sub> (g/l)	0	0	0.05	0.1
	UV (W)	0	20	20	20
Experiment-I					
			UVT <sub>15</sub>	UVT <sub>42</sub>	UVT <sub>80</sub>
	Water volume (l)		5	5	5
	Water flow rate (cc/min)		15	42	80
	Concentration of		984.03	984.03	984.03
	Trimethoprim (µg/l)		$\pm 21.27$	$\pm 21.27$	$\pm 21.27$
	Concentration of Sulphamethoxazole		877.37	877.37	877.37
	(µg/l)		$\pm 3.15$	$\pm 3.15$	$\pm 3.15$
	Concentration of TiO <sub>2</sub> (g/l)		0.1	0.1	0.1
	UV (W)		20	20	20

Table 1. Experimental conditions followed in the study



Figure 1. Chemical structure of trimethoprim



Figure 2. Chemical structure of sulphamethoxazole.



Figure 3. Schematic diagram of UV-water flow system (UVWFS) used in the experiments. 1. Reservoir; 2. Pump; 3. UV chamber. (Arrow indicates water flow direction).



Figure 4. Temporal responses of antibiotic concentration (a) trimethoprim and (b) sulphamethoxazole in different treatments employed in experiment -I. Inset shows the mean concentration decrease.



Figure 5. Temporal responses of antibiotic concentration (a) trimethoprim and (b) sulphamethoxazole in different treatments employed in experiment -II. Inset shows the mean concentration decrease.



Figure 6. Antibiotic degradation efficiency of different treatments employed in experiment-I (a) and in experiment-II (b). Same script on the same bar types of different treatments revealed lack of significant difference. Inset shows the trend lines of antibiotic degradation in different treatments (X axis indicates times and Y axis indicates antibiotic degradation efficiency).