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Efficiency and Mechanism of 17α - Methyltestosterone Degradation by UVC

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***Abstract*—17α–Methyltestosterone or MT is a synthetic androgenic steroid hormone. It is commonly used in Nile tilapia farming in order to induce the gender of all tilapia fry to be male. MT is also an endocrine disruptor (EDCs) that can disturb the function of the reproductive system of human and animals. Therefore, to prevent the contamination of MT in the environment, wastewater containing MT has to be treated prior to be discharged. In this study, synthetic wastewater containing MT at the initial concentrations of 100, 500 and 1000 ppb was treated by irradiation with UVC lamp at the power of 20 watts. The concentration of MT was monitored using HPLC. For 100 ppb, it was found that MT concentration reduced to less than 5 ppb within 15 minutes of UVC irradiation. The degradation of MT is found to be first order reaction. The degradation rate constant of MT was slightly decreased with the increase of the initial concentration. Degradation mechanism was investigated by adding Tert-butyl alcohol. From the experimental results, the main mechanism of MT degradation by UVC irradiation was direct photolysis.**

***Keywords*—17α–Methyltestosterone, Photolysis, Tert-butyl alcohol (TBA) and Ultraviolet Radiation**

I. Introduction

In Nile tilapia farming, all fry are changed to be male fish because it has higher growth rate and production yield by treating tilapia fry after hatched with 17α – Methyltestosterone (MT) [1]. MT, a synthetic androgenic steroid hormone is mixed with fry feedat the concentration of 60 mg of MT per one kilogram of feed. The tilapia fry will be fed excessively with feed containing MT for 21 days. After the fry are treated with MT, uneaten feed containing MT remains in the water of the fish pond. When the fish is moved to another pond and the fish pond is cleaned, dischraged water containing MT is released to the environment. This wastewater may contaminate the surrounded environment and cause effects on creatures in that area. MT has endocrine disrupting property to human and animals. MT was reported to have an effect on vitellogenin synthesis and steroid levels of the reproductive system of male zebrafish at very low concentration (4.5 ng/L) in short term [2] and effect on egg-laying rate of female Japanese quail [3]. MT was also reported to have a chronicaleffect on hepatotoxicity and fetotoxicity of human[4]. Hence, it is necessary to remove MT from the wastewater prior to discharge in order to protect natural water resources and creatures by effective methods.

Nowadays, Advanced Oxidation Process (AOPs) is proven to be effective technology for wastewater treatment. Some examples of AOPs are Fenton's reagent, ultrasound, electron beam irradiation and UV. Oxidation processes were used for MT degradation such as fenton process [5], sunlight and UVC. There was a report that MT was degraded about 48 – 62 % when exposed to sunlight and was completely degraded by UVC irradiation [6]. Another research also reported that the degradation of MT by UVC was much more effective than UVA and UVB at the same power level [7]. There are two possible degradation mechanisms when applying UVC, photolysis and oxidation. For photolysis, contaminants were degraded by UV light absorption at their corresponding wavelengths. For oxidation, contaminants were degraded by oxidation of radicals especially hydroxyl radical ($OH^{\bullet}$). Oxidation mechanism may be investigated by adding a radical scavenger such as Tert-butyl alcohol (TBA) [8].

The objective of this research was to study degradation of MT by UVC and kinetic of degradation including major mechanism that occurs during degradation.

II. Materials and Methods

A. *Reagents and Chemicals*

17α – Methyltestosterone (MT) (>97%) was purchased from Aldemex limited. Physical and chemical properties of MT were presented in TABLE I. Tert-butyl alcohol (TBA) was AR grade and was purchased from Panreac. Acetonitrile, etylacetate and methanol were purchased from ACI Labscan and were HPLC grade.

MT stock solution was prepared in methanol. Synthetic wastewater was prepared by diluted MT stock solution with de-ionized water to make up the MT initial concentration of 100, 500 and 1000 ppb in water.

B. *Experimental Set-up*

All experiments were done in a closed rectangular reactor equipped with a 20-watt UVC lamp at the cover. Dimension of reactor is 30 cm. x 70 cm. x 25 cm. (Figure 1). Ten liters of synthetic wastewater was added into the reactor then the UV

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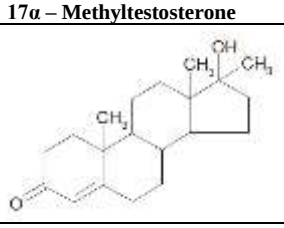
lamp was turned on. Samples were collected at 0, 3, 6, 9, 12 and 15 minutes after irradiation.

To inhabit the oxidation mechanism, 0.5 mM of Tert-butyl alcohol (TBA) was added as a radical scavenger at prior to turn on the UV lamp.

TABLE I. PROPERTIES OF 17A – METHYLTESTOSTERONE

Property	17α – Methyltestosterone
Structure	
Formula	$C_{20}H_{30}O_2$
Molecular Weight	302.45
CAS. No.	58 – 18 – 4
Appearance	white to off-white crystalline powder
Melting point	162-168 °C
Solubility	soluble in methanol, ethanol, ether and other organic solvents
Water solubility	3.39 mg/lat 25 °C
Vapor pressure	1.85 x 10^{-8} mmHg at 25 °C
log Kow	3.36

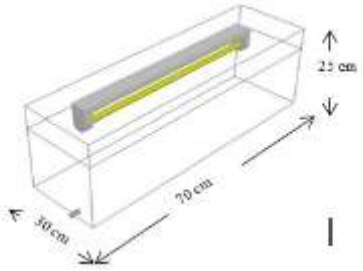


Figure 1. Schematic representation of the UV reactor

C. Sample Preparation and Analysis

MT concentration was analyzed by using HPLC technique with solid phase extraction. For the solid phase extraction (SPE), PEP cartridges were selected and were conditioned by rinsing with acetonitrile (5 ml), ethylacetate (5 ml) and milli-Q water (5 ml), respectively. 20 ml of a sample was loaded through the conditioned cartridge. Then, the cartridge was washed by 5 ml milli-Q water and eluted with 5 ml of methanol. Finally, the sample was purged with nitrogen gas until the remained volume was 1 ml.

All samples were filtered through a 0.45 µm filter to remove suspended particle matters.

MT concentrations were measured by HPLC (Agilent 1100 series) equipped with UV detector at the wavelength of 245 nm. Reversed-phase column was C8 (150 mm x 4.6 mm x 5 µm) at 40 ± 0.5°C.

Mobile phase was operated in gradient mode. At the beginning the ratio of acetonitrile to water was 20:80, then increased the ratio to be 96:4 at 19 minutes and reduced to 20:80 at 20 minutes and kept constant until 30 minutes. The flow rate of the mobile phase was 0.5 ml/min.

Detection limit of MT concentration was 5 ppb.

III. Results and Discussions

A. Degradation of MT by UVC Irradiation

MT at the initial concentration of 100 ppb was degraded to be lower than 5 ppb within 15 minutes after UVC irradiation shown in Figure 2. When the initial concentration was increased to be 500 and 1000 ppb, percentage of MT reduction slightly changed. Percentage of MT reduction after 15 minutes of UVC irradiation was in the range of 95 – 96% shown in TABLE II.

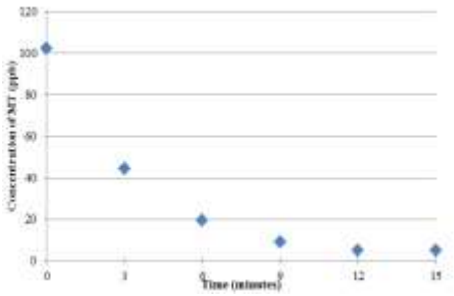


Figure 2. Degradation of MT at initial concentration 100 ppb

B. Kinetic of MT degradation

When plotted ln (C/C_0) versus time, the curve was linear as shown in Figure 3 to Figure 5. This indicated that the degradation of MT followed first order kinetic. This is similar to the degradation of quinestrol by UVC irradiation that was also reported to be first order reaction [9]. At the initial concentrations of MT 100, 500 and 1000 ppb, the degradation rate constants were 0.25, 0.22 and 0.21 min^{-1} respectively at R^2 more than 0.98 as shown in TABLE III

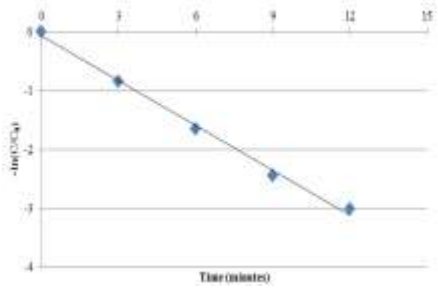


Figure 3. Kinetic of MT degradation at initial concentration 100 ppb

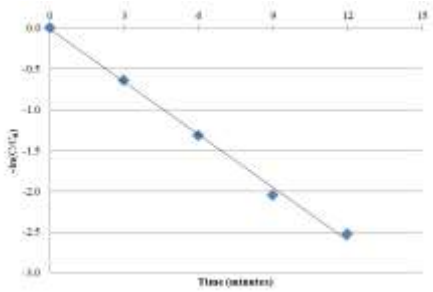


Figure 4. Kinetic of MT degradation at initial concentration 500 ppb

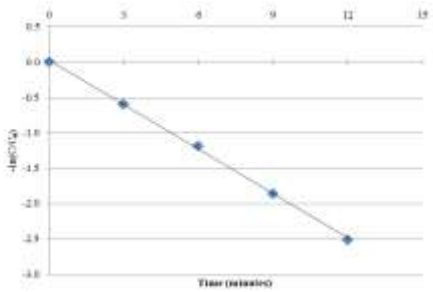


Figure 5. Kinetic of MT degradation at initial concentration 1000 ppb

C. *Mechanism of MT Degradation*

TBA was added as a radical scavenger. If the oxidation mechanism played a significant role of MT degradation, the addition of a radical scavenger would result in the reduction of degradation efficiency. However, it was found that percentage of MT reduction and degradation rate constants changed slightly. Percentage of reduction was 95 – 97% as shown in TABLE II and degradation rate constants (k) were 0.26, 0.24 and 0.22 min^{-1} at initial concentration of MT 100, 500 and 1000 ppb, respectively as shown in TABLE III. It could be concluded that during the MT degradation by UVC irradiation, oxidation reaction might not occur. Therefore, the main mechanism of MT degradation in this study should be photolysis.

TABLE II. PERCENTAGE OF MT REDUCTION

Time	% Reduction					
	MT 100 ppb	***MT 500 ppb***	***MT 1000 ppb***	***MT 100 ppb+TBA***	***MT 500 ppb+TBA***	***MT 1000 ppb+TBA***
0	0	0	0	0	0	0
3	58.75	47.37	45.09	56.46	51.26	45.12
6	82.36	73.16	69.39	81.34	75.26	69.57
9	91.90	87.11	84.45	93.00	88.71	85.38
12	95.23	92.02	91.90	95.05	94.42	92.46
15	95.23	96.07	95.30	95.05	97.05	95.97

TABLE III. DEGRADATION RATE CONSTANT OF MT

Conditions	k (min^{-1})	R^2
100	0.25	0.9954
500	0.22	0.9964
1000	0.21	0.9991
100 +TBA	0.26	0.9834
500+TBA	0.24	0.9996
1000+TBA	0.22	0.9982

IV. Conclusion

MT at the initial concentration of 100 ppb was degraded to be lower than 5 ppb within 15 minutes of UVC irradiation. Increase of MT initial concentrations was not much effect on degradation. Kinetic of the degradation followed the first order reaction with the degradation rate constants of 0.25, 0.22 and 0.21 min^{-1} at the initial concentration of MT 100, 500 and 1000 ppb, respectively ($R^2 > 0.98$). By the addition of TBA, it was found that the major mechanism of MT degradation was photolysis.

V. Acknowledgment

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UVC can degrade androgenic steroid hormone in wastewater more than 95% in short time so it can apply this method in Nile tilapia farming to remove androgenic steroid hormone before release wastewater to environment.