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Removal of 17alpha-methyltestosterone and Its Metabolites by Photo-Fenton Process

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Abstract

17alpha-methytestosterone (MT) has been used as a synthetic androgenic hormone to induce male population in Nile tilapia fry by feeding the MT at 60 mg/kg. The effluent wastewater containing residue MT from masculinizing ponds without any treatment may effect on the endocrine system of exposed human and wildlife. The MT is classified as an endocrine disrupting compound. Thus, this research aims to study the efficiency of MT removal by Photo-Fenton and the effect of initial MT concentration and dissolved organic carbon (DOC) on the removal efficiency. The result showed that the optimal pH, amount of ferric iron, and ratio of Fe^{2+}:H_2O_2 of Photo-Fenton process was 3, 0.003 mM, and 1:100 (mM:mM), respectively. The degradation rate constant decreased with the increasing of the initial MT concentration because of the limitation of hydroxyl radical generation. The DOC could inhibit the MT removal in the first 40 min due to the competition between the existing organic compounds. However, MT did not undergo mineralization as the DOC was not significantly decreased.

Keywords: 17aplha-methyltestosterone: Photo-Fenton: Endocrine disrupting compounds: Masculinization

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1. Introduction

In aquaculture industry, 17alpha-methytestosterone (MT) has been used as the synthetic androgen hormone to induce male sex population in Nile tilapia fry by mixing with fish feed at 60 mg/kg. In Thailand, the effluent wastewater from small aquaculture can be discharged directly into the receiving water without any treatment. The residue MT which is classified as the endocrine disrupting compounds, can pose the risk to human and wildlife exposing to it. The MT can interfere the normal function of sex organ in living organisms as low as nanogram level. There are several reports

FEAT JOURAL
January - June 2018 ; 4(1) : 35 - 41

about the adverse effects of MT; for example, MT at the level higher than 46.8 ng/L could decrease the vitellogenin protein which is generally produced in yolk only by female fish [1]. Moreover, MT reduced the laying rate of female bird when exposed to MT at ppm level for 3 weeks [2]. Additionally, the male sex organ was generated in female fresh water ramshorn snail when exposed to MT at 100-1000 ng/L for 6 months [3]. However, the study of effect of MT on human has been limited.

There are relatively few studies on the treatment of MT. Homklin [4] found that MT can be degraded by microorganisms under both aerobic and anaerobic conditions except under anoxic condition. However, under anaerobic condition, the intermediate by-products of MT had higher endocrine disrupting potential and were more recalcitrant than the parent compound. In the study of Sagulsawasdipan [5], MT could be degraded by photolysis with UV-C (10 w) approximately 88% within 30 min. However, there are no study on the degradation of MT under the other advanced oxidation processes. Therefore, this research aims to study the optimal condition for MT removal by Photo-Fenton process and to study the effect of initial MT concentration and dissolved organic carbon (DOC) on the removal efficiency of MT and its metabolites by Photo-Fenton process.

2. Methodology

2.1 Chemicals

MT (≥97%, HPLC, Sigma Aldrich, USA) was prepared in methanol at 1000 mg/L as a stock solution. The initial concentration of MT in this study was at 3 mg/L due to the maximum water solubility of MT. H_2SO_4 (98%, AR, Labscan, Thailand) and NaOH (98%, AR, Labscan, Thailand) were used to adjust the pH of synthetic wastewater. Ferric chloride ($FeCl_3$) (>99%, ACS, Acros organic, USA) and 37% H_2O_2 (AR, Fisher scientific, USA) were used as the main chemical reagents in Fenton and Photo-Fenton treatment processes. Na_2SO_3 (98%, ACS, Acros organic, USA) was prepared at the concentration of 0.40 mM, which was used as a reaction-stopping solution.

2.2 Reactor and the experiments

Reactor was made from a 2-liter glass cylinder. In the reactor, UV-C lamp was installed as a light source (light intensity around 1.927 mW/cm^2) and covered with quartz tube. The reactor was placed on a magnetic stirrer for complete mixing of the solution. The outer part of the reactor was covered with mirror to increase the light reflection and protect the UV-C exposure.

The experiments were divided into 4 sets. The first set was to study the efficiency of Fenton and Photo-Fenton for MT treatment. The pH of the synthetic wastewater was adjusted to 3 with the addition of ferrous iron and H_2O_2 at 0.006 mM and 0.6 mM, respectively. UV-C was added as the light source in the Photo-Fenton. The second set was to study the optimal amounts of pH, ferrous iron and the ratio of Fe^{2+}:H_2O_2 for MT treatment by Photo-Fenton. pH was studied at 2, 3 and 5. The concentrations of ferrous iron was at 0.003, 0.006, 0.009 and 0.012 mM. The ratio of Fe^{2+}:H_2O_2 was studied at 1:100, 1:200, 1:300, 1:400 and 1:500. The third set was to study the effect of initial concentration of MT on treatment efficiency. The initial MT concentrations used in this study were 0.5, 3.0 and 5.0 mg/L. The reaction was done following the optimal condition. The fourth set was to study

the effect of organic compound in each source of water on the treatment efficiency of MT. There were three sources of water including deionized water, river water, and fish farming water. All sources of water were analyzed for the dissolved organic compounds by TOC analyzer. The experiment was set at the optimal condition for Photo-Fenton.

The reaction time was set at 60 min for all experiments. At each sampling time, 1.0 mL of water sample was collected before adding 1.0 mL of methanol for preservation. The water sample was filtrated by syringe filter (Nylon, 0.45 um) prior to the analysis by HPLC.

2.3 Analytical method for MT by HPLC-UV

MT was analyzed by High performance liquid chromatography (HPLC) (Shimadzu, LC-20A, Japan) with C18 column (4.60 mm x 250 mm x 5 um). Mobile phase was Acetonitrile (ACN) and water. The mobile phase was used gradient condition with 50% of ACN at 0 min, then 96% of ACN at 19 min and 50% of ACN at 20 min. Analytical time was 27 min. The detector was Ultraviolet detector at 245 nm. Sample volume was 40 uL.

2.4 Analytical method for DOC by TOC analyzer

Sample was prepared by filtration through GF/C pore size 0.45 um prior to analysis by TOC analyzer. TOC analyzer (Shimadzu, TOC-L, Japan) was set at the temperature of 680 C. Volume of sample was 30 mL.

3. Results and discussion

3.1 Efficiency of MT removal by Fenton and Photo-Fenton

In this research, Fenton and Photo-Fenton are the advanced oxidation processes applied to remove MT. The water sample was a synthetic wastewater containing 3 mg/L of MT, which is close to the maximum water solubility of MT. The condition for Fenton process consisted of pH at 3, Fe^{2+} concentration of 0.006 mM, and ratio of Fe^{2+}:H_2O_2 of 1:100 (mM:mM), respectively. For Photo-Fenton, the light source was UV-C with light intensity about 1.927 mW/cm^2.

The results showed that Fenton and Photo-Fenton could remove 41 and 98% of MT, respectively within 60 min (Figure 1). Photo-Fenton showed higher removal efficiency than Fenton about 3 times because there were the degradation from photolysis and oxidation reaction. Moreover, UV-C can activate the formulation of hydroxyl radical and increase the photo reduction of ferric ion to ferrous ion as described in Equation 1 – 3 [6]. In the same manner of the other studies, it was found that Photo-Fenton can remove ibuprofen [7], p-Nitroaniline [6], formaldehyde [8], Methomyl [9] better than Fenton.

$$Fe^{2+} + H_2O \rightarrow Fe^{3+} + OH^{\bullet} \quad (1)$$

$$Fe^{3+}\ H_2O_2 + UV \rightarrow Fe^{2+} + OH^{-} + OH^{\bullet} \quad (2)$$

$$Fe^{2+} + OH^{-} + UV \rightarrow Fe^{2+} + OH^{\bullet} \quad (3)$$

FEAT JOURAL
January - June 2018 ; 4(1) : 35 - 41

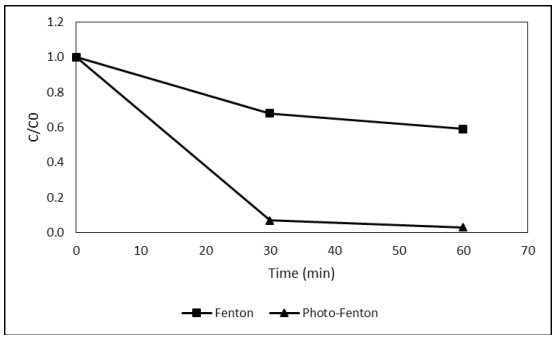

Figure 1: MT removal by Fenton and Photo-Fenton processes

3.2 The optimal condition of Photo-Fenton process for MT removal

pH has effects on the solubility and form of iron. Thus, the optimal pH for MT removal by Photo-Fenton process was studied. Values of pH in this study were 2, 3, and 5. The result showed that MT can be degraded more than 99% at all pH. However, the by-products of MT were observed in the HPLC chromatogram at pH 5 whereas no metabolite peak was presented in HPLC chromatogram at the other pH (data not shown). Moreover, pH >3 can inhibit the oxidation reaction. At pH> 3, ferrous is not stable and changes to ferric iron. Ferric hydroxide ($Fe(OH)_3$) is then formed and obstructed the reaction between ferrous iron and hydrogen peroxide [10]. In addition, high pH value causes the cleavage of hydrogen peroxide into oxygen and water [10]. Thus, pH at 3 was selected as the optimal pH in this study. According to other studies, the optimal pH for Photo-Fenton to eliminate the other compounds such as Methomyl [9], p-Nitraniline [6], Formaldehyde and Methanol [8] was also at 3.

The high amount of ferrous iron increases the efficiency of treatment and also causes the residue of ferric iron. Therefore, the determination of suitable ferrous iron concentration is of concern. In this study, the range of ferrous iron was 0.003 – 0.0012 mM. The result showed that MT can be degraded more than 99% at all ferrous iron concentrations within 60 min of reaction. Therefore, the most suitable condition of ferrous iron was at 0.003 mM due to the lowest usage and to prevent the residue of ferric iron. Moreover, high dose of ferrous iron can react with hydroxyl radical resulting in the decrease of treatment efficiency as shown in Equation 4 [11, 12, 13]. However, ferric iron can react with hydrogen peroxide (Equation 5) to produce the superoxide radical ($OOH^{\bullet}$) that has low oxidizing potential. Additionally, high concentration of ferrous iron is costly for treatment [6].

$$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^{-} \quad (4)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\bullet} + OH^{+} \quad (5)$$

According to the stoichiometry (Equation 6), 3 mg/L or 0.01 mM of MT can react sufficiently with hydrogen peroxide at 0.5 mM. Because the most suitable ferrous concentration was 0.003 mM, the appropriate ratio of Fe^{2+}: H_2O_2 was estimated to be 1:200. Therefore, we decided to apply the rage of Fe^{2+}: H_2O_2 ratio used in this study to be 1:100 - 1:500 (mM:mM). The result showed that degradation of MT decreased when ratio of Fe^{2+}: H_2O_2 increased from 1:100 to 1:500. Increasing hydrogen peroxide can induce the formation of hydroxyl radical, which in turn can react with excess hydrogen peroxide to produce superoxide that has low oxidizing potential as shown in Equation 7 [6, 14, 15, 16]. Moreover, the superoxide can continuously react with the hydroxyl radical and convert to water and oxygen reducing oxidizing power (Equation 8). The hydroxyl radical can be

self combined to form hydrogen peroxide back to the system. (Equation 9).

$$C_{20}H_{30}O_2 + 53H_2O_2 \rightarrow 2CO_2 + 68H_2O \quad (6)$$

$$H_2O_2 + OH^{\bullet} \rightarrow OOH^{\bullet} + H_2O \quad (7)$$

$$OOH^{\bullet} + OH^{\bullet} \rightarrow H_2O + O_2 \quad (8)$$

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \quad (9)$$

In conclusion for this part, the optimal pH, ferric iron concentration, and ratio of Fe^{2+}:H_2O_2 were 3, 0.003 and 1:100, respectively. MT removal efficiency was 100% within 40 min of reaction period. The degradation rate constant was 0.22 min^{-1} following the first order kinetic reaction. The half-life of MT in this process was 3.10 min showing that MT can be degraded more rapidly than biodegradation under aerobic, anaerobic and anoxic, in which MT has half-life about 1.80, 1.30 and 181 days, respectively [4]. The result indicated that Photo-Fenton has high efficiency to remove MT more than the Fenton process and biological processes.

3.3 Effect of initial concentration of MT

In this part, the effect of initial concentration of MT on the removal efficiency was studied. The initial concentrations of MT introduced here were 0.5, 3.0 and 5.0 mg/L. The result showed that MT can be removed more than 99% at all concentrations within 40 min of reaction. However, the degradation of MT obeyed the first order kinetics. The degradation rate constants were 0.35, 0.22, and 0.12 min^{-1} at MT concentration of 0.5, 3.0, and 5.0 mg/L, respectively (Figure 2). The degradation rate constant decreased when the initial concentration of MT increased because of the limitation of hydroxyl radical generation. The hydroxyl radical was stably generated whereas the concentration of MT increased resulting in the decline of degradation rate constant.

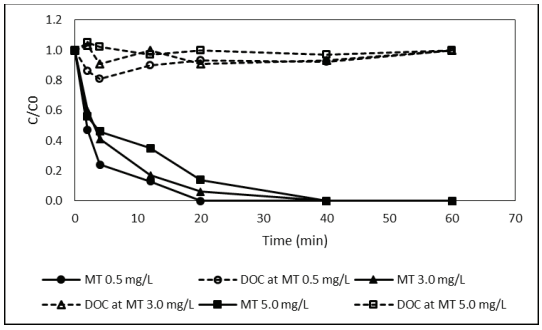

Figure 2: Effect of initial MT concentration on removal efficiency by Photo-Fenton

3.4 Effect of organic compounds

The organic compounds may interfere the removal efficiency of MT by Photo-Fenton process because the hydroxyl radical can react with all organic compounds. To apply in the real site treatment, this process may be encountered with various organic compounds existing in the natural water. Thus, this part aims to study the effect of organic compound in water from various sources such as deionized water, river water and fish farming water on the degradation of MT. Each source of water contained dissolved organic compounds (DOC) about 1.5, 26.0, and 55.3 mg/L, respectively. The result showed that Photo-Fenton can degrade MT up to 100% at all sources of water. The degradation rate followed the first order kinetics. The degradation rate constants were 0.34, 0.23, and 0.22 min^{-1} in the deionized water, river water, and fish farming water, respectively (Figure 3). This result indicated that the DOC can interrupt the MT removal in the first 40 min due to the competition between existing organic compounds

FEAT JOURAL
January - June 2018 ; 4(1) : 35 - 41

and MT. However, MT was not mineralized because the DOC was not significantly decreased (data not shown).

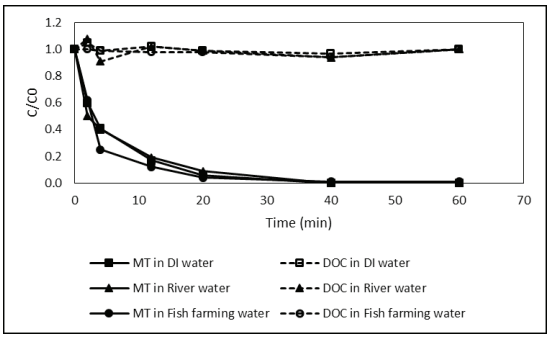

Figure 3: Effect of organic compounds in term of DOC on the MT removal efficiency by Photo-Fenton

3.5 DOC removal

Normally, organic compounds are mineralized to CO_2 and H_2O. In this study, the MT as a parent compound decreased whereas the DOC concentration was insignificantly reduced (Figure 2 and 3). The result indicated that MT was not completely degraded but transformed to other metabolites. As shown in the study of Hu et al. [17], MT was oxidized by $OH^{\bullet}$ at the position of O3 while cleaving the structure of MT together with producing $OH^{\bullet}$ radical. Occasionally, $OH^{\bullet}$ can cleave MT at the position of C5 and C4. The transformation of MT always occurs at the functional group position. However, the main structure of MT is still remained. Thus, MT cannot be mineralized by Photo-Fenton process. Moreover, it was not clear on the endocrine disrupting potential of the metabolites of MT. Homklin [4] found that some biological treatment processes of MT showed that its metabolite had higher disrupting potential than the parent compound.

4. Conclusions

Methyltestosterone (MT) was effectively degraded by Photo-Fenton. The most suitable condition was pH at 3, Fe^{2+} concentration of 0.003 mM, and ratio of Fe^{2+}:H_2O_2 of 1:100, respectively, even though it was not mineralized to CO_2 and H_2O. More details should be studied on the androgenic activity of the intermediate by-products of MT degradation before it can be applied to treat wastewater from masculinizing farm.

5. Acknowledgement

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