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Degradation of Tetracycline in Synthesized Wastewater Using Rotating TiO_2-coated Corrugated Aluminum Drum

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Abstract*—The persistence and abundance of antibiotics in surface waters pose health risks by developing resistance genes in microorganisms, which cannot be treated by conventional processes. One of the most commonly found antibiotics in the wastewater is tetracycline (TC). Studies on advance oxidation processes are being conducted to remove TC from wastewater. This study investigates the adsorption and surface reaction of TC with alumina and titania. Photocatalytic activity was also explored using UV irradiation. The experiments were done by immersing a portion of a rotating corrugated aluminum drum (CAD) to TC solution. Bare and TiO_2-coated CADs were used to expose adhering TC solution to UV lamps above the reactor to evaluate the photon effects. The TC concentration in the solution was measured using spectrophotometry. The degradation byproducts were determined using liquid chromatography-mass spectrometry (LCMS). The antimicrobial assays of the samples were determined by disc diffusion method using *Pseudomonas aeruginosa* as test organism. Results show that TC adsorption on drum surface follows Langmuir isotherm, which implies monolayer adsorption. There is rapid adsorption in the first 10 min, which accounts for about 50% TC removal, followed by slower adsorption and surface reaction. The photocatalytic reaction is effective in sweeping and degrading adhering TC on alumina and titania. The two major TC byproducts in the treatment were m/z 234.95 and m/z 195.00. Disc diffusion assay proves the abrupt decline of antimicrobial potency of TC solution after 10 min and potency loss after 20 min. The LCMS results confirm that degradation resulted to reduction in mass of TC by possible defragmentation and cleaving of TC core structure.

Keywords*—adsorption, aluminum, pseudomonas, tetracycline, photocatalysis

I. INTRODUCTION

Antibiotics are one of the largest groups in the pharmaceutical industry used in humans and animals to treat bacterial infections. Tetracycline (TC) is one of the most commonly used antibiotics to treat urinary tract and intestinal infections. It was reported that the human body absorbs only 60-80% of ingested TC and the animals absorb only 20-50% of TC intake [1]-[2]. The unabsorbed TC is therefore excreted and goes to wastewater. The microorganisms with prolonged exposure to accumulated TC in the aquatic environment can develop antimicrobial resistance. Existing antibiotics may no longer be effective in treating these resistant microorganisms, which can pose greater health risks to humans and animals. It was reported that one child dies every five minutes in Southeast Asia due to resistant microorganism-related infections [3]. Therefore, there is a need to remove and degrade TC in the wastewater before discharge.

For these reasons, studies are conducted to treat TC in wastewater including photolysis, adsorption, biochemical reactions, and advanced oxidation processes (AOPs). The common adsorbents used are activated carbon, zeolite, kaolinite, clay, and aluminum oxide while the most common AOPs are ozonation, electrochemical oxidation and photocatalysis. The AOPs produce highly reactive chemicals such as hydroxyl radicals (•OH) that will degrade the pollutants. In photocatalysis, a semiconductor such as titanium dioxide (TiO_2) or titania is irradiated to create an electron-hole pair that will undergo redox reactions with chemical species present in the solution. The hole (h^+) can either react directly with the pollutant to initiate radical degradation reactions or react with H_2O or ^-OH to produce •OH, which is highly reactive and is effective in the degradation of pollutants.

Fig. 1 shows the tetracycline structure, which is composed of the four-ring core and the functional groups attached to it. The functional groups are electron-rich and can act as a good binding site with metal ions.

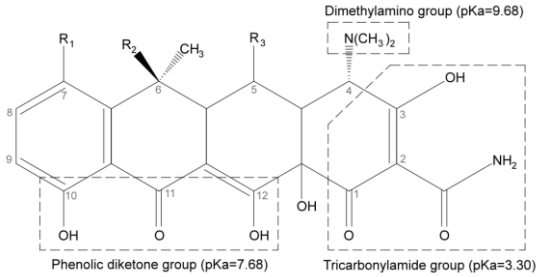

Fig. 1. Chemical structure of tetracycline.

This study was conducted to investigate the adsorption of TC to alumina and titania and to determine the degree of TC degradation by photocatalysis using TiO_2. The aluminum drums serve as both the adsorbent and support for the immobilization of TiO_2 particles. The adsorption of TC on Al and Al-TiO_2 systems, UV photon absorption and photocatalytic effects were determined. The antimicrobial potency tests of the treated tetracycline solutions were also conducted.

II. Methods

A. Materials

Tetracycline (≥98%, Sigma Aldrich) was dissolved in deionized water to prepare the solutions for the calibration curves and samples for the batch experiments. Fig. 2 illustrates the configuration of the reactor setup with the following basic components: aluminum drum with 60-degree angle corrugation, trough, motor drive and the UV lamp. The diameter and length of the drum are 77 mm and 204 mm, respectively. The agar used in the disc diffusion assay was the Difco™ R2A Agar. The bacterium used to measure the potency of the treated solutions was Pseudomonas aeruginosa. The pure culture was grown in Nutrient Broth No. 1 (Fluka® Analytical).

B. TiO_2 Immobilization

The corrugated aluminum drum (CAD) was washed with 20% sulfuric acid. The clean and dried drum was then heated in the oven up to 200°C for 2h, and cooled down to room temperature. It was used in the bare aluminum adsorption experiments. The immobilization of TiO_2 was adopted from a previous study [4]. The aluminum drum was heated to 450°C for 2h after acid wash to remove organic impurities and to produce aluminum oxide film, which allows better bonding with TiO_2 particles. Aeroxide® P25 TiO_2 catalyst was suspended in water (180g/L) and was painted on the surface of the drums. After air-drying, the coated drums were baked in the oven at 200°C for 4h. After cooling, these drums were used in the Al-TiO_2 experiments.

C. Batch Reaction

During batch operations, corrugated aluminum drum (CAD) was partially immersed in the trough that contained 160mL TC solution (Fig. 2). The motor was used to rotate the drums along the shaft. The two 40W UV lamps (Philips, TLK 40W/10R R-UVA) were used to irradiate the top portion of the drums with adhering TC solutions.

The calibration curve was established using UV-Vis spectrophotometer (Hewlett Packard, HP 8452). This was used to measure the absorbance of the solutions at 360nm. The calibration curve was obtained using varying aqueous TC concentrations of 0.5, 1.0, 5.0, 10, 30, 50, 60, 80, 100, and 120 ppm. The adsorption isotherm was determined by running the setup using the smooth-surfaced aluminum drum with different TC solution concentrations of 10, 20, 40, 60, 80 and 100 ppm. All the batch runs were measured every 20 min until saturation or equilibrium was achieved. Initial and equilibrium concentrations were, then, determined and the difference was the TC removed from the solution. Values were analyzed to determine the effects of adsorption, photolysis and surface reactions.

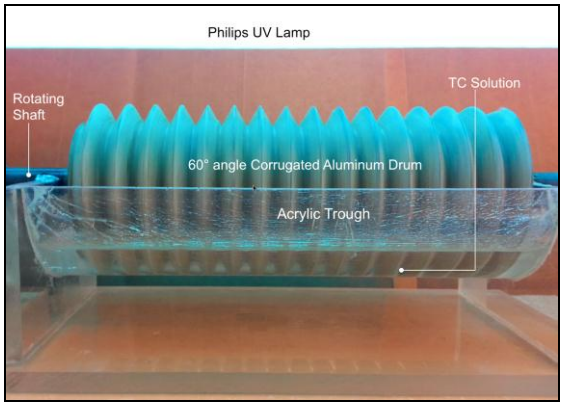

Fig. 2. Rotating corrugated drum reactor setup.

D. LCMS Analysis

An LCMS method was developed to detect tetracycline and its degradation byproducts. The equipment used was Shimadzu LCMS-8030. The chromatography conditions used in the analyses were as follows: C18 50mm L x 2.1mm ID x 1.9µm particle size column, dual ionization source (DUIS) ionization mode, 50-50 split of methanol as organic phase and 5mM oxalic acid solution as aqueous phase flowing at 0.2 mL/min carrying 2 µL of injected sample.

E. Antimicrobial Potency Test

A 200-mL nutrient broth was prepared in a 500-mL Erlenmeyer flask where a loop of cultured *Pseudomonas aeruginosa* was added to obtain an approximately 0.05 optical density at 600 nm (OD600). The culture was then grown aerobically for 24 h at 37°C with rotary shaking at 300 rpm.

About 20 µL of *Pseudomonas aeruginosa* bacteria from the culture flask was spread evenly on the surface of the agar. This is to allow uniform growth of a lawn of bacteria on the surface of the agar. Then, filter discs of about 7 mm in diameter were used as reservoir of samples for the test. Each

disc absorbed approximately 30 µL of TC solutions that were taken from the reactor trough at times 0, 10, 20, 40, 60, and 120 min. The discs were then placed on top of the agar covered with a layer of bacteria spread. After putting all the discs, the plates were incubated at 35°C for 24 h. The bacteria grew on the agar surface except at the area containing potent tetracycline, which is referred to as the inhibition zone (IZ). The diameter of these zones was measured and compared among all the samples. This procedure was also performed to test different freshly prepared TC solutions with the following concentration: 0, 5, 10, 13, 15, 20, 25 ppm and 80 ppm as a control. A relationship between the TC concentration and the IZ diameter was correlated.

III. Results and Discussion

A. Adsorption Isotherm

The relationship between TC concentration (C_{TC}) and the absorbance readings (ABS) in the spectrophotometer at 360 nm is linear with the following equation ABS=0.031(C_{TC}), R^2=0.99438.

Fig. 3 shows the initial TC concentration versus equilibrium TC loading (W_e) curve using a smooth aluminum surface. W_e is defined as the TC adsorbed on the entire drum surface. The figure implies that the available surface area of the drum has not been saturated until 80ppm initial TC concentration. It has been found that the data fitted the linearized Langmuir isotherm with the following equation:

$$1/W_e = 777.46\ (C_{TC}) + 4.6794,\ R^2 = 0.9922. \quad (1)$$

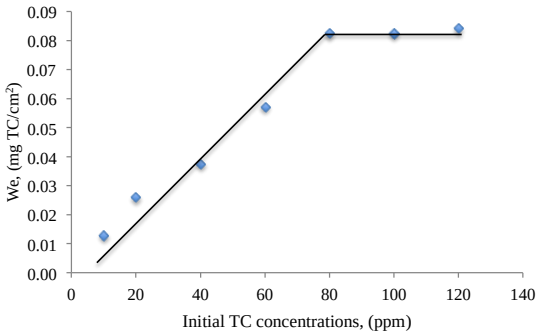

Fig. 3. Adsorption of tetracycline on the aluminum drum.

Adsorption isotherm implies that TC forms a monolayer of adsorbed TC molecules on the surface of the aluminum drum. During acid wash in the preparation of the drum, the reaction might have formed $Al_2(SO_4)_3$. In the batch adsorption experiment, where the drum surface was submerged in the aqueous TC solution, dissociation of $Al_2(SO_4)_3$ resulted to the availability of Al^{3+} sites for the attachment of electron-rich moieties of TC.

B. Batch Adsorption and Tetracycline Removal

The spectra of initial and treated solutions are shown in Fig. 4. It can be seen that the initial spectrum of tetracycline has two characteristic wavelengths or absorbance bands (ca. 270 and 360 nm). The initial TC solution concentration used was about 42 ppm. The drum rotation was maintained at 3 rpm. The solution underwent adsorption on aluminum drum surface, adsorption on TiO_2-coated aluminum drum surface, and reaction on TiO_2-coated aluminum drum exposed to UV irradiation. It can be seen that as TC undergoes different reactions, the UV-Vis spectra also changes. During adsorption of TC to aluminum surface, a bathochromic shift from 360 nm to ca. 376 nm on the second absorbance band was observed, while the first band slightly shifted downwards. The notable bathochromic shifting may imply a possible oxidation of TC core, increasing the its mass. The sudden deepening of the "valley" between the absorbance bands may have resulted from the consumption or detachment of moiety/chromophore responsible for the ca. 314nm wavelength. Consumption of TC in the solution was observed after 600 min of operation, as seen in the lowering of the peaks of TC, proving that TC was degraded to its products. Al^{3+} can be used as a Lewis acid center and may catalyze oxidation, dehydration and isomerization reactions [5]. Al^{3+} is also capable of complexation with O-containing functional groups of an aromatic compound, making it susceptible to oxidation and possible ring opening [6].

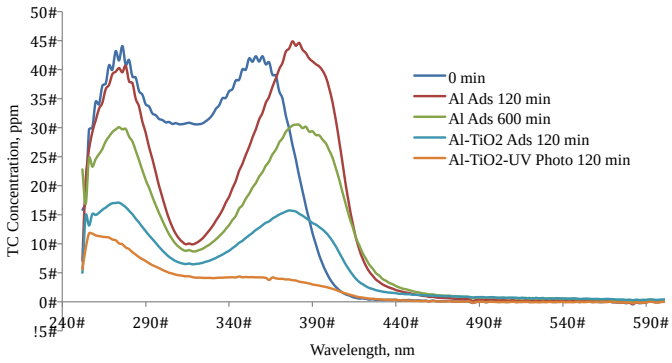

Fig. 4. Spectra of tetracycline solutions after treatments.

Meanwhile, the consumption of TC in the solution was rapid in the presence of TiO_2. The bathochromic effect of aluminum surface was still evident as the second band shifted toward the longer wavelength but the removal was faster at about 68% removal at 360 nm. The carboxyl group can attach and transfer its charge with the oxide surface of TiO_2 [7]. It was discussed how the Ti^{4+} ion behaves as Lewis acid and the amine group as the Lewis base upon adsorption and formation of N-Ti bond. Thus, the apparent number of adsorption sites in

the TiO_2-coated drum is more than that of the bare aluminum drum.

With UV irradiation on TiO_2-coated drum, the second absorption band was retained at about 366 nm through the time of operation until 120 min. Thus the band shifting effect of the aluminum was less due to greater effects of sorption and surface reaction of TC to TiO_2. The TC consumption after 120 min was 90%. Different from other treatments, the absorbance bands did not shift downward proportionally. This suggests that TC was directly attacked and degraded by additional reactive species such as the hole (h^+) on the TiO_2 surface or the hydroxyl radicals that were produced during photocatalysis. It can be assumed that the TC core was degraded and produced byproducts that have at least one ring that gave a characteristic wavelength of about 260 nm. The absorption peak of benzene is 255 nm [8].

Fig. 5 shows the TC concentrations of treated samples through time. The curves suggest that there is a rapid removal of TC from the solution within 10 min of operation and followed by decreasing removal rates towards the end.

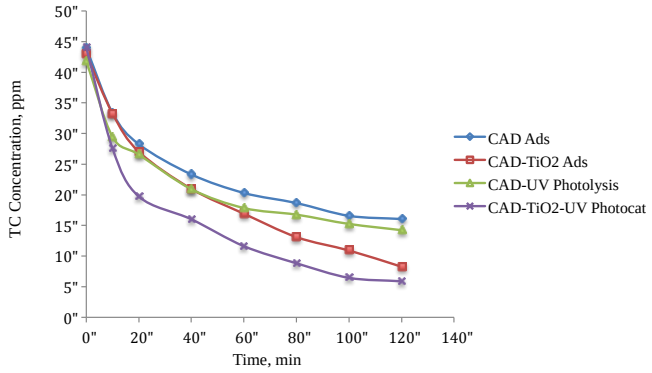

Fig. 5. TC concentration through batch operation time.

With adsorption alone, the drum surface was almost saturated and the TC concentration in the solution begins to become constant. In the presence of UV, a decline in the TC concentration was observed, which might be due to the positive effect of UV irradiation in desorbing attached oxidized TC to produce more vacant sites for surface degradation reactions. The presence of TiO_2 further increased the TC removal in the solution. This indicates that there were more sites for sorption and surface reaction at a given time. The figure also shows that in CAD-TiO_2-UV photocatalysis performed with the fastest rate of degradation in the first 20 min. The figure suggests that photocatalysis creates more vacant sites on TiO_2. This was possibly achieved by initiating radical reactions in photocatalysis, which were faster than the TC-Ti^{4+} catalytic reaction alone.

An optimization of the operation was performed and is presented in another paper. The reactor was operated at 17.66rpm drum speed using corrugated aluminum drum exposed to 0.08 mW/cm^2 to degrade 160 mL of 40ppm initial TC concentration. The treatment resulted to 94.21% TC removal.

C. LCMS Analysis

A product ion scan of TC was performed and the mass-to-charge (m/z) values of the detected ions are 410 and 154. Precursor scans of the treated samples reveal two major byproducts: m/z 234.95 and 195.00. Ion monitoring of TC and its byproducts shows that the TC concentration decreases and the relative abundance of the byproducts increases through operation time.

D. Disc Diffusion Assay

A calibration curve was established to relate the TC concentration and the inhibition zone (IZ) created by the solution against *Pseudomonas aeruginosa*. Fig. 6 shows the discs and their corresponding IZ. It can be seen that the IZ increases with increasing TC concentration. This calibration proposes that the solution will be potent until no TC is present in the solution. The 5-ppm solution is still potent to the bacteria. Fig. 7 illustrates the linear relationship between the TC concentration and the diameter of the inhibition zones up to 25 ppm TC solution.

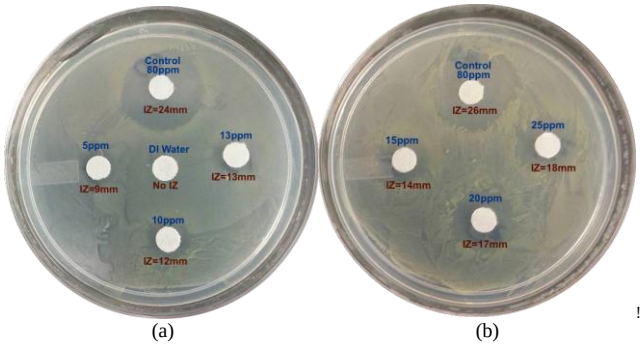

Fig. 6. Disc diffusion plates for the calibration procedure: (a) 0, 5, 10, 13, and 80 ppm; (b) 15, 20, 25 and 80 ppm.

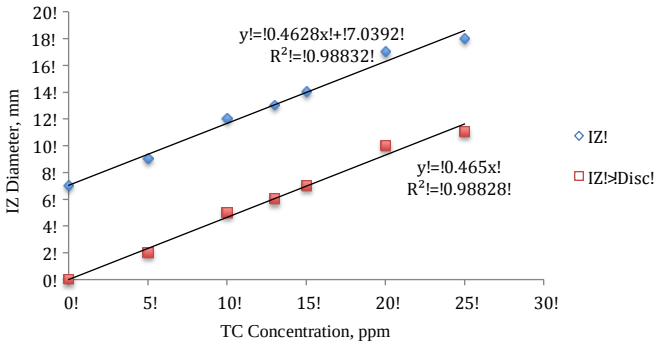

Fig. 7. Calibration curve of TC concentration versus IZ diameter.

The optimized condition was used in this assay. Fig. 8 shows the IZ on the disc diffusion plates of the samples. The

sample taken after 10 min of reaction shows an inhibition zone of 9 mm diameter, creating a 1 mm clearance from the disc. This proves that the sample still possessed antimicrobial activity. The sample was measured and found to contain 13.30 ppm of TC and the LCMS analysis found that the sample contains TC degradation byproducts. This concentration was still potent but after 20 min, the TC concentration was measured to be 11.23 ppm and the sample was considered not potent to the bacteria.

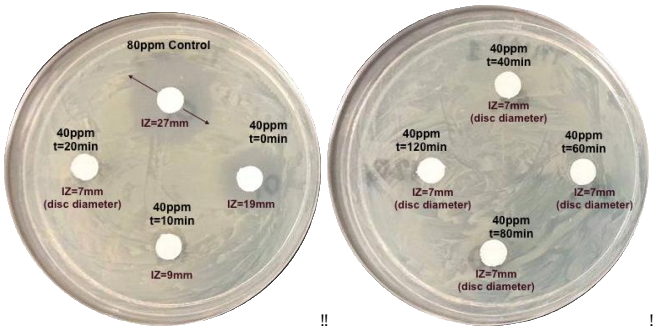

Fig. 8. Disc diffusion test plates using the optimized condition: (a) 0, 10 and 20 min and 80-ppm control; (b) 40, 60, 80 and 120 min.

Based on the calibration curve, the IZ must be at least 12 mm but the sample did not show any antimicrobial activity. The possible reason is that the degradation byproducts might have the same absorption bands as TC but have no antimicrobial potency. The loss of potency could be attributed to possible rearrangement and defragmentation of moieties of the core and further treatment could result to core cleaving and mineralization.

IV. CONCLUSIONS

The adsorption of TC on aluminum surface follows Langmuir isotherm, which suggests that TC forms a monolayer of adsorbed TC molecules on the metal surface with Al^{3+} sites. These sites were responsible for binding with and oxidation of the electron-rich TC core and its moieties. The oxidation of TC was described by the bathochromic shift of the 360 nm absorbance band. The TC degradation was observed later at a slow rate due to saturation of aluminum surface.

The presence of TiO_2 increased the rate of TC removal in the solution due to additional sorption sites. During the adsorption of TC to either SSD or TiO_2-coated SSD, the two characteristic bands of TC shifted downwards proportionally. However, in the presence of TiO_2 and UV, the downshift of 360 nm was greater than that of the 270 nm. This could be attributed to more aggressive surface reactions resulting from photocatalysis.

An LCMS method was developed and used to detect TC and its byproduct during treatment. The analysis shows that the two major byproducts were m/z 234.95 and 195.00. Their increasing relative abundance corresponds to the decreasing TC concentration. This proves that TC is degraded into smaller compounds during treatment.

The disc diffusion assay shows that the antimicrobial potency of TC solution after 10 min of treatment time declined and eventually lost its potency after 20 min. The degradation products of TC were found to have no potency against *Pseudomonas aeruginosa*. The loss of potency can be attributed to core cleaving and defragmentation and possible rearrangement of moieties.

Acknowledgment

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