

## Degradation and Microbiological Validation of Meropenem Antibiotic in Aqueous Solution Using UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> Processes.

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### ABSTRACT

Aqueous UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> mediated degradation/oxidation of the carbapenem antibiotic, meropenem (MERO) was experimentally studied. Degussa P-25 titanium dioxide was used as photocatalyst and UV-light source was used for activation of TiO<sub>2</sub>. The nanosized titanium dioxide was immobilized on the glass support for improving the efficiency and economics of the photocatalytic processes. The immobilized film of titanium dioxide has been characterized, using X-ray diffraction (XRD) and scanning electron microscopy (SEM). The study of antibiotic degradation was conducted in the specific Batch Photocatalytic Reactor. MERO standard solution was used at 500 µg/ml concentration, which degraded up to 99% of antibiotics. Microbiological assay showed that the loss of antibacterial activity is directly proportional to the time of UV-irradiation. The experiment also showed that the UV-irradiation itself causes the degradation of antibiotics, but in very slow manner in comparison to the photocatalysis process. The experimental study showed that UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system is effective and efficient for the treatment of antibiotic waste.

**Keywords-** Photocatalysis, Meropenem, Antibiotics, TiO<sub>2</sub>, UV-irradiation.

### I. INTRODUCTION

Carbapenem are a class of β-lactam broad-spectrum antibacterial agents used for human and veterinary application. It act by inhibiting the cell wall synthesis and are known to be most effective against gram negative infections. As a result of their rising popularity, overuse and misuse of antibiotics has fuelled a rise in drug-resistant infections and experts are particularly alarmed about bacteria that cannot be killed with carbapenems [1-3], the foremost powerful category of antibiotics. Being persistent compounds [4-6], antibiotics pass the several treatment plants intact and accumulate in the environment [7-11]. Higher concentration of these compounds in the environment causes damage to the micro-flora and fauna [12,13], and its lower concentration in the environment promotes the development of antibiotic resistant bacteria [14-17].

In recent decades, the emergence of antibiotic resistant bacteria has increased and many specialists believe that the increase is due to the inappropriate use of antibiotics [18, 19]. Furthermore, the presence of antibiotics in aqueous effluent has also increased and their removal will be a challenge in the near future.

Therefore, physical-chemical removal technologies are needed such as advanced oxidation processes (AOPs) like photolysis, photocatalysis

and incorporation of strong oxidizing agents like H<sub>2</sub>O<sub>2</sub>. Among the different AOPs, TiO<sub>2</sub> photocatalysis emerges as a promising treatment technology due to its specific advantages like lack of mass transfer limitations, operation at ambient conditions and catalyst is inexpensive, commercially accessible, non-toxic and photochemically stable. One of the most widely used carbapenem antibiotics is meropenem (MERO, (4R,5S,6S)-3-[(3S,5S)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]sulfanyl-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, see Fig. 1). To the best of author's knowledge, there are no works dealing with the AOPs application to the MERO degradation. The application of microbiological assay for the determination of MERO concentration in its treated solutions makes the novelty of the present research.

The UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> processes are the UV based advanced oxidation processes (AOPs) and generates the hydroxyl radical (·OH) which has strong oxidizing capabilities [20]. Many studies have illustrated the effectiveness of these processes in the oxidation and mineralization of various pharmaceutical compounds and these processes has been widely studied for the remediation of both ground and drinking waters [21-25].

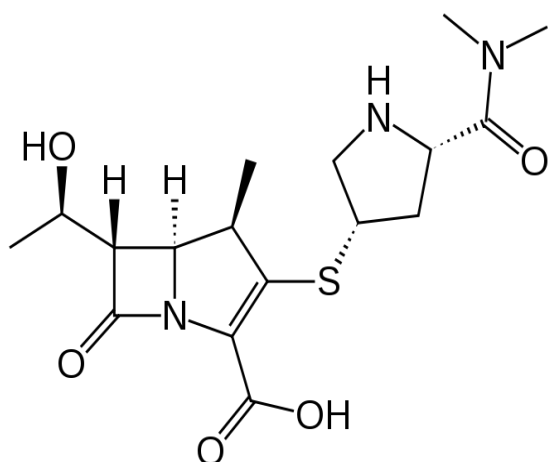


Fig. 1. Meropenem (MERO) molecule.

Although these AOPs are efficient in the mineralization of various antibiotics [26-50], however, it is uncertain whether carbapenem antibiotics like MERO can be completely mineralized by these processes. It is also unknown whether the process renders MERO and its potential photocatalytic transformation products biologically inactive, as bioactivity is an important parameter to consider when assessing the feasibility of an advanced degradation system.

The aim of this study is to investigate the elimination of MERO and look at the changes in its antibacterial activity using UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> processes. Microbial growth inhibition bioassay will be performed to determine the loss of antibacterial activity as well as concentration of intact MERO in treated solutions. In this study, we also examine and compare the degradation kinetics for these processes.

## II. MATERIALS AND METHODS

### 2.1 Chemicals

Meropenem, ADH2031, was provided by Cipla (India). Hydrogen peroxide (> 30%) was obtained from Merck (India). Ethanol (99.9%) and Whatman filter paper no.-1 were purchased from s d Fine Chem Ltd (India). Mueller Hinton agar and LB broth media, used for microbiological assay, were obtained from HIMEDIA (India). Degussa P25 TiO<sub>2</sub>; commercial photocatalyst provided by Evonik Industries, Germany was used throughout the current investigation. According to manufacturer's specifications, its BET surface area was 50±10 m<sup>2</sup>/g, average particle size was around 25 nm, purity was 99.5 %, and anatase to rutile ratio was 3:1. Any other chemicals used in this study were of analytical grade. Stock solution of MERO and other solution were prepared in distilled water (DW) and diluted as required.

### 2.2 Coating of Degussa P25 film on petri-plate by Dip-Coating method

The catalyst (TiO<sub>2</sub>) was immobilized on the petri plate using Dip-Coating method according to the process mentioned by Negishi et al. 1998 [51], with some modifications. The TiO<sub>2</sub> suspension was prepared by adding 2gm of TiO<sub>2</sub> in 40 ml of distilled water in a beaker. The resulting solution was stirred continuously for 15-20 min until all the TiO<sub>2</sub> powder gets mixed up properly. After the suspension was prepared, the petri-plates (100 mm diameter) were dipped in a beaker containing the suspension for 2-3 min and again taken out from the suspension (i.e. simply consider it as one time dip-coating). After dip-coating, the plates were dried in an oven for 1 hour at 108°C. If layer deposited on the plates was not uniform, then plates were dipped again in suspension to obtain uniform layer and above procedure was repeated. After drying, the plates were carried to muffle furnace where the coated supports were annealed for 3-4 hours at 500°C. Annealing provides better mechanical strength to films. Coated petri-plates having active catalyst surface area of 78.54 cm<sup>2</sup> were used as such for photocatalytic experiments.

### 2.3 Characterization of coated films

The morphology of the coated titanium dioxide was studied using SEM. The test sample was analyzed in the scanning electron microscope JSM 6100 (JEOL), which operated at 25 kV. The XRD analysis of the titanium dioxide coating was done by the plate XRD technique to examine the crystalline structure of it. The X-ray diffraction pattern was established on a Phillips PW-1710 X-ray diffractometer using Cu-K $\alpha$  radiation as X-ray source at an angle of 2 $\theta$  ranging from 20° to 80°. The scanning rate of 0.034(2 $\theta$ )/s was used for the measurements.

### 2.4 Experimental procedure

Photocatalytic degradation of MERO was carried out in a Batch Photocatalytic Reactor (BPR) at room temperature (23 ± 2 °C), manufactured by Labco India limited, having dimension (52cm x 28cm x 56cm). The reaction chamber contains two UV Tube light (Philips, TUV, 15W/G15 T8) placed at the top of the reactor, one exhaust fan (to control inside temperature) fitted at the back side of the reactor, a temperature probe fitted at the top of reactor, a magnetic stirrer placed at the bottom of reactor with a height adjustable stand as shown in Fig. 2. Inner walls of the reactor were fully coated with silver paint in order to minimize the irradiation losses. A MERO solution of 20 ml with concentration of 500  $\mu$ g/ml was poured into petri plates having a similar cross-sectional area of 78.54 cm<sup>2</sup>.

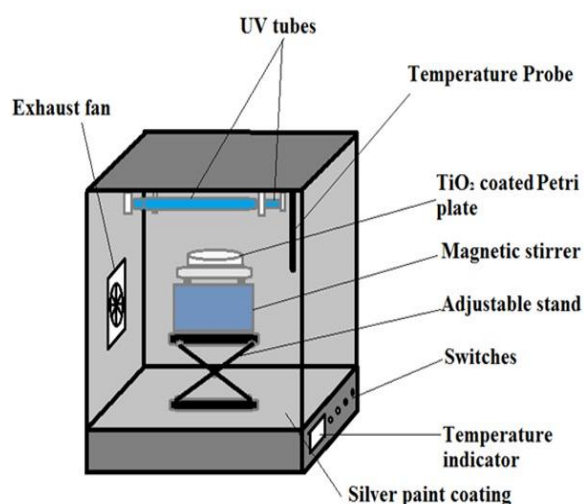


Fig. 2. Batch Photocatalytic Reactor.

Four different modes of treatment were studied for the degradation of MERO solution. UV irradiation and operating conditions were similar for each mode of degradation. But, they differ only in the presence of the  $\text{TiO}_2$  as photocatalyst and  $\text{H}_2\text{O}_2$  as strong oxidizing agent. **UV/ $\text{TiO}_2$  system** involves UV-irradiation in the presence of  $\text{TiO}_2$  photocatalyst, MERO solution was poured into a  $\text{TiO}_2$  coated petri plate; **UV system** uses UV-irradiation only, MERO solution was poured into an uncoated petri plate; **UV/ $\text{H}_2\text{O}_2$  system** uses UV-irradiation in the presence of hydrogen peroxide, MERO solution was poured into an uncoated petri plate containing  $\text{H}_2\text{O}_2$  (100 mg/L); **UV/ $\text{TiO}_2$ / $\text{H}_2\text{O}_2$  system** involves the combination of UV-irradiation,  $\text{TiO}_2$  photocatalyst and oxidizing agent hydrogen peroxide. MERO solution was poured into a  $\text{TiO}_2$  coated petri plate containing  $\text{H}_2\text{O}_2$  (100 mg/L). These plates were placed into the reaction chamber of BPR for degradation. During the experiment, aliquots of 0.2 ml of MERO solution were withdrawn from the petri plates at regular intervals to observe the degradation of MERO. The aliquots withdrawn were then analyzed by Microbiological assay.

### 2.5 Microbiological assay

The antibacterial activity of the MERO solution, before, during and after treatment by UV, UV/ $\text{H}_2\text{O}_2$ , UV/ $\text{TiO}_2$  and UV/ $\text{TiO}_2$ / $\text{H}_2\text{O}_2$  processes were measured using non-pathogenic bacterial strain, *E. coli* MTCC 443 as a test microorganism, according to the protocol based on Disk diffusion method [52]. In brief, 0.2 ml of standard solution of MERO and the solution treated with UV, UV/ $\text{H}_2\text{O}_2$ , UV/ $\text{TiO}_2$  and

UV/ $\text{TiO}_2$ / $\text{H}_2\text{O}_2$  processes were taken. From the collected samples, 7  $\mu\text{L}$  aliquots were used for the impregnation of filter paper discs having diameter of 5 mm. The test microorganism was cultured in LB broth, adjusting its concentration to the 0.5 McFarland standards, containing approximately  $1.5 \times 10^8$  CFU/mL. The dried surface of a Mueller Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. The antibiotic impregnated discs were dispensed onto the surface of the inoculated agar plate. In addition, the positive and negative growth controls were also used. The plates were incubated at 37 °C for 16 hours. Each assay was realized in triplicate. After the incubation, the inhibition zones (halos) were measured and correlated with MERO degradation.

## III. RESULTS AND DISCUSSION

### 3.1 Characterization of $\text{TiO}_2$ coated film

The XRD studies were made to notice any change in the phase composition and photocatalytic properties of the titanium dioxide after immobilization on the plate. The XRD measurements were done directly on coated catalyst resulting in noisy patterns. The XRD pattern of  $\text{TiO}_2$  coated film is presented in Fig. 3. The XRD patterns of the coated  $\text{TiO}_2$  surface did not show any variation in the structure and phase composition due to coating process. The diffraction peaks observed at  $2\theta = 25.31^\circ, 37.91^\circ, 48.15^\circ, 53.92^\circ, 55.16^\circ, 62.84^\circ, 69.12^\circ, 70.41^\circ$  and  $75.02^\circ$  correspond to the known diffraction maxima of anatase phase of  $\text{TiO}_2$ . The peaks at  $2\theta = 27.45^\circ, 36.10^\circ, 41.66^\circ, 54.34^\circ, \text{ and } 56.61^\circ$  correspond to rutile phase of  $\text{TiO}_2$ . The intensity of the peaks reveals that the major phase available in the coated  $\text{TiO}_2$  is anatase while the rutile is the minor phase. The anatase  $\text{TiO}_2$  is necessary to achieve the required electronic band gap of 3.2 eV. The photocatalytic reaction is very efficient on the mixture of anatase and rutile particles [53].

To study the morphology of  $\text{TiO}_2$  coating, the SEM images of the prepared film were taken at  $\times 10,000$  magnifications, and shown in Fig. 4. SEM image demonstrate the nanostructure of the catalyst, and rough surface of the film, which is quite necessary for the good photocatalytic activity of the film. It is obvious from the micrographs that  $\text{TiO}_2$  (white spots) is more uniformly distributed on the film surface, which indicate the availability of larger surface area of titanium dioxide for photocatalytic reactions.

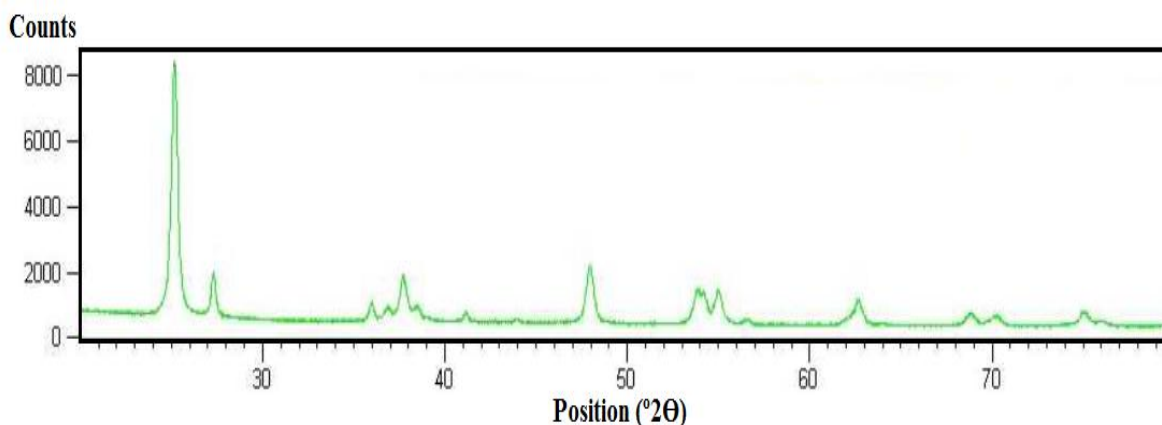


Fig. 3. The XRD pattern of TiO<sub>2</sub> coated film.

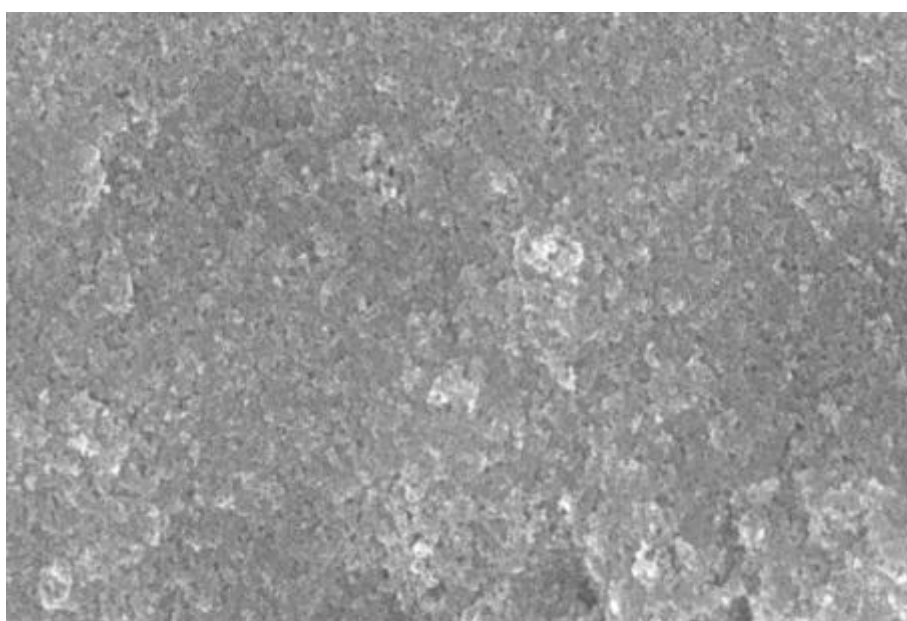


Fig. 4. SEM image of TiO<sub>2</sub> coated film.

### 3.2. Degradation of MERO

Total four modes were studied for MERO degradation which includes UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system. Fig. 5 and 6 shows the results for these modes of MERO degradation.

The decrease in MERO concentration was recorded with respect to time as shown in Fig. 5. UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system shows a degradation of 41.14, 85.65, 78.52 and 99.91% respectively during the first hour of irradiation. UV, UV/H<sub>2</sub>O<sub>2</sub> and UV/TiO<sub>2</sub> system take about three hours of UV-irradiation to give degradation in the range of 90%. In case of UV/H<sub>2</sub>O<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system, a sudden degradation of about 80% was observed within the first half hour of irradiation. It was occurred due to the fast chemical oxidation of MERO with the help of strong oxidizing agent, hydrogen peroxide.

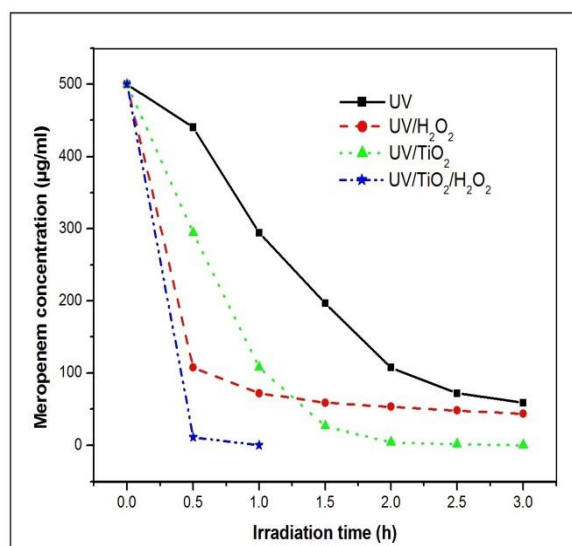
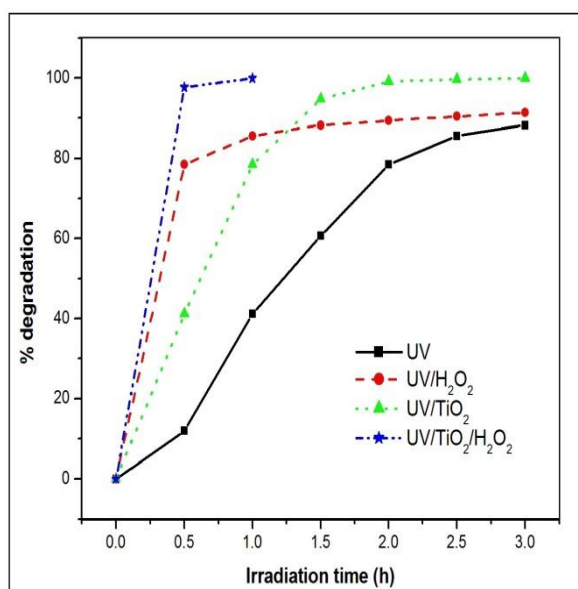


Fig. 5. MERO concentration as function of time for different modes of degradation.



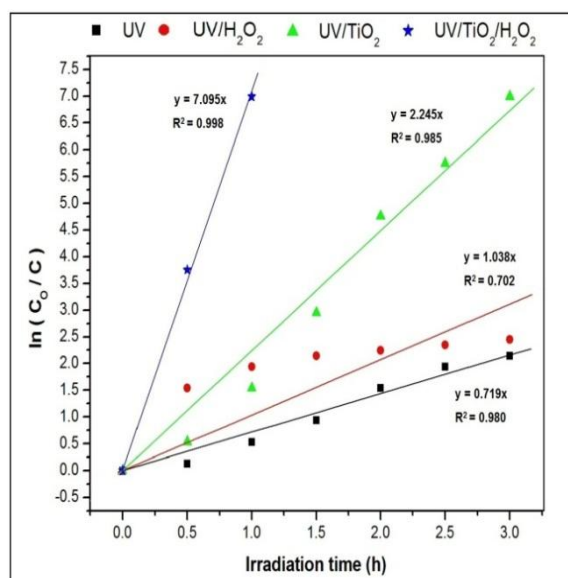
**Fig. 6.**Percentage degradation as function of time for different modes of degradation.

The degradation efficiency was found to be higher for the UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> treatment of MERO as compared to other studied treatment. Fig. 5 and 6 clearly shows that the UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> treatment is the most efficient treatment method for antibiotic degradation. Nevertheless, UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system allows the elimination of an equivalent quantity of MERO with in 1hour, where the abatement reaches 99% approximately. It clearly supports the higher economy and effectiveness of this system for antibiotic degradation [54].

Chemical oxidation of MERO antibiotic through hydrogen peroxide alone gives 67.86% degradation, which is not sufficient for resistance prevention in pathogens.

### 3.3 Kinetics of the degradation of MERO

The kinetics of photocatalytic and photolytic degradation of MERO was also studied. The application of the Langmuir-Hinshelwood model for the photocatalytic degradation of MERO was confirmed by the straight line obtained from the representation of the  $\ln(C_0/C)$  versus time plots. Fig. 7 shows the plot of  $\ln(C_0/C)$  versus Irradiation time (t) for UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> processes of MERO degradation. The linearity of the plots as shown in Fig. 7 suggests that the photocatalytic and photolytic degradation follows the pseudo-first order kinetics. Degradation of MERO exhibited the highest rate constant 7.095 h<sup>-1</sup> for UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system followed by 2.245 h<sup>-1</sup> for UV/TiO<sub>2</sub>, 1.038 h<sup>-1</sup> for UV/H<sub>2</sub>O<sub>2</sub> and 0.719 h<sup>-1</sup> for UV system.



**Fig. 7.**Kinetic analysis for different modes of MERO degradation.

The values of half-life time for the degradation of MERO through UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system were calculated and tabulated in Table 1. The half-life time values for UV, UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub> and UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system was 0.964 h, 0.668 h, 0.309 h and 0.098, respectively. The lowest half-life time and highest reaction constant for the degradation of MERO were obtained for UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system which is an indicative of highest photocatalytic activity.

**Table 1.**Half-life time and rate constant for different modes of degradation.

Mode of Degradation	Rate Constant, h <sup>-1</sup>	Half- life time, h
UV	0.719	0.964
UV/H <sub>2</sub> O <sub>2</sub>	1.038	0.668
UV/TiO <sub>2</sub>	2.245	0.309
UV/TiO <sub>2</sub> /H <sub>2</sub> O <sub>2</sub>	7.095	0.098

## IV. CONCLUSIONS

Degradation of MERO has been successfully conducted by photocatalytic process using an immobilized TiO<sub>2</sub> catalyst. It had been additionally compared with photolysis and chemical oxidation using H<sub>2</sub>O<sub>2</sub>. It is a promising and Eco-friendly technique; its advantage is that it does not require any kind of separation or filtration prior to treatment, compared to other treatment methods. The degradation potency was found to be maximum for the UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system followed by UV/TiO<sub>2</sub> system among the studied methods. Highest

photocatalytic activity is shown by UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> system. The kinetics obeys Langmuir-Hinshelwood model from which kinetic constant were obtained. It shows that the degradation of MERO follows the pseudo-first order kinetics. For identical UV dose, the rate constants were greatly increases with increasing H<sub>2</sub>O<sub>2</sub> concentration.

## V. ACKNOWLEDGMENTS

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## VI. ACRONYMS

AOPs	Advance oxidation processes
BPR	Batch Photocatalytic Reactor
MERO	Meropenem
SEM	Scanning electron microscopy
UV	Ultra-violet light
XRD	X-ray diffraction

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