Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

Biological treatment of swine manure with microalgae-bacteria consortium. Removal and fate of emerging pollutants present in pig manure wastewater

Presentada por:
Johanna Vanessa Zambrano Flores
para optar al grado de
Doctor por la Universidad de Valladolid

Dirigida por:
Dr. Rubén Irusta-Mata
Dr. Juan José Jiménez

A mis padres

Table of contents

Resumen

España es el segundo país de Europa con mayor consumo de carne y el cuarto productor mundial de porcino, con alrededor de 30 millones de cabezas y una generación de cerca de 44 Mt de purín al año (223 Mt en la Unión Europea). Los purines presentan concentraciones de materia orgánica, nitrógeno y fósforo muy elevadas que los hacen altamente contaminantes. La contaminación por nitratos de los acuíferos debido a las actividades agrícolas y ganaderas es una de las principales preocupaciones ambientales en la UE. Asimismo, los purines contienen antibióticos veterinarios que debido a la inadecuada gestión de estos residuos pueden ser liberados e ingresar al entono circundante a través de múltiples vías, como plantas de tratamiento de aguas residuales, escorrentía de los campos a las aguas superficiales, percolación a aguas subterráneas, entre otras. Distintos estudios en los últimos años han demostrado que el uso de microalgas para el tratamiento de aguas residuales puede depurar aguas contaminadas con purines, reduciendo la concentración de antibióticos y disminuyendo su efecto. Además, la biomasa de microalgas producida contiene proteínas, carbohidratos, lípidos y algunos productos minoritarios de alto valor añadido, lo que puede contribuir a la creación de una economía sostenible, utilizando recursos renovables. En este sentido, esta tesis se centra en el estudio de los mecanismos involucrados en la eliminación de antibióticos presentes en purines porcinos al ser tratados mediante un tratamiento biológico con un consorcio de microalgas-bacterias. Por otra parte, se propone un tratamiento alterativo mediante un proceso de oxidación avanzada (POA), como es la fotocatálisis, para reducir, y si es posible eliminar, la presencia de antibióticos.

El estado del arte de las tecnologías de tratamiento de antibióticos veterinarios se presenta en la sección de **Introduction**. Los objetivos, estrategias y desarrollo seguidos en esta tesis se resumen en la sección **Aims and Scope**.

En el **Capítulo 3** se estudiaron los mecanismos (hidrólisis, fotólisis, biosorción y biodegradación) implicados en la eliminación de una mezcla de cuatro antibióticos veterinarios (AV) – tetraciclina (TET), ciprofloxacina (CIP), sulfadiazina (SDZ) y sulfametoxazol (SMX) – en aguas residuales sintéticas utilizando consorcios de microalgas-bacterias (MBC) dominados por *Scenedesmus almeriensis* a diferentes concentraciones iniciales de 1000, 500, 100 y 20 µg/L de cada antibiótico. Se utilizó cromatografía líquida de ultra alta resolución y espectrometría de masas en tándem (UHPLC-MS/MS) para determinar la eliminación de antibióticos veterinarios para cada mecanismo. Para un tiempo de retención hidráulica de 4 días, la eliminación de antibióticos por parte del consorcios de microalgas-bacterias fue del 99,9 % para tetraciclina, 78,0 % para ciprofloxacina, 52,6 % para sulfadiazina y 5,0 % para sulfametoxazol. Se aplicó un modelo de cinética irreversible de pseudo primer orden para ajustar mejor los datos experimentales. Las constantes de degradación fueron 0,136 h^{-1} para tetraciclina, 0,012 h^{-1} para ciprofloxacina, 0,010 h^{-1} para sulfadiazina y 0,0007 h^{-1} para sulfametoxazol. Bajo todas las condiciones evaluadas, ciprofloxacina y tetraciclina exhibieron la mayor eficiencia de remoción. La biosorción fue el principal mecanismo para los cuatro antibióticos, seguida de la biodegradación en los casos de tetraciclina y sulfadiazina. La ciprofloxacina no mostró eliminación por biodegradación, mientras que la sulfametoxazol no mostró eliminación por hidrólisis o fotólisis. Este estudio i) integró y evaluó individualmente los mecanismos involucrados en la remoción de antibióticos veterinarios utilizando un consorcios de microalgas-bacterias; ii) determinó la constante cinética de la tasa de eliminación en el intervalo de concentraciones 0 – 1000 µg/L de

tetraciclina, ciprofloxacina, sulfadiazina y sulfametoxazol; y iii) demostró la alta capacidad de eliminación, y por tanto, el potencial uso de las tecnologías basadas en microalgas den el tratamiento de aguas residuales.

En el **Capítulo 4** se estudió la adsorción de los antibióticos veterinarios tetraciclina, ciprofloxacino, sulfadiazina y sulfametoxazol en un consorcio liofilizado de bacterias y microalgas, principalmente constituido por *Scenedesmus almeriensis*, a varias concentraciones (20, 100, 500 y 1000 μg/L). Se realizaron análisis de microscopía electrónica de barrido (SEM), y espectroscopía infrarroja transformada de Fourier (FTIR) para investigar la morfología de la superficie del consorcio microalgas/bacterias e identificar el efecto de los grupos funcionales de los antibióticos en la superficie de este consorcio. Se utilizó cromatografía líquida de ultra alta resolución acoplada a espectrometría de masas para determinar la viabilidad de este consorcio para la eliminación de antibióticos mediante biosorción. Se utilizaron modelos de adsorción de Freundlich y Langmuir para la descripción matemática del equilibrio de adsorción. Se aplicaron modelos cinéticos de pseudo-primer orden y pseudo-segundo orden para ajustar los datos experimentales de biosorción. La eliminación relativa del antibiótico fue mayor a bajas concentraciones de equilibrio. En el rango de la concentración inicial de antibióticos veterinarios estudiada, la ciprofloxacina y la tetraciclina exhibieron la mayor eficacia de eliminación, del 43 al 100 % y del 75 al 82 %, respectivamente. Asimismo, ciprofloxacina y tetraciclina presentaron las mayores tasas de adsorción, de 0,11 a 26,66 y de 1,78 a 27,09 $mg \cdot \mu g^{-1} \cdot h^{-1}$, respectivamente. Este estudio reveló que el consorcio *S. almeriensis*-bacteria tiene un alto poder de biosorción y demostró que la biosorción es un mecanismo importante en la eliminación de ciprofloxacina y tetraciclina mediante un proceso de tratamiento de agua basado en microalgas. Sin embargo, las eficacias de eliminación para la sulfadiazina y el sulfametoxazol no superaron el 32 %.

En el **Capítulo 5** se investigó la eliminación de una mezcla de los cuatro antibióticos veterinarios referidos en el Capítulo 4 –tetraciclina, ciprofloxacina, sulfadiazina y sulfametoxazol– mediante fotodegradación (UVC) y fotocatálisis con TiO_2 (UVC/TiO_2) en un reactor discontinuo, bajo diferentes concentraciones iniciales de los fármacos (20, 100, 500 y 1000 μg/L por antibiótico). Se utilizó cromatografía líquida de ultra alta resolución acoplada a espectrometría de masas para determinar la eliminación de estos antibióticos veterinarios, observándose que las eficacias de eliminación de todos los antibióticos por fotólisis fue de alrededor del 98 - 99 % después de 100 horas para tetraciclina, 122 horas para ciprofloxacina, 212 horas para sulfadiazina y 240 horas para sulfametoxazol. Sin embargo, la eliminación de todos los antibióticos por fotocatálisis fue de alrededor del 99 - 100 % después de 4,2 horas para tetraciclina, 3,5 horas para ciprofloxacina, 7,1 horas para sulfadiazina y 16,5 horas para sulfametoxazol. La cinética de fotólisis de los cuatro antibióticos veterinarios siguió preferentemente un modelo irreversible de primer orden. En cambio, la fotocatálisis de tetraciclina, ciprofloxacina y sulfadiazina siguió un modelo cinético de Langmuir-Hinshelwood en el que la adsorción reveló ser el paso limitante, mientras que el sulfametoxazol siguió un modelo cinético irreversible de primer orden. La constante de velocidad de degradación fotolítica (k_1) fue de $7{,}3 \cdot 10^{-4}$ min^{-1} para la tetraciclina, $5{,}5 \cdot 10^{-4}$ min^{-1} para la ciprofloxacina, $3{,}1 \cdot 10^{-4}$ min^{-1} para la sulfadiazina y $2{,}7 \cdot 10^{-4}$ min^{-1} para el sulfametoxazol. Sin embargo, la constante cinética de fotocatálisis (k_1) del sulfametoxazol fue de $4{,}4 \cdot 10^{-3}$ min^{-1}; las constantes de adsorción ($k_{L\text{-}H}$) fueron $2{,}8 \cdot 10^{-2}$ min^{-1} para la tetraciclina, $3{,}79 \cdot 10^{-2}$ min^{-1} para la ciprofloxacina y $1{,}41 \cdot 10^{-2}$ min^{-1} para la sulfadiazina. La degradación de los antibióticos veterinarios mejoró mediante el uso de un catalizador. El catalizador ofrece sitios activos y la formación de huecos de electrones que conducen a una mayor producción de radicales hidroxilo y superóxidos, que facilitan la degradación de antibióticos veterinarios.

Adicionalmente, se evaluó la energía eléctrica por orden (E_{EO}) para estimar la eficiencia energética de cada proceso. E_{EO} se define como la energía eléctrica requerida para reducir la concentración de un contaminante en un orden de magnitud (90%) en un volumen fijo de agua contaminada. Los valores de E_{EO} para fotólisis fueron 339,06 kWh/m^3/orden para la tetraciclina, 449,84 kWh/m^3/orden para la ciprofloxacina, 795,31 kWh/m^3/orden para la sulfadiazina y 897,71 kWh/m^3/orden para el sulfametoxazol. Por otro lado, los valores de E_{EO} para fotocatálisis fueron sensiblemente menores, 14,96 kWh/m^3/orden para la tetraciclina, 12,07 kWh/m^3/orden para la ciprofloxacina, 20,39 kWh/m^3/orden para la sulfadiazina y 62,10 kWh/m^3/orden para el sulfametoxazol. Por tanto, el consumo de energía requerido para la fotocatálisis resultó ser considerablemente menor que para la fotólisis. En este estudio se determinaron las constantes de velocidad de degradación para un amplio intervalo de concentraciones de tetraciclina, ciprofloxacina, sulfadiazina y sulfametoxazol. Además, al trabajar con un pH de 8 (pH típico de aguas residuales de explotaciones ganaderas) y una mezcla de antibióticos veterinarios cuyas concentraciones se asemejan a las características de muestras de agua reales, este estudio demostró que fotólisis y fotocatálisis son procesos potenciales para el tratamiento de aguas residuales con bajo consumo energético.

En el **Capítulo 6** se evaluó la eliminación de una mezcla de tetraciclina, ciprofloxacina y sulfadiazina operando con alimentaciones reales correspondientes a la fracción líquida de purín de cerdo, mediante un consorcio microalgas *Scenedesmus almeriensis* y bacterias, en un fotobiorreactor a escala piloto. Después de 15 días de operación, el reactor se dopó con una mezcla de 100 μg/L de cada antibiótico. Una vez dopado el reactor, se operó por 20 días adicionales. Los parámetros analizados antes y después de la adición de antibióticos mostraron que los antibióticos no tuvieron un efecto negativo sobre la biomasa del reactor. Los antibióticos presentes en las fases líquida y

sólida se determinaron mediante cromatografía líquida de ultra alta resolución y espectrometría de masas en tándem. En la fase líquida del efluente, la remoción de antibióticos fue de 77 ± 5 %, 90 ± 14 % y 60 ± 27 % para tetraciclina, ciprofloxacina y sulfadiazina, respectivamente. En la fase sólida del efluente, no se encontraron antibióticos antes de dopar el reactor; al final de la operación del reactor los antibióticos encontrados fueron 979 ± 382 ng/g para la tetraciclina y 192 ± 69 ng/g para la sulfadiazina; en cambio, no se detectó ciprofloxacina en la biomasa. Además, se evaluó el desempeño del fotobiorreactor, encontrándose las siguientes eficacias de remoción de los parámetros analizados: 64,6 ± 0,6 % para TOC, 56,9 ± 0,6 % para CI, 63,9 ± 0,6 % para NT, 88,6 ± 0,9 % para $N\text{-}NH_4^+$, 64,9 ± 0,6 % para $N\text{-}NO_3^-$ y 30,1 ± 0,3 % para $P\text{-}PO_4^{3-}$. Este estudio demostró el buen desempeño de la tecnología basada en microalgas para el tratamiento de aguas residuales de purín porcino, no solo en términos de eliminación de materia orgánica y nutrientes, sino también en cuanto a la eliminación de antibióticos.

También se simuló el balance de materia de todo el proceso introduciendo las diferentes constantes cinéticas de degradación y adsorción encontradas en los ensayos batch previamente descritos, con el objeto de validar estos valores en un sistema que operaba en modo quasi-continuo.

Los resultados obtenidos en la presente tesis confirmaron el potencial del tratamiento de aguas residuales generadas en explotaciones porcinas mediante consorcios de algas-bacterias. Así pues, esta tecnología basada en microalgas se revela como una herramienta sostenible y eficiente para la remoción de antibióticos de uso veterinario. Esta tesis doctoral proporcionó un mejor entendimiento de los mecanismos que intervienen en la remoción de los antibióticos investigados, gracias al estudio de las

respetivas cinéticas de eliminación involucradas. Además, aportó una comparación con un proceso de oxidación avanzada, como es la fotocatálisis heterogénea con TiO_2, que podría ser usado complementariamente con tratamiento biológico para mejorar la eficacia de remoción de los antibióticos de uso veterinario.

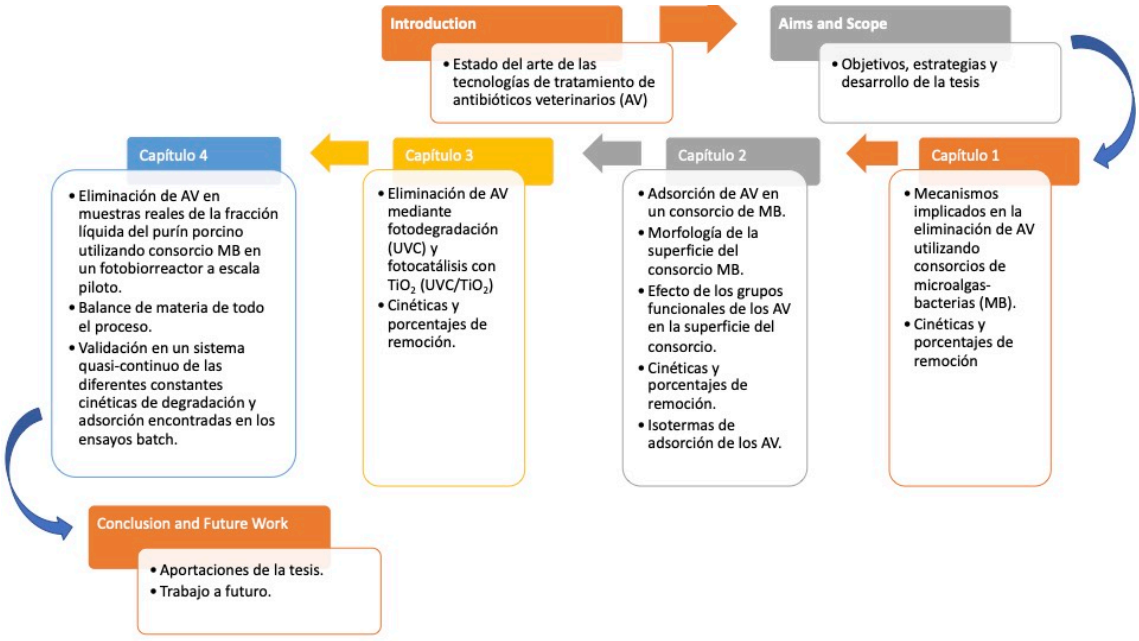

Figura 1. Diagrama esquemático de la estructura de la tesis

Abstract

Spain is the second country in Europe with the highest consumption of meat and the fourth largest producer of pigs in the world, with around 30 million heads and a generation of around 44 Mt of pig manure per year (223 Mt in the European Union). Manure has very high concentrations of organic matter, nitrogen and phosphorus that make it highly polluting. Nitrate contamination of aquifers due to agricultural and livestock activities is one of the main environmental concerns in the EU. In addition, pig manure contains antibiotics that due to its improper management can be released and enter the environment through multiple routes, such as wastewater treatment plants, runoff from fields to surface water, percolation to groundwater, among others. In recent years, different studies have shown that the use of microalgae for wastewater treatment can purify water contaminated with pig manure, reducing antibiotics concentration and reducing their effect. Additionally, the microalgae biomass contains proteins, carbohydrates, lipids and some minor products with high added value, which can contribute to the creation of a sustainable economy, using renewable resources. In this sense, this thesis focuses on the study of the mechanisms involved in the removal of antibiotics present in pig manure when treated by biological treatment with microalgae-bacteria consortium. Furthermore, an alternative treatment is proposed through an advanced oxidation process (AOP), such as photocatalysis, to reduce, and if possible eliminate, the presence of antibiotics.

The state-of-the-art of veterinary antibiotic treatment technologies is presented in the Introduction section. The objectives, approach and strategies followed in this thesis are summarized in the Aims and Scope section.

In **Chapter 3**, the mechanisms (hydrolysis, photolysis, biosorption and biodegradation) involved in the removal of a mixture of four veterinary antibiotics (VA) – tetracycline (TET), ciprofloxacin (CIP), sulfadiazine (SDZ) and sulfamethoxazole (SMX) – in synthetic wastewater using microalgae-bacteria consortia (MBC) dominated by Scenedesmus almeriensis was studied at different initial concentrations of 1000, 500, 100 and 20 μg/L of each antibiotic. Ultra-high performance liquid chromatography and tandem mass spectrometry (UHPLC-MS/MS) were used to determine the removal of the veterinary antibiotics for each mechanism. For a hydraulic retention time of 4 days, the overall removal of antibiotics by the microalgae-bacteria consortia was 99.9% for tetracycline, 78.0% for ciprofloxacin, 52.6% for sulfadiazine and 5.0% for sulfamethoxazole. A pseudo-first order irreversible model was applied to best fit the experimental data. The degradation constant rates were 0.136 h^{-1} for tetracycline, 0.012 h^{-1} for ciprofloxacin, 0.010 h^{-1} for sulfadiazine and 0.0007 h^{-1} for sulfamethoxazole. Under all the evaluated conditions, ciprofloxacin and tetracycline exhibited the highest removal efficiency. Biosorption was the main mechanism for all four antibiotics, followed by biodegradation in the cases of tetracycline and sulfadiazine. Ciprofloxacin did not show removal via biodegradation, while sulfamethoxazole did not show removal via hydrolysis or photolysis. This study i) integrates and evaluates individually the mechanisms involved in veterinary antibiotics removal using an microalgae-bacteria consortia; ii) determines an overall removal rate constant in the concentration range of 0 to 1000 μg/L for tetracycline, ciprofloxacin, sulfadiazine and sulfamethoxazole; and iii) demonstrates the high removal capacity and therefore, the potential use of microalgae-based technologies in wastewater treatment process.

In **Chapter 4**, the adsorption of the veterinary antibiotics tetracycline, ciprofloxacin, sulfadiazine and sulfamethoxazole onto a dried *Scenedesmus almeriensis*

microalgae-bacteria consortium was studied at several concentrations (20 to 1000 µg/L). Scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR) analysis were performed to investigate the surface morphology of the microalgae-bacteria consortia and to identify the effect of the antibiotics´ functional groups on the surface of this consortia. Ultra-high performance liquid chromatography tandem mass spectrometry was used to determine the feasibility of this consortium for the removal of antibiotics via biosorption. Freundlich and Langmuir adsorption models were used for the mathematical description of the adsorption equilibrium. Pseudo-first order and pseudo-second order kinetic models were applied to fit the biosorption experimental data. Relative antibiotic removal was higher at low equilibrium concentrations. In the range of the initial veterinary antibiotics concentration studied, ciprofloxacin and tetracycline exhibited the highest removal efficiency of 43-100% and 75-82%, respectively. Likewise, ciprofloxacin and tetracycline presented the highest adsorption rates of 0.11 to 26.66 and 1.78 to 27.09 $mg \cdot \mu g^{-1} \cdot h^{-1}$, respectively. This study revealed that the *S. almeriensis*-bacteria consortium has a high biosorption power and proved that biosorption is an important mechanism in the removal of ciprofloxacin and tetracycline using a microalgae-based water treatment process. However, the removal efficiencies of sulfadiazine and sulfamethoxazole did not exceed 32%.

In **Chapter 5**, the removal of a mixture of four veterinary antibiotics referred to in Chapter 4 – tetracycline, ciprofloxacin, sulfadiazine and sulfamethoxazole – via photo-degradation (UVC) and photocatalysis with TiO_2 (UVC/TiO_2) was investigated in a batch reactor under different initial antibiotics concentrations (20, 100, 500 and 1000 µg/L per antibiotic). Ultra-high performance liquid chromatography coupled to a mass spectrometry was used to determine the removal of these veterinary antibiotics. The removal of all antibiotics via photolysis was around 98 - 99% after 100 hours for

tetracycline, 122 hours for ciprofloxacin, 212 hours for sulfadiazine and 240 hours for sulfamethoxazole. Nevertheless, the removal of all antibiotics via photocatalysis was around 99 - 100% after 4.2 hours for tetracycline, 3.5 hours for ciprofloxacin, 7.1 hours for sulfadiazine and 16.5 hours for sulfamethoxazole. The photolysis kinetics for the four veterinary antibiotics preferably followed a first-order irreversible kinetic model. Instead, the photocatalysis of tetracycline, ciprofloxacin and sulfadiazine followed a Langmuir-Hinshelwood kinetic model in which adsorption was revealed to be the limiting step, while sulfamethoxazole followed a first-order irreversible kinetic model. The photolytic degradation rate constant (k_1) was 0.00073 min^{-1} for tetracycline, 0.00055 min^{-1} for ciprofloxacin, 0.00031 min^{-1} for sulfadiazine and 0.00027 min^{-1} for sulfamethoxazole. While, the photocatalytic rate constant (k_1) for sulfamethoxazole was 0.0044 min^{-1}; the adsorption constants (k_{L-H}) were 0.0284 min^{-1} for tetracycline, 0.0379 min^{-1} for ciprofloxacin and 0.0141 min^{-1} for sulfadiazine. The veterinary antibiotics degradation was enhanced by the use of a catalyst. The catalyst offers active sites and electron-hole formation leading to boosted hydroxyl and superoxide radicals production, which facilitate the degradation of veterinary antibiotics. Additionally, electrical energy per order (E_{EO}) was assessed to estimate the electrical energy efficiency of each process. E_{EO} is defined as the electrical energy required to reduce the concentration of a pollutant by one order of magnitude (90%) in a fixed volume of polluted water. E_{EO} values for photolysis were 339.06 kWh/m^3/order for tetracycline, 449.84 kWh/m^3/order for ciprofloxacin, 795.31 kWh/m^3/order for sulfadiazine and 897.71 kWh/m^3/order for sulfamethoxazole. On the other hand, E_{EO} values for photocatalysis were significantly lower, 14.96 kWh/m^3/order for tetracycline, 12.07 kWh/m^3/order for ciprofloxacin, 20.39 kWh/m^3/order for sulfadiazine and 62.10 kWh/m^3/order for sulfamethoxazole. Therefore, the energy consumption required for photocatalysis was found to be considerably lower

than for photolysis. This study determined an overall degradation rate constant for a wide range of tetracycline, ciprofloxacin, sulfadiazine and sulfamethoxazole concentrations. Furthermore, when working with a pH of 8 (a typical pH from wastewater from livestock farms) and a veterinary antibiotics mixture whose concentrations resemble the characteristics of real water samples, that photolysis and photocatalysis are potential processes for wastewater treatment with low energy consumption.

In **Chapter 6**, the removal of a mixture of tetracycline, ciprofloxacin and sulfadiazine was evaluated operating with real samples corresponding to the liquid fraction of pig slurry, using *Scenedesmus almeriensis* microalgae-bacteria consortia in a pilot scale photobioreactor. After 15 days of operation the reactor was spiked with a mixture of 100 μg/L of each antibiotic. The experiment run for 20 additional days. The parameters analyzed before and after the antibiotics addition showed that the antibiotics did not have a negative effect on the reactor biomass. Antibiotics present in the liquid and solid phase were determined by ultra-high performance liquid chromatography and tandem mass spectrometry. In the liquid phase of the effluent, the antibiotics removal were 77 ± 5 %, 90 ± 14 % and 60 ± 27 % for tetracycline, ciprofloxacin and sulfadiazine, respectively. In the solid phase of the effluent, no antibiotics were found before doping the reactor; at the end of the reactor operation the antibiotics were 979 ± 382 ng/g for tetracycline and 192 ± 69 ng/g for sulfadiazine; instead, ciprofloxacin was not detected in the biomass. Additionally, the performance of the photobioreactor was evaluated, finding the following removal efficiencies of the analyzed parameters: 64.6 ± 0.6 % for TOC, 56.9 ± 0.6 % for IC, 63.9 ± 0.6 % for TN, 88.6 ± 0.9 % for $N\text{-}NH_4^+$, 64.9 ± 0.6 % for $N\text{-}NO_3^-$ and 30.1 ± 0.3 % for $P\text{-}PO_4^{3-}$. This study demonstrated the good performance of microalgae-based technology for swine manure wastewater treatment, not only in

terms of organic matter and nutrient removal, but also regarding the removal of antibiotics.

The mass balance analysis of the entire process was also simulated by introducing the different kinetic constants of degradation and adsorption found in the previously described batch tests, to validate these values in a system that operated in quasi-continuous mode.

The results obtained in the present thesis confirmed the potential of treating wastewater generated in pig farms by algae-bacteria consortia. Thus, this microalgae-based technology is revealed as a sustainable and efficient tool for the removal of antibiotics for veterinary use. This doctoral thesis provided a better understanding of the mechanisms involved in the removal of the investigated antibiotics, thanks to the study of the respective removal kinetics involved. In addition, it provided a comparison with an advanced oxidation process, such as heterogeneous photocatalysis with TiO_2, which could be used in addition to biological treatment to improve the removal efficiency of veterinary antibiotics.

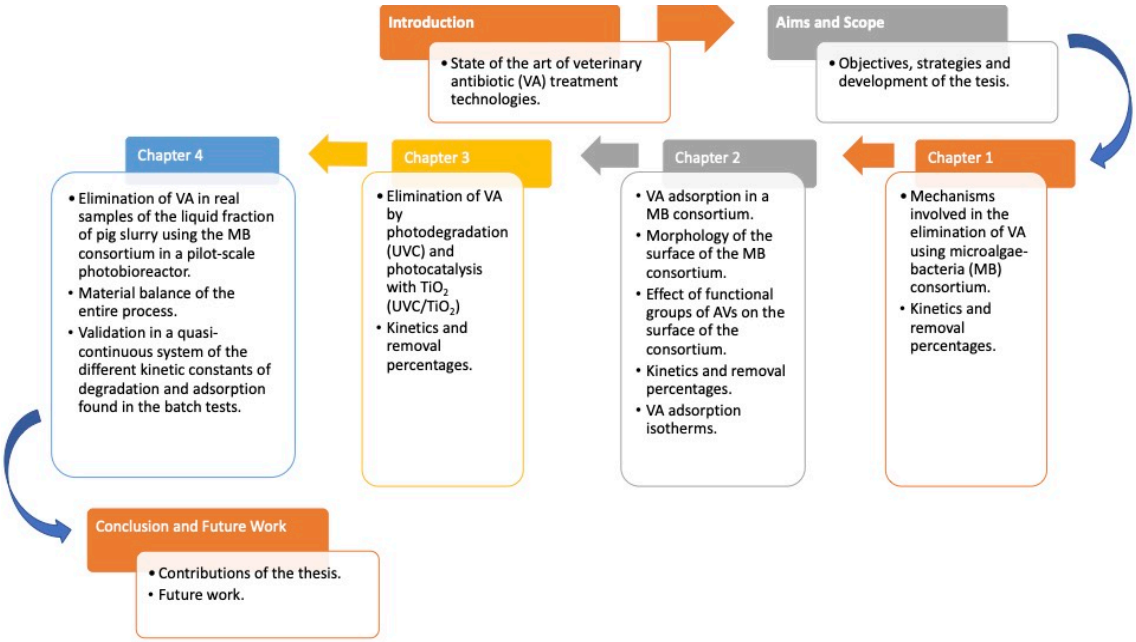

Figure 1. Schematic diagram of the thesis structure

List of publications

The following publications are presented as part of the present thesis. All of them are published in international journals indexed in Journal Citation Reports.

Paper I. Zambrano, J., García-Encina, P. A., Hernández, F., Botero-Coy, A. M., Jiménez, J. J., & Irusta-Mata, R. (2023). Kinetics of the removal mechanisms of veterinary antibiotics in synthetic wastewater using microalgae-bacteria consortia. *Environmental Technology & Innovation*, 103031.

Paper II. Zambrano, J., García-Encina, P. A., Hernández, F., Botero-Coy, A. M., Jiménez, J. J., & Irusta-Mata, R. (2021). *Removal of a mixture of veterinary medicinal products by adsorption onto a Scenedesmus almeriensis microalgae-bacteria consortium.* Journal of Water Process Engineering, 43, 102226.

Paper III. Zambrano, J., García-Encina, P. A., Jiménez, J. J., López-Serna, R., & Irusta-Mata, R. (2022). Photolytic and photocatalytic removal of a mixture of four veterinary antibiotics. Journal of Water Process Engineering, 102841.

Paper IV. Zambrano, J., García-Encina, P. A., Jiménez, J. J., Ciardi, M., Bolado-Rodríguez, S., & Irusta-Mata, R. (2023). *Removal of veterinary antibiotics in swine manure wastewater using microalgae-bacteria consortia in a pilot scale photobioreactor.* (Submitted to Environmental Technology & Innovation)

Contribution to the papers included in the thesis

Paper I. In this work, I was responsible of the design, start-up and operation of the experimental set-up under the supervision of Dr. Rubén Irusta, Dr. Pedro García and Dr. Juan José Jiménez. I performed the mass balance calculations, results evaluation and manuscript writing under the supervision of Dr. Rubén Irusta, Dr. Pedro García and Dr. Juan José Jiménez. Dr. Félix Hernández and Dr. Ana María Botero were responsible of the UHPLC-MS/MS analysis, where I contributed in the results evaluation and discussion.

Paper II. In this work, I was responsible of the design, start-up and operation of the experimental set-up under the supervision of Dr. Rubén Irusta, Dr. Pedro García and Dr. Juan José Jiménez. I performed the mass balance calculations, results evaluation and manuscript writing under the supervision of Dr. Rubén Irusta, Dr. Pedro García and Dr. Juan José Jiménez. Dr. Félix Hernández and Dr. Ana María Botero were responsible of the UHPLC-MS/MS analysis, where I contributed in the results evaluation and discussion.

Paper III. In this work, I was responsible of the design, start-up and operation of the experimental set-up under the supervision of Dr. Rubén Irusta, Dr. Pedro García and Dr. Juan José Jiménez. I performed the mass balance calculations, results evaluation and manuscript writing under the supervision of Dr. Rubén Irusta, Dr. Pedro García and Dr. Juan José Jiménez. Dr. Rebeca Lopez was responsible of the UHPLC-MS/MS analysis, where I contributed in the data analysis and discussion.

Paper IV. In this work, I was responsible of the UHPLC-MS/MS analysis, results evaluation and discussion under the supervision of Dr. Juan José Jiménez. I performed

the mass balance calculations, results evaluation and manuscript writing under the supervision of Dr. Rubén Irusta and Dr. Pedro García. Dr. Martina Ciardi was responsible of the start-up and operation of the experimental set-up. Dr. Silvia Bolado-Rodríguez was responsible of the funding acquisition, resources and conceptualization.

Chapter 1. Introduction

1. Emerging contaminants

Water scarcity is nowadays one of the major global concerns. Attempts have been done to convert wastewater into water that can be reused for other purposes. However, traditional methods are not suitable for water reclamation as not all contaminants present in wastewater are easily removed. A group of contaminants that have raise researchers´ attention are emerging contaminants (ECs). ECs are synthetic or naturally occurring chemicals, and their transformation products, or any microorganisms (virus as SARS-CoV-2 could be included) which are not commonly monitored in the environment but have a high potential to enter the environment causing known or suspected harmful effects on human health and ecosystems (Kumar et al., 2022; Tong et al., 2022; Yap et al., 2019).

ECs are typically present in trace amounts, ranging from ng/L to mg/L. These contaminants encompass a range of substances such as pharmaceuticals and personal care products (PPCPs), endocrine disrupting chemicals (EDCs), perfluorinated compounds (PFCs), surfactants, pesticides, flame retardants, industrial additives, and viruses, including human polyomaviruses, hepatitis E virus, and human adenoviruses (Priya et al., 2022; Puri et al., 2023; Zhao et al., 2018).

ECs are biologically active compounds that pose significant risks to both human health and natural ecosystems due to their recalcitrance, low biodegradability and short- and long-term toxicities. These compounds can cause ecological risk such as interfering with the normal endocrine functions of living organisms, microbiological resistance, and bioaccumulation. Additionally, ECs tend to accumulate in lipid-rich tissues and can be

active across the food chain (Abdel-Maksoud et al., 2018; Samal et al., 2022; Sanganyado and Kajau, 2022).

Endocrine disrupting compounds (EDCs) are particularly concerning, as they can cause abnormal physiological processes, reproductive damage and increase the incidence of cancer. Even at low concentrations detected in environmental samples, EDCs can have biological effects (Gogoi et al., 2018; Wilkinson et al., 2017). Despite these risks, there is still a lack of knowledge regarding the occurrence, proliferation, impacts and fate of these compounds. Few ECs have been inventoried or regulated as contaminants (Puri et al., 2023; Wilkinson et al., 2017). Overall, there is a pressing need to further understand and address the risks posed by ECs and EDCs to safeguard human health and natural ecosystems.

Emerging contaminants (ECs) are commonly found in various water sources, such as wastewater, surface water, ground water, and drinking water, and arise from numerous sources, including personal care products, pharmaceuticals, and improper disposal of unused medications (Fig.1). ECs can come from point sources, such as industrial and agricultural activities, or diffuse sources, such as stormwater runoff from highways and agricultural land. Wastewater treatment plants are a primary point source of ECs, with processed wastewater containing pharmaceuticals, natural hormones, and synthetic steroids. Landfill sites can also contribute ECs to groundwater due to their toxicity. Biosolids from wastewater treatment plants can affect groundwater sources when utilized for land applications, while pesticides are a major contributor to agricultural contamination. Finally, livestock farms are a significant source of veterinary antibiotics (Kumar et al., 2022; Tong et al., 2022; Zhou et al., 2023). The present study will specifically focus on these contaminants.

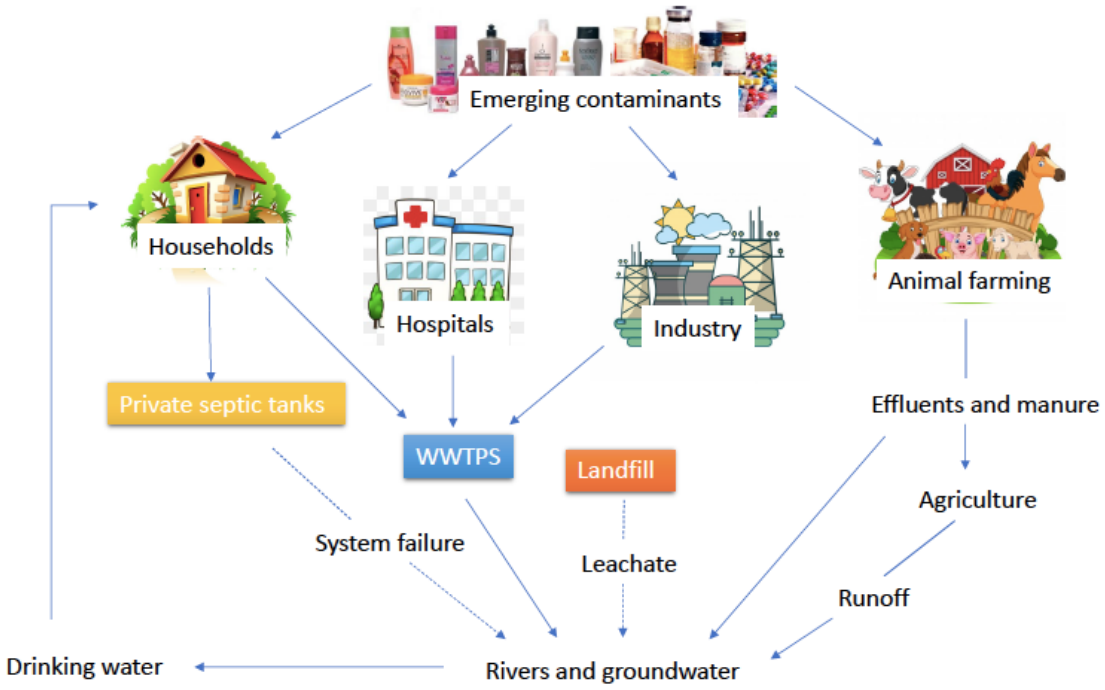

Figure 1. Potential sources and pathways of emerging compounds in the environment.

Source: Own elaboration using information from (Grassi et al., 2012)

1.1. Veterinary antibiotics

Antibiotics are a class of organic molecule that specifically interact with bacterial targets, inhibiting or killing microbes (Michael et al., 2012; Samal et al., 2022). However, the widespread and intensive use of antibiotics in human, veterinary, and agriculture settings has resulted in their emergence as a new class of water contaminant of emerging concern. Worldwide, the total use of antibiotics is estimated to be in the range of 100,000–200,000 tons per year (Fig.2) with approximately 70% used for animal husbandry (Bloem et al., 2017; Rocha et al., 2021).

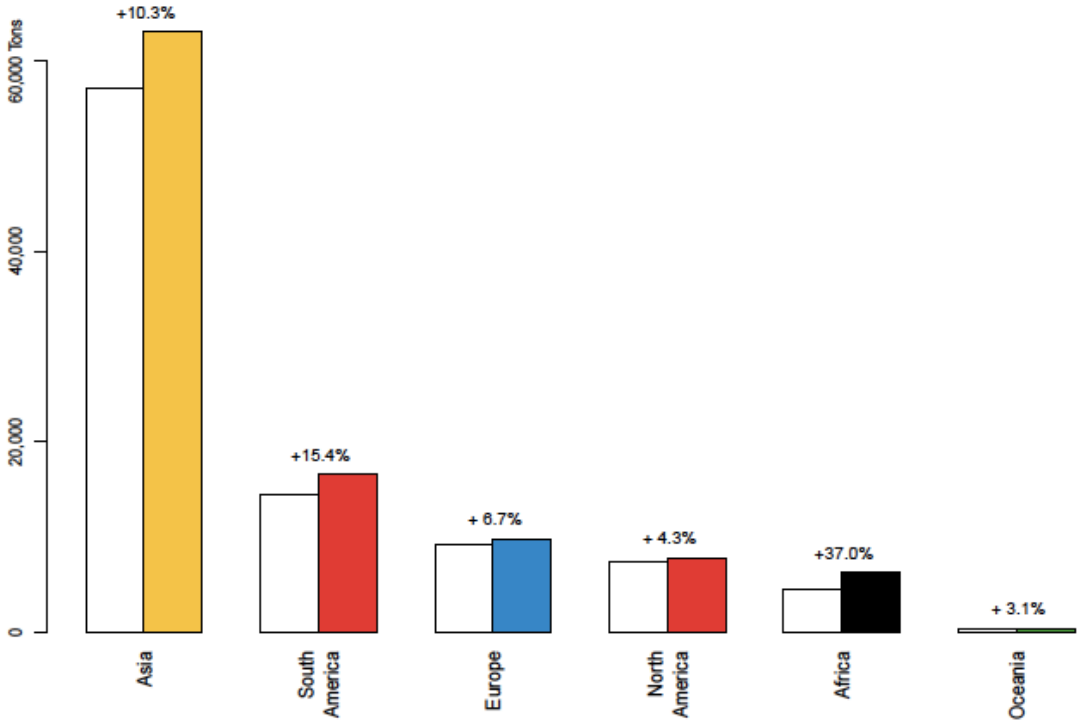

Figure 2. Estimated increase in antimicrobial use by continent in 2017 (white columns) and 2030 (colored columns). Source:(Tiseo et al., 2020)

In 2016, global consumption of veterinary antibiotics (VA) was 97,784 tons, with 61,170 tons consumed in Asia, the Far East, and Oceania, and 24,035 tons consumed in America (Michelon et al., 2022). This highlights the significant impact of veterinary antibiotic use on the global antibiotic load and the potential for VA to enter the environment as a water contaminant. Overall, the widespread use and subsequent release of antibiotics into the environment underscores the importance of effective monitoring and management strategies to mitigate their impact on the environment and public health.

The main groups of antibiotics used in various settings include aminoglycosides, β-lactams, ionophores, peptides, peptidomimetics, cephalosporines, fluorochinolones, lincosamides, macrolides, sulphonamides and tetracyclines (Kuppusamy et al., 2018). In swine production specifically, Cheng et al. (2020), reported that sulfonamides and

tetracyclines were the most commonly used VA (Tiseo et al., 2020). Similarly, another study found that sulfonamides and tetracyclines accounted for the largest amounts of VA sales in European countries in 2018 (European Medicines Agency, 2019). Studies conducted in China demonstrated that fluoroquinolones, sulfonamides and tetracyclines were widely used as veterinary medicines (Li et al., 2022; Tong et al., 2009).

The majority of VA are not completely metabolized by animals and are excreted unchanged or as their metabolites in feces and urine, which are commonly used as fertilizers (Kuppusamy et al., 2018; Li et al., 2022; Rocha et al., 2021). Moreover, water bodies contaminated by VA runoff from agricultural soils are often used for crop irrigation, raising concerns about the potential impacts of VA on agro-ecosystems (Kuppusamy et al., 2018; Rocha et al., 2021). The increasing use of swine manure in agriculture is becoming a major environment threat as it can lead to antibiotic resistance (Chaturvedi et al., 2021; Rocha et al., 2021). In 2019, antibiotic resistance has been linked to 5 million human deaths by The Food and Agriculture Organization of the United Nations (FAO), and it is projected to increase to 10 million by 2050 (Chaturvedi et al., 2021; Gundran et al., 2019).

To address this issue, responsible and sustainable use of antibiotics in animal husbandry and agriculture is crucial to minimize the release of VA into the environment and to prevent the development of antibiotic resistance in bacteria. Proper manure management practices, such as composting and treatment, can reduce the release of VA into the environment while also producing nutrient-rich fertilizers. Moreover, improved wastewater treatment and regulation of agricultural practices can help to reduce the potential environmental and health impacts of VA.

2. Treatment technologies for VA removal

Treatment technologies for VA include physical, chemical, biological, and advanced oxidation processes. Physical methods involve the use of naturally occurring forces, such as van der Waals forces, electrical attraction, and gravity, or physical barriers to remove the pollutants. Chemical methods rely on the properties of the pollutant, such as volatility or solubility, or the tendency to react between treated chemicals and contaminants. Biological methods involve breaking down organic pollutants using normal cellular processes (Phoon et al., 2020). Advanced oxidation processes (AOPs) are based on generating reactive oxygen species (ROS), particularly hydroxyl radicals (OH•), which are highly reactive and non-selective. These ROS can oxidize pollutants into less harmful and more biodegradable compounds, and some organic compounds can be fully oxidized into carbon dioxide (CO_2) and water (H_2O) (Fast et al., 2017). The effectiveness of VA removal will depend on their physicochemical properties, biological persistency, and operational conditions (Zambrano et al., 2022).

2.1. Physical processes

Physical processes including sedimentation, coagulation, membrane treatment, distillation, adsorption, filtration and reverse osmosis, have been used to treat VA. Saucier et al. (2015) used cocoa shell to produce activated carbon for treating simulated hospital effluents and achieved a 95.58% removal efficiency for a mixture of different organic compounds, including diclofenac and nimesulide. Ben et al. (2014) studied the adsorption behaviour of sulfamethazine using activated sludge to treat swine wastewater and obtained a removal efficiency of around 40% after 6 hours, when the adsorption of sulfamethazine to the activated sludge reached equilibrium. Taheran et al. (2016) demonstrated that reverse osmosis can remove 75% of pharmaceutically active compounds while membrane bioreactors showed diverse removal efficiencies from 0 to

90%. Although these processes are technologically simple and flexible, physical processes have been shown to be unable to break down ECs and only transfer the pollutants from one phase to another. Adsorption onto activated carbons is one of the most widely used methods as it gives the best results in terms of efficiency and technical feasibility at the industrial scale (Ahmed et al., 2021; Crini and Lichtfouse, 2019).

2.2. Chemical processes

Chemical processes include catalysis, electrolysis, ion exchange, neutralization, ozonation, Fenton oxidation, among others. López-Ortiz et al. (2018) used anion exchange resins and achieved removals of 66% for bisphenol A and lower than 15 % for carbamazepine (CBZ), atrazine, simazine and estrone. Iakovides et al. (2019) obtained over 99% removal of a mixture of eight antibiotics (ampicillin, azithromycin, erythromycin, clarithromycin, ofloxacin, sulfamethoxazole, trimethoprim and tetracycline) by ozonation with low ozone doses applied for 40 min. De Souza Santos et al. (2015) used Fenton oxidation and UV/H_2O_2 for the removal of norfloxacin, obtaining degradation rates of 100% and 60%, respectively, and mineralization rates of 55% and 32%. Chemical processes are simple, rapid and efficient processes that have been well-established and widely tested. However, their requirement of pretreatment, the addition of chemicals, sludge production or the generation of unknown intermediates are some of their disadvantages (Ahmed et al., 2021; Crini and Lichtfouse, 2019).

2.3. Biological processes

Biological processes used for wastewater treatment include anaerobic digestion, aerated lagoons, activated sludge, fungal treatment, trickling filters, stabilization, among others. In a study by Feng et al. (2017), the removal of sulfonamides (sulfadiazine, sulfamethizole, sulfamethoxazole), macrolides (clarithromycin, erythromycin) and

trimethoprim during the anaerobic digestion of pig manure was investigated. The study found no biodegradation of sulfadiazine and sulfamethizole, while sulfamethoxazole, erythromycin and trimethoprim were degraded rapidly. In another study, Lucas et al. (2016) treated veterinary hospital effluent containing forty-seven antibiotics from seven different groups (β-lactams, fluoroquinolones, macrolides, metronidazoles, sulfonamides, tetracyclines, and trimethoprim) in a fungal bioreactor. The study found that 77% of antibiotics were removed. A et al. (2021) evaluated the removal efficiency of sulfonamides, quinolones, tetracyclines, and macrolides antibiotics from sewage treatment plant effluent in a hybrid constructed wetland (HCW) and a layered biological filter (LBF). The study showed that the removal efficiencies of antibiotics were 70–95% by HCW and 62-64% by LBF for quinolones; 58–77% by HCW and 13-25% by LBF for macrolides; 59–67% of tetracyclines in both systems; and 0% of sulfonamides in both systems. Biological treatment is a more cost-effective well-accepted method for wastewater treatment than chemical or physical treatments (Ahmed et al., 2021; Crini and Lichtfouse, 2019). However, even though biological oxidation is one of the most widely used technologies for ECs removal and is highly advantageous for wastewater remediation, some ECs can inhibit biodegradation due to their toxicity and resistance to microbial growth (Ahmed et al., 2017; Mirzaei et al., 2016; Zhao et al., 2018).

2.3.1. Microalgae based technology

Microalgae-based technology has gained the attention of researchers as an eco-friendly wastewater treatment process. It has lower operational and capital costs, provides natural disinfection, and is more efficient in removing nutrients compared to traditional wastewater treatment processes (Ahmed et al., 2022; Sutherland and Ralph, 2019; Zhou et al., 2023). Algae-bacteria consortium can fix CO_2, reducing the emission of greenhouse gases, and assimilate nutrients from wastewater for their growth. Additionally, the algae

biomass can be harvested to produce algae-derived products such as biofuel, biofertilizer, proteins, carbohydrates, pigments, and vitamins (Abdelfattah et al., 2023; Leng et al., 2020; Xiong et al., 2021).

Microalgae-based technology has been proven to be efficient for the removal of heavy metals through surface sorption, bioaccumulation, and precipitation mechanisms. Yang et al. (2015) investigated the removal of Zn, Mn, Cd and Cu by *Chlorella minutissima* UTEX2341 and obtained removal rates of 62.05%, 83.68%, 74.34% and 83.60%, respectively. The oleaginous microalgae efficiently removed these metals mainly through intracellular accumulation and partly through extracellular immobilization. Additionally, Balaji et al. (2015) found that the removal of heavy metals by microalgal biomass involves bio-reduction and bio-sorption mechanisms. They used microalgal species, namely Anabaena, Oscillatoria, Phormidium, and Spirogyra, for Cr(VI) removal and obtained removal rates of 70.96%, 80.64%, 76.12%, and 74.83% for bio-reduction, respectively; and 75.48%, 80.64%, 79.35%, and 77.41% for bio-sorption, respectively.

Additionally, microalgae-based technology has demonstrated its effectiveness in the removal of ECs. Ferrando and Matamoros, (2020) obtained 50 and 65% removal of antibiotics (sulfacetamide, sulfamethazine, and sulfamethoxazole) and pesticides (bromacil, atrazine, diuron, bentazone, and mecoprop) in batch mode and continuous feeding, respectively. Villar-Navarro et al. (2018) detected 64 pharmaceuticals in the influent of a wastewater treatment plant (WWTP) with an average concentration of 223 µg/L and treated it in a high-rate algae pond (HRAP), obtaining 94% removal. Through a literature review, Liu et al. (2021) evaluated 114 micropollutants and 16 microalgae

species and determined that 94 were removed by ≥ 50%. Moreover, macrolides, hormone active substances and cephalosporins presented removals higher than 80%.

2.3.1.1. Removal mechanisms in ECs removal by microalgae

The main mechanisms involved in the removal of VA by microalgae include hydrolysis, photodegradation, biosorption and biodegradation (Fig. 3). The efficacy of an algal treatment system in removing antibiotics was evaluated by de Wilt et al. (2016), who obtained removal rates of 60–100% for diclofenac, ibuprofen, paracetamol and metoprolol. However, the removal rates for carbamazepine and trimethoprim were only around 30% and 60%, respectively. De Wilt et al. (2016) determined that the biodegradation and photolysis were the main mechanisms involved in the removal of these antibiotics, while sorption to algal biomass accounted for less than 20% of their removal. In a study by Yu et al. (2017), *C. pyrenoidosa* was used to remove ceftazidime and 7-aminocephalosporanic acid, with removal rates of 92.70% and 96.07%, respectively. Yu et al. (2017) identified a three-step mechanism involved in the algal removal of these antibiotics: first, a rapid adsorption; second, slow cell wall-transmission; and finally biodegradation.

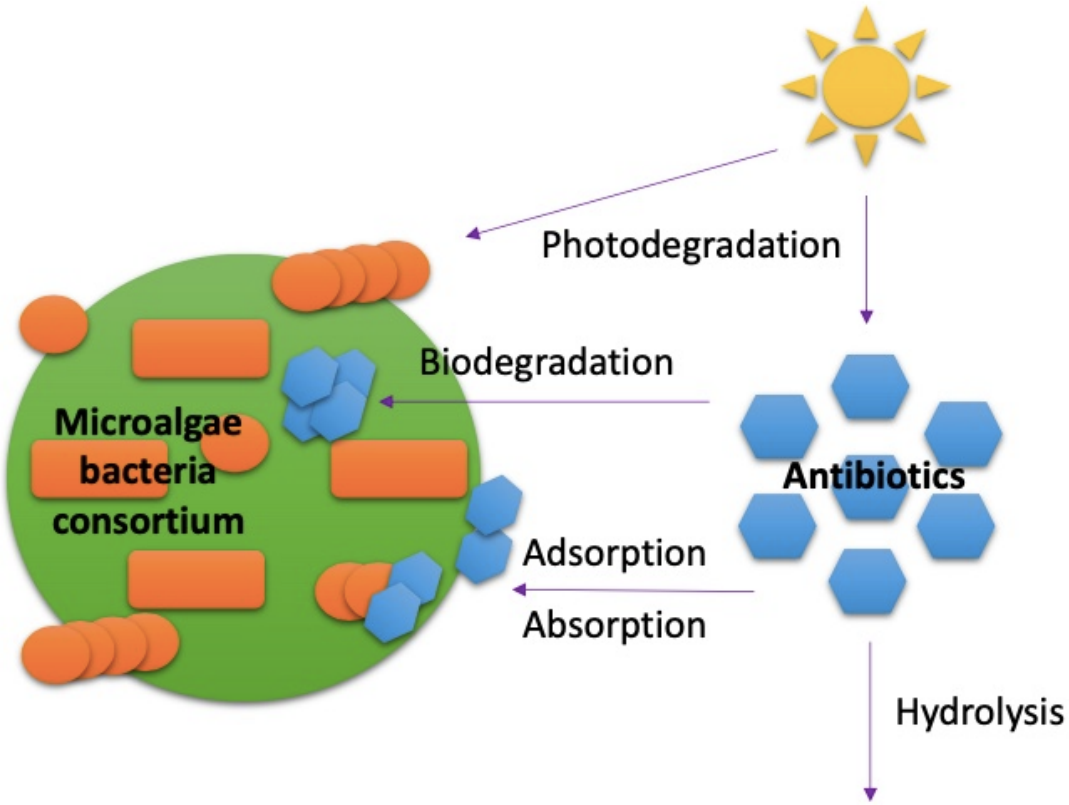

Figure 3. Mechanisms involved in the removal of VA by microalgae-bacteria consortium. Source: own elaboration based on (Leng et al., 2020; Wang et al., 2017).

Diverse literature exists on the mechanisms involved in VA removal (Table 1). However, most of these studies focus on the presence or absence of microalgae in the media or if the experiments were performed in darkness, without distinguishing between mechanisms. For instance, biodegradation, bioaccumulation and biosorption are consider biotic factors/alga-mediate processes, while hydrolysis and photolysis are considered abiotic factors. Xiong et al. (2017b) used by *C. mexicana, C. pitchmannii, C. vulgaris* and *O. multisporus* to remove ciprofloxacin and obtained removal rates of 13%, 1.6%, 0% and 2% removal, respectively. The authors found that the removal was due to biodegradation, bioaccumulation and biosorption. Xiong et al. (2019) studied the elimination of sulfamethoxazole by *S. obliquus* and achieved 15.7 – 17.3% removal by biotic factors and less than 2% removal by abiotic factors. Bai and Acharya (2016)

investigated the removal of sulfamethoxazole and triclosan by *Nannochloris sp.* The algae mediated processes resulted in 32-37% removal of sulfamethoxazole, while photolysis and biosorption led to complete removal of triclosan. Bai and Acharya (2019) used *Nannochloris sp.* to remove of 17β-estradiol, 17α-ethinylestradiol, and salicylic acid and achieved 60% removal by photodegradation and biodegradation, and 63-100% removal of triclosan by photodegradation, biosorption and bioaccumulation. Guo et al. (2016) studied the removal of 7-amino cephalosporanie acid by *Chlorella* sp., *Chlamydomonas* sp. and *Mychonastes* sp and obtained 75% removal by hydrolysis, biosorption and photolysis.

Table 1. Mechanisms involved in the removal of emerging contaminants by microalgae.

Antibiotic	**Microalgae**	**Operation Time**	**Degradation Efficiency (%)**	**Degradation mechanism**	**References**
Tetracycline	Algae-bacteria consortia from a HRAP	14 h	97	Biosorption	(Norvill et al., 2017)
Tetracycline	*C. vulgaris*	43 h	81 50-71	Photolysis Biosorption	(de Godos et al., 2012)
Ciprofloxacin	*C. mexicana* *C. pitchmannii* *C. vulgaris* *O. multisporus*	11 d	13.0±1.3 1.6±0.3 Not observed 2.0±0.2	Biodegradation, bioaccumulation, biosorption	(Xiong et al., 2017b)
Ciprofloxacin Sulfamethoxazole	*Nannochloris sp.*	7 d 14 d	100 11	Photolysis Biosorption	(Bai and Acharya, 2017)
Ciprofloxacin	Algae-bacteria consortia from a HRAP	111 h 48 h 48 h	52.88 97.25 83.78	Biosorption Photolysis Biodegradation	(Hom-Diaz et al., 2017)
Ciprofloxacin	*Chlamydomonas sp.*	9 d	2.38 27.07 5.50 65.05	Hydrolysis Photolysis Biosorption Biodegradation	(Xie et al., 2020)
Sulfadiazine	*Chlamydomonas sp.*	9 d	1.34 35.60 0.52 17.05	Hydrolysis Photolysis Biosorption Biodegradation	(Xie et al., 2020)
Sulfamethoxazole	*S. obliquus*	12 d	15.7-17.3 0-2	Biotic factors Abiotic factors	(Xiong et al., 2019)
Climbazole	*Scenedesmus obliquus*	12 d	88	Biodegradation	(Pan et al., 2018)
Sulfamethoxazole Triclosan	*Nannochloris sp.*	14 d	32-37 100	Algae - mediated process Photolysis and biosorption	(Bai and Acharya, 2016)

17β-estradiol, 17α-ethinylestradiol, and salicylic acid	*Nannochloris sp.*	7 d	60	Photodegradation and biodegradation	(Bai and Acharya, 2019)
Triclosan	*Nannochloris sp.*	7 d	63-100	Photodegradation Biosorption Bioaccumulation	(Bai and Acharya, 2019)
7-amino cephalosporanie acid	*Chlorella sp.*, *Chlamydomonas sp.*, *Mychonastes sp*	24 h	75	Biosorption, hydrolysis, photolysis	(Guo et al., 2016)
Bisphenol A	*Chlamydomonas mexicana* *Chlorella vulgaris*	10 d	23 24	Biodegradation	(Ji et al., 2014)
Carbamazepine Diclofenac Ibuprofen Metoprolol Paracetamol	*Chlorella sorokiniana*	30 d	10-30 40-60 100 100 100	Biosorption Photolysis Photolysis Biodegradation Biodegradation, photolysis	(de Wilt et al., 2016)
Levofloxacin	*Chlorella vulgaris*	11 d	12-90	Biodegradation	(Xiong et al., 2017a)
Progesterone	*S. obliquus* *C. pyrenoidosa*	5 d	95	Biodegradation	(Peng et al., 2014)
Tramadol	*Scenedesmus obliquus* modified using alkaline solution	45 min	91	Biosorption	(Ali et al., 2018a)
Triclosan	*Chlorella pyrenoidosa*	96 h	77.2	Biosorption	(Wang et al., 2013)

2.3.1.1.1. Hydrolysis

Hydrolysis plays an important role in predicting the environmental persistence of antibiotics. For amides and esters, hydrolysis can be a significant degradation pathway (Zhou et al., 2022). Therefore, antibiotics containing these functional groups may bioaccumulate less than the parent compound due to their high polarity, resulting in low octanol-water partition coefficients and highwater solubility (Phoon et al., 2020).

Hydrolysis can breakdown some ionic antibiotics, and its efficacy will depend on the pH and temperature of the aquatic system. Additionally, hydrolysis can be induced by algal metabolites such as enzymes. In a microalgae-based technology, hydrolysis can increase the polarity and hydrophilicity of antibiotics, facilitating their solubilization and biodegradation by microalgae (Leng et al., 2020).

Xie et al. (2020) used *Chlamydomonas sp*. and achieved a 100% and 54.53% removal of ciprofloxacin and sulfadiazine, respectively. The results showed that hydrolysis only represented 2.38% and 1.34% of the total removal, respectively. Additionally, Norvill et al. (2017) and de Godos et al. (2012) investigated the removal of tetracycline by microalgae and determined that hydrolysis was not significant.

2.3.1.1.2. Photodegradation

Photodegradation of antibiotics can occur through two pathways: direct photolysis and indirect photolysis. Direct photolysis involves the direct breakdown of the target compound by absorbing incident light. Indirect photolysis, on the other hand, is induced by reactive components produced by algae, such as hydroxyl radicals, peroxyl radicals, and singlet oxygen, in the presence of light (Leng et al., 2020; Zhou et al., 2022).

In natural waters and wastewater, indirect photolysis is more important for the removal of antibiotics due to the presence of photosensitizer such as chromophoric dissolved organic matters, NO_3^-, carbonate, and certain metal ions that promote the generation of free radicals (Leng et al., 2020; Wang et al., 2017).

Bai and Acharya (2017) and Hom-Diaz et al. (2017) achieved complete removal of ciprofloxacin while using microalgae. In both cases, the results showed that the main removal mechanism was photolysis. Wilt et al. (2016) achieved complete removal of ibuprofen and 40-60% removal of diclofenac by photolysis using *Chlorella sorokiniana*. De Godos et al. (2012) investigated the removal of tetracycline by C. *vulgaris*, reaching 81% removal by photolysis after 43 hours.

2.3.1.1.3. Biosorption

Biosorption occurs when antibiotics are adsorbed onto the cell wall of algae and bacteria. Polysaccharides such as cellulose, chitin, alginate, and glycan are present in the cell wall of algae, while peptidoglycan, teichoic acids and lipoteichoic acids are found on the surface of bacteria. Additionally, adsorption can be accomplished by organic substances excreted by algae and bacteria, known as extracellular polymeric substances (EPS), which include polysaccharides, proteins, nucleic acids, and lipids (Leng et al., 2020; Xiong et al., 2021; Zhou et al., 2023).

The process of biosorption occurs through hydrogen bonds, electrostatic attraction, partitioning, and the hydrophobic effect. The effectiveness of this process varies significantly depending on the physical and chemical properties of the biomass, such as surface chemistry and surface area, as well as the hydrophobicity, structure and functional groups of the antibiotics. Moreover, environmental conditions also play a crucial role (Leng et al., 2020; Wang et al., 2017; Xiong et al., 2021).

Biosorption processes transfer antibiotics residues from wastewater to biomass instead of breaking them down. Therefore, antibiotics residues should be considered if biomass from algae-based treatment technology is used to produce other valuable products, as they can still be hazardous (Abdelfattah et al., 2023; Wang et al., 2017).

Norvill et al. (2017) studied the biosorption of tetracycline using an algae-bacteria consortium from a HRAP and obtained 97% removal after 14 hours. Ali et al. (2018b) obtained 91% removal of tramadol by biosorption using modified *Scenedesmus obliquus* in just 45 minutes. Wang et al. (2013) achieved 77.2% removal of triclosan by biosorption onto *Chlorella pyrenoidosa* after 96 hours.

2.3.1.1.4. Biodegradation

Biodegradation is the process by which organic compounds are broken down through biotransformation, leading to the generation of metabolic intermediates or complete mineralization to CO_2 and H_2O. Microalgae-bacteria consortia contain enzymes that are capable of metabolizing a variety of pollutants, including antibiotics. Antibiotic degradation can occur through metabolic degradation, in which antibiotics are used as the sole carbon and energy source, or co-metabolic degradation, in which the degradation of antibiotics depends on the presence of non-specific enzymes catalyzing the metabolism of other substrates (Leng et al., 2020; Wang et al., 2017; Xiong et al., 2021).

The photosynthesis of algae releases O_2, which supports the aerobic degradation of antibiotics by bacteria. In turn, bacterial respiration produces CO_2 that is used by algae for photosynthesis. Additionally, algae produce organic compounds like amino acids and carbohydrates, which can support the heterotrophic metabolism and provide substrates for the co-metabolism of antibiotics. Bacteria also accelerate the regeneration of nutrients and trace elements, and release phytohormones that stimulate algae growth (Abdelfattah et al., 2023; Wang et al., 2017).

There is a wide range of results reported in the literature regarding the ability of microalgae to biodegrade antibiotics. For instance, Pan et al. (2018) investigated the biodegradation of climbazole by *Scenedesmus obliquus* and achieved an efficiency of 88% after 12 days. Ji et al. (2014) studied the removal of Bisphenol A by *Chlamydomonas Mexicana* and *Chlorella vulgaris*, achieving a biodegradation of 23% and 24%, respectively, after 10 days. Xiong et al. (2017a) reported biodegradations rates between 12% and 90% for the microalgae treatment of levofloxacin by *Chlorella vulgaris* after 11

days. Peng et al. (2014) reached a 95% biodegradation of progesterone after 5 days using *S. obliquus* and *C. pyrenoidosa.*

2.4. Advanced oxidation processes (AOPs)

Advanced oxidation processes (AOPs) such as photo-Fenton oxidation, electrochemical degradation and photocatalytic processes can effectively eliminate ECs, avoiding the production of secondary waste in the environment (Zambrano et al., 2022). Fiorentino et al. (2019) studied the removal of ten different antibiotics (azithromycin, ciprofloxacin, clarithromycin, erythromycin, lincomycin, levofloxacin, enrofloxacin, doxycycline, clindamycin and metronidazole) by solar photo-Fenton process and found that 7 out of 10 antibiotics were efficiently removed (60–100%), after 3 hours. Ormeno-Cano and Radjenovic (2022) studied the removal of antibiotics using a flow-through electrochemical system equipped with a boron-doped reduced graphene oxide sponge anode and a nitrogen-doped RGO sponge cathode, and achieved removal efficiencies of 82% for sulfamethoxazole, 85% for ofloxacin, 74.7% for erythromycin and 46% for trimethoprim. Kanakaraju et al. (2015) demonstrated high degradation efficiency (99%) in different water matrices using photocatalysis with TiO_2, with an enhacement of photocatalytic degradation in the presence of chloride. Although AOPs can remove significant quantities of ECs from wastewater, they are not yet applied at an industrial scale due to high energy consumption, high materials costs, and the use of additional chemicals (Dong et al., 2022; Xiong et al., 2021).

2.4.1. Photocatalysis

Photocatalysis involves the interaction between a semiconductor and a light source to generate reactive oxygen species (ROS) capable of deactivating microorganisms and transforming pollutants. The process of photocatalytic oxidation is

triggered by UV illumination of a semiconductor catalyst. However, utilizing sunlight radiation as the light source would considerably decrease the amount of electric power required, thus lowering water treatment expenses. Catalyst fouling and recovery are the primary drawbacks of photocatalysis, which can be mitigated through doping or immobilization techniques. The most commonly used photocatalysts are single metal oxides, including titanium dioxide (TiO_2), zinc oxide (ZnO), zirconia (ZrO_2), iron (III) oxide (Fe_2O_3), vanadium (V) oxide (V_2O_5), and tungsten trioxide (WO_3) , either alone or in combination with graphene oxide (GO) or carbon nanotubes (CNT) (Aoudj et al., 2019; Fast et al., 2017; Gomes et al., 2019).

Photocatalytic reactions are initiated by exciting a semiconductor photocatalyst (SP) through irradiation with photons that have an equal or greater amount of energy than its band gap (Fig. 4). Photo-generated electrons in the conduction band excite electrons from the valence band to the conduction band, producing holes (h^+) in the valence band (Eq.1). The electron holes produced can directly oxidize organic matter or react with either water or hydroxyl ions to generate hydroxyl radicals ($OH^\bullet$) which are strong, highly reactive, and non-selective oxidants (Eq.2-3). The conduction band electron can reduce dissolved oxygen species to form superoxide radical anions (Eq.4). Superoxide radical anions can then react with water to produce hydrogen peroxide and hydroxyl ions (Eq.5). In addition, further hydroxyl radicals and hydroxyl ions are generated by the cleavage of hydrogen peroxide (Eq.6) (Arora et al., 2022; Lofrano et al., 2017; Nguyen et al., 2020).

$$SP + hv \rightarrow SP(e^- + h^+) \quad (1)$$

$$SP\ (h^+) + H_2O \rightarrow H^+ + OH^\bullet \quad (2)$$

$$SP\ (h^+) + OH^- \rightarrow OH^\bullet \quad (3)$$

$$SP\ (e^-) + O_2 \rightarrow O_2^{\bullet -} \quad (4)$$

$$O_2^{\bullet -} + H_2O + H^+ \rightarrow H_2O_2 + OH^- \quad (5)$$

$$H_2O_2 + e^- \rightarrow OH^\bullet + OH^- \quad (6)$$

The photocatalytic degradation of organic substances, such as EC, can be achieved through direct electron transfer between the pollutant and the positive holes or indirectly through the generation of oxidizing species. In real wastewater, other species, such as Cl^-, HCO_3^-, CO_3^{2-} and/or SO_4^{2-} are present, which can be reduced and produce radicals, thereby improving the degradation of pollutants. In addition, several authors have described the recombination of photo-generated electrons and holes, which can cause a pre-adsorption of substrate onto the photocatalyst, thereby enhancing the photocatalytic degradation (Aoudj et al., 2019; Gomes et al., 2019; Lofrano et al., 2017; Nguyen et al., 2020).

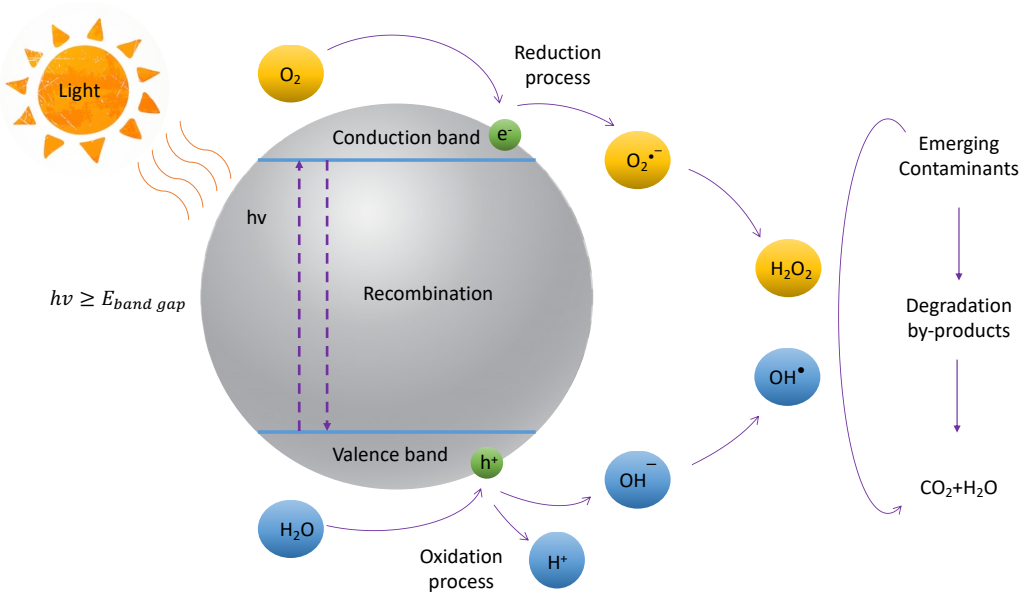

Figure 4. Photocatalysis mechanism for emerging contaminants removal. Source: own elaboration based on (Nguyen et al., 2020)

TiO_2 is the most widely investigated photocatalysts due to its cost effectiveness, abundance in earth, photo stability, low-toxicity, and good performance. TiO_2 is considered the most efficient photocatalyst for the removal of bacteria and organic contaminants, and it can operate at natural pH, ambient temperature and pressure (Ahmed et al., 2017; Ahmed and Haider, 2018; Arfanis et al., 2017; Davididou et al., 2017; Philippe et al., 2016). Since the 1970s, TiO_2 has been used for environmental purposes. However, the application of TiO_2 in the photodegradation of emerging contaminants dates form 2000s (Belver et al., 2020). For instance, Cai and Hu (2017) obtained a 90% removal of sulfamethoxazole and trimethoprim within 20 min with the initial concentration of 400 ppb for both antibiotics. Ounnar et al. (2016) achieved a 98% removal of the spiramycin macrolide antibiotic by determining its optimum conditions at low UV power, small SPM concentrations, natural pH and a catalyst dose of 1g/L. Eskandarian et al. (2016a) analyzed the photolytic decomposition of acetaminophen, diclofenac, ibuprofen, and sulfamethoxazole and determined that the decomposition kinetics followed the order sulfamethoxazole > diclofenac > ibuprofen > acetaminophen with removals from 26% to 58% in three hours of operation. However, more research remains to be done as some discordant results have been observed between authors (Table 2). For example, N-TiO_2 was used for the removal of methylene blue, and Than et al. (2017) obtained 99.4% within 90 min, while Liu et al. (2016) achieved only 71.1% removal after 180 min, even though N-TiO_2 was coupled with graphene to improve its performance. Similarly, for the removal of bisphenol A, López-Serna et al. (2017) obtained only 50% using visible light after 3 hours.

One of the major drawbacks of TiO_2 is that it can only utilize the UV spectrum, limiting its efficiency in wastewater treatment processes. Moreover, the higher concentration of contaminants, the presence of other ions and oxidants can cause catalyst

deactivation, slow kinetics, and low photo-efficiencies. However, these disadvantages can be overcome by coupling TiO_2 with other semiconductors oxides or by doping the catalyst (Arora et al., 2022; Fast et al., 2017; Kanakaraju and Chandrasekaran, 2023). Ioannidou et al. (2017) found that WO_3 modifies the properties of TiO_2 and enhances its photocatalytic activity towards the degradation of sulfamethoxazole. Ananpattarachai et al. (2016) showed that co-doping TiO_2 with carbon and nitrogen leads to a higher formation rate of $OH^{\bullet}$ and photocatalytic activity than those only doped with carbon or nitrogen. C,N co-doped TiO_2 achieved 95% of 2-chlorophenol degradation compared to 80% for C-doped TiO_2 and 70% for N-doped TiO_2. Petala et al. (2015) demonstrated the efficiency of N-doped TiO_2 by obtaining up to 100% removal of ethyl paraben in different water matrices, including ultrapure water, water spiked with humic acids or bicarbonates, drinking water and secondary treated wastewater.

The increasing concern on ECs in the environment has raised researcher's attention on environmentally friendly process which can be cost effective for the removal of these organic pollutants. Among AOPs, photocatalytic processes has shown to be a suitable choice since catalysts can present good activity under sunlight radiation, reducing operational costs.

Table 2. Comparative review of TiO_2 application for the removal of emerging contaminans.

Catalyst	Contaminant	Water Matrix	Light source	Irradiation Time	Degradation Efficiency (%)	pH	References
1 g/L **N-TiO_2** **C- TiO_2** **C,N TiO_2**	100 mg/L 2-chlorophenol	Deionized water	Visible light	30 min	70 80 95	7	(Ananpattarachai et al., 2016)
0.1 and 1 g/L **N- TiO_2**	150 and 900 □g/L Ethyl paraben	Ultrapure water, water spiked with humic acids or bicarbonates, drinking water and secondary treated wastewater	Solar and visible light	120 min	Up to 100	3-9	(Petala et al., 2015)
2 g/L **Sm-TiO_2** **N-TiO_2**	50 mg/L 4-Acetamidophenol	Double distilled water	UV and visible light	180 min	60 63	6.8	(Rajoriya et al., 2019)
1 g/L **Graphene oxide- TiO_2 composites**	100 mg/L Diphenhydramine	Synthetic solution	UV/Vis radiation	60 min	92–94	-	(Pedrosa et al., 2018)
0.5 g/L **N-TiO_2**	5 mg/L Clopyralid, triclopyr and picloram	Milli-Q water	Black light	180 min	95	4	(Solís et al., 2016)
0.05 g/L **TiO_2**	400 ug/L Sulfamethoxazole and trimethoprim	Milli-Q water	UVA/LED	20 min	90	5.6	(Cai and Hu, 2017)
0.8 g/L **TiO_2/SnO_2**	20 mg/L Diclofenac	Synthetic solution	UV irradiation	300 min	90	5	(Mugunthan et al., 2019)
10 mg **TiO_2/SnO_2**	10 mg/L Rhodamine B	Synthetic solution	UV irradiation	15 min	100	-	(Yuan et al., 2015)
1 g/L **TiO_2**	10 mg/L Spiramycin macrolide antibiotic	Synthetic solution	UV irradiation	300 min	98	6.68	(Ounnar et al., 2016)
0.5 g/L **TiO_2**	20 mg/L Acetaminophen Diclofenac Ibuprofen Sulfamethoxazole	Milli-Q water	UV-LED	3 h	26 50 35 58	natural pH	(Eskandarian et al., 2016a)
150 mg **N-TiO_2**	10 mg/L Methylene blue	Synthetic solution	Visible light	90 min	99.4	-	(Than et al., 2017)
80 mg **TiO_2/G** **N- TiO_2/G** **N-TiO_2/NG**	10 mg/L Methylene blue	Synthetic solution	Visible light	180 min	59.8 71.1 87.9	-	(Liu et al., 2016)
100 mg **N–TiO_2**	30 mg/L Bisphenol A	Synthetic solution	Visible light	3 h	50	5.5	(López-Serna et al., 2017)

0.1 g Cu, N co-doped TiO_2	3 x 10^{-5} M Methyl orange	Synthetic solution	Visible light	6 h	92	6	(Reda et al., 2020)
TiO_2 nanotubular films	15 mg/L Caffeine 17.3 mg/L Salicylic acid	Synthetic solution	UV irradiation	240 min	50	Natural pH	(Arfanis et al., 2017)
50 mg/L N-TiO_2	5 mg/L Saccharin Bisphenol-A	Synthetic solution	UV irradiation	90 min	98 99	4.6 6.4	(Davididou et al., 2017)
1.5 g/L N,F-TiO_2	10 mg/L Acid red B	Synthetic solution	Visible light	4 h	100	5.6	(Guo et al., 2017)
300 mg/L carbon modified TiO_2	5 mg/L Tetracycline hydrochloride	Synthetic solution	White-LED light	2 h	74	7	(Oseghe and Ofomaja, 2018)
100 mg NeTiO_2	20 mg/L Rhodamine B dye	Synthetic solution	Sunlight irradiation	300 min	97.36	7	(Barkul et al., 2016)
250 mg/L TiO_2/AC[1]	5 mg/L Acetaminophen	Synthetic solution	Simulated sunlight	6 h	100	6.9	(Peñas-Garzón et al., 2019)
1 g/L Bi-TiO_2 / PAC[2]	20 mg/L Sulfamethazine	Ultra-pure water	Visible light	300 min	81.18	6	(Wang et al., 2019)
0.43 g Zeolite-TiO_2	20 mg/L Reactive black 5, cefiximetrihydrate, and phenazopyridine	Synthetic solution	UV-LED	40 min	60	7	(Eskandarian et al., 2016b)
0.5 mg/L TiO_2–RGO[3]	10 mg/l Bisphenol A	Simulated wastewater	UV	45 min	98.4	-	(Liao et al., 2016)

[1]AC: Activated carbon; [2]PAC: Powder activated carbon; [3]RGO: Reduced graphene oxide

References

A, D., Chen, C., Zou, M., Deng, Y., Zhang, X., Du, J., Yang, Y., 2021. Removal efficiency, kinetic, and behavior of antibiotics from sewage treatment plant effluent in a hybrid constructed wetland and a layered biological filter. J. Environ. Manage. 288, 112435. https://doi.org/10.1016/J.JENVMAN.2021.112435

Abdel-Maksoud, Y.K., Imam, E., Ramadan, A.R., 2018. Sand supported TiO2 photocatalyst in a tray photo-reactor for the removal of emerging contaminants in wastewater. Catal. Today 313, 55–62. https://doi.org/10.1016/j.cattod.2017.10.029

Abdelfattah, A., Ali, S.S., Ramadan, H., El-Aswar, E.I., Eltawab, R., Ho, S.H., Elsamahy, T., Li, S., El-Sheekh, M.M., Schagerl, M., Kornaros, M., Sun, J., 2023. Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. Environ. Sci. Ecotechnology 13, 100205. https://doi.org/10.1016/j.ese.2022.100205

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J., 2017. Progress

in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: A critical review. J. Hazard. Mater. 323, 274–298. https://doi.org/10.1016/j.jhazmat.2016.04.045

Ahmed, S.F., Mofijur, M., Nuzhat, S., Chowdhury, A.T., Rafa, N., Uddin, A., Inayat, A., Mahlia, T.M.I., Chyuan Ong, H., Chia, W.Y., Loke Show, P., 2021. Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. https://doi.org/10.1016/j.jhazmat.2021.125912

Ahmed, S.F., Mofijur, M., Parisa, T.A., Islam, N., Kusumo, F., Inayat, A., Le, V.G., Badruddin, I.A., Khan, T.M.Y., Ong, H.C., 2022. Progress and challenges of contaminate removal from wastewater using microalgae biomass. Chemosphere 286, 131656. https://doi.org/10.1016/j.chemosphere.2021.131656

Ahmed, S.N., Haider, W., 2018. Heterogeneous photocatalysis and its potential applications in water and wastewater treatment: A review. Nanotechnology 29, 342001. https://doi.org/10.1088/1361-6528/aac6ea

Ali, M.E.M., Abd El-Aty, A.M., Badawy, M.I., Ali, R.K., 2018a. Removal of pharmaceutical pollutants from synthetic wastewater using chemically modified biomass of green alga Scenedesmus obliquus. Ecotoxicol. Environ. Saf. 151, 144–152. https://doi.org/10.1016/j.ecoenv.2018.01.012

Ali, M.E.M., Abd El-Aty, A.M., Badawy, M.I., Ali, R.K., 2018b. Removal of pharmaceutical pollutants from synthetic wastewater using chemically modified biomass of green alga Scenedesmus obliquus. Ecotoxicol. Environ. Saf. 151, 144–152. https://doi.org/10.1016/J.ECOENV.2018.01.012

Ananpattarachai, J., Seraphin, S., Kajitvichyanukul, P., 2016. Formation of hydroxyl radicals and kinetic study of 2-chlorophenol photocatalytic oxidation using C-doped TiO2, N-doped TiO2, and C,N Co-doped TiO2 under visible light. Environ. Sci. Pollut. Res. 23, 3884–3896. https://doi.org/10.1007/s11356-015-5570-8

Aoudj, S., Drouiche, N., Khelifa, A., 2019. Emerging contaminants remediation by heterogeneous photocatalysis, in: Emerging and Nanomaterial Contaminants in Wastewater: Advanced Treatment Technologies. Elsevier Inc., pp. 245–275. https://doi.org/10.1016/B978-0-12-814673-6.00009-7

Arfanis, M.K., Adamou, P., Moustakas, N.G., Triantis, T.M., Kontos, A.G., Falaras, P.,

2017. Photocatalytic degradation of salicylic acid and caffeine emerging contaminants using titania nanotubes. Chem. Eng. J. 310, 525–536. https://doi.org/10.1016/j.cej.2016.06.098

Arora, I., Chawla, H., Chandra, A., Sagadevan, S., Garg, S., 2022. Advances in the strategies for enhancing the photocatalytic activity of TiO2: Conversion from UV-light active to visible-light active photocatalyst. Inorg. Chem. Commun. 143, 109700. https://doi.org/10.1016/j.inoche.2022.109700

Bai, X., Acharya, K., 2019. Removal of seven endocrine disrupting chemicals (EDCs) from municipal wastewater effluents by a freshwater green alga. Environ. Pollut. 247, 534–540. https://doi.org/10.1016/J.ENVPOL.2019.01.075

Bai, X., Acharya, K., 2017. Algae-mediated removal of selected pharmaceutical and personal care products (PPCPs) from Lake Mead water. Sci. Total Environ. 581–582, 734–740. https://doi.org/10.1016/j.scitotenv.2016.12.192

Bai, X., Acharya, K., 2016. Removal of trimethoprim, sulfamethoxazole, and triclosan by the green alga Nannochloris sp. J. Hazard. Mater. 315, 70–75. https://doi.org/10.1016/j.jhazmat.2016.04.067

Balaji, S., Kalaivani, T., Shalini, M., Gopalakrishnan, M., Rashith Muhammad, M.A., Rajasekaran, C., 2015. Sorption sites of microalgae possess metal binding ability towards Cr(VI) from tannery effluents—a kinetic and characterization study. New pub Balaban 57, 14518–14529. https://doi.org/10.1080/19443994.2015.1064032

Barkul, R.P., Koli, V.B., Shewale, V.B., Patil, M.K., Delekar, S.D., 2016. Visible active nanocrystalline N-doped anatase TiO2 particles for photocatalytic mineralization studies. Mater. Chem. Phys. 173, 42–51. https://doi.org/10.1016/j.matchemphys.2016.01.035

Belver, C., Bedia, J., Peñas-Garzón, M., Muelas-Ramos, V., Gómez-Avilés, A., Rodriguez, J.J., 2020. Structured photocatalysts for the removal of emerging contaminants under visible or solar light, in: Visible Light Active Structured Photocatalysts for the Removal of Emerging Contaminants. Elsevier, pp. 41–98. https://doi.org/10.1016/b978-0-12-818334-2.00003-1

Ben, W., Qiang, Z., Yin, X., Qu, J., Pan, X., 2014. Adsorption behavior of sulfamethazine in an activated sludgeprocess treating swine wastewater. J. Environ. Sci. 26, 1623–1629.

https://doi.org/https://doi.org/10.1016/j.jes.2014.06.002

Bloem, E., Albihn, A., Elving, J., Hermann, L., Lehmann, L., Sarvi, M., Schaaf, T., Schick, J., Turtola, E., Ylivainio, K., 2017. Contamination of organic nutrient sources with potentially toxic elements, antibiotics and pathogen microorganisms in relation to P fertilizer potential and treatment options for the production of sustainable fertilizers: A review. Sci. Total Environ. 607–608, 225–242. https://doi.org/10.1016/j.scitotenv.2017.06.274

Cai, Q., Hu, J., 2017. Decomposition of sulfamethoxazole and trimethoprim by continuous UVA/LED/TiO2 photocatalysis: Decomposition pathways, residual antibacterial activity and toxicity. J. Hazard. Mater. 323, 527–536. https://doi.org/10.1016/j.jhazmat.2016.06.006

Chaturvedi, P., Shukla, P., Giri, B.S., Chowdhary, P., Chandra, R., Gupta, P., Pandey, A., 2021. Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: A review on emerging contaminants. Environ. Res. 194, 110664. https://doi.org/10.1016/j.envres.2020.110664

Cheng, D., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Wei, Q., Wei, D., 2020. A critical review on antibiotics and hormones in swine wastewater: Water pollution problems and control approaches. J. Hazard. Mater. 387, 121682. https://doi.org/10.1016/j.jhazmat.2019.121682

Crini, G., Lichtfouse, E., 2019. Advantages and disadvantages of techniques used for wastewater treatment. Environ. Chem. Lett. 17, 145–155. https://doi.org/10.1007/s10311-018-0785-9

Davididou, K., Hale, E., Lane, N., Chatzisymeon, E., Pichavant, A., Hochepied, J.F., 2017. Photocatalytic treatment of saccharin and bisphenol-A in the presence of TiO2 nanocomposites tuned by Sn(IV). Catal. Today 287, 3–9. https://doi.org/10.1016/j.cattod.2017.01.038

de Godos, I., Muñoz, R., Guieysse, B., 2012. Tetracycline removal during wastewater treatment in high-rate algal ponds. J. Hazard. Mater. 229–230, 446–449. https://doi.org/10.1016/j.jhazmat.2012.05.106

de Souza Santos, L.V., Meireles, A.M., Lange, L.C., 2015. Degradation of antibiotics norfloxacin by Fenton, UV and UV/H2O2. J. Environ. Manage. 154, 8–12. https://doi.org/10.1016/J.JENVMAN.2015.02.021

de Wilt, A., Butkovskyi, A., Tuantet, K., Leal, L.H., Fernandes, T. V., Langenhoff, A., Zeeman, G., 2016. Micropollutant removal in an algal treatment system fed with source separated wastewater streams. J. Hazard. Mater. 304, 84–92. https://doi.org/10.1016/J.JHAZMAT.2015.10.033

Dong, G., Chen, B., Liu, B., Hounjet, L.J., Cao, Y., Stoyanov, S.R., Yang, M., Zhang, B., 2022. Advanced oxidation processes in microreactors for water and wastewater treatment: Development, challenges, and opportunities. Water Res. 211, 118047. https://doi.org/10.1016/j.watres.2022.118047

Eskandarian, M.R., Choi, H., Fazli, M., Rasoulifard, M.H., 2016a. Effect of UV-LED wavelengths on direct photolytic and TiO2 photocatalytic degradation of emerging contaminants in water. Chem. Eng. J. 300, 414–422. https://doi.org/10.1016/j.cej.2016.05.049

Eskandarian, M.R., Fazli, M., Rasoulifard, M.H., Choi, H., 2016b. Decomposition of organic chemicals by zeolite-TiO2 nanocomposite supported onto low density polyethylene film under UV-LED powered by solar radiation. Appl. Catal. B Environ. 183, 407–416. https://doi.org/10.1016/j.apcatb.2015.11.004

European Medicines Agency, 2019. Sales of veterinary antimicrobial agents in 31 European countries in 2017: Trends from 2010-2017. Ninth ESVAC Rep. - EMA/294674/2019 106.

Fast, S.A., Gude, V.G., Truax, D.D., Martin, J., Magbanua, B.S., 2017. A Critical Evaluation of Advanced Oxidation Processes for Emerging Contaminants Removal. Environ. Process. 4, 283–302. https://doi.org/10.1007/s40710-017-0207-1

Feng, L., Casas, M.E., Ottosen, L.D.M., Møller, H.B., Bester, K., 2017. Removal of antibiotics during the anaerobic digestion of pig manure. Sci. Total Environ. 603–604, 219–225. https://doi.org/10.1016/J.SCITOTENV.2017.05.280

Ferrando, L., Matamoros, V., 2020. Attenuation of nitrates, antibiotics and pesticides from groundwater using immobilised microalgae-based systems. Sci. Total Environ. 703. https://doi.org/10.1016/j.scitotenv.2019.134740

Fiorentino, A., Esteban, B., Garrido-Cardenas, J.A., Kowalska, K., Rizzo, L., Aguera, A., Pérez, J.A.S., 2019. Effect of solar photo-Fenton process in raceway pond reactors at neutral pH on antibiotic resistance determinants in secondary treated

urban wastewater. J. Hazard. Mater. 378, 120737. https://doi.org/10.1016/J.JHAZMAT.2019.06.014

Gogoi, A., Mazumder, P., Tyagi, V.K., Tushara Chaminda, G.G., An, A.K., Kumar, M., 2018. Occurrence and fate of emerging contaminants in water environment: A review. Groundw. Sustain. Dev. 6, 169–180. https://doi.org/10.1016/j.gsd.2017.12.009

Gomes, J., Lincho, J., Domingues, E., Quinta-Ferreira, R.M., Martins, R.C., 2019. N-TiO2 photocatalysts: A review of their characteristics and capacity for emerging contaminants removal. Water (Switzerland) 11, 373. https://doi.org/10.3390/w11020373

Grassi, M., Kaykioglu, G., Belgiorno, V., Lofrano, G., 2012. Emerging Compounds Removal from Wastewater Natural and Solar Based Treatments, Emerging Compounds Removal from Wastewater.

Gundran, R.S., Cardenio, P.A., Villanueva, M.A., Sison, F.B., Benigno, C.C., Kreausukon, K., Pichpol, D., Punyapornwithaya, V., 2019. Prevalence and distribution of bla CTX-M, bla SHV, bla TEM genes in extended- spectrum β-Lactamase- producing E. coli isolates from broiler farms in the Philippines. BMC Vet. Res. 15, 1–8. https://doi.org/10.1186/s12917-019-1975-9

Guo, Q., Zhang, Z., Ma, X., Jing, K., Shen, M., Yu, N., Tang, J., Dionysiou, D.D., 2017. Preparation of N,F-codoped TiO2 nanoparticles by three different methods and comparison of visible-light photocatalytic performances. Sep. Purif. Technol. 175, 305–313. https://doi.org/10.1016/j.seppur.2016.11.041

Guo, W.Q., Zheng, H.S., Li, S., Du, J.S., Feng, X.C., Yin, R.L., Wu, Q.L., Ren, N.Q., Chang, J.S., 2016. Removal of cephalosporin antibiotics 7-ACA from wastewater during the cultivation of lipid-accumulating microalgae. Bioresour. Technol. 221, 284–290. https://doi.org/10.1016/j.biortech.2016.09.036

Hom-Diaz, A., Norvill, Z.N., Blánquez, P., Vicent, T., Guieysse, B., 2017. Ciprofloxacin removal during secondary domestic wastewater treatment in high rate algal ponds. Chemosphere. https://doi.org/10.1016/j.chemosphere.2017.03.125

Iakovides, I.C., Michael-Kordatou, I., Moreira, N.F.F., Ribeiro, A.R., Fernandes, T., Pereira, M.F.R., Nunes, O.C., Manaia, C.M., Silva, A.M.T., Fatta-Kassinos, D., 2019. Continuous ozonation of urban wastewater: Removal of antibiotics,

antibiotic-resistant Escherichia coli and antibiotic resistance genes and phytotoxicity. Water Res. 159, 333–347. https://doi.org/10.1016/J.WATRES.2019.05.025

Ioannidou, E., Frontistis, Z., Antonopoulou, M., Venieri, D., Konstantinou, I., Kondarides, D.I., Mantzavinos, D., 2017. Solar photocatalytic degradation of sulfamethoxazole over tungsten – Modified TiO2. Chem. Eng. J. 318, 143–152. https://doi.org/10.1016/j.cej.2016.06.012

Ji, M.K., Kabra, A.N., Choi, J., Hwang, J.H., Kim, J.R., Abou-Shanab, R.A.I., Oh, Y.K., Jeon, B.H., 2014. Biodegradation of bisphenol A by the freshwater microalgae Chlamydomonas mexicana and Chlorella vulgaris. Ecol. Eng. 73, 260–269. https://doi.org/10.1016/J.ECOLENG.2014.09.070

Kanakaraju, D., Chandrasekaran, A., 2023. Recent advances in TiO2/ZnS-based binary and ternary photocatalysts for the degradation of organic pollutants. Sci. Total Environ. 868, 161525. https://doi.org/10.1016/j.scitotenv.2023.161525

Kanakaraju, D., Motti, C.A., Glass, B.D., Oelgemöller, M., 2015. TiO2 photocatalysis of naproxen: Effect of the water matrix, anions and diclofenac on degradation rates. Chemosphere 139, 579–588. https://doi.org/10.1016/j.chemosphere.2015.07.070

Kumar, R., Qureshi, M., Vishwakarma, D.K., Al-Ansari, N., Kuriqi, A., Elbeltagi, A., Saraswat, A., 2022. A review on emerging water contaminants and the application of sustainable removal technologies. Case Stud. Chem. Environ. Eng. 6, 100219. https://doi.org/10.1016/j.cscee.2022.100219

Kuppusamy, S., Kakarla, D., Venkateswarlu, K., Megharaj, M., Yoon, Y.E., Lee, Y.B., 2018. Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: A critical view. Agric. Ecosyst. Environ. 257, 47–59. https://doi.org/10.1016/j.agee.2018.01.026

L Opez-Ortiz, C.M., Sentana-Gadea, I., Var O-Galva, P.J., Maestre-P Erez, S.E., Prats-Rico, D., 2018. Effect of magnetic ion exchange (MIEX ®) on removal of emerging organic contaminants. https://doi.org/10.1016/j.chemosphere.2018.05.194

Leng, L., Wei, L., Xiong, Q., Xu, S., Li, W., Lv, S., Lu, Q., Wan, L., Wen, Z., Zhou, W., 2020. Use of microalgae based technology for the removal of antibiotics from

wastewater: A review. Chemosphere 238, 124680. https://doi.org/10.1016/j.chemosphere.2019.124680

Li, W., Li, Y., Zheng, N., Ge, C., Yao, H., 2022. Occurrence and distribution of antibiotics and antibiotic resistance genes in the guts of shrimp from different coastal areas of China. Sci. Total Environ. 815, 152756. https://doi.org/10.1016/j.scitotenv.2021.152756

Liao, G., Zhu, D., Zheng, J., Yin, J., Lan, B., Li, L., 2016. Efficient mineralization of bisphenol A by photocatalytic ozonation with TiO2–graphene hybrid. J. Taiwan Inst. Chem. Eng. 67, 300–305. https://doi.org/10.1016/j.jtice.2016.07.035

Liu, C., Zhang, L., Liu, R., Gao, Z., Yang, X., Tu, Z., Yang, F., Ye, Z., Cui, L., Xu, C., Li, Y., 2016. Hydrothermal synthesis of N-doped TiO2 nanowires and N-doped graphene heterostructures with enhanced photocatalytic properties. J. Alloys Compd. 656, 24–32. https://doi.org/10.1016/j.jallcom.2015.09.211

Liu, R., Li, S., Tu, Y., Hao, X., 2021. Capabilities and mechanisms of microalgae on removing micropollutants from wastewater: A review. J. Environ. Manage. 285. https://doi.org/10.1016/j.jenvman.2021.112149

Lofrano, G., Libralato, G., Brown, J., 2017. Nanotechnologies for environmental remediation: Applications and implications, Nanotechnologies for Environmental Remediation: Applications and Implications. https://doi.org/10.1007/978-3-319-53162-5

López-Serna, D., Suárez-Vázquez, S.I., Durán-Álvarez, J.C., Zanella, R., Guerra-Cobián, V.H., Cruz-López, A., 2017. Kinetic study of photocatalytic degradation of the emerging contaminant bisphenol A using N–TiO2 in visible light: a study of the significance of dissolved oxygen. React. Kinet. Mech. Catal. 122, 655–670. https://doi.org/10.1007/s11144-017-1200-4

Lucas, D., Badia-Fabregat, M., Vicent, T., Caminal, G., Rodríguez-Mozaz, S., Balcázar, J.L., Barceló, D., 2016. Fungal treatment for the removal of antibiotics and antibiotic resistance genes in veterinary hospital wastewater. Chemosphere 152, 301–308. https://doi.org/10.1016/J.CHEMOSPHERE.2016.02.113

Michael, I., Rizzo, L., Mcardell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2012. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review.

https://doi.org/10.1016/j.watres.2012.11.027

Michelon, W., Matthiensen, A., Viancelli, A., Fongaro, G., Gressler, V., Soares, H.M., 2022. Removal of veterinary antibiotics in swine wastewater using microalgae-based process. Environ. Res. 207, 112192. https://doi.org/10.1016/j.envres.2021.112192

Mirzaei, A., Chen, Z., Haghighat, F., Yerushalmi, L., 2016. Removal of pharmaceuticals and endocrine disrupting compounds from water by zinc oxide-based photocatalytic degradation: A review. Sustain. Cities Soc. 27, 407–418. https://doi.org/10.1016/j.scs.2016.08.004

Mugunthan, E., Saidutta, M.B., Jagadeeshbabu, P.E., 2019. Photocatalytic degradation of diclofenac using TiO2–SnO2 mixed oxide catalysts. Environ. Technol. (United Kingdom) 40, 929–941. https://doi.org/10.1080/09593330.2017.1411398

Nguyen, D.N., Bui, H.M., Nguyen, H.Q., 2020. Heterogeneous photocatalysis for the removal of pharmaceutical compounds, in: Current Developments in Biotechnology and Bioengineering. Elsevier B.V., pp. 161–183. https://doi.org/10.1016/B978-0-12-819594-9.00007-3

Norvill, Z.N., Toledo-Cervantes, A., Blanco, S., Shilton, A., Guieysse, B., Muñoz, R., 2017. Photodegradation and sorption govern tetracycline removal during wastewater treatment in algal ponds. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.02.011

Ormeno-Cano, N., Radjenovic, J., 2022. Electrochemical degradation of antibiotics using flow-through graphene sponge electrodes. J. Hazard. Mater. 431, 128462. https://doi.org/10.1016/J.JHAZMAT.2022.128462

Oseghe, E.O., Ofomaja, A.E., 2018. Study on light emission diode/carbon modified TiO2 system for tetracycline hydrochloride degradation. J. Photochem. Photobiol. A Chem. 360, 242–248. https://doi.org/10.1016/j.jphotochem.2018.04.048

Ounnar, A., Favier, L., Bouzaza, A., Bentahar, F., Trari, M., 2016. Kinetic study of spiramycin removal from aqueous solution using heterogeneous photocatalysis. Kinet. Catal. 57, 200–206. https://doi.org/10.1134/S0023158416020087

Pan, C.G., Peng, F.J., Ying, G.G., 2018. Removal, biotransformation and toxicity variations of climbazole by freshwater algae Scenedesmus obliquus. Environ.

Pollut. 240, 534–540. https://doi.org/10.1016/J.ENVPOL.2018.05.020

Pedrosa, M., Pastrana-Martínez, L.M., Pereira, M.F.R., Faria, J.L., Figueiredo, J.L., Silva, A.M.T., 2018. N/S-doped graphene derivatives and TiO2 for catalytic ozonation and photocatalysis of water pollutants. Chem. Eng. J. 348, 888–897. https://doi.org/10.1016/j.cej.2018.04.214

Peñas-Garzón, M., Gómez-Avilés, A., Bedia, J., Rodriguez, J.J., Belver, C., 2019. Effect of activating agent on the properties of TiO 2 /activated carbon heterostructures for solar photocatalytic degradation of acetaminophen. Materials (Basel). 12, 378. https://doi.org/10.3390/ma12030378

Peng, F.Q., Ying, G.G., Yang, B., Liu, S., Lai, H.J., Liu, Y.S., Chen, Z.F., Zhou, G.J., 2014. Biotransformation of progesterone and norgestrel by two freshwater microalgae (Scenedesmus obliquus and Chlorella pyrenoidosa): Transformation kinetics and products identification. Chemosphere 95, 581–588. https://doi.org/10.1016/J.CHEMOSPHERE.2013.10.013

Petala, A., Frontistis, Z., Antonopoulou, M., Konstantinou, I., Kondarides, D.I., Mantzavinos, D., 2015. Kinetics of ethyl paraben degradation by simulated solar radiation in the presence of N-doped TiO2 catalysts. Water Res. 81, 157–166. https://doi.org/10.1016/j.watres.2015.05.056

Philippe, K.K., Timmers, R., Van Grieken, R., Marugan, J., 2016. Photocatalytic Disinfection and Removal of Emerging Pollutants from Effluents of Biological Wastewater Treatments, Using a Newly Developed Large-Scale Solar Simulator. Ind. Eng. Chem. Res. 55, 2952–2958. https://doi.org/10.1021/acs.iecr.5b04927

Phoon, B.L., Cheen Ong, C., Shuaib, M., Saheed, M., Show, P.-L., Chang, J.-S., Ling, C., Lam, S.S., Juan, C., My, J.C., Juan,), 2020. Conventional and emerging technologies for removal of antibiotics from wastewater. https://doi.org/10.1016/j.jhazmat.2020.122961

Priya, A.K., Gnanasekaran, L., Rajendran, S., Qin, J., Vasseghian, Y., 2022. Occurrences and removal of pharmaceutical and personal care products from aquatic systems using advanced treatment- A review. Environ. Res. 204, 112298. https://doi.org/10.1016/j.envres.2021.112298

Puri, M., Gandhi, K., Kumar, M.S., 2023. Emerging environmental contaminants: A global perspective on policies and regulations. J. Environ. Manage. 332, 117344.

https://doi.org/10.1016/j.jenvman.2023.117344

Rajoriya, S., Bargole, S., George, S., Saharan, V.K., Gogate, P.R., Pandit, A.B., 2019. Synthesis and characterization of samarium and nitrogen doped TiO2 photocatalysts for photo-degradation of 4-acetamidophenol in combination with hydrodynamic and acoustic cavitation. Sep. Purif. Technol. 209, 254–269. https://doi.org/10.1016/j.seppur.2018.07.036

Reda, S.M., Khairy, M., Mousa, M.A., 2020. Photocatalytic activity of nitrogen and copper doped TiO2 nanoparticles prepared by microwave-assisted sol-gel process. Arab. J. Chem. 13, 86–95. https://doi.org/10.1016/j.arabjc.2017.02.002

Rocha, D.C., da Silva Rocha, C., Tavares, D.S., de Morais Calado, S.L., Gomes, M.P., 2021. Veterinary antibiotics and plant physiology: An overview. Sci. Total Environ. 767, 144902. https://doi.org/10.1016/j.scitotenv.2020.144902

Samal, K., Mahapatra, S., Hibzur Ali, M., 2022. Pharmaceutical wastewater as Emerging Contaminants (EC): Treatment technologies, impact on environment and human health. Energy Nexus 6, 100076. https://doi.org/10.1016/j.nexus.2022.100076

Sanganyado, E., Kajau, T.A., 2022. The fate of emerging pollutants in aquatic systems: An overview, Emerging Freshwater Pollutants: Analysis, Fate and Regulations. Elsevier Inc. https://doi.org/10.1016/B978-0-12-822850-0.00002-8

Saucier, C., Adebayo, M.A., Lima, E.C., Cataluña, R., Thue, P.S., Prola, L.D.T., Puchana-Rosero, M.J., Machado, F.M., Pavan, F.A., Dotto, G.L., 2015. Microwave-assisted activated carbon from cocoa shell as adsorbent for removal of sodium diclofenac and nimesulide from aqueous effluents. J. Hazard. Mater. 289, 18–27. https://doi.org/10.1016/J.JHAZMAT.2015.02.026

Solís, R.R., Javier Rivas, F., Gimeno, O., Pérez-Bote, J.L., 2016. Photocatalytic ozonation of pyridine-based herbicides by N-doped titania. J. Chem. Technol. Biotechnol. 91, 1998–2008. https://doi.org/10.1002/jctb.4791

Sutherland, D.L., Ralph, P.J., 2019. Microalgal bioremediation of emerging contaminants - Opportunities and challenges. Water Res. 164, 114921. https://doi.org/10.1016/J.WATRES.2019.114921

Taheran, M., Brar, S.K., Verma, M., Surampalli, R.Y., Zhang, T.C., Valero, J.R., 2016.

Membrane processes for removal of pharmaceutically active compounds (PhACs) from water and wastewaters. Sci. Total Environ. 547, 60–77. https://doi.org/10.1016/j.scitotenv.2015.12.139

Than, L.D., Luong, N.S., Ngo, V.D., Tien, N.M., Dung, T.N., Nghia, N.M., Loc, N.T., Thu, V.T., Lam, T.D., 2017. Highly Visible Light Activity of Nitrogen Doped TiO2 Prepared by Sol–Gel Approach. J. Electron. Mater. 46, 158–166. https://doi.org/10.1007/s11664-016-4894-6

Tiseo, K., Huber, L., Gilbert, M., Robinson, T.P., Van Boeckel, T.P., 2020. Global trends in antimicrobial use in food animals from 2017 to 2030. Antibiotics 9, 1–14. https://doi.org/10.3390/antibiotics9120918

Tong, L., Li, P., Wang, Y., Zhu, K., 2009. Analysis of veterinary antibiotic residues in swine wastewater and environmental water samples using optimized SPE-LC/MS/MS. Chemosphere 74, 1090–1097. https://doi.org/10.1016/j.chemosphere.2008.10.051

Tong, X., Mohapatra, S., Zhang, J., Tran, N.H., You, L., He, Y., Gin, K.Y.H., 2022. Source, fate, transport and modelling of selected emerging contaminants in the aquatic environment: Current status and future perspectives. Water Res. 217, 118418. https://doi.org/10.1016/j.watres.2022.118418

Villar-Navarro, E., Baena-Nogueras, R.M., Paniw, M., Perales, J.A., Lara-Martín, P.A., 2018. Removal of pharmaceuticals in urban wastewater: High rate algae pond (HRAP) based technologies as an alternative to activated sludge based processes. Water Res. 139, 19–29. https://doi.org/10.1016/j.watres.2018.03.072

Wang, N., Li, X., Yang, Y., Guo, T., Zhuang, X., Ji, S., Zhang, T., Shang, Y., Zhou, Z., 2019. Enhanced photocatalytic degradation of sulfamethazine by Bi-doped TiO2 nano-composites supported by powdered activated carbon under visible light irradiation. Sep. Purif. Technol. 211, 673–683. https://doi.org/10.1016/j.seppur.2018.10.040

Wang, S., Wang, X., Poon, K., Wang, Y., Li, S., Liu, H., Lin, S., Cai, Z., 2013. Removal and reductive dechlorination of triclosan by Chlorella pyrenoidosa. Chemosphere 92, 1498–1505. https://doi.org/10.1016/J.CHEMOSPHERE.2013.03.067

Wang, Y., Liu, J., Kang, D., Wu, C., Wu, Y., 2017. Removal of pharmaceuticals and

personal care products from wastewater using algae-based technologies: a review. Rev. Environ. Sci. Biotechnol. 16, 717–735. https://doi.org/10.1007/s11157-017-9446-x

Wilkinson, J., Hooda, P.S., Barker, J., Barton, S., Swinden, J., 2017. Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field. Environ. Pollut. 231, 954–970. https://doi.org/10.1016/j.envpol.2017.08.032

Xie, P., Chen, C., Zhang, C., Su, G., Ren, N., Ho, S.H., 2020. Revealing the role of adsorption in ciprofloxacin and sulfadiazine elimination routes in microalgae. Water Res. 172, 115475. https://doi.org/10.1016/j.watres.2020.115475

Xiong, J.Q., Kim, S.J., Kurade, M.B., Govindwar, S., Abou-Shanab, R.A.I., Kim, J.R., Roh, H.S., Khan, M.A., Jeon, B.H., 2019. Combined effects of sulfamethazine and sulfamethoxazole on a freshwater microalga, Scenedesmus obliquus: toxicity, biodegradation, and metabolic fate. J. Hazard. Mater. 370, 138–146. https://doi.org/10.1016/j.jhazmat.2018.07.049

Xiong, J.Q., Kurade, M.B., Jeon, B.H., 2017a. Biodegradation of levofloxacin by an acclimated freshwater microalga, Chlorella vulgaris. Chem. Eng. J. 313, 1251–1257. https://doi.org/10.1016/J.CEJ.2016.11.017

Xiong, J.Q., Kurade, M.B., Kim, J.R., Roh, H.S., Jeon, B.H., 2017b. Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga Chlamydomonas mexicana. J. Hazard. Mater. 323, 212–219. https://doi.org/10.1016/j.jhazmat.2016.04.073

Xiong, Q., Hu, L.X., Liu, Y.S., Zhao, J.L., He, L.Y., Ying, G.G., 2021. Microalgae-based technology for antibiotics removal: From mechanisms to application of innovational hybrid systems. Environ. Int. 155. https://doi.org/10.1016/j.envint.2021.106594

Yang, J.S., Cao, J., Xing, G.L., Yuan, H.L., 2015. Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae Chlorella minutissima UTEX2341. Bioresour. Technol. 175, 537–544. https://doi.org/10.1016/J.BIORTECH.2014.10.124

Yap, H.C., Pang, Y.L., Lim, S., Abdullah, A.Z., Ong, H.C., Wu, C.H., 2019. A comprehensive review on state-of-the-art photo-, sono-, and sonophotocatalytic

treatments to degrade emerging contaminants. Int. J. Environ. Sci. Technol. 16, 601–628. https://doi.org/10.1007/s13762-018-1961-y

Yu, Y., Zhou, Y., Wang, Z., Torres, O.L., Guo, R., Chen, J., 2017. Investigation of the removal mechanism of antibiotic ceftazidime by green algae and subsequent microbic impact assessment. Sci. Rep. 7, 1–11. https://doi.org/10.1038/s41598-017-04128-3

Yuan, J., Zhang, X., Li, H., Wang, K., Gao, S., Yin, Z., Yu, H., Zhu, X., Xiong, Z., Xie, Y., 2015. TiO2/SnO2 double-shelled hollow spheres-highly efficient photocatalyst for the degradation of rhodamine B. Catal. Commun. 60, 129–133. https://doi.org/10.1016/j.catcom.2014.11.032

Zambrano, J., Irusta-Mata, R., Jiménez, J.J., Bolado, S., A.García-Encina, P., 2022. Chapter 24 - Photocatalytic removal of emerging contaminants in water and wastewater treatments: a review, in: Development in Wastewater Treatment Research and Processes. pp. 543–572. https://doi.org/https://doi.org/10.1016/B978-0-323-85583-9.00023-5

Zhao, L., Deng, J., Sun, P., Liu, J., Ji, Y., Nakada, N., Qiao, Z., Tanaka, H., Yang, Y., 2018. Nanomaterials for treating emerging contaminants in water by adsorption and photocatalysis: Systematic review and bibliometric analysis. Sci. Total Environ. 627, 1253–1263. https://doi.org/10.1016/j.scitotenv.2018.02.006

Zhou, J.L., Yang, L., Huang, K.X., Chen, D.Z., Gao, F., 2022. Mechanisms and application of microalgae on removing emerging contaminants from wastewater: A review. Bioresour. Technol. 364, 128049. https://doi.org/10.1016/j.biortech.2022.128049

Zhou, T., Zhang, Z., Liu, H., Dong, S., Nghiem, L.D., Gao, L., Chaves, A. V., Zamyadi, A., Li, X., Wang, Q., 2023. A review on microalgae-mediated biotechnology for removing pharmaceutical contaminants in aqueous environments: Occurrence, fate, and removal mechanism. J. Hazard. Mater. 443, 130213. https://doi.org/10.1016/j.jhazmat.2022.130213

Chapter 2. Aims and Scope

1. Justification

In recent years, improper management of agro-industrial waste, particularly slurry, has become a serious threat to the environment due to it its high organic matter, nitrogen, and phosphorus content. The use of microalgae and bacteria consortia for water treatment has emerged as a potential solution to this issue, as it has demonstrated significant potential for nutrient recovery. As well, high concentrations of emerging contaminants, particularly antibiotics, have been detected in slurry, and their behavior in microalgae-based treatment technologies is not well understood. A thorough analysis is necessary to quantify the removal of these contaminants and determine the extent to which they remain in the treated water or accumulate in the biomass. The composition and concentration of the antibiotics can significantly affect the species present and the composition of the algal biomass, which may determine their feasibility for various applications.

Although biological treatment is widely used for the removal of emerging contaminants and is one of the most advantageous technologies for wastewater remediation, some emerging contaminants can inhibit biodegradation due to their toxicity and resistance to microbial growth. In this context, advanced oxidation processes such as photolysis and photocatalysis can effectively eliminate emerging contaminants and avoid the production of secondary waste in the environment. Photocatalysis has been widely used for water and wastewater purification. It has been proven effective in treating various types of emerging contaminants in wastewater, and even for the treatment of seawater in case of oil spills, for their removal from water-soluble fractions of crude oil.

Photocatalysis proved to be a suitable option for the removal of emerging contaminants from water, safeguarding ecosystems and human health.

2. Main Objectives

The overall objective of the present thesis was to determine the removal and fate of veterinary antibiotics in a biological wastewater treatment process based on the use of microalgae-bacteria consortium (lab and pilot scale) and to compare its effectiveness with an advanced oxidation process (AOP). This goal will be addressed using multidisciplinary approach involving both biological (microalgae-bacteria consortium) and advanced oxidation process (heterogeneous photocatalysis with TiO_2). More specifically, the individual goals to achieve this overall objective were:

1. To determine the efficiency and kinetics of the process for removing veterinary antibiotics using a microalgae-bacteria consortium at the lab scale.
2. To study the mechanisms involved in the removal of veterinary antibiotics by the microalgae-bacteria consortium and determine the kinetics of each mechanism at the lab scale.
3. To study the biosorption capacity of the microalgae-bacteria consortium for veterinary antibiotics and determine the adsorption isotherms at the lab scale.
4. To study the photolytic removal of veterinary antibiotics and its kinetics at the lab scale.
5. To determine the feasibility of using heterogeneous photocatalysis with TiO_2 as an alternative for veterinary antibiotics removal and study its kinetics at the lab scale.

6. To verify the removal efficiency of veterinary antibiotics by the microalgae-bacteria consortium at a pilot scale to validate the results obtained at the lab scale.

3. Development of the thesis

To achieve the specific objectives mentioned above, six sets of experiments were conducted over the course of this four-year thesis. More specifically:

First, at the lab scale, three types of reactors were used to distinguish between biosorption (Reactor A), hydrolysis (Reactor B), and photolysis (Reactor C). Reactor A (biosorption) contained a lyophilized microalgae-bacteria consortium (dead biomass) and was not exposed to light. Reactor B (hydrolysis) did not contain a microalgae-bacteria consortium or light exposure. Reactor C (photolysis) was exposed to light but did not contain a microalgae-bacteria consortium.

The process of removing antibiotics using a microalgae-bacteria consortium was then studied in batch experiments at the lab scale, using active biomass. To determine the biodegradation efficiency of veterinary antibiotics by the microalgae-bacteria consortium, the results obtained from hydrolysis, photolysis and biosorption were subtracted from the results obtained for the whole process.

Photolytic and photocatalytic degradation experiments were conducted in a photoreactor system at the lab scale, using the same antibiotics and initial concentrations as those used in the microalgae-bacteria experiments.

Finally, a pilot-scale two-channel thin-layer cascade reactor, operating in continuous mode, was inoculated with a microalgae-bacteria consortium and fed with pig slurry spiked with a mixture of antibiotics.

The first objective was achieved in **Chapter 3,** where the removal efficiency of veterinary antibiotics by a microalgae-bacteria consortium was calculated and the process kinetics over a wide range of concentrations was determined. The second objective was also accomplished in **Chapter 3**, which analyzed each removal mechanism, determined its efficiency and kinetics, and studied biosorption and photolysis as the most important mechanisms for the removal of veterinary antibiotics. Biosorption was studied in **Chapter 4**, while photolysis was studied in **Chapter 5**.

Chapter 4 addressed the third objective by evaluating the adsorption capacity of microalga-bacteria consortia for the removal of veterinary antibiotics and determining the adsorption isotherms of four compounds from the three families of antibiotics most commonly found in water samples.

The fourth and fifth objective were accomplished in **Chapter 5**, where a comparison between photolysis and photocatalysis was performed, and the removal efficiency and kinetics of both processes were determined.

Finally, **Chapter 6** focuses in the sixth objective by corroborating batch experiments performed at lab scale regarding veterinary antibiotics removal efficiencies and validating the proposed kinetic model of pseudo-first order irreversible kinetics. Additionally, this chapter determines the antibiotics elimination in both the aqueous and solid phase by microalgae-bacteria consortium at a pilot scale using real wastewater.

Kinetics of the removal mechanisms of veterinary antibiotics in synthetic wastewater using microalgae-bacteria consortia

Johanna Zambrano[a,b], Pedro Antonio García-Encina[a,b], Félix Hernández[c], Ana M. Botero-Coy[c], Juan J. Jiménez[a,d], Rubén Irusta-Mata[a,b,*]

[a] *Institute of Sustainable Processes. University of Valladolid. Dr. Mergelina s/n, 47011 Valladolid. Spain*

[b] *Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr Mergelina s/n 47011 Valladolid, Spain.*

[c] *Environmental and Public Health Analytical Chemistry, Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat s/n, 12071 Castellón, Spain*

[d] *Department of Analytical Chemistry, University of Valladolid, Campus Miguel Delibes, Paseo Belen 7, 47011 Valladolid, Spain*

**Corresponding author. E-mail: ruben.irusta@uva.es*

https://doi.org/10.1016/j.eti.2023.103031

Removal of a mixture of veterinary medicinal products by adsorption into a *Scenedesmus almeriens*is microalgae-bacteria consortium

Johanna Zambrano[a,b], Pedro Antonio García-Encina[a,b], Félix Hernández[c], Ana M. Botero-Coy[c], Juan J. Jiménez[a,d], Rubén Irusta-Mata[a,b,*]

[a] *Institute of Sustainable Processes. University of Valladolid. Dr. Mergelina s/n, 47011 Valladolid. Spain*

[b] *Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr Mergelina s/n 47011 Valladolid, Spain.*

[c]*Environmental and Public Health Analytical Chemistry, Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat s/n, 12071 Castellón, Spain*

[d] *Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Campus Miguel Delibes, Paseo de Belén 7, 47011 Valladolid, Spain*

**corresponding author.* Phone: (+34) 983423693; Fax: (+34) 983423310

E-mail: rubiru@eii.uva.es

https://doi.org/10.1016/j.jwpe.2021.102226

Photolytic and photocatalytic removal of a mixture of four veterinary antibiotics

Johanna Zambrano[a,b], Pedro Antonio García-Encina[a,b], Juan José Jiménez[a,c], Rebeca López-Serna[a,c], Rubén Irusta-Mata[a,b,*]

[a] *Institute of Sustainable Processes. University of Valladolid. Dr. Mergelina s/n, 47011 Valladolid. Spain*

[b] *Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr Mergelina s/n 47011 Valladolid, Spain.*

[c] *Department of Analytical Chemistry, University of Valladolid, Campus Miguel Delibes, Paseo Belen 7, 47011 Valladolid, Spain*

**corresponding author*

https://doi.org/10.1016/j.jwpe.2022.102841

Removal of veterinary antibiotics in swine manure wastewater using microalgae-bacteria consortia in a pilot scale photobioreactor

Johanna Zambrano[a,b], Pedro Antonio García-Encina[a,b], Juan J. Jiménez[a,c], Martina Ciardi[d,e], Silvia Bolado-Rodríguez[a,b], Rubén Irusta-Mata[a,b,*]

[a] *Institute of Sustainable Processes. University of Valladolid. Dr. Mergelina s/n, 47011 Valladolid. Spain.*

[b] *Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina s/n, 47011 Valladolid, Spain.*

[c] *Department of Analytical Chemistry, University of Valladolid, Campus Miguel Delibes, Paseo Belen 7, 47011 Valladolid, Spain.*

[d] *Department of Chemical Engineering, University of Almería, 04120, Almería, Spain.*

[e] *CIESOL Solar Energy Research Centre, Joint Centre University of Almería-CIEMAT, 04120, Almería, Spain.*

** Corresponding author.*

Submitted to Environmental Technology & Innovation

Chapter 7. Conclusion and Future Work

The potential use of a microalgae-bacteria consortium for the removal of veterinary antibiotics was evaluated in this thesis, as well as the description of the mechanisms involved in the process. Additionally, photocatalysis was analyzed as an alternative process for the removal of veterinary antibiotics.

In Chapter 3, the mechanisms involved in the removal of TET, CIP, SDZ, and SMX using a *Scenedesmus almeriensis* microalgae-bacteria consortium were analyzed, as well as the determination of their rate constants by fitting experimental data over a wide range of concentrations to a single general equation. The microalgae-bacteria consortium proved to be highly efficient in the removal of a mixture of VA, working with a pH of 8 and a mixture of low concentrations of four VA that resemble the characteristics of real water samples. The overall removal of the four veterinary antibiotics followed a pseudo-first order irreversible kinetic. Biosorption was the most important removal process for the four antibiotics, while hydrolysis was insignificant. Hydrolysis and photolysis occurred in the removal of the VA except for SMX. Biodegradation was an essential mechanism for TET, SDZ, and SMX, but it was not present in CIP removal.

In Chapter 4, the biosorption capacity of the *Scenedesmus almeriensis* microalgae-bacteria consortium was analyzed and proven to be highly efficient in the removal of a mixture of four widely used VMP. Fluoroquinolones and tetracyclines showed a better affinity to the *S. almeriensis* microalgae-bacteria consortium, demonstrating high adsorption removal rates. Sulfonamides, on the other hand, presented limited biosorption in the range of 20 to 1000 µg/L. The adsorption process of tetracycline, ciprofloxacin, sulfadiazine and sulfamethoxazole was better described by the Langmuir isotherm, defining a monolayer adsorption with no interaction between adsorbed species.

In Chapter 5, the removal efficiencies, kinetics, and energy consumption of a mixture of four antibiotics were evaluated for photo-degradation (UVC) and photocatalytic-degradation with TiO_2 (UVC/TiO_2) at different initial concentrations. TET, CIP, SDZ, and SMX were found to be photosensitive and capable of being removed by either photolysis or photocatalysis, with photocatalysis achieving higher degradation percentages. The photolysis for the four veterinary antibiotics followed a first-order irreversible kinetic model, while the photocatalysis of TET, CIP, and SDZ followed a Langmuir-Hinshelwood kinetic model, with adsorption being considered as the limiting step. SMX followed a first-order irreversible kinetic model. The calculation of the electrical energy consumed by both processes showed that photocatalysis is more energy-efficient compared to other systems. These findings provide helpful insights into the extent of degradation of VAs in water treatment processes using photolysis and photocatalysis, with UVC photocatalysis with TiO_2 proving to be efficient and cost-effective in the removal of VAs.

In Chapter 6, the effectiveness of microalgae-based technology as an alternative for wastewater treatment process was evaluated. A thin-layer cascade reactor inoculated with a microalgae-bacteria consortium, mainly composed of *Scenedesmus almeriensis*, was tested for the treatment of real liquid fraction of pig slurry and the removal of antibiotics. This treatment process was highly efficient in the removal of organic matter, nutrients, and antibiotics. Additionally, the antibiotics did not have a negative effect on the *Scenedesmus almeriensis* microalgae-bacteria consortium. These results agreed with previous research, supporting the findings of laboratory-scale batch experiments and confirming the proposed irreversible pseudo-first-order kinetics for the removal of veterinary antibiotics. The use of photobioreactors containing microalgae-bacteria consortia proved to be a highly effective and promising approach for treating swine

manure wastewater and removing antibiotics. This technology was able to remove up to 77% of TET, 90% of CIP, and 60% of SDZ without the need for additional chemicals, which helps prevent the generation of further waste.

While this thesis has provided valuable insights into the fate of antibiotics in algal-bacterial photobioreactors and the potential of photocatalysis for their removal, further research is needed to advance in this field. Specifically, future studies should focus on:

1. Optimizing operating parameters to enhance the efficiency of photolysis and biodegradation for increased veterinary antibiotic degradation.
2. Investigating the formation of byproducts in order to facilitate the implementation of microalgae-based technology for veterinary antibiotic removal at an industrial scale.
3. Exploring potential uses of the harvested biomass to maximize its value and contribute to a circular economy.
4. Developing a combined approach using both biological processes and advanced oxidation processes (AOPs) to improve the removal of veterinary antibiotics.

Chapter 9. About the author

Biography

Johanna Vanessa Zambrano Flores (Quito, Ecuador, 1989) studied Environmental Engineering at Universidad San Francisco de Quito. In September 2012, Johanna was awarded a scholarship from Ecuador's Government to conduct a master's degree in environmental management and technology at University of Valladolid. Her Master Thesis focused on the ecotoxicity and biodegradability of ibuprofen in a continuous stirred tank bioreactor under the supervision of Dr. Rubén Irusta and Dr. Pedro García.

Back in Ecuador Johanna worked in environmental consulting companies with different industries such as banana, shrimp, oil, mining, tourism, among others. As well, she worked as an environmental specialist at the Environmental Ministry. In February 2017, Johanna joined the Environmental Biotechnology Laboratory for Water and Energy (EBLWE) – Kyung Hee University as a researcher for two years. Her research was focused on electrochemical processes for wastewater treatment.

In April 2019, Johanna joined the Institute of Sustainable Processes within the Environmental Technology Group of the Department of Chemical Engineering and Environmental Technology – University of Valladolid. In August 2020, Johanna was awarded with a PhD contract by the Regional Government of Castilla y León. Her PhD thesis was focused on the mechanisms involved in the removal of veterinary antibiotics (April 2019-April 2023). During her PhD, Johanna carried out a research stay of three months at NIREAS International water research Center (2021, Cyprus).

Publications in International Journals

- Zambrano, J., García-Encina, P. A., Jiménez, J. J., Ciardi, M., Bolado-Rodríguez, S., & Irusta-Mata, R. (2023). *Removal of veterinary antibiotics in swine manure wastewater using microalgae-bacteria consortia in a pilot scale photobioreactor.* (Submitted to Environmental Technology & Innovation).
- Zambrano, J., García-Encina, P. A., Hernández, F., Botero-Coy, A. M., Jiménez, J. J., & Irusta-Mata, R. (2023). *Kinetics of the removal mechanisms of veterinary antibiotics in synthetic wastewater using microalgae-bacteria consortia*. Environmental Technology & Innovation, 103031.
- Zambrano, J., García-Encina, P. A., Jiménez, J. J., López-Serna, R., & Irusta-Mata, R. (2022). *Photolytic and photocatalytic removal of a mixture of four veterinary antibiotics*. Journal of Water Process Engineering, 102841.
- Johanna, Z., & Johnny, Z. (2021). *Bioleaching: Validation of the Extraction of Precious Metals Through Selective Recovery of Iron, Copper and Zinc*. International Journal of Mineral Processing and Extractive Metallurgy, 6(4), 73-78. DOI: 10.11648/j.ijmpem.20210604.11
- Zambrano, J., García-Encina, P. A., Hernández, F., Botero-Coy, A. M., Jiménez, J. J., & Irusta-Mata, R. (2021). *Removal of a mixture of veterinary medicinal products by adsorption onto a Scenedesmus almeriensis microalgae-bacteria consortium.* Journal of Water Process Engineering, 43, 102226.

- Zambrano, J., & Min, B. (2020). *Electrochemical treatment of leachate containing highly concentrated phenol and ammonia using a Pt/Ti anode at different current densities.* Environmental Technology & Innovation, 18, 100632. DOI: https://doi.org/10.1016/j.eti.2020.100632
- Zambrano, J., Park, H., & Min, B. (2020). *Enhancing electrochemical degradation of phenol at optimum pH condition with a Pt/Ti anode electrode.* Environmental technology, 41(24), 3248-3259. DOI: https://doi.org/10.1080/09593330.2019.1649468
- Zambrano, J., Kovshov, S. V., & Lyubin, E. (2019). *Correlation of Viscosities for Biofuels mixtures.* Revista Mexicana de Ingeniería Química, 18(2), 759-776. DOI: https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Zambrano
- Zambrano, J., & Min, B. (2019). *Comparison on efficiency of electrochemical phenol oxidation in two different supporting electrolytes (NaCl and Na_2SO_4) using Pt/Ti electrode*. Environmental Technology & Innovation, 15, 100382. DOI: https://doi.org/10.1016/j.eti.2019.100382
- Johanna, Z., & Johnny, Z. (2019). *Effluents Treatment Generated by Biolixiviation in the Extraction of Precious Metals through Selective Recovery of Iron, Copper and Zinc*. International Journal of Mineral Processing and Extractive Metallurgy, 4(2), 44. DOI: 10.11648/j.ijmpem.20190402.12

Book chapters

- Zambrano, J., Irusta-Mata, R., Jiménez, J. J., Bolado, S., & García-Encina, P. A. (2022). *Photocatalytic removal of emerging contaminants in water and wastewater treatments: a review.* Development in Wastewater Treatment Research and Processes, 543-572.

Conference Participation

Oral presentation

Zambrano, J., García-Encina, P. A., Jiménez, J. J., López-Serna, R., & Irusta-Mata, R. (2022). Kinetics of the photolytic and photocatalytic degradation of veterinary antibiotics. IWA 4th Regional Conference on Diffuse Pollution & Eutrophication. Istambul, Turkey. 24-28 October.

Zambrano, J., García-Encina, P. A., Jiménez, J. J., López-Serna, R., & Irusta-Mata, R. (2022). Kinetic analysis for the removal of a mixture of veterinary antibiotics by photocatalysis. 18th Annual Workshop on Emerging High-Resolution Mass Spectrometry (HRMS) and LC-MS/MS Applications in Environmental Analysis and Food Safety. Barcelona, Spain. 10-11 October.

Johanna Zambrano, Pedro García, Félix Hernández, Ana María Botero-Coy, Juan José Jiménez, Rubén Irusta (2022). *Scenedesmus Almeriensis* microalgae-bacteria consortium as a potential biosorbent of veterinary antibiotics. 12th Micropol & Ecohazard Conference 2022. Santiago de Compostela, Spain. 6-10 June.

Johanna Zambrano, Johnny Zambrano (2020). Effluents treatment generated by biolixiviation in the extraction of precious metals through selective recovery of iron,

copper and zinc. XVI International Forum-Contest of Students and Young Researchers "Tropical Issues of Rational Use of Natural Resources". Saint-Petersburg, Rusia. 17-19 June.

Johanna Zambrano, Booki Min (2018). Electrochemical treatment of leachate containing phenols using a Pt/Ti anode. Clean Technology Institute conference. Gwang-Ju, South Korea. 12-14 September.

Johanna Zambrano, Booki Min (2018). Effect of pH on the electrochemical degradation of phenol using Pt/Ti anode electrode. Clean Technology Institute conference. Gyeong-Ju, South Korea. 28-30 March

Poster Participation

Johanna Zambrano, Pedro García, Félix Hernández, Ana María Botero-Coy, Juan José Jiménez, Rubén Irusta (2022). Removal of veterinary medicinal products by adsorption into microalgae-bacteria consortium. 3rd BioIberoAmerica 2022. Braga, Portugal. 7-9 April.

Johanna Zambrano, Booki Min (2018). Electrochemical degradation of phenol in presence of chloride with a Pt/Ti anode. 19th UK-IWA Young Water Professionals Conference. Cranfield, UK. 16-18 April.

Johanna Zambrano, Booki Min (2017). Effect of pH on the electrochemical degradation of phenol using Pt/Ti anode electrode. 11th international symposium on Green Energy. Yongin, South Korea. 22-23 September.

Johanna Zambrano, Sheyla Ortiz, Pedro García, Rubén Irusta (2016). Ecotoxicity and biodegradability of ibuprofen in a continuous stirred tank bioreactor. Congreso

Iberoamericano de Biotecnología BIOIBEROAMERICA 2016. Salamanca, Spain. 5-8 Junio.

Research Stays

NIREAS International water research Center (Cyprus) May 2021 – August 2021. Supervisor: Dr. Despo Fatta-Kassinos Scope: During this stay Johanna participated in the project Nanoplastics as carriers for the spread of chemicals and antimicrobial resistance in the aquatic environment.

Conferences Committee

Member of the Organizing Committee of the "IWA Conference on Algal Technologies and Stabilization Ponds for Wastewater Treatment and Resource Recovery - IWAlgae2019". 01-02 July 2019, Valladolid, Spain.

Student mentoring

Co-supervisor of a master student Final Degree Project from the master's in advanced techniques in chemistry. Chemical analysis and quality control at Valladolid University (2021). Student: Angelli Pérez. Title: Photocatalytic degradation of tetracycline hydrochloride using ZNO and N-ZnO nanoparticles and visible light radiation.

Internship supervisor for 150 hours internship at the chemical engineering and environmental technology laboratory of Valladolid University. Student: Marta Santos.

Internship supervisor for 150 hours internship at the chemical engineering and environmental technology laboratory of Valladolid University. Student: Miguel Almarza.

Acknowledgments

"Solo aquellos que se arriesgan a ir demasiado lejos pueden descubrir hasta dónde se puede llegar". T.S. Eliot

Gracias a mi tutor y directores, Pedro, Rubén y JuanJo, por su dedicación, guía y consejos. Especialmente a Rubén y Pedro por confiar en mi desde la primera vez que vine a Valladolid a hacer el máster y darme la oportunidad de ser parte de su grupo de investigación.

A la Dra. Despo Fatta-Kassinos, por darme la oportunidad de realizar la estancia de investigación en NIREAS y a Kyriakos por todas sus enseñanzas.

A todos los profesores del departamento, técnicos y compañeros del laboratorio por siempre estar dispuestos a prestar su ayuda y por el gran ambiente de trabajo.

A todos mis amigos, que más que amigos ya son familia. Nas, Caro, Luis, Liz, Bolita y Esteban por ser esa recarga de energía cada que viajaba a Ecuador. Jorge y Karlita que sin importar el tiempo y la distancia han estado siempre allí apoyándome y aconsejándome. Caro, Isi, Rodolfo, Ferrer, Borja, Yogurin, Lois, Javi y Pelitos que han hecho que me sienta en Valladolid como es casa. Especialmente a David por ser la persona que me ayudó desde el primer día que llegué a Valladolid, tanto dentro del laboratorio como fuera de él, gracias por ser ese amigo incondicional.

A las personas más importantes en mi vida, mi familia, que a pesar de la distancia siempre me han dado el cariño y la fuerza para seguir adelante. A mis muchachos, que siempre eché de menos, por llenarme de alegría. A mis padres, que son mi inspiración, gracias por su apoyo incondicional y por siempre creer en mí. Especialmente a mi madre

por ser mi fan número uno y nunca dejarme desmayar. Y a mis abuelos, que me criaron, que sé que siempre estarán cuidándome. Los quiero.

Mil gracias a todas aquellas personas que han contribuido de una u otra forma a esta Tesis Doctoral.