USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

ESCUELA DE DOCTORADO INTERNACIONAL EN CIENCIAS Y TECNOLOGÍAS DE LA USC

María Jesús
Abeledo Lameiro

Tesis doctoral

Cryptosporidium in water reuse. Evaluation of new disinfection technologies

Santiago de Compostela, 2020

PD en Avances en Biología Microbiana y Parasitaria

DOCTORAL THESIS

Cryptosporidium in water reuse. Evaluation of new disinfection technologies

María Jesús Abeledo Lameiro

ESCUELA DE DOCTORADO INTERNACIONAL EN CIENCIAS DE LA SALUD
PROGRAMA DE DOCTORADO EN AVANCES EN BIOLOGÍA MICROBIANA Y PARASITARIA

SANTIAGO DE COMPOSTELA
2020

DECLARACIÓN DE LA AUTORA DE LA TESIS

***Cryptosporidium* in water reuse. Evaluation of new disinfection technologies.**

Dña. María Jesús Abeledo Lameiro

Presenta su Tesis Doctoral, siguiendo el procedimiento adecuado al Reglamento, y declara que:

1) La Tesis abarca los resultados de la elaboración de mi trabajo.

   De ser el caso, en la Tesis se hace referencia a las colaboraciones que tuvo este trabajo.

2) La Tesis es la versión definitiva presentada para su defensa y coincide con la versión enviada en formato electrónico.
3) Confirmo que la Tesis no incurre en ningún tipo de plagio de otros autores ni de trabajos presentados por mí para la obtención de otros títulos.
4) Todas las figuras de la presente Tesis Doctoral son originales de la autora o del Grupo de Investigación (GI-2109), excepto aquellas en las que se incluye la correspondiente licencia.

En Santiago de Compostela, a 31 de enero de 2020

Fdo. María Jesús Abeledo Lameiro

AUTORIZACIÓN DE LOS DIRECTORES DE LA TESIS

***Cryptosporidium* in water reuse. Evaluation of new disinfection technologies.**

D. Hipólito Gómez Couso (Director y Tutor)

Dña. Elvira Ares Mazás (Directora)

INFORMAN:

Que la presente Tesis Doctoral, se corresponde con el trabajo realizado por Dña. María Jesús Abeledo Lameiro, bajo nuestra dirección, y autorizamos su presentación, considerando que reúne los requisitos exigidos en el Reglamento de Estudios de Doctorado de la USC, y que como directores de esta no incurre en las causas de abstención establecidas en la Ley 40/2015.

De acuerdo con el artículo 41 del Reglamento de Estudios de Doctorado, declaramos también que la presente Tesis Doctoral es idónea para ser defendida en base a la modalidad de COMPENDIO DE PUBLICACIONES, en los que la participación de la doctoranda fue decisiva para su elaboración.

La utilización de estos artículos en esta memoria, está en conocimiento de los coautores, tanto doctores como no doctores. Además, estos últimos tienen conocimiento de que ninguno de los trabajos aquí reunidos podrá ser presentado en ninguna otra Tesis Doctoral.

En Santiago de Compostela, a 31 de enero de 2020

Fdo. Hipólito Gómez Couso　　　　　　Fdo. Elvira Ares Mazás

CONFLICTO DE INTERÉS

***Cryptosporidium* in water reuse. Evaluation of new disinfection technologies.**

Dña. María Jesús Abeledo Lameiro

Declara no tener ningún conflicto de interés en relación con su Tesis Doctoral.

En Santiago de Compostela, a 31 de enero de 2020

Fdo. María Jasús Abeledo Lameiro

A mamá

'When we save one resource, such as water, we save on all others'

Hans Bruyninckx

Executive Director of the European Environment Agency

AGRADECIMIENTOS

La Tesis Doctoral ha sido una etapa muy importante que estará siempre presente en mi vida y en mi pensamiento, nunca la podré olvidar, y durante este camino me han acompañado unas personas maravillosas a las que les quiero a agradecer con palabras todo el cariño que he recibido.

En primer lugar, a mis directores, Dra. Dña. Elvira Ares Mazás y Dr. D. Hipólito Gómez Couso por darme la oportunidad de conocer, explorar y aprender el apasionante universo de la investigación. Elvira, gracias por ser la mejor profesora, y no solo en las aulas, tu pasión por la Parasitología hizo que muchos nos enamorásemos de ella. Y este amor es para siempre. Los dos me habéis encaminado en este camino, demostrándome y haciéndome crecer como persona e investigadora. Ahora sé que no hay 'imposibles'. No habrá agradecimientos que compensen todo el bagaje que me llevo conmigo. *¡Gracias!*

A mi familia, mil vidas no serían suficientes para devolveros todo los que me dais cada día. José Antonio Abeledo Abeledo, Mª del Carmen Lameiro Varela, Laura Abeledo Lameiro y Fi sois mi gran apoyo, acompañándome en mis decisiones, dándome fuerzas y haciéndome ver las dos caras de la moneda. No habría sido capaz sin vosotros.

También quiero agradecer, a las Doctoras Dña. Inmaculada Polo López y Dña. Pilar Fernández Ibáñez, dos grandes investigadoras a las que admiro, su gran acogida en la Platafoma Solar de Almería, mi segunda casa. No tengo más que buenos e inolvidables recuerdos, rodeada de personas extraordinarias que han hecho que me sienta una más de ellos. A todos vosotros: Agustín, Laura, María, Isa Fernández, Eli, Isa Oller, Panchi, Isaac, Marcelo, Gracia, Ana, Melina, Azahara, Teresa, Arianna. En especial a Ángela y a mis tres mosqueteras Irene, Leonor y Samira con las que comparto una gran amistad. *¡Gracias y hasta luego Mª Carmen!*

Le agradezco al Dr. D. Fernando Martínez Castillejo y a la Dra Dña. Yolanda Segura Urraca, Catedrático y Profesora Titular, respectivamente, del Departamento de Tecnología Química y Ambiental de la Universidad Rey Juan Carlos, por su asesoramiento en los ensayos de evaluación de la tecnología de ultrasonidos, y al D. Carlos Aymerich Rico, Jefe de Explotación de Instalaciones y Laboratorio de la empresa Espina y Delfín S.L., por autorizar la recogida de las muestras de agua y la realización de los correspondientes análisis, así como al personal de la Estación Depuradora de Aguas Residuales de Bertamiráns (Ames, A Coruña).

Agradecer a los propietarios, capataces, veterinarios y al resto de personal de las granjas *As Travesas Agropecuaria* (Carral, A Coruña), *A Casa Grande de Xanceda* (Xanceda, A Coruña) y *Finca Arosa* (Xanceda, A Coruña), por permitir la recogida de las muestras fecales de terneros.

Agradecer a Seila Couso Pérez, mi compañera de laboratorio, estos años juntas, en las cuales hemos compartido muchas horas de trabajo y risas. Has sido una gran compañera, amiga y consejera, formando un gran tándem en el trabajo del día a día que nos ayudó a crecer y aprender la una de la otra, tanto científica como personalmente. También quisiera dedicar unas palabras de agradecimiento a todas las personas que han pasado por el laboratorio, y en especial a Rainer Campo Ramos y a Antonio Cañizo Outeriño, por su carisma y ganas de aprender.

También quiero agradecer a todos los profesores del laboratorio de Parasitología, a Anabel Vidal Varela, y a todos los compañeros ya Doctores por su cariño y acogida.

Finalmente, y no por ello menos importantes, quiero darles la gracias a mis amigos, mi segunda familia. Mi especial cariño y agradecimiento a Guillermo Varela Iglesias por dedicar su tiempo y aportar la ilustración del ciclo de *Cryptosporidium*. Gracias por apoyarme, por comprenderme, por aguantarme, por vuestras risas y consejos, por los cafés (Dra. Neko Aguín) y las cenas (Iztiar, Marta, Gali, Dani, David, Alex, Cristina y Pablo), por estar a mi lado cuando os necesito, aquí o desde la distancia (Ruth y Juan). Por último, a mi Rocío Álvarez Pérez, sabes que mis palabras no reflejarán ni la mitad de lo que somos nosotras dos, juntas o separadas. Y como tú me repites muchas veces: '*nada hay bajo el sol que no tenga solución, nunca una noche venció a un amanecer*' (Warcry).

¡Gracias!

This Doctoral Thesis was funded by the following projects:

Cryptosporidium *as a model of emerging waterborne enteropathogen in the evaluation of photocatalytic processes for water regeneration* (CRYPTOREGWATER), granted by the Spanish Ministry of Economy and Competitiveness (CTM2011-29143-C03-02).

Drug delivery systems and immunoparasitology of waterborne protozoan zoonoses granted by the Programme for the Consolidation and Structuring of Competitive Research Units in the Galician University System and Autonomous Government of Galicia (GPC2014-069 and ED431C 2017/31).

Water-Sustainable Point-of-Use Treatment Technologies (WATERSPOUTT) granted by the European Union´s Horizon 2020 Research and Innovation Programme (688928).

The results of the present Doctoral Thesis have been published in the following manuscripts:

Abeledo-Lameiro, M.J., Ares-Mazás, E. and Gómez-Couso, H. 2016. Evaluation of solar photocatalysis using TiO_2 slurry in the inactivation of *Cryptosporidium parvum* oocysts in water. *Journal of Photochemistry and Photobiology, B: Biology,* **163:** 92-99. doi: 10.1016/j.jphotobiol.2016.08.016.

Abeledo-Lameiro, M.J., Reboredo-Fernández, A., Polo-López, M.I., Fernández-Ibáñez, P., Ares-Mazás, E. and Gómez-Couso, H. 2017. Photocatalytic inactivation of the waterborne protozoan parasite *Cryptosporidium parvum* using TiO_2/H_2O_2 under simulated and natural solar conditions. *Catalysis Today,* **280:** 132-138. doi: 10.1016/j.cattod.2016.05.046.

Abeledo-Lameiro, M.J., Ares-Mazás, E. and Gómez-Couso, H. 2018. Use of ultrasound irradiation to inactivate *Cryptosporidium parvum* oocysts in effluents from municipal wastewater treatment plants. *Ultrasonic Sonochemistry,* **48:** 118-126. doi: 10.1016/j.ultsonch.2018.05.013.

Abeledo-Lameiro, M.J., Polo-López, M.I., Ares-Mazás, E. and Gómez-Couso, H. 2019. Inactivation of the waterborne pathogen *Cryptosporidium parvum* by photo-Fenton process under natural solar conditions. *Applied Catalysis B: Environmental,* **253:** 341-347. doi: 10.1016/j.apcatb.2019.04.049.

TABLES OF CONTENTS

LIST OF ABBREVIATIONS

AD	*Anno Domini*
AHMC	Australian Health Minister´s Conference
AIDS	Adquired immune deficiency syndrome
AOP	Advanced oxidation process
BC	Before Christ
BOD	Biological oxygen demand
BOD_5	Biological oxygen demand by 5 days
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
COD	Ordinary legislative procedure (ex-codecision procedure)
DNA	Deoxyribonucleic acid
DW	Distilled water
DWTP	Drinking water treatment plant
EC	European Comission
ECHA	European Chemicals Agency
EEC	European Economic Community
EPHC	Environment Protection and Heritage Council
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GEC	Global Environment Centre Foundation
HIV	Human immunodeficiency virus
ICZN	International Code of Zoological Nomenclature
NRMMC	Natural Resource Management Ministerial Council
NTU	Nephelometric turbidity unit

NTZ	Nitazoxanide
PV	Parasitophorous vacuole
ROS	Reactive oxygen species
SDG	Sustainable Development Goal
SODIS	Solar disinfection process
TOC	Total organic carbon
TSS	Total suspended solids
UN	United Nations
UNESCO	United Nations Educational, Scientific and Culture Organization
UNEP	United Nations Environment Programme
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
vs	*versus*
WHO	World Health Organization
WWAP	United Nations World Water Assessment Programme
WWTP	Wastewater treatment plant

LIST OF FIGURES

LIST OF TABLES

RESUMEN

En estas últimas décadas, el crecimiento experimentado por la población mundial (establecido en una tasa del 1,2% al año y estimándose que alcance los 9000 millones en el año 2030); el notable aumento de la urbanización; el empleo de agua en agricultura (aproximadamente un 70% del agua dulce disponible); el calentamiento global y la aparición de periódicas sequías (consecuencia del cambio climático y que podrían conducir a que alrededor de 2000 millones de personas carezcan de la suficiente cantidad de agua para satisfacer sus necesidades básicas); y, el progresivo deterioro de la calidad del agua (presencia de contaminantes químicos emergentes, fertilizantes agrícolas…) son circunstancias que han ejercido una enorme presión sobre los recursos hídricos disponibles en el planeta [Organización Mundial de la Salud (WHO), 2019].

Ante esta situación, diversos organismos internacionales defienden la política de la no utilización de recursos hídricos de mayor calidad en usos que pudiesen admitir el empleo de aguas con propiedades más bajas. De esta forma, la reutilización de las aguas depuradas en diversos fines, como el regadío o la recarga artificial de acuíferos, permite la recuperación parcial de los costes derivados del proceso de depuración y proporciona un recurso de agua alternativo, especialmente en aquellas zonas áridas y semiáridas.

A nivel de la Unión Europea, no existen directrices o reglamentos sobre la reutilización de las aguas residuales; sin embargo, el artículo 12 de la Directiva 91/271/CEE sobre las aguas residuales establece que "las aguas residuales tratadas se reutilizarán siempre que sea apropiado" y que "las vías de eliminación minimizarán los efectos adversos sobre el medio ambiente" (Directiva 91/271/ECC del Parlamento Europeo y del Consejo de 21 de mayo de 1991). La Directiva Marco del Agua (2000/60/CE) menciona la reutilización del agua como una posible medida complementaria con el fin de proporcionar una protección adicional o una mejora de las aguas (Directiva 2000/60/EC del Parlamento Europeo y del Consejo de 23 de octubre de 2000). En mayo de 2018, se presentó una Propuesta de Reglamento del Parlamento

Europeo y del Consejo sobre las exigencias mínimas para la reutilización del agua en el riego agrícola (2018/0169/COD). Sin embargo, varios Estados Miembros han elaborado reglamentos propios sobre la reutilización del agua residual: Chipre, Francia, Grecia, Italia, Portugal y España. Todos ellos se basan en las directrices y normas de referencia de diferentes organismos internacionales, incluyendo varias modificaciones para algunos usos. Aunque las normativas de los citados países no son homogéneas, en todas ellas se consideran los siguientes criterios: i) usos previstos; ii) parámetros analíticos y valor límite máximo permitido para cada parámetro; iii) protocolos de vigilancia; y, iv) medidas preventivas adicionales para la protección del medio ambiente y la salud (Alcalde-Sanz y Gawlik, 2014; Paranychianakis *et al.*, 2015, 2017).

En España, la publicación de una normativa referente a la reutilización de aguas data del año 2007. En el Real Decreto 1620/2007, de 7 de diciembre, se establece el régimen jurídico de la reutilización de las aguas depuradas y se incorpora el concepto de "agua regenerada" definida como "agua residual depurada que, en su caso, ha sido sometida a un proceso de tratamiento adicional o complementario que permite adecuar su calidad al uso al que se destina". Además, en el citado Real Decreto 1620/2007, se distinguen los siguientes usos del agua regenerada: urbano, agrícola, industrial, recreativo y ambiental. En su Anexo I, también se recogen los criterios de calidad diferenciados según los destinos, estableciendo límites de obligado cumplimiento. Los parámetros considerados son: *Escherichia coli* y nematodos intestinales (indicadores microbiológicos) y sólidos en suspensión y turbidez (indicadores físicoquímicos). Asimismo, dependiendo del tipo de aplicación, es necesario controlar otros parámetros, como *Legionella* spp., cuando existe la posibilidad de aerosolización, y huevos de *Taenia saginata* y *Taenia solium*, si se destinan al riego de pastos para consumo de animales productores de carne. Sin embargo, a pesar de la inclusión de los citados parámetros biológicos, la reutilización de las aguas residuales conlleva riesgos sanitarios asociados a la transmisión de ciertos protozoos parásitos no contemplados en la legislación vigente.

A este respecto, *Cryptosporidium* es un género de protozoos pertenecientes al filo Apicomplexa, cuyas especies ocasionan en población humana y animal una enfermedad diarreica, la cryptosporidiosis, de carácter autolimitado en individuos inmunocompetentes pero que puede cronificarse e incluso ser mortal en aquellos inmunocomprometidos. Estos enteroparásitos emergentes presentan unas formas infectantes particularmente robustas (ooquistes), estando considerados como el segundo agente infeccioso tras los priones en la pirámide de resistencia a los tratamientos de desinfección (Rutala y Weber, 2004), siendo la transmisión hídrica su principal mecanismo de transmisión desde el punto de vista numérico. De esta forma, el origen de los dos brotes hídricos de cryptosporidiosis más importantes reportados a nivel mundial, acontecidos en las ciudades de Milwaukee (Wisconsin, Estados Unidos) y Östersund (Suecia) que afectaron a unas 403.000 y 27.000 personas, respectivamente, fue la contaminación por aguas residuales urbanas conteniendo ooquistes de *Cryptosporidium hominis*, procedentes de aguas superficiales utilizadas como afluentes en las plantas de tratamiento de agua potable, junto con la ineficacia de los tratamientos destinados a la eliminación del parásito. De hecho, *Cryptosporidium* es uno de los agentes infecciosos más frecuentemente detectado en los brotes hídricos de etiología parasitaria, estando *Cryptosporidium parvum* y *C. hominis* implicados en un 63% de los brotes reportados en el periodo comprendido entre los años 2011 y 2016 (Efstratiou *et al.*, 2017).

Un factor condicionante en los tratamientos de regeneración de aguas es el nivel de desinfección, generalmente alcanzado mediante tratamientos con cloro, ozono o radiación ultravioleta. Sin embargo, los hechos referidos anteriormente demuestran la necesidad real de evaluar nuevas tecnologías que garanticen, desde el punto de vista parasitológico, el empleo de las aguas regeneradas, ya que los métodos convencionales de desinfección son ineficaces en la total eliminación de enteroparásitos de transmisión hídrica.

En consecuencia, la Organización Mundial de la Salud seleccionó a rotavirus, *Campylobacter* y *Cryptosporidium* como patógenos de referencia para virus, bacterias y protozoos parásitos, respectivamente en la evaluación de métodos de tratamiento, tanto para el agua de bebida como para el agua regenerada [WHO, 2006a, 2017; United States Environmental Protection Agency (USEPA), 2012; Alcalde-Sanz y Gawlik, 2017].

En los últimos años, los procesos de oxidación avanzados, definidos como aquellos que implican la formación de radicales hidroxilo ($HO^{\bullet}$) cuyo potencial de oxidación es muy superior al de otros agentes oxidantes tradicionales, se han revelado como una tecnología importante al acelerar las reacciones de oxidación pudiendo ser utilizados en numerosas áreas de descontaminación y desinfección. La versatilidad de estos procesos radica en el hecho de que existen diferentes formas de producir radicales $HO^{\bullet}$, siendo los métodos más habitualmente empleados la fotocatálisis heterogénea, generalmente con dióxido de titanio (TiO_2), sola o combinada con la fotolisis directa de ciertos oxidantes [peróxido de hidrogeno (H_2O_2)] y la fotocatálisis homogénea mediante foto-Fenton, que permiten incluso el empleo de la radiación solar, un recurso natural, abundante y renovable.

El objetivo de la presente Tesis Doctoral es evaluar la eficacia de ciertos procesos de oxidación avanzados, tanto fotoquímicos (la fotocatálisis heterogénea empleando TiO_2, sola o en combinación con el oxidante H_2O_2, y la fotocatálisis homogénea mediante foto-Fenton) en condiciones simuladas y/o naturales de radiación solar, como no fotoquímicos (la tecnología de ultrasonidos), frente al protozoo parásito de transmisión hídrica *C. parvum* en distintos tipos de agua.

1. Evaluación de la fotocatálisis heterogénea con TiO_2

Se estudió la eficacia de la fotocatálisis solar con TiO_2 en la regeneración de aguas experimentalmente contaminadas con ooquistes de *C. parvum*. Para ello, vasos de precipitados conteniendo agua destilada o un efluente simulado de estación depuradora de aguas residuales urbanas (EDAR) con distintas concentraciones de TiO_2 (0; 63; 100; y 200 mg/L) y contaminadas con $15{\times}10^6$ ooquistes purificados de *C. parvum*, se expusieron durante 2,5 y 5 horas a la radiación solar simulada. La viabilidad ooquística se determinó mediante la técnica de inclusión/exclusión del colorante vital fluorogénico ioduro de propidio. Durante la realización de los ensayos se monitorizó la temperatura, alcanzando esta unos valores máximos de 36,1 °C y 37,1 °C en agua destilada y en efluente simulado de EDAR, respectivamente.

El análisis de los resultados obtenidos en agua destilada tras 5 horas de exposición a la radiación solar, demostró que las concentraciones de 100 y 200 mg/L de TiO_2 provocaron una drástica reducción de la viabilidad ooquística en comparación con el correspondiente valor determinado en el control de exposición en oscuridad (4,16±2,35% y 15,02±4,54% *vs* 92,28±4,01%, respectivamente). Los resultados obtenidos con la mínima concentración del catalizador ensayada (63 mg/L) demostraron un menor descenso en la viabilidad ooquística que, si bien es significativamente inferior al obtenido en el control de oscuridad, no mostró diferencias estadísticamente significativas con el correspondiente valor determinado en las muestras sin catalizador (0 mg/L) (57,86±10,72% *vs* 51,06±9,38%, respectivamente).

En los estudios realizados utilizando efluente simulado de EDAR y al máximo tiempo de exposición, se comprobó que el empleo del fotocatalizador a las distintas concentraciones ensayadas, si bien disminuye la viabilidad ooquística con respecto al control en oscuridad, esta reducción es significativamente menor a la alcanzada en las muestras sin fotocatalizador (70,96±7,18%; 73,08±4,93% y 81,64±4,07% *vs* 93,18±1,75% *vs* 48,98±12,31 para concentraciones de 63; 100; y 200 mg/L *vs* control de exposición en oscuridad *vs* 0 mg/L, respectivamente).

Ante la observación realizada microscópicamente de la formación de agregados entre las partículas de TiO_2 y los ooquistes de *C. parvum*, se valoró la capacidad de retención ooquística por parte del fotocatalizador. Así, tras el tiempo requerido para su sedimentación en tubos graduados de 10 mL de fondo cónico (establecido en una hora), se comprobó que los porcentajes de ooquistes retenidos en agua destilada y en efluente simulado de EDAR empleando la máxima concentración de TiO_2 (200 mg/L) fueron de 56,57±1,33% y 78,41±2,92%, respectivamente, disminuyendo de esta forma la carga parasitaria de las aguas tratadas.

La interpretación de los resultados obtenidos, concretamente, en el ensayo realizado con agua destilada utilizando una concentración de TiO_2 de 100 mg/L y expuesta durante 5 horas a la radiación solar simulada (4,16±2,35% de viabilidad ooquística y 46,97±6,17% de retención ooquística), permite afirmar que el riesgo de transmisión de la cryptosporidiosis por el agua tratada bajo estas condiciones es prácticamente nulo ante el carácter conservador de la técnica utilizada en la valoración de la viabilidad ooquística. Sin embargo, los resultados hallados plantean que la eficacia del tratamiento con TiO_2 en la regeneración de aguas depende de muchas variables que deben ser estudiadas y optimizadas.

2. Evaluación de la incorporación del H_2O_2 a la fotocatálisis heterogénea con TiO_2

Se estudió el efecto de la adición del oxidante H_2O_2 en la eficacia de la desinfección fotocatalítica con TiO_2 frente a los ooquistes de *C. parvum* bajo condiciones simuladas y naturales de radiación solar. La concentración de fotocatalizador seleccionada fue de 100 mg/L, al comprobarse una mayor reducción de la viabilidad ooquística en los estudios anteriores realizados en agua destilada. Por otra parte, se estableció una concentración de H_2O_2 de 50 mg/L, concentración mínima a la cual se observa una ligera mejora en la inactivación solar de *C. parvum* determinada en estudios previos realizados por nuestro grupo de investigación.

Tanto en los experimentos realizados utilizando radiación solar simulada como en los llevados a cabo en la Plataforma Solar de Almería (Tabernas, Almería), se siguió el mismo diseño experimental del estudio anterior y se aplicó la técnica de inclusión/exclusión del colorante vital fluorogénico ioduro de propidio para la valoración de la viabilidad ooquística. Durante la realización de los ensayos también se monitorizó la temperatura, alcanzando unos valores máximos de 34,2 °C y 36,5 °C en condiciones simuladas y naturales de radiación solar, respectivamente.

En los ensayos realizados empleando radiación solar simulada, si bien no se observaron diferencias estadísticamente significativas entre los valores de viabilidad ooquística determinados en las muestras en oscuridad y en las expuestas en presencia/ausencia de H_2O_2, se detectó una fuerte reducción de la viabilidad ooquística en aquellas con TiO_2 y TiO_2/H_2O_2 tras 2,5 horas de exposición (22,09±10,83% y 24,64±14,95%, respectivamente, *vs* 90,44±5,87%, viabilidad ooquística inicial). Al final de los ensayos (5 horas de exposición), los porcentajes de viabilidad determinados en las muestras con TiO_2 y TiO_2/H_2O_2 fueron notablemente inferiores sin mostrar diferencias estadísticamente significativas entre sí (4,16±2,35% y 3,82±4,26%, respectivamente).

Bajo condiciones naturales de radiación solar y a tan sólo 2,5 horas de exposición, se observó una drástica disminución de la viabilidad ooquística en las muestras con TiO_2 y TiO_2/H_2O_2 (4,45±3,55% y 1,58±0,48%, respectivamente), comprobándose la existencia de diferencias estadísticamente significativas con los correspondientes valores determinados en muestras expuestas sin fotocatalizador. Tras 5 horas de exposición, las viabilidades ooquísticas detectadas en las muestras que contenían TiO_2 y TiO_2/H_2O_2 fueron 2,29±1,99% y 0,92±0,71%, respectivamente, observándose también una notable disminución de la viabilidad ooquística en aquellas muestras expuestas en presencia/ausencia de H_2O_2 (22,63±13,31% y 33,85±5,82%, respectivamente).

La comparación de los resultados obtenidos bajo ambas condiciones de radiación solar, permitió comprobar mayores reducciones de la viabilidad ooquística en los experimentos de campo. Así, tras un tiempo de exposición de 2,5 horas, los porcentajes de viabilidad ooquística determinados en muestras con TiO_2 y TiO_2/H_2O_2 expuestas a la radiación solar natural fueron significativamente inferiores a los correspondientes valores obtenidos en condiciones simuladas (4,45±3,55% y 1,58±0,48% *vs* 22,09±10,83% y 24,64±14,95%, respectivamente). Sin embargo, al final de los ensayos, los porcentajes de viabilidad ooquística obtenidos en ambas condiciones de trabajo fueron similares (2,29±1,99% y 0,92±0,71% *vs* 4,16±2,35% y 3,82±4,26% en muestras conteniendo TiO_2 y TiO_2/H_2O_2 bajo luz natural y artificial, respectivamente). Sin embargo, al máximo tiempo de exposición, los valores de viabilidad ooquística determinados en las muestras expuestas a la radiación solar natural en presencia de H_2O_2 fueron significativamente inferiores con respecto a los correspondientes valores obtenidos bajo luz solar simulada (22,63±13,31% *vs* 82,48±3,46%, respectivamente).

Los resultados obtenidos confirman la eficacia de la fotocatálisis heterogénea en la inactivación de ooquistes de *C. parvum* en agua destilada, disminuyendo el tiempo necesario para alcanzar la inactivación ooquística con respecto a los valores determinados en muestras expuestas a la radiación solar sin fotocatalizador. Sin embargo, la adición de H_2O_2 a baja concentración no mejoro la eficacia de la fotocatálisis con TiO_2 frente a este protozoo parásito.

3. Evaluación de la fotocatálisis homogénea mediante el proceso de foto-Fenton

Se estudió la eficacia de la fotocatálisis homogénea mediante el proceso de foto-Fenton en la inactivación de los ooquistes de *C. parvum* en agua destilada bajo condiciones naturales de radiación solar. Mediante un diseño factorial 3×3 de primer orden se evaluó la influencia de varias concentraciones de Fe^{2+}/H_2O_2 (5/10, 10/20 y 20/50 mg/L), tres valores de pH (3, 5,5 y 8) y diferentes tiempos de exposición (2, 4 y 6 horas) en la supervivencia de las formas infectantes de este protozoo parásito. Para ello, vasos de precipitados conteniendo 30 mL de agua destilada con las distintas concentraciones de Fe^{2+}/H_2O_2 a los valores de pH establecidos, se contaminaron con 6×10^6 ooquistes de *C. parvum* y se expusieron a la luz solar natural durante un tiempo de exposición máximo de 6 horas en la Plataforma Solar de Almería.

Los menores valores de viabilidad ooquística, determinada mediante la técnica de inclusión/exclusión del colorante vital fluorogénico ioduro de propidio, se obtuvieron a la mayor concentración de Fe^{2+}/H_2O_2 (20/50 mg/L), el menor valor de pH (3) y tiempos de exposición de 4 y 6 horas (3,68±1,38% y 6,39±2,65%, respectivamente, *vs* 91,67±3,63%, viabilidad ooquística inicial). Además, bajos valores de viabilidad ooquística se observaron en el ensayo realizado con una concentración de Fe^{2+}/H_2O_2 de 20/50 mg/L, a pH 5,5 y tras un tiempo de exposición de 6 horas (9,35±2,26%). Mediante el análisis de la significación del efecto de cada parámetro evaluado en el diseño experimental, se comprobó que la concentración de Fe^{2+}/H_2O_2, y el tiempo de exposición, así como la interacción del pH y la concentración de Fe^{2+}/H_2O_2 son factores que influyen de forma estadísticamente significativa sobre la viabilidad de los ooquistes de *C. parvum*. El análisis de regresión múltiple se obtuvo la siguiente ecuación empírica:

$$\textit{Viabilidad ooquística (\%)} = 50{,}139 - 11{,}515C - 17{,}867T + 10{,}746PC + 1{,}868P$$

donde *C*, es la concentración de Fe^{2+}/H_2O_2; *T*, el tiempo de exposición; *PC*, la interacción del pH y la concentración de Fe^{2+}/H_2O_2; y *P*, el pH. Los valores estadísticos de R^2 indican que el modelo explica el 76,76% de la variabilidad de la viabilidad ooquística (P = 0,0033). El diseño factorial no incluye los parámetros temperatura e intensidad de radiación, los cuales varían durante los experimentos llevados a cabo en condiciones naturales de radiación solar. Así, la temperatura de las muestras osciló entre 24,6±1,73 °C y 44,4±4,10 °C, alcanzando una temperatura máxima de 47,3 °C a un tiempo de exposición de 5 horas. De esta forma, además del proceso de foto-Fenton, las reducciones observadas en la viabilidad ooquística pudieron ser también consecuencia de las altas temperaturas registradas en las muestras de agua.

Los resultados obtenidos demuestran la eficacia del proceso de foto-Fenton para inactivar los ooquistes de *C. parvum.* Sin embargo, se requieren altas concentraciones de Fe^{2+}/H_2O_2 (20/50 mg/L), bajos valores de pH (≤5,5) y largos tiempos de exposición (4 y 6 horas) para obtener porcentajes elevados de inactivación ooquística.

4. Evaluación de la tecnología de ultrasonidos

Se estudió la eficacia de la tecnología de ultrasonidos en la regeneración de aguas experimentalmente contaminadas con ooquistes de *C. parvum*. Para ello, vasos de precipitados de 100 mL de capacidad conteniendo 75 mL de distintos tipos de agua (agua destilada, efluentes simulado, real y filtrado de EDAR) se contaminaron con 7×10^6 ooquistes y se expusieron al proceso de sonicación a potencias de 60, 80 y 100 W, en modo pulsado/continuo, durante diferentes tiempos de exposición. Al igual que ensayos anteriores, la viabilidad ooquística también se determinó mediante la técnica de inclusión/exclusión del colorante vital fluorogénico ioduro de propidio.

Los estudios iniciales realizados con agua destilada demostraron descensos significativos en la viabilidad ooquística cuando los ooquistes de *C. parvum* se sometieron a potencias de 60, 80 y 100 W en modo de trabajo pulsado, obteniéndose valores de 15,54±2,90%; 0,00±0,00%; y, 15,00±2,06% tras la aplicación de las distintas potencias durante 60, 30 y 5 minutos, respectivamente (*vs* 98,57±0,01%, viabilidad ooquística inicial). De igual forma, en modo continuo se observó un brusco descenso de la viabilidad ooquística hasta llegar a anularse a las potencias de 60 y 80 W después de tiempos de exposición de 60 y 20 minutos, respectivamente. A la mayor potencia ensayada (100 W) y tras 5 minutos de exposición, la viabilidad ooquística fue de 5,38±1,49%. Se comprobó la existencia de diferencias estadísticamente significativas entre los porcentajes de viabilidad ooquística obtenidos a distintas potencias y en ambos modos de trabajo, siendo estos menores cuando se aplican las mayores potencias y el modo de trabajo continuo (P<0,01). Sin embargo, a igual potencia y liberando la misma cantidad de energía, no se detectaron diferencias estadísticamente significativas entre los valores de viabilidad ooquística determinados en ambos modos de trabajo.

Utilizando distintos tipos de agua residual e independientemente del modo de trabajo, se comprobaron mayores descensos en la viabilidad ooquística, con respecto a los correspondientes valores determinados en agua destilada, probablemente debido al importante papel que desempeña la composición química de las mismas. Así, a la potencia de 80 W en modo continuo y tras 5 minutos de exposición, se observaron diferencias estadísticamente significativas entre los valores de viabilidad ooquística obtenidos en muestras de efluentes simulado, real y filtrado de EDAR con respecto al correspondiente valor hallado en agua destilada (11,79±2,13%; 14,16±1,96%; 14,78±2,66% *vs* 23,21±4,84%, respectivamente). Sin embargo, tras 10 minutos de exposición, los valores de viabilidad ooquística observados en los distintos tipos de agua ensayados fueron similares (1,29±0,86%; 3,16±0,69%; 3,15±0,87% ; 4,16±1,93% en efluentes simulado, real y filtrado de EDAR y agua destilada, respectivamente), reduciéndose drásticamente la viabilidad ooquística.

Aunque en el presente estudio los reactores se mantuvieron en baño de hielo con el fin de disipar el calor de la muestra, los valores de temperatura aumentaron de forma significativa a medida que se incrementó la potencia aplicada y el tiempo de exposición, siendo significativamente superiores en modo continuo *vs* pulsado (P<0,05). Sin embargo, no se observaron diferencias estadísticamente significativas entre los valores de temperatura obtenidos en los distintos tipos de agua a las tres potencias ensayadas, alcanzándose valores máximos próximos a 40 °C.

La tecnología de los ultrasonidos representa una importante alternativa a los métodos de desinfección actualmente empleados en la regeneración de aguas, al reducir drásticamente la supervivencia de *Cryptosporidium* sin modificar la composición química del medio ni generar subproductos tóxicos.

En conclusión, la interpretación global de los resultados obtenidos tras la evaluación de diferentes procesos de oxidación avanzados frente al protozoo parásito de transmisión hídrica *C. parvum*, considerado un patógeno de referencia en la evaluación de los tratamientos de agua, demuestra la eficacia

de estas tecnologías en la mejora de la calidad microbiológica del agua regenerada, aunque es necesario la realización de estudios para optimizar las condiciones de trabajo en función de la composición química del efluente de las aguas residuales. La inactivación de este enteropatógeno conlleva la eliminación de otros agentes infecciosos menos resistentes, permitiendo por tanto, un uso seguro del agua regenerada al proteger el medio ambiente y consecuentemente la salud humana y animal.

Palabras clave: *Cryptosporidium;* Desinfección de aguas residuales; Procesos de oxidación avanzados; Dióxido de titanio; Peróxido de hidrógeno; Foto-Fenton; Ultrasonidos; Aguas regeneradas.

RESUMO

O crecemento da poboación mundial, o notábel aumento das áreas urbanas, o uso da auga na agricultura, o quecemento global e as recorrentes secas, e o progresivo deterioro da calidade da auga son circunstancias que nas recentes décadas están exercendo unha enorme presión sobre os recursos hídricos do planeta. Ante esta situación, varios organismos internacionais defenden o uso de augas rexeneradas para diferentes propósitos que permite recuperar os custes parciais dos procesos de tratamento e proporcionan recursos hídricos alternativos. Sen embargo, a pesar do control dalgúns parámetros biolóxicos, a reutilización das augas residuais implica riscos para a saúde asociados a transmisión de certos patóxenos. *Cryptosporidium* é un xénero de protozoos entéricos emerxentes que causan brotes hídricos en todo o mundo. Este parasito ten unha forma inféctante resistente (ooquiste), cuxa presenza reportouse en efluentes de estacións depuradoras augas residuais, o que demostra que os tratamentos convencionais empregados para a reutilización da auga non son suficientes para a súa eliminación.

O obxectivo da presente Tese de Doutoramento é investigar a eficacia de novas tecnoloxías baseadas nos procesos de oxidación avanzados, concretamente a fotocatálise heteroxénea empregando dióxido de titanio (TiO_2) en suspensión, só ou combinada con peróxido de hidróxeno (H_2O_2), e a fotocatálise homoxénea mediante o proceso de foto-Fenton en condicións solares simuladas e/ou naturais, e a tecnoloxía de ultrasóns fronte o parasito de transmisión hídrica *Cryptosporidium parvum*. A viabilidade dos ooquistes determinouse mediante a aplicación da técnica inclusión/exclusión do colorante vital fluoroxénico ioduro de propidio.

A fotocatálise solar heteroxénea empregando TiO_2 en suspensión (0, 63, 100 e 200 mg/L) avaliouse en auga destilada ou nun efluente simulado de estación depuradora de augas residuais (EDAR) contaminados con ooquistes de *C. parvum* e expostos a radiación simulada nun tempo máximo de 5 horas. O emprego de TiO_2 en suspensión a concentración de 100 mg/L en auga destilada causou unha forte inactivación dos ooquistes despois de 5 horas de

exposición (4,16±2,35% *vs* 99,33±0,58%, viabilidade ooquística inicial). Sen embargo, nos ensaios realizados con efluentes simulados de EDAR, a incorporación do fotocatalizador non ofreceu mellores resultados. Despois do proceso de desinfección fotocatalítica, a recuperación do TiO_2 en suspensión por sedimentación proporcionou unha redución substancial da carga parasitaria nas mostras de auga tratada (57,81±1,10% e 82,10±2,64% para 200 mg/L de TiO_2 en auga destilada e en efluente simulado de EDAR, respectivamente).

Para acelerar o proceso de desinfección solar da auga, avaliouse a fotocatálise heteroxénea empregando unha combinación de TiO_2 e H_2O_2 baixo condicións solares simuladas e naturais. Desta forma, mostras de auga destilada contendo 100 mg/L de TiO_2 e 50 mg/L de H_2O_2 foron contaminadas con ooquistes de *C. parvum* e expostas a radiación solar durante 5 horas. Baixo condicións solares simuladas observouse unha forte diminución da viabilidade ooquística nas mostras que contiñan TiO_2 e TiO_2/H_2O_2 (4,16±2,35% e 3,82±4,26%, respectivamente, *vs* 90,44±5,87%, viabilidade ooquística inicial). Baixo luz solar natural, unha redución similar da viabilidade ooquística detectouse nas mesmas mostras pero tan só as 2,5 horas, a metade do tempo de exposición (4,45±3,55% e 1,58±0,48% nas mostras que contiñan TiO_2 e TiO_2/H_2O_2, respectivamente, *vs* 99,45±0,95%, viabilidade ooquística inicial). Sen embargo, a adición de H_2O_2 a baixas concentracións (50 mg/L) non mellorou o proceso de fotocatálise con TiO_2 fronte a *Cryptosporidium*.

Ademais, na presente Tese de Doutoramento avaliouse por primeira vez a eficacia da fotocatálise homoxénea polo proceso de foto-Fenton fronte a *C. parvum*. Para iso empregouse un deseño factorial que estuda os efectos combinados da concentración de Fe^{2+}/H_2O_2 (5/10, 10/20 e 20/50 mg/L), o pH (3, 5,5 e 8) e o tempo de exposición (2, 4 e 6 horas) sobre a supervivencia ooquística en auga destilada baixo luz solar natural. Os parámetros concentración de Fe^{2+}/H_2O_2 e tempo de exposición, así como a interacción do pH coa concentración de Fe^{2+}/H_2O_2 mostraron efectos estatisticamente significativos sobre a viabilidade ooquística. As maiores inactivacións ooquísticas corresponderon a combinación de maior concentración de Fe^{2+}/H_2O_2 (20/50 mg/L), o menor pH (3) e os maiores tempos de exposición (4 e 6 horas) (3,68±1,38% e 6,39±2,65%, respectivamente, *vs* 91,67±3,63%, viabilidade ooquística inicial).

Finalmente, a eficacia da tecnoloxía de ultrasóns foi avaliada empregando tres niveis de potencia (60, 80 e 100 W), en modo descontinuo o 50% ou en continuo, na inactivación dos ooquistes de *C. parvum* en auga destilada e en efluentes simulado, real e filtrado de EDAR. A aplicación dos ultrasóns a 80 W de potencia en modo continuo diminuíu fortemente a viabilidade de *C. parvum* tras exposición de 10 minutos, obténdose viabilidades ooquísticas de 4,16±1,93%; 1,29±0,86%; 3,16±0,69% e 3,15±0,87% en auga destilada e en efluentes simulado, real e filtrado de EDAR, respectivamente (*vs* 98,57±0,01%; viabilidade ooquística inicial). Independentemente do modo de traballo empregado (descontinuo/continuo) e a potencia de 80 W, o niveis de inactivación ooquística observados nos distintos efluentes de EDAR empregados foron maior que en auga destilada.

A interpretación global dos resultados obtidos trala avaliación dos diferentes procesos de oxidación avanzados para inactivar o parasito de transmisión hídrica *C. parvum*, considerado un patóxeno de referencia para a avaliación dos tratamentos de auga, demostra a eficacia destas tecnoloxías para mellorar a calidade microbiolóxica da auga rexenerada. Non obstante é necesario realizar máis estudos para optimizar as condicións de traballo en función de composición química do efluente das augas residuais. A inactivación deste patóxeno entérico probabelmente asegurará a eliminación doutros axentes infecciosos menos resistentes, permitindo o uso seguro da auga rexenerada para diferentes propósitos e proporcionando una valiosa protección do medio ambiente e, polo tanto da saúde humana e animal.

Palabras chave: *Cryptosporidium;* Desinfección de augas residuais; Procesos de oxidación avanzados; Dióxido de titanio; Peróxido de hidróxeno; Foto-Fenton; Ultrasóns; Augas rexeneradas.

ABSTRACT

The water resources available on Earth have been placed under enormous stress during the last few decades by the rapid growth of the world's population, the extraordinary increase in urbanization, the overuse of water in agriculture, global warming, the periodic occurrence of droughts and the gradual deterioration of water quality. In view of this situation, several international agencies have defended the use of reclaimed water for different purposes, as this enables partial recovery of the cost of treatment processes and provides alternative sources of water. However, although some biological parameters are controlled in relation to the reuse of treated wastewater, there are some health risks associated with the transmission of pathogens. *Cryptosporidium* is one genus of emerging enteropathogens responsible for important waterborne outbreaks of disease worldwide. This protozoan parasite has a robust infectious form (oocyst), which has been reported to occur in wastewater treatment plant effluents, indicating that conventional wastewater treatments do not remove the pathogen.

The aim of this Doctoral Thesis is to evaluate disinfectant efficacy of new technologies based on advanced oxidation processes (AOPs) to inactivate *Cryptosporidium parvum*, specifically heterogeneous photocatalysis with titanium dioxide (TiO_2) slurry, alone or in combination with hydrogen peroxide (H_2O_2), homogeneous photocatalysis by the photo-Fenton process, and ultrasound technology. The oocyst viability was determined by inclusion/exclusion of the fluorogenic vital dye propidium iodide.

Heterogeneous solar photocatalysis with TiO_2 slurry (0, 63, 100 and 200 mg/L) was evaluated using distilled water (DW) or a simulated wastewater treatment plant (WWTP) effluent spiked with *C. parvum* oocysts and exposed to simulated solar radiation for a maximum exposure time of 5 hours. The use of TiO_2 slurry at a concentration of 100 mg/L in DW yielded a high level of oocyst inactivation after 5 hours of exposure (4.16±2.35%

vs 99.33±0.58%, initial oocyst viability). However, in the assays carried out with simulated WWTP effluent, addition of the photocatalyst did not increase the efficacy of the process. After the photocatalytic disinfection process, the TiO_2 slurry was recovered by sedimentation and a substantial reduction in the parasitic load was observed in the treated water samples (57.81±1.10% and 82.10±2.64% for 200 mg/L of TiO_2 in DW and in simulated WWTP effluent, respectively).

In order to accelerate the solar-induced disinfection of water, heterogeneous photocatalysis with TiO_2 and H_2O_2 was conducted under simulated and natural solar radiations. Samples of DW containing 100 mg/L of TiO_2 and 50 mg/L of H_2O_2 were spiked with *C. parvum* oocysts and exposed to solar radiation for 5 hours. A strong decrease in the oocyst viability was observed in samples containing TiO_2 or TiO_2/H_2O_2 under simulated solar conditions (4.16±2.35% and 3.82±4.26%, respectively, *vs* 90.44±5.87%, initial oocyst viability). Under natural sunlight, and after 2.5 hours, similar drastic reductions in the oocyst viability were observed in samples containing TiO_2 or TiO_2/H_2O_2 (4.45±3.55% and 1.58±0.48%, respectively *vs* 99.45±0.95%, initial oocyst viability). However, the addition of a low concentration of H_2O_2 (50 mg/L) did not enhance the photocatalytic activity of TiO_2 against *Cryptosporidium*.

The efficacy of homogeneous photocatalysis by the photo-Fenton process for inactivating *C. parvum* was evaluated for the first time. For this purpose, a factorial design was used to study the combined effects of Fe^{2+}/H_2O_2 concentration (5/10, 10/20 and 20/50 mg/L), pH (3, 5.5 and 8) and exposure time (2, 4 and 6 hours) on oocyst survival in DW under natural sunlight. The variables Fe^{2+}/H_2O_2 concentration and exposure time had statistically significant effects on the oocyst viability, as did the interaction between pH and Fe^{2+}/H_2O_2 concentration. The maximum oocyst inactivation rates were obtained with the combination of the highest concentration of Fe^{2+}/H_2O_2

(20/50 mg/L), the lowest pH value (3) and the longest exposure times (4 and 6 hours) (3.68±1.38% and 6.39±2.65%, respectively, *vs* 91.67±3.63%, initial oocyst viability).

Finally, the efficacy of ultrasound technology was evaluated at three power levels (60, 80 and 100 W), pulsed at 50% or in continuous mode, for inactivating *C. parvum* oocysts in DW and simulated, real and filtered WWTP effluents. Application of ultrasound irradiation at 80 W power in continuous mode for an exposure time of 10 minutes greatly reduced the viability of *C. parvum*. Thus, oocyst viabilities of 4.16±1.93%, 1.29±0.86%, 3.16±0.69% and 3.15±0.87% were obtained in DW and simulated, real and filtered WWTP effluents, respectively (*vs* 98.57±0.01%, initial oocyst viability). Independently of the mode used (pulsed/continuous) and at 80 W power, the level of oocyst inactivation was higher in WWTP effluents than in DW.

The overall interpretation of the results obtained after evaluation of the efficacy of different AOPs for inactivating the waterborne parasite *C. parvum*, considered a reference pathogen in the evaluation of water treatments, demonstrates the effectiveness of these technologies for improving the microbiological quality of reclaimed water, although further studies are needed in order to optimize the working conditions regarding the chemical composition of wastewater effluents. Inactivation of this enteropathogen will probably ensure elimination other less resistant infectious agents, thus ensuring the safe of reclaimed water for different purposes and providing a valuable protection of the environment and consequently for the human and animal health.

Keywords: *Cryptosporidium;* Wastewater disinfection; Advanced oxidation processes; Titanium dioxide; Hydrogen peroxide; Photo-Fenton; Ultrasound; Reclaimed water.

INTRODUCTION

1 WATER

Water is an essential resource for life. It is vital for human health and well-being, for supporting ecosystems and for the development of agriculture and industry. Although the United Nations General Assembly (UN) has explicitly recognized the human right to water and sanitation, more than 663 million people throughout the world lack access to safe drinking water and 2,000 million people do not have sanitation facilities (World Bank, 2017). The World Health Organization (WHO) has estimated that unsafe drinking water, inadequate sanitation and poor hygiene lead to more than 800,000 diarrhoeal deaths annually (WHO, 2019a, 2019b). For these reasons, the 2030 agenda for Sustainable Development Goals (SDGs) addresses the comprehensive, inclusive and integrated improvement of water resources. SDG 6 focuses on the following targets: safe drinking water, sanitation and hygiene; water and wastewater quality; water use efficiency and scarcity; integrated water management; ecosystem protection; international cooperation; capacity building; and stakeholder participation (UN, 2015).

Freshwater represents 2.5% of the total water on earth. However, more than two-thirds of this water (68.6%) is frozen, occurring as snow and ice, and approximately one third is stored below ground as groundwater (30.1%). Thus, only 1.3% of freshwater on the planet is readily available as surface water in lakes, swamps, rivers and streams, which are the main source of water used for human consumption (Figure 1). Furthermore, water is not homogeneously distributed throughtout the world as some regions suffer greatly from water scarcity and excessive variability in supplies, and most water sources are not available for human use or consumption (Shiklomanov, 1993). The availability and sustainability of freshwater in sufficient quantity for humans and the functioning of the biosphere is therefore a global problem (Lehr and Keeler, 2005).

Moreover, several factors such as the growth of the world population, the large increase in urbanization, the use of water in agriculture (which currently consumes 70% of the resources); global warming and extreme weather events (floods and droughts); and the progressive deterioration of water quality (pollution and eutrophication) (Winpenny *et al.*, 2013; World Bank, 2016 continue to undermine the quality and availability of water (European Environment Agency, 2018).

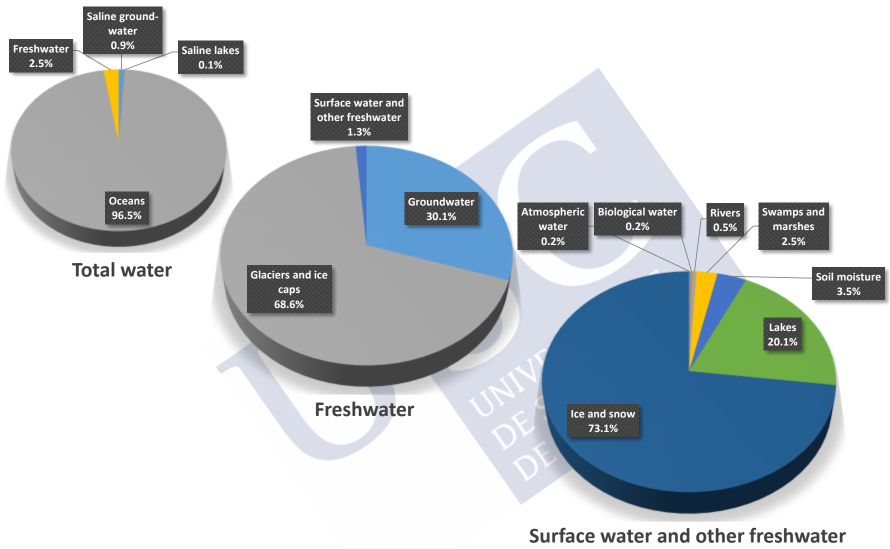

Figure 1. Water reserves on Earth.

Water stress is currently affecting millions of people around the world: two-thirds of the global population (4,000 million people) live under conditions of severe water scarcity during at least 1 month a year and approximately 500 million people face severe water scarcity all year round (Mekonnen and Hoekstra, 2016). By 2025, about 1,800 million people will be living in regions or countries with absolute water scarcity (World Bank, 2016).

In order to combat the problems of scarcity, water resources must be given more attention directed towards sustainable management in the coming years. This implies the need for greater efficiency, innovation, minimization of waste, reuse and recycling, all key components of a circular economy (Chen *et al.*, 2016; European Environment Agency, 2018). Consequently, water reuse has been introduced as an eco-efficient way of reducing water stress and it is a growing practice in many countries (United Nations Environment Programme [UNEP] and Global Environment Centre Foundation [GEC], 2004; Hochstrat *et al.*, 2008; Sa-nguanduan and Nititvattananon, 2011; Nitivttananon and Sa-nguanduan, 2016). Various international organizations such as the World Bank, the WHO and the United Nations Educational, Scientific and Cultural Organization (UNESCO) promote the reuse of wastewater as an alternative and reliable resource. In addition, water reuse is beneficial in both developed and developing countries because (Nitivttananon and Sa-nguanduan, 2016): i) increased water availability; ii) integrated and sustainable use of water resources; iii) drinking water is used only for drinking and reclaimed water is used for other purposes; iv) reduced over-abstraction of surface and groundwater; v) lower energy consumption than associated with the use of deep groundwater resources, water importation or desalination; vi) reduced nutrient loads to receive waters; vii) reduced manufacturing cost of using high quality reclaimed water; viii) increased agricultural production and reduced application of fertilisers; ix) enhanced environmental protection by restoration of streams, wetlands and ponds; x) and increased employment and development of local economies (Alcalde-Sanz and Gawlik, 2014).

1.1 Water reuse

The UNESCO defines wastewater as a combination of one or more domestic effluents that consist of blackwater (excrement, urine and faecal sludge) and greywater (sink and bath water); water from commercial establishments and institutions (including hospitals); industrial effluents; storm water and other urban run-off; and run-off from agriculture, horticulture and aquaculture (Raschid-Sally and Jayakody, 2009).

Water reuse or wastewater use is a comprehensive concept that involves the use of untreated, partially treated or treated wastewater in different applications. The terms ‘reused’, ‘recycled’ and ‘reclaimed’ are difficult to define as they are sometimes considered synonymous, whereas in other cases each is specifically defined.

The United Nations World Water Assessment Programme (WWAP) defines recycled water and/or reclaimed water as treated (‘fit-for-purpose’) wastewater that can be used under controlled conditions for beneficial purposes within the same establishment or in other facilities, respectively (WWAP, 2017).

Treated or untreated wastewater is a critical component of the water cycle and should be taken into account during all water management cycles - capture of freshwater (surface or groundwater), water treatment (drinking water treatment in drinking water treatment plants), distribution, use, collection and treatment of wastewater (wastewater treatment plant) - until its possible reuse and discharge in the environment, where the sources are recharged for the following capture freshwater.

It has been estimated that more of 80% of wastewater worldwide is discharged directly to the environment without adequate treatment (WWAP, 2012; UN-Water, 2015). This has direct impacts on human health, the environment and economic productivity. Indeed, the level of treatment of wastewater usually reflect the level of income in a country (70%, 38%, 28% and 8% of treated wastewater from countries characterised respectively by high, upper-middle, lower-middle and low incomes) (Sato *et al.*, 2013). Consideration of wastewater as a resource is aimed at reducing pollution as well as decreasing the cost of managing wastewater and improving the sustainable economy of a system. This will have a positive impact on freshwater supplies, human and environmental health and will generate income by promoting new business opportunities and supporting the advancement of a green economy. In this respect, in the previously mentioned SDGs, target 6.3 is closely related to wastewater management and focuses on the necessary increase in safe water recycling and reuse worldwide (UN-Water, 2016; WWAP, 2017).

1.2 Historical perspective

The treatment and reuse of wastewater is a concept that it has evolved and advanced throughout human history. The application of human waste to land is an ancient practice that has undergone different stages of development, leading to better knowledge of the process, technological advances and development of water quality standards (Angelakis and Snyder, 2015; Paranychianakis *et al.*, 2015), from ancient to contemporary times (Rose and Angelakis, 2014; Angelakis *et al.*, 2018). The practice of reusing of raw sewage in order to remove waste from human settlements has been carried out for centuries. The reuse of wastewater underwent a decline in the late 19th and early 20th centuries with the development of wastewater treatment methods, before regaining popularity due to population growth, urban development, climate change, rapid development of technologies, and the recognition that freshwater is a finite resource. In order to understand how water is distributed and to identify the problematic situation today, we must know how wastewater, and the associated problems, have been addressed throughout the centuries (Salgot and Folch, 2018).

1.2.1 Prehistoric times: the Neolithic and the Bronze Age (10,000 BC-8th century BC)

Until the Bronze Age and the birth of the first advanced civilizations, human excreta was removed when necessary as explained by the Mosaic Law of Sanitation (Deuteronomy 23.9-14) to an area of the ground or to a hole dug in the ground and covered after use. Growth of the population led to development of collection systems for wastewater and rainwater (Lofrano and Brown, 2010; Angelakis *et al.*, 2018).

Prehistoric civilizations developed advanced systems to move sewage to rivers, and the sea, or to use it to irrigate and fertilizate agricultural land. The first historical evidence of reuse of wastewater effluent reuse was found in Crete (Hellas) and Mohenjo-Daro (Indus Valley). The latter city received water from at least 700 wells and had bathrooms inside the house and sewage system in the streets. In the Harappa city (Indus Valley), sewage was removed from all houses to a main sewer, which was connected to larger sewers for transportion and disposal on agricultural land. Other great civilizations, such as the Minoan and Mesopotamian civilizations, which also flourished in this period, used similar water use systems and developed advanced sewerage and drainage systems (aqueducts, cisterns, filtration systems, sedimentation basins, rain collection systems, terracotta pipes for water and wastewater supply, as well as sewage and drainage systems) (Angelakis *et al.*, 2005; Angelakis and Zheng, 2015).

1.2.2 Classical antiquity (8th century BC-5th century AD)

Ancient Greece was one of the first civilizations to use the wastewater in agriculture, and archaeological and historical sources of evidences show advanced wastewater technologies that extended throughout the Archaic (*ca*. 750-480 BC) and Classical (*ca*. 480-336 BC) periods (Angelakis and Zheng, 2015). During the Classical period, the sewage produced from public toilets and residences was eliminated, along with rainwater, through combined sewerage and drainage systems to collection basins outside the city through the river Eridanos (De Feo *et al.*, 2013). A sewerage and drainage system found in southeast of the Acropolis consisted of a central sewer that transported the wastewater collected from surrounding houses and shops throught to clay drainpipes. In addition, the great drain of the ancient Agora (Athens) delivered rainwater and sewage to a collection basin (Antoniou,

2010) (Figure 2). From the basin, the wastewater was transported through brick-lined conduits to agricultural land in Elaionas, where it was used to irrigate and fertilize orchards and field crops (Angelakis and Gikas, 2014; Tzanakakis *et al.*, 2014; Yannopoulos *et al.*, 2015).

Figure 2. Photograph of a section of the Hadrianic aqueduct near the Ancient Agora (Athens) (🅭 Carole Raddato).

The Romans inherited and further developed the Greek water systems throughout the Roman Empire (1st century BC-5th century AD), increasing the scale of the application and implementing water projects in almost all cities (De Feo *et al.*, 2013; Angelakis and Zheng, 2015).

1.2.3 Medieval times (6th-15th century AD)

Water technology and knowledge during medieval times in Europe made little progress, and sanitation in most cities even become very primitive or reverted to basic sanitation. In European cities, the inhabitants dumped wastwater in the middle of the streets or where it was collected by sewage carts and finally discharged close to water bodies. The inhabitants of densely populated towns and villages were thus subjected to bad odours and were susceptible to illnesses and disease outbreaks that sometimes decimated the population (Salgot and Folch, 2018). As a result, at least 25% of the European population died during this period as a result of waterborne diseases (Schladweiler, 2001).

Islamic culture religiously mandated high levels of personal hygiene, along with highly developed water supplies and adequate sanitation systems, which in some cases were the same as Greek and Roman facilities (Mays, 2008; Angelakis and Zheng, 2015; Salgot and Folch, 2018).

At the end of the Medieval Age (14th and 15th centuries), in the Mediterranean region and in some countries in northern Europe, sewage was used to irrigate agricultural land (Durham *et al.*, 2005). In Central and South America, innovative ways of reusing water, such as the *chinampa* system were already in use before these countries were colonized. *Chinampa* is a Mesoamerican agricultural practice described as floating gardens, consisting of small but highly productive plots constructed on wetlands, swamps, shallow lakes and floodplains with sediments, manure, compost and plant debris (Smith, 1996). The Aztec civilizations (around 1200-1500 AD) were the first to document the use of *chinampa,* observing the benefits of adding animal and human waste to soil land agricultural lands (Coe, 1964; Angelakis *et al.*, 2018).

1.2.4 Modern and contemporary times (16th-20th century AD)

The disposal of human waste became an important problem in major cities during this period as the population increased and waste management was unregulated. Recognition by authorities for the need for sanitation led to development of means of disposing and reusing effluents, know as ‘sewage farms’ to protect the public health and control contamination (Stanbridge, 1976). The first ‘sewage farms’ were operated in 1531 in Bunzlau (Silesia, Poland) and in 1650 in Edinburgh (Scotland, United Kingdom), where wastewater was used to irrigate and fertilize agricultural land. At the end of the 18th century, large sewage farms were established in large cities in Europe, the United States of America (USA) and, at the end of 19th century, in Australia (Tzanakakis *et al.*, 2014; Angelakis and Snyder, 2015).

In the 19th century, sanitation practices remerged after the great epidemics that occurred in various parts of the world. Thus, the great cholera and typhoid fever epidemic that resulted in the death of 10,000 people in England between 1830 and 1850 caused by contamination of water sources with raw sewage, revealing the need for better sanitation and protection of water sources. In the summer of 1858, central London was affected the 'Great Stink', with the overpowering smell occurring as a consequence of the discharge of untreated human sewage into the River Thames. Scientists observation of the relationship between sewage disposal and the health of the population led to the establishment of sanitation regulations by public health and environmental policy agencies (Stanbridge, 1976; Angelakis and Gikas, 2014; Angelakis and Snyder, 2015).

Moreover, new methods of treating sewage (large septic tanks, contact beds, and trickling filters) before it was discharged on land or in freshwater bodies were developed in both Germany and England (Angelakis *et al.,* 2018). Reuse as a planning activity was implemented in San Francisco (California, USA) in the 19th century as treated effluent was used to irrigate the Golden Gate park (Hyde, 1937; Angelakis and Snyder, 2015).

The 20th century brought important technological and scientific advances, in addition to a large increase in the number of wastewater treatment plants (WWTP), where were widely adopted in the major cities of the world because they were compact, mechanized and could handle large volumes of sewage for direct discharge in to rivers or the sea (Jiménez and Asano, 2008; Lazarova *et al.*, 2013). The direct discharge of treated effluents in water bodies led to decrease in the interest in recovering nutrients and organic matter to fertilize land and improve soil characteristics. However, California was a pioneering state in promoting the regeneration and reuse of water. Thus, in 1918, the California State Board of Health established the water quality requirements needed for the reuse of effluents from a septic tank system and regulated their use for agricultural irrigation (California State Board of Health, 1918). Nevertheless, it was not until the latter part of the 20th century and early 21th century that water reclamation and reuse regained popularity (Asano *et al.*, 2007; Angelakis *et al.*, 2018). Thus, the concept of purified/regenerated water although not new, arise in the 1970s in many countries (Israel, Spain, USA, among others). The concept is applicable to treated water, the quality of which makes it suitable for reuse, with an approach considered from the health point of view (Ayers and Westcot, 1994).

1.3 Conventional wastewater treatments

As already mentioned, wastewater treatment is important for human and environmental health, and reuse of the effluent is also appropriate for domestic, agricultural and industrial applications. This process is carried out in WWTP, where the sewage is subjected to physical, chemical, biological and disinfection processes in order to remove organic matter, pollutants and microorganisms. The guidelines for physical-chemical and microbiological parameters that treated wastewater must meet are very diverse and depend on the country of application. However, wastewater treatment process are very similar, involving primary, secondary and tertiary treatments (Kumar-Singh *et al.,* 2016).

In primary treatment, the raw sewage is subjected to physical and chemical processes (passed through screens, coagulation, flocculation, flotation…) in order to separate large objects such as rags, plastic, oil fatty acids, and suspended solids. Initial screening remove material of different size (10 mm to >50 mm). The filtered raw sewage is then passed through a grit chamber, where the influent is slowed down so that the suspended solid particles fall to the bottom, whereas fats, oil or greases float on the top. During the coagulation process, the colloidal particles are destabilized by addition of coagulants, most commonly aluminium sulphate. Several reagents are added to the influent during the flocculation process to promote the formation of agglomerates of destabilized colloidal particles. Finally, flotation occurs on solid-liquid or liquid-liquid separation when the density of the particles is less than the density of the liquid. This initial treatment removes approximately 55% of suspended solids and reduces the biochemical oxygen demand (BOD) by 35%, because some of these particles are biodegradable. This treatment can remove bacteria, viruses and protozoa, although the proportion removed from the different microorganisms varies widely: 25% of faecal coliforms; 29% of faecal streptococci; 12% of *Escherichia coli*; 51% of *Clostridium perfringens*; 76% of *Giardia* spp.; 27% of *Cryptospodium* spp.; the treatment is totally ineffective against enteric viruses (Payment *et al.*, 2001).

Secondary treatment usually uses microorganisms to convert most contaminants from the soluble and non-settling solids into settleable solids (wastewater biosolids) (Hartley, 2006). Biological treatments are very diverse, although activated sludge is commonly used. The process involves aeration and agitation of a bacterial culture in tanks fed with the influent. This process can remove up to 95% of the BOD and suspended solids, as well as significant amounts of heavy metals, some organic compounds and approximately 2-3 log of microbial indicators (Wen *et al.*, 2009; Voulvoulis, 2018).

Conventional wastewater treatment usually ends with secondary treatment, after which the water is discharged into a stream or water body. However, secondary treatment cannot efficiently remove all of the different compounds found in sewage, and treated effluents therefore constitute one of the main sources of persistent micropollutants in the environment (Rowsell *et al.*, 2010). Tertiary treatment effluents of better quality, as it removes other contaminants such as microbial pathogens, particulates and nutrients (nitrogen and phosphorus). This allows effluents to be discharged into areas where the requirements are stricter or allow them to be reused in other applications. Tertiary disinfection treatment can use chemicals (chlorine or ozone), membrane systems (reverse osmosis), or physical process (ultraviolet radiation) (Kumar-Singh *et al.*, 2016).

Chlorination has been widely used as a disinfectant in water and to treat wastewater to inactivate pathogens (Adeyemo *et al.*, 2019). However, several waterborne protozoa, such as *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts, which are resistant to the concentrations of chlorine usually used, constitute a serious challenge in standard water treatment processes (Korich *et al.*, 1990; Rennecker *et al.*, 2000; Craun *et al.*, 2010). Moreover, the use of high doses of chlorine may have ecological side effects due to the formation of chlorinated hydrocarbons (Redwan and Abdullah, 2012).

Ozone disinfection is effective against pathogens that are resistant to chlorine (Campos-Reales-Pineda *et al.*, 2008), as ozonisation significantly reduces the viability and infectivity of *Cryptosporidium* spp. oocysts (Quintero-Betancourt *et al.*, 2003; Pereira *et al.*, 2008). However, high concentrations and high exposure times are necessary for effective inactivation of this protozoa. Rennecker *et al.* (2000) used a concentration of 5 mg/L for exposure times of 5 minutes, and achieved of 99% of inactivation of *Cryptosporidium parvum* oocysts.

Ultraviolet (UV) radiation is a physical method widely used in WWTPs for additional treatment of secondary effluents (Adeyemo *et al.*, 2019) and several studies have demonstrated the efficiency of this process to inactivate resistant forms of *Giardia* spp. and *Cryptosporidium* spp. (Morita *et al.*, 2002; Hijnen *et al.*, 2006; Adeyemo *et al.*, 2019).

1.4 New technologies for wastewater treatment based on advanced oxidation processes

Advanced oxidation processes (AOPs) are oxidative processes that involve the formation of hydroxyl radical ($HO^{\bullet}$), which has the second oxidizing potential after fluorine. These radicals are capable of non-selective oxidizing and mineralizing a wide variety of organic molecules, allowing the degradation of recalcitrant and emerging contaminants and the inactivation of different microorganisms in water. AOPs investigated for application in water treatment include photochemical and non-photochemical processes (Table 1) (Kanakaraju *et al.*, 2018).

Table 1. Classification of advanced oxidation processes.

Non-photochemical	Photochemical
Fenton	UV/H_2O_2
O_3/H_2O_2	UV/O_3
Electrochemical oxidation	$UV/H_2O_2/O_3$
Ultrasound irradiation	UV/TiO_2
Sub/super critical water	Photo-Fenton

The term photocatalysis was first defined by Carey *et al.* in 1976 as the acceleration of a photoreaction through the presence of a catalyst, with light and a catalyst being essential (Carey *et al.*, 1976). In this way, chemical species are altered as a result of the absorption of UV-visible radiation by a photosensitive species, the catalyst. Heterogeneous photocatalysis is based on the use of a solid semiconductor (e.g. titanium dioxide, zinc oxide, zinc sulphide, cadmium sulphide and iron oxides) irradiated with photons of the appropriate wavelength to generate a reaction at the solid-liquid or solid-gas interface. By definition, the catalyst must be able to be reused after acting in the oxidation-reduction system without undergoing significant changes (Herrmann, 2005). On the contrary, in homogeneous photocatalysis, all of the components are at the same phase, generally dissolved in the liquid phase, and copper and iron salts are often used (Malato *et al.*, 2009).

1.4.1 Heterogeneous photocatalysis with titanium dioxide (TiO_2)

In the last few decades, the degradation of chemical compounds by photocatalytic or photochemical processes has gained importance in the area of wastewater treatment. Water disinfection is very importance in photocatalytic processes. Among the AOPs, heterogeneous photocatalysis with TiO_2 is the most widely investigated, particularly as a tertiary treatment for the degradation of chemical pollutants present in wastewater. The main advantages are as follows: i) the absence of toxicity; ii) the high stability and possible reuse of the treated wastewater; iii) the low cost of the photocatalyst; iv) the gentle working conditions (natural pH, environmental temperature and pressure); v) the absence of additional chemical agents; and, vi) the possible use of sunlight as a source of radiation (Malato *et al.*, 2009; Byrne *et al.*, 2015).

In addition, TiO_2 photocatalysis can be carried out by maintaining the catalyst in aqueous suspension or immobilized in a solid support. The choice of one method or the other will depend on the final destination of the treated water. Thus, for purifying of drinking water, the TiO_2 must be immobilized, whereas in wastewater treatment, the photocatalyst can be used in suspension, thus providing a larger surface area of contact. In addition to latter reuse, it is possible to recover TiO_2 by different methods, some as simple as sedimentation (Fernández Ibáñez, 2004) and others based on the use of filters and/or coagulating agents (Xi and Geissen, 2001; Gustafsson *et al.*, 2003).

Titanium dioxide occurs in an oxidized state as three different crystalline forms: brookite, rutile and anatase, with the latter being the most active from a photocatalytic point of view (Fernández Ibáñez, 2004). The commercial Aeroxide P-25 (Degussa, Evonik Industries, Essen, Germany) is characterized by a 70:30 ratio of anatase:rutile (Tech. Bulle Pigm., Degussa, 1991), a mean particle size of 20-40 nm and a specific area (surface area per sample mass unit) of 55±5 m^2/g (Fernández Ibáñez, 2004). Moreover, TiO_2 Degussa P-25 is the most commonly used form, providing the highest degradation efficiency (Sakthivel *et al.*, 2000; Yamazaki *et al.*, 2001).

In heterogeneous photocatalysis with TiO_2, organic compounds (M) are oxidized (M_x) through the valence band opening while oxygen is reduced (Thiruvenkatachari *et al.*, 2008; Chong *et al.*, 2010). The positive opening can also react with water, forming the $HO^{\bullet}$ (Malato *et al.*, 2009; Foster *et al.*, 2011), which can further oxidize organic compounds (Thiruvenkatachari *et al.*, 2008) (Figure 3).

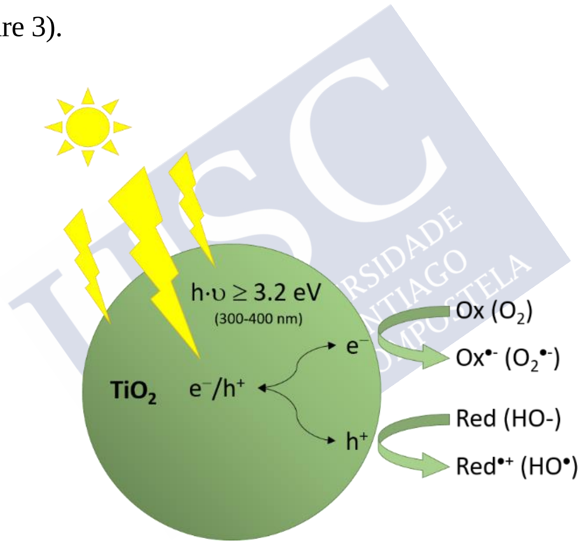

Figure 3. Diagram illustrating an advanced oxidation process involving use of UV radiation of 300-400 nm in a particle of TiO_2 to excite an electron to the conduction band, creating a positive opening in the valence band (h^+).

The chemical reactions that occur during this process are summarized by equations 1-5 (Wu and Englehardt, 2015; Gassie *et al.*, 2016; Gassie and Englehardt, 2017):

$$TiO_2 + hv \rightarrow e^- + h^+ \tag{1}$$

$$e^- + O_2 \rightarrow O_2^- \tag{2}$$

$$h^+ + M \rightarrow M_x \quad (3)$$

$$h^+ + H_2O \rightarrow HO^\bullet \quad (4)$$

$$HO^\bullet + M \rightarrow M_x \quad (5)$$

Matsunaga *et al.* (1985) first reported on the potential use of TiO_2 in disinfection processes by verifying the total or partial inactivation of *Lactobacillus acidophilus*, *E. coli*, *Saccharomyces cerevisiae* and *Chlorella vulgaris* after 120 minutes of incubation. Subsequently, Ireland *et al.* (1993) observed the total reduction of bacterial flora in non-chlorinated samples of surface water exposure to TiO_2 in a continuous flow reactor. Herrera Melián *et al.* (2000) proved the efficacy of the use of TiO_2 in urban wastewater disinfection, significantly reducing the bacterial load of faecal coliforms by using UV-C radiation lamps and solar light. More recently, Rincón and Pulgarin (2004) demonstrated a residual disinfectant effect by the lack of re-growth of *E. coli* K12 within 60 hours of TiO_2 treatment in the presence of sunlight. Other studies shown the efficacy of TiO_2 against more resistant microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. cerevisiae*, *Candida albicans* and *Fusarium* spp. (Seven *et al.*, 2004; Sichel *et al.*, 2007; Polo-López *et al.*, 2012). A significant reduction in the infectivity of infectious biomolecules (prions) was even demonstrated after heterogeneous photocatalysis treatment of the samples with TiO_2 (Paspaltsis *et al.*, 2009).

The mechanism underlying photocatalytic inactivation is not yet clear. However, numerous studies have investigated the generation of reactive oxygen species (ROS) (hydroxyl radical, superoxide radical anion, hydroperoxyl radical and hydrogen peroxide) and their interaction with biological structures in an attempt to elucidate the inactivation mechanisms, concluding that the leading cause of loss of microorganism viability is not yet completely understood (Dalrymple *et al.*, 2010; Byrne *et al.*, 2015).

1.4.2 Homogeneous photocatalysis by photo-Fenton process

Henry J. Fenton described the Fenton reaction in 1894, demonstrating that hydrogen peroxide (H_2O_2) could be activated by Fe^{2+} salts to oxidize tartaric acid in aqueous solution (Fenton, 1894). In 1934, $HO^{\bullet}$ was suggested to be the main compound responsible for the oxidative capacity of the Fenton reaction (Haber and Weiss, 1934). However, during the 1950s, Barb *et al.* (1949, 1951a, 1951b) reported a number of reactions, which today still describe the classical Fenton reaction, considered a key stage in the process of production of $HO^{\bullet}$. The mechanism proposed by Barb *et al.* (1951a, 1951b) includes the main reactions involved in the decomposition of H_2O_2 in darkness, pure acid solution and in absence of organic compounds (Rigg *et al.*, 1954; Pignatello *et al.*, 2006; Polo-López *et al.*, 2018):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\bullet} \qquad (6)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \qquad (7)$$

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \qquad (8)$$

$$HO^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + OH^- \qquad (9)$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+ \qquad \text{pH 3} \qquad (10)$$

$$Fe^{2+} + HO_2^{\bullet} + H^+ \rightarrow Fe^{3+} + H_2O_2 \qquad \text{pH 3} \qquad (11)$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 \qquad (12)$$

In the absence of other oxidizing agent, the reaction involves the conversion of H_2O_2 to molecular oxygen (O_2) and water catalysed by iron (reaction 13).

$$2H_2O_2 \xrightarrow{Fe} O_2 + H_2O \qquad (13)$$

Generation of $HO^{\bullet}$ during the Fenton reaction is limited because reaction 7 is extremely slow relative to reaction 6. However, in the 1990s, it was shown that the process can be accelerated by irradiation with UV or visible light, and this was investigated as a novel method for water treatment (Bauer, 1994;

Oppenländer, 2003). This process, known as photo-Fenton, leads to much faster rates of generation of $HO^{\bullet}$ and therefore a higher degree of oxidation than the Fenton reaction in darkness (Polo-López *et al.*, 2018). Briefly, when the Fe^{3+} aqua complexes are irradiated at wavelengths below 580 nm, these complexes are reduced to Fe^{2+} complexes (reaction 10), generating an extra $HO^{\bullet}$ and enabling the iron cycle to restart, thus increasing the efficiency of the process (Pignatello *et al.*, 2006).

$$Fe^{3+}(L)_n + hv \rightarrow Fe^{2+}(L)_{n-1} + L_{ox}^{\bullet} \tag{14}$$

This reaction is beneficial for the photo-Fenton process as reduced iron can react with H_2O_2 to produce more $HO^{\bullet}$ (reaction 6). However, generation of the radical by reaction 6 produces large stoichiometric quantities of Fe^{3+} that precipitate as ferric oxyhydroxides when the pH varies from acid to neutral (the optimal pH to prevent iron precipitation is 2.8) (Tang and Huang, 1996; Kwon *et al.*, 1999). The precipitation of oxyhydroxides reduces the efficiency of the photo-Fenton process (Pignatello *et al.*, 2006). The Fe^{3+} complexes usually generated in acid solution are $Fe(OH)^{2+}$ and $[Fe_2(OH)_2]^{4+}$, which absorb UV or visible light. These complexes undergo photoreduction to yield $HO^{\bullet}$ and Fe^{2+} (reaction 15). The most important iron species in the photo-Fenton process is the $Fe(OH)^{2+}$ complex due to the combination of a high coefficient of absorption and greater concentration relative to Fe^{3+} species.

$$Fe(OH)^{2+} + hv \rightarrow Fe^{2+} + HO^{\bullet} \tag{15}$$

Since the 1960s, use of the Fenton reaction to degrade toxic organic compounds in water has been investigated. However, evaluation of this process to inactivate waterborne pathogens only began in the last decade (Polo-López *et al.*, 2018). The first demonstration of the capacity of the photo-Fenton process to disinfect water was reported by Rincón and Pulgarin (2006). These authors showed that the use of low concentrations of reagent (0.3 mg/L of Fe and 10 mg/L of H_2O_2) greatly enhanced the inactivation kinetics of

E. coli in water (Rincón and Pulgarin, 2006). Since then, the efficiency of the photo-Fenton process against other pathogens and the related chemical and biological parameters have been investigated in several types of water (Giannakis *et al.*, 2016a; Polo-López *et al.*, 2018).

Sciacca *et al.* (2010) described the benefits associated with the addition of H_2O_2 to the solar disinfection process (SODIS) for treatment of surface waters that contain dissolved iron salts. These authors observed that solar disinfection does not inactivate of *Salmonella* sp. after 6 hours of exposure to natural solar radiation, while the addition of 10 mg/L of H_2O_2 strongly increases the inactivation of bacteria.

1.4.3 Photolysis of hydrogen peroxide

Hydrogen peroxide is an oxidant that has been used as additive in AOPs together with ozone, UV-C radiation, TiO_2 and the photo-Fenton process (Malato *et al.*, 2009). Irradiation of H_2O_2 with photons of wavelength less than 300 nm generates photolysis of H_2O_2, producing two molecules of $HO^{\bullet}$ (reaction 16) (Malato *et al.*, 2009; Gassie *et al.*, 2016).

$$H_2O_2 + hv \rightarrow 2HO^{\bullet} \tag{16}$$

The first evidence of the lethal synergistic effect between H_2O_2 and solar radiation was described for the bacteriophage T7 (Ananthaswamy and Eisenstark, 1976, 1977; Ananthaswamy *et al.*, 1979) and subsequently for *E. coli* k12 (Hartman and Eisenstark, 1980). Although the germicide mechanism is not known, different authors suggest that the increase in oxidative stress cannot be controlled by the enzymatic mechanisms of the microorganisms. Rincón and Pulgarin (2006) demonstrated the inactivation of *E. coli* by H_2O_2 and UV-A radiation, which was increased in the presence of iron salts. In addition, Spuhler *et al.* (2010) showed the bactericidal effect of the combination of H_2O_2 and simulated solar radiation, demonstrating the inactivation of *E. coli* at a concentration of 10 mg/L of H_2O_2.

Moreover, Sichel *et al.* (2009) proved, for the first time, the lethal synergistic effect of low concentrations of H_2O_2 (≤5 mg/L) and solar natural radiation for inactivating *Fusarium solani* spores. Finally, Polo-López *et al.* (2011) showed the fungicidal capacity of the combination of H_2O_2 (10 mg/L) and natural solar radiation against *Fusarium equiseti* spores in different types of water (distilled water, well water and simulated wastewater treatment plant effluent) in borosilicate glass bottles and solar reactors fitted with compound parabolic collectors.

1.4.4 Ultrasound irradiation

Ultrasound comprises sound waves of frequency above the threshold perceived by the human ear (20-20,000 Hz), in a range from 20 kHz to 20 MHz (Quesada Peñate, 2009). The waves are generated by mechanical or electrical energy in an ultrasonic transductor and can be classified into different categories depending on their frequency and intensity. Low frequency ultrasound ranges from 20 to 100 kHz, whereas high frequency ultrasound ranges from 100 kHz to 1 MHz. On the other hand, low intensity ultrasound generates power of less than one watt. However, high intensity ultrasound is capable of generating tens of watts (Van der Walt, 2002).

For more than one hundred years, ultrasound technology has been used for numerous applications in several fields: i) synthesis and chemical processing (reducing/increasing the time and the yield of chemical reactions to increase the paths of reaction and the efficacy of catalyst, chemical of polymers, solid-liquid extraction and crystallization); ii) the oil industry (for refining fossil fuels, determinating the composition and extraction of coal tars); iii) the textile industry (improving the efficiency of dyeing techniques); iv) medicine (diagnostic techniques); v) the naval industry (cleaning and detection of structural defects in the hulls of ships); vi) biotechnology (in cellular lysis and release of intracellular compounds); and, vii) water treatment (degradation of emerging chemical pollutants, water disinfection, homogenization of sludge) (Thompson and Doraiswamy, 1999; Ashokkumar *et al.*, 2003; Piyasena *et al.*, 2003; Antoniadis *et al.*, 2007; Olvera *et al.*, 2008; Drakopoulou *et al.*, 2009; Wang *et al.*, 2011).

The several applications of the ultrasound are based on the phenomenon of acoustic cavitation, which has physical, mechanical and chemical effects on solids as well as in aqueous solutions. In the latter media, the cavitation phenomenon can be differentiated in three successive phases: the first phase consists of the process of nucleation, in which a cavitation core is generated from microbubbles trapped in microfractures of the particles suspended in the aqueous solution; in the second phase, the microbubbles grow and expand depending on the intensity of the sound wave; and, in the final phase of the cavitation process, the microbubbles collapse, although only if the intensity of sound wave exceeds the threshold of acoustic cavitation (usually a few watts per square centimetre at 20 kHz). Under these conditions, the microbubbles expand until they cannot absorb more energy and implode violently. In this phase of collapse, extreme temperature and pressure values are reached, so that the gas trapped inside the microbubble is submitted to molecular fraction, the phenomenon on which sonochemistry is based (Figure 4) (Ince *et al.*, 2001; Zupanc *et al.*, 2019).

The collapse of microbubbles can be explained by the 'hot spot' theory, which establishes that microbubbles collapse so quickly that the process is adiabatic, i.e. no heat exchange occurs with the medium. Consequently, the temperature and the pressure registered inside the microbubbles during the collapse can reached extremely high values of up to 3900-5000 ºC and 200-500 atm, respectively. The hot spot generated by the rapid collapse of the acoustic cavities is produced in a short period of time, and the diameter of the bubble is approximately 170 μm at a frequency of 20 kHz (Figure 4).

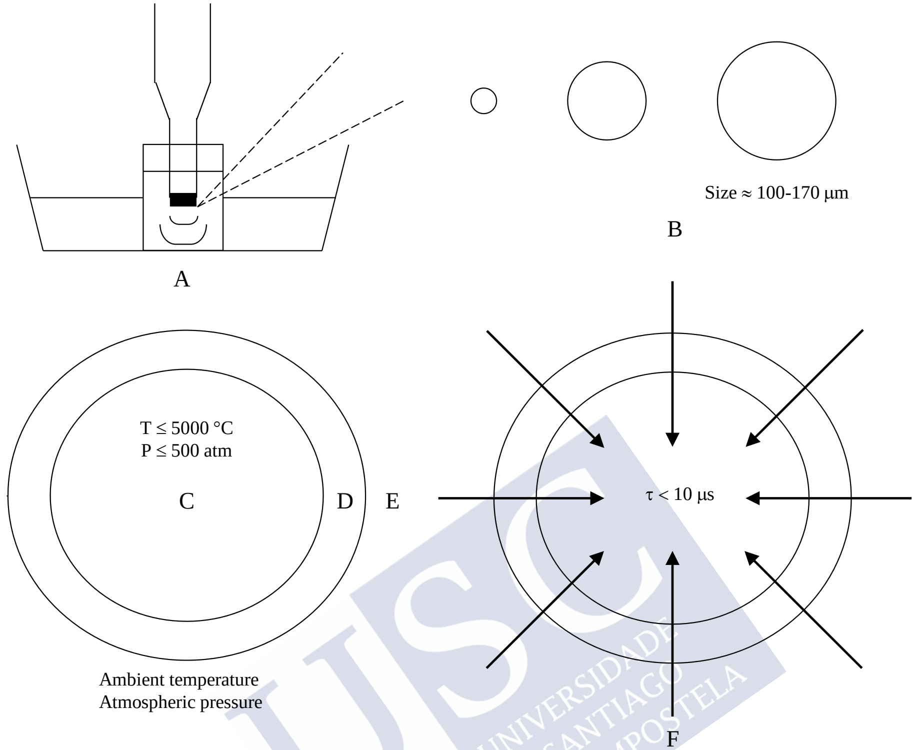

Figure 4. Representative diagram of the successive phases of the cavitation phenomenon.

A, ultrasound irradiation of aqueous solution; B, core and growth phase of microbubbles of cavitation; C, hot core gas, site where extreme values of temperature and pressure are reached; D, interphase or middle region, where a temperature gradient occurs; E, aqueous dissolution, with ambient temperature and atmospheric pressure values; and, F, collapse of cavitation microbubble. τ, half-life of microbubbles of cavitation.

The extreme conditions that occur during the collapse of the microbubbles have catalytic effects that lead to various sonochemical reactions. Thus, in pure aqueous systems and as a consequence of the fragmentation of water molecules in gaseous phase, ROS are generated, in combination with H_2O_2 and O_3, as shown in reactions 17-29 (Ince *et al.*, 2001; Adewuyi, 2005).

$$H_2O + ultrasound \rightarrow H^{\bullet} + HO^{\bullet} \quad (17)$$

$$HO^{\bullet} + OH^{\bullet} \rightarrow H_2O + O^{\bullet} \quad (18)$$

$$HO^{\bullet} + H_2O \rightarrow H_2O_2 + O^{\bullet} \quad (19)$$

$$H^{\bullet} + HO^{\bullet} \rightarrow H_2O \quad (20)$$

$$H^{\bullet} + H^{\bullet} \rightarrow H_2 \quad (21)$$

$$O^{\bullet} + O^{\bullet} \rightarrow O_2 \quad (22)$$

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2 + O_2 \quad (23)$$

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2 \quad (24)$$

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \quad (25)$$

$$HO_2^{\bullet} + H^{\bullet} \rightarrow H_2O_2 \quad (26)$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad (27)$$

$$O_2 \rightarrow 2^{\bullet}O \quad (28)$$

$$O_2 + O^{\bullet} \rightarrow O_3 \quad (29)$$

The radicals generated react with each other to form new molecules and radicals or they diffuse into the medium, acting as oxidants. In aqueous solutions that contain solutes and organic volatile gas, collapse of the microbubbles creates $HO^{\bullet}$ and $H^{\bullet}$ by fragmentation of the water molecules and can also generate inorganic radicals (Ince *et al.*, 2001). The production of free radicals and H_2O_2 depends on the frequency and intensity of the ultrasound irradiation, the properties of aqueous solution and the nature of dissolved gas (Van der Walt, 2002).

Water treatments based on ultrasound technology have been investigated in relation to the inactivation of different microorganisms using different frequencies and power levels. Using high frequency ultrasonic equipment (maximum 20 kHz), Gao *et al.* (2014) inactivated up to 90% of *Enterobacter aerogenes* at power levels of 4, 8 and 12 W after respectively 45, 14 and 11 minutes. However, the yeast species *Aureobasidium polluans* needed longer exposure times to achieve the same level of inactivation at power levels of 4, 8, 10 and 12 W after 364, 92, 26 and 23 minutes, respectively (Gao *et al.*, 2014). Furthermore, at the same frequency of 20 kHz, the inactivation rates for *E. coli* and *S. aureus* were 98.14±0.89% and 91.68±1.06%, respectively, after 20 minutes at 60 W/cm^2 (Li *et al.*, 2016).

1.5 Guidelines and regulations for water reuse

Water reclamation strategies take into consideration efforts to minimize environmental and health risks that require high-level guidance based on a majority consensus (Alcalde-Sanz and Gawlik, 2014). Thus, in order to ensure safe and efficient consideration of wastewater reclamation, recycling and reuse, strong and effective legislation on water reuse is required (Thakur and Kabo-bah, 2016). In this way, several international and national organizations have developed guidelines or regulations for water reuse (Tables 2-4) (Ayers and Westcot, 1994; UNEP, 2005; WHO, 2006b; Alcalde-Sanz and Gawlik, 2014).

Table 2. Water reuse guidelines developed by international organizations.

Organization	Guidelines (year of publication)
Food and Agriculture Organization of United Nations (FAO)	Water quality for agriculture (1994)
United Nations Environment Programme (UNEP)	Guidelines for municipal wastewater reuse in the Mediterranean region (2005)
World Health Organization (WHO)	Guidelines for the safe use of wastewater, excreta and greywater (2006)

Source: Ayers and Westcot (1994); UNEP (2005); WHO (2006b)

Table 3. Several criteria and guidelines for water reuse in non-European Countries.

Country	Guidelines (year of publication)
United States of America	Guidelines for water reuse (2012)
Australia	Guidelines for water recycling: managing health and environmental risks (2006)
Canada	Canadian guidelines for domestic reclaimed water for use in toilet and urinal flushing (2010)
China	National reclaimed water quality standards (GB/T 18920-2002, GB/T 18921-2002, GB/T 19923-2005, GB/T 19772-2005 and, GB 20922-2007)
Israel	Effluent irrigation disposal (2005)
Japan	Report of the microbial water quality project on treated sewage and reclaimed wastewater (2008)
Jordan	Jordanian technical base n. 893/2006 from Jordan water reuse management Plan (2006)
Mexico	Mexican Standards NOM-001-ECOL-1996 governing wastewater reuse in Agriculture (1996)
South Africa	The South Africa guide for the permissible utilization and disposal of treated effluent (1978)
Tunisia	Standard for use of treated wastewater in agriculture (NT 106-109 of 1989) and list of crops that can be irrigated with treated wastewater (1994)
Turkey	Water Pollution Control Regulations (1991)

Source: Alcalde-Sanz and Gawlik (2014)

Table 4. Most representative legislation on water reuse in European Union Members States.

Country	Legislation
Cyprus	Law 106 (I)/2002, control of the water and soil pollution and associated regulations: KDP 407/2002, 772/2003, and 269/2005, KD 379/2015. The Ministry of Agriculture, Natural Resources and Environment, and Water development Department.
France	Decree from 2 August 2010, use of water from treated urban wastewater for irrigation of crops and green areas (amended in 2014 - JORF num. 0153 of 4 July 2014). The Ministry of Public Health; Agriculture, Food and Fisheries; and Energy and Sustainability.
Greece	Joint Ministerial Decree 14516/11 or CMD No 145116, measures, limits and procedures for reuse of treated wastewater. The Ministry of Environment and Energy and Climate Change.
Italy	Ministry Decree 185/2003, technical measures for reuse of wastewater. The Ministry of Environment; Agriculture; and Public Health.
Portugal	Standard NP 4434, reuse of reclaimed urban water for irrigation. The Portuguese Institute for Quality.
Spain	Royal Decree 1620/2007, legal framework for the reuse of treated wastewater. The Ministry of Environment; Agriculture, Food and Fisheries, and Health.

Source: Alcalde-Sanz and Gawlik (2014, 2017)

The available guidelines are very well structured and provide information on several aspects of water reuse such as permitted uses, treatments, quality requirements, water and environmental monitoring, on-site preventive measures and communication strategies. Nevertheless, most regulations are limited regarding the necessary water quality for different end uses, and some regulations written years ago should be updated in order to reflect the current water crisis, while also considering current technologies (Alcalde-Sanz and Gawlik, 2017).

The WHO was the first organization to recognize the potential risks involved in the use of untreated wastewater and to develop guidelines for policy makers to legislate the safe use of wastewater for agriculture and aquaculture (WHO, 1989, 2006b). These standards were adopted by several countries in order to make their own regulations, especially developed countries (WHO, 2006a, 2006b, 2006c, 2006d; Alcalde-Sanz and Gawlik, 2017).

Australia has a long experience in water reuse, as it is one of the driest inhabited continents on the Earth. As water consumption rates are considered unsustainable either because they exceed or are close to extraction limits (Thakur and Kabo-bah, 2016), the country has begun to shift its focus of water management towards seeking new strategies for the use of wastewater. The Australian government developed guidelines for water recycling based on risk management framework following the WHO guidelines, providing a generic framework for management of reclaimed water quality and uses that applies to all combinations of reclaimed water and end uses (includes agricultural irrigation and aquifer recharge) (Alcalde-Sanz and Gawlik, 2017). However, most of the Australian states have their own regulations for the reuse of treated wastewater (Alcalde-Sanz and Gawlik, 2014, 2017) (see Tables 2 and 3).

In USA, the regulations on water reuse have been developed in the different states and the underlying objectives of regulations and guidelines vary considerably from state to state. Arizona, California, Florida and Washington have developed the most comprehensive regulations or guidelines that specify the water quality requirements and/or treatment process, for the full spectrum of reuse applications, which aim to maximize the benefits of reclaimed water while protecting environmental and public health. As there are no federal regulations on practices for the reuse of treated wastewater, in 2004, the United States Environmental Protection Agency (USEPA) elaborated the national guidelines for water reuse, which include a wide range of reuse applications, following a similar approach to that used in the WHO guidelines and the Australian guidelines (Natural Resource Management Ministerial Council - Environment Protection and Heritage Council - Australian Health Ministers Conference [NRMMC-EPHC-AHMC], 2006; USEPA, 2012; Alcalde-Sanz and Gawlik, 2017).

At European Union (EU) level, there are no explicit guidelines or regulations on wastewater reuse (European Union and the Committee of the Regions, 2018); however, article 12 of the wastewater Directive 91/271/EEC states that 'treated wastewater shall be reused whenever appropriate' and 'disposal routes shall minimize the adverse effects on the environment' (Directive 91/271/ECC of European Parliament and of the Council of 21 May 1991). The Water Framework Directive (2000/60/EC) mentions water reuse as a possible supplementary measure, in order to provide additional protection or improvement of the waters (Directive 2000/60/EC of European Parliament and of the Council of 23 October 2000). In May 2018, a Proposal for a Regulation of the European Parliament and of the Council on the minimum requirements for water reuse in agricultural irrigation was submitted (2018/0169/COD). However, several Member States have developed their own water reuse regulations and recommendations: Cyprus (Law 106 (I)/2002), France (Decree from 2 August, 2010), Greece (Joint Ministerial Decree 14516/11), Italy (Ministry Decree 185/2003), Portugal (Standard NP 4434) and Spain (Royal

Decree 1620/2007) (see Table 4). All of these are based on the previously mentioned reference guidelines and regulations, including several modifications for some uses. Although, the different regulations are not homogeneous, they all consider the following criteria: intended uses, analytical parameters, maximum limit value permitted for each parameter, monitoring protocols and additional preventive measures for protection of the environment and health (Alcalde-Sanz and Gawlik, 2014; Paranychianakis *et al.*, 2015; 2017) (Table 5).

Table 5. Main applications of reclaimed water included in the legislation of European Union Members States.

Category of use	Types of use
Urban	Irrigation of private gardens; landscape irrigation of urban areas; street cleaning; fire hydrants; and supply to sanitary appliances.
Agricultural	Irrigation of crops eaten raw or not eaten raw; irrigation of pastures for milk or meat producing animals; and irrigation of trees without contact of reclaimed water with fruit for human consumption; and aquaculture.
Industrial	Industrial washing of vehicles; cooling towers and evaporative condensers; and soil compaction.
Recreational	Irrigation of golf course.
Environmental	Maintenance of wetlands; minimum stream flows and similar; and silviculture.

Spain presents the most comprehensive legislation and includes the greatest number of permitted uses: urban, agricultural, industrial, recreational and environmental (Royal Decree 1620/2007). In addition, the Spanish legislation forbids the use of the reclaimed water for human consumption, food industry, hospital facilities, cultivation of filter feeding molluscs in aquaculture, bathing water, cooling towers and evaporative condensers, and fountains in public spaces. In this respect, Royal Decree 1620/2007 of 7 December establishes the legal framework for the reuse of wastewater, and defines the reclaimed water as 'treated wastewater that, in its case, has been submitted to additional or complementary treatment processess which permit to adapt its quality at intended use'. Moreover, the proposed regulation

2018/0169/COD presented in 2018 to the European Parliament and the Council also describe reclaimed water as: 'waste water that has been treated in compliance with the requirements set out in Directive 91/271/EEC and which results from further treatment in a reclamation plant'.

The physico-chemical parameters that are usually contemplated in the regulations for reclaimed water include biological oxygen demand (BOD_5), total suspended solids (TSS) and turbidity. However, other parameters should also be controlled, such as the total phosphorus and nitrogen, total organic carbon (TOC) and residual chlorine, although monitoring is only carried out for certain types of intended uses and some regulations (Alcalde-Sanz and Gawlik, 2014, 2017; European Union and the Committee of the Regions, 2018) (Table 6).

Table 6. Microbiological and physico-chemical parameters included in legislation for reclaimed water in European Union Members States.

Type of parameter	Analytical parameters
Microbiological	*Escherichia coli*; faecal coliforms; total coliforms; faecal enterococci; *Legionella* sp.; *Salmonella* sp.; sulphate-reducing bacteria; helminth eggs; and F-specific bacteriophages.
Physico-chemical	Total suspended solids (TSS); turbidity; biochemical oxygen demand (BOD_5); chemical oxygen demand (COD); pH; heavy metals and metalloids; electrical conductivity; total dissolved solids (TDS); sodium adsorption ratio; chlorine (Cl and Chlorides); nitrogen forms (total, $N\text{-}NO_3$, $N\text{-}NH_4$); total phosphorus; bicarbonate (HCO_3); and toxic substances including priority substances.

On the other hand, due to the potential transmission of infectious diseases, the microbiological parameters are the most important for human and animal health (Table 7). All regulations established by EU Members States include a bacterial indicator to evaluate the quality of reclaimed water: *E. coli*, faecal coliforms, total coliforms, and total enterococci. Moreover, Spanish legislation requires analysis to detect *Legionella*, if there is a risk of water aerosolisation, and *Salmonella* sp. in water destined for irrigating crops for human or animal consumption and use in the food industry when *E. coli* counts are above the maximum limit. As compulsory parameters, Italian regulations include detection of *Salmonella* for all intended uses and the Spanish, Cypriot and Portuguese requirements contemplate the determination or helminth eggs or intestinal nematode eggs for most intended uses. Furthermore, the French regulations consider the presence of pathogenic viruses and protozoan parasites in reclaimed water by including the detection of indicators of viral and protozoan parasites (Table 6) (Ministry Decree 185/2003; Standard NP 4434; Royal Decree 1620/2007; Decree from 2 August, 2010). The microbiological parameters for water reuse established by Spanish Royal Decree 1620/2007, the future European Proposal for Regulation for water reuse in agriculture 2018/0169 (COD), and the WHO (2006a, 2006b) guidelines for the safe use of wastewater, excreta and greywater in agriculture are compared in Table 8.

Table 7. Some pathogenic microorganisms and concentrations detected in raw sewage.

Pathogen		Disease	Concentration (number per litre)*
Bacteria	*Escherichia coli*	Gastroenteritis, haemolytic uremic syndrome	10^5-10^{10}
	Enterococci	Endocarditis, urinary and intra-abdominal infections, prostatitis, cellulitis and wound infections, as well as concurrent bacteramia	10^6-10^7
	Shigella spp.	Dysentery	10-10^4
	Campylobacter spp.	Gastroenteritis; Guillain–Barré syndrome Pathogenic	10^2-10^5
	Salmonella spp.	Gastroenteritis, reactive arthritis	10^3-10^5
	Clostridium perfringens	Necrotic enteritis or gas gangrene	10^5-10^6
Viruses	Enterovirus	Gastroenteritis, respiratory illness, nervous disorders, myocarditis	10^2-10^6
	Adenovirus	Gastroenteritis, respiratory illness, eye infections	10-10^4
	Norovirus	Gastroenteritis	10-10^4
	Rotavirus	Gastroenteritis	10^2-10^5
Protozoan and helminths	*Cryptosporidium* spp.	Cryptosporidiosis	0-10^4
	Giardia duodenalis	Giardiosis	10^2-10^5
	Taenia spp.	Taeniosis/Cysticercosis	0-10^4
	Ascaris lumbricoides	Ascariosis	0-10^4
	Trichuris trichiura	Trichurosis	0-10^4

*Bacteria, colony forming units (CFU); viruses, plaque forming units (PFU); protozoa, oo/cysts; helminths, eggs. Source: Feacham *et al.*, 1983; Geldreich, 1990; National Research Council (NRC), 1996; Bitton, 1999; NRMMC-EPHC-AHMC, 2006.

Table 8. Microbiological parameters for water reuse according to the Spanish Royal Decree 1620/2007; regulation proposed by the European Parliament and European Council 2018/0169 (COD); and the WHO Guidelines for the safe use of wastewater, excreta and greywater.

Parameter	Spanish RD 1620/2007 (2007)	European Union Proposal regulation 2018/0169 COD	WHO (2006a; 2006b)
Intestinal nematodes (helminth eggs/10 L)	Uses: -Agricultural: < 1 -Urban: < 1 -Industrial: not limit/ < 1 -Recreational: not limit/ < 1 -Environmental: < 1	Agricultural use: ≤ 0.1	Agricultural use: -Un/restricted irrigation: ≤ 0.1 when direct contact with persons (children under the age of 15 years) and food. Other uses: no recommendations
Escherichia coli (CFU/100 mL)	Uses: -Agricultural: < 10^2-10^4 -Urban: 0/< 200 -Industrial: 0 < 10^4 -Recreational: < 200-10^4 -Environmental: 0/< 10^3	Agricultural use: -Class A (food consumed raw): ≤ 0 -Class B (food consumed raw without skin): ≤ 10^2 -Class C (food consumed raw without skin watered straight into the ground): ≤ 10^3 -Class D (non-food crops): ≤ 10^4	Agricultural use: -Un/restricted irrigation: ≤ 10^3, ≤ 10^4 and ≤ 10^5 for root crops, leaf crops and drip irrigation (high growing crops), respectively. -Restricted irrigation: ≤ 10^4 and ≤ 10^5 for high human exposure (children under the age of 15 years exposed) and highly mechanized agriculture, respectively. Other uses: no recommendations
Legionella spp. (CFU/L)	Uses: -Agricultural and urban: < 10^3 and < 10^2 where there is risk of aerosolisation, respectively -Industrial: < 10^2 -Recreational: < 10^2 -Environmental: no recommendations	Agricultural use: Class A, B, C or D: < 10^3 where there is risk of aerosolisation in greenhouses	No recommendations
Taenia saginata *Taenia solium* (eggs/L)	Uses: -Agricultural: < 1 pastures irrigation of meat-producing animals	No recommendations	No recommendations
Cryptosporidium spp.	No recommendations	No recommendations	No recommendations

2 *CRYPTOSPORIDIUM* AND CRYPTOSPORIDIOSIS

2.1 The genus *Cryptosporidium*

The genus *Cryptosporidium* was first described in 1907 by Ernest Edward Tyzzer, who frequently observed developing forms of this protozoan parasite in the gastric glands of common mice (*Mus musculus*). The parasite was designated *Cryptosporidium muris* (Tyzzer, 1907, 1910). A new species described in 1912 by Tyzzer and named *C. parvum* differed from the first species in the location inside the host and in the morphology of the oocyst (Tyzzer, 1912). In 1955, the species *Cryptosporidium meleagridis* was observed in turkeys (*Meleagris gallopavo*) and was associated with illness and death of the birds (Slavin, 1955). In the 1970s, *Cryptosporidium* began to be considered of veterinary importance when this parasite was associated with infections in cattle (Panciera *et al*., 1971). Moreover, in 1976, the first cases of human cryptosporidiosis were diagnosed in a 3 year-old child and a 39 year-old immunosuppressed patient (Meisel *et al.*, 1976; Nime *et al.*, 1976). Although human cases of cryptosporidiosis continued to be reported in the following years, they only attracted medical interest when many cases of severe diarrhoea caused by *Cryptosporidium* were described in patients with acquired immune deficiency syndrome (AIDS) (Anonymous, 1982). However, it was not until 1993, after a massive waterborne outbreak in Milwaukee (USA), with 403,000 people infected, when the importance of *Cryptosporidium* in public health was recognized, and it was no longer considered exclusively as an opportunistic pathogen (Mac Kenzie *et al.*, 1994).

2.2 Biology

Cryptosporidium has a complex and monoxenous life cycle that completes in a single host (sexual and asexual reproduction) (Current and Garcia, 1991; Bouzid *et al.*, 2013) (Figure 5). Infection is initiated by the ingestion of sporulated oocysts, which contain four sporozoites. The sporozoites are released through a suture in the oocyst wall due to the response to body temperature, gastric acids, trypsin and biliary salts. Infectious sporozoites then attach to the apical surface of epithelia cells where they are internalized within the cell plasmalemma by an active invasion mechanism until they become enclosed within a parasitophorous vacuole (PV).

In this intracellular but extracytoplasmic location, the parasite develops into spherical trophozoites, which undergo asexual replication (merogony) to produce type I meronts containing 6-8 merozoites. When the PV breaks, type I merozoites are released and can infect adjacent cells, where they undergo asexual multiplication to produce additional type I meronts, or type II meronts, which contain 4 merozoites. Upon infecting new host cells, type II merozoites differentiate to microgamonts or macrogamonts (O'Donoghue, 1995; Tzipori and Griffiths, 1998). Each microgamont becomes multinucleate and each nucleus is incorporated into a microgamete. Microgametes are released and fertilize the quiescent macrogamete. Fertilization produce a zygote, which undergoing meiosis to produces four sporozoites. The sporulated oocysts are released to the intestinal lumen as thin-walled oocysts of only a single layer membrane or a thick-walled oocysts with two-layered membranes (Bouzid *et al.*, 2013). The thin-walled oocysts (approximately 20%) excyst inside the same host and enable maintenance of the infection, obviating the need for a new oral infection (Tzipori and Ward, 2002). However, 80% of the thick-walled oocysts are released with the faeces and, as these are environmentally resistant forms, they are responsible for the transmission of infection from one host to the other. In the life cycle of *Cryptosporidium*, the existence of thin-walled oocysts led to autoinfection phenomena whereby acute diarrhoea is prolonged and large quantities of oocysts are released by infected hosts (Figure 5).

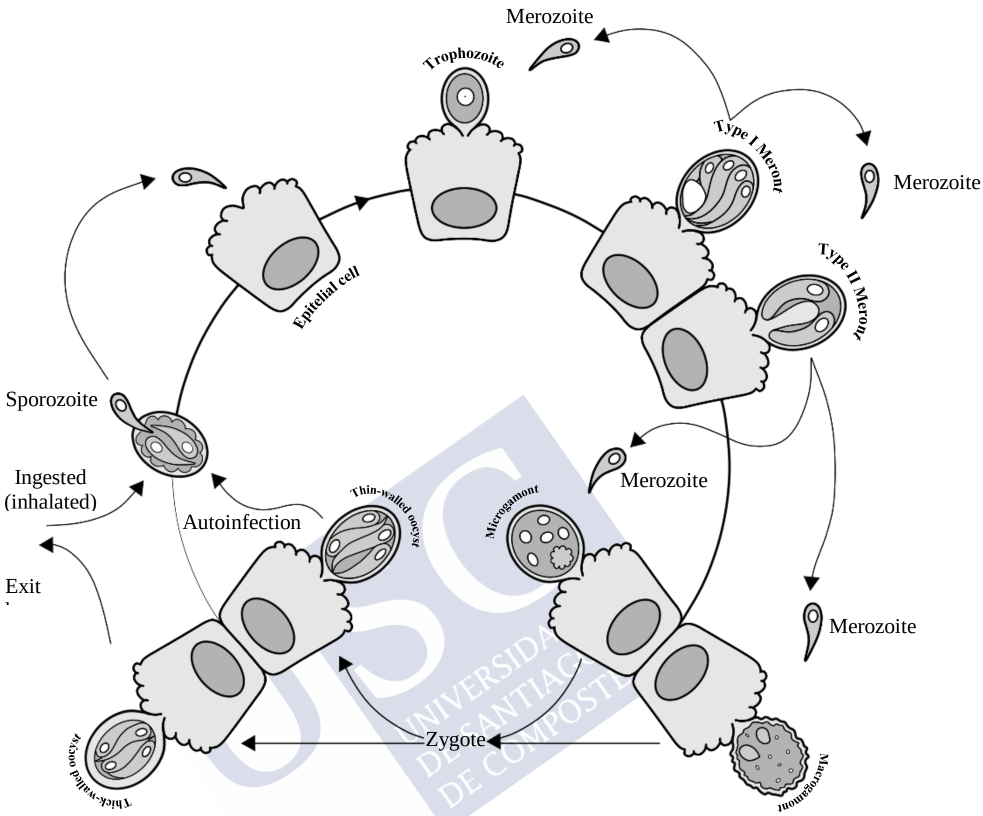

Figure 5. Schematic representation of life cycle of *Cryptosporidium* species (created by Guillermo Varela Iglesias)

2.3 Taxonomy

The genus *Cryptosporidium* was traditionally included in the order Eucoccidioria, class Coccidia, within the phylum Apicomplexa (Levine, 1984). However, biochemical and genetic data indicate that *Cryptosporidium* differs from the other organisms of the phylum Aplicomplexa in several characteristics. In addition, from a phylogenetic point of view, it has some features that are more closely related to gregarines than to coccidian parasites (Carreno *et al.*, 1999; Leander *et al.*, 2003; Wetzel *et al.*, 2005;

Ryan and Hijjawi, 2015): i) the intracellular but extracytoplasmic location of the endogenous developmental stages on the apical surfaces of the host cell (Thompson *et al.*, 2005; Karanis and Aldeyarbi, 2011); ii) attachment of the parasite to the host cell by a multi-membranous feeder organelle situated at the base of the PV which facilitates the uptake of nutrients from the host cell (Huang *et al.*, 2004); iii) the existence of two different morpho-functional types of oocysts (Karanis and Aldeyarbi, 2011); iv) the spherical shape and small size of the oocysts (3-8 μm depending on the species), which lack morphological structures such as sporocysts, micropyle and polar granules (Tzipori and Widmer, 2000; Petry, 2004); v) resistance to anti-coccidial agents tested to date (Blagburn and Soave, 1997; Cabada and White, 2010); vi) cross-reaction between anti-cryptosporidial monoclonal antibodies and gregarines (Bull *et al.*, 1998); and, vii) observation of the presence of novel gamont-like extracellular stages similar to those found in gregarine life cycles (Rosales *et al.*, 2005; Aldeyarbi and Karanis, 2016).

Therefore, taking into account the similarities between the species of *Cryptosporidium* and the gregarine parasites, various researchers have suggested that cryptosporidia may represent a 'lost link' between the more primitive gregarines and coccidians. A new classification was therefore proposed for the genus *Cryptosporidium* in the subclass Cryptogregaria, which comprises epicellular parasites of vertebrates possessing a gregarine-like feeder organelle but lacking an apicoplast organelle (Carreno *et al.*, 1999; Hijjawi *et al.*, 2002; Cavalier-Smith, 2014; Ryan *et al.*, 2016). Consequently, *Cryptosporidium* is officially considered a gregarine according to the International Code of Zoological Nomenclature (ICZN) (Ryan *et al.*, 2016).

In recent decades, the number of species described as belonging to genus *Cryptosporidium* has increased considerably. In order to clarify the confusing taxonomy of the species of the genus *Cryptosporidium* and validate new species, minimum requirements have been proposed. New species of *Cryptosporidium* are named on basis of several parameters: i) morphometric studies of the oocyst; ii) genetic characterization with sequence information deposited in the GenBank® database; iii) demonstration of natural and, whenever feasible, at least some experimental host specificity; and, iv) compliance with ICZN (Xiao *et al.*, 2004; Jirků *et al.*, 2008; Cacciò and Widmer, 2014; Ryan *et al.*, 2014). Currently, 39 species of *Cryptosporidium* which meet these requirements have been formally designated and are therefore considered valid (Table 9). More than 70 genotypes have also been described, and further morphological and biological characterization for recognizing them as separate species are required (Ryan *et al.*, 2014; Holubová *et al.*, 2016; Couso-Pérez *et al.*, 2018, 2019; Chalmers *et al.*, 2018).

2.4 Human cryptosporidiosis

Cryptosporidiosis is a common disease that represents a public health problem worldwide, producing high morbidity and mortality in developing countries and immunocompromised patients. Due to the impact of this disease on the socio-economic development of some countries, in 2004 the WHO included cryptosporidiosis in the Neglected Diseases Initiative (Savioli *et al.*, 2006). Clinical manifestations of human infection depend on the immune status of individuals, and three categories have been established: acute or self-limiting, chronic and fulminant disease (Warren and Guerrant, 2007).

Acute and self-limiting cryptosporidiosis is the most frequent clinic disease in immunocompetent people. The incubation period is usually 4-7 days, after which the clinical presentations include acute diarrhoea during a maximum of 7 days and 8-20 faecal depositions daily. However, oocysts may be excreted for up to one month after remission of the symptoms. The hallmark of infection is watery diarrhoea, but the parasite can cause other symptoms such as malaise or fatigue, abdominal pain, anorexia and low-grade fever (Chappell *et al.*, 2003; Ryan and Hijjawi, 2015).

Chronic cryptosporidiosis usually reported in immunocompromised patients and people suffering malnutrition (Blanshard *et al.*, 1992; Cárcamo *et al.*, 2005). The clinical manifestations are also characterized by watery diarrhoea, nausea, vomiting, headaches, anorexia, fever and weight loss. Cryptosporidiosis is considered one of the original AIDS- defining illnesses due to its high association with mortality in this group of patients (Colford *et al.*, 1996; Hunter and Nichols, 2002).

Fulminant infection has only been described in AIDS patients or patients with drug-induced immunosuppression. In these cases, the illness presents as a 'cholera-like' syndrome that can cause the death of the patient by hypovolemic shock (Hunter and Nichols, 2002).

2.4.1 Treatment

Widespread prophylactic and therapeutic treatment options for cryptosporidiosis remain limited. In the last three decades, several treatment strategies have been proposed. However, although almost one thousand chemotherapeutic agents have been evaluated, none of these therapies were able to clear the infection (Mead and Arrowood, 2014). Despite, the identification of a number of candidate drug targets, which are currently under evaluation, none of these has advanced to clinical trials (Sparks *et al.*, 2015). This is of particular concern, considering the benefits that an available treatment will have for risk groups, i.e. children, the elderly and immunocompromised individuals, including organ transplant recipients, patients undergoing cancer chemotherapy, patients with congenital or induced immunodeficiency, and especially people infected by human immunodeficiency virus (HIV) (Mead and Arrowood, 2014; Cacciò and Chalmers, 2016).

Table 9. Species of *Cryptosporidium* currently recognized.

Species	Major host	Anatomical location/ Site of infection	References
Cryptosporidium muris	Rodents	Gastric	Tyzzer (1907; 1910)
Cryptosporidium parvum	Ruminants	Intestinal	Tyzzer (1912)
Cryptosporidium meleagridis	Birds and humans	Intestinal	Slavin (1955)
Cryptosporidium wrairi	Guinea pigs	Intestinal	Vetterling *et al.* (1971)
Cryptosporidium felis	Felines	Intestinal	Iseki (1979)
Cryptosporidium cuniculus	Rabbits	Intestinal	Inman and Takeuchi (1979)
Cryptosporidium serpentis	Snakes and lizards	Gastric	Levine (1980)
Cryptosporidium baileyi	Birds	Intestinal	Current *et al.* (1986)
Cryptosporidium varanii	Lizards	Gastric	Pavlásek *et al.* (1995)
Cryptosporidium galli	Birds	Gastric, proventriculus	Pavlásek (1999)
Cryptosporidium andersoni	Cattle	Gastric	Lindsay *et al.* (2000)
Cryptosporidium canis	Dogs	Intestinal	Fayer *et al.* (2001)
Cryptosporidium molnari	Fish	Gastric	Álvarez-Pellitero and Sitjà-Bobadilla (2002)
Cryptosporidium hominis	Humans	Intestinal	Morgan-Ryan *et al.* (2002)
Cryptosporidium suis	Pigs	Intestinal	Ryan *et al.* (2004)
Cryptosporidium bovis	Cattle	Unknown	Fayer *et al.* (2005)
Cryptosporidium fayeri	Marsupials	Intestinal	Ryan *et al.* (2008)
Cryptosporidium fragile	Toads	Gastric	Jirků *et al.* (2008)
Cryptosporidium macropodum	Marsupials	Intestinal	Power and Ryan (2008)
Cryptosporidium ryanae	Cattle	Unknown	Fayer *et al.* (2008)
Cryptosporidium xiaoi	Sheep and goats	Unknown	Fayer and Santín (2009)
Cryptosporidium ubiquitum	Ruminants, rodents, primates	Intestinal	Fayer *et al.* (2010)
Cryptosporidium tyzzeri	Rodents	Intestinal	Ren *et al.* (2012)
Cryptosporidium viatorum	Humans	Intestinal	Elwin *et al.* (2012a)
Cryptosporidium scrofarum	Pigs	Intestinal	Kváč *et al.* (2013)

Table 9. (Continued)

Species	Major host	Anatomical location/ Site of infection	References
Cryptosporidium erinacei	Hedgehogs and horses	Intestinal	Kváč *et al.* (2014)
Cryptosporidium rubeyi	Squirrels	Intestinal	Li *et al.* (2015)
Cryptosporidium huwi	Fish	Gastric	Ryan *et al.* (2015)
Cryptosporidium avium	Birds	Intestinal	Holubová *et al.* (2016)
Cryptosporidium proliferans	Rodents	Gastric	Kváč *et al.* (2016)
Cryptosporidium testudinis	Tortoises	Intestinal	Ježková *et al.* (2016)
Cryptosporidium ducismarci	Tortoises	Intestinal	Ježková *et al.* (2016)
Cryptosporidium homai	Guinea pigs	Intestinal	Zahedi *et al.* (2017)
Cryptosporidium apodemi	Rodents	Intestinal	Čondlová *et al.* (2018)
Cryptosporidium ditrichi	Rodents	Intestinal	Čondlová *et al.* (2018)
Cryptosporidium occultus	Rodents	Intestinal	Kváč *et al.* (2018)
Cryptosporidium alticolis	Rodents	Intestinal	Horčičková *et al.* (2019)
Cryptosporidium microti	Rodents	Intestinal	Horčičková *et al.* (2019)
Cryptosporidium proventriculi	Birds	Proventriculus and ventriculus	Holubová *et al.* (2019)

Currently, only one drug, nitazoxanide (NTZ) (Alinia®, Romark Laboratories), a thiazoline compound with broad antiparasitic activity, has been approved by the USA Food and Drug Administration (FDA) for treatment of cryptosporidiosis in children and immunocompetent patients. However, the drug is considered ineffective in immunocompromised patients (the results obtained are no better than the corresponding result observed using a placebo) and malnourished children (positive response in approximately half of treated patients) (Abubakar *et al.*, 2007; Anderson and Curran, 2007; Imboden *et al.*, 2010; Cacciò and Chalmers, 2016; Chavez and White, 2018; Khan *et al.*, 2018). In Europe, NTZ is not licenced for the treatment of human cryptosporidiosis (Cacciò and Chalmers, 2016).

The ineffectiveness of chemotherapeutic agents against *Cryptosporidium* is due to the high resistance of this protozoan enteropathogen to anti-parasitic drugs and related to several factors: i) lack of specific targets or differences in targets either at molecular or structural levels relative to the host; ii) differences in biochemical pathways; iii) the parasite´s unique location in the host cell, which may affect drug concentration; and, iv) the existence of transport proteins or efflux pumps that move drugs out of the parasite to the lumen or back into the host (Mead and Arrowood, 2014). In addition, the main limitation that hinders progress in the discovery and development of new anti-parasitic therapeutics against *Cryptosporidium* is the lack of systems for continuous *in vitro* culture, cryopreservation of the parasite, adequate animal models and tools for manipulation of the parasite genome (Cacciò and Widmer, 2014).

Nonetheless, new drugs based on metabolic pathways of *Cryptosporidium* are being investigated, including the enzyme inosine-5´-monophosphate dehydrogenase (conversion of adenosine into guanine) (Umejiego *et al.*, 2008; Jefferies *et al.*, 2015); calcium-dependent protein kinase (essential for host cell invasion) (Murphy *et al.*, 2010a, 2010b); and, clan CA papain-like (group of cysteine proteases important for cell invasion and survival) (Caffrey and Steverding, 2009; Ndao *et al.*, 2013). The pyrazolopyridines are one of a promising group of drugs whose target is a lipid kinase PI(4)K (Manjunatha *et al.*, 2017). In immunocompromised mice and neonatal calves, oral treatment with pyrazolopyridine KDU731 significantly reduces the parasite burden and induces rapid resolution of diarrhoea (Chavez and White, 2018; Khan *et al.*, 2018).

On the other hand, the development of vaccines against human cryptosporidiosis has been hampered by the lack of a reliable method for continuous culture of the parasites *in vitro* and the lack of a robust animal model (Vinayak *et al.*, 2015). Numerous immunogenic antigens are immunodominant in the invasive stages of *C. parvum*, some of which have demonstrated therapeutic efficacy in animal models and can therefore be used as vaccine candidates (Tilley *et al.*, 1991; He *et al.*, 2004; Ehigiator *et al.*, 2007; Mead and Arrowood, 2014; Khan *et al.*, 2018). Several studies have also attempted to generate vaccines based on DNA immunization by inducing antigen specific B and T cell responses in various infection model systems (Tilley *et al.*, 1991; Jenkins *et al.*, 1995; Sagodira *et al.*, 1999a; Sagodira *et al.*, 1999b; He *et al.*, 2004). Vaccines expressing the CP15/60, CpP2 and CP23 genes induced an immune response that produced a reduction of 60% in oocyst shedding after challenge in mouse model (Mead and Arrowood, 2014).

2.4.2 Epidemiology

Some biological characteristics of *Cryptosporidium* species determine the general epidemiological aspects of infection: i) the small size of the oocysts and their low specific gravity, which facilitate their dispersion through water; ii) the ability to remain infectious for long periods, especially in cold and humid environments; iii) the high resistance to most disinfectants commonly used in water treatments; iv) the wide range of hosts; v) the ability to multiply in a single host, which eliminates, over long periods of time, large numbers of parasitic forms already infectious in susceptible hosts; and, vi) the low minimum infective dose (<10 oocysts) (Fayer, 2004; Gajadhar and Allen, 2004; Hunter and Thompson, 2005; Smith and Nichols, 2006; Smith *et al.*, 2006; Thompson and Smith, 2011).

Oocysts are transmitted among hosts by the main transmission mechanisms of intestinal pathogens involving multiple routes. However, *Cryptosporidium* species differ from other enteropathogens in their inability to multiply outside the host. The transmission routes include direct person-to-person, animal-to-animal and animal-to-person contact, or indirectly transmission through the ingestion of contaminated food and water (Figure 6) (Nichols, 2007).

Approximately 20 *Cryptosporidium* species and genotypes have been reported to infect humans. Among these, *C. parvum* and *C. hominis* are identified in most cases. The species *C. meleagridis* is the third most important species and is considered an emerging parasite in some regions (Xiao, 2010). Other species and genotypes, such as *Cryptosporidium canis*, *Cryptosporidium felis*, *Cryptosporidium suis*, *Cryptosporidium muris*, *Cryptosporidium ubiquitum*, *Cryptosporidium cuniculus*, *Cryptosporidium viatorum*, *Cryptosporidium fayeri*, *Cryptosporidium andersoni*, *Cryptosporidium bovis*, *Cryptosporidium scrofarum*, *Cryptosporidium xiaoi*, *Cryptosporidium tyzzeri*, *Cryptosporidium erinacei* and the *Cryptosporidium* horse, skunk and chipmunk I genotypes have also been detected in immunocompetent and immunocompromised people (Xiao, 2010; Waldron *et al.*, 2011; Elwin *et al.*, 2012b; Ng *et al.*, 2012; Kváč *et al.*, 2013; Rašková *et al.*, 2013; Liu *et al.*, 2014; Khan *et al.*, 2018).

Cryptosporidiosis has a worldwide distribution. However, the prevalence is assumed to be higher in developing countries (Putignani and Menichella, 2010). Thus, the prevalence in these countries ranged between 0 and 13.7%. Unussually, in developed countries, prevalence rates range from 0.3 to 54.2%, although this is probably because of the existence of surveillance systems for routine detection of *Cryptosporidium* (Cacciò and Putignani, 2014). In addition, most outbreaks of infection associated with recreational or drinking water have been reported in these countries (Abeywardena *et al.*, 2015). In this respect, 99% of the waterborne parasitic protozoan outbreaks occurred in New Zealand (49%), North America (41%) and in Europe (9%), with *Cryptosporium* spp. being the most common aetiological agent, as it was reported in 63% (239/381) of outbreaks (Efstratiou *et al.*, 2017).

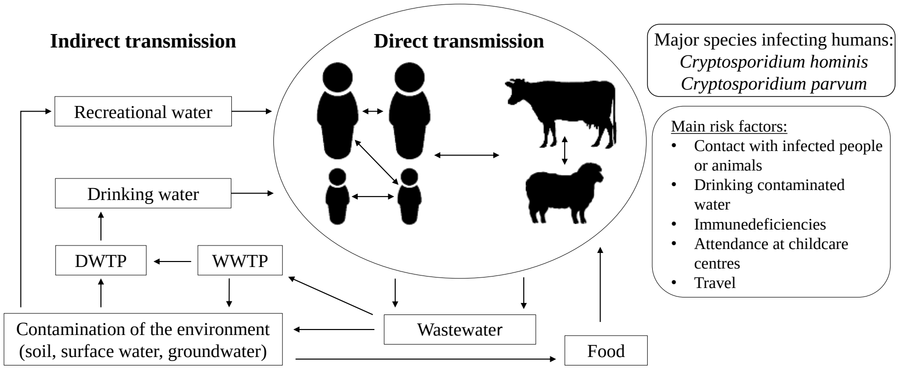

Figure 6. Diagram illustrating the routes of transmission of cryptosporidiosis.
DWTP, drinking water treatment plant; WWTP, wastewater treatment plant.

In the USA data provided by Centers for Disease Control and Prevention (CDC) related to the situation of cryptosporidiosis during 2009-2017 showed a notable increase in the number of cases reported each year with an average of 12.8% per year. During the period 2009-2017, 444 cryptosporidiosis outbreaks were reported, resulting in 7465 cases in 40 states and Puerto Rico, in which 156 (35.1%) outbreaks were associated with exposure to recreational water (swimming pools and water playgrounds); 65 (14.6%) outbreaks were cause by contact with cattle; and 57 (12.8%) outbreaks were originated by infected people in child care settings. Moreover, the highest seasonal peaks were reached during the summer months (July-August), coinciding with the main period of recreational water activities (Gharpure *et al.*, 2019).

In the European Union, cryptosporidiosis is a notifiable disease. During 2016, 13,623 cases of infection were confirmed in the 21 Member States. The global incidence was 3.8 cases per 100,000 habitants, although there are significant differences in the percentages reported by the different Member States: from 0% in Cyprus, Lithuania, Luxembourg, Romania to 11.8% detected in Ireland. Moreover, 87% cases of cryptosporidiosis were reported from Belgium, Germany, the Netherlands and the United Kingdom, with the

United Kingdom alone accounting for 49% of all cases. Furthermore, the reported data revealed that the highest incidence of infection occurred in children aged 0-4 years (16.3 cases per 100,000 habitants). The analysis and investigation carried out after outbreaks of cryptosporidiosis related the infection to drinking water, travel and/or exposure to recreational water as the main risk factors (Fournet *et al.*, 2013; Cacciò and Chalmers, 2016). In this respect, the largest waterborne outbreak of cryptosporidiosis reported in Europe was caused by *C. hominis* in 2010 and affected 27,000 people in the Swedish city of Östersund (Widerström *et al.*, 2014).

In Spain, cryptosporidosis has also been a notifiable disease since 2015 (Order SSI/445/2015), when 653 human cases were reported, with children of age 1-4 years being the group most commonly affected (approximately 21 per 100,000 inhabitants). Furthermore, *Cryptosporidium* outbreaks were documented after recreational activities in swimming pools and day-care centres, affecting children, caregivers and tourists (Galmes *et al.*, 2003; Artieda *et al.*, 2012; Fuentes *et al.*, 2015).

In developing countries, *Cryptosporidium* is one of the top three pathogens causing diarrhoeal disease in children under two years old, and it is responsible for 30-50% of childhood mortality in these regions (Kotloff *et al.*, 2013). Sow *et al.* (2016) estimated that 2.9 million diarrhoeal cases can be attributed to *Cryptosporidium* annually in children aged <24 months in Sub-Saharan region, and 455,000 are deaths attributable to the disease. The incidence of cryptosporidiosis in moderate-to-severe cases of diarrhoea were estimated to be 3.48 and 1.41 per 100 child in the 0-11 and 12-23 month age group, respectively in Africa (Sow *et al.*, 2016). The importance of this protozoan parasite has increased among vulnerable groups of malnourished children and immunecompromised individuals, especially children infected with HIV: approximately 500,000 to 700,000 new cases are identified each year in this region (Mahin and Peletz, 2009; Aldeyarbi *et al.*, 2016).

In India/Pakistan/Bangladesh/Nepal/Afghanistan region, it was estimated that 4.7 million cases of moderate-to severe diarrhoea can be attributed annually to *Cryptosporidium* in children <24 months, and approximately 254,000 annual deaths. The incidence in Asian countries of moderate-to-severe diarrhoea attributable to *Cryptosporidium* was estimated to be 3.18 and 1.36 per 100 children in infants and toddlers, respectively (Mølbak *et al.*, 1993; Kotloff *et al.*, 2013; Striepen, 2013; Sow *et al.*, 2016; Mahmoudi *et al.*, 2017).

As already mentioned, waterborne cryptosporidiosis is a globally emerging public health issue (Karanis *et al.*, 2007). Several studies have demonstrated the presence of oocysts of *Cryptosporidium* spp. in different types of water (surface waters, drinking water, recreational waters and wastewater treatment plant effluents) (Hamilton *et al.*, 2018). Water bodies may be directly contaminated with faecal residues from animals or indirectly by run-off from contaminated surfaces (Lu *et al.*, 2011; Ahmed *et al.*, 2013; Sidhu *et al.*, 2013). Moreover, water systems may also be contaminated by sewage or effluents from WWTP, which treatments are insufficient to totally remove *Cryptosporidium* oocysts (Ahmed *et al.*, 2010; Schneeberger *et al.*, 2015; Vermeulen *et al.*, 2019). In this way, and taking into account that the average overall human excretion rate is estimated to be 1×10^{8} oocysts/person/year and 5×10^{7} oocysts/person/year, for developing and developed countries, respectively, the total global human emissions were 1.6×10^{17} oocysts/year, with the urban population being responsible of the 89% of emissions (Hofstra and Vermeulen, 2016). The concentration of oocysts of *Cryptosporidium* in river water was predicted using a mathematical model developed by Vermeulen *et al.* (2019), which established values of between 10^{-6} and 10^{2} oocysts/L worldwide. Furthermore, Hofstra and Vermeulen (2016) predicted an increase of up to 70% in human *Cryptosporidium* emissions, with higher concentrations of this waterborne protozoan in surface waters due to population growth in developing countries.

Cryptosporidium is also considered of vital importance in foodborne transmission (FAO and WHO, 2014). Thus, in 2010, this protozoan parasite, was responsible for 8.6 million cases of foodborne illness, 3,759 deaths and 296,156 disability-adjusted life years. The literature describes foodborne outbreaks linked to the consumption of salads, unpasteurized milk, unpeeled carrots, fresh parsley, beef tartare, raw liver, apple cider, green onion, fruits and milk kefir (Robertson, 2014; Dixon, 2015; Ryan *et al.*, 2018). These foods can be contaminated by irrigation of crops with faecally contaminated water, the application of manure to crops such as fertiliser and the use of contaminated water to mix pesticides or wash produce (Budu-Amoako *et al.*, 2011; Iqbal *et al.*, 2015). In Spain, lettuces and cabbages irrigated with contaminated water have been found to be contaminated with *Cryptosporidium* oocysts and *Giardia* cysts (Amorós *et al.*, 2010). Studies in Costa Rica and Peru (Monge and Arias, 1996; Ortega *et al.*, 1997) have demonstrated contamination of numerous raw vegetables, including basil, cabbage, celery, cilantro, green onions, leeks, lettuce and parsley. In Vietnam, *Cryptosporidium* oocysts and *Giardia* cysts have been detected found in water spinach, lettuce and coriander irrigated with sewage contaminated water (Nguyen *et al.*, 2016). In Norway, Robertson and Gjerde (2001) detected *Cryptosporidium* oocysts and *Giardia* cysts in samples of water used to irrigate bean sprouts. Although foodborne outbreaks of cryptosporidiosis are extensively reported, many are never identified, and those that are recognised are poorly investigated and often not reported (Robertson, 2014).

2.5 Cryptosporidiosis and climate change

As already mentioned, climate change may affect the availability and quality of water due to multiple process such as the gradual increase in temperature, change in the seasonal patterns and increase in the frequency and intensity of extremely climatic events (heavy rainfall, floods and droughts) (Ebi *et al.*, 2006; Semenza *et al.*, 2012; Cann *et al.*, 2013; Schijven *et al.*, 2013; Young *et al.*, 2014). Thus, climate variability can directly affect the survival and dissemination of the pathogens through water, food and the environment (Lafferty, 2009; Rosenthal, 2009; Lal *et al.*, 2013). In fact, worldwide increase in temperature of between 1.5 and 2 degrees predicted by the Intergovernmental Panel on Climate Change to occur by the end of the 21st century and the effects on the hydrological system due to climate change, have led to waterborne illness being included among the main risks to human health (Luber *et al.*, 2014; Patz *et al.*, 2014; Smith *et al.*, 2014). All causes of diarrhoeal diseases have recently been positively associated with temperature and rainfall by different authors (Carlton *et al.*, 2015; Levy *et al.*, 2016). Furthermore, floods can increase the presence of *Cryptosporidium* in surface waters by up to 2.6 times during and after heavy rain (Young *et al.*, 2014). The consequence of the increase in cryptosporidiosis due to climate change is clearly observed on the African continent, where climate change increases the variability in rainfall patterns and extreme drought and reduces freshwater resources. It thus also increases levels of malnutrition, which in turn undermines the resistance of vulnerable populations to *Cryptosporidium* infections, reducing their ability to cope with cryptosporidiosis and the adaptation to the climate change (Ringler *et al.*, 2010).

Jagai *et al.* (2009) provided a quantitative link between the incidence of cryptosporidiosis and meteorological parameters on a global scale, particularly in warm and wet areas of Africa. These authors confirmed an increase in the incidence of *Cryptosporidium* during warm, rainy seasons, as previously reported by other authors (Adegbola *et al.*, 1994; Newman *et al.*, 1999; Perch *et al.*, 2001), and concluded that temperature and precipitation are significant predictors of the incidence of cryptosporidiosis, particularly in tropical climates.

In a meta-analysis carried out by Lal *et al.* (2019), cryptosporidiosis has been related to local factors of precipitation and density of population. These researchers showed distinct spatio-temporal patterns of the disease in regions at different latitudes, with lower latitudes more likely to have high population densities, lower socioeconomic development and therefore a higher risk of suffering diseases due to other transmission pathways, such as poor hygiene, water and sanitation infrastructure. Consequently, the increase in the morbidity of cryptosporidiosis can affect the growth and cognitive development of children, increasing the susceptibility to other infectious and chronic diseases, aggravating the individual and community vulnerabilities to the effects of climate change (Guerrant *et al.*, 2013; Levy *et al.*, 2016).

Finally, climate change also has serious consequences in developed countries (Semenza *et al.*, 2012). For example, although in northern Europe precipitation is expected to increase by 5-20%, experts forecast a decrease of 5-30% in southern Europe (Semenza *et al.*, 2012). The decrease in rainfall and increase in water scarcity will favour the use of reclaimed water. However, due the presence of the protozoan parasite *Cryptosporidium* in WWTP effluents and the ineffectiveness of the treatments used in water reclamation, new treatments that effectively eliminate this enteropathogen from water must be developed in order to prevent public and environmental health risks.

OBJECTIVE

The aim of this Doctoral Thesis is to evaluate new water reclamation technologies based on advanced oxidation processes against *Cryptosporidium*, an enteroprotozoan parasite frequently involved in waterborne outbreaks of disease and considered a resistance model due to the robust nature of its infective forms (oocysts). For this purpose, two photochemical processes (heterogeneous photocatalysis with TiO_2 slurry, alone or in combination with H_2O_2, and homogeneous photocatalysis by photo-Fenton process) and ultrasound technology were assessed to inactivate *C. parvum* oocysts and thus improve the microbiological quality of reclaimed water and reduce the risk of transmission of infectious diseases.

PUBLICATIONS

Evaluation of solar photocatalysis using TiO_2 slurry in the inactivation of *Cryptosporidium parvum* oocysts in water

María Jesús Abeledo-Lameiro, Elvira Ares-Mazás
and Hipólito Gómez-Couso

Journal of Photochemistry and Photobiology, B: Biology 163 (2016) 92-99

Journal Citation Reports, impact factor: Q2 in Biophysics

https://www.sciencedirect.com/science/article/pii/S1011134416302755

Photocatalytic inactivation of the waterborne protozoan parasite *Cryptosporidium parvum* using TiO_2/H_2O_2 under simulated and natural solar conditions

María Jesús Abeledo-Lameiro, Aurora Reboredo-Fernández,
María Inmaculada Polo-López, Pilar Fernández-Ibáñez,
Elvira Ares-Mazás and Hipólito Gómez-Couso

Catalysis Today 280 (2017) 132-138

Journal Citation Reports, impact factor: Q1 in Chemistry, applied

https://www.sciencedirect.com/science/article/abs/pii/S0920586116304072

Use of ultrasound irradiation to inactivate *Cryptosporidium parvum* oocysts in effluents from municipal wastewater treatment plants

María Jesús Abeledo-Lameiro, Elvira Ares-Mazás
and Hipólito Goméz-Couso

Ultrasonics Sonochemistry 48 (2018) 118-126
Journal Citation Reports, impact factor: Q1 in Acoustics

https://www.sciencedirect.com/science/article/abs/pii/S1350417718307466?via%3Dihub

Inactivation of the waterborne pathogen *Cryptosporidium parvum* by photo-Fenton process under natural solar conditions

María Jesús Abeledo-Lameiro, María Inmaculada Polo-López,
Elvira Ares-Mazás and Hipólito Gómez-Couso

Applied Catalysis B: Environmental 253 (2019) 341-347

Journal Citation Reports, impact factor: Q2 in Chemistry, physical

https://www.sciencedirect.com/science/article/pii/S0926337319303716

DISCUSSION

Surface water and groundwater are the major resources used to meet water needs in both urban and rural areas. In this context, the hydrological cycle is very important for maintaining water through precipitation, runoff and filtration (Tan and Wang, 2010). Water stress and sustainability depend directly on the available water resources and on water withdrawal and consumption. Thus, currently, the land overuse and the increasing water demands due to population growth (the rapid urbanization has led to augmentation of the water demands cause tension in urban water supplies) are major drivers of the increasing global water stress. Furthermore, the climate change is an additional challenge in relation to water stress, because higher temperatures lead to higher rates of evaporation and the variability in the rainwater increases the risk of droughts, thus affecting the availability of surface and groundwater resources (Gallopín, 2012).

On the other hand, the quantity of water available for human consumption is closely associated with water quality, as polluted water cannot be used for drinking, bathing, industry, or agriculture. In this respect, wastewater discharge from cities and industrial installations is the main source of contamination (either chronically or accidentally spills) and is responsible for polluting of approximately half of the rivers and lakes worldwide. Furthermore, over-exploitation of surface waters increases the concentration of harmful compounds in water, not only due to urban pollution or leaching of minerals, but also to the excessive use of chemical fertilizers in agriculture. In addition, groundwater abstraction increases the concentration of natural compounds in water, which can reach dangerous levels. In this respect, salinity can be increased by the movement of seawater into coastal aquifers, reducing the amount of freshwater available for human, agricultural and industrial uses (Chen *et al.*, 2016).

For all of the above reasons, water stress is a real problem that affects millions of people worldwide. The European Environment Agency estimates that around one third of the EU territory (over 100 million people) can suffer the consequences of water stress either permanently or temporarily. Although countries such as Greece, Portugal and Spain are already affected by severe droughts during the summer months, water scarcity is also becoming a problem in northern regions, including parts of the United Kingdom and Germany. Furthermore, agricultural areas where intensive irrigation is carried out, busy touristic islands in southern Europe and large urban agglomerations are currently considered water stress hotspots (Alcalde-Sanz and Gawlik, 2014; European Environment Agency, 2018).

Water reuse has been identified as one of the most important priorities worldwide and is the mainstay of the circular economy. Several national and international organisations have considered using reclaimed water for urban, agricultural, industrial, recreational, and environmental purposes (Winpenny *et al.*, 2013; Alcalde-Sanz and Gawlik, 2014; Kirhensteine *et al.*, 2016). However, the quality of reclaimed water should comply with several physico-chemical and microbiological parameters. The latter are the most important for human and animal heath due to the risk of transmission of infectious diseases. Different pathogenic agents have been identified in raw sewage (viruses, bacteria, protozoa and helminths), and although diverse microorganisms are used to determine the quality of reclaimed water, reference pathogens representing groups of pathogens have been selected for validating water treatments. The WHO guidelines for water reuse and drinking water consider rotavirus, *Campylobacter* and *Cryptosporidium* (presumably *C. parvum*) as reference pathogens for respectively viruses, bacteria and protozoan parasites on the basis of the following criteria: i) infectivity, incidence and severity of disease associated with waterborne transmission; ii) sufficient data on dose-response relationships in humans and disease burden; iii) occurrence and persistence in source waters and in the environment; and, iv) sensitivity to removal or inactivation by treatment processes (NRMMC-EPHC-AHMC, 2006; WHO, 2006b, 2017; USEPA, 2012; Alcalde-Sanz and Gawlik, 2017). Moreover, reference pathogens should be conservative in relation to dose-response and infectivity, so that if a reference pathogen is controlled, other pathogens within the same group are also expected to be controlled. However, no reference pathogens have been selected for the helminth because helminth infections are not endemic in developed countries, information about the occurrence of helminths in water is scarce and there are no human dose-response models available. Nevertheless, it is generally considered that protozoan reference pathogens can be used as reference pathogens for helminths (NRMMC-EPHC-AHMC, 2006; Alcalde-Sanz and Gawlik, 2017).

Studies carried out in recent years demonstrated the presence of high concentrations of *Cryptosporidium* spp. oocysts in WWTP effluents and in reclaimed waters worldwide, which are resistant to the treatments commonly used in water disinfection process of wastewater (Ajonina *et al.*, 2012; Sroka *et al.*, 2013; Galván *et al.*, 2014; Kitajima *et al.*, 2014; Ehsan *et al.*, 2015; Ma *et al.*, 2016; Nasser, 2016; Santos *et al.*, 2016; King *et al.*, 2017; Razzolini *et al.*, 2020). Consequently, the aim of the present Doctoral Thesis, is to evaluate new technologies based on advances oxidation processes against the waterborne protozoan parasite *C. parvum*, considered as one of the most resistant infectious agents, after prions, to the commonly used disinfectants (Rutala and Weber, 2004).

In the first study, heterogeneous photocatalysis with TiO_2 slurry (63, 100 and 200 mg/L) was evaluated for its capacity to inactivate *C. parvum* oocysts under simulated solar conditions in distilled water (DW), which used as a reference solution for observing and comparing the inactivation kinetics under controlled laboratory conditions, to exclude the influence of or interference from compounds found in other types of water. The lowest oocyst viability (4.16±2.35%) was observed at concentration of 100 mg/L of TiO_2

photocatalyst after 5 hours of exposure to simulated solar radiation. This represented an improvement relative to the results obtained with samples exposed without photocatalyst and with 63 mg/L of TiO_2 (51.06±9.35% and 57.86±10.72%, respectively), optimal concentration estimated on the basis of the reactor dimensions, so that suspended catalyst uses 99% of the incident radiation (Fernández Ibáñez, 2004).

In order to minimize the variability in the composition of the WWTP effluents observed from different WWTPs and also on a daily basis, a simulated WWTP effluent (commonly used in studies of solar decontamination and disinfection) was used as a model of treated discharge from a municipal WWTP (Klamerth *et al.*, 2010; Polo-López *et al.*, 2010; Polo-López *et al.*, 2012; Márquez *et al.*, 2014; Rodríguez-Chueca *et al.*, 2014; Pouran *et al.*, 2015). In this respect, this is the first study to evaluate the efficacy of solar photocatalysis with TiO_2 slurry to inactivate *C. parvum* oocysts in wastewater. The decreases in the oocyst viability detected in simulated WWTP effluent were significantly lower than the corresponding values observed in DW, even in comparison with the samples exposed without photocatalyst (48.98±12.31%; 70.96±7.18%; 73.05±4.93%; and 81.64±4.07% for samples containing 0, 63, 100 and 200 mg/L of TiO_2 in simulated WWTP effluent, respectively).

Numerous studies have demonstrated that solar photocatalysis with TiO_2 is effective for inactivating a wide range of microorganisms present in water, air and on surfaces: Gram-negative and Gram-positive bacteria, unicellular and filamentous fungi, mammalian bacteriophages and viruses, algae and protozoa. However, bacterial endospores, fungal spores and protozoan oo/cysts are very resistant to TiO_2 photocatalytic process because they have a robust cell wall (Chong *et al.*, 2010; Foster *et al.*, 2011; Byrne *et al.*, 2015; Malato *et al.*, 2016; Nasser, 2016). In this respect, the few studies involving this photocatalyst and *Cryptosporidium* used immobilized TiO_2 (Otaki *et al.*, 2000; Curtis *et al.*, 2002; Méndez-Hermida *et al.*, 2007; Navalon *et al.*, 2009; Sunnotel *et al.*, 2010; Fontán Sainz, 2012). As far as we are aware, only two studies have evaluated the efficacy of TiO_2 slurry, at concentrations of 100, 500 and 1000 mg/L, for inactivating *C. parvum* oocysts (Cho and Yoon, 2008; Ryu *et al.*, 2008).

With the aim of accelerating the solar water disinfection process, heterogeneous photocatalysis with TiO_2 has been combined with the addition of readily available, inexpensive and safe oxidant compounds, such as H_2O_2. Thus, several authors have demonstrated that the TiO_2 photocatalytic disinfection process is enhanced by the addition of H_2O_2, effectively killing several bacterial species such as *E. coli*, *Staphylococcus epidermidis* and *Staphylococcus mutans* (Malato *et al.*, 2009; Pablos *et al.*, 2013; Unosson *et al.*, 2013). All live cells are exposed to intracellular H_2O_2 due to cellular metabolic activity, and the concentration of the compound depends on the production rate, diffusion in the cytosolic space and/or mitochondrial matrix and elimination by antioxidant compounds and specific enzymes (catalase, superoxide dismutase, glutathione peroxidase and glutathione S-transferase) (Entrala *et al.*, 1997; Cadenas and Davies, 2000). The introduction of exogenous H_2O_2 provides an additional input of a highly oxidizing product.

In previous studies performed by our research group, the effectiveness of different concentrations of H_2O_2 (0 to 500 mg/L) for inactivating *C. parvum* oocysts was evaluated in DW and simulated WWTP effluent under artificial sunlight. The results showed the enhancement of solar disinfection when H_2O_2 is incorporated, speeding up the inactivation of *C. parvum* oocysts and establishing 50 mg/L as the minimum concentration at which a slight improvement in oocyst inactivation was detected (Couso Pérez, 2013; Gómez-Couso *et al.*, 2013).

Considering that oocyst inactivation was most effective at 100 mg/L of TiO_2, a combination of 100 mg/L of TiO_2 and 50 mg/L of H_2O_2 was selected to evaluate the addition of this oxidant to the photocatalytic disinfection against *C. parvum* in DW. However, in both simulated and natural solar conditions, the results obtained in the water samples containing TiO_2/H_2O_2 were not statistically significant different from the corresponding values observed in samples exposed exclusively with TiO_2, despite of the quick consumption of H_2O_2 throughout the experiments (oocyst viabilities of 4.16±2.35% *vs* 3.82±4.26% and 2.29±1.99% *vs* 0.92±0.71% determined in samples containing TiO_2 and TiO_2/H_2O_2, under simulated and natural solar radiations, respectively).

Among AOPs, the photo-Fenton process is another catalytic process that uses H_2O_2 and solar light, and has been widely evaluated for ability to inactivate viruses, bacteria, fungi and the nematode parasite *Ascaris suum*. Thus, several compounds and concentrations of iron, different ratios of Fe^{2+}/H_2O_2 and pH values, diverse types of water (DW, MilliQ water, simulated and real municipal WWTP effluents) have been tested under simulated and natural solar conditions (Polo-López *et al.*, 2012, 2013; Ortega-Gómez *et al.*, 2014; Giannakis *et al.*, 2016a; Polo *et al.*, 2018; García-Fernández *et al.*, 2019).

In this Doctoral Thesis, the effect of the photo-Fenton process on the survival of *Cryptosporidium* is also reported for the first time. A factorial 3×3 first order design was applied to study the combined effects of the Fe^{2+}/H_2O_2 concentration (5/10; 10/20 and 20/50 mg/L), pH value (3, 5.5 and 8) and exposure time (2, 4 and 6 hours) for inactivating *C. parvum* oocysts in DW under natural solar radiation. The use of factorial designs offers important advantages, in comparison with one-factor-at-a-time experiments, since they allow effects to be investigated with many fewer experiments without changing reliability in the obtained results, the detection of factor interactions and the identification of the response-variable maxima, in addition to facilitate system-modelling (Quinn and Keough, 2002). This type of experimental design is most often used for manipulative experiments in order to evaluate the influence of several factors in the survival of different organisms, including *Cryptosporidium* (Leiro *et al.*, 1995; Freire-Santos *et al.*, 2000; Quinn and Keough, 2002; Box *et al.*, 2005; Gómez-Couso *et al.*, 2009b).

The parameters Fe^{2+}/H_2O_2 concentration and exposure time, as well as the interaction between pH and Fe^{2+}/H_2O_2 concentration had a statistically significant influence on the viability of this waterborne enteropathogen. Thus, the minimum values of oocyst viability corresponded to the combination of the highest concentration of Fe^{2+}/H_2O_2 (20/50 mg/L), the lowest pH value (3) and longest exposure times (4 and 6 hours) (3.68±1.38% and 6.39±2.65%, respectively). Similarly, other authors proved that the highest inactivation for different microorganisms were achieved using the maximum concentration of Fe^{2+}/H_2O_2 tested (Polo-López *et al.*, 2012; Ortega-Gómez *et al.*, 2014; Polo *et al.*, 2018). According to the literature reviewed, the concentration of Fe^{2+}/H_2O_2 evaluated in most of the water disinfection studies ranged from a minimum of 5/10 mg/L to a maximum of 20/50 mg/L, using a Fe^{2+}/H_2O_2 ratio of approximately 1:2, as this ratio produced the highest rate of inactivation (Giannakis *et al.*, 2016b).

Likewise the experiments carried out with TiO_2/H_2O_2, in assays of photo-Fenton, H_2O_2 concentrations of 10, 20 and 50 mg/L did not affect the survival of *C. parvum* oocysts. Moreover, in both photocatalytic processes H_2O_2 was completely consumed, independently of the initial concentration employed. The rapid reduction in H_2O_2 levels may have mainly been caused by reaction with the catalyst, excluding H_2O_2 decomposition at temperatures above 30 °C, which increase 2-3 times for every 10 °C increase in temperature (Jones, 1999; Giannakis *et al.*, 2016b).

In the empirical equation obtained, the largest coefficient with a negative sign corresponded to the exposure time. Previous studies of solar disinfection carried out with simulated solar radiation have shown that exposure time is an important variable at high irradiation intensities, but that at low irradiation intensities, this parameter has a null effect on the survival of *C. parvum* oocysts (Gómez-Couso *et al.*, 2009b). In the present study, the UV irradiance (295-385 nm) ranged between a minimum of 29.65±1.62 W/m^2 and a maximum of 46.08±0.56 W/m^2, equivalent to global solar radiation intensities of approximately 700 and 1100 W/m^2, respectively, corresponding to hazy and strong sunlight in equatorial areas (Joyce *et al.*, 1996; McGuigan *et al.*, 1998; Gómez-Couso *et al.*, 2009b). The aforementioned equation is valid for predicting the viability of *C. parvum* oocysts under natural sunlight in the range of conditions assayed. However, the factorial design does not include the parameters temperature or intensity of solar radiation, which vary throughout the experiments carried out under field conditions. These variations can generate imbalances in the proposed model and the values provided by the model therefore do not entirely correspond to the observed data on oocyst viability. Despite this, the equation obtained proved to be valid for predicting at 76.76% the oocyst viability under the range of conditions assayed.

Solar radiation can inactivate diverse microorganisms, being the UV-A and UV-B radiations are the most important components of the electromagnetic spectrum responsible for the germicidal activity (Pichel *et al.*, 2019). Although several authors have reported the efficacy of UV radiation for inactivating *Cryptosporidium* in different water matrices (drinking water and wastewater), the mechanisms of solar inactivation of the oocysts remain unknown (Méndez-Hermida *et al.*, 2005, 2007; King *et al.*, 2008; Gómez-Couso *et al.*, 2009b, 2009c, 2010, 2012a, 2012b; Heaselgrave and Kilvington, 2011; Fontán-Sainz *et al.*, 2012; Polo-López *et al.*, 2019). Liu *et al.* (2015) observed that inactivation of the oocysts by UV-A/visible light mainly involved an indirect endogenous mechanism, which was unpredictable and other factors, such as oocyst source and temperature, could affected

Cryptosporidium inactivation. However, under solar full-spectrum the oocyst inactivation was dominated by a mechanism of direct inactivation induced by UV-B radiation, being more effective and independent of external factors (Liu *et al.*, 2015). For this reason, the experiments carried out under natural solar conditions (i.e. using the full-spectrum of sunlight) provided the best results regarding inactivation of *C. parvum* oocysts. Moreover, these differences can also be explained by the larger surface area of exposure in assays carried out under real sunlight: under simulated solar conditions, the top of the reactor receives most of radiation, whereas under natural conditions, sunlight reaches both the top and lateral sides of the reactor.

In addition to direct damage caused by solar radiation, the mechanisms of photocatalytic inactivation of microorganisms are based on the generation of hydroxyl radicals, which damage biological structures, rendering microorganisms unviable (Foster *et al.*, 2011; Byrne *et al.*, 2015). Cho and Yoon (2008) investigated the inactivation of *C. parvum* oocysts by TiO_2 slurry in phosphate buffer solution in a reactor irradiated with UV-A light, reporting the concentration×contact time (CT) value required for 2-log *C. parvum* inactivation with the hydroxyl radical of 7.9×10^{-5} mg minutes/L. Moreover, these authors estimated that $HO^{\bullet}$ was approximately 10^4-10^7 times more effective for inactivating *Cryptosporidium* than other popular chemical disinfectants such as ozone, chlorine dioxide and free chlorine (Cho and Yoon, 2008). Furthermore, in photocatalytic processes involving the exogenous addition of H_2O_2, which can enter the cells, endogenous photo-Fenton processes can produce as presence of iron clusters in *Cryptosporidium* have been reported by several authors (Ali and Nozaki, 2013; Miller *et al.*, 2018; Nelson *et al.*, 2018).

The chemical composition of the water is also an important factor affecting the efficacy of photocatalysis with TiO_2 and the photo-Fenton process. Inorganic species such as carbonates and bicarbonates and organic matter present in water are natural competitors of $HO^{\bullet}$ and thus reduce the efficacy of the processes (Otaki *et al.*, 2000; Giannakis *et al.*, 2016b). Moreover absorption, shading, and dispersion of the light by dissolved material and particles in suspension may also reduce the efficacy of the photocatalytic process. Thus, Rincón and Pulgarin (2004) observed that bicarbonates present in water samples absorb some of the light, protecting microorganisms from the action of solar radiation by a screening effect that may limit the efficacy of the disinfection procedures. For this reason, the chemical composition of the simulated WWTP effluent (with an abundant content of bicarbonates, meat extract and peptone) used to evaluate the heterogeneous photocatalysis with TiO_2 may negatively affect the efficacy of the process and thus explain why the disinfection process is not as effective as in DW. Moreover, the turbidity of the simulated WWTP effluent (6.2 NTU) may also have had a slightly negative effect on *C. parvum* inactivation, as observed in previous studies (Gómez-Couso *et al.*, 2009a).

On the other hand, previous studies carried out by our research group in DW under simulated solar radiation showed that the variable pH by itself did not influence the inactivation of *C. parvum* oocysts (unpublished data), but it is a very important parameter in photo-Fenton process, as it strongly influences the speciation and solubility of iron in water, being the optimal pH≤3 (Pignatello *et al.*, 2006; Giannakis *et al.*, 2016a). At neutral and basic pH values, iron species can precipitate and form complexes in solution, causing changes in the colour of the sample and increasing the number of suspended particles in the water that absorb and scatter the light, thus negatively affecting the penetration of sunlight and reducing the efficacy of inactivation of microorganisms (Polo-López *et al.*, 2018). Consequently, the interaction between pH and Fe^{2+}/H_2O_2 concentration is important in the proposed model of *Cryptosporidium* inactivation by photo-Fenton process, being the best results observed at lower pH values, due the strong influence of pH in the solubility/precipitation of iron salts. Similarly, Polo-López *et al.* (2014) observed >2-log reductions in the concentration of *F. solani* spores in simulated WWTP effluent after photo-Fenton process using 10/20 mg/L of Fe^{2+}/H_2O_2 during 2.5 hours at pH 3, whereas at pH 8 and after 5 hours, the concentration of fungal spores remained almost constant under the same experimental conditions.

Moreover, pH affects the zeta potential of the particles in suspension. In the present Doctoral Thesis, examination of the oocyst-spiked water samples under bright field microscopy revealed the existence of aggregates of iron particles on the surface of the oocysts as a consequence of the different surface charges: at neutral-alkaline pH, the oocyst wall has a strong negative surface charge (Hsu and Huang, 2002), whereas iron species are positively charged (Pignatello *et al.*, 2006). Similarly, aggregates of TiO_2 particles were also observed on the surface of the oocysts, and the number of these increased with the concentration of TiO_2 and the exposure time. In DW (pH 6.5), the surface of TiO_2 particles is positively charged, whereas the surface of *C. parvum* oocysts is negatively charged (Drozd and Schwartzbrod, 1996; Fernández Ibáñez, 2004). Aggregation favours photocatalytic disinfection as the short-lived hydroxyl radicals are generated directly on the surface of the microorganisms (Herrmann, 2005).

After use of TiO_2 suspensions for photocatalysis, TiO_2 particles can be recovered by rapid, efficient and economic methods for possible reuse. Fernández Ibáñez (2004) described a recovery method *via* sedimentation based on modification of the medium pH to the isoelectric point of TiO_2 (≈7.0), at which the net surface charge is nil, as the particles aggregate to form larger particles that settle rapidly. In consequence, the capacity of the photocatalyst to retain oocysts was also evaluated in the present study. After sedimentation for one hour, the proportions of oocysts retained in DW and simulated WWTP effluent samples treated with the maximum concentration of

TiO_2 tested (200 mg/L) were approximately 60% and 80%, representing a substantial reduction in the parasitic load of the treated water.

Ultrasound technology is a non-photochemical advance oxidation process, that can be used alone or in combination with conventional methods and other AOPs for treating WWTP effluents, thus increasing the efficacy of disinfection (Blume and Neis, 2004; Oyane *et al.*, 2005; Naddeo *et al.*, 2009; Al-Hashimi *et al.*, 2015; Wei *et al.*, 2016; Madhavan *et al.*, 2019).

Studies evaluating the use of ultrasound technology to inactivate the infective forms of *Cryptosporidium* are scarce, and the type of equipment used and the experimental conditions vary widely, with oocyst inactivation rates of 90-95% reported (Ashokkumar *et al.*, 2003; Oyane *et al.*, 2005; Olvera *et al.*, 2008). In the present work the efficacy of ultrasound technology to inactivate *C. parvum* oocysts was evaluated at three power levels (60, 80 and 100 W), pulsed at 50% or in continuous mode, in four types of water: DW, simulated, real and filtered WWTP effluents. Overall interpretation of the results showed that the application of ultrasound irradiation at 80 W power in continuous mode for an exposure time of 10 minutes drastically reduced the survival of *Cryptosporidium* in all types of water tested (approximately 95-99% of reduction in the oocyst viability).

The observation of high proportions of partially or totally empty oocysts is noteworthy. The existence of empty oocysts may be consequence of a damage to the oocyst wall as a result of mechanical fatigue caused by pressure gradients generated by collapse of the gas microbubbles that enter the solution during acoustic cavitation. Therefore, and as other authors suggested, we conclude that the main mechanism of oocyst inactivation is the mechanical effect generated during the cavitation events induced by ultrasound (Ashokkumar *et al.*, 2003; Oyane *et al.*, 2005). Nevertheless, chemical attack due to the formation of free radicals and further recombination of these to form other strong oxidants can also contributes to the oocyst inactivation, as the oxidants may alter the chemical structure of the oocyst wall and penetrate the cell (Antoniadis *et al.*, 2007; Olvera *et al.*, 2008).

Comparison of the results obtained in both modes showed that use of continuous mode yielded significantly lower values of oocyst viability, as it was previously proved by Ashokkumar *et al.* (2003). Thus, after application of 80 W power in continuous mode by 5 minutes, the oocyst viability was approximately half of the corresponding values obtained in the pulsed mode. However, calculation of the *Dose* parameter (energy per volume unit) did not reveal any statistically significant differences in the oocyst viability in relation to the mode used.

Taking into account the water matrix, higher levels of oocyst inactivation were detected in WWTP effluents than in DW. These differences in the efficacy of ultrasound irradiation may be explained by the variable chemical composition of the samples, as unlike TiO_2 photocatalysis and the photo-Fenton process, dissolved salts and suspended solids increase the action of ultrasound, as they can act as cavitational nuclei. Even organic matter does not adversely affect, but it may favour the efficacy of ultrasonic disinfection (Madge and Jensen, 2002; Drakopoulou *et al.*, 2009). With respect to the influence of pH on the efficacy of the ultrasound technology to inactivate different microorganisms is unclear, but it suggested this parameter can affect to the degradation kinetics of organic pollutants because pH alters cavitation effects including collapse temperature and $HO^{\bullet}$ formation (Wei *et al.*, 2016).

On the other hand, temperature does not only influence on the reaction kinetics of AOPs but is also one of the most important abiotic factors affecting survival of *Cryptosporidium* spp. in the environment (King and Monis, 2007; Peng *et al.*, 2008; Deng and Zhao, 2015). The viability of *C. parvum* decrease at temperature of 30-50 °C due to the melting point of the fatty acids and hydrocarbons present in the oocyst wall and the increase in the metabolic activity of amylopectin (Fayer and Nerad, 1996; King *et al.*, 2005; Peng *et al.*, 2008; Jenkins *et al.*, 2010). Furthermore, high temperatures can induce the phenomenous of spontaneous excystation of *C. parvum* oocysts (Gómez-Couso *et al.*, 2009a). Thus, a small percentage of the sporozoites may excyst when the oocysts are incubated at 37 °C in the absence of any other stimulus making their survival impossible because the environment is different from that provided by the host (Smith *et al.*, 2005). Moreover, a strong synergistic effect between the optical and thermal effects of the solar radiation has been described at temperatures above 45 °C (Wegelin *et al.*, 1994; McGuigan *et al.*, 1998). In experiments carried out to evaluate the efficacy of the heterogeneous photocatalysis with TiO_2 (alone and in combination with H_2O_2), maximum temperature values of the water samples were around 37 ºC under simulated and natural solar conditions, which did not affect to the viability of *C. parvum* oocysts. However, in the evaluation of the photo-Fenton process, the water temperature ranged from 24.60±1.73 °C to 44.40±4.10 °C, reaching a maximum value of 47.30 °C after 5 hours of exposure to sunlight. Therefore, observed reductions in oocyst viability were not only due to the photo-Fenton process, but also to higher temperature reached inside the water samples, as demonstrated by Gómez-Couso *et al.* (2009c). Regarding ultrasound experiments, although the reactor was maintained in an ice-water bath to dissipate the heat of the sample, in the assays carried out at maximum power (100 W) in continuous mode, the temperature reached approximately 40 °C in the different types of water samples. However, it is not clear whether this would cause inactivation of the *C. parvum* oocysts, because of the short exposure time (Gómez-Couso *et al.*, 2009a, 2010).

All of the experiments carried out in this Doctoral Thesis, the potential viability of *C. parvum* oocysts was determined by inclusion/exclusion of the fluorogenic vital dye propidium iodide and a further modification that includes an immunofluorescence antibody test to verify oocyst identification (Campbell *et al.*, 1992; Dowd and Pillai, 1997). This staining method is quick, simple and relatively inexpensive and provides useful information in studies of the influence of environmental factors on the survival of *Cryptosporidium* oocysts, but this technique overestimates the oocyst infectivity in comparison with cell culture methods and bioassays with murine models (Robertson and Gjerde, 2007; Rousseau *et al.*, 2018; Adeyemo *et al.*, 2019). Therefore, the values of oocyst viability obtained are conservative and correspond to lower levels of infectivity (Gómez-Couso *et al.*, 2009b).

Because of its physicochemical properties, TiO_2 is widely used in paints, coatings, plastics, papers, inks, and medical, pharmaceutical, cosmetic and food products, among others. Moreover, studies of TiO_2 photocatalytic disinfection have demonstrated that the process has potentially widespread applications: in indoor air and environmental health, for biological, medical, laboratory and hospital purposes, in the pharmaceutical, food and agriculture industries, and to treat drinking water and wastewater (Baranowska-Wojcik *et al.*, 2020). However, use of this photocatalyst to treat water has been questioned in relation to possible toxic effects include in microorganisms, algae, plants, invertebrates and vertebrates (Hou *et al.*, 2019). Although the possible risk to human health by TiO_2 particles has scarcely been explored, the toxicity is assumed to depend on the morphology (size and shape), rate of migration and amount consumed (Baranowska-Wojcik *et al.*, 2020). A number of recent studies have shown that TiO_2 nanoparticles (1-100 nm) can accumulate in diverse organs, such as the lungs, digestive tract, liver, spleen, kidney, heart, and even the central nervous system (Baranowska-Wojcik *et al.*, 2020), whereas another earlier studies did not indicate any such results (Shi *et al.*, 2013). On 9 of June 2017, the Committee for Risk Assessment of European Chemical Agency (ECHA) classified TiO_2 as a substance suspected of causing cancer *via* inhalation (ECHA, 2017). As consequence of several studies indicating that TiO_2 nanoparticles can induce an oxidative stress, which damage lipids, carbohydrates, proteins and DNA in mammals (see Hou *et al.*, 2019). In the present study, we did not observe any negative effects on the potential viability of *C. parvum* oocysts after 5 hours of exposure to the maximum concentration of TiO_2 tested (200 mg/L) in absence of light, independently of the type of water used.

Homogeneous Fenton process have mainly been used to oxidize organic compounds presented in many types of wastewater (of municipal, hospital, pharmaceutical, industrial origin) because of their simplicity (operated at room temperature and atmospheric pressure) and safety (H_2O_2 decomposes to environmentally safe species such as H_2O and O_2) (Wang *et al.*, 2016; Mirzaei *et al.*, 2017). However, this AOP has some limitations such as high operational cost, narrow optimum pH range (around pH 3), the large volume of iron-containing sludge produced, and difficulties in recycling iron ions, so that the final effluent must be treated to meet the discharge standards for iron concentration (the required concentration range of the iron ion is 50-80 mg/L for processes removing organic compounds and 0.3-20 mg/L for water disinfection, whereas the limit imposed by the EU directives for direct discharge of wastewater to the environment is 2 mg/L). In the photo-Fenton process, the use of UV and/or visible light enhances the disinfection efficacy and reduces the required quantity of catalyst and generation of waste. Moreover, the use of sunlight can minimize energy costs and improve sustainable processes for contaminant removal (Wang *et al.*, 2016; Mirzaei *et al.*, 2017; Durán *et al.*, 2018).

Because optimal pH in photo-Fenton process is around 3 (lethal for most of the microorganisms), and it is required the adjustment of the pH to approximately 7 before effluent discharge, neutral photo-Fenton has received more attention in the last years. However, lower inactivation efficiencies at neutral pH have been reported for real and synthetic secondary effluents. In despite of this, the neutral photo-Fenton process proved more efficient than TiO_2 to inactivate several microorganisms such as *E. coli*, *Salmonella* spp., *Shigella* spp., *E. faecalis*, MS2 coliphage and spores of *Fusarium* spp. (Ajonina *et al.*, 2012; Giannakis *et al.*, 2016b).

Ultrasound technology can be used alone or in combination with conventional methods for treating WWTP effluents and can increase the disinfection capacity of some treatments (Blume and Neis, 2004; Oyane *et al.*, 2005; Naddeo *et al.*, 2009; Al-Hashimi *et al.*, 2015). Furthermore, this technology is currently being applied to treat sludge in WWTPs and to mitigate and inhibit the massive proliferation of algae in reservoirs such as irrigation ponds and lakes, and other applications (Ashokkumar *et al.*, 2003; Olvera *et al.*, 2008). Among the AOPs evaluated in the present Doctoral Thesis, the application of ultrasound technology to water samples spiked with *C. parvum* oocysts yielded the highest inactivation rates, as it greatly reduced the oocyst viability in a short exposure time, irrespective of the chemical composition of the water. Ultrasound provides advantages over other methods of disinfection: i) addition of chemical substances or disinfectants is not required; ii) suspended solids do not decrease the power of action; iii) ultrasound energy is released directly into the sample; and iv) strong oxidant power due to the generation of free radicals. Nonetheless, this technology has some disadvantages: i) the intensity decreases exponentially with distance from the transmitting source, depending on the maximum power input and operating frequency of the equipment, until finally disappearing; ii) it is more expensive than conventional chemical methods of disinfection; and, iii) the transmission source can become eroded, contaminating the medium and blocking cavitation (Gogate, 2008; Matafonova and Batoev, 2019).

The application of these technologies in water reuse must take the operational costs into consideration, but economic data are scarcely reported and planning of water reclamation plants is therefore limited. The cost by heterogeneous photocatalysis with TiO_2 and homogeneous photo-Fenton process for wastewater treatment is affected by factors related to the catalyst and associated reagents (source, modification/preparation, activity, stability, and dose), the wastewater (nature of the organic compounds, concentration and removal level) and the combination with other technologies at the input energy used. As the experimental conditions may vary widely for different pollutants, direct comparison of operational costs is very difficult (Wang *et al.*, 2011). In a review of the cost of solar photocatalytic degradation of pharmaceutical contaminants, Durán *et al.* (2018) estimate a cost of 0.74-0.85 €/m^3 for mineralization of pure compounds in the range 18-21% by homogeneous solar photo-Fenton process. These authors pointed out that heterogeneous photocatalysis with TiO_2 (both immobilized and as slurry) cannot yet compete with homogeneous photo-Fenton process as regards the cost. However, reports of the operational cost associated with use of these AOPs for disinfecting WWTP effluents are very scarcer (almost non-existent). Soriano-Molina *et al.* (2019) recently evaluated, for the first time, the effect of the main operating variables on the solar photo-Fenton at neutral pH for the simultaneous disinfection and removal of contaminants of emerging concern in low-cost raceway pond reactors (open reactors in which the liquid depth can be varied according to the availability of solar radiation). These authors estimated costs ranging from 0.13 to 0.49 €/m^3 depending on application and the planned use of the treated water. Regarding the cost associated with ultrasound technology, Olvera *et al.* (2008) estimated a cost of 0.86 and 14.13 \$/m^3 of water for application of frequency of 1 MHz for 2 and 10 minutes, respectively.

The global interpretation of the results obtained after evaluation of different AOPs to inactivate the waterborne parasite *C. parvum*, considered a reference pathogen for assessing water treatments, demonstrates the effectiveness of these technologies for improving of the microbiological quality of reclaimed water, although further studies are needed in order to optimize the working conditions according with the chemical composition of

WWTP effluent. Inactivation of this enteropathogen will probably ensure elimination other less resistant infectious agents, allowing the safe use of reclaimed water for different purposes and providing a valuable protection of the environment and consequently for the human and animal health.

CONCLUSIONS

First

The addition of TiO_2 slurry to oocyst-spiked samples of distilled water exposed to artificial sunlight yielded a high level of oocyst inactivation. However, the reduction in oocyst viability was significantly lower in simulated municipal wastewater treatment plant effluent than in distilled water. The chemical composition of the simulated wastewater may negatively affect the efficacy of the process by limiting attack of the *C. parvum* oocyst wall by hydroxyl radicals.

Second

As no negative effects on the survival of *C. parvum* oocysts were observed after incubation at the maximum concentration of TiO_2 tested in the absence of light, and as temperature inside the samples did not reach levels that are lethal for the infective forms, it can be concluded that the substantial reduction in oocyst viability detected in distilled water after exposure to both simulated and natural solar radiation was exclusively due to the photocatalytic process.

Third

The incorporation of H_2O_2 at low concentration did not improve the efficacy of heterogeneous photocatalysis with TiO_2 for inactivating *C. parvum* oocysts, as indicate by the lack of statistically significant differences between the values obtained for samples treated with TiO_2/H_2O_2 and those obtained in samples exposed to solar radiation in presence of TiO_2 slurry.

Fourth

The high values of oocyst retention obtained during the disposal and recovery of the photocatalyst *via* sedimentation demonstrate the capacity of TiO_2 slurry to eliminate infectious forms of *Cryptosporidium* by aggregation of TiO_2 particles with *C. parvum* oocysts, leading to a significant reduction in the parasitic load in the treated water samples.

Fifth

The research findings demonstrate the efficacy of homogeneous photocatalysis by photo-Fenton process for inactivating *C. parvum* oocysts in distilled water under natural solar radiation. However, high Fe^{2+}/H_2O_2 concentrations (20/50 mg/L), low pH values (≤5.5) and long exposure times (4 and 6 hours) are required to yield good inactivation rates. Further studies are needed to optimize the photo-Fenton process against this protozoan parasite according to the water matrix.

Sixth

Although, the 3×3 first-order full factorial design used to study the combined effects of several Fe^{2+}/H_2O_2 concentrations, pH values and different exposure times on the survival of *C. parvum* in distilled water subjected to the photo-Fenton process did not include the parameters temperature and intensity of radiation, as these varied throughout the experiments carried out under natural sunlight, the equation obtained proved to be valid for predicting at approximately 75% the oocyst viability under the range of conditions assayed.

Seventh

Ultrasound technology represents a promising alternative to the disinfection methods currently used in water reclamation as it drastically reduces the survival of *C. parvum* oocysts, without altering the chemical composition of the water or producing toxic by-products. Moreover, in contrast to heterogeneous photocatalysis with TiO_2 slurry, the chemical composition of the water does not adversely affect the efficacy of ultrasound irradiation, as dissolved salts and suspended solids act as cavitational nuclei, thereby increasing the disinfectant action of the process.

Eighth

Considering the energy received by the sample during ultrasound treatment, the oocyst inactivation process is most efficient when the ultrasound irradiation is applied at high power levels in continuous mode, as this shortens the exposure time required. Consequently, application of ultrasound irradiation under these conditions will have a favourable influence on the water reclamation capacity by enabling treatment of larger volumes of water per unit of time.

Ninth

The overall interpretation of the research findings obtained after evaluation of the efficacy of different advanced oxidation processes for inactivating *C. parvum* oocysts, considered a reference organism for protozoan pathogens in the validation of water treatments, suggests that these methods are promising alternatives for improving the microbiological quality of reclaimed water. Inactivation of this waterborne enteropathogen will probably ensure elimination of other less resistant infectious agents, providing a valuable protection for environment and, consequently, human and animal health.

REFERENCES

Abeywardena, H., Jex, A.R. and Gasser, R.B., 2015. A perspective on *Cryptosporidium* and *Giardia*, with an emphasis on bovines and recent epidemiological findings. In: *Advances in Parasitology*. Rollinson, D. and Stothard, J.R. (Eds.). Academia Press, pp. 243-301.

Abubakar, I., Aliyu, S.H., Arumugam, C., Hunter, P.R. and Usman, N.K., 2007. Prevention and treatment of cryptosporidiosis in immunocompromised patients. *Cochrane Database of Systematic Reviews*, Cd004932.

Adegbola, R., Demba, E., De, G.V. and Todd, J., 1994. *Cryptosporidium* infection in Gambian children less than 5 years of age. *The Journal of Tropical Medicine and Hygiene*, **97:** 103-107.

Adewuyi, Y.G., 2005. Sonochemistry in environmental remediation. 1. Combinative and hybrid sonophotochemical oxidation processes for the treatment of pollutants in water. *Environmental Science and Technology*, **39:** 3409-3420.

Adeyemo, F.E., Singh, G., Reddy, P., Bux, F. and Stenström, T.A., 2019. Efficiency of chlorine and UV in the inactivation of *Cryptosporidium* and *Giardia* in wastewater. *PLoS One*, **14:** e0216040.

Ahmed, W., Goonetilleke, A. and Gardner, T., 2010. Human and bovine adenoviruses for the detection of source-specific fecal pollution in coastal waters in Australia. *Water Research*, **44:** 4662-4673.

Ahmed, W., Sritharan, T., Palmer, A., Sidhu, J. and Toze, S., 2013. Evaluation of bovine feces-associated microbial source tracking markers and their correlations with fecal indicators and zoonotic pathogens in a Brisbane, Australia, reservoir. *Applied and Environmental Microbiology*, **79:** 2682-2691.

Ajonina, C., Buzie, C., Ajonina, I.U., Basner, A., Reinhardt, H., Gulyas, H., Liebau, E. and Otterpohl, R., 2012. Occurrence of *Cryptosporidium* in a wastewater treatment plant in North Germany. *Journal of Toxicology and Environmental Health, Part A*, **75:** 1351-1358.

Al-Hashimi, A.M., Mason, T.J. and Joyce, E.M., 2015. Combined effect of ultrasound and ozone on bacteria in water. *Environmental Science and Technology*, **49:** 11697-11702.

Alcalde-Sanz, L. and Gawlik, B.M., 2014. Water Reuse in Europe-Relevant guidelines, needs for and barriers to innovation. Publications Office of the European Union, Luxembourg, 49 pp.

Alcalde-Sanz, L. and Gawlik, B.M., 2017. Minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge - Towards a water reuse regulatory instrument at EU level. Publications Office of the European Union, Luxembourg, 57 pp.

Aldeyarbi, H.M., El-Ezz, N.M.A. and Karanis, P., 2016. *Cryptosporidium* and cryptosporidiosis: the African perspective. *Environmental Science and Pollution Research*,**23:** 13811-13821.

Aldeyarbi, H.M. and Karanis, P., 2016. The ultra-structural similarities between *Cryptosporidium parvum* and the gregarines. *Journal of Eukaryotic Microbiology*, **63:** 79-85.

Ali, V. and Nozaki, T., 2013. Iron-sulphur clusters, their biosynthesis, and biological functions in protozoan parasites. *Advances in Parasitology*, **83:** 1-92.

Álvarez-Pellitero, P. and Sitjà-Bobadilla, A., 2002. *Cryptosporidium molnari* n. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. *International Journal for Parasitology*, **32:** 1007-1021.

Amorós, I., Alonso, J.L. and Cuesta, G., 2010. *Cryptosporidium* oocysts and *Giardia* cysts on salad products irrigated with contaminated water. *Journal of Food Protection*, **73:** 1138-1140.

Ananthaswamy, H. and Eisenstark, A., 1976. Near-UV-induced breaks in phage DNA: sensitization by hydrogen peroxide (a tryptophan photoproduct). *Photochemistry and Photobiology*, **24:** 439-442.

Ananthaswamy, H. and Eisenstark, A., 1977. Repair of hydrogen peroxide-induced single-strand breaks in *Escherichia coli* deoxyribonucleic acid. *Journal of Bacteriology*, **130:** 187-191.

Ananthaswamy, H., Hartman, P. and Eisenstark, A., 1979. Synergistic lethality of phage T7 by near-UV radiation and hydrogen peroxide: an action spectrum. *Photochemistry and Photobiology*, **29:** 53-56.

Anderson, V.R. and Curran, M.P., 2007. Nitazoxanide: a review of its use in the treatment of gastrointestinal infections. *Drugs*, **67:** 1947-1967.

Angelakis, A. and Gikas, P., 2014. Water reuse: overview of current practices and trends in the world with emphasis on EU states. *Water Utility Journal*, **8:** 67-78.

Angelakis, A.N., Koutsoyiannis, D. and Tchobanoglous, G., 2005. Urban wastewater and stormwater technologies in ancient Greece. *Water Research*, **39:** 210-220.

Angelakis, A.N. and Snyder, S.A., 2015. Wastewater treatment and reuse: past, present, and future. *Water*, **7:** 4887-4895.

Angelakis, A.N. and Zheng, X.Y., 2015. Evolution of water supply, sanitation, wastewater, and stormwater technologies globally. *Water*, **7:** 455-463.

Angelakis, A.N., Asano, T., Bahri, A., Jimenez, B.E. and Tchobanoglous, G., 2018. Water reuse: from ancient to modern times and the future. *Frontiers in Environmental Science*, **6:** 26.

Anonymous, 1982. Cryptosporidiosis: assessment of chemotherapy of males with acquired immune deficiency syndrome (AIDS). *Morbidity and Mortality Weekly Report*, **31:** 89-102.

Antoniadis, A., Poulios, I., Nikolakaki, E. and Mantzavinos, D., 2007. Sonochemical disinfection of municipal wastewater. *Journal of Hazardous Materials*, **146:** 492-495.

Antoniou, G.P., 2010. Ancient Greek lavatories: operation with reused water. In: *Ancient Water Technologies*. Mays, L. (Ed.) Springer, Dordrecht, the Netherlands, pp. 67-86.

Artieda, J., Basterrechea, M., Arriola, L., Yagüe, M., Albisua, E., Arostegui, N., Astigarraga, U., Botello, R. and Manterola, J., 2012. Outbreak of cryptosporidiosis in a child day-care centre in Gipuzkoa, Spain, October to December 2011. *Eurosurveillance*, **17:** 20070.

Asano, T., Burton, F.L., Leverenz, H., Tsuchihashi, R. and Tchobanoglous, G., 2007. Water reuse: issues, technologies, and applications. McGraw-Hill Professional Publishing, New York, USA, 1541 pp.

Ashokkumar, M., Vu, T., Grieser, F., Weerawardena, A., Anderson, N., Pilkington, N. and Dixon, D.R., 2003. Ultrasonic treatment of *Cryptosporidium* oocysts. *Water Science and Technology*, **47:** 173-177.

Ayers, R.S. and Westcot, D.W., 1994. Water quality for agriculture. Food and Agriculture Organization of the United Nations, Rome, 174 pp.

Baranowska-Wojcik, E., Szwajgier, D., Oleszczuk, P. and Winiarska-Mieczan, A., 2020. Effects of titanium dioxide nanoparticles exposure on human health-a review. *Biological Trace Element Research*, **193:** 118-129.

Barb, W.G., Baxendale, J.H., George, P. and Hargrave, K.R., 1949. Reactions of ferrous and ferric ions with hydrogen peroxide. *Nature*, **163:** 692-694.

Barb, W.G., Baxendale, J.H., George, P. and Hargrave, K.R., 1951a. Reactions of ferrous and ferric ions with hydrogen peroxide. Part I. - The ferrous ion reaction. *Transactions of the Faraday Society*, **47:** 462-500.

Barb, W.G., Baxendale, J.H., George, P. and Hargrave, K.R., 1951b. Reactions of ferrous and ferric ions with hydrogen peroxide. Part II. - The ferric ion reaction. *Transactions of the Faraday Society*, **47:** 591-616.

Bauer, R., 1994. Applicability of solar irradiation for photochemical wastewater treatment. *Chemosphere*, **29:** 1225-1233.

Bitton, G., 1999. Wastewater Microbiology, 2nd Edition. John Wiley & Sons Inc., Hoboken, New Jersey, USA, 750 pp.

Blagburn, B.L. and Soave, R., 1997. Prophylaxis and chemotherapy: human and animal. In: Cryptosporidium *and cryptosporidiosis*. Fayer, R. (Ed.) CRC Press, Boca Raron, Florida, USA, pp. 111-128.

Blanshard, C., Jackson, A.M., Shanson, D.C., Francis, N. and Gazzard, B.G., 1992. Cryptosporidiosis in HIV-seropositive patients. *Quarterly Journal of Medicine*, **85:** 813-823.

Blume, T. and Neis, U., 2004. Improved wastewater disinfection by ultrasonic pre-treatment. *Ultrasonics Sonochemistry*, **11:** 333-336.

Bouzid, M., Hunter, P.R., Chalmers, R.M. and Tyler, K.M., 2013. *Cryptosporidium* pathogenicity and virulence. *Clinical Microbiology Reviews*, **26:** 115-134.

Box, G.E., Hunter, J.S. and Hunter, W.G., 2005. Statistics for experimenters: design, innovation, and discovery. John Wiley & Sons Inc., Hobeken, New Jersey, USA, 639 pp.

Budu-Amoako, E., Greenwood, S.J., Dixon, B.R., Barkema, H.W. and McClure, J., 2011. Foodborne illness associated with *Cryptosporidium* and *Giardia* from livestock. *Journal of Food Protection*, **74:** 1944-1955.

Bull, S., Chalmers, R., Sturdee, A.P., Curry, A. and Kennaugh, J., 1998. Cross-reaction of an anti-*Cryptosporidium* monoclonal antibody with sporocysts of monocystis species. *Veterinary Parasitology*, **77:** 195-197.

Byrne, J.A., Dunlop, P.S., Hamilton, J.W., Fernández-Ibáñez, P., Polo-López, M.I., Sharma, P.K. and Vennard, A.S., 2015. A review of heterogeneous photocatalysis for water and surface disinfection. *Molecules*, **20:** 5574-5615.

Cabada, M.M. and White, A.C.J., 2010. Treatment of cryptosporidiosis: do we know what we think we know? *Current Opinion in Infectious Diseases*, **23:** 494-499.

Cacciò, S.M. and Putignani, L., 2014. Epidemiology of human cryptosporidiosis. In: Cryptosporidium*: parasite and disease*. Cacciò, S.M. and Widmer, A. (Eds.). Springer Science & Business Media, Vienna, Austria, pp. 43-79.

Cacciò, S.M. and Widmer, G., 2014. *Cryptosporidium*: parasite and disease. Springer Science & Business Media, Vienna, Austria, 564 pp.

Cacciò, S.M. and Chalmers, R.M., 2016. Human cryptosporidiosis in Europe. *Clinical Microbiology and Infection*, **22:** 471-480.

Cadenas, E. and Davies, K.J., 2000. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine*, **29:** 222-230.

Caffrey, C.R. and Steverding, D., 2009. Kinetoplastid papain-like cysteine peptidases. *Molecular and Biochemical Parasitology*, **167:** 12-19.

California State Board of Health, 1918. Regulations governing use of sewage for irrigation purposes. California State Board of Health. Sacramento, California, USA.

Campbell, A.T., Robertson, L.J. and Smith, H.V., 1992. Viability of *Cryptosporidium parvum* oocysts: correlation of *in vitro* excystation with inclusion or exclusion of fluorogenic vital dyes. *Applied and Environmental Microbiology*, **58:** 3488-3493.

Campos-Reales-Pineda, A., de Velásquez, M.O. and Rojas-Valencia, M., 2008. The use of ozone during advanced primary treatment of wastewater for its reuse in agriculture: an approach to enhance coagulation, disinfection and crop productivities. *Water Science and Technology*, **57:** 955-962.

Cann, K., Thomas, D.R., Salmon, R., Wyn-Jones, A. and Kay, D., 2013. Extreme water-related weather events and waterborne disease. *Epidemiology and Infection*, **141:** 671-686.

Cárcamo, C., Hooton, T., Wener, M.H., Weiss, N.S., Gilman, R., Arévalo, J., Carrasco, J., Seas, C., Caballero, M. and Holmes, K.K., 2005. Etiologies and manifestations of persistent diarrhea in adults with HIV-1 infection: a case-control study in Lima, Peru. *Journal of Infectious Diseases*, **191:** 11-19.

Carey, J.H., Lawrence, J. and Tosine, H.M., 1976. Photodechlorination of PCB's in the presence of titanium dioxide in aqueous suspensions. *Bulletin of Environmental Contamination and Toxicology*, **16:** 697-701.

Carlton, E.J., Woster, A.P., DeWitt, P., Goldstein, R.S. and Levy, K., 2015. A systematic review and meta-analysis of ambient temperature and diarrhoeal diseases. *International Journal of Epidemiology*, **45:** 117-130.

Carreno, R.A., Martin, D.S. and Barta, J.R., 1999. *Cryptosporidium* is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences. *Parasitology Research*, **85:** 899-904.

Cavalier-Smith, T., 2014. Gregarine site-heterogeneous 18S rDNA trees, revision of gregarine higher classification, and the evolutionary diversification of Sporozoa. *European Journal of Protistology*, **50:** 472-495.

Coe, M.D., 1964. The chinampas of Mexico. *Scientific American*, **211:** 90-99.

Colford, J.M.J., Tager, I.B., Hirozawa, A.M., Lemp, G.F., Aragon, T. and Petersen, C., 1996. Cryptosporidiosis among patients infected with human immunodeficiency virus: factors related to symptomatic infection and survival. *American Journal of Epidemiology*, **144:** 807-816.

Čondlová, Š., Horčičková, M., Sak, B., Květoňová, D., Hlásková, L., Konečný, R., Stanko, M., McEvoy, J. and Kváč, M., 2018. *Cryptosporidium apodemi* sp. n. and *Cryptosporidium ditrichi* sp. n. (Apicomplexa: Cryptosporidiidae) in *Apodemus* spp. *European Journal of Protistology*, **63:** 1-12.

Couso-Pérez, S., Ares-Mazás, E. and Gómez-Couso, H., 2018. Identification of a novel piscine *Cryptosporidium* genotype and *Cryptosporidium parvum* in cultured rainbow trout (*Oncorhynchus mykiss*). *Parasitology Research*, **117:** 2987-2996.

Couso-Pérez, S., Ares-Mazás, E. and Gómez-Couso, H., 2019. First report of *Cryptosporidium* molnari-like genotype and *Cryptosporidium parvum* zoonotic subtypes (IIaA15G2R1 and IIaA18G3R1) in brown trout (*Salmo trutta*). *Journal of Parasitology*, **105:** 170-179.

Couso Pérez, S., 2013. Empleo del peróxido de hidrógeno y de la radiación solar en la regeneración de aguas. Evaluación frente a *Cryptosporidium*. Degree Thesis. 74 pp.

Craun, G.F., Brunkard, J.M., Yoder, J.S., Roberts, V.A., Carpenter, J., Wade, T., Calderon, R.L., Roberts, J.M., Beach, M.J. and Roy, S.L., 2010. Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. *Clinical Microbiology Reviews*, **23:** 507-528.

Current, W.L., Upton, S.J. and Haynes, T.B., 1986. The life cycle of *Cryptosporidium baileyi* n. sp. (Apicomplexa, Cryptosporidiidae) infecting chickens. *The Journal of Protozoology*, **33:** 289-296.

Current, W.L. and Garcia, L.S., 1991. Cryptosporidiosis. *Clinical Microbiology Reviews*, **4:** 325-358.

Curtis, T.P., Walker, G., Dowling, B.M. and Christensen, P.A., 2002. Fate of *Cryptosporidium* oocysts in an immobilised titanium dioxide reactor with electric field enhancement. *Water Research*, **36:** 2410-2413.

Chalmers, R.M., Pérez-Cordón, G., Cacció, S.M., Klotz, C. and Robertson, L.J., 2018. *Cryptosporidium* genotyping in Europe: the current status and processes for a harmonised multi-locus genotyping scheme. *Experimental Parasitology*, **191:** 25-30.

Chappell, C.L., Okhuysen, P.C. and White, A.C.J., 2003. *Cryptosporidium parvum*: infectivity, pathogenesis, and host-parasite relationship. In: Cryptosporidium*: from molecules to disease*. Thompson, R.C.A., Armsony, A. and Ryan, U.M. (Eds.). Elsevier B.V., Amsterdam, The Netherlands, pp. 19-49.

Chavez, M.A. and White, A.C., Jr., 2018. Novel treatment strategies and drugs in development for cryptosporidiosis. *Expert Review of Anti-infective Therapy*, **16:** 655-661.

Chen, Z., Ngo, H.H., Guo, W. and Eslamian, S., 2016. Water shortages. In: *Urban Water Reuse (Handbook)*. Eslamian, S. (Ed.) CRC Press Tailor & Francis Group, Boca Raton, Florida, USA, pp. 3-13.

Cho, M. and Yoon, J., 2008. Measurement of OH radical CT for inactivating *Cryptosporidium parvum* using photo/ferrioxalate and photo/TiO_2 systems. *Journal of Applied Microbiology*, **104:** 759-766.

Chong, M.N., Jin, B., Chow, C.W. and Saint, C., 2010. Recent developments in photocatalytic water treatment technology: a review. *Water Research*, **44:** 2997-3027.

Dalrymple, O.K., Stefanakos, E., Trotz, M.A. and Goswami, D.Y., 2010. A review of the mechanisms and modeling of photocatalytic disinfection. *Applied Catalysis B: Environmental*, **98:** 27-38.

De Feo, G., Angelakis, A.N., Antoniou, G.P., El-Gohary, F., Haut, B., Passchier, C.W. and Zheng, X.Y., 2013. Historical and technical notes on aqueducts from prehistoric to medieval times. *Water*, **5:** 1996-2025.

Decree from 2 August 2010 related to the use of water from treated urban wastewater for irrigation of crops and green areas (amended in 2014 - JORF num. 0153 of 4 July 2014). Ministry of Public Health, Ministry of Agriculture, Food and Fisheries, Ministry of Ecology and Energy and Sustainability. Goverment of France.

Deng, Y. and Zhao, R., 2015. Advanced oxidation processes (AOPs) in wastewater treatment. *Current Pollution Reports,* **1:** 167-176.

Directive 91/271/EEC of European Parliament and of the Council of 21 May 1991 concerning urban wastewater treatment. *Official Journal of the European Communities* L **135:** 40-52.

Directive 2000/60/EC of European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* L **327:** 1-73.

Dixon, B., 2015. Transmission dynamics of foodborne parasites on fresh produce. In: *Foodborne Parasites in the Food Supply Web*. Gajadhar, A.A. (Ed.) Woodhead Publishing, Oxford, United Kingdom, pp. 317-353.

Dowd, S.E. and Pillai, S.D., 1997. A rapid viability assay for *Cryptosporidium* oocysts and *Giardia* cysts for use in conjunction with indirect fluorescent antibody detection. *Canadian Journal of Microbiology,* **43:** 658-662.

Drakopoulou, S., Terzakis, S., Fountoulakis, M., Mantzavinos, D. and Manios, T., 2009. Ultrasound-induced inactivation of gram-negative and gram-positive bacteria in secondary treated municipal wastewater. *Ultrasonics Sonochemistry,* **16:** 629-634.

Drozd, C. and Schwartzbrod, J., 1996. Hydrophobic and electrostatic cell surface properties of *Cryptosporidium parvum*. *Applied and Environmental Microbiology,* **62:** 1227-1232.

Durán, A., Montegudo, J.M. and San Martín, I., 2018. Operation costs of the solar photo-catalytic degradation of pharmaceuticals in water: a mini-review. *Chemosphere,* **211:** 482-488.

Durham, B., Angelakis, A., Wintgens, T., Thoeye, C. and Sala, L., 2005. Water recycling and reuse in EUREAN countries: trends and challenges. Technical workshop: the integration of reclaimed water in water resource management. Lloret de Mar, Girona. España.

Ebi, K.L., Kovats, R.S. and Menne, B., 2006. An approach for assessing human health vulnerability and public health interventions to adapt to climate change. *Environmental Health Perspectives,* **114:** 1930-1934.

Efstratiou, A., Ongerth, J.E. and Karanis, P., 2017. Waterborne transmission of protozoan parasites: review of worldwide outbreaks-an update 2011–2016. *Water Research*, **114:** 14-22.

Ehigiator, H.N., Romagnoli, P., Priest, J.W., Secor, W.E. and Mead, J.R., 2007. Induction of murine immune responses by DNA encoding a 23-kDa antigen of *Cryptosporidium parvum*. *Parasitology Research*, **101:** 943-950.

Ehsan, A., Geurden, T., Casaert, S., Paulussen, J., De Coster, L., Schoemaker, T., Chalmers, R., Grit, G., Vercruysse, J. and Claerebout, E., 2015. Occurrence and potential health risk of *Cryptosporidium* and *Giardia* in different water catchments in Belgium. *Environmental Monitoring and Assessment*, **187:** 1-12.

Elwin, K., Hadfield, S.J., Robinson, G., Crouch, N.D. and Chalmers, R.M., 2012a. *Cryptosporidium viatorum* n. sp. (Apicomplexa: Cryptosporidiidae) among travellers returning to Great Britain from the Indian subcontinent, 2007–2011. *International Journal for Parasitology*, **42:** 675-682.

Elwin, K., Hadfield, S.J., Robinson, G. and Chalmers, R.M., 2012b. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000–2008. *Epidemiology and Infection*, **140:** 673-683.

Entrala, E., Mascaró, C. and Barrett, J., 1997. Anti-oxidant enzymes in *Cryptosporidium parvum* oocysts. *Parasitology*, **114:** 13-17.

European Chemicals Agency (ECHA), 2017. Titanium dioxide proposed to be classified as suspected of causing cancer when inhaled. Available in: https://echa.europa.eu/fr/-/titanium-dioxide-proposed-to-be-classified-as-suspected-of-causing-cancer-when-inhaled (last accessed 15 on January 2020).

European Environment Agency, 2018. Water is life. Europe's rivers, lakes and seas are under pressure from pollution, over-exploitation and climate change. How can we ensure a sustainable use of this vital resource? European Environmental Agency, Copenhagen, Denmark, 79 pp.

European Union and the Committee of the Regions, 2018. Water Reuse – Legislative Framework in EU Regions. European Union, Brussels, 55 pp.

Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), 2014. Multicriteria-based ranking for risk management of food-borne parasites. Microbiological risk assessment series No. 23. FAO and WHO, Rome, Italy, 302 pp.

Fayer, R. and Nerad, T., 1996. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, **62:** 1431-1433.

Fayer, R., Trout, J., Xiao, L., Morgan, U., Lal, A. and Dubey, J., 2001. *Cryptosporidium canis* n. sp. from domestic dogs. *The Journal of Parasitology*, **87:** 1415-1423.

Fayer, R., 2004. *Cryptosporidium*: a water-borne zoonotic parasite. *Veterinary Parasitology*, **126:** 37-56.

Fayer, R., Santín, M. and Xiao, L., 2005. *Cryptosporidium bovis* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). *The Journal of Parasitology*, **91:** 624-629.

Fayer, R., Santín, M. and Trout, J.M., 2008. *Cryptosporidium ryanae* n. sp. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). *Veterinary Parasitology*, **156:** 191-198.

Fayer, R. and Santín, M., 2009. *Cryptosporidium xiaoi* n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (*Ovis aries*). *Veterinary Parasitology*, **164:** 192-200.

Fayer, R., Santín, M. and Macarisin, D., 2010. *Cryptosporidium ubiquitum* n. sp. in animals and humans. *Veterinary Parasitology*, **172:** 23-32.

Feacham, R., Bradley, D., Garelick, H. and Mara, D., 1983. Sanitation and disease: health aspects of excreta and wastewater management. John Wiley & Sons Inc., Washington D.C., USA, 501 pp.

Fenton, H., 1894. LXXIII.—Oxidation of tartaric acid in presence of iron. *Journal of the Chemical Society, Transactions*, **65:** 899-910.

Fernández Ibáñez, P., 2004. Propiedades coloidales de partículas de TiO_2: aplicación al tratamiento fotocatalítico solar de agua. PhD Thesis. University of Granada. 337 pp.

Fontán-Sainz, M., Gómez-Couso, H., Fernández-Ibáñez, P. and Ares-Mazás, E., 2012. Evaluation of the solar water disinfection process (SODIS) against *Cryptosporidium parvum* using a 25-L static solar reactor fitted with a compound parabolic collector (CPC). *The American Journal of Tropical Medicine and Hygiene*, **86:** 223-228.

Fontán Sainz, M., 2012. *Cryptosporidium* en la desinfección solar del agua de bebida. PhD Thesis. University of Santiago de Compostela. 174 pp.

Foster, H.A., Ditta, I.B., Varghese, S. and Steele, A., 2011. Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. *Applied Microbiology and Biotechnology*, **90:** 1847-1868.

Fournet, N., Deege, M., Urbanus, A., Nichols, G., Rosner, B., Chalmers, R., Gorton, R., Pollock, K., van der Giessen, J. and Wever, P., 2013. Simultaneous increase of *Cryptosporidium* infections in the Netherlands, the United Kingdom and Germany in late summer season, 2012. *Eurosurveillance*, **18:** 20348.

Freire-Santos, F., Oteiza-Lopez, A., Vergara-Castiblanco, C. and Ares-Mazas, E., 2000. Study of the combined influence of environmental factors on viability of *Cryptosporidium* parvum oocysts in water evaluated by fluorogenic vital dyes and excystation techniques. *Veterinary Parasitology*, **89:** 253-259.

Fuentes, I., Martín, C., Beristain, X., Mazón, A., Saugar, J., Blanco, A., Cenoz, M.G., Valle-Cristia, M., Ezpeleta, C. and Castilla, J., 2015. *Cryptosporidium hominis* genotypes involved in increased incidence and clusters of cases, Navarra, Spain, 2012. *Epidemiology and Infection*, **143:** 1033-1036.

Gajadhar, A.A. and Allen, J.R., 2004. Factors contributing to the public health and economic importance of waterborne zoonotic parasites. *Veterinary Parasitology*, **126:** 3-14.

Galmes, A., Nicolau, A., Arbona, G., Smith-Palmer, A., Hernández Pezzi, G., Soler, P., Gomis, E., Guma, M. and Baleares, I., 2003. Cryptosporidiosis outbreak in British tourists who stayed at a hotel in Majorca, Spain. *Eurosurveillance*, **7:** 2275.

Galván, A., Magnet, A., Izquierdo, F., Vadillo, C.F., Peralta, R., Angulo, S., Fenoy, S. and del Aguila, C., 2014. A year-long study of *Cryptosporidium* species and subtypes in recreational, drinking and wastewater from the central area of Spain. *Science of the Total Environment*, **468:** 368-375.

Gallopín, G.C., 2012. Five stylized scenarios. United Nations Educational, Scientific and Cultural Organization, Paris, France, 19 pp.

Gao, S., Lewis, G.D., Ashokkumar, M. and Hemar, Y., 2014. Inactivation of microorganisms by low-frequency high-power ultrasound: 2. A simple model for the inactivation mechanism. *Ultrasonics Sonochemistry*, **21:** 454-460.

García-Fernández, I., Miralles-Cuevas, S., Oller, I., Malato, S., Fernández-Ibáñez, P. and Polo-López, M.I., 2019. Inactivation of *E. coli* and *E. faecalis* by solar photo-Fenton with EDDS complex at neutral pH in municipal wastewater effluents. *Journal of Hazardous Materials*, **372:** 85-93.

Gassie, L.W., Englehardt, J.D., Wang, J., Brinkman, N., Garland, J., Gardinali, P. and Guo, T., 2016. Mineralizing urban net-zero water treatment: phase II field results and design recommendations. *Water Research*, **105:** 496-506.

Gassie, L.W. and Englehardt, J.D., 2017. Advanced oxidation and disinfection processes for onsite net-zero greywater reuse: a review. *Water Research*, **125:** 384-399.

Geldreich, E., 1990. Microbiological quality of source waters for water supply. In: *Drinking water microbiology: progress and recent developments*. McFeters, G. (Ed.) Springer-Verlag, New York, USA, pp. 3-31.

Gharpure, R., Perez, A., Miller, A.D., Wikswo, M.E., Silver, R. and Hlavsa, M.C., 2019. Cryptosporidiosis Outbreaks-United States, 2009-2017. *Morbidity and Mortality Weekly Report*, **68:** 568.

Giannakis, S., Polo-López, M.I., Spuhler, D., Sánchez-Pérez, J.A., Fernández-Ibáñez, P. and Pulgarin, C., 2016a. Solar disinfection is an augmentable, *in situ*-generated photo-Fenton reaction—part 1: a review of the mechanisms and the fundamental aspects of the process. *Applied Catalysis B: Environmental*, **199:** 199-223.

Giannakis, S., Polo-López, M.I., Spuhler, D., Sánchez-Pérez, J.A., Fernández-Ibáñez, P. and Pulgarin, C., 2016b. Solar disinfection is an augmentable, in situ-generated photo-Fenton reaction—Part 2: A review of the applications for drinking water and wastewater disinfection. *Applied Catalysis B: Environmental*, **198:** 431-446.

Gogate, P.R., 2008. Cavitational reactors for process intensification of chemical processing applications: a critical review. *Chemical Engineering and Processing*, **47:** 515-527.

Gómez-Couso, H., Fontán-Sainz, M., Fernández-Alonso, J. and Ares-Mazás, E., 2009a. Excystation of *Cryptosporidium parvum* at temperatures that are reached during solar water disinfection. *Parasitology*, **136:** 393-399.

Gómez-Couso, H., Fontán-Sainz, M., McGuigan, K.G. and Ares-Mazás, E., 2009b. Effect of the radiation intensity, water turbidity and exposure time on the survival of *Cryptosporidium* during simulated solar disinfection of drinking water. *Acta Tropica*, **112:** 43-48.

Gómez-Couso, H., Fontán-Sainz, M., Sichel, C., Fernández-Ibáñez, P. and Ares-Mazás, E., 2009c. Efficacy of the solar water disinfection method in turbid waters experimentally contaminated with *Cryptosporidium parvum* oocysts under real field conditions. *Tropical Medicine and International Health*, **14:** 620-627.

Gómez-Couso, H., Fontán-Sainz, M. and Ares-Mazás, E., 2010. Thermal contribution to the inactivation of *Cryptosporidium* in plastic bottles during solar water disinfection procedures. *The American Journal of Tropical Medicine and Hygiene*, **82:** 35-39.

Gómez-Couso, H., Fontán-Sainz, M., Fernández-Ibáñez, P. and Ares-Mazás, E., 2012a. Speeding up the solar water disinfection process (SODIS) against *Cryptosporidium parvum* by using 2.5 l static solar reactors fitted with compound parabolic concentrators (CPCs). *Acta Tropica*, **124:** 235-242.

Gómez-Couso, H., Fontán-Sainz, M., Navntoft, C., Fernández-Ibáñez, P. and Ares-Mazás, E., 2012b. Comparison of different solar reactors for household disinfection of drinking water in developing countries: evaluation of their efficacy in relation to the waterborne enteropathogen *Cryptosporidium parvum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **106:** 645-652.

Gómez-Couso, H., Couso-Pérez, S., Abeledo-Lameiro, M.J. and Ares-Mazás, E., 2013. Speeding up the solar water disinfection process against *Cryptosporidium parvum* oocysts by using H_2O_2. 3rd European Conference on Environmental Applications of Advanced Oxidation Processes. Almería (Spain).

Guerrant, R.L., DeBoer, M.D., Moore, S.R., Scharf, R.J. and Lima, A.A., 2013. The impoverished gut—a triple burden of diarrhoea, stunting and chronic disease. *Nature Reviews Gastroenterology and Hepatology*, **10:** 220-229.

Gustafsson, J., Nordenswan, E. and Rosenholm, J.B., 2003. Consolidation behavior in sedimentation of TiO_2 suspensions in the presence of electrolytes. *Journal of Colloid and Interface Science*, **258:** 235-243.

Haber, F. and Weiss, J., 1934. The catalytic decomposition of hydrogen peroxide by iron salts. *Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences*, **147:** 332-351.

Hamilton, K.A., Waso, M., Reyneke, B., Saeidi, N., Levine, A., Lalancette, C., Besner, M.-C., Khan, W. and Ahmed, W., 2018. *Cryptosporidium* and *Giardia* in wastewater and surface water environments. *Journal of Environmental Quality*, **47:** 1006-1023.

Hartley, T.W., 2006. Public perception and participation in water reuse. *Desalination*, **187:** 115-126.

Hartman, P. and Eisenstark, A., 1980. Killing of *Escherichia coli* K-12 by near-ultraviolet radiation in the presence of hydrogen peroxide: role of double-strand DNA breaks in absence of recombinational repair. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **72:** 31-42.

He, H., Zhao, B., Liu, L., Zhou, K., Qin, X., Zhang, Q., Li, X., Zheng, C. and Duan, M., 2004. The humoral and cellular immune responses in mice induced by DNA vaccine expressing the sporozoite surface protein of *Cryptosporidium parvum*. *DNA and Cell Biology*, **23:** 335-339.

Heaselgrave, W. and Kilvington, S., 2011. The efficacy of simulated solar disinfection (SODIS) against *Ascaris*, *Giardia*, *Acanthamoeba*, *Naegleria*, *Entamoeba* and *Cryptosporidium*. *Acta Tropica*, **119:** 138-143.

Herrera Melián, J., Dona Rodríguez, J., Viera Suárez, A., Tello Rendón, E., Valdés Do Campo, C., Arana, J. and Pérez Peña, J., 2000. The photocatalytic disinfection of urban waste waters. *Chemosphere*, **41:** 323-327.

Herrmann, J.M., 2005. Heterogeneous photocatalysis: state of the art and present applications. *Topics in Catalysis*, **34:** 49-65.

Hijjawi, N., Meloni, B., Ryan, U., Olson, M. and Thompson, R.C.A., 2002. Successful *in vitro* cultivation of *Cryptosporidium andersoni*: evidence for the existence of novel extracellular stages in the life cycle and implications for the classification of *Cryptosporidium*. *International Journal for Parasitology*, **32:** 1719-1726.

Hijnen, W., Beerendonk, E. and Medema, G.J., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research*, **40:** 3-22.

Hochstrat, R., Wintgens, T. and Melin, T., 2008. Development of integrated water reuse strategies. *Desalination*, **218:** 208-217.

Hofstra, N. and Vermeulen, L.C., 2016. Impacts of population growth, urbanisation and sanitation changes on global human *Cryptosporidium* emissions to surface water. *International Journal of Hygiene and Environmental Health*, **219:** 599-605.

Holubová, N., Sak, B., Horčičková, M., Hlásková, L., Květoňová, D., Menchaca, S., McEvoy, J. and Kváč, M., 2016. *Cryptosporidium avium* n. sp. (Apicomplexa: Cryptosporidiidae) in birds. *Parasitology Research*, **115:** 2243-2251.

Holubová, N., Zikmundová, V., Limpouchová, Z., Sak, B., Konečný, R., Hlásková, L., Rajský, D., Kopacz, Z., McEvoy, J. and Kváč, M., 2019. *Cryptosporidium proventriculi* sp. n. (Apicomplexa: Cryptosporidiidae) in Psittaciformes birds. *European Journal of Protistology*, **69:** 70-87.

Horčičková, M., Čondlová, Š., Holubová, N., Sak, B., Květoňová, D., Hlásková, L., Konečný, R., Sedláček, F., Clark, M. and Giddings, C., 2019. Diversity of *Cryptosporidium* in common voles and description of *Cryptosporidium alticolis* sp. n. and *Cryptosporidium microti* sp. n. (Apicomplexa: Cryptosporidiidae). *Parasitology*, **146:** 220-233.

Hou, J., Wang, L., Wang, C., Zhang, S., Liu, H., Li, S. and Wang, X., 2019. Toxicity and mechanisms of action of titanium dioxide nanoparticles in living organisms. *Journal of Environmental Sciences*, **75:** 40-53.

Hsu, B.M. and Huang, C., 2002. Influence of ionic strength and pH on hydrophobicity and zeta potential of *Giardia* and *Cryptosporidium*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **201:** 201-206.

Huang, B.Q., Chen, X.M. and LaRusso, N.F., 2004. *Cryptosporidium parvum* attachment to and internalization by human biliary epithelia *in vitro*: a morphologic study. *The Journal of Parasitology*, **90:** 212-221.

Hunter, P.R. and Nichols, G., 2002. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. *Clinical Microbiology Reviews*, **15:** 145-154.

Hunter, P.R. and Thompson, R.C.A., 2005. The zoonotic transmission of *Giardia* and *Cryptosporidium*. *International Journal for Parasitology*, **35:** 1181-1190.

Hyde, C.G., 1937. The beautification and irrigation of golden gate park with activated sludge effluent. *Sewage Works Journal*, **9:** 929-941.

Imboden, M., Riggs, M.W., Schaefer, D.A., Homan, E.J. and Bremel, R.D., 2010. Antibodies fused to innate immune molecules reduce initiation of *Cryptosporidium parvum* infection in mice. *Antimicrobial Agents and Chemotherapy*, **54:** 1385-1392.

Ince, N., Tezcanli, G., Belen, R. and Apikyan, İ.G., 2001. Ultrasound as a catalyzer of aqueous reaction systems: the state of the art and environmental applications. *Applied Catalysis B: Environmental*, **29:** 167-176.

Inman, L. and Takeuchi, A., 1979. Spontaneous cryptosporidiosis in an adult female rabbit. *Veterinary Pathology*, **16:** 89-95.

Iqbal, A., Labib, M., Muharemagic, D., Sattar, S., Dixon, B.R. and Berezovski, M.V., 2015. Detection of *Cryptosporidium parvum* oocysts on fresh produce using DNA aptamers. *PLoS One*, **10:** e0137455.

Ireland, J.C., Klostermann, P., Rice, E.W. and Clark, R.M., 1993. Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. *Applied and Environmental Microbiology*, **59:** 1668-1670.

Iseki, M., 1979. *Cryptosporidium felis* sp. n. (Protozoa Eimeriorina) from the domestic cat. *Japanese Journal of Parasitology*, **28:** 285-307.

Jagai, J.S., Castronovo, D.A., Monchak, J. and Naumova, E.N., 2009. Seasonality of cryptosporidiosis: a meta-analysis approach. *Environmental Research*, **109:** 465-478.

Jefferies, R., Yang, R., Woh, C.K., Weldt, T., Milech, N., Estcourt, A., Armstrong, T., Hopkins, R., Watt, P., Reid, S., Armson, A. and Ryan, U.M., 2015. Target validation of the inosine monophosphate dehydrogenase (IMPDH) gene in *Cryptosporidium* using Phylomer® peptides. *Experimental Parasitology*, **148:** 40-48.

Jenkins, M., Kerr, D., Fayer, R. and Wall, R., 1995. Serum and colostrum antibody responses induced by jet-injection of sheep with DNA encoding a *Cryptosporidium parvum* antigen. *Vaccine*, **13:** 1658-1664.

Jenkins, M.B., Eaglesham, B.S., Anthony, L.C., Kachlany, S.C., Bowman, D.D. and Ghiorse, W.W., 2010. Significance of wall structure, macromolecular composition, and surface polymers to the survival and transport of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, **76:** 1926-1934.

Ježková, J., Horčičková, M., Hlásková, L., Sak, B., Květoňová, D., Novák, J., Hofmannová, L., McEvoy, J. and Kváč, M., 2016. *Cryptosporidium testudinis* sp. n., *Cryptosporidium ducismarci* Traversa, 2010 and *Cryptosporidium* tortoise genotype III (Apicomplexa: Cryptosporidiidae) in tortoises. *Folia Parasitologica*, **63:** 35.

Jiménez, B. and Asano, T., 2008. Water reclamation and reuse around the world. In: *Water Reuse: an international survey of current practice, issues and needs*. Jiménez, B. and Asano, T. (Eds.). IWA Puplishing, London, United Kingdom, pp. 3-26.

Jirků, M., Valigurová, A., Koudela, B., Krízek, J., Modrý, D. and Slapeta, J., 2008. New species of *Cryptosporidium* Tyzzer, 1907 (Apicomplexa) from amphibian host: morphology, biology and phylogeny. *Folia Parasitologica*, **55:** 81-94.

Joint Ministerial Decree 14516/11 2012 or CMD No 145116 Measures, limits and procedures for reuse of treated wastewater. Ministry of Environment and Energy and Climate Change. Goverment of Greece.

Jones, C.W., 1999. Applications of hydrogen peroxide and derivatives. Royal Society of Chemistry, Cambrigde, United Kingdom, 264 pp.

Joyce, T.M., McGuigan, K.G., Elmore-Meegan, M. and Conroy, R.M., 1996. Inactivation of fecal bacteria in drinking water by solar heating. *Applied and Environmental Microbiology*, **62:** 399-402.

Kanakaraju, D., Glass, B.D. and Oelgemöller, M., 2018. Advanced oxidation process-mediated removal of pharmaceuticals from water: a review. *Journal of Environmental Management*, **219:** 189-207.

Karanis, P., Plutzer, J., Halim, N.A., Igori, K., Nagasawa, H., Ongerth, J. and Liqing, M., 2007. Molecular characterization of *Cryptosporidium* from animal sources in Qinghai province of China. *Parasitology Research*, **101:** 1575-1580.

Karanis, P. and Aldeyarbi, H.M., 2011. Evolution of *Cryptosporidium in vitro* culture. *International Journal for Parasitology*, **41:** 1231-1242.

Khan, A., Shaik, J.S. and Grigg, M.E., 2018. Genomics and molecular epidemiology of *Cryptosporidium* species. *Acta Tropica*, **184:** 1-14.

King, B., Hoefel, D., Daminato, D., Fanok, S. and Monis, P., 2008. Solar UV reduces *Cryptosporidium parvum* oocyst infectivity in environmental waters. *Journal of Applied Microbiology*, **104:** 1311-1323.

King, B., Fanok, S., Phillips, R., Lau, M., Van Den Akker, B. and Monis, P., 2017. *Cryptosporidium* attenuation across the wastewater treatment train: recycled water fit for purpose. *Applied and Environmental Microbiology*, **83:** e03068-03016.

King, B.J., Keegan, A.R., Monis, P.T. and Saint, C.P., 2005. Environmental temperature controls *Cryptosporidium* oocyst metabolic rate and associated retention of infectivity. *Applied and Environmental Microbiology*, **71:** 3848-3857.

King, B.J. and Monis, P.T., 2007. Critical processes affecting *Cryptosporidium* oocyst survival in the environment. *Parasitology*, **134:** 309-323.

Kirhensteine, I., Cherrier, V., Jarritt, N., Farmer, A., de Paoli, G., Delacamara, G. and Psomas, A., 2016. EU-level instruments on water reuse. Final report to support the Commission´s Impact Assessment. Amec Foster Wheeler Environment & Infrastructure UK Ltd, IEEP, ACTeon, IMDEA and NTUA. Publications Office of the European Union, Luxembourg, 292 pp.

Kitajima, M., Haramoto, E., Iker, B.C. and Gerba, C.P., 2014. Occurrence of *Cryptosporidium*, *Giardia*, and *Cyclospora* in influent and effluent water at wastewater treatment plants in Arizona. *Science of the Total Environment*, **484:** 129-136.

Klamerth, N., Rizzo, L., Malato, S., Maldonado, M.I., Agüera, A. and Fernández-Alba, A.R., 2010. Degradation of fifteen emerging contaminants at μgL^{-1} initial concentrations by mild solar photo-Fenton in MWTP effluents. *Water Research*, **44:** 545-554.

Korich, D., Mead, J., Madore, M., Sinclair, N. and Sterling, C.R., 1990. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied and Environmental Microbiology*, **56:** 1423-1428.

Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S., Wu, Y., Sow, S.O., Sur, D. and Breiman, R.F., 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet*, **382:** 209-222.

Kumar-Singh, C., Jha, N. and Eslamian, S., 2016. Reuse, potable water, and possibilities. In: *Urban water reuse (Handbook)*. Eslamian, S. (Ed.) CRC Press, Tailor and Francis Group, Boca Raton, Florida, USA, pp. 113-125.

Kváč, M., Kestřánová, M., Pinková, M., Květoňová, D., Kalinová, J., Wagnerová, P., Kotková, M., Vítovec, J., Ditrich, O. and McEvoy, J., 2013. *Cryptosporidium scrofarum* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic pigs (*Sus scrofa*). *Veterinary Parasitology*, **191:** 218-227.

Kváč, M., Hofmannová, L., Hlásková, L., Květoňová, D., Vítovec, J., McEvoy, J. and Sak, B., 2014. *Cryptosporidium erinacei* n. sp. (Apicomplexa: Cryptosporidiidae) in hedgehogs. *Veterinary Parasitology*, **201:** 9-17.

Kváč, M., Havrdová, N., Hlásková, L., Daňková, T., Kanděra, J., Ježková, J., Vítovec, J., Sak, B., Ortega, Y. and Xiao, L., 2016. *Cryptosporidium proliferans* n. sp. (Apicomplexa: Cryptosporidiidae): molecular and biological evidence of cryptic species within gastric *Cryptosporidium* of mammals. *PLoS One,* **11:** e0147090.

Kváč, M., Vlnatá, G., Ježková, J., Horčičková, M., Konečný, R., Hlásková, L., McEvoy, J. and Sak, B., 2018. *Cryptosporidium occultus* sp. n. (Apicomplexa: Cryptosporidiidae) in rats. *European Journal of Protistology,* **63:** 96-104.

Kwon, B.G., Lee, D.S., Kang, N. and Yoon, J., 1999. Characteristics of p-chlorophenol oxidation by Fenton's reagent. *Water Research,* **33:** 2110-2118.

Lafferty, K.D., 2009. The ecology of climate change and infectious diseases. *Ecology,* **90:** 888-900.

Lal, A., Baker, M.G., Hales, S. and French, N.P., 2013. Potential effects of global environmental changes on cryptosporidiosis and giardiasis transmission. *Trends in Parasitology,* **29:** 83-90.

Lal, A., Fearnley, E. and Wilford, E., 2019. Local weather, flooding history and childhood diarrhoea caused by the parasite *Cryptosporidium* spp.: a systematic review and meta-analysis. *Science of the Total Environment,* **674:** 300-306.

Law 106 (l)/2002 the control of the water and soil pollution and associated regulations: KDP 407/2002, 772/2003, and 269/2005, KD 379/2015. Ministry of agriculture, Natural Resources and Environment and Water Development Department. Goverment of Cyprus.

Lazarova, V., Asano, T., Bahri, A. and Anderson, J., 2013. Milestones in water reuse. The best success stories. IWA publishing, London, United Kingdom, 375 pp.

Leander, B.S., Clopton, R.E. and Keeling, P.J., 2003. Phylogeny of gregarines (Apicomplexa) as inferred from small-subunit rDNA and β-tubulin. *International Journal of Systematic and Evolutionary Microbiology,* **53:** 345-354.

Lehr, J.H. and Keeler, R., 2005. Domestic, municipal, and industrial water supply and water disposal. Water Encyclopedia. John Wiley and Sons, New Jersey, USA, 923 pp.

Leiro, J., Siso, M.I.G., Ortega, M., Santamarina, M.T. and Sanmartín, M.L. 1955. A factorial experimental design for investigation of the temperatura of the incubation time and pathogen-to-phagocyte ratio of *in vitro* phagocytosis by turbot adherent cells. *Comparative Biochemistry and Physiology,* **112:** 215-220.

Levine, N.D., 1980. Some corrections of coccidian (Apicomplexa: Protozoa) nomenclature. *The Journal of Parasitology*, **66:** 830-834.

Levine, N.D., 1984. Taxonomy and review of the coccidian genus *Cryptosporidium* (Protozoa, Apicomplexa). *Journal of Protozoology*, **31:** 94-98.

Levy, K., Woster, A.P., Goldstein, R.S. and Carlton, E.J., 2016. Untangling the impacts of climate change on waterborne diseases: a systematic review of relationships between diarrheal diseases and temperature, rainfall, flooding, and drought. *Environmental Science and Technology*, **50:** 4905-4922.

Li, J., Ahn, J., Liu, D., Chen, S., Ye, X. and Ding, T., 2016. Evaluation of ultrasound-induced damage to *Escherichia coli* and *Staphylococcus aureus* by flow cytometry and transmission electron microscopy. *Applied and Environmental Microbiology*, **82:** 1828-1837.

Li, X., Pereira, M.d.G.C., Larsen, R., Xiao, C., Phillips, R., Striby, K., McCowan, B. and Atwill, E.R., 2015. *Cryptosporidium rubeyi* n. sp. (Apicomplexa: Cryptosporidiidae) in multiple *Spermophilus* ground squirrel species. *International Journal for Parasitology: Parasites and Wildlife*, **4:** 343-350.

Lindsay, D.S., Upton, S.J., Owens, D.S., Morgan, U.M., Mead, J.R. and Blagburn, B.L., 2000. *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporiidae) from cattle, *Bos taurus*. *Journal of Eukaryotic Microbiology*, **47:** 91-95.

Liu, H., Shen, Y., Yin, J., Yuan, Z., Jiang, Y., Xu, Y., Pan, W., Hu, Y. and Cao, J., 2014. Prevalence and genetic characterization of *Cryptosporidium*, *Enterocytozoon*, *Giardia* and *Cyclospora* in diarrheal outpatients in China. *BMC Infectious Diseases*, **14:** 25.

Liu, Y., Dong, S., Kuhlenschmidt, M.S., Kuhlenschmidt, T.B., Drnevich, J. and Nguyen, T.H., 2015. Inactivation mechanisms of *Cryptosporidium parvum* oocysts by solar ultraviolet irradiation. *Environmental Science: Water Research and Technology*, **1:** 188-198.

Lofrano, G. and Brown, J., 2010. Wastewater management through the ages: a history of mankind. *Science of the Total Environment*, **408:** 5254-5264.

Lu, J., Ryu, H., Hill, S., Schoen, M., Ashbolt, N., Edge, T.A. and Santo Domingo, J., 2011. Distribution and potential significance of a gull fecal marker in urban coastal and riverine areas of southern Ontario, Canada. *Water Research*, **45:** 3960-3968.

Luber, G., Knowlton, K., Balbus, J., Frumkin, H., Hayden, M., Hess, J., McGeehin, M., Sheats, N., Backer, L., Beard, C.B., Ebi, K.L., Maibach, E., Ostfeld, R.S., Wiedinmyer, C., Zielinski-Gutiérrez, E. and Ziska, L., 2014. Human health. In: *Climate change impacts in the United States: the Third National Climate Assessment*. Melillo, J.M., Richmond, T.C. and Yohe, G.W. (Eds.). U.S. Global Change Research Program, Washington, D.C., USA, pp. 220-256.

Ma, J., Feng, Y., Hu, Y., Villegas, E.N. and Xiao, L., 2016. Human infective potential of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in urban wastewater treatment plant effluents. *Journal of Water and Health*, **14:** 411-423.

Mac Kenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmierczak, J.J., Addiss, D.G., Fox, K.R. and Rose, J.B., 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine*, **331:** 161-167.

Madge, B.A. and Jensen, J.N., 2002. Disinfection of wastewater using a 20-kHz ultrasound unit. *Water Environment Research*, **74:** 159-169.

Madhavan, J., Theerthagiri, J., Balaji, D., Sunitha, S., Choi, M.Y. and Ashokkumar, M., 2019. Hybrid advanced oxidation processes involving ultrasound: an overview. *Molecules*, **24:** 3341.

Mahin, T. and Peletz, R., 2009. *Cryptosporidium* contamination of water in Africa: the impact on mortality rates for children with HIV/AIDS. Water, sanitation and hygiene - Sustainable development and multisectoral approaches: Proceedings of the 34th Water, Engineering and Development Centre (WEDC) International Conference. Addis Ababa, Ethiopia.

Mahmoudi, M.-R., Ongerth, J.E. and Karanis, P., 2017. *Cryptosporidium* and cryptosporidiosis: the Asian perspective. *International Journal of Hygiene and Environmental Health*, **220:** 1098-1109.

Malato, S., Fernández-Ibáñez, P., Maldonado, M.I., Blanco, J. and Gernjak, W., 2009. Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. *Catalysis Today*, **147:** 1-59.

Malato, S., Maldonado, M.I., Fernández-Ibáñez, P., Oller, I., Polo-López, I. and Sánchez-Moreno, R., 2016. Decontamination and disinfection of water by solar photocatalysis: the pilot plants of the Plataforma Solar de Almeria. *Materials Science in Semiconductor Processing*, **42:** 15-23.

Manjunatha, U.H., Vinayak, S., Zambriski, J.A., Chao, A.T., Sy, T., Noble, C.G., Bonamy, G.M.C., Kondreddi, R.R., Zou, B., Gedeck, P., Brooks, C.F., Herbert, G.T., Sateriale, A., Tandel, J., Noh, S., Lakshminarayana, S.B., Lim, S.H., Goodman, L.B., Bodenreider, C., Feng, G., Zhang, L., Blasco, F., Wagner, J., Leong, F.J., Striepen, B. and Diagana, T.T., 2017. A *Cryptosporidium* PI(4)K inhibitor is a drug candidate for cryptosporidiosis. *Nature*, **546:** 376-380.

Márquez, G., Rodríguez, E.M., Maldonado, M.I. and Álvarez, P.M., 2014. Integration of ozone and solar TiO_2-photocatalytic oxidation for the degradation of selected pharmaceutical compounds in water and wastewater. *Separation and Purification Technology*, **136:** 18-26.

Matafonova, G. and Batoev, V., 2019. Review on low- and high-frequency sonolytic, sonophotolytic and sonophotochemical processes for inactivating pathogenic microorganisms in aqueous media. *Water Research*, **166:** 115085.

Matsunaga, T., Tomoda, R., Nakajima, T. and Wake, H., 1985. Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiology Letters*, **29:** 211-214.

Mays, L.W., 2008. A very brief history of hydraulic technology during antiquity. *Environmental Fluid Mechanics*, **8:** 471-484.

McGuigan, K.G., Joyce, T.M., Conroy, R.M., Gillespie, J.B. and Elmore-Meegan, M., 1998. Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. *Journal of Applied Microbiology*, **84:** 1138-1148.

Mead, J.R. and Arrowood, M.J., 2014. Treatment of cryptosporidiosis. In: Cryptosporidium*: parasite and disease*. Cacciò, S.M. and Widmer, G. (Eds.). Springer, Vienna, Austria, pp. 455-486.

Meisel, J., Perera, D., Meligro, C. and Rubin, C., 1976. Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patient. *Gastroenterology*, **70:** 1156-1160.

Mekonnen, M.M. and Hoekstra, A.Y., 2016. Four billion people facing severe water scarcity. *Science Advances*, **2:** e1500323.

Méndez-Hermida, F., Castro-Hermida, J.A., Ares-Mazás, E., Kehoe, S.C. and McGuigan, K.G., 2005. Effect of batch-process solar disinfection on survival of *Cryptosporidium parvum* oocysts in drinking water. *Applied and Environmental Microbiology*, **71:** 1653-1654.

Méndez-Hermida, F., Ares-Mazás, E., McGuigan, K.G., Boyle, M., Sichel, C. and Fernández-Ibáñez, P., 2007. Disinfection of drinking water contaminated with *Cryptosporidium parvum* oocysts under natural sunlight and using the photocatalyst TiO_2. *Journal of Photochemistry and Photobiology B: Biology*, **88:** 105-111.

Miller, C.N., Jossé, L. and Tsaousis, A.D., 2018. Localization of Fe-S biosynthesis machinery in *Cryptosporidium parvum* mitosome. *Journal of Eukaryotic Microbiology*, **65:** 913-922.

Ministry Decree 185/2003 of technical measures for reuse of wastewate. Ministry of Environment, Ministry of Agriculture, Ministry of Public and Health. Goverment of Italy.

Mirzaei, A., Chen, Z., Haghighat, F. and Yerushalmi, L., 2017. Removal of pharmaceuticals from water by homo/heterogonous Fenton-type processes – a review. *Chemosphere*, **174:** 665-688.

Mølbak, K., Højlyng, N., Gottschau, A., Sa, J.C., Ingholt, L., Da Silva, A.P. and Aaby, P., 1993. Cryptosporidiosis in infancy and childhood mortality in Guinea Bissau, west Africa. *British Medical Journal*, **307:** 417-420.

Monge, R. and Arias, M.L., 1996. Presence of various pathogenic microorganisms in fresh vegetables in Costa Rica. *Archivos Latinoamericanos de Nutrición*, **46:** 292-294.

Morgan-Ryan, U.M., Fall, A., Ward, L.A., Hijjawi, N., Sulaiman, I., Fayer, R., Thompson, R.C.A., Olson, M., Lal, A. and Xiao, L., 2002. *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. *Journal of Eukaryotic Microbiology*, **49:** 433-440.

Morita, S., Namikoshi, A., Hirata, T., Oguma, K., Katayama, H., Ohgaki, S., Motoyama, N. and Fujiwara, M., 2002. Efficacy of UV irradiation in inactivating *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, **68:** 5387-5393.

Murphy, R.C., Ojo, K.K., Larson, E.T., Castellanos-Gonzalez, A., Perera, B.G., Keyloun, K.R., Kim, J.E., Bhandari, J.G., Muller, N.R., Verlinde, C.L., White, A.C., Jr., Merritt, E.A., Van Voorhis, W.C. and Maly, D.J., 2010a. Discovery of potent and selective inhibitors of calcium-dependent protein kinase 1 (CDPK1) from *C. parvum* and *T. gondii*. *ACS Medicinal Chemistry Letters*, **1:** 331-335.

Murphy, R.C., Ojo, K.K., Larson, E.T., Keyloun, K.R., Castaneda, L.J., Derocher, A.E., Inampudi, K.K., Kim, J.E., Arakaki, T.L., Murphy, R.C., Zhang, L., Napuli, A.J., Maly, D.J., Verlinde, C.L., Buckner, F.S., Parsons, M., Hol, W.G., Merritt, E.A. and Van Voorhis, W.C., 2010b. *Toxoplasma gondii* calcium-dependent protein kinase 1 is a target for selective kinase inhibitors. *Nature Structural and Molecular Biology*, **17:** 602-607.

Naddeo, V., Landi, M., Belgiorno, V. and Napoli, R., 2009. Wastewater disinfection by combination of ultrasound and ultraviolet irradiation. *Journal of Hazardous Materials*, **168:** 925-929.

Nasser, A.M., 2016. Removal of *Cryptosporidium* by wastewater treatment processes: a review. *Journal of Water and Health*, **14:** 1-13.

National Research Council (NRC), 1996. Use of reclaimed water and sludge in food crop production. National Academy Press, Washington, D.C., USA, 178 pp.

Natural Resource Management Ministerial Council - Environment Protection and Heritage Council - Australian Health Ministers Conference (NRMMC-EPHC-AHMC), 2006. Australian guidelines for water recycling: managing health and environmental risks (Phase 1). Canberra, Australia, 415 pp.

Navalon, S., Alvaro, M., Garcia, H., Escrig, D. and Costa, V., 2009. Photocatalytic water disinfection of *Cryptosporidium parvum* and *Giardia lamblia* using a fibrous ceramic TiO_2 photocatalyst. *Water Science and Technology*, **59:** 639-645.

Ndao, M., Nath-Chowdhury, M., Sajid, M., Marcus, V., Mashiyama, S.T., Sakanari, J., Chow, E., Mackey, Z., Land, K.M., Jacobson, M.P., Kalyanaraman, C., McKerrow, J.H., Arrowood, M.J. and Caffrey, C.R., 2013. A cysteine protease inhibitor rescues mice from a lethal *Cryptosporidium parvum* infection. *Antimicrobial Agents and Chemotherapy*, **57:** 6063-6073.

Nelson, K.L., Boehm, A.B., Davies-Colley, R.J., Dodd, M.C., Kohn, T., Linden, K.G., Liu, Y., Maraccini, P.A., McNeill, K. and Mitch, W.A., 2018. Sunlight-mediated inactivation of health-relevant microorganisms in water: a review of mechanisms and modeling approaches. *Environmental Science: Processes and Impacts*, **20:** 1089-1122.

Newman, R.D., Sears, C.L., Moore, S.R., Nataro, J.P., Wuhib, T., Agnew, D.A., Guerrant, R.L. and Lima, A.A., 1999. Longitudinal study of *Cryptosporidium* infection in children in northeastern Brazil. *The Journal of Infectious Diseases*, **180:** 167-175.

Ng, J.S.Y., Eastwood, K., Walker, B., Durrheim, D.N., Massey, P.D., Porigneaux, P., Kemp, R., McKinnon, B., Laurie, K., Miller, D., Bramley, E. and Ryan, U., 2012. Evidence of *Cryptosporidium* transmission between cattle and humans in northern New South Wales. *Experimental Parasitology*, **130:** 437-441.

Nguyen, T.T., Traub, R., Pham, P.D., Nguyen, H.V., Nguyen, K.C., Phung, C.D. and Dalsgaard, A., 2016. Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia* spp. in environmental samples in Hanam province, Vietnam. *Food and Waterborne Parasitology*, **3:** 13-20.

Nichols, G., 2007. Epidemiology. In: Cryptosporidium *and cryptosporidiosis*. Fayer, R. and Xiao, L. (Eds.). CRC Press, Boca Raton, Florida, USA, pp. 79-118.

Nime, F.A., Burek, J.D., Page, D.L., Holscher, M.A. and Yardley, J.H., 1976. Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. *Gastroenterology*, **70:** 592-598.

Nitivttananon, V. and Sa-nguanduan, N., 2016. Urbanization and water reuse. In: *Urban water reuse (Handbook)*. Eslamian, S. (Ed.) CRC Press, Boca Raton, Florida, USA, pp. 15-24.

O'Donoghue, P.J., 1995. *Cryptosporidium* and cryptosporidiosis in man and animals. *International Journal for Parasitology*, **25:** 139-195.

Olvera, M., Eguía, A., Rodríguez, O., Chong, E., Pillai, S. and Ilangovan, K., 2008. Inactivation of *Cryptosporidium parvum* oocysts in water using ultrasonic treatment. *Bioresource Technology*, **99:** 2046-2049.

Oppenländer, T., 2003. Photochemical purification of water and air. Principles, Reaction Mechanisms and Reactor Concepts. Wiley, Weinheim, Germany, 368 pp.

Order SSI/445/2015 of 9 March amending Annexes I, II and III of Royal Decree 2210/1995 of 28 December establishing the National Network for Epidemiological Surveillance, concerning the list of notifiable diseases, methods of notification and regionally endemic diseases. Ministry of Health, Social Services and Equality. Goverment of Spain.

Ortega-Gómez, E., García, B.E., Martín, M.B., Ibáñez, P.F. and Pérez, J.S., 2014. Inactivation of natural enteric bacteria in real municipal wastewater by solar photo-Fenton at neutral pH. *Water Research,* **63:** 316-324.

Ortega, Y.R., Roxas, C.R., Gilman, R.H., Miller, N.J., Cabrera, L., Taquiri, C. and Sterling, C.R., 1997. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *The American Journal of Tropical Medicine and Hygiene*, **57:** 683-686.

Otaki, M., Hirata, T. and Ohgaki, S., 2000. Aqueous microorganisms inactivation by photocatalytic reaction. *Water Science and Technology,* **42:** 103-108.

Oyane, I., Furuta, M., Stavarache, C., Hashiba, K., Mukai, S., Nakanishi, M., Kimata, I. and Maeda, Y., 2005. Inactivation of *Cryptosporidium parvum* by ultrasonic irradiation. *Environmental Science and Technology,* **39:** 7294-7298.

Pablos, C., Marugán, J., van Grieken, R. and Serrano, E., 2013. Emerging micropollutant oxidation during disinfection processes using UV-C, UV-C/H_2O_2, UV-A/TiO_2 and UV-A/TiO_2/H_2O_2. *Water Research,* **47:** 1237-1245.

Panciera, R., Thomassen, R. and Garner, F., 1971. Cryptosporidial infection in a calf. *Veterinary Pathology,* **8:** 479-484.

Paranychianakis, N., Salgot, M., Snyder, S. and Angelakis, A., 2015. Quality criteria for recycled wastewater effluent in EU-countries: need for a uniform approach. *Critical Reviews in Environmental Science and Technology,* **45:** 1409-1468.

Paspaltsis, I., Berberidou, C., Poulios, I. and Sklaviadis, T., 2009. Photocatalytic degradation of prions using the photo-Fenton reagent. *Journal of Hospital Infection,* **71:** 149-156.

Patz, J.A., Frumkin, H., Holloway, T., Vimont, D.J. and Haines, A., 2014. Climate change: challenges and opportunities for global health. *Journal of the American Medical Association*, **312:** 1565-1580.

Pavlásek, I., Lávicková, M., Horák, P., Král, J. and Král, B., 1995. *Cryptosporidium varanii* n. sp (Apicomplexa: Cryptosporidiidae) in Emerald monitor (*Varanus prasinus* Schlegal, 1893) in captivity in Prague zoo. *Gazella,* **22:** 99-108.

Pavlásek, I., 1999. Cryptosporidia: biology, diagnosis, host spectrum, specificity, and the environment. *Remedia Klinicka Mikrobiologie*, **3:** 290-301.

Payment, P., Plante, R. and Cejka, P., 2001. Removal of indicator bacteria, human enteric viruses, *Giardia* cysts, and *Cryptosporidium* oocysts at a large wastewater primary treatment facility. *Canadian Journal of Microbiology*, **47:** 188-193.

Peng, X., Murphy, T. and Holden, N.M., 2008. Evaluation of the effect of temperature on the die-off rate for *Cryptosporidium parvum* oocysts in water, soils, and feces. *Applied and Environmental Microbiology*, **74:** 7101-7107.

Perch, M., Sodemann, M., Jakobsen, M.S., Valentiner-Branth, P., Steinsland, H., Fischer, T.K., Lopes, D.D., Aaby, P. and Mølbak, K., 2001. Seven years' experience with *Cryptosporidium parvum* in Guinea-Bissau, West Africa. *Annals of Tropical Paediatrics*, **21:** 313-318.

Pereira, J.T., Costa, A.O., de Oliveira Silva, M.B., Schuchard, W., Osaki, S.C., de Castro, E.A., Paulino, R.C. and Soccol, V.T., 2008. Comparing the efficacy of chlorine, chlorine dioxide, and ozone in the inactivation of *Cryptosporidium parvum* in water from Parana State, Southern Brazil. *Applied Biochemistry and Biotechnology*, **151:** 464-473.

Petry, F., 2004. Structural analysis of *Cryptosporidium parvum*. *Microscopy and Microanalysis*, **10:** 586-601.

Pichel, N., Vivar, M. and Fuentes, M., 2019. The problem of drinking water access: a review of disinfection technologies with an emphasis on solar treatment methods. *Chemosphere*, **218:** 1014-1030.

Pignatello, J.J., Oliveros, E. and MacKay, A., 2006. Advanced oxidation processes for organic contaminant destruction based on the Fenton reaction and related chemistry. *Critical Reviews in Environmental Science and Technology*, **36:** 1-84.

Piyasena, P., Mohareb, E. and McKellar, R., 2003. Inactivation of microbes using ultrasound: a review. *International Journal of Food Microbiology*, **87:** 207-216.

Polo-López, M., Oller, I. and Fernández-Ibáñez, P., 2013. Benefits of photo-Fenton at low concentrations for solar disinfection of distilled water. A case study: *Phytophthora capsici*. *Catalysis Today*, **209:** 181-187.

Polo-López, M., Castro-Alférez, M., Oller, I. and Fernández-Ibáñez, P., 2014. Assessment of solar photo-Fenton, photocatalysis, and H_2O_2 for removal of phytopathogen fungi spores in synthetic and real effluents of urban wastewater. *Chemical Engineering Journal*, **257:** 122-130.

Polo-López, M.I., Fernández-Ibáñez, P., García-Fernández, I., Oller, I., Salgado-Tránsito, I. and Sichel, C., 2010. Resistance of *Fusarium* sp. spores to solar TiO_2 photocatalysis: influence of spore type and water (scaling-up results). *Journal of Chemical Technology and Biotechnology*, **85:** 1038-1048.

Polo-López, M.I., García-Fernández, I., Oller, I. and Fernández-Ibáñez, P., 2011. Solar disinfection of fungal spores in water aided by low concentrations of hydrogen peroxide. *Photochemical and Photobiological Sciences*, **10:** 381-388.

Polo-López, M.I., García-Fernández, I., Velegraki, T., Katsoni, A., Oller, I., Mantzavinos, D. and Fernández-Ibáñez, P., 2012. Mild solar photo-Fenton: an effective tool for the removal of *Fusarium* from simulated municipal effluents. *Applied Catalysis B: Environmental*, **111-112:** 545-554.

Polo-López, M.I., Nahim-Granados, S. and Fernández-Ibáñez, P., 2018. Homogeneous Fenton and photo-Fenton disinfection of surface and groundwater. In: *Applications of Advanced Oxidation Processes (AOPs) in drinking water treatment. The Handbook of Environmental Chemistry*. Gil, A., Galeano, L.A. and Vicente, M.Á. (Eds.). Springer, Cham, pp. 155-177.

Polo-López, M.I., Martínez-García, A., Abeledo-Lameiro, M.J., Gómez-Couso, H., Ares-Mazás, E., Reboredo-Fernández, A., Morse, T.D., Buck, L., Lungu, K. and McGuigan, K.G., 2019. Microbiological evaluation of 5 L-and 20 L-transparent polypropylene buckets for solar water disinfection (SODIS). *Molecules*, **24:** 2193-2206.

Polo, D., García-Fernández, I., Fernández-Ibáñez, P. and Romalde, J.L., 2018. Hepatitis A virus disinfection in water by solar photo-Fenton systems. *Food and Environmental Virology*, **10:** 159-166.

Pouran, S.R., Aziz, A.A. and Daud, W.M.A.W., 2015. Review on the main advances in photo-Fenton oxidation system for recalcitrant wastewaters. *Journal of Industrial and Engineering Chemistry*, **21:** 53-69.

Power, M.L. and Ryan, U.M., 2008. A new species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) from eastern grey kangaroos (*Macropus giganteus*). *The Journal of Parasitology*, **94:** 1114-1118.

Proposal 2018/0169/COD for a Regulation of the European Parliament and of the Council on minimum requirements for water reuse COM (2018) 337 Final, 2018/0169 (COD). Available in: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52018PC0337&from=EN (last accessed 29 January 2020).

Putignani, L. and Menichella, D., 2010. Global distribution, public health and clinical impact of the protozoan pathogen *Cryptosporidium*. *Interdisciplinary Perspectives on Infectious Diseases*, **2010:** 39.

Quesada Peñate, I., 2009. Métodos no convencionales para el tratamiento de aguas contaminadas con productos farmacéuticos. PhD Thesis. Universidad de Toulouse. 177 pp.

Quinn, G.P. and Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, United Kingdom, 557 pp.

Quintero-Betancourt, W., Gennaccaro, A., Scott, T. and Rose, J., 2003. Assessment of methods for detection of infectious *Cryptosporidium* oocysts and *Giardia* cysts in reclaimed effluents. *Applied and Environmental Microbiology*, **69:** 5380-5388.

Raschid-Sally, L. and Jayakody, P., 2009. Drivers and characteristics of wastewater agriculture in developing countries: results from a global assessment. International Water Management Institute (IWMI), Colombo, Sri Lanka, 35 pp.

Rašková, V., Květoňová, D., Sak, B., McEvoy, J., Edwinson, A., Stenger, B. and Kváč, M., 2013. Human cryptosporidiosis caused by *Cryptosporidium tyzzeri* and *C. parvum* isolates presumably transmitted from wild mice. *Journal of Clinical Microbiology*, **51:** 360-362.

Razzolini, M.T.P., Breternitz, B.S., Kuchkarian, B. and Bastos, V.K., 2020. *Cryptosporidium* and *Giardia* in urban wastewater: a challenge to overcome. *Environmental Pollution*, **257:** 113545.

Redwan, N. and Abdullah, A., 2012. Molecular detection of hepatitis A virus in wastewater in Jeddah province, Kingdom of Saudi Arabia. *Journal of Applied Sciences Research*, **8:** 1-9.

Ren, X., Zhao, J., Zhang, L., Ning, C., Jian, F., Wang, R., Lv, C., Wang, Q., Arrowood, M.J. and Xiao, L., 2012. *Cryptosporidium tyzzeri* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). *Experimental Parasitology*, **130:** 274-281.

Rennecker, J.L., Driedger, A.M., Rubin, S.A. and Mariñas, B.J., 2000. Synergy in sequential inactivation of *Cryptosporidium parvum* with ozone/free chlorine and ozone/monochloramine. *Water Research*, **34:** 4121-4130.

Rigg, T., Taylor, W. and Weiss, J., 1954. The rate constant of the reaction between hydrogen peroxide and ferrous ions. *The Journal of Chemical Physics*, **22:** 575-577.

Rincón, A.G. and Pulgarin, C., 2004. Effect of pH, inorganic ions, organic matter and H_2O_2 on *E. coli* K12 photocatalytic inactivation by TiO_2: implications in solar water disinfection. *Applied Catalysis B: Environmental*, **51:** 283-302.

Rincón, A.G. and Pulgarin, C., 2006. Comparative evaluation of Fe^{3+} and TiO_2 photoassisted processes in solar photocatalytic disinfection of water. *Applied Catalysis B: Environmental*, **63:** 222-231.

Ringler, C., Zhu, T., Cai, X., Koo, J. and Wang, D., 2010. Climate change impacts on food security in sub-Saharan Africa: insights from comprehensive climate change scenarios (No. 1042). International Food Policy Research Institute (IFPRI), Washington D.C., USA, 20 pp.

Robertson, L. and Gjerde, B., 2001. Occurrence of parasites on fruits and vegetables in Norway. *Journal of Food Protection*, **64:** 1793-1798.

Robertson, L.J. and Gjerde, B.K., 2007. *Cryptosporidium* oocysts: challenging adversaries? *Trends in Parasitology*, **23:** 344-347.

Robertson, L.J., 2014. *Cryptosporidium* as a foodborne pathogen. Springer, New York, USA, 74 pp.

Rodríguez-Chueca, J., Polo-López, M., Mosteo, R., Ormad, M. and Fernández-Ibáñez, P., 2014. Disinfection of real and simulated urban wastewater effluents using a mild solar photo-Fenton. *Applied Catalysis B: Environmental*, **150:** 619-629.

Rosales, M.J., Cordón, G.P., Moreno, M.S., Sánchez, C.M. and Mascaró., C., 2005. Extracellular like-gregarine stages of *Cryptosporidium parvum*. *Acta Tropica*, **95:** 74-78.

Rose, J. and Angelakis, A.N., 2014. The evolution of sanitation and wastewater management throughout the centuries: past, present, and future. In: *Evolution of sanitation and wastewater management through the centuries*. Angelakis, A. and Rose, J. (Eds.). IWA Publishing, London, United Kingdom, pp. 507-528.

Rosenthal, J., 2009. Climate change and the geographic distribution of infectious diseases. *Ecohealth*, **6:** 489-495.

Rousseau, A., La Carbona, S., Dumètre, A., Robertson, L.J., Gargala, G., Escotte-Binet, S., Favennec, L., Villena, I., Gérard, C. and Aubert, D., 2018. Assessing viability and infectivity of foodborne and waterborne stages (cysts/oocysts) of *Giardia duodenalis*, *Cryptosporidium* spp., and *Toxoplasma gondii*: a review of methods. *Parasite*, **25:** 14.

Rowsell, V.F., Tangney, P., Hunt, C. and Voulvoulis, N., 2010. Estimating levels of micropollutants in municipal wastewater. *Water, Air, and Soil Pollution*, **206:** 357-368.

Royal Decree 1620/2007, of 7 December, establishing the legal framework for the reuse of treated wastewater. Ministry of Environmental, Ministry of Agriculture, Food and Fisheries and Ministry of Health. Goverment of Spain.

Rutala, W.A. and Weber, D.J., 2004. Registration of disinfectants based on relative microbicidal activity. *Industrial and Engineering Chemistry Research*, **25:** 333-341.

Ryan, U., Monis, P., Enemark, H.L., Sulaiman, I., Samarasinghe, B., Read, C., Buddle, R., Robertson, I., Zhou, L., Thompson, R. and Xiao, L., 2004. *Cryptosporidium suis* n. sp. (Apicomplexa: Cryptosporidiidae) in pigs (*Sus scrofa*). *The Journal of Parasitology*, **90:** 769-774.

Ryan, U.M., Power, M. and Xiao, L., 2008. *Cryptosporidium fayeri* n. sp. (Apicomplexa: Cryptosporidiidae) from the Red Kangaroo (*Macropus rufus*). *Journal of Eukaryotic Microbiology*, **55:** 22-26.

Ryan, U.M., Fayer, R. and Xiao, L., 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology*, **141:** 1667-1685.

Ryan, U.M. and Hijjawi, N., 2015. New developments in *Cryptosporidium* research. *International Journal for Parasitology*, **45:** 367-373.

Ryan, U.M., Paparini, A., Tong, K., Yang, R., Gibson-Kueh, S., O'Hara, A., Lymbery, A. and Xiao, L., 2015. *Cryptosporidium huwi* n. sp. (Apicomplexa: Eimeriidae) from the guppy (*Poecilia reticulata*). *Experimental Parasitology*, **150:** 31-35.

Ryan, U.M., Paparini, A., Monis, P. and Hijjawi, N., 2016. It's official - *Cryptosporidium* is a gregarine: what are the implications for the water industry? *Water Research*, **105:** 305-313.

Ryan, U.M., Hijjawi, N. and Xiao, L., 2018. Foodborne cryptosporidiosis. *International Journal for Parasitology*, **48:** 1-12.

Ryu, H., Gerrity, D., Crittenden, J.C. and Abbaszadegan, M., 2008. Photocatalytic inactivation of *Cryptosporidium parvum* with TiO_2 and low-pressure ultraviolet irradiation. *Water Research*, **42:** 1523-1530.

Sa-nguanduan, N. and Nititvattananon, V., 2011. Strategic decision making for urban water reuse application: a case from Thailand. *Desalination*, **268:** 141-149.

Sagodira, S., Buzoni-Gatel, D., Iochmann, S., Naciri, M. and Bout, D., 1999a. Protection of kids against *Cryptosporidium parvum* infection after immunization of dams with CP15-DNA. *Vaccine*, **17:** 2346-2355.

Sagodira, S., Iochmann, S., Mevelec, M.N., Dimier-Poisson, I. and Bout, D., 1999b. Nasal immunization of mice with *Cryptosporidium parvum* DNA induces systemic and intestinal immune responses. *Parasite Immunology*, **21:** 507-516.

Sakthivel, S., Neppolian, B., Arabindoo, B., Palanichamy, M. and Murugesan, V., 2000. TiO_2 catalysed photodegradation of leather dye, Acid Green 16. *Indian Journal of Science and Industrial Research*, **59:** 556-562.

Salgot, M. and Folch, M., 2018. Wastewater treatment and water reuse. *Current Opinion in Environmental Science and Health*, **2:** 64-74.

Santos, S.F.O., Silva, H.D., Wosnjuk, L.A.C., Anunciação, C.E., Silveira-Lacerda, E.P., Peralta, R.H.S., Cunha, F.S., Ferreira, T.D.-S. and García-Zapata, M.T.A., 2016. Occurrence and evaluation of methodologies to detect *Cryptosporidium* spp. in treated water in the central-west region of Brazil. *Exposure and Health*, **8:** 117-123.

Sato, T., Qadir, M., Yamamoto, S., Endo, T. and Zahoor, A., 2013. Global, regional, and country level need for data on wastewater generation, treatment, and use. *Agricultural Water Management*, **130:** 1-13.

Savioli, L., Smith, H. and Thompson, A., 2006. *Giardia* and *Cryptosporidium* join the 'neglected diseases initiative'. *Trends in Parasitology*, **22:** 203-208.

Sciacca, F., Rengifo-Herrera, J.A., Wéthé, J. and Pulgarin, C., 2010. Dramatic enhancement of solar disinfection (SODIS) of wild *Salmonella* sp. in PET bottles by H_2O_2 addition on natural water of Burkina Faso containing dissolved iron. *Chemosphere*, **78:** 1186-1191.

Schijven, J., Bouwknegt, M., de Roda Husman, A.M., Rutjes, S., Sudre, B., Suk, J.E. and Semenza, J.C., 2013. A decision support tool to compare waterborne and foodborne infection and/or illness risks associated with climate change. *Risk Analysis*, **33:** 2154-2167.

Schladweiler, J.C., 2001. Tracking down the roots of our sanitary sewers. *Proceedings of the Water Environment Federation*, **2001:** 1-27.

Schneeberger, C.L., O'Driscoll, M., Humphrey, C., Henry, K., Deal, N., Seiber, K., Hill, V.R. and Zarate-Bermudez, M., 2015. Fate and transport of enteric microbes from septic systems in a coastal watershed. *Journal of Environmental Health*, **77:** 22-31.

Semenza, J.C., Herbst, S., Rechenburg, A., Suk, J.E., Höser, C., Schreiber, C. and Kistemann, T., 2012. Climate change impact assessment of food-and waterborne diseases. *Critical Reviews in Environmental Science and Technology*, **42:** 857-890.

Seven, O., Dindar, B., Aydemir, S., Metin, D., Ozinel, M. and Icli, S., 2004. Solar photocatalytic disinfection of a group of bacteria and fungi aqueous suspensions with TiO_2, ZnO and Sahara desert dust. *Journal of Photochemistry and Photobiology A: Chemistry*, **165:** 103-107.

Shi, H., Magaye, R., Castranova, V. and Zhao, J., 2013. Titanium dioxide nanoparticles: a review of current toxicological data. *Particle And Fibre Toxicology*, **10:** 10-15.

Shiklomanov, I.A., 1993. World freshwater resources. In: *Water in crisis: a guide to the world's fresh water resources*. Gleck, P.H. (Ed.) Oxford University Press, New York, USA, pp. 13-24.

Sichel, C., De Cara, M., Tello, J., Blanco, J. and Fernández-Ibáñez, P., 2007. Solar photocatalytic disinfection of agricultural pathogenic fungi: *Fusarium* species. *Applied Catalysis B: Environmental*, **74:** 152-160.

Sichel, C., Fernández-Ibáñez, P., De Cara, M. and Tello, J., 2009. Lethal synergy of solar UV-radiation and H_2O_2 on wild *Fusarium solani* spores in distilled and natural well water. *Water Research*, **43:** 1841-1850.

Sidhu, J., Ahmed, W., Gernjak, W., Aryal, R., McCarthy, D., Palmer, A., Kolotelo, P. and Toze, S., 2013. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. *Science of the Total Environment*, **463:** 488-496.

Slavin, D., 1955. Cryptosporidium meleagridis (sp. nov.). Journal of Comparative Pathology and Therapeutics, **65:** 262-266.

Smith, H. and Nichols, R., 2006. Zoonotic protozoa-food for thought. *Parassitologia*, **48:** 101-104.

Smith, H.V., Nichols, R.A. and Grimason, A.M., 2005. *Cryptosporidium* excystation and invasion: getting to the guts of the matter. *Trends in Parasitology*, **21:** 133-142.

Smith, H.V., Cacciò, S.M., Tait, A., McLauchlin, J. and Thompson, R.C.A., 2006. Tools for investigating the environmental transmission of *Cryptosporidium* and *Giardia* infections in humans. *Trends in Parasitology*, **22:** 160-167.

Smith, K.R., Woodward, A., Campbell-Lendrum, D., Chadee, D.D., Honda, Y., Liu, Q., Olwoch, J.M., Revich, B. and Sauerborn, R., 2014. Human health: impacts, adaptation and co-benefits. In: *Climate Change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change*. Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R. and White, L.L. (Eds.). Cambridge University Press, Cambridge, United Kingdom and New York, USA, pp. 709-754.

Smith, M.B., 1996. The aztec silent majority: William, T. Sanders and the study of the aztec peasantry. In: *Arqueología Mesoamericana: Homenaje a William T. Sanders*. Mastache, A.G., Parsons, J.R., Santley, R.S. and Serra Puche, M.C. (Eds.). Instituto Nacional de Antropología e Historia, Mexico D.F., pp. 375-386.

Soriano-Molina, P., Mirralles-Cuevas, S., García, B.E., Plaza-Bolaños, P. and Sánchez-Pérez, J.A., 2019. Two strategies of solar photo-Fenton at neutral pH for the simultaneous disinfection and removal of contaminants of emerging concern. Comparative assessment in raceway pond reactors. *Catalysis Today*, Available online 28 November 2019 (in press).

Sow, S.O., Muhsen, K., Nasrin, D., Blackwelder, W.C., Wu, Y., Farag, T.H., Panchalingam, S., Sur, D., Zaidi, A.K.M., Faruque, A.S.G., Saha, D., Adegbola, R., Alonso, P.L., Breiman, R.F., Bassat, Q., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., Kanungo, S., Ahmed, S., Qureshi, S., Quadri, F., Hossain, A., Das, S.K., Antonio, M., Hossain, M.J., Mandomando, I., Nhampossa, T., Acácio, S., Omore, R., Oundo, J.O., Ochieng, J.B., Mintz, E.D., O'Reilly, C.E., Berkeley, L.Y., Livio, S., Tennant, S.M., Sommerfelt, H., Nataro, J.P., Ziv-Baran, T., Robins-Browne, R.M., Mishcherkin, V., Zhang, J., Liu, J., Houpt, E.R., Kotloff, K.L. and Levine, M.M., 2016. The burden of *Cryptosporidium* diarrheal disease among children < 24 months of age in moderate/high mortality regions of Sub-Saharan Africa and south Asia, utilizing data from the Global Enteric Multicenter Study (GEMS). *PLoS Neglected Tropical Diseases*, **10:** e0004729.

Sparks, H., Nair, G., Castellanos-Gonzalez, A. and White, A.C., Jr., 2015. Treatment of *Cryptosporidium*: what we know, gaps, and the way forward. *Current Tropical Medicine Reports*, **2:** 181-187.

Spuhler, D., Rengifo-Herrera, J.A. and Pulgarin, C., 2010. The effect of Fe^{2+}, Fe^{3+}, H_2O_2 and the photo-Fenton reagent at near neutral pH on the solar disinfection (SODIS) at low temperatures of water containing *Escherichia coli* K12. *Applied Catalysis B: Environmental*, **96:** 126-141.

Sroka, J., Stojecki, K., Zdybel, J., Karamon, J., Cencek, T. and Dutkiewicz, J., 2013. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in effluent from sewage treatment plant from eastern Poland. *Annals of Agricultural and Environmental Medicine*, **1:** 57-62.

Stanbridge, H., 1976. History of sewage treatment in Britain, Part 5, Land treatment. Institute of Water Pollution Control, Maidstone, Kent, England, 35 pp.

Standard NP 4434 on the reuse of reclaimed urban wastewater for irrigation. Portuguese Institute for Quality. Goverment of Portugal.

Striepen, B., 2013. Parasitic infections: time to tackle cryptosporidiosis. *Nature News*, **503:** 189-191.

Sunnotel, O., Verdoold, R., Dunlop, P.S., Snelling, W.J., Lowery, C.J., Dooley, J.S., Moore, J.E. and Byrne, J.A., 2010. Photocatalytic inactivation of *Cryptosporidium parvum* on nanostructured titanium dioxide films. *Journal of Water and Health*, **8:** 83-91.

Tan, Y.Y. and Wang, X., 2010. An early warning system of water shortage in basins based on SD model. *Procedia Environmental Sciences*, **2:** 399-406.

Tang, W. and Huang, C., 1996. 2, 4-dichlorophenol oxidation kinetics by Fenton's reagent. *Environmental Technology*, **17:** 1371-1378.

Thakur, J.K. and Kabo-bah, A.T., 2016. State legislation on water reuse. In: *Urban water reuse (Handbook)*. Eslamian, S. (Ed.) CRC Press, Boca Raton, Florida, USA, pp. 201-206.

Thiruvenkatachari, R., Vigneswaran, S. and Moon, I.S., 2008. A review on UV/TiO_2 photocatalytic oxidation process. *Korean Journal of Chemical Engineering*, **25:** 64-72.

Thompson, L. and Doraiswamy, L., 1999. Sonochemistry: science and engineering. *Industrial and Engineering Chemistry Research*, **38:** 1215-1249.

Thompson, R. and Smith, A., 2011. Zoonotic enteric protozoa. *Veterinary Parasitology*, **182:** 70-78.

Thompson, R.C.A., Olson, M.E., Zhu, G., Enomoto, S., Abrahamsen, M.S. and Hijjawi, N.S., 2005. *Cryptosporidium* and cryptosporidiosis. *Advances in Parasitology*, **59:** 77-158.

Tilley, M., Upton, S.J., Fayer, R., Barta, J.R., Chrisp, C.E., Freed, P.S., Blagburn, B.L., Anderson, B.C. and Barnard, S.M., 1991. Identification of a 15-kilodalton surface glycoprotein on sporozoites of *Cryptosporidium parvum*. *Infection and Immunity*, **59:** 1002-1007.

Tyzzer, E., 1907. A sporozoan found in the peptic glands of the common mouse. *Proceedings of the Society for Experimental Biology and Medicine*, **5:** 12-13.

Tyzzer, E., 1910. An extracellular coccidium, *Cryptosporidium muris* (gen. et sp. nov.), of the gastric glands of the common mouse. *The Journal of Medical Research*, **23:** 487-509.

Tyzzer, E., 1912. *Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. *Archiv der Protistenkunde*, **26:** 394-412.

Tzanakakis, V., Koo-Oshima, S., Haddad, M., Apostolidis, N., Angelakis, A., Angelakis, A. and Rose, J., 2014. The history of land application and hydroponic systems for wastewater treatment and reuse. IWA Publishing, London, United Kingdom, 457 pp.

Tzipori, S. and Griffiths, J.K., 1998. Natural history and biology of *Cryptosporidium parvum*. *Advances in Parasitology*, **40:** 5-36.

Tzipori, S. and Widmer, G., 2000. The biology of *Cryptosporidium*. *Contributions to Microbiology and Immunology*, **6:** 1-32.

Tzipori, S. and Ward, H., 2002. Cryptosporidiosis: biology, pathogenesis and disease. *Microbes and Infection*, **4:** 1047-1058.

Umejiego, N.N., Gollapalli, D., Sharling, L., Volftsun, A., Lu, J., Benjamin, N.N., Stroupe, A.H., Riera, T.V., Striepen, B. and Hedstrom, L., 2008. Targeting a prokaryotic protein in a eukaryotic pathogen: identification of lead compounds against cryptosporidiosis. *Chemistry and Biology*, **15:** 70-77.

United Nations (UN)-Water, 2015. Wastewater management-A UN-water analytical brief. UN-Water, New York, USA, 54 pp.

United Nations (UN)-Water, 2016. Metadata on suggested indicators for global monitoring of the sustainable development goal 6 on water and sanitation. UN-Water, New York, USA, 46 pp.

United Nations (UN), 2015. Transforming our world: the 2030 Agenda for Sustainable Development. UN, Available in: https://www.un.org/ga/search/view_doc.asp?symbol=A/RES/70/1&Lang=E (last accessed 29 January 2020).

United Nations Environment Programme (UNEP) and Global Environment Centre Foundation (GEC), 2004. Water and wastewater reuse. An environmentally sound approach for sustainable urban water management. UNEP, Osaka, Japan, 48 pp.

United Nations Environment Programme (UNEP), 2005. Guidelines for municipal water reuse in the Mediterranean region. UNEP, Athens, Greece, 27 pp.

United States Environmental Protection Agency (USEPA), 2012. Guidelines for water reuse (EPA/600/R-12/618). USEPA, Washington, D.C., USA, 643 pp.

Unosson, E., Tsekoura, E.K., Engqvist, H. and Welch, K., 2013. Synergetic inactivation of *Staphylococcus epidermidis* and *Streptococcus mutans* in a TiO_2/H_2O_2/UV system. *Biomatter*, **3:** e26727.

Van der Walt, E., 2002. The effect of ultraviolet light, cavitational flow and ultrasound on protozoan cysts and oocysts, bacteriophages and *Clostridium*. Durban, South Africa, 16-22.

Vermeulen, L.C., van Hengel, M., Kroeze, C., Medema, G., Spanier, J.E., van Vliet, M.T. and Hofstra, N., 2019. *Cryptosporidium* concentrations in rivers worldwide. *Water Research*, **149:** 202-214.

Vetterling, J.M., Jervis, H.R., Merrill, T.G. and Sprinz, H., 1971. *Cryptosporidium wrairi* sp. n. from the guinea pig *Cavia porcellus*, with an emendation of the genus. *The Journal of Protozoology*, **18:** 243-247.

Vinayak, S., Pawlowic, M.C., Sateriale, A., Brooks, C.F., Studstill, C.J., Bar-Peled, Y., Cipriano, M.J. and Striepen, B., 2015. Genetic modification of the diarrhoeal pathogen *Cryptosporidium parvum*. *Nature*, **523:** 477-480.

Voulvoulis, N., 2018. Water reuse from a circular economy perspective and potential risks from an unregulated approach. *Current Opinion in Environmental Science and Health*, **2:** 32-45.

Waldron, L.S., Dimeski, B., Beggs, P.J., Ferrari, B.C. and Power, M.L., 2011. Molecular epidemiology, spatiotemporal analysis, and ecology of sporadic human cryptosporidiosis in Australia. *Applied and Environmental Microbiology*, **77:** 7757-7765.

Wang, J., Guo, Y., Liu, B., Jin, X., Liu, L., Xu, R., Kong, Y. and Wang, B., 2011. Detection and analysis of reactive oxygen species (ROS) generated by nano-sized TiO_2 powder under ultrasonic irradiation and application in sonocatalytic degradation of organic dyes. *Ultrasonics Sonochemistry*, **18:** 177-183.

Wang, N., Zheng, T., Zhang, G. and Wang, P., 2016. A review on Fenton-like processes for organic wastewater treatment. *Journal of Environmental Chemical Engineering*, **4:** 762-787.

Warren, C.A. and Guerrant, R.L., 2007. Clinical disease and pathology. In: *Cryptosporidium and Cryptosporidiosis, Second Edition*. Fayer, R. and Xiao, L. (Eds.). CRC Press, Boca Raton, Florida, USA, pp. 235-253.

Wegelin, M., Canonica, S., Mechsner, K., Fleischmann, T., Pesaro, F. and Metzler, A., 1994. Solar water disinfection: scope of the process and analysis of radiation experiments. *Journal of Water Supply Research and Technology-AQUA*, **43:** 154-169.

Wei, Z., Spinney, R., Ke, R., Yang, Z. and Xiao, R., 2016. Effect of pH on the sonochemical degradation of organic pollutants. *Environmental Chemistry Letters*, **14:** 163-182.

Wen, Q., Tutuka, C., Keegan, A. and Jin, B., 2009. Fate of pathogenic microorganisms and indicators in secondary activated sludge wastewater treatment plants. *Journal of Environmental Management*, **90:** 1442-1447.

Wetzel, D.M., Schmidt, J., Kuhlenschmidt, M.S., Dubey, J.P. and David Sibley, L., 2005. Gliding motility leads to active cellular invasion by *Cryptosporidium parvum* sporozoites. *Infection and Immunity*, **73:** 5379-5387.

Widerström, M., Schönning, C., Lilja, M., Lebbad, M., Ljung, T., Allestam, G., Ferm, M., Björkholm, B., Hansen, A. and Hiltula, J.I., 2014. Large outbreak of *Cryptosporidium hominis* infection transmitted through the public water supply, Sweden. *Emerging Infectious Diseases*, **20:** 581-589.

Winpenny, J., Heinz, I., Koo-Oshima, S., Salgot, M., Collado, J., Hérnandez, F. and Torricelli, R., 2013. Reutilización del agua en la agricultura: ¿beneficios para todos? Food and Agriculture Organization of United Nations, Rome, Italy, 124 pp.

World Bank, 2016. High and dry: climate change, water, and the economy. World Bank, Washington, D.C., USA, 55 pp.

World Bank, 2017. Water supply. Available in: https://www.worldbank.org/en/topic/watersupply (last accessed 29 January 2019)

World Health Organization (WHO), 1989. Health guidelines for the use of wastewater in agriculture and aquaculture. WHO, Geneva, Switzerland, 74 pp.

World Health Organization (WHO), 2006a. Guidelines for the safe use of wastewater, excreta and greywater. Volume 1: policy and regulatory aspects. WHO, Geneva, Switzerland, 114 pp.

World Health Organization (WHO), 2006b. Guidelines for the safe use of wastewater, excreta and greywater. Volume 2: wastewater use in agriculture. WHO, Geneva, Switzerland, 222 pp.

World Health Organization (WHO), 2006c. Guidelines for the safe use of wastewater, excreta and greywater. Volume 3: wastewater and excreta use in aquaculture. WHO, Geneva, Switzerland, 140 pp.

World Health Organization (WHO), 2006d. Guidelines for the safe use of wastewater, excreta and greywater. Volume 4: excreta and greywater use in agriculture. WHO, Geneva, Switzerland, 182 pp.

World Health Organization (WHO), 2017. Guidelines for drinking-water quality. WHO, Geneva, Switzerland, 541 pp.

World Health Organization (WHO), 2019a. Drinking-water. WHO, Available in: https://www.who.int/en/news-room/fact-sheets/detail/drinking-water (last accessed 29 January 2019).

World Health Organization (WHO), 2019b. Sanitation. WHO, Available in: https://www.who.int/en/news-room/fact-sheets/detail/sanitation (last accessed 29 January 2019).

Wu, T. and Englehardt, J.D., 2015. Peroxone mineralization of chemical oxygen demand for direct potable water reuse: kinetics and process control. *Water Research*, **73:** 362-372.

United Nations World Water Assessment Programme (WWAP), 2012. The United Nations world water development report 2012: managing water under uncertainty and risk. UNESCO, Paris, France, 380 pp.

United Nations World Water Assessment Programme (WWAP), 2017. The United Nations world water development report 2017: Wastewater: the untapped resource. UNESCO, Paris, France, 180 pp.

Xi, W. and Geissen, S., 2001. Separation of titanium dioxide from photocatalytically treated water by cross-flow microfiltration. *Water Research*, **35:** 1256-1262.

Xiao, L., Fayer, R., Ryan, U. and Upton, S.J., 2004. *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clinical Microbiology Reviews*, **17:** 72-97.

Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: an update. *Experimental Parasitology*, **124:** 80-89.

Yamazaki, S., Matsunaga, S. and Hori, K., 2001. Photocatalytic degradation of trichloroethylene in water using TiO_2 pellets. *Water Research*, **35:** 1022-1028.

Yannopoulos, S.I., Lyberatos, G., Theodossiou, N., Li, W., Valipour, M., Tamburrino, A. and Angelakis, A.N., 2015. Evolution of water lifting devices (pumps) over the centuries worldwide. *Water*, **7:** 5031-5060.

Young, I., Smith, B.A. and Fazil, A., 2014. A systematic review and meta-analysis of the effects of extreme weather events and other weather-related variables on *Cryptosporidium* and *Giardia* in fresh surface waters. *Journal of Water and Health*, **13:** 1-17.

Zahedi, A., Durmic, Z., Gofton, A.W., Kueh, S., Austen, J., Lawson, M., Callahan, L., Jardine, J. and Ryan, U., 2017. *Cryptosporidium homai* n. sp. (Apicomplexa: Cryptosporidiiae) from the guinea pig (*Cavia porcellus*). *Veterinary Parasitology,* **245:** 92-101.

Zupanc, M., Pandur, Ž., Stepišnik Perdih, T., Stopar, D., Petkovšek, M. and Dular, M., 2019. Effects of cavitation on different microorganisms: the current understanding of the mechanisms taking place behind the phenomenon. A review and proposals for further research. *Ultrasonics Sonochemistry*, **57:** 147-165.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

CAMPUS VIDA CAMPUS DE EXCELENCIA INTERNACIONAL

Water reclamation is considered as one of the most important priorities worldwide. However, the reuse of treated wastewater involves health risks related with the transmission of certain pathogens. This Doctoral Thesis evaluates the efficacy of new technologies based on advanced oxidation processes against *Cryptosporidium*, which infectious forms have a robust nature, and they are often detected in wastewater treatment plant effluents, indicating that conventional sewage treatments do not remove this waterborne enteroparasite. The results obtained suggest that these methods are promising alternatives for improving the microbiological quality of reclaimed water, reducing the risk of transmission of infectious diseases.