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Performance of sewage treatment technologies for the removal of *Cryptosporidium* sp. and *Giardia* sp.: Toward water circularity

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Highlights

- Aerobic and ultrasound technologies present the greatest removal of *Cryptosporidium* sp.
- Anaerobic technologies and combined disinfection present the greatest removal of *Giardia* sp.
- Treated sewage still presents risky concentrations of *Cryptosporidium* sp. and *Giardia* sp.
- To achieve circularity of treated sewage, exhaustive monitoring must quantify and measure the viability of *Cryptosporidium* sp. and *Giardia* sp.

Abstract

Cryptosporidium sp. and *Giardia* sp. are parasites that cause diseases in the population, affecting its quality of life. It has been found that, worldwide, *Cryptosporidium* sp. and *Giardia* sp. cause 63% and 32%, respectively, of outbreaks related to consumption of drinking water contaminated by wastewater, demonstrating problems in the removal of these parasites by the technologies used for wastewater treatment. Therefore, in this work the removal or inactivation efficiency of different treatment technologies presented by around 40 scientific studies was evaluated, with a view to water circularity. For *Cryptosporidium* sp., we conclude that the most efficient secondary technologies are aerobic technologies, which remove between 0.00 and 2.17 log units (Ulog), with activated sludge presenting the greatest efficiency, and that the tertiary technologies with the greatest removal are those that use ultrasound, which reach removal values of 3.17 Ulog. In the case of *Giardia* sp., the secondary technologies with the greatest removal are anaerobic technologies, with values between 0.00 and 3.80 Ulog, and the tertiary technologies with the greatest removal are those that combine filtration with UV or a chemical disinfection agent.

Despite the removal values obtained, the greatest concern remains detecting and cuantifying the infectious forms of both parasites in effluents; therefore, although the technologies perform adequately, discharge effluents must be monitored with more sensitive techniques, above all aiming for circularity of the treated water in a context of the water scarcity that affects some parts of the world.

Keywords: *Cryptosporidium*, *Giardia*, Sewage, Disinfection, water reuse

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Declarations

The authors declare no conflicts of interest

1. Introduction

Domestic sewage is a mixture of black and gray water. Black water contains matter resulting from human metabolism, while gray water is produced by household washing (WHO, 2015). Due to its origins, sewage has complex physical-chemical and microbiological characteristics (Vera et al., 2016; García-Aljaro et al., 2019; Praus, 2019; Alisawi, 2020). In microbiological terms, the organisms present in raw sewage are mainly intestinal microbiota (García-Aljaro et al., 2019; Kuramae et al., 2021). Sewage contains a great variety of microorganisms that can be classified as harmless or pathogenic (Kuramae et al., 2021). Among the pathogenic microorganisms are fungi, viruses, and parasites; this last group includes eggs and larvae of helminths and protozoa. Parasite concentrations depend on their prevalence in the population and its sanitation level, among other factors (Robertson et al., 2006; Wang et al., 2014). Parasitic protozoa are eukaryotic unicellular microorganisms; the genera *Cryptosporidium* sp. and *Giardia* sp. have been linked to infectious outbreaks due to contamination of drinking water with sewage, affecting thousands of people throughout the world (Baldursson and Karanis, 2011; Efstratiou et al., 2017). Therefore, during sewage treatment, it is necessary to apply technologies capable of removing and/or inactivating these parasites to avoid environmental contamination, thereby reducing the probability of transmission to the population (Purnell et al., 2020). There are several sewage treatment technologies, which reduce the parasite load of the influent; however, parasite concentrations present in the effluent are still a risk (Nasser, 2016; Ziaei Hezarjaribi et al., 2020). Therefore, one must consider the removal and/or inactivation efficiencies of these technologies before

implementing them, since it is estimated that, due to climate change, the outbreaks produced by microorganisms will increase, especially in cities that have treatment plants with operational deficiencies (Hofstra et al., 2013; Ikiroma and Pollock, 2021). Moreover, in the same context of climate change, water reuse is proposed as an alternative amid water scarcity (Vera et al., 2016). Therefore, the removal and/or inactivation of these protozoa should be considered to avoid the contamination of crops by irrigation with potentially contaminated water (King et al., 2017; Shoushtarian and Negahban-Azar, 2020; Ryu et al., 2021).

The objective of this work is to evaluate the performance of the technologies used for the treatment and disinfection of wastewater and their efficiency in the removal and/or inactivation of *Cryptosporidium* sp. and *Giardia* sp. parasites. This study also shows the importance of wastewater monitoring considering the circularity of wastewater, and how to promote the interruption of the transmission cycle of these parasites in environmental contamination by wastewater.

2. The effect on human health of the presence of *Cryptosporidium* sp. and *Giardia* sp. in the environment

Cryptosporidium sp. and *Giardia* sp. are parasitic microorganisms that cause diarrhea of varying severity in people and whose main transmission mechanism is fecal contamination through sewage. The World Health Organization has declared these parasites to be among the waterborne pathogens of worldwide concern and has designed guidelines to avoid contamination of water for human consumption with sewage (WHO, 2006; Mehlhorn, 2015). For this same reason, risk assessment and monitoring programs in which these microorganisms are evaluated in the effluents discharged from sewage treatment plants to

the environment have also been designed in countries such as the USA and Australia (WHO, 2016; Petterson et al., 2021).

Cryptosporidium sp. is a protozoan belonging to the phylum Apicomplexa, an intracellular parasite that affects children under 5 years of age, severely immunosuppressed people, and those with adjacent chronic diseases, causing cryptosporidiosis (Dong et al., 2020). The severity of cryptosporidiosis depends on the person's immune status (Chalmers et al., 2019). Asymptomatic cases have been described in immunocompetent people, who, despite not presenting symptoms, eliminate infectious forms in their excreta (Vanathy et al., 2017). Symptomatic immunocompetent individuals may present profuse watery diarrhea, anorexia, nausea, and vomiting, leading to weight loss; however, the biggest problem is the dehydration that occurs, since the clinical picture can last from 3 to 21 days, requiring palliative treatments and hospitalization (Current et al., 1983; Vanathy et al., 2017).

In immunosuppressed people, the symptoms can be more serious and chronic. Profuse diarrhea with water loss between 3 to 10 L day^{-1} is described, which generates serious weight loss that compromises the general state of the person (Costa et al., 2018, 2020). Symptoms can appear over periods of months to years; therefore, the infection can be the cause of death in patients with AIDS (Ryan et al., 2018). Extraintestinal infections have also been described, in which the respiratory system or organs such as the gallbladder and pancreas are involved (Shrivastava et al., 2017). In addition, there is evidence that relates infection by *Cryptosporidium* sp. and intestinal cancer in patients with chronic cryptosporidiosis (Sawant et al., 2020). Another consideration is that cryptosporidiosis does not have a specific treatment and the most widely used drug is not available in many countries, especially in countries in which this type of infection is more frequent (Diptyanusa and Sari, 2021).

The life cycle of *Cryptosporidium* sp. begins when a parasitized person eliminates thick-walled oocysts through deposition, which reach water and from there spread to new hosts (Bouzid et al., 2013). Regarding infection, it is estimated that a parasitized person can begin to eliminate between $1x10^3$ to $1x10^5$ oocysts ml^{-1} 5 to 7 days post infection, for a period of 2 to 3 weeks (Zahedi and Ryan, 2020). The infectious dose is low compared to that of other microorganisms; to infect a new host, only 10 to 100 oocysts are needed (Boyer et al., 2009). Water, raw fruits and vegetables that have been irrigated with contaminated water, and shellfish are described as the main transmission vehicles, the last because they concentrate oocysts (Ryan et al., 2018). To date, 42 species of *Cryptosporidium* sp. have been identified, 9 of which parasitize humans (Zahedi and Ryan, 2020). Among the species that have human beings as a reservoir are *C. hominis*, *C. parvum*, *C. meleagridis*, *C. viatorum,* and *C. ubiquitum* (Zahedi and Ryan, 2020). Morphologically, *Cryptosporidium* sp. has two stages: sporozoite and oocyst. The sporozoite is arch-shaped and corresponds to the reproductive stage of the parasite. (Bouzid et al., 2013). The oocyst, meanwhile, is spherical and contains 4 sporozoites, and has epidemiological importance due to the resistance it presents to adverse conditions (Bouzid et al., 2013; Zahedi and Ryan, 2020). It measures between 4 to 6 µm and has the particularity of being flexible, folding on itself; therefore, its size decreases even more (Jenkins et al., 2010). In molecular terms, the wall of the oocyst is made up of acid-alcohol resistant lipids, proteins rich in cysteine and histidine, and a layer of glycoproteins formed by N-acetyl-galactosamide molecules, which gives it impermeability and resistance to adverse environmental conditions (Jenkins et al., 2010; Samuelson et al., 2013). In addition, a stress-associated metabolic response has been identified in *Cryptosporidium parvum*, such as that generated against UV radiation, triggering a protective response against it (Zhang et al., 2012). Furthermore, *Cryptosporidium* sp. in the environment can produce biofilms or

attaching bacterial biofilms, such as those generated by *Pseudomonas aeruginosa*, further favoring its persistence in the environment (Luo et al., 2017; Lefebvre et al., 2021).

Giardia sp. is a flagellated protozoan belonging to the phylum Sarcomastigophora. It is the etiological agent that causes giardiasis, which mainly affects children and people with nutritional deficiencies (Coffey et al., 2021). There are also asymptomatic people infected with giardiasis who eliminate cysts through the stool. The infection can present in an acute or chronic form (Leung et al., 2019). Among the described symptoms are the generation of watery diarrhea, nausea, anorexy, vomiting, flatulence, and meteorism (Allain and Buret, 2020). In chronic cases, it can produce periods of diarrhea followed by periods of constipation, altering the absorption of nutrients, which links giardiasis to malabsorption syndrome (Singer et al., 2020). In addition, extraintestinal complications such as cognitive deficiencies and irritable bowel syndrome have been described (Halliez and Buret, 2013; Allain and Buret, 2020). *Giardia duodenalis* is considered a species complex consisting of eight assemblages (A-H) with varying degrees of host specificity, which can only be separated by molecular genotyping (Zajaczkowski, et al., 2021; Heyworth, 2016).

Morphologically, it presents two parasitic stages: trophozoite and cyst. The trophozoite is pyriform and measures approximately 20 µm; its importance is related to infection in the intestine. The cyst is oval and measures between 8 to 18 µm and is the form of transmission of the parasite (Ryan et al., 2019).

The transmission cycle is very similar to that of *Cryptosporidium* sp., beginning with a parasitized person shedding concentrations of 10^6 cysts g^{-1}. The cysts remain in the environment, contaminating water and foods such as fruits and vegetables, and concentrating in shellfish. The infectious dose is also between 10 to 100 cysts (Ryan et al., 2019). Molecularly, the cyst also has a complex wall, composed of ß 1,3 -N Acetyl-galactosamide

polymers, without the presence of lipids and wall proteins rich in cysteine (Konrad et al., 2010; Samuelson et al., 2013). The composition of the layers of the cystic wall is said to be what gives it water resistance (Konrad et al., 2010). The problem of the existence of asymptomatic and chronically ill people and the fact that diagnostic techniques have little sensitivity make it difficult to quantify the actual prevalence in the population (Shaposhnik et al., 2019). The worldwide human prevalence of *Cryptosporidium* sp. is 7.6%, but in some countries, can reach values of 69% (Dong et al., 2020). This implies that there is likely to be a greater presence of the infectious forms of these parasites in the environment.

Figure 1 shows the transmission of these parasites through water or food that has had contact with sewage-contaminated water (Ryan et al., 2018, 2019). The infectious forms, due to their molecular composition, are characterized by resistance to adverse environmental conditions such as those created by water (lack of nutrients and change in pH, among others) or resistance to the action of disinfectant agents. Also worth mentioning are the parasite load that infected people eliminate, which varies between $1x10^3$ to $1x10^6$ (oo) cysts, and the low infectious dose, which fluctuates between 10 to 100 (oo) cysts, which also favors infection. It also must be remembered that there are asymptomatic people; therefore, the prevalence of infection in people is underestimated (Bouzid et al., 2013). *Cryptosporidium* sp. and *Giardia* sp. have caused 63 and 32%, respectively, of outbreaks due to contaminated drinking water between 2011 and 2016 (Baldursson and Karanis, 2011; Efstratiou et al., 2017). Therefore, considering that the main source of transmission is sewage, it is necessary to evaluate the efficiency of sewage treatment technologies to eliminate and/or irreversibly inactivate these microorganisms, reduce infection in the population, and avoid their dissemination, as shown in Figure 1.

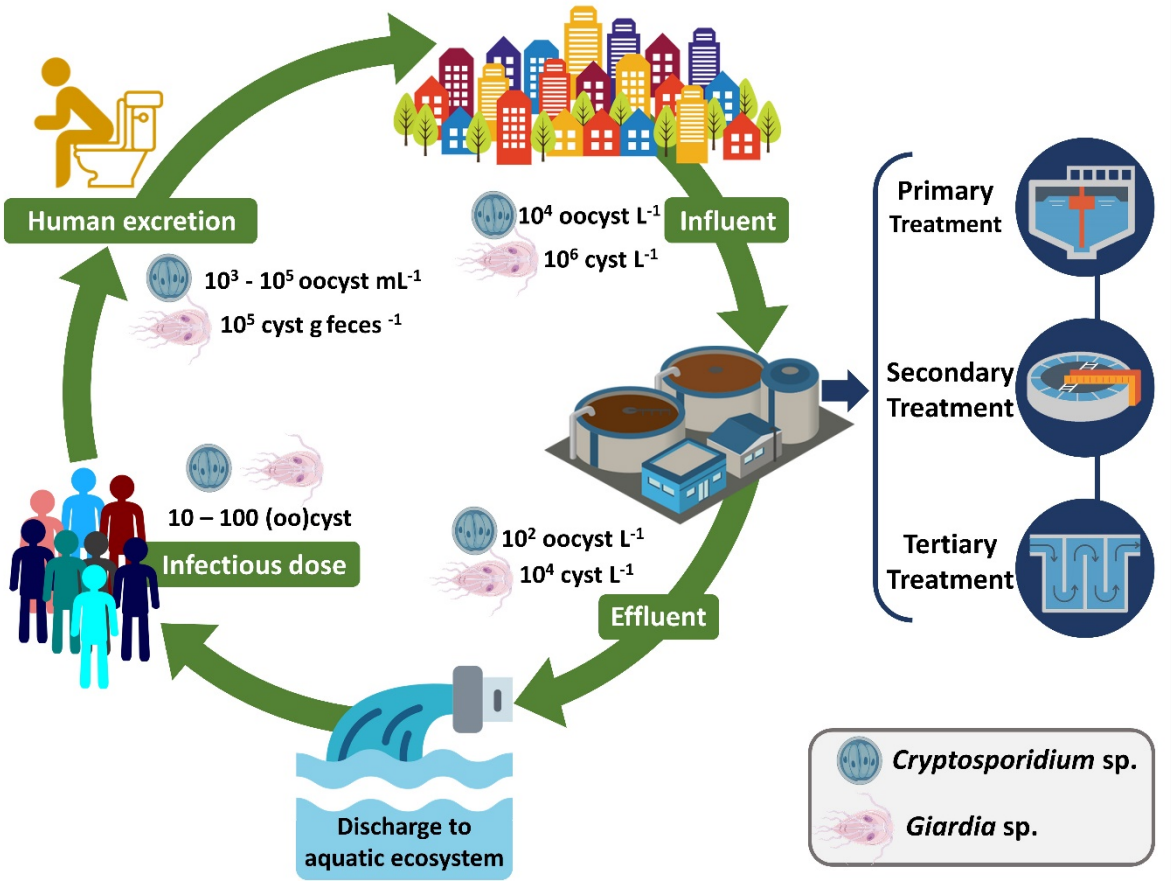


Fig.1 Schematic representation of the transmission mechanisms of *Cryptosporidium* sp. and *Giardia* sp. through sewage to people.

3. Treatment technologies for the removal of *Cryptosporidium* sp. and *Giardia* sp. in sewage

The treatment stages that sewage undergoes depend on its composition. In general, processes are considered to reduce fats and solids, organic matter, nutrients, and pathogenic microorganisms (WHO, 2015; García-Aljaro et al., 2019; Purnell et al., 2020; Praus, 2019; Kuramae et al., 2021). Figure 1 shows wastewater collection by a sewer network. The influent is treated by a treatment plant and processed sequentially in primary, secondary, and tertiary phases. In the primary treatment, the removal of grease and oils and solids is reduced. Subsequently, the secondary treatment reduces organic matter through biological processes

196 and the tertiary treatment, in most cases, consists of the disinfection of the influent (WHO,
197 2015, Vera et al., 2013). The treated effluent is generally discharged to surface ecosystems
198 such as rivers, lakes, and lagoons (Wei et al., 2018). In addition, a sludge stream is generated
199 as a by-product that must be stabilized and sanitized (Vera et al., 2013, Neumann et al., 2018,
200 Cartes et al., 2018). In terms of eliminating physical, chemical and microbiological
201 pollutants, the wastewater treatment process is crucial to avoiding environmental impacts
202 and health problems in the population due to the discharge of effluent into ecosystems
203 (Amoueyan et al., 2017; Purnell et al., 2020).
204 Next, various technologies and operating conditions and the removal efficiency of
205 *Cryptosporidium* sp. and *Giardia* sp. by the different sewage treatment technologies will be
206 analyzed.
207
208 **3.1. Biological technologies and their efficiency in the removal of *Cryptosporidium* sp.**
209 **and *Giardia* sp.**
210 Secondary technologies or biological systems for sewage treatment are based on the
211 transformation of organic matter through biological processes (Jakubaszek and Stadnik,
212 2018). Although the objective of these technologies is not the removal of microorganisms,
213 they have been shown to affect the removal of *Cryptosporidium* sp. and *Giardia* sp. in the
214 influent.
215 Table 1 shows biological technologies (aerobic, anaerobic) and/or a combination of
216 biological and chemical treatments and different degrees of parasite removal. Aerobic
217 secondary treatments are based on the transformation of organic matter by bacterial biomass
218 under aerobic conditions. This process generates an effluent, which requires disinfection, and
219 a by-product, the secondary sludge that is not stabilized or sanitized (Gherghel et al., 2019).

220 Using this type of technology, it is possible to transform between 60 and 90% of the organic
221 matter present in the influent (Gherghel et al., 2019). Particularly, the activated sludge
222 technology, which consists of the elimination of organic matter using bacterial flocs, removes
223 parasites through the adsorption of the parasite in the floc and subsequently sediments inside
224 the reactor (Nasser, 2016). Under these conditions it has been found that a load of
225 *Cryptosporidium* sp. in bacterial flocs can reach concentrations between $4.9x10^2$ oocyst g^{-1}
226 (Amorós et al., 2016) and $2.3x10^4$ -$1.7x10^5$ oocyst g^{-1} (Schlindwein et al., 2010). Meanwhile,
227 for *Giardia* sp. the measured concentrations canbetween $2.4x10^2$ cyst g^{-1} (Amorós et al.,
228 2016) and $3.2x10^5$ - $6.0x10^5$ cyst g^{-1} (Medeiros and Daniel, 2018).
229 The suspended biomass aerobic technologies shown in Table 1 are activated sludge, aerated
230 lagoons, and oxidation ditches, while trickling filter technology consists of adhered biomass.
231 *Cryptosporidium* sp. removal with different aerobic technologies reported by various authors
232 varies between 0.00 to 2.17 Ulog (log Unit), with activated sludge being the technology with
233 the highest removal efficiency and aerated lagoons and trickling those the lowest. In the case
234 of *Giardia* sp., removal reported by these technologies fluctuates between - 0.77 and 2.93
235 Ulog. The technology with the highest removal efficiency was oxidation trenches, with 2.09
236 Ulog, and that with the lowest was aerated lagoons, with 0.77 Ulog.
237 The mechanism through which anaerobic biological technologies transform organic matter
238 is metabolic transformation by facultative and/or anaerobic bacteria. Organic matter is
239 transformed to CH_4 and CO_2 by different bacterial groups and only about 10% of organic
240 matter is transformed into bacterial biomass. Table 1 shows an analysis of different anaerobic
241 technologies to eliminate *Cryptosporidium* sp. and *Giardia* sp. Anaerobic systems such as
242 upflow anaerobic sludge blankets (UASB) and UASB combined with dissolved air flotation

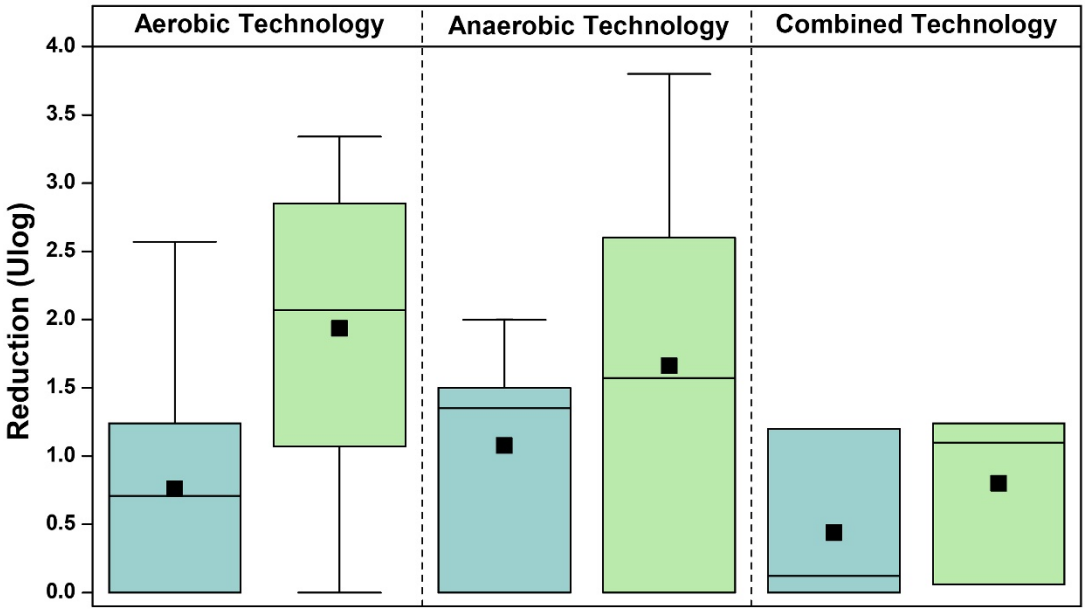
(DAF), flocculation and DAF technology, and an SBBGR reactor are described. BARDENPHO technology for nitrogen removal from sewage is also analyzed.

In general, it was found that for these technologies, the removal values for *Cryptosporidium* sp. ranged from 0.00 to 2.00 Ulog. The technologies with the greatest removal values were the SBBGR reactor and BARDENPHO, with values of 2.00 and 1.59 Ulog, respectively. The lowest-efficiency anaerobic technology would be a UASB reactor, with a value of 0.28 Ulog. For *Giardia* sp. removal by anaerobic technologies ranges from 0.39 Ulog to 3.80 Ulog. The technology with the highest removal efficiency is the SBBGR reactor, with 3.80 Ulog, and that with the lowest efficiency is the UASB reactor, with 0.39 Ulog. The mechanism involved in removal is thought to be related to biomass sedimentation under anaerobic conditions (Reinoso et al., 2011).

Combined technologies (aerobic and anaerobic treatments) such as facultative lagoons, UASB reactors with activated sludge, and anaerobic-anoxic aerated filters, have been shown to have *Cryptosporidium* sp. removal efficiencies from 0.00 Ulog to 1.95 Ulog, while *Giardia* sp. removal ranges from 0.06 to 1.24 Ulog, with a mean of 0.80 Ulog. The UASB reactor technology with activated sludge appears to be inefficient in the removal of *Cryptosporidium* sp., presenting a minimum removal value, unlike the aerated anaerobic-anoxic filter technology, which has a value of 1.20 Ulog. Meanwhile, the facultative lagoon technology presents a minimum *Giardia* sp. removal with a value of 0.06 Ulog, unlike the combination of the UASB reactor with activated sludge, which has a *Giardia* sp. removal value of 1.24 Ulog.

Nasser (2016) also evaluated secondary biological technologies, indicating that activated sludge presents a low *Cryptosporidium* sp. removal efficiency and that facultative lagoons have a greater efficiency given the retention time of the technology (approximately 20 days).

267 In this type of treatment there could be several associated mechanisms, including
268 sedimentation, elimination by anaerobiosis and the predation of *Cryptosporidium* sp. by other
269 species (Reinoso et al., 2011).
270 Table 1 also shows the concentration of these parasites in effluent treated by the respective
271 technologies. It is shown that although some technologies remove a large proportion of these
272 parasites, they can still be present in the effluent, with values higher than the infectious doses
273 for people if they are discharged without tertiary treatment. In the works cited, these
274 technologies were the last stage before the discharge of the effluent to the receiving bodies
275 (Lim et al., 2007; Ribeiro dos Santos and Daniel, 2016; Ramo et al., 2017). Therefore,
276 considering that the values fluctuate between $1x10^0$ to $1x10^2$ oocysts ml^{-1} and that the treated
277 volumes fluctuate between 0.59 to $7.9x10^9$ m^3 d^{-1}, it can be concluded that the analyzed
278 technologies are sufficient to avoid making the population sick if the infectious dose of both
279 parasites is 10-100 (oo)oocyst. (Bouzid et al., 2013).
280 Figure 2 shows the calculated removal efficiency averages presented by the different
281 secondary technologies described above.



282

283 **Fig.2** Removal efficiency of *Cryptosporidium* sp. (¢) and *Giardia* sp. (¢) by secondary

284 sewage treatment technologies.

285

286 **3.2. Disinfection technologies and their efficiency in the removal of *Cryptosporidium* sp.**

287 **and *Giardia* sp.**

288 The disinfection of sewage (tertiary treatment) seeks to eliminate or reduce the presence of

289 pathogenic microorganisms in the effluent; therefore, it uses physical, chemical and/or

290 physicochemical treatments for this purpose (WHO, 2015). Table 2 shows the efficiency of

291 different treatments for the removal or inactivation of *Cryptosporidium* sp and *Giardia* sp.

292 The physical treatments presented in Table 2 consist of UV radiation, ultrasound and/or

293 filtration. UV radiation applied at a length of 254 nm, with an exposure time of 10 minutes

294 and at different doses, reached a removal efficiency of 0.00 to 1.77 Ulog in the case of

295 *Cryptosporidium* sp. and 2 to 4 Ulog for *Giardia* sp. (Shin et al., 2001; Neto et al., 2006;

296 Hassaballah et al., 2020). The UV radiation inactivation mechanism at 254 nm for

297 *Cryptosporidium* sp. and *Giardia* sp. results from the formation of pyrimidine dimers in the

298 parasite's DNA (Shin et al., 2001; Zhang et al., 2012; Shin and Linden, 2015). Shin et al.

299 (2001) reported that *Giardia* sp. cannot photo-repair DNA; however, it has been shown

300 through transcriptomic analysis that *Cryptosporidium* sp. has genes that regulate a protective

301 metabolic response against radiation damage (Zhang et al., 2012). This means that, once the

302 damage has begun, it can initiate photo-repair, making the application of UV radiation

303 inefficient. Therefore, it is recommended to quantify the parasite concentrations in the final

304 effluent and perform a feasibility analysis. Another analyzed physical effect is the action of

305 ultrasound irradiation on *Cryptosporidium* sp., in which an inactivation under different

306 conditions had values of 2.96 Ulog and 3.17 Ulog (Olvera et al., 2008; Abeledo-Lameiro et
307 al., 2018). Ultrasound technology generates waves that hit the oocystic wall of
308 *Cryptosporidium* sp. by cavitation, which generates disruption of the wall, causing
309 irreversible inactivation of the oocysts. Olveda et al. (2008) showed that under a frequency
310 of 1 MHz, an exposure time of 4 min, and a sample volume of 100 ml, oocyst inactivation
311 presented a value of 2.46 Ulog.
312 Table 2 shows the effect of membrane filtration in a full-scale treatment plant. It was
313 confirmed that with a pore size of 0.02 μm, a removal values of *Cryptosporidium* sp. and
314 *Giardia* sp., respectively (Fu et al., 2010). The filtration method allows a more precise
315 calculation of parasite removal (due to the pore size of the membrane). However, although
316 *Giardia* sp. is known to have an oval cyst measuring approximately 8 to 18 μm and
317 *Cryptosporidium* sp. has a spherical cyst measuring 4 to 6 μm, the *Cryptosporidium* sp. cyst
318 can overfold, reducing its size and allowing it to pass through some membranes (Jenkins et
319 al., 2010).
320 The physical treatments included in Table 2 remove minimum values of 0.88 Ulog and 2.40
321 Ulog and maximum values of 3.06 and 3.00 Ulog, respectively, for *Cryptosporidium* sp. and
322 *Giardia* sp., respectively.
323 Table 2 also shows the effect of technologies based on chemical agents such as chlorine,
324 sodium hypochlorite, and peracetic acid to eliminate or inactivate *Cryptosporidium* sp.
325 oocysts (Dungeni and Momba, 2010; Hassaballah et al., 2020). However, these technologies
326 act on parasites and their oocysts in a nonspecific way. It is important to note that the oocyst
327 is resistant to disinfectant agents due to its molecular composition. In the case of *Giardia* sp.,
328 there was only removal by chlorination, with a removal value of 2.35 Ulog (Dungeni and
329 Momba et al., 2010).

In addition, Table 2 shows combined treatments that remove (oo)cysts through physicochemical methods using, for example, filtration and a disinfectant agent (Dungeni and Momba, 2010; Taran-Benshoshan et al., 2015; Nasser et al., 2017), or a disinfectant agent and UV radiation (Clancy et al., 2004; Hassaballah et al., 2020). The use of coagulating agents such as aluminum sulfate ($Al_2(SO_4)_3$), ultrafiltration, and UV radiation is also described (De Lima Isaac et al., 2014).

The combined treatments that appear in Table 2 remove (oo)cysts through physicochemical methods such as ultrafiltration with a chemical agent (Dungeni and Momba, 2010; Taran-Benshoshan et al., 2015; Nasser et al., 2017), or a chemical agent and UV radiation (Clancy et al., 2004; Hassaballah et al., 2020). The use of coagulating agents such as $Al_2(SO_4)_3$ is also described, along with ultrafiltration and the use of UV radiation (De Lima Isaac et al., 2014). Finally, it is observed that when combining two table technologies such as physical ultrafiltration and UV radiation, *Cryptosporidium* sp. removal reaches values ranging from 0.00 Ulog to 1.50 Ulog. Meanwhile, *Giardia* sp. removal presents a minimum value of 0.62 Ulog and a maximum of 3.60 Ulog (Nasser et al., 2017). The combination providing the greatest *Cryptosporidium* sp. removal was ultrafiltration technology with chlorine disinfection (Dungeni and Momba, 2010). The combination with the highest *Giardia* sp. removal efficiency was ultrafiltration technology with UV radiation or chlorine (Nasser et al., 2017). Despite these results, it is important to consider that the final effluent concentrations of *Cryptosporidium* sp. present infectious doses of approximately $1x10^3$ oocysts L^{-1}. The same occurs in the case of *Giardia* sp.; the removal described is high, but the effluent is left with concentrations of approximately $1x10^3$ cysts L^{-1}. Both doses are sufficient to cause human infections.

Consequently, with respect to the obtained results, it is worth mentioning that the technologies based on physical treatments have the greatest removal efficiencies for *Cryptosporidium* sp. and *Giardia* sp. Those with the lowest efficiencies are chemical treatments, for which values of 0.11 Ulog have been calculated for *Cryptosporidium* sp. In the case of *Giardia* sp., their removal efficiency of the chemical treatments is not possible to determine due to the lack of data.

Moreover, Nasser (2016) evaluated the *Cryptosporidium* sp. removal efficiencies of tertiary technologies, indicating that the highest efficiency was achieved by UV radiation and ultrafiltration technologies. Meanwhile that filtration and chlorination are inefficient for removing oocysts from effluent. According to all the analyses presented in this article, it can be concluded that each technology presents differences in the removal of these parasites, with some achieving complete removal and others having no effects. It should be considered that the determination of *Cryptosporidium* sp. and *Giardia* sp. contained in the influent being treated may be underestimated due to the complexity of the wastewater matrix.

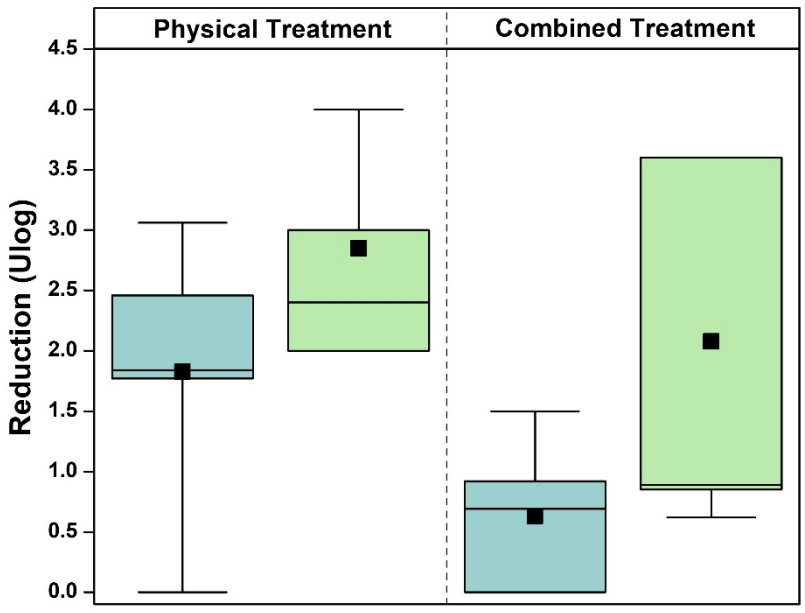


Fig 3 Removal efficiencies of *Cryptosporidium* sp. (¢) and *Giardia* sp. (¢) presented by tertiary sewage treatment technologies. (Chemical disinfection results were not included).

4. Detection, identification, and quantification of *Cryptosporidium sp.* oocysts and *Giardia sp.* cysts in sewage

The detection, identification, and quantification of *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts have been a challenge due to their morphology and size and because both protozoa parasitize one or a wide variety of hosts (Adeyemo et al., 2018, Praus, 2019, Costa et al., 2021). Wastewater samples are no exception; in several works the difficulty of identifying the species and quantifying the actual concentrations of these protozoa, especially *Cryptosporidium* sp., has been stressed (Ramo et al., 2017; Yamashiro et al., 2019). Thus, there is a percentage of samples in which only the genus of the protozoan is identified and concentrations of *Cryptosporidium* sp. oocysts are underestimated. The importance of an adequate identification of the species lies in the fact that the presence of species or subspecies of (oo)cysts that parasitize only animals in the treated wastewater would not affect the human population and, while species that parasitize humans would pose a health risk (Amorós et al., 2016). Meanwhile, (oo)cyst concentrations could be higher than those reported in most studies, also posing a risk (Andreoli and Sabogal-Paz, 2017). Therefore, the detection, identification, and quantification technique used is important in wastewater monitoring studies and in environmental matrices (Robertson et al., 2006). Figure 4 shows a classification of techniques used for the detection of protozoa. These have been mainly used in the field of clinical diagnosis, but in some cases are applicable to the study of environmental matrices such as wastewater (Adeyemo et al., 2018). There are three main

399 classifications: microscopy staining, immunodetection or enzyme activity, and DNA
400 detection. Microscopic observation is based on optical microscope observations with or
401 without dyes, in which a protozoan genus is morphologically identified (O'Leary et al., 2021).
402 The technique is based on the the dye penetrating the envelope and homogeneously or
403 differentially staining certain structures, allowing identification of the genus of the parasite.
404 The techniques used in optical microscopy are cheap and frecuently implemented in the
405 laboratory (O´leary et al., 2021). However, the sensitivity of these techniques is usually lower
406 compared to molecular techniques, with detection limits of 10^4 to 10^5 (oo) cysts L^{-1} in the
407 samples. Another difficulty is that morphological recognition depends on the observer;
408 therefore, the person in charge of the observation must be trained (O'leary et al., 2021). In
409 addition, these techniques only indicate the presence or absence of the protozoa, but not their
410 viability or the identity of the species. Therefore, in certain studies are usually used for
411 sample screening; however, in the clinical area they are the most used for diagnosis (Jerez et
412 al., 2020).

413 Meanwhile, immunodetection or enzymatic activity techniques are based on the detection of
414 antigens that make up the envelope of the (oo) cyst with antibodies directed at molecules
415 (Christy et al., 2012; Adeyemo et al., 2018). This detection can be specific or non-specific,
416 depending on the form of antibody production (mono- or polyclonal). The detection of
417 *Cryptosporidium* sp. and *Giardia* sp. in sewage samples it is carried out mainly by
418 immunofluorescence techniques, which use antibodies linked to fluorophores that, when
419 excited by light, produce fluorescence. This technique has a detection limit of 10^3 (oo) cysts
420 L^{-1}, with higher specificity than light microscopy (98-100%) but can generate cross-reaction
421 between species (Ahmed and Karanis, 2018).

Another aspect to consider is that in wastewater studies, immunomagnetic separation is often used to purify (oo)cysts. Although not a detection technique, the use of antibodies specifically helps with the purification from the (oo)cyst sample. However, during the process there is a percentage between 65.5 and 90% of oocysts of *Cryptosporidium* sp. and 5.0 and 80% of *Giardia* sp. that is not detected. Thus, this technique detects lower values than the actual number (Kitajima et al., 2014; Taran-Benshoshan et al., 2015; Ribeiro dos Santos and Daniel, 2017; Andreoli and Sabogal-Paz, 2019; Medeiros et al., 2019; Yamashiro et al., 2019).

DNA detection techniques are more sensitive, specific, and faster in obtaining results than those previously mentioned (Adeyemo et al., 2018; Ahmed and Karanis, 2018; O'Leary et al., 2021). However, achieving validation of a molecular technique is cumbersome, especially for protozoan parasites. From the sample pretreatment stage to the analysis of the parasite, the stages must be fully validated. In addition, more expensive equipment and qualified personnel are needed; therefore, this clinical diagnosis approach has not achieved widespread implementation (Ahmed and Karanis et al., 2018). The genetic analysis of parasitic protozoa has also presented problems, both in the preservation and extraction of DNA and the search for genes that allow the identification of species or subspecies (Lalonde and Gajadhar, 2009; Wilke and Robertson, 2009; Abdelsalam et al., 2016; Costa et al., 2021; Köster et al., 2021).

Currently, the molecular analysis of environmental samples is leading to the analysis of the microbial community in the sample, with these parasites included in the study (Moreno et al., 2018; Zahedi et al., 2019). The advancement in metagenomic techniques has provided novel tools for profiling human parasites in environmental matrices, such as water and wastewater (Mthethwa et al., 2021). Two main approaches have been employed for

445 metagenomic profiling: (1) amplicon-based sequencing, which involves Polymerase Chain
446 Reaction (PCR) amplification of a target gene marker before NGS sequencing (Moreno et
447 al., 2018; Maloney et al., 2021), and (2) the shotgun metagenomic approach, which involves
448 sequencing of total nucleic acid present in a sample by first shearing the DNA sequences into
449 short fragments (Quince et al., 2017). Advances in NGS technologies such as sequencing
450 longer reads (> 20 Kbp) (for example, Pacific Biosciences's SMRT 3rd generation NGS)
451 (Vernet, 2017), simplified DNA sample preparation process, PCR-free library preparation,
452 and fewer PCR-induced errors, have allowed the shortcomings of conventional NGS
453 platforms to be overcome (Quail et al., 2012; Raza and Ahmad, 2019). However, work must
454 be done to standardize the preservation, processing, and storage of the sample for comparison
455 studies and to determine the appropriate metagenomic approach (Mthethwa et al., 2021).
456 Finally, the techniques used for wastewater analysis are important for public health, since it
457 is important to determine the species and the concentration of (oo)cysts in a sample. It should
458 also be mentioned that measure the viability of the parasites is a relevant parameter when
459 detecting them and must be reported.
460

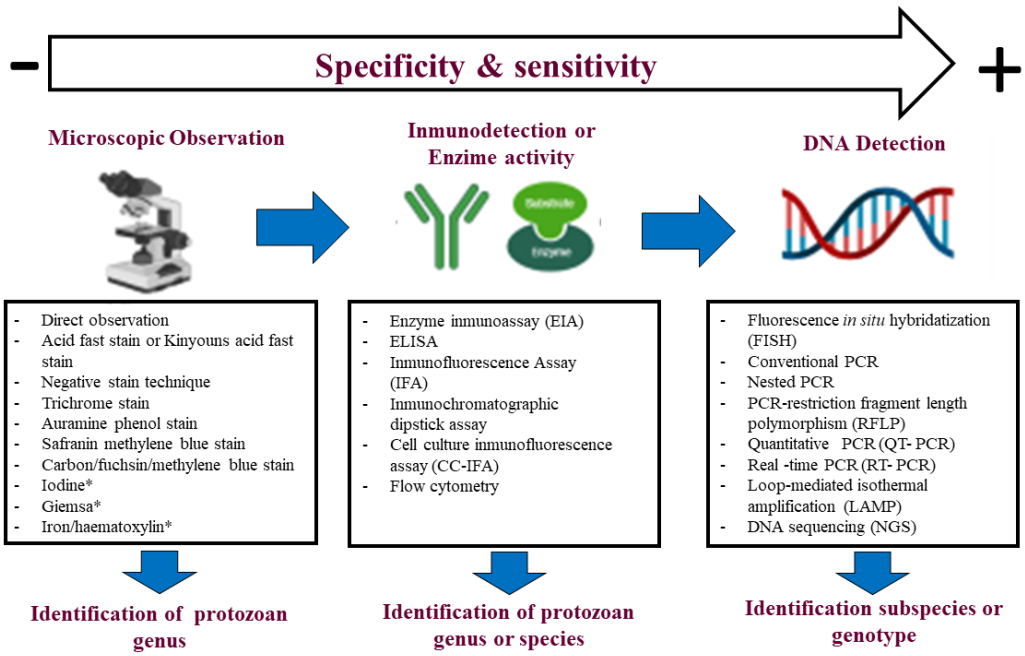


Fig 4. *Cryptosporidium* sp. oocyst and *Giardia* sp. cyst identification techniques in environmental and clinical matrices

5. On *Cryptosporidium* sp. and *Giardia* sp: what has been studied and future prospects

5.1. Bibliometric study

Figure 5 shows the network using the publications of the Web of Science platform in which the concepts searched were: "Wastewater" or "Sewage," "*Cryptosporidium*" or "*Giardia,*" or "Disinfection," "Sludge," and "Biosolid" in investigations between 1975 - 2022 (CVTS, 2019). A total of 11,252 articles was found, of which only those related to sewage were kept, filtering out those with words related to the clinical study of the infection and animals. As shown in Figure 5, 6 clusters with 275 items were formed. Cluster 1 is red; it contains concepts such as *Cryptosporidium* pathogens, sewage, quantitative microbial risk, outbreak, and irrigation, among others. This cluster connects co-occurrences that involve *Cryptosporidium* as a pathogen in water considering used for irrigation. Cluster 2 is green and has 71 items, where *Giardia* is the most important concept. Here the co-occurrences are related to genome and genetic expression, and some *Giardia* species appear.

The concept of resistance is also present. Cluster 3, in blue, contains 66 items, in which the concepts of taxonomy, genotype, PCR, assemblage b, subtyping, basic molecular biology, and genetic identification are observed. The yellow cluster contains 20 items and connects concepts such as antigens, disease, and association, among others. The purple cluster has only one item, wetland, as does the light blue cluster, which contains the word "Milwaukee," which is the city where the first massive outbreak of *Cryptosporidium* is described, a result of the contamination of drinking water with sewage. Scientific studies have been focused on detecting both parasites in surface water, attaching importance to taxonomy, and thus defining whether the contamination is human or animal. It should also be noted that in the *Cryptosporidium* cluster several concepts are related to efficiency, removal, and outbreaks, as this parasite is of great concern not only because it affects people's health, but also because the economic losses that result from hospitalizations, treatment, and work absences, among other factors, are considerable. The great Milwaukee (Wisconsin, U.S.A) outbreak of 1993, which affected 403,000 residents, has been estimated to cost 96.2 million dollars, including 31.7 million dollars for hospitalizations and 64.6 million dollars in lost labor productivity (Corso et al., 2003). This experience shows the relevance of the technologies performance and their operation for removing pathogenic microorganisms, in order to monitor the discharges of the treated wastewater to surface aquatic ecosystems for avoiding massive infectious outbreaks (Chamorro et al., 2013).

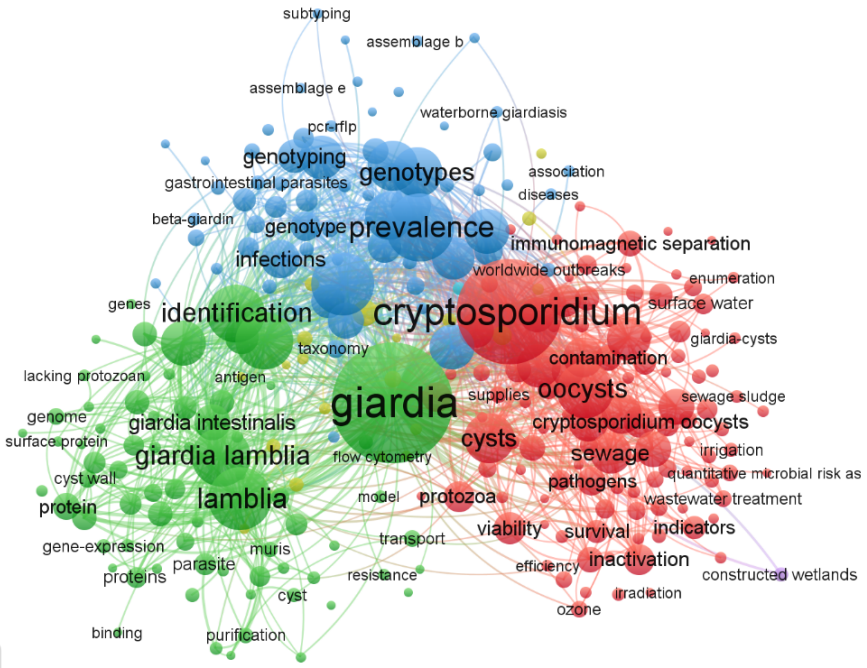


Fig. 5. Representation of the bibliographic network of co-occurrence (Vosviewer 1.6.17, 2021). The words used were "Wastewater," "Sewage," "*Cryptosporidium,*" "*Giardia,*" "Sludge," and "biosolid." The included studies were from 1975 to 2022, and a total of 246 words was indicated.

5.2. Reuse possibilities and health risk

Faced with the challenges of water scarcity, different strategies have been proposed, among which is the reuse of treated sewage (Jiménez-Cisneros, 2014 a; Jiménez Cisneros, 2014b; Vera et al., 2016). Stabilized biosolids from sewage treatment plants can also be a source of nutrients for agriculture and soil amendments (Neumann et al., 2018, Cartes et al., 2018). However, the removal and inactivation processes in plants are not yet reliable and, therefore, their reuse could represent a health problem depending on their final purpose (agricultural, environmental, industrial, recreational, or urban).

In this sense, Benito et al. (2020) present a study of parasites and cysts in effluents and sludge from five sewage treatment plants. They found at least one of the studied pathogenic parasites in four out of five effluents from the evaluated treatment plants. Similar studies carried out by Razzolini et al. (2020) show how the presence of *Cryptosporidium* sp. and *Giardia* sp. can be minimized through biological technologies (UASB + activated sludge) followed by disinfection by chlorination and later a final filtration. The effluents from the process contain concentrations below the detection limit (<0.03 (oo) cysts L^{-1}). However, these results could not be maintained over time, as there were periods where the sampling detected concentrations from 2 to 25.8 (oo) cysts L^{-1} (Razzolini et al., 2020). In addition, Ryu et al. (2021) analyzed an effluent stored after tertiary treatment by UV radiation and chlorination and found *Cryptosporidium* sp. concentrations of 0.34, 0.23, and 0.17 oocysts L^{-1}. However, in the same study, *Giardia* sp. presented values of 10, 1.45 and 4.74 cysts L^{-1}, which seem low, but the infectious dose for humans is 10 to 100 cysts (Bouzid et al., 2013).

When thinking about discharges of treated effluents to surface ecosystems, it is important to keep in mind that infections in people are usually linked to certain seasons of the year; thus, it is necessary to study the discharge area and/or the area of water reuse. King et al. (2017a) recommend seasonal and integrated monitoring together with notification outbreak data to improve monitoring with a view to direct reuse. With the results of such efforts, a more robust and accurate database can be built to manage the risk of contamination due to reuse of treated sewage (King et al., 2017b).

6. Conclusions

The performance of wastewater treatment technologies for the removal of *Cryptosporidium* sp. and *Giardia* sp. shows that secondary treatment can eliminate parasites. It is concluded

534 that the technology with the highest removal of *Cryptosporidium* sp. oocysts is activated
535 sludge, while for *Giardia* sp. cysts the secondary technologies with the highest removal were
536 anaerobic technologies that used UASB reactors. The lowest removal efficiencies were
537 presented by combined technologies (aerobic and anaerobic treatments). In all the studied
538 cases, the concentrations of *Cryptosporidium* sp. in the effluents generated were still a risk
539 for the population.
540 Regarding the technologies used for the disinfection of wastewater, those with the highest
541 removal of *Cryptosporidium* sp. were physical technologies, specifically UV radiation and
542 ultrasound (not full-scale operation). In the case of *Giardia* sp. the tertiary technologies with
543 the highest removal of cysts were those that combined filtration with UV radiation or a
544 chemical agent. In both cases, the use of disinfectant agents such as chlorine as the only
545 disinfection technology leaves (oo) cysts in doses that pose a risk to the population.
546 Among the future perspectives raised, the circularity of treated wastewater is proposed due
547 to water scarcity in some parts of the world. It is necessary to advance in monitoring programs
548 before and after considering the reuse of treated waters, especially in devoloping countries,
549 allowing the most appropriate technologies to be chosen to ensure that treated wastewater
550 circularity is not a public health problem.
551

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555 ***Authors contributions***

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557 Review and Editing. **Alonso, J.L**: Validation, Conceptualization, Review and Editing.

Gomez, G.: Layout and graphics designe, image editing. **Vidal, G**.: Conceptualization, Validation, Investigation, Resources, Original Draft, Writing, Review and Editing, Visualization, Supervision, Project administration

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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908 Tables:

909 **Table 1:** Efficiency of *Cryptosporidium* sp. and *Giardia* sp. removal by secondary

910 treatment

911 **Table 2:** Effiency of *Cryptosporidium* sp. and *Giardia* sp. removal by tertiary treatment

912

Technology	Flow ($m^3 day^{-1}$)	Hydraulic Retention Time (days)	*Cryptosporidium* sp. oocyst			*Giardia* sp. cyst			Authors
			Influent concentration (oocyst/L)	Effluent concentration (oocyst/L)	Log removal (Ulog)	Influent concentration (cyst/L)	Effluent concentration (cyst/L)	Log removal (Ulog)	
Aerobic Secondary Treatment									
Activated Sludge	$3.5x10^1$ - $2.0x10^9$	0.25 – 21	$<1.0x10^1$* - $6.0x10^4$	$<1.0x10^0$ – $1.2x10^2$	0 – 2.57	$7.0x10^0$ - $1.0x10^5$	0.4 $x10^0$ - $1.0x10^3$	0.54 - 2.85	[2], [3], [5], [7], [10], [10],[11],[12],[15], [16],[17],[18].
Biological Trickling Filter	$9.4x10^4$ – $2.2x10^9$	NI	$1.0x10^0$ - $1.0x10^2$	$2.0x10^0$ -$1.2x10^1$	0.00 - >0.96	0.00 - $6.4x10^3$	$6.0x10^0$ - $1.9x10^2$	-0.77- >1.52	[2],[3],[7],[15]
Aerated Lagoon	NI	1 – 25	$8.0x10^2$	$8.0x10^2$	0.00	$8.4x10^3$	$1.4x10^3$	0.77	[9]
Oxidation Ditch	$1.5x10^3$ -$3x10^5$	10 – 15	$1.4x10^1$- $5.9x10^2$	$1.0x10^0$-$1.2x10^2$	0.69 - 2.17	$3.2x10^2$ - $3.1x10^3$	$1.4x10^0$ - $5.6x10^1$	0.75- 2.93	[3],[6],[17]
Anaerobic Secondary Treatment									
UASB Reactor	0.18- $2.9x10^2$	0.25– 0.55	$1.0x10^1$ - $1.35x10^2$	$1.1x10^1$- $3.2x10^1$	0 - 1.13	$8.0x10^2$- $1.89x10^4$	$6.0x10^2$ - $4.0x10^3$	0.00 - 1.57	[8],[14],[19]
UASB Reactor + DAF[1,2,3]	LD - $7.9x10^9$	0.25 – 0.3	$3.3x10^1$- $1.35x10^2$	0 - $5.9x10^0$	1.35- 1.50	$4.3x10^3$ - $4.3x10^4$	$1.5x10^0$– $2.3x10^2$	0.47– 3.20	[8], [14]
CF+DAF[4,5]	LD	LD	$6.4x10^2$- $1.3x10^3$	$5.7x10^1$-$7.5x10^1$	0.82-1.42	$4.5x10^2$- $1.4x10^3$	$2.4x10^1$- $2.5x10^1$	1.44 - 1.78	[1]
DAF+ BARDENPHO[6]	$1.2x10^5$ - $1.8x10^5$	NI	$2.4x10^2$- $3.0x10^2$	$4.0x10^0$ -$8.0x10^0$	>1.52 - >1.67	$1.8x10^3$ - $2.4x10^3$	$8.0x10^0$ - $8.8x10^0$	>2.44 - >2.63	[15]
SBBGR Reactor	LD	0.9	$3.1x10^2$	$1.3x10^0$	2.00	$6.2x10^4$	$6.2x10^0$	3.80	[4]
Aerobic and Anaerobic Secondary Treatment									
Facultative Pond	20.04	75.98	$4.5x10^1$	$3.4x10^1$	0.12	$2.8x10^2$	$2.4x10^2$	0.06	[13]
AA filter + aerated biofilter	0.59	0.5 (1) 0.0 (2) 0.35 (3) 0.45 (4)	$4.1x10^1$	$2.5x10^0$	1.2*	$1.0x10^3$	$1.8x10^1$	1.1	[20]

UASB + Activated sludge	37.6	02. [5] 7-21 [6]	$3.1x10^2$	$3.4x10^0$	1.95	$1.5x10^4$	$1.3x10^3$	1.24	[10]

DAF1: $FeCl_3$ 100 mg L^{-1}; DAF2: PACl 75 mg L^{-1}; DAF3: PACl 200 mg L^{-1}; DAF4: PACl 25mg L^{-1}; DAF5: PACl 25mg L^{-1}; Filtration: sand grain 0.42 mm; CF: Coagulation – Flotation; AA: Anaerobic Anoxic; (*) copies L^{-1} (ª): hours; (1): Anaerobic; (2): Anoxic; (3): Aeriation; (4): decatation; (5) time in UASB reactor; (6): time in activated sludges. [1]: Andreoli and Sabogal-Paz, 2017; [2]: Berglund et al., 2017;[3]: Cheng et al., 2009;[4]: De Sanctis et al., 2016;[5]: Dungeni and Momba et al., 2010;[6]: Fu et al., 2010;[7]: Kitajima et al., 2014;[8]: Laila de Olivera et al., 2021;[9]: Lim et al., 2007;[10]: Medeiros et al., 2019;[11]: Neto et al., 2006;[12]: Ramo et al., 2017;[13]: Reinoso et al., 2008;[14]: Ribeiro dos Santos and Daniel, 2017;[15]: Shmitz et al., 2018;[16]: Taran-Benshoshan et al., 2015;[17]: Teel et al., 2021;[18]: Tonani et al., 2013;[19]: Valdez et al., 2021;[20]: Yamashiro et al., 2019

Tabla 1

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Physical Disinfection Treatment										
	Treatment Conditions			*Cryptosporidium* sp. oocyst			*Giardia* sp. cyst			
Type of treatment	**Dose ($mJcm^2$)**	**Hydraulic retention time (s)**	**Exposure time(min)**	**Influent Concentration (oocyst/ml)**	**Effluent Concentration (oocyst/ml)**	**Log Removal (Ulog)**	**Influent Concentration (cyst/ml)**	**Effluent Concentration (cyst/ml)**	**Log Removal (Ulog)**	**Authors**
Irradiation (UV [(1)])	14.7 - 30	7 - 48	10	$1.5x10^3$ - $6.0x10^4$	0 – $1.6x10^3$	0.00 - 1.77	$1.0x10^5$ - $1x10^6$	$1x10^2$ - $1.0x10^3$	2 - 4	[6],[10], [11]
Irradiation (Ultrasound)	**Dose (W)**	**Pulse Power (%)**	**Exposition time(min)**							
	60 - 80*	50	4-10	$3.3x10^2$ - $1.5x10^3$	0 – $8.8x10^{-1}$	2.46 – 3.17	(-)	(-)	(-)	[1],[9]
Filtration	**Matrix**	**Size pore (µm)**	**Flow**							
	Membrane	0.02 – 0.1	NI	0 – $1.4x10^2$	0	1.84	$5.50x10^0$ - $9.1x10^2$	0	0.47 - 2.40	[5][7]
	Treatment Conditions			**Chemical Disinfection Treatment**						
Disinfectant	**Disinfectant agent**	**concentration (mgL^{-1})**	**Exposition time(min)**							
	Peracetic Acid	2	10 - 30	$1.4x10^3$ - $1.7x10^3$	$1.2x10^3$ – $2.0x10^3$	-0.03 - 0	(-)	(-)	(-)	[6]
	NaOCl	2	10 - 30	$1.7x10^3$ -$1.4x10^3$	$1.6x10^3$-$1.9x10^3$	0	(-)	(-)	(-)	
	Chlorine	1.5– 2.0	Prolong	$2.6x10^1$	$7.0x10^0$	0.56	$1.8x10^3$	$8.0x10^0$	2.35	[4]
	Treatment Conditions			**Physicochemical Disinfection Treatment**						
		Operational parameters	**Exposition time(min)**							
Filtration/ Disinfectant	Sand	NI	NI	$1.7x10^1$ - $3.7x10^1$	$9.9x10^0$ - $1.0x10^0$	0.47 - 1.5	$8.1x10^2$ - $3.6x10^4$	0 - $0.9x10^0$	0.62 - 3.60	[4],[7],[12]
	Chlorine	1.5– 2.0								
Irradiation/ Disinfectant	UV[(1)]	3		$2.0x10^0$ – $2.4x10^1$	0	0.3 – 1.38	(-)	(-)	(-)	[2]
	Chlorine	-	30							
	$Al_2(SO_4)_3$	2.5- 15.0 [(4)]	NI	$5.0x10^1$	0	0.69	$5.0x10^2$	$3.7x10^1$	0.48	[3]

Coagulation/ Filtration/ Irradiation	Sand [2]	4.0-7.5 [5] 300 [3]					– $8.0x10^2$	– $2.6x10^2$	– 1.13	
	UV[1]	95/ 9[6]								
Disinfectant/ Irradiation	Peracetic Acid	2	10- 30	$1.3x10^3$ – $2.4x10^3$	$1.0x10^3$ – $1.5x10^3$	0	(-)	(-)	(-)	[6]
	UV [1]	14.7								
CF/ Filtration	NI	NI	NI	$2.4x10^2$	0	0.92	$1.6x10^3$	0	0.89	[5]
Filtration/ Irradiation	Sand	NI	NI	(-)	(-)	(-)	$3.6x10^4$	0	3.6	[8]
	UV [1]	63								

(-): No determinated. [1]: 254 nm; [2]: grain size 4.2mm; [3]: m^3 $m^{-2}d$; [4]: mg L^{-1}; [5] pH; [6]: seconds. Authors:[1]: Abeledo-Lameiro et al., 2018;[2]: Clancy et al., 2004;[3]: De Lima Isaac et al., 2014;[4]: Dungeni and Momba et al., 2010;[5]: Fu et al., 2010;[6]: Hassaballah et al., 2020;[7]: Lonigro et al., 2006; [8] Nasser et al., 2017;[9]: Neto et al., 2006;[10]: Olvera et al., 2008;[11]: Shi et al., 2002;[12]: Taran-Benshoshan et al., 2015.

Table

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