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BIOREMEDIATION AS A PROMISING STRATEGY FOR MICROPLASTICS REMOVAL IN WASTEWATER TREATMENT PLANTS

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Microplastics (MPs) attract ever-increasing attention due to environmental concerns. Nowadays, they are ubiquitous across ecosystems, and research demonstrates that the origin is mainly terrestrial. Wastewater treatment plants (WWTPs) are a major source of MPs, especially fibres, in water masses. This review is focused on understanding the evolution and fate of microplastics during wastewater treatment processes with the aim of identifying advanced technologies to eliminate microplastics from the water stream. Amongst them, bioremediation has been highlighted as a promising tool, but confinement of microorganisms inside the WWTP is still a challenge. The potential for MPs bioremediation in WWTPs of higher aquatic eukaryotes, which offer the advantages of low dispersion rates and being easy to contain, is reviewed. Animals, seagrasses and macrophytes are considered, taking into account ecoethical and biological issues. Necessary research and its challenges have been identified.

Keywords: bioremediation, eukaryotes, microplastics, sludge, technologies, wastewater

Acronyms: A^2O: Anaerobic, anoxic, aerobic; CAS: Conventional activated sludge; DAF: Dissolved air flotation; DM: Dynamic membrane; HRT: Hydraulic retention time; MBR: Membrane bioreactor; MPs: Microplastics; PAH: Polycyclic aromatic hydrocarbon; PCB: Polychlorinated biphenyl; PES: Polyether sulfone; RO: Reverse osmosis; RSF: Rapid sand filtration; SBR: Sequencing batch reactor; UV: Ultraviolet irradiation; WWTP: Wastewater treatment plant.

Highlights

- WWTPs are main sources of marine MP pollution
- Efficient technologies to avoid MPs presence in sludges are still a challenge
- Higher eukaryotes are explored for MP bioremediation in WWTP
- Animal welfare is a concern for MP bioremediation, with few exceptions
- Seagrasses and aquatic macrophytes seem optimal candidates for MP bioremediation

1. Fate and evolution of microplastics in WWTPs

The aim of this review being to explore new ecologically acceptable ways to prevent MPs pollution by WWTPs, we will examine first the points where MPs can escape from WWTPs, the treatments currently employed for the capture and elimination of MPs, and then focus on bioremediation and specifically on higher eukaryotes as species that can be more easily contained inside WWTPs than unicellular organisms.

1.1. Legislation on microplastics

It has been estimated that 245 tonnes of MPs, whose final destination is the aquatic environment, are generated every year. There is a lack of international regulation governing the production of microplastics, especially in the field of personal care and cosmetic products (Auta et al., 2017), but some countries such as Canada, Ireland, the United Kingdom and the USA are banning microbead production and products that contain them (Prata 2018a). In the case of the USA, "The Microbead-Free Waters Act of 2015" is the legislation that bans the addition of plastic microbeads to products. This law came into force for manufacturers in July 2017 and for retail sales in July 2018 (H. Rept. 114-371, 2015). Following the same line, in 2019, the European Chemicals Agency (ECHA) submitted a proposal to forbid the intentional addition of microplastics to different products. It has been estimated that the enforcement of this proposal would reduce the emission of these microcontaminants to the environment in the European Union by 85-95%, which means it would avoid the emission of approximately 400 thousand tonnes over 20 years. The legislation is expected to be ready by June 2020 and it would be subsequently sent to the European Commission for evaluation (ECHA, 2019). In addition, also in 2019, the European Parliament submitted a proposal (TA/2019/0071) to tackle microplastic pollution in wastewater treatment and those issues derived from sludge use as a fertiliser in agriculture (European Parliament, 2019). Proposing legislation on WWTPs is a good starting point for the implementation of measures that minimize dispersion of MPs in the environment.

1.2. Main sources of microplastics

Water is the main means by which MPs are transported (Alimi et al., 2018), so WWTPs receive millions of microplastic fragments every day (Okoffo et al., 2019). There is no linear correlation between population density and MPs concentrations in the inlet stream of WWTPs, but agricultural and industrial activities seem to be strong determinants (Eerkes-Medrano et al., 2015; Long et al., 2019). Recently, Bayo et al. (2020) demonstrated that another important factor is seasonal variability, the highest concentrations being found during hot periods, since temperature contributes to the acceleration of plastic degradation and fragmentation. Additionally, high concentrations of microplastics were also observed after rainfall events, due to urban runoff.

1.3. Evolution of microplastic at each stage of WWTP

The few studies that have analysed the evolution of microplastics in WWTPs report that, although these facilities do not completely remove these pollutants from wastewater, in some cases removal efficiency values higher than 90% are achieved (Bayo et al., 2020; Blair et al., 2020; Edo et al., 2020). MPs are defined as particles between 5 mm and 0.1 µm (Picó et al., 2019), and although investigation has been carried out, completion of the task of establishing sampling, extraction and quantification protocols for microplastics smaller than 20 µm is still a huge challenge (Poerio et al., 2019).

The MPs classified as fibres and fragments are the most frequently observed types, making up, respectively, 57 and 34% of the total. In addition, fibres are the most difficult MPs to remove in WWTPs, due to their morphological characteristics (Ngo et al., 2019).

Microplastics removal efficiency depends on treatment, operating conditions, sludge characteristics and microplastic buoyancy (Lusher et al., 2018; Nemerow, 2006). To understand MPs behaviour and fate during wastewater treatment processes, it is necessary to study in depth each stage of waste treatment in the processing plant. A scheme for a conventional WWTP is shown in Figure 1.

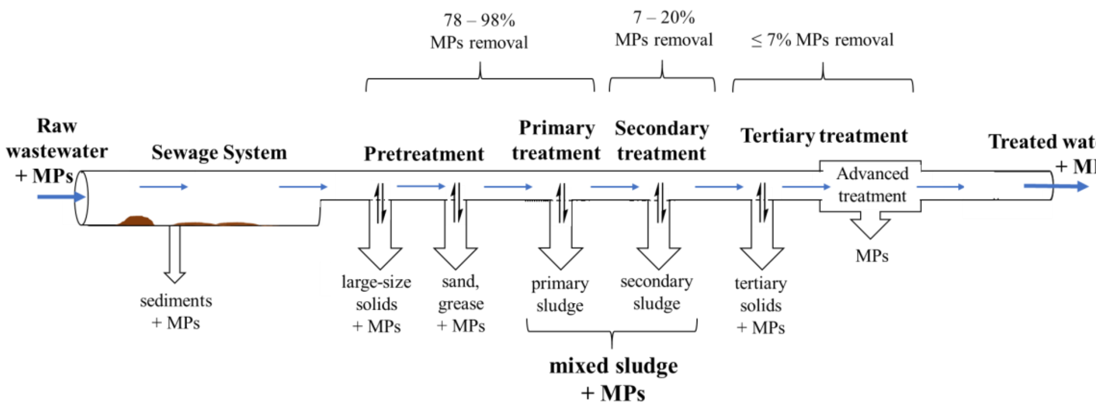

Figure 1. A schematic representation of WWTP processes and percentages of MPs removal during processing.

High variability of influent flow and composition makes it more difficult to obtain representative samples. This leads to underestimation of MPs concentrations because they can be retained in the sewage system by sedimentation (Lepot et al., 2017).

Bigger microplastic particles in WWTP influent are removed by screening systems (screens, meshes) (Zhang et al., 2020). After that, the process for grit and grease removal takes place by means of sand sinking and grease floating, and during this step MPs are also separated from the wastewater stream by sinking and floating. It is noticeable that Murphy (2016) reported that the highest MPs elimination efficiencies are achieved in the grit and grease removal systems. Lusher et al. (2018) indicated that 62% of microplastics extracted during WWTPs processes probably come from this specific separation phase.

Primary clarifier is used for removing settleable solids from wastewater, so during this sedimentation process, based on a pseudo-equilibrium approach (solid-liquid), some microplastics settle and others remain suspended in wastewater. The structure of suspended solids and their concentration have an important effect in solid-liquid separation (Sheng et al., 2008). A circular tank is the most frequently employed design, in which water enters centrally and radial flow towards the periphery allows sedimentation.

Sedimentation efficiency depends on different factors, such as retention time, temperature, type of flow and speed, system design, size and particle densities, etc. (Nemerow, 2006). For example, higher retention times increase the amount of settled solids (so it is also expected that the amount of settled MPs increases); high temperatures decrease the density of the medium, which also favours sedimentation.

Secondary treatment is a biological process that allows the biodegradation of organic matter. It is usually carried out under aerobic conditions, so it is necessary to supply oxygen to the microorganisms by wastewater aeration. During this aeration process, which is also a pseudo equilibrium (solid-liquid-gas), some microplastics could pass to the atmosphere since it is well known that MPs can be found in air (Chen et al., 2020; Enyoh et al., 2019; Prata 2018b).

The conventional activated sludge process (CAS), which basically involves the biological oxidation of carbonaceous organic matter, and subsequent separation of treated water from solid particles through sedimentation in a secondary clarifier, are commonly employed to treat municipal wastewaters. It has been reported that secondary treatment can reduce the MPs concentration in water by between 96% and 98% (Lares et al., 2018; Michielssen et al., 2016).

Tertiary treatment is the final cleaning step that improves wastewater quality before it is reused, recycled or discharged to the environment. It usually consists of a disinfection process to kill or inactivate pathogenic organisms, and chlorination and UV irradiation are the most common processes (Zhuang et al., 2015). In general, tertiary treatment has no effect on MPs removal (Prata 2018a; Sun et al., 2019), but in some cases microplastics can be degraded by this treatment, i.e., it has been described that chlorination can reduce MPs concentration by 7% (Liu et al., 2019).

Microplastics that remain in treated water are discharged into the environment in rivers or oceans (Galafassi et al., 2019; Uurasjärvi et al., 2020; Wang et al., 2019; Waring et al., 2018) and different studies have estimated that globally WWTPs discharge millions of microplastics particles every day. For example, Edo et al. (2020) reported the release of 300 million into the Henares river (Madrid) per day, while three WWTPs located in South Carolina discharged between 500 and 1000 million per day into Charleston Harbor estuary (Conley et al., 2019), despite the fact that the MPs removal efficiencies of the plants were 93% and 85-98%, respectively. In addition, it has been found that in rivers, MPs concentration is higher downstream of WWTPs than upstream (Li et al., 2020; McCormick et al., 2014; Shruti et al., 2019; Vermaire et al., 2017).

Most microplastics removed in WWTPs are accumulated in the sewage sludge produced at each stage of the treatment process, especially in the primary and secondary clarifiers (Prata 2018a; Sun et al., 2019). This sludge is widely applied to soils, so it can be an important source of pollutants, including microplastics (Barbosa Jr. et al., 2020; Gherghel et al., 2019; Lassen et al., 2015). For example, 50% of annual sludge wastes generated in Europe and North America are employed as agricultural fertilizer and it has been estimated that these wastes contain a total amount of MPs of between 44000 and 430000 tonnes (Hurley et al., 2018; Lu et al., 2019). Furthermore, stabilization processes such as lime addition or anaerobic digestion do not reduce MPs concentration in sludge (Gatidou et al., 2019; Rolsky et al., 2020).

The effects of microplastics on soils have scarcely been studied, but some studies have indicated their ability to absorb toxic contaminants such as metals, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Caruso et al., 2019; Rodrigues et al., 2019; Xu et al., 2019), which increases the pollutant risks of MPs (Al-Odaini et al., 2015).

Today, technologies to totally avoid the presence of MPs in sewage sludge are unrealistic, but the amount of microplastics in sludge can be reduced by, for example, improving the elimination of microplastics in grit and grease removal systems (Sun et al., 2019). Anaerobic digestion can also be considered as a potential way to reduce MPs in stabilized sludge, but further research on this topic is needed, investigating areas such as bioremediation (Enfrin et al., 2019).

1.4. Advanced wastewater treatment

Although, in general, conventional WWTPs have high MPs removal efficiencies (≥ 90%), it is a fact that a large amount of microplastics is still discharged into the environment. For this reason, the use of advanced treatments, especially during the tertiary phase, could provide an alternative for reducing the concentration of MPs in treated water before it is discharged. A summary of different wastewater treatments that, according to the literature, have been proved to effectively remove microplastics from wastewater is seen in Table 1.

Table 1. A list of different treatment processes used in recent studies that analysed the microplastic removal efficiency of wastewater in WWTPs.

Treatment process	Removal efficiency (%)	Reference
Conventional activated process (CAS)	96-98	Lares et al., 2018; Michielssen et al., 2016
Oxidation ditch	97	Lv et al., 2019
Chlorination disinfection	7	Liu et al., 2019.
Ozone	90	Hidayaturrahman et al., 2019.
Coagulation/Flocculation	47-82	Hidayaturrahman et al., 2019.
Rapid Sand Filtration (RSF)	45-97	Magni et al., 2019; Michielssen et al., 2016; Murphy et al., 2016; Talvitie et al., 2017.
Anaerobic, anoxic, aerobic (A^2O)	72-98	Lee et al., 2018; Yang et al., 2019
Sequencing batch reactor (SBR)	98	Lee et al., 2018
Discfilter	40-98	Hidayaturrahman et al., 2019; Simon et al., 2019; Talvitie et al., 2017
Dissolved Air Flotation (DAF)	95	Talvitie et al., 2017
Reverse Osmosis (RO)	90	Ziajahromi et al., 2017
Dynamic membrane (DM)	99	Li et al., 2018
Membrane Bioreactor (MBR)	≥ 99	Lares et al., 2018; Michielssen et al., 2016; Talvitie et al., 2017.
Ultrafiltration (UF)	42	Ziajahromi et al., 2017

As can be seen in Table 1, dynamic membranes (DM) and membrane bioreactors (MBR) are, so far, the most efficient processes in terms of removing microplastics from wastewater, achieving MPs removal values as high as 99.9% (Li et al., 2018; Talvitie et al., 2017). The main disadvantages of MBR are membrane costs, energy demand, fouling control and low flux. In comparison, dynamic membranes offer lower costs and energy consumption, but, in this case, the filter is easily clogged (Ersahin et al., 2012; Li et al., 2018; Poerio et al., 2019).

MPs removal by means of different organisms such as bacteria, fungi and algae has been recently investigated, and bioremediation is a very interesting challenge (Shahnawaz et al., 2019). Eukaryotic species have received much less attention and their efficiency is unknown, even though they can accumulate MPs.

2. Use of higher Eukaryotes for MPs bioremediation in WWTPs

Microplastics are present in all the oceans and in marine organisms worldwide (e.g. Andrady et al., 2011; Wright et al., 2014; Zhao et al., 2014; Lusher et al., 2015). As we have commented above, WWTPs are major sources of MPs pollution (e.g. Eerkes-Medrano et el., 2015; Murphy et al., 2016; Ziajahromi et al., 2017). Fibres and small fragments escape the filtering processes and are not efficiently retained in WWTPs; consequently, coastal cities are hotspots for MPs entering the ocean (Browne et al., 2011; Murphy et al., 2016). WWTPs discharging to rivers also contribute to ocean pollution because MPs transported by the current finally enter the sea; river mouths are also hotspots for MPs pollution (Leslie et al., 2017). Finding efficient and ecologically friendly ways of retaining MPs in WWTPs is urgently needed to prevent marine MPs pollution. Here we will focus on bioremediation, which is already employed for removing pollutants such as hydrocarbons or phosphates from WWTPs (Kshirsagar 2013; Gargouri et al., 2014).

The use of living organisms for MPs bioremediation is still a challenge. Most research has been done on bacteria and lower eukaryotes (fungi). The main problem with these small organisms is their containment within WWTPs to prevent their unwanted release into the ecosystems (Nuzzo et al., 2020). Containing bigger organisms like higher eukaryotes could, in theory, be easier, but their application in MPs bioremediation is still an alternative which has received little attention. This section focusses on the potential of aquatic higher plants and animals for MPs bioremediation.

2.1. Characteristics relevant to the use of higher eukaryotes for bioremediation

Candidate species should possess several features (Figure 2). First, to comply with animal welfare legislation (European Directive 2010/63/EU http://data.europa.eu/eli/dir/2010/63/oj), species that suffer as a result of exposure to MPs cannot be employed. Under this Directive, vertebrates, decapods and cephalopods, whose capacity for suffering is recognized, should be disregarded for use in bioremediation. Secondly, the capture, retention and filtration/ingestion rates of MPs should be high, as should be their digestion/elimination, and, furthermore, they should not be returned to the environment. Species should be employed only within their native range, since geographical transfers must be absolutely disregarded for biodiversity conservation reasons (Molnar et al., 2008). Species with a broader distribution, easy control and management, would be more suitable. Finally, since the use of a species for WWTP treatments implies growing it inside or near treatment plants, containment measures for preventing releases to the environment should be efficient.

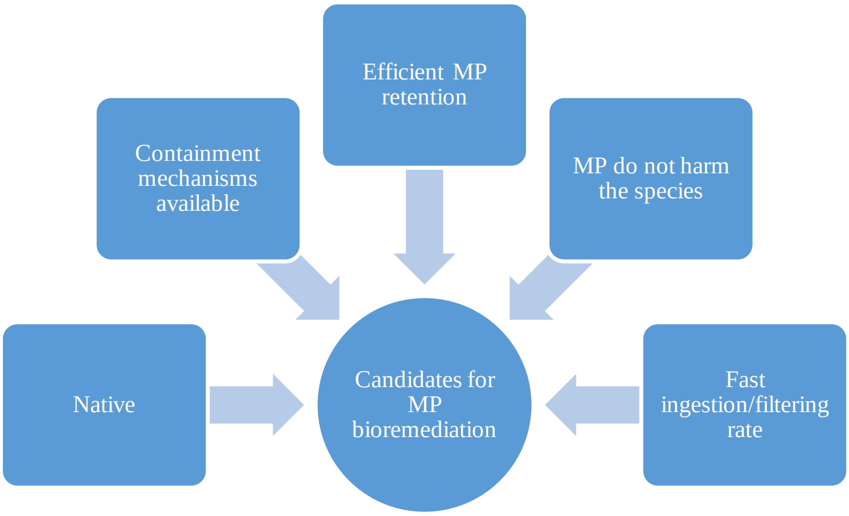

Figure 2. Characteristics required by candidate species for MPs bioremediation in WWTP.

2.2. Potential of marine animals

Marine animals, from zooplankton (Frydkjær et al., 2017) to top predators (Alomar & Deudero, 2017; Masiá et al., 2019; Zhu et al., 2019), ingest MPs. MPs are harmful to them (Anbumani et al., 2018), although their toxic effect in the wild is still unknown because most experiments conducted in laboratories use higher MPs densities that those observed in the environment (Lenz et al., 2016; de Sá et al., 2018). Further studies with higher ecological validity, i.e. with realistic amounts of MPs, are needed.

Among the marine invertebrates (excluding Decapods and Cephalopods), many active-feeding species are not suitable for bioremediation purposes due to low retention rates. Gastropods rapidly expel MPs in faecal pellets (Gutow et al., 2015). Copepods also expel MPs in faecal pellets efficiently (Cole et al., 2013), as do amphipods (Au et al., 2015; Blarer and Burkhardt-Holm, 2016), while the Cladocera *Daphnia magna* expels MPs at different rates depending on their shape (Frydkjær et al., 2017). However, species that combine deposit feeding and predation, like the echinoderm *Ophiomusium lymani*, accumulate more MPs fragments and fibres (1.96 ± 0.66 to 3.43 ± 1.35 microplastics/g) than exclusive predators like *Hymenaster pellucidus* (0.48 ± 0 to 9.10 ± 4.21 microplastics/g) (Courtene-Jones et al., 2019). This suggests better ingestion and retention of MPs by filter-feeding or deposit-feeding organisms.

Filter-feeding organisms seem to have some potential for MPs retention. *Mytilus* mussels retain pollutants, and thus serve for bioremediation in natural ecosystems (Broszeit et al., 2016). MPs fragments can be retained in their circulatory system for 48 days (Browne et al., 2008); however, most MPs fibres, which are abundant, are excreted after 24h, reducing their eliminatory efficiency (Chae et al., 2020). Other filter-feeders like cnidarians gained interest among the scientific community because adhesion to the coral surface seems to be an effective mechanism for MPs retention (ingestion rates of 0.25 x 10^{-3} to 14.8 x 10^{-3} microplastic particles hour-1 were observed, while adhesion to the surface was 40 times greater; Martin et al., 2019). However, although the responses to MPs vary among species (Reichert et al., 2018) it seems that MPs alter coral feeding behaviour (Hall et al., 2015; Murphy and Quinn 2018), and cause a reduction in anti-stress capacity and the immune system activity (Tang et al., 2018). So, unfortunately, since tropical coral reefs are highly affected by climate change (Hoegh-Guldberg et al., 2007), tropical corals should not be used for bioremediation.

The sandworm *Arenicola marina* has a retention rate of 240 – 700 MPs over its lifetime (1.2 ± 2.8 particles/g), apparently without impacts on its metabolism (Van Cauwenberghe et al., 2015); it could be a possible candidate for bioremediation in sea and brackish waters because it tolerates salinities down to 12 ppt. More studies should be carried out into possible harm caused by MPs in this species. Another promising organism is the echinoderm sea cucumber, which has been proposed for pollution monitoring (Mohsen et al., 2019), and may be suitable for removing PCB-contaminated plastic as it selectively ingests plastic particles over other types of sediment particles (Graham and Thompson, 2009). Thus, these sediment feeders would be suitable during the bioremediation process for the solid phase in wastewater treatment plants. However, MPs affect the embryonic development of other echinoderms like sea urchins (Nobre et al., 2015) and therefore, without an analysis of the impact of MPs on holothurian health, their use for bioremediation cannot be proposed.

In summary, sandworms and holothurians are promising for MPs bioremediation, but animal welfare issues are still a concern. Further experiments should investigate the impacts of MPs on them before proposing applications for WWTP treatment.

2.3. Potential of aquatic higher plants

The greatest advantage of higher plants over animals is that there is no evidence of suffering. Algae, specifically microalgae, have been tested for bioremediation potential in water. Roccuzzo et al. (2020) described unicellular microalgae that, alone or combined with bacteria, can degrade endocrine disrupting chemicals in wastewaters. Seaweeds like *Fucus vesiculosus* can retain suspended MPs on their surface (Gutow et al., 2015). Removal of other pollutants, such as heavy metals, by means of bioremediation has already been studied. Phytoextraction is a technology proposed in 1995 by Salt et al. (1995) whereby plants that can accumulate metals and store them in harvestable parts are used to extract these pollutants from soil. Rhizofiltration is another method proposed by the same authors to eliminate heavy metals from water, instead of soils, through their roots. Therefore, the same approach could be used for MPs extraction both in the solid and in the liquid phase, by growing them in WWTPs.

Seagrasses are of interest for treating effluents near the sea because they can grow in marine and brackish waters. Soumya et al. (2015) proposed the smooth ribbon seagrass *Cymodocea rotundata* for bioremediation on textile dye effluent. Recently, attention has been paid to the relation between seagrasses and MPs. The first evidence *in situ* showed MPs adherence to seagrasses by encrustation, to epibionts associated with the macrophyte, and by adhesion to the polysaccharide mucus layer (Goss et al., 2018; Seng et al., 2020). Seagrasses can thus act as a trap or sink of MPs (Huang et al., 2020; Jones et al., 2020), suggesting they have potential for sludge treatment – if they can be grown on sludge. For example, the Caribbean angiosperm *Thalassia testudinum* has MPs encrusted by epibionts on its blades (average of 0.75 ± 0.25 beads/blade; and 3.69 ± 0.99 microfibres/blade) (Goss et al., 2018), and far away in Scotland, *Zostera marina* beds accumulate MPs in higher concentrations than bare sandy sites (average of 4.25 ± 0.59 MPs in blades; and 4.50 ± 0.96 MPs in seagrass-associated biota) (Jones et al., 2020). Herbivores eating these seagrasses will introduce MPs in their diet and transfer them to higher levels in the trophic chain; but perhaps growing this plant in controlled conditions on WWTP sludge could help to prevent MPs from reaching the open sea. Seagrasses are generally very sensitive to pollution, especially of nitrogen (e.g. Fernandes et al., 2019), but some species are more resistant (O'Brien et al., 2018) and could theoretically grow in disturbed areas like the outfall or the sludge of WWTPs.

Regarding other higher plants, Ali et al. (2020) proposed several freshwater Magnoliophyta for removing heavy metals in WWTPs: water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and Duck weed (*Lemna minor*), amongst others. It seems that at environmentally realistic concentrations, nano- and microplastics do not pose ecological risks to aquatic macrophytes. Some macrophytes like *Egeria densa* and their associated microbiome can accumulate and transform gold nanoparticles (Avellan et al., 2018); these systems could be investigated for MPs bioremediation. Since few macrophytes would grow well in brackish or seawater (Haller et al. 1974), their use for MPs retention, yet to be investigated, would be recommended for freshwater WWTPs.

Summarising, seagrasses could be the optimal candidate for MPs bioremediation in marine and brackish WWTP, and aquatic macrophytes with their associated microbiota in freshwater WWTP. Further investigations should target the best methods for growing resistant seagrasses in sludge waters, the capacity of local species for MPs retention, and methods to ensure containment of species propagules. The latter objective is important for avoiding diversity disturbances outside the WWTP.

2.4. Fate of MPs in eukaryotes

As mentioned above, one of the characteristics that a species should possess in order to be a candidate for use in bioremediation is for it to efficiently digest and/or eliminate MPs, without returning them to the environment. Many species have been dismissed for this reason, as their retention rate is low (e.g. active-feeding species), and others because MPs cause the animal harm. Translocation of MPs from the digestive tract to other organs may occur in aquatic animals, as reported in fish-brain and liver (Collard et al., 2017; Mattsson et al., 2017), but it is probably uncommon (Jovanović, 2017) and hardly damages the animal; if it did, removing MPs from an organ without killing the individual would be very difficult. For animals that retain MPs in the digestive tract without apparent harm, such as sandworms and echinoderms (Graham and Thompson, 2009; Van Cauwenberghe et al., 2015), the optimal accumulation time for efficient retention without harming the animal should first be studied carefully. For the elimination of MPs retained in the digestive tract, after a time in the WWTPs, individuals could be removed and placed in a clean environment where they could eliminate gut MPs by defecation; and then returned to the WWTP while the defecated MPs are disposed of. Ideally, the organisms would be grown in aquaculture facilities, transferred to WWTPs, and left there for the time considered optimal for MPs accumulation without animal harm. Then the individuals could be transported back to culture facilities for MPs disposal. The whole process should be conducted in such a way as to avoid animal suffering.

In seagrasses and higher plants, MPs retention may take place in different ways, with the particles accumulating on the blades and also their associated microbiota. In *Thalassia testudinum*, MPs are retained in the epibiont communities on the blades (Goss et al., 2018), while MPs, especially microfibres, have been found attached to blades without epibiont communities in the seaweed *Fucus vesiculosus* (Gutow et al., 2015). As no relation between epibiont communities and MPs density have been found (Seng et al., 2020), a wide range of seagrasses and algae species could be valid for bioremediation. Moreover, not only blades retain MPs. Mangrove rhizospheres have been shown to act as a sink of MPs (Li et al., 2019), and sediments of seagrasses like *Enhalus acoroides* and *Zostera marina,* can trap MPs as well (Huang et al., 2020; Jones et al., 2020). Information about patterns and efficiency of MPs accumulation in seagrasses and macroalgae is scarce, and further studies in this field should be done.

Given the diversity of retention mechanisms in higher aquatic plants, MPs elimination could be approached differently depending on the species. Generally, plants could be grown in WWTPs from the stage at which MPs retention is efficient, then the parts of plants where MPs are retained, sediments, or the whole plants, could be harvested for disposal of the MPs. Systems for preventing dispersal of small propagules (seeds, spores, others) should be designed in order not to disturb surrounding ecosystems, something that may be caused by artificial propagation even if the species are local.

3. Conclusions

WWTPs are not intentionally designed for the removal of MPs, and despite having an efficiency of retention ≥ 90%, millions of microplastics are still released into the environment every day, not only by treated water discharge, but also by sewage sludge use for soil improvement. Consequently, these facilities are considered to be an important source of release of MPs into aquatic environments. Some higher eukaryotes have potential for elimination of MPs from WWTPs. Animal candidates may be annelids (sandworms), echinoderms (sea cucumbers) and perhaps other groups still not investigated. Seagrasses and macrophytes seem to be good candidates, with certain precautions for containment of species propagules. The results of this review suggest that the following research and management actions could be recommended:

a) Targeting of WWTPs as priority hotspots for the avoidance of microplastics discharge into the environment.

b) The improvement and implementation of advanced processes in tertiary treatments to remove a greater amount of MPs from treated water.

c) Exploring bioremediation as a potential alternative in order to degrade or accumulate microplastics in wastewater treatment, depending on the species considered.

d) Investigation of new technologies and biotechnologies to efficiently eliminate MPs from sludges.

e) Assessment of the efficiency of candidate species for retaining MPs at realistic environmental concentrations.

f) The improvement of cultivation, manipulation and management of choice species, with special attention to containment inside WWTPs, and animal welfare if animals are employed.

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Suplemetary material:

Treatment process	Removal efficiency (%)	References
Conventional Activated Process (CAS)	96-98	Lares et al., 2018; Michielssen et al., 2016
Oxidation ditch	97	Lv et al., 2019
Chlorination disinfection	7	Liu et al., 2019
Ozone	90	Hidayaturrahman et al., 2019
Coagulation/Flocculation	47-82	Hidayaturrahman et al., 2019
Rapid Sand Filtration (RSF)	45-97	Magni et al., 2019; Michielssen et al., 2016; Murphy et al., 2016; Talvitie et al., 2017
Anaerobic, Anoxic, Aerobic (A^2O)	72-98	Lee et al., 2018; Yang et al., 2019
Sequencing Batch Reactor (SBR)	98	Lee et al., 2018
Discfilter	40-98	Hidayaturrahman et al., 2019; Simon et al., 2019; Talvitie et al., 2017
Dissolved Air Flotation (DAF)	95	Talvitie et al., 2017
Reverse Osmosis (RO)	90	Ziajahromi et al., 2017
Dynamic Membrane (DM)	99	Li et al., 2018
Membrane Bioreactor (MBR)	≥ 99	Lares et al., 2018; Michielssen et al., 2016; Talvitie et al., 2017
Ultrafiltration (UF)	42	Ziajahromi et al., 2017

Supplementary table 1. A summary of different treatment processes reported in recently studies that have analysed the microplastic removal efficiency from wastewater in WWTP