
Biological Removal of Micropollutants

1.1. Introduction

Wastewater treatment plants treat wastewater from a wide variety of sources including domestic, industrial, commercial or healthcare activities. The effluents contain numerous chemical substances, some of which are classified as “micropollutants” because they are present at very low concentrations (nanograms to micrograms per liter). Micropollutants are mineral or organic molecules originating from the chemical, pharmaceutical or agricultural industries, which, beyond a certain threshold, can have negative impacts on ecosystems and the environment. However, due to these human activities, micropollutants end up dispersed in aquatic environments as well as in the various environmental compartments (Kümmerer 2001).

Even at very low concentrations, some substances can significantly disrupt ecosystems, for example, by causing endocrine-disrupting effects (hormonal system dysfunction), or, in the case of antibiotics, by playing a major role in the spread of antibiotic resistance (Stalder 2012). Controlling the release of micropollutants into the environment has become a major issue for resource conservation and public health.

European regulations, particularly through directives 2000/60/EC and 2013/39/EU, define a list of 45 priority substances for which environmental quality standards must be met. These substances include trace metal elements (TMEs) and organic trace compounds (OTCs). OTCs are organic compounds present in very small quantities, but which pose a significant ecotoxicological risk. They are also called xenobiotics, organic micropollutants or, for some, persistent organic pollutants (POPs). OTCs include various families of compounds, such as polycyclic aromatic

hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, dioxins, furans, detergent derivatives, plasticizers and natural and synthetic hormones. Pharmaceutical residues are also classified as organic micropollutants, but they are not currently regulated, despite their proven presence and impact on living organisms. Thus, a distinction is made between regulated and emerging pollutants.

1.2. Micropollutant origin

1.2.1. Trace metal elements (TMEs)

Trace elements are defined as elements present in the Earth's crust at less than one part per thousand. This concept should be distinguished from that of a microelement, which characterizes an element whose average dry matter concentration in living organisms is less than 100 mg.kg^{-1} . Certain major elements of the Earth's crust act like trace elements in their interactions with living organisms, such as iron and manganese (ADEME 1995). The metal functional group characterizes mineral elements that, in ionic form, exhibit a high affinity for the hydroxyl groups of water, with which they bind. The term "heavy metals" is frequently used to refer to mineral micropollutants. However, it encompasses both metals and metalloids, as well as elements with a density greater than 6 g.mL^{-1} (ADEME 1995).

TMEs therefore include:

- trace elements (metals or non-metals) essential in small doses for living organisms: copper (Cu), zinc (Zn), boron (B), manganese (Mn), iron (Fe), nickel (Ni), vanadium (V), molybdenum (Mo), chromium (Cr), tin (Sn), arsenic (As), etc.;
- heavy metals for which no positive role in biological activity has been identified: mercury (Hg), lead (Pb) and cadmium (Cd).

Many metals, such as cadmium, chromium, copper, iron, lead, manganese, mercury, nickel and zinc, are naturally present in trace amounts in water. Most of these elements (cadmium, lead, mercury, nickel) are classified as priority pollutants in the Water Framework Directive 2000/60/EC from October 23, 2000. However, most of them are also essential elements for biological growth, and insufficient quantities can limit the growth of certain organisms, such as algae. Conversely, in excessive quantities and depending on their chemical forms, their toxicity can pose health problems and limit the use of water resources. It is therefore necessary to measure and control the concentrations of these substances.

Urban wastewater contains metal micropollutants in concentrations far exceeding those typically found in clean water (drinking water, groundwater, surface water). These pollutants originate from both domestic use and runoff from

impermeable surfaces exposed to atmospheric pollution (rainwater, wash water) (ADEME 1995; Karvelas et al. 2003). The most common metallic micropollutants found in wastewater are iron, zinc, manganese, copper and nickel. These are followed by chromium, lead and cadmium (Lassachagne 2014).

1.2.2. Organic trace compounds (OTCs)

Organic trace compounds are organic compounds present in very small quantities, but which pose a significant ecotoxicological risk (Martín 2012a). They are also called xenobiotics, organic micropollutants or persistent organic pollutants (POPs). OTCs include different families of compounds such as PAHs (polycyclic aromatic hydrocarbons), PCBs (polychlorinated biphenyls), pesticides, dioxins, furans, detergent derivatives, plasticizers, natural and synthetic hormones, etc., as well as drug residues, otherwise known as pharmaceutical compounds.

For over 50 years, the production of organic chemical compounds for industrial and domestic uses has been steadily increasing (Rogers 1996). In addition to pesticides, residues of medicines and pharmaceutical substances are being studied with increasing frequency (Hirsch et al. 1998). They are considered emerging pollutants, residues from the use of chemicals present in the environment, not yet regulated but a topic with much scientific and public attention (Petrovic et al. 2003). This new concern is linked to two major phenomena: firstly, the emergence of modern analytical tools capable of measuring drug molecule concentrations at increasingly lower levels and, secondly, the greater scientific evidence of biological effects even at very low doses (Halling-Sørensen et al. 1998; Hirsch et al. 1998; Beausse 2004). Traces of these residues can subsequently be detected and quantified in different aquatic water cycle compartments: urban wastewater (Lindberg et al. 2005), the inlet and outlet effluents of wastewater treatment plants (Miao et al. 2002), etc.

Today, more than 100,000 chemical substances are listed in Europe, of which more than 30,000 are produced in quantities exceeding one ton. Among these, pharmaceutical compounds have been a major concern for several years and have also been identified as emerging environmental contaminants. Medicine is making significant progress thanks to medications with constantly evolving active substances that allow for the treatment of an ever-increasing number of human and animal diseases. Pharmaceutical compounds from human and animal medicines represent more than 4,000 molecules. After being consumed, the active compounds are often only partially metabolized and are then excreted via urine or feces. Thus, hospital, domestic or industrial wastewater sent to treatment plants is one of the largest sources (Hirsch et al. 1998; Beausse 2004; Mullot 2009; Deblonde et al. 2011; Verlicchi et al. 2012a).

1.2.2.1. *Pesticides*

Agriculture is by far the largest consumer of pesticides; the majority of active substances released into the environment originate from agriculture to combat pests and weeds. There are three main categories of biocides: insecticides, fungicides and herbicides.

Insecticides can affect the nervous system (neurotransmission), respiration or molting. Fungicides act selectively on respiration, biosynthesis (sterols, RNA, melanin), growth or cell permeability. Finally, herbicides can have a detrimental impact on photosynthesis, lipid and amino acid synthesis, cell division or growth. The most common pesticide families are listed as follows:

- Organophosphates (chlorpyrifos, malathion) have an insecticidal effect by blocking acetylcholinesterase (disrupting the transmission of nerve impulses).

- Organochlorines (DDT, chlordecone, lindane) are insecticides that act on the nervous system by disrupting nerve impulses at the synapses. These compounds are now all banned in France but are very persistent in the environment (the chlordecone problem in the French West Indies, for example).

- Carbamates (aldicarb, carbofuran) act on the nervous system in the same way as organophosphates.

- Triazines (atrazine, simazine) have a herbicidal effect by blocking electron transport and light energy.

- Phenylureas (diuron, isoproturon) have a mode of action comparable to that of triazines.

- Chloroacetanilides (metolachlor, acetochlor) act on plants by inhibiting elongases, enzymes involved in lipid synthesis (long-chain fatty acids).

- Aryloxy acids (2,4-D, mecoprop, dichlorprop) disrupt the regulation of auxin IAA (indole-3-acetic acid), a phytohormone essential for plant growth.

- Sulfonylureas (chlorsulfuron, nicosulfuron) act on the synthesis of branched-chain amino acids (valine, leucine, isoleucine) via the inhibition of acetolactate synthetase.

Agricultural activity is not the only source of pesticide emissions. Secondary sources include the maintenance of public spaces, golf courses, use by private individuals, annual mosquito control in wetlands, the veterinary sector, road maintenance, etc.

Main families	Compounds
Drug molecules	Anxiolytics, analgesics, anti-inflammatories, antibiotics, lipid-lowering drugs, antihypertensives, antiepileptics, anticancer drugs, iodinated contrast agents, etc.
Cosmetic and personal hygiene products	Musks (galaxolide, tonalide), triclosan, etc.
Endocrine disruptors	Hormones, alkylphenols, BPA, phthalates, flame retardants, etc.
Illegal drugs	Cocaine and metabolites, THC, amphetamines, etc.
Pesticides	Insecticides, herbicides
Priority substances (Annex X of the WFD) and hazardous substances (RSDE circular 050109)	PAHs, dichloromethane
Disinfection byproducts	Trihalomethanes, haloacetic acids

Table 1.1. *Main families and organic compounds*

1.2.2.2. *Pharmaceutical products and industrial waste*

France is one of the world's largest consumers of pharmaceuticals. More than 3,000 pharmaceutical molecules are commonly used in medicines, and 300 veterinary medicines are currently available on the French market. The biologically active substances contained in each pharmaceutical product have a wide diversity of chemical structures.

Drug residues are excreted primarily in feces and urine in their original form or as one or more active or inactive metabolites, and then released into sewage systems, wastewater and onto the soil, particularly in the case of most veterinary drugs.

The main sources of pharmaceutical water pollution include diffuse sources, primarily from human and animal excrement, which account for the majority of medications released into the environment, and point sources, which result in much more concentrated but geographically limited emissions.

Diffuse sources include improper storage and disposal of expired or unused medications and metabolic excretion via urine or the digestive tract of medications consumed by humans and animals.

Drug substances are grouped into several classes, which themselves encompass a wide range of chemical families as well as degradation metabolites produced by humans or in the environment. These include:

- analgesics and anti-inflammatories, which are the most commonly used molecules in France for human use, such as diclofenac and ibuprofen, acetylsalicylic acid and paracetamol;
- psychotropic drugs, which include antidepressants, anxiolytics and sleeping pills;
- anticonvulsants;
- lipid-lowering drugs of the fibrate family, which are intended to lower the plasma concentration of cholesterol and triglycerides;
- beta-blockers, which lower blood pressure by minimizing the binding of adrenaline to its receptors. They are cardioselective because they act specifically by reducing nerve impulses to the heart;
- antibiotics, which aim to destroy or block the action of bacteria, disrupt, for example, protein synthesis associated with bacterial growth;
- bronchodilators, which act by stimulating beta-2 receptors in the bronchi, causing their short-term dilation.

Other classes that can be mentioned include diuretics, antifungals, antivirals and laxatives.

On the other hand, the use of various antibiotics in animal production and human medicine may explain the observation of distinct correlations between the composition of the bacterial community and antibiotic-resistant populations, and the concentration of tetracycline, sulfonamide, penicillin and fluoroquinolone in wastewater. Indeed, antibiotic-resistant bacteria have been detected in several wastewater sources and clarified wastewater.

Point source contamination includes the direct discharge of pharmaceuticals (and chemicals used in their manufacture) into wastewater from pharmaceutical and chemical industries; the direct or indirect disposal of pharmaceutical agents used in healthcare facilities; the direct dispersal of veterinary drugs into the environment in aquaculture or during the treatment of livestock; indirect dispersal through the spreading of slurry and liquid manure on agricultural land; and the direct dispersal of therapeutic molecules in the form of feed supplements added to fish farms.

These substances are found in different environmental compartments (water, air, soil), through direct or indirect emission (runoff, drainage, atmospheric deposition, etc.), with potential direct or indirect effects on human health and ecosystems, notably via contamination of the food chain (see Figure 1.1). These molecules have a very wide diversity of chemical structures.

When molecules used for therapeutic or diagnostic purposes are not completely degraded in the body, they are excreted primarily in feces and urine in their initial form or as one or more metabolites. These human or veterinary drug residues can, after several stages, be released into the environment. Concentration levels in natural waters vary depending on the chemical stability, biodegradability and physicochemical characteristics of the molecules, as well as the performance of wastewater treatment plants.

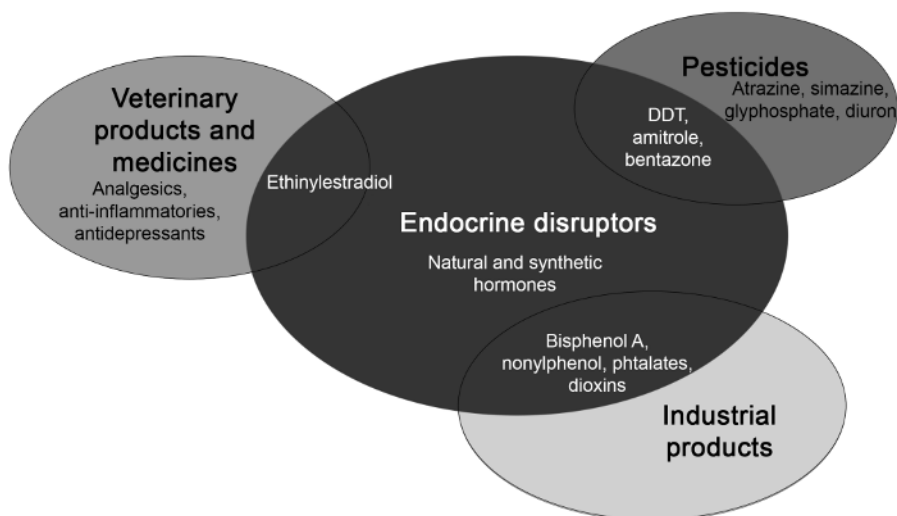


Figure 1.1. *Different categories of micropollutants*

In general, the concentrations of pesticides and pharmaceuticals found in the environment vary from one compound to another, as well as from one site to another. Regional differences in drug use, as well as the biodegradability of pharmaceuticals and their metabolites, could explain these differences in concentrations.

Analysis campaigns conducted at wastewater treatment plant outlets demonstrate the presence of these substances, often in significant concentrations.

	0.001–0.1 µg.L ⁻¹	0.1–1 µg.L ⁻¹	1–10 µg.L ⁻¹	> 10 µg.L ⁻¹
> 70%	Terbutaline, diazepam, salbutamol, bromazepam, 17 estradiol, estrone, fluoxetine, nordiazepam	Amitriptyline, metoprolol, roxithromycin, bisoprolol, propranolol, estriol, carbamazepine, diclofenac, gemfibrozil, solalol, sulfamethoxazole Diuron, fluoranthene	Naproxen, atenolol, acebutolol, caffeine, ketoprofen	45 substances (WFD)
30–70%	Atrazine, simazine, DEA, DIA, tributyltin, pentachlorophenol Indeno(1,2,3-pyrene), endosulfan, isoroturon, benzo(g,h,i)perylene, isodrine, CCl ₄	Dichlorophenol, benzothiazole, 4 ter-butylphenol Naphthalene, trichlorobenzene, benzo(b)fluoranthene AMPA, triclosan, tributylphosphate	Di- and trichloromethane, tetrachloroethylene Decabromodiphenyl ether	–
30%	1, dichloroethane, anthracene, benzo(a)pyrene, benzene, dieldrin, hexachlorobenzene Hexabromodiphenyl ether, di- and tribromophenol, tri- and tetrachlorophenol	Benzo(k)fluoranthene, chlorpyrifos, hexachlorocyclohexane, trichloroethylene, pentabromodiphenyl ether Monochlorophenol, glyphosate, tetrabromodiphenylene	C10–13 chloroalkanes, tribromodiphenyl ether	–

Table 1.2. *Example of assessment of water micropollutant concentration*

Due to the diversity and complexity of the substances involved, data on the effects of pharmaceutical residues and endocrine disruptors in the environment, and their prevalence in resources, remain limited. Given the wide variety of molecules considered and the difficulties associated with their analysis, sometimes linked to their low concentrations, studies conducted to date to assess the presence of these compounds in the environment have been limited to a few groups of molecules. These were selected based on production quantities and estimates of their persistence in the environment for pharmaceutical residues and substances (PRS) and were based on potential endocrine activity for endocrine disruptors (EDs), which are molecules that affect the hormonal balance of living species. Recent toxicological studies have demonstrated that, in humans, EDs influence sperm count, genital malformations, impaired sexual function and neuronal development, obesity and even cancer.

Substance	Concentration ($\mu\text{g.L}^{-1}$)	Substance	Concentration ($\mu\text{g.L}^{-1}$)
Acebutolol	0.3–5.9	Galaxolide	0.1–1.4
Atenolol	0.15–3.2	Gemfibrozil	0.3–1.2
Clofibric acid	0.9	Ibuprofen	0.4–3.5
Bromazepam	0.02–0.12	Iopromide	2–2.9
Carbamazepine	0.1–0.9	Ketoprofen	0.2–3.1
Ciprofloxacin	0.05–1.4	Metoprolol	0.2–1.6
Cyproconazole	0.2–1.9	Naproxen	0.5–4
Diclofenac	0.9–1.2	Nordazepam	0.05–1.1
Estrone	0.05–1.1	Propranolol	0.6–3.8
17- β -estradiol	0.2–1.4	Roxithromycin	0.05–1.5
17- α -estradiol	0.2–1.2	Sulfamethoxazole	0.5–1.3
17- α -ethinylestradiol	0.2–1.2	Sotalol	0.3–1.5
Estriol	0.05–1.1	Terbutaline	0.05–0.1
Erythromycin	0.2–2.9	Triadimefon	0.3–4.2
Fluoxetine	0.3–2.1	Tebuconazol	0.1–3.8

Table 1.3. *Example of micropollutant concentrations in clarified wastewater*

The harmful effects of releases of PE and RSPs into the environment on aquatic and terrestrial organism populations (from simple gastropods to mammals) are now largely undisputed. Numerous examples demonstrate the impact of these substances on fish (incomplete development of genital organs, hermaphroditism, alteration of the male/female ratio, etc.), birds (e.g. thinning of eggshells) and mammals (e.g. decline in seal populations feeding on fish contaminated with PCBs) that are affected. Many questions remain in the scientific community regarding the cause-and-effect relationship, which is often difficult to establish (knowledge is still incomplete regarding the toxic and ecotoxic effects of metabolites, cocktails of substances, mechanisms of disruption, etc.).

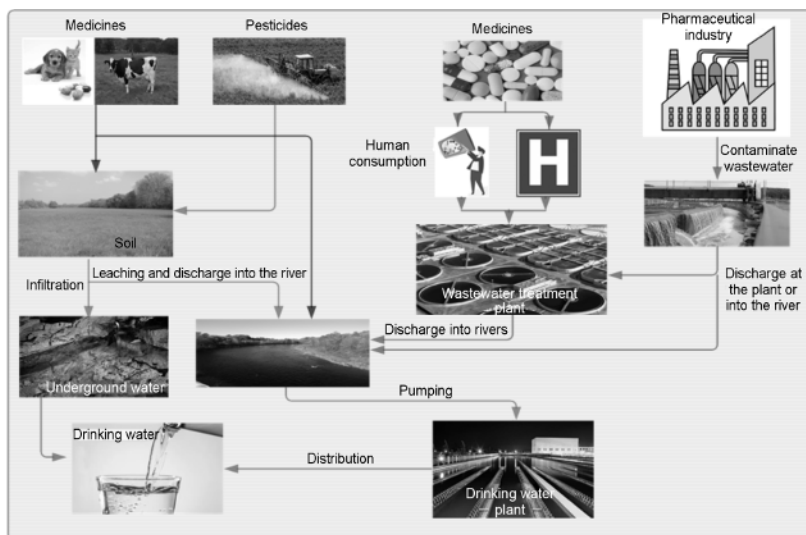


Figure 1.2. Simplified diagram of the water cycle containing pesticides, drug residues and endocrine disruptors

According to the European Commission, an endocrine disruptor is an exogenous substance or mixture which alters the functions of the endocrine system and thereby induces adverse health effects in an intact organism, its offspring or subpopulations.

In humans, the links between the presence of pharmaceutical residues and endocrine disruptors in the environment are a cause for concern among the population. European authorities appear to have acknowledged that an association between endocrine disruptors and increasingly common pathologies, such as testicular, breast and prostate cancers, decreased sperm count, malformations of reproductive organs and thyroid disorders, can be confirmed.

Their massive use, as well as the emissions generated during their manufacture or use, result in their presence at concentration levels ranging from a few tens of nanograms per liter to a few micrograms per liter.

The presence of pharmaceutical residues in drinking water is a major concern for the scientific community and public authorities. For several years, the scientific community, public authorities and the public have been questioning the presence in aquatic environments (surface water, groundwater, transfer media, soils) and in drinking water, even in trace amounts, of drug residues and their effects on the environment and human health. This inquiry is part of a broader context of

environmental and water resource preservation, particularly against micropollutants, substances that can have a toxic effect on low doses in a given environment (nanograms or micrograms per liter for water).

French public authorities have declared source reduction a priority, even aiming for the complete elimination of emissions of certain substances. Source reduction measures are essential and prevent the spread of substances in the environment, but it will take many years for the effects to be seen, and it will not be possible to control all the risks associated with micropollutants. This is due to the very large number of molecules, uses and practices, as well as to the fact that many micropollutants mainly originate from diffuse pollution emitted by individuals (pharmaceutical residues, hormones, biocides, etc.), which are therefore difficult to eliminate completely.

In Europe, the implementation of a regulatory framework aims to reduce the release of hazardous substances into the natural environment and to help study their fate within these environments. The numerous efforts undertaken, particularly at the European level, contribute to a better understanding of the environmental (chemical) quality of the various available water resources and their evolution.

In France, regulatory requirements aimed at reducing emissions of chemical substances and their presence in the natural environment stem from European regulations and directives, such as the REACH regulation, regulations on biocidal and plant protection products, the Water Framework Directive (WFD) or the Industrial Emissions Directive (IED). These regulations primarily aim to control the placing of potentially hazardous substances on the market, reduce their emissions into various environmental compartments and achieve environmental quality objectives. These increasingly stringent requirements are likely to strengthen in the coming years as scientific knowledge evolves and health and environmental impacts are demonstrated.

Beyond transposing these fundamental European texts into French law, the government has implemented a micropollutant plan to preserve water quality and biodiversity. This plan aims to reduce emissions of micropollutants with proven toxicity, consolidate knowledge and establish lists of pollutants requiring action.

Municipal and industrial wastewater treatment has evolved considerably over the past few decades, discharging effluents according to the increasingly stringent water quality requirements. Unplanned indirect potable water reuse is common worldwide.

However, regarding micropollutants such as emerging contaminants, studies monitoring their presence in surface water or wastewater are becoming increasingly common in the literature.

Wastewater treatment plants, although much improved from a technical aspect, were not designed to eliminate all pharmaceutical molecules. For example, even though paracetamol is broken down by more than 90%, traces can still be found in the clarified wastewater discharged into surface waters. Some compounds, such as diclofenac (an anti-inflammatory) or carbamazepine (an anti-epileptic), are only broken down a little.

These pharmaceutical residues released into surface waters can therefore present a significant environmental risk, as can their metabolites or degradation byproducts, which are sometimes more dangerous than the original product.

1.3. Mechanisms governing the fate of micropollutants in wastewater treatment plants

When transported to wastewater treatment plants, metallic and organic micropollutants are not completely removed from the water by these facilities, which were not designed to eliminate very low pollutant concentrations.

Recent studies show that during treatment, some of these micropollutants are likely to be found in the extracted sludge (Besnault and Martin 2011; Tahar 2013).

Indeed, domestic, industrial, agricultural and medical activities result in the discharge of numerous chemical substances into wastewater treatment plants. Most of these substances are discharged in low concentrations, hence the term “micropollutants” (concentrations of ng.L^{-1} to $\mu\text{g.L}^{-1}$).

Micropollutants are thus defined as mineral or organic molecules, originating from agricultural, chemical or industrial processes, which can have negative impacts on ecosystems and the environment beyond a certain threshold.

Wastewater treatment involves transferring organic and mineral pollution from the liquid phase (water) to a solid phase (sludge) and a gaseous phase (emission of CO_2 , N_2 , etc.). The sludge produced thus constitutes a direct waste product from the treatment process.

The activated sludge treatment process is currently the most common method for treating urban wastewater. Figure 1.4 shows the main stages of an activated sludge treatment process.

Municipal wastewater treatment plants most often include pretreatment, a primary settling stage, biological treatment and a clarifier. Sometimes, tertiary treatment is added when the effluent quality must meet stricter criteria.

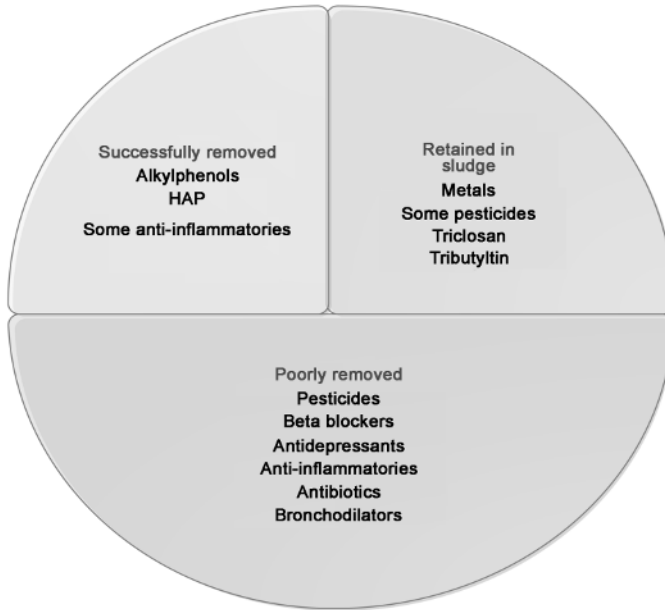


Figure 1.3. *Fate of some substances in wastewater treatment plants*

The biological treatment stage relies on a natural microbiological process, in which microorganisms convert conventional pollutants into end products such as carbon dioxide, water and nitrogen gas and create new bacterial cells.

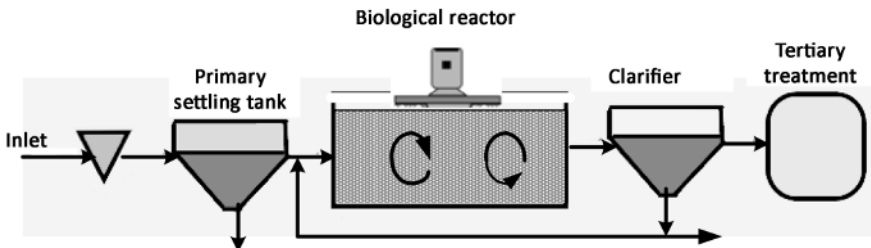


Figure 1.4. *Biological treatment process using activated sludge*

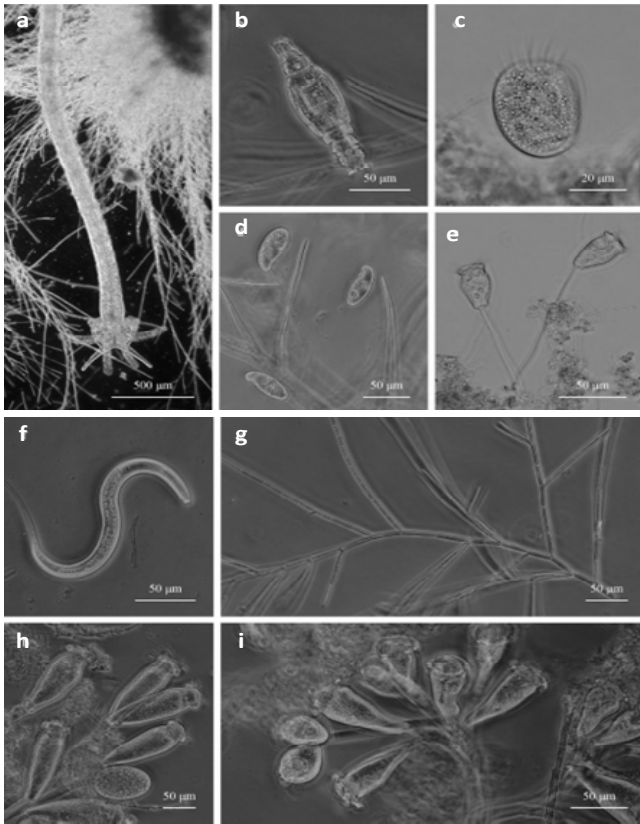


Figure 1.5. Metazoa (a, b, f), protozoa (c, d, e, h, i) and fungi (g)

The biological reactor biomass includes bacteria, archaea, fungi, protozoa and metazoa (see Figure 1.5). Protozoa and metazoa are the main predators of bacteria and, along with bacteriophages, contribute to the regulation of the bacterial population (Feng et al. 2017). These organisms have characteristic shapes and are larger than bacteria. The length of bacterial cells is generally approximately 1 µm but can reach, in some cases, up to 5 µm (Eikelboom 2000).

Figure 1.5 shows the different types of microorganisms (fungi, protozoa and metazoa) observed in biological reactors. The degradation and mineralization of nutrients and organic matter occur through a series of enzymatic reactions. Enzymes are intracellular or extracellular proteins that act as biological catalysts. The breakdown of organic matter is carried out by heterotrophic organisms that metabolize organic carbon as a source of energy and growth.

1.3.1. Molecular characteristics of micropollutants

Significant variations in the biological elimination of micropollutants (MPs) are reported, ranging from complete removal of some compounds such as ibuprofen to much only slight removal for carbamazepine. The physicochemical properties of MPs help determine the mechanisms of their biological removal. These properties are linked to molecular structure: molar mass, availability of functional groups, branching (Tadkaew et al. 2011). Being chemically complex molecules, the molecular weight, structure, functionality and shape of MPs vary considerably (Rivera-Utrilla et al. 2013). Molecular structure controls the chemical and biochemical reactions that a compound undergoes in a biological reactor. It is generally accepted that the persistence of an organic pollutant is linked to the presence of halogenated elements, nitro-aromatic groups and highly branched molecules in its structure. Conversely, biodegradability increases with compounds containing straight aliphatic chains, esters, acids and hydroxyl functional groups (Howard and Muir 2010). The polarity of molecules also influences the fate of the compound and its hydrophobicity, hence the tendency to favor a hydrophobic compound. The octanol–water parameter is a reference system that provides a commonly recognized measure of hydrophobicity: the logarithm of the partition coefficient, $\log P$ (or $\log K_{ow}$). Many MPs include acids, bases, ampholytes and salts.

1.3.2. Micropollutant removal mechanisms

The accumulation and transport of micropollutants are governed by various physico-chemical and biological mechanisms (Chipasa 2003):

- biosorption onto cells or extracellular polymers contained in bacterial flocs or biofilms;
- bioaccumulation: this refers to the active interaction between cells and micropollutants;
- sorption onto particulate matter present in wastewater.

Biotransformation induced by biological processes leads to the modification of the molecule and its structure into metabolites or end products (CO_2 , H_2O). Intermediate metabolites can be more persistent and less biodegradable than the parent molecule.

While biotransformation alters the structure of the compound, sorption leads to phase transfer. Non-biodegradable substances with a high sorption potential can be discharged with both the clarified effluent and the extracted sludge.

Adsorption onto bacterial flocs results in a longer residence time in the reactor and may lead to subsequent removal via biodegradation in the clarifier or other sludge

storage facilities. Other, less significant mechanisms involve the adsorption of particulate matter onto colloidal and inert particles. This phenomenon occurs during pretreatment, primary settling and even clarification stages. Elimination by stripping is rarely observed. This pathway is considered negligible because MPs have a Henry coefficient (H) $< 10^{-5}$.

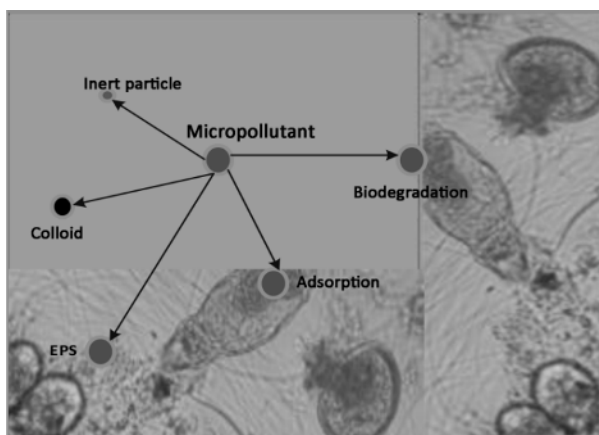


Figure 1.6. *Micropollutant removal types during biological treatment*

1.3.2.1. *Biotransformation*

Microbial biotransformation refers to the microbial conversion of a compound into a transformed product called a “metabolite” or “metabolic intermediate”. Different intermediates can be formed either by the degradation of the parent substance or by its modification. These metabolic reactions can be intracellular or extracellular (Fischer and Majewsky 2014; Stadlmair et al. 2018). Biotransformation is considered an important pathway for the neutralization of microplastics, even though it is still poorly understood and the compounds can be completely mineralized or transformed into metabolites, which could be even more problematic for the environment than the parent molecule, especially when the biologically active part remains intact during degradation (Boxall et al. 2004; Magdeburg et al. 2014).

MPs are generally recognized as non-growth substrates because their concentrations are too low to participate in bacterial metabolism. Co-metabolous biodegradation is thought to be the dominant factor in the biodegradation process (Nsenga Kumwimba 2019). This means that the MPs are biodegraded by non-specific enzymes generated by the metabolism of the main substrates in wastewater (Fischer and Majewsky 2014).

1.3.2.2. *Biosorption on biomass*

Biosorption is a process that involves the retention of organic substances on solid materials of biological origin (Yuncu et al. 2006; Pagnanelli et al. 2009).

Various mechanisms are involved, such as surface complexation where carboxyl, hydroxyl and phosphoric functional groups form binding sites on bacterial surfaces, biosorption onto cell walls and external surfaces, and then active transport across the cell membrane.

Biosorption can be defined as a metabolically passive process, physico-chemical in nature, involving ionic or molecular bonds. The attraction of micropollutant molecules towards the solid biomass matrix is governed by intramolecular interactions between the compound and the biomass surface. They are hydrophobic and/or electrostatic, also involving mechanisms such as van der Waals interactions, hydrogen bonding, electron donors, electrostatic interactions of cations and anions with charged sites in biomass, cation exchange, cation bridging and ligand exchange with surface hydroxyl groups (OH⁻) (Trapp et al. 2010; Hyland et al. 2012; MacKay and Vasudevan 2012; Oberoi et al. 2019; Burzio 2022).

Hydrophobicity and the ionic state of MPs help to determine the most predominant sorption mechanisms (Rybacka and Andersson 2016). The characteristics of the liquid medium, in terms of pH and ionic strength, influence the surface chemistry of the adsorbent and the activity of the compound, and therefore play a role in sorption dynamics. Since these interactions involve several functional groups, the characteristics of the biomass in the biological reactor (surface loading, specific surface area, EPS content, mineral content, degree of oxidation of the organic matter) determine the degree of adsorption (Barret 2010a; Alvarino et al. 2018a, 2018b; Zhang et al. 2018).

Since biomass is generally considered to be negatively charged at the surface (Hermansson 1999), and since MPs are positively charged, micropollutants are likely to exhibit a high sorption potential due to electrical attraction (Franco and Trapp 2008). The lipophilic cell membrane, the cell wall of microorganisms and the lipid fraction of sludge attract hydrophobic compounds (Verlicchi et al. 2012a, 2012b). Different sorption capacities are therefore observed with different types of biomasses (primary and secondary sludge, digested sludge, biofilms, flocs, anaerobic sludge, etc.).

The distribution of MP between the aqueous phase and the biomass is generally assumed to be instantaneous (Pomiès 2013) and involves two opposing mechanisms: sorption from the aqueous phase to the solid phase and desorption from the solid phase to the aqueous phase (Joss et al. 2006). Equilibrium is reached when the sorption kinetics are identical to those of desorption.

The hydraulic residence time in the biological reactor affects the contact time between the biomass and the MPs. Considering the slow kinetics of MP removal, a long residence time is beneficial and positively influences the degradation of the compounds (Gros et al. 2010).

Similarly, increasing the age of sludge has a beneficial impact on the removal of particulate matter. Microbial diversity increases the number of metabolic pathways (Falås et al. 2016) and promotes the growth of slow-growing microorganisms such as nitrifiers, which contribute to the degradation of a wide range of MPs (Park et al. 2017).

Temperature affects microbial activity and the sorption of several MPs in biomass. (Xu et al. 2008; Zeng et al. 2009; Tran et al. 2013). The pH of the environment determines the protonation state of the compounds, affecting their solubility and sorption capacity on biomass (Trapp et al. 2010).

The biomass configuration, whether in free or fixed culture, can differentiate the performance of the biological reactor. Higher MP removal rates have been observed in fixed culture biofilters (MBBRs) compared to activated sludge (Falås et al. 2012a, 2012b). Longer biomass retention in the biofilm favors slow-growing organisms, as does the presence of microniches with redox conditions that stimulate the development of specific microbial communities (Wolff et al. 2021). In a biofilm, microorganisms integrated into an EPS matrix, which is more developed than in activated sludge, facilitate the bio-absorption of MPs (Schmidt et al. 2012). EPSs, being a complex mixture of polymers, offers rich bonding sites due to the presence of anionic, cationic and nonpolar groups (Wunder et al. 2011; Zhang et al. 2018). EPSs have been observed to be key components in the sorption elimination of OMPs (Xu et al. 2013; Zhang et al. 2018).

1.3.2.3. *Biokinetics of micropollutant elimination*

Various studies show that in wastewater treatment plants, certain organic micropollutants can be directly degraded by specific bacteria. The overall mass of organic matter is eliminated by the diverse biomass present in the biofilm or activated sludge.

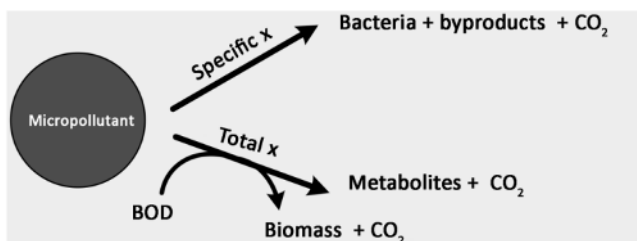


Figure 1.7. Schematic diagram of the degradation of micropollutants and BOD

The fate of micropollutants in wastewater treatment plants is governed by various processes, including solubilization (characterized by $\log K_{ow}$, water/octanol partition coefficient, for organic trace compounds), sorption/desorption to the biological matrix (characterized by the K_d coefficient), degradation for organic micropollutants (characterized by the k_{biol}) and finally, for some compounds, volatilization (stripping) characterized by the Henry's constant (H) of the molecule.

1.3.2.3.1. Sorption constant (K_d)

The mechanisms of sorption of organic compounds onto solid matrices (wastewater) involve two processes: adsorption of the compound from the liquid phase onto the surface of the solid and distribution of the compounds between the aqueous phase and the organic matter contained in the solid phase.

The equilibrium exchanges between the aqueous and solid phases of the adsorbent are represented by the sorption distribution coefficient $K_{d_{solid}}$, defined according to the following equation (Tolls 2001):

$$K_{d_{solid}} = C_s / C_{aq}$$

where C_s is the concentration of the adsorbed compound ($\mu\text{g.g}^{-1}$) and C_{aq} is the concentration of the compound in the aqueous phase ($\mu\text{g.L}^{-1}$).

Regarding the sorption phenomenon, the MP (micropollutant) concentration sorbed on the biomass (C_{bio} , $\mu\text{g.L}^{-1}$) is proportional to the dissolved concentration (C , $\mu\text{g.L}^{-1}$).

The distribution coefficient between biomass and effluent (K_d) is expressed in the form (Torresi et al. 2017):

$$K_d = \frac{C_{bio}}{C_X} = \frac{C_o - C_f}{C_f X}$$

The concentration absorbed by the biomass (C_{bio} , $\mu\text{g.L}^{-1}$) is estimated from the MP concentration at the treatment plant inlet (C_o , $\mu\text{g.L}^{-1}$) and the final concentration (C_f , $\mu\text{g.L}^{-1}$). This linear equation is a simplified case of the empirical Freundlich isotherm (Schwarzenbach et al. 2003).

In theory, the sorption of substances onto the sludge with $\log K_d < 2$ is negligible, whereas the sorption of substances onto the sludge with $\log K_d$ values > 4 is one of the main removal processes (Clara et al. 2004; Joss et al. 2005). Clara et al. (2004) and Joss et al. (2005) claimed that for compounds whose sorption coefficient (K_d) is below 300 L/kg, adsorption onto the sludge is not significant, and the

transformation can be easily assessed by a balance calculation comparing the concentrations at the inlet and outlet of the process.

Sorption models have been established, proposing an approach that takes into account the role of dissolved and colloidal matter (Barret 2010a). A micropollutant present in the bacterial floc can be distributed according to three possible phases (see Figure 1.8): the dissolved free phase (concentration C_{free} in $\mu\text{g.L}^{-1}$), the phase sorbed to the dissolved and colloidal matter (concentration C_{col} in $\mu\text{g.g}^{-1}$) and the particulate phase (concentration C_{part} in $\mu\text{g.g}^{-1}$).

Thus, at equilibrium, the system can be described by the equations:

$$K_{\text{part}} = C_{\text{part}}/C_{\text{free}}$$

$$K_{\text{col}} = C_{\text{col}}/C_{\text{free}}$$

K_{part} is the sorption equilibrium constant of the micropollutant to particles (in L.g^{-1}) and K_{col} is that of sorption to colloidal matter (in L.g^{-1}).

The concentration C_{free} is difficult to obtain experimentally, unlike concentrations C_{part} and C_{aq} (concentration of dissolved micropollutants, which corresponds to the addition of the dissolved and sorbed phases to the colloidal matter, in $\mu\text{g.L}^{-1}$).

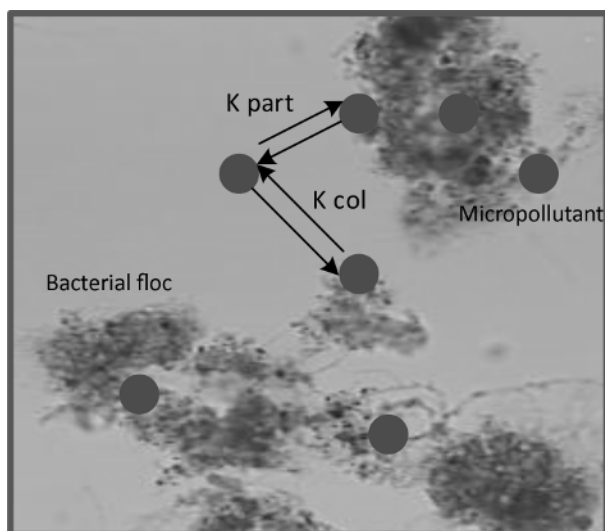


Figure 1.8. Representation of the adsorption of a micropollutant by a bacterial floc (Barret 2010a)

After determining C_{aq} and C_{part} , the global partition coefficient K can be calculated, and then K_{part} and K_{col} can be estimated:

$$K_{part} = \frac{C_{part}}{C_{aq}} = \frac{K_{part} C_{free}}{K_{col} C_{free} (C_{col}) + C_{free}} = \frac{K_{part}}{K_{col} (C_{col}) + 1}$$

where C_{col} is the concentration of the dissolved and colloidal matter ($g.L^{-1}$).

1.3.2.3.2. Biodegradation constant (K_{biol})

The degradation or transformation rates of micropollutants are calculated using the following equation, assuming pseudo-first-order kinetics (Joss et al. 2006):

$$dC/dt = k_{bio} X C$$

where C is the concentration of the compound ($\mu g.L^{-1}$), t is the time (d), k_{bio} is the rate constant of degradation or transformation ($L g^{-1} SS.d^{-1}$) and X is the concentration of suspended matter ($g SS.L^{-1}$). The k_{bio} values include sorption, desorption and biological transformation.

Three groups of micropollutants can be identified according to their biodegradation constant k_{biol} (Joss et al. 2006):

- Compounds with $k_{biol} < 0.1 L/g/d$ are not eliminated in significant quantities (< 20%).
- More than 90% of compounds with $k_{biol} > 10 L/g/d$ are transformed.
- For compounds with $0.1 L/g/d < k_{biol} < 10 L/g/d$, a moderate transformation is expected.

On the other hand, the biodegradation of organic micropollutants is often described by a first-order law (Pomiès 2013):

$$(dLmp/dt)_{biodegradation} = -k_{bmp} Lmp(t) X_a$$

or:

$$(dLmp/dt)_{biodegradation} = -k'_{bmp} Lmp(t)$$

where Lmp is the concentration of micropollutants in the dissolved phase ($M.L^{-3}$), k_{bmp} is the biodegradation rate constant of the micropollutant ($L^3.M^{-1}.T^{-1}$), k'_{bmp} is the kinetic biodegradation constant of the micropollutant (T^{-1}) and X_a is the concentration of active biomass ($M.L^{-3}$). Most of the methods described for determining biodegradation are based on measuring the disappearance of the

compound over time in batch mode, after boosting the biomass and adding wastewater, either under aerobic or anoxic conditions to reproduce the operating conditions of a wastewater treatment plant (Pomiès 2013; Seira 2013). The kinetic constants of biodegradation are then determined from the slopes of change in micropollutant concentrations.

1.3.2.3.3. Octanol–water partition coefficient (Kow)

During the primary and secondary settling stages, hydrophobic compounds can adsorb onto suspended solids and bacterial flocs via a mechanism linked to van der Waals forces and interactions between the micropollutant and the bacterial surface.

Solubility and hydrophobicity allow us to estimate the feasibility of molecule adsorption. The adsorption of micropollutants is primarily governed by hydrophobicity and can therefore be predicted using the log Kow.

The octanol/water partition coefficient (Kow) is defined as the ratio of the concentration of a chemical in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system. Log Kow is equal to the logarithm of the ratio of the concentrations of the substance in octanol to those in water. This parameter allows us to understand the hydrophilic or hydrophobic nature of a molecule. Indeed, if the log Kow is positive and very high, this indicates that the molecule in question is much more soluble in octanol than in water, reflecting its hydrophobic nature. Conversely, if the log Kow is negative, it means that the molecule in question is hydrophilic. Kow values are dimensionless. In terms of log Kow, the range varies between -3 and 7 . The log Kow is generally inversely related to water solubility and directly proportional to the molecular weight of a substance. Due to its increasing use in estimating the adsorption of organic molecules on activated carbon, Kow is considered a necessary parameter.

Log D is a more refined measure that takes into account its change depending on the pH of the aqueous phase and the pKa of the compound being studied. For neutral (uncharged) compounds, log D and log Kow are equal. A positively or negatively charged molecule has a log D value lower than log Kow.

Table 1.4 presents log Kow values for pharmaceuticals, endocrine disruptors, industrial products, pesticides, disinfection byproducts, etc. Substances with a log Kow greater than 3 will be very well adsorbed. Those with a log Kow between 1 and 3 will be fairly well adsorbed. Molecules with a log Kow between 0 and 0.5 will be poorly adsorbed, and those with a negative log Kow will not be adsorbed at all.

The structure of the molecule influences adsorption kinetics. When a molecule is not symmetrical, adsorption is less efficient than with a symmetrical molecule. The

OH, NH₂, SO₂ and COOH functional groups tend to reduce the adsorption mechanism as well as the ionized molecules. On the other hand, the functional groups NO₂, NOH, COOR and X, molecules with double bonds or delocalized electrons facilitate adsorption mechanisms.

The solubility of organic micropollutants varies over a wide range of 0.15 mg.L⁻¹ (antidepressant maprotiline) to 270,000 mg.L⁻¹ (artificial sweetener acesulfame).

Several correlations have been observed between the log K_{ow} and the sorption of organic compounds onto bacterial flocs. The compounds can be classified according to their adsorption capacity based on their K_{ow}:

- log K_{ow} < 2.5: low sorption potential;
- 2.5 < log K_{ow} < 4.0: average sorption potential;
- log K_{ow} > 4.0: high sorption potential.

Compounds and log K _{ow}		
Acenaphthene 3.90	Diazinon 3.01	Metolachlor 3.25
Acetochlor 3.17	Dibromochloromethane 2.16	Metolachlor EZA -0.03
Acetochlor EZA 1.82	Dibutyltin cation 4.44	Metolachlor OSA -0.03
Acetochlor OSA 1.82	Dicamba -1.88	Metamitron 0.85
Aclonifen 3.09	Diclofenac 0.70	Metazachlor 2.50
Alachlor 2.64	1,2-Dichlorobenzene 3.38	Metsulfuron -1.70
Aldicarb 1.13	1,4-Dichlorobenzene 3.38	MIB 3.31
Aldrin 3.01	1,2-Dichloroethane 1.45	Methylparathion 3.32
Ametryn 2.98	Dichloromethane 1.25	Methoxychlor 5.08
Aminotriazole -0.97	Dichlorprop 2.29	2,4-MCPA -0.81
AMPA -2.17	Dichlorvos 1.90	Monuron 1.46

Compounds and log Kow		
Anthracene 4.54	Dicofol 4.30	Naphthalene 3.36
Atrazine 2.33	Dieldrin 5.40	Napropamide 3.36
Azithromycin 4.02	Diflufenican 4.20	Nicosulfuron 1.00
Azoxystrobin 2.50	Dimethenamid 2.47	Nonylphenol 5.00
Benazolin 1.34	Dimethachlor 2.17	Octylphenols 5.30
Bentazone −0.46	Dinoseb 3.15	Oxadiazon 5.30
Benzene 2.11	Diquat −4.60	Parathion 3.81
Benzo (a) pyrene 6.35	Diuron 2.81	Paracetamol 0.46
Benzo (b) fluoranthene 6.67	Endosulfan 3.62	PCB-24 5.10
Benzo (k) fluoranthene 6.30	Endrin 3.20	PCB-44 5.58
Benzo (g,h,j) perylene 6.61	Erythromycin 4.51	PCB 118 4.4–5.0
Benzothiazol 2.10	Esteron 3.13	Pendimethalin 5.20
Bezafibrate 4.25	Estriol E3 2.45	Pentachlorobenzene 5.20
BDE 047 6.77	Ethephon −1.89	Pentachlorophenol 3.00
Bifenox 3.64	Ethofumesate 2.70	PEFOS 3.5–5.8
Biphenyl 4.09	Epoxiconazole 3.30	Phenanthrene 4.57
Boscalid 2.96	17-alpha-ethinyloestradiol 3.67	Phenmedipham 3.59
Bromacil 2.02	17-beta-estradiol 4.01	Prochloraz 4.30

Compounds and log K _{ow}		
Bromobenzene 2.99	Fenuron 1.00	Prometon 2.99
Monobutyltin cation 3.31	Fenoprop 3.80	Propamocarb HCl -1.20
Carbamazepine 2.45	Flufenacet 3.20	Propyzamide 3.00
Carbendazim 1.52	Fluorobenzene 2.27	Propazine 2.94
Carbaryl 2.81	Fluoranthene 5.10	Prosulfocarb 4.48
Carbofuran 2.07	Fluorene 4.20	Prothioconazole 3.82
Clarithromycin 3.16	Fluoxetine 4.05	Pyrene 5.18
Chlordane 3.32	Gemfibrozil 4.77	Quinoxifen 4.70
Chloroalkanes (C10-13) 7.00	Genistein 2.84	Quinmerac 1.76
Chlorobenzene 2.84	Geosmin 3.10	Simazine 2.16
Chloroform 1.97	Glyphosate -3.20	Spiroxamine 2.80
Chlormequat chloride -1.56	Heptachlor 4.41	Sulcotrione -1.70
Chlorotoluron 2.41	Heptachlor epoxide 2.70	Sulfamethazine 3.11
Chlorpyrifos 3.31	Heptachlorobenzene 5.73	Sulfamethoxazole 0.89
Chlopropham 3.06	Hexachlorobutadiene 4.78	Tebuconazole 3.70
Chlorsulfuron -0.99	YHCH lindane 3.72	Terbacil 1.89
Chlorothalonil 2.94	Hydroxyalachlor 2.74	Terbutryn 3.65
Clofenvinphos 4.22	Hydroxymetholachlor 2.81	1,2,4-Trichlorobenzene 4.02

Compounds and log Kow		
Chlorpyrifos 4.23	Ibuprofen 3.97	Trietazine 3.35
Ciprofloxacin 0.28	Indeno (1,2,3-cd) pyrene 7.60	1,1,2,2-Tetrachloroethane 2.39
Cyanazine 2.24	Iodobenzene 3.25	1,1,1-Trichloroethane 2.47
Cybutryne 2.70-4.00	Iodosulfuron 1.59	Triclosan 4.76
Cypermethrin 5.50	Isodrine 6.50	Tetrachloroethylene 2.53
Cyprodinil 4.00	Ioxynil 2.20	Carbon Tetrachloride 2.60
DDT 6.90	Imazapyr 0.11	Thiram 1.73
2,4, D 2.81	Imidaclopride 0.57	Tributylphosphate 4.00
Deethylatrazine 1.51	Isoproturon 2.50	Triclopyr -0.45
DEHP 7.60	Linuron 2.19	Trichlorobenzene 4.05
Deisopropylatrazine 1.15	Malathion 2.89	Trichloroethylene 2.99
Deschloroalachlor 3.08	Mancozeb 1.30	Trifuran 5.07
Deschlorometholachlor 3.10	Mecoprop -0.19	Trifluralin 5.34
Desethylhydroxyatrazine -2.70	Mesotrione 0.11	Tylosin 0.14
	Metaldehyde 0.12	

Table 1.4. Partition coefficient values Kow for various organic micropollutants

Name	Active ingredient	Effluent (ng.L ⁻¹)	Solubility (mg.mL ⁻¹)	pKa	Henry's constant (atm. m ⁻³ .mole)
Atenolol	Beta blocker	843	0.3	9.6	1.37 E-18
Azithromycin	Antibiotic	175	< 1–25°C	8.74	5.30 E-29
Bezafibrate	Hypolipidemic	139	0.00155	3.83	–
Carbamazepine	Anticonvulsant	482	0.152	15.96	1.08×10^{-10}
Carbamazepine-10, 11-dihydro-10, 11-dihydroxy	Transformation product	1,551	–	–	–
Clarithromycin	Antibiotic	276	0.00033	8.99–25°C	1.73 E-29
Diatrizoate (amidotrizoic acid)	Contrast agent	598	0.107	2.17	–
Diclofenac	Analgesic	647	0.00447	4	4.73 E-12
Erythromycin	Antibiotic	42	0.459	12.44	1.46 E-29
Ethinylestradiol	Estrogen	2	0.00677	10.33	7.94 E-12
Ibuprofen	Analgesic	394	0.0684	4.85	1.50 E-07
Iomeprol	Contrast agent	380	–	–	–
Iopamidol	Contrast agent	377	0.117	4.15	1.14 E-25
Iopromide	Contrast agent	876	0.0238	–	1.00 E-28
Mefenamic acid	Analgesic	870	0.0137	3.89	2.57 E-11
Metformin	Antidiabetic	10,347	1.38	12.4	–
Metoprolol	Beta blocker	166	0.402	14.09	1.40 E-13
Naproxen	Analgesic	462	0.0511	4.19	3.39 E-10
Sotalol	Beta blocker	435	0.782	10.07	2.49 E-14
Sulfamethoxazole	Antibiotic	238	0.459	6.16	6.42 E-13
N4-acetyl-sulfame-thoxazole	Transformation product	67	–	–	–
Trimethoprim	Antibiotic	100	0.615	17.33	2.39 E-14
Penicillin V	Personal care	28.7	0.454	3.39	4.42 E-15
Irbesartan	Antihypertensive	479.5	0.00884	7.4	–
Tramadol	Analgesic	255.8	0.75	13.8	1.54 E-11
Risperidone	Neuroleptic	6.9	0.171	8.76	–
Trihexyphenidyl	Anti-dementia	0.2	0.00314	13.84	4.73 E-10

Name	Active ingredient	Effluent (ng.L ⁻¹)	Solubility (mg.mL ⁻¹)	pKa	Henry's constant (atm. m ³ .mole)
Venlafaxine	Antidepressant	118.9	0.23	14.42	—
Codeine	Morphine derivative	70.6	0.577	13.78	7.58 E-14
Fluconazole	Antifungal	108.2	1.39	12.71	—
Diphenhydramine	Antihistamine	11.7	0.0752	8.98	3,70 E-09
Repaglinide	Antidiabetic	3.1	0.00294	3.68	—
Flecainide	Antiarrhythmic	45.5	0.0324	13.68	5.75 E-13
Bisoprolol	Beta blocker	41.6	0.0707	14.09	2.89 E-15
Alfuzosin	Alpha-blocker	2.8	0.282	14.64	—
Bupropion	Antidepressant	1.0	312	18.29	—
Ciprofloxacin	Antibiotic	96.3	1.35	6.09	5.09 E-19
Oxazepam	Anxiolytic	161.7	0.0881	10.61	5.53 E-10
Carbamazepine	Antiepileptic	832.3	0.152	15.96	1.08 E-10
Diclofenac	Analgesic	647	0.00447	4	4.73 E-12
Orphenadrine	Antihistamine	3.9	0.03	8.91	4.08 E-09
Sulfamethoxazole (VITO)	Antibiotic	280.2	0.459	6.16	—
Haloperidol	Psychiatric medication	32.2	0.00446	8.66	2.26 E-14
Citalopram	Antidepressant	3.8	—	—	—
Sulfamethoxazole (JRC)	Antibiotic	142.3	0.459	6.16	—
Fexofenadine	Antihistamine	165	0.00266	4.04	—
Diltiazem	Antiarrhythmic	10.7	0.0168	12.86	8.61 E-17
Fluoxetine	Antidepressant	2.1	0.0017	9.8	8.90 E-08
Terbutaline	Anti-asthmatic	1.1	5.84	8.86	1.65 E-18
Clindamycin	Antibiotic	70.4	3.1	12.16	2.89 E-22
Telmisartan	Antihypertensive	367.5	0.0035	3.65	—
Eprosartan	Antihypertensive	226.8	0.00866	3.63	—
Gemfibrozil	Hypolipidemic	137.7	0.0278	4.42	—
Zolpidem	Hypnotic	1.5	0.0313	6.2	—
Hydroxyzine	Antihistamine	1.1	0.0914	15.12	—

Name	Active ingredient	Effluent (ng.L ⁻¹)	Solubility (mg.mL ⁻¹)	pKa	Henry's constant (atm. m ³ .mole)
Ketoprofen	Analgesic	86.0	0.0213	4.45	2.12 E-11
Ranitidine	Antihistamine	68	0.0795	8.08	3.42 E-15
Triclosan	Disinfectant	74.8	0.00605	7.9	4.99 E-09
Levamisole	Anthelmintic	40.6	1.44	6.98	4.03 E-10
Lincomycin	Antibiotic	31.2	29.3	12.37	3.00 E-23
Rosuvastatin	Statin	31.0	0.0886	4	–
Mianserin	Antidepressant	1.5	0.232	6.92	–
Clofibric acid	Hypolipidemic	5.3	0.583	– 4.9	2.19 E-08
Iohexol	Radioactive contrast agent	158	0.796	11.73	2.66 E-29
Memantine	Anti-dementia	22.8	0.0455	10.7	1.47 E-05
Sertraline	Antidepressant	2.1	0.000145	9.85	–
Tiamulin	Antibiotic	3.3	–	–	–
Clonazepam	Anticonvulsant	1.6	0.0106	11.89	7.02 E-13
Alprazolam	Antidepressant	1.3	0.0324	18.3	9.77 E-12
Fenofibrate	Hypolipidemic	1.1	0.000707	– 4.9	–
Sulfadiazine	Antibiotic	3.5	0.601	6.36	1.58 E-10
Tilmicosin	Antibiotic	3.1	–	–	–
Cyproheptadine	Chemotherapy	3.9	0.0136	8.05	9.20 E-09
Methylbenzotriazole	Personal care	2900	0.366	8.55	4.13 E-07
Gadolinium	Personal care	115.0	–	–	–
Loperamide	Personal care	29.3	0.00086	13.96	–
Buprenorphine	Personal care	3.9	0.0168	8.31–25°C	1.76 E-17
Maprotiline	Personal care	0.4	0.00015	10.54	–
Duloxetine	Personal care	0.1	0.00296	9.7	–
Miconazole	Personal care	0.2	0.000763	6.77	–
Chlorpromazine	Personal care	0.1	0.00417	9.3–25°C	3.95 E-11
Flutamide	Personal care	0.1	0.00566	13.17	3.73 E-10
DEET, N,N'-diethyl-toluamide	Personal care	678.1	0.912	–	2.08 E-08

Name	Active ingredient	Effluent (ng.L ⁻¹)	Solubility (mg.mL ⁻¹)	pKa	Henry's constant (atm. m ³ .mole)
Caffeine	Food additive	191.1	11.0	10.4–40°C	1.90 E-19
Acesulfame	Food additive	22,500	588	5.67	–
Sucralose	Food additive	4,600	22.7	4.2	–
Diazinon	Insecticide	173	0.04	2.6	1.13 E-07
Diethyl-toluamide (DEET)	Insecticide	593	0.912	–	2.08 E-08
Dimethoate	Insecticide	–	25	–	1.05 E-10
MCPA	Insecticide	149.9	0.63	3.13	1.33 E-09
Carbaryl	Insecticide	1.6	0.11	10.4	–
2,4-D	Herbicide	13	0.012	2.73	1.59 E-07
Carbendazim	Fungicide	81	0.029	4.2	2.12 E-11
Diuron	Herbicide	201	0.042	–	5.04 E-10
Glyphosate	Herbicide	–	12	0.8	4.08 E-19
Irgarol (cybutryne)	Herbicide	30	–	–	–
Isoproturon	Herbicide	12	0.065	–	1.12 E-10
MCPA	Herbicide	25	0.63	3.13	1.33 E-09
Mecoprop-P	Herbicide	424	0.62	3.1	1.82 E-08
Triclosan	Microbicide	116	0.010	7.9	4.99 E-09
Terbuthylazine	Herbicide	90.6	0.0085	2	3.72 E-08
Atrazine	Herbicide	4.2	0.0347	1.7	2.36 E-09
Terbutylazine-desethyl	Herbicide	68.8	–	–	–
Isoproturon	Herbicide	10.1	0.065	–	1.12 E-10
Bentazone	Herbicide	9.6	0.5	2.92	2.18 E-09
Metolachlor	Herbicide	12.4	0.53	–	9 E-09
Dichlorprop	Herbicide	9.6	0.35	3.1	8.68 E-11
Simazine	Herbicide	26.3	0.0062	1.62	9.42 E-10
Atrazine-diethyl	Herbicide	13.8	3.2	–	1.53 E-09
Chlortoluron	Herbicide	3.2	0.07	–	–
Hexazinone	Herbicide	0.8	33	–	2.26 E-12
Linuron	Herbicide	40.1	0.075	–	–

Name	Active ingredient	Effluent (ng.L ⁻¹)	Solubility (mg.mL ⁻¹)	pKa	Henry's constant (atm. m ³ .mole)
2,4,5-T	Herbicide	0.3	0.248	2.88	6.83 E-09
Bisphenol A (BPA)	Additive	331	0.12	9.6	1 E-11
Estradiol	Estrogen	3	0.0213	10.33	3.64 E-11
Estrone	Estrogen	15	0.00394	10.33	3.8 E-10
Nonylphenol	Additive	267	0.00635	10.25	1.1 E-06
Perfluorooctane sulfonate (PFOS)	Surfactant	—	3.1	0.14	—

Table 1.5. Concentration of organic micropollutants at the outlet of wastewater treatment plants (activated sludge)

1.4. Effectiveness of biological treatments

The biological activated sludge process with extended aeration shows that approximately 50% of substances are removed, with highly variable efficiencies ranging from 20% to 95%. Some substances are primarily removed by biodegradation (triclosan, estrogenic hormones, analgesics), but most are removed by adsorption (particularly PBDEs, PAHs, or certain metals such as Hg, Fe, Cu, Cr, Zn, Cd, Pb).

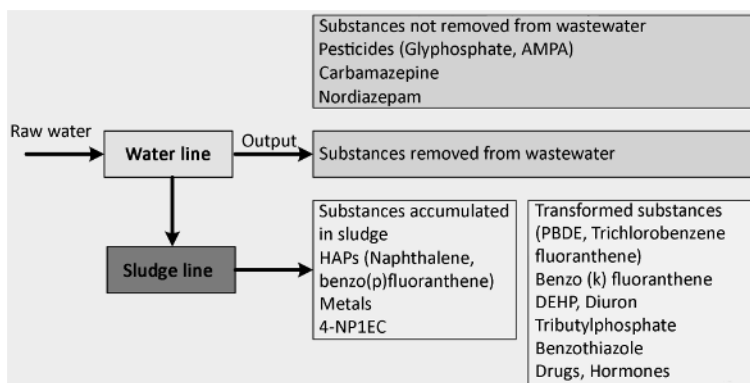


Figure 1.9. Fate of micropollutants in a conventional biological wastewater treatment process (Besnault et al. 2009)

Some substances are eliminated at a rate of less than 20%, meaning that biological treatment has proven ineffective or only marginally effective. These include polar

pesticides (glyphosate, AMPA (a glyphosate degradation product), diuron), some pharmaceutical substances (carbamazepine, diclofenac, propranolol) and alkylphenol carboxylates, the latter showing negative yields because they are produced by the biological oxidation of alkylphenols.

In total, 15% of priority substances, 30% of organic molecules and 90% of pharmaceutical substances are found in conventional wastewater treatment plant discharge in concentrations exceeding 100 ng.L^{-1} due to their physicochemical properties and their high concentration at the inlet of the wastewater treatment plants (Besnault and Martin 2011).

1.4.1. Organic micropollutants

An activated sludge floc is characterized by a microstructure, resulting from bioflocculation by microorganisms (small and mechanically fragile flocs), and a macrostructure, where filamentous microorganisms create a three-dimensional network, a support to which the small flocs can adhere, thus forming larger and stronger flocs (see Figure 1.10).

The cells and extracellular matter that make up the sludge clump together and adopt a structure resembling a multi-dimensional fractal geometry (Chu 2004). An initial organization into flocs approximately $50\text{--}100 \text{ }\mu\text{m}$ in diameter, 60% porosity and a pore diameter of approximately $10 \text{ }\mu\text{m}$ can be observed, and at a lower level, much denser microflocs can be distinguished.

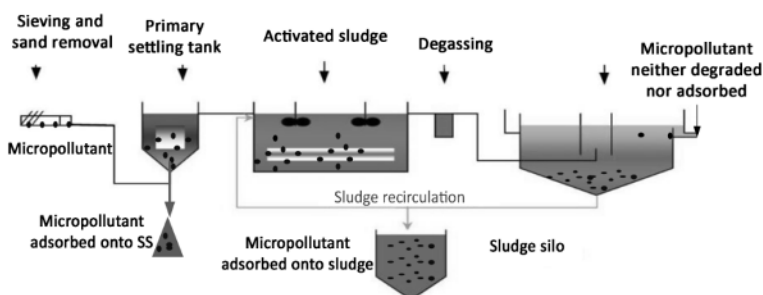


Figure 1.10. *Fate of micropollutants in a wastewater treatment plant*

Adsorption onto suspended solids in primary and secondary treatment units is an important process for PM removal. Adsorption can occur due to hydrophobic interactions between the aliphatic and aromatic groups of compounds with the fatty

and lipid fractions in primary sludge and the lipophilic cell membrane of microorganisms in secondary sludge, respectively. Electrostatic interactions also occur between positively charged micropollutant groups and negatively charged microorganisms in the secondary sludge. Many acidic pharmaceuticals are negatively charged at neutral pH, and their sorption onto the sludge is negligible.

With a nonpolar core and a polar fraction, the properties of pharmaceuticals and antibiotics vary considerably, making it difficult to estimate their sorption onto sludge. Nine different biosolids are produced by municipal wastewater treatment plants in seven US states. Sludge is a complex biological matrix containing numerous microorganisms, bacteria (10^{12} – 10^{13} bacteria/g) primarily, as well as protozoa and fungi. This living portion represents only 5–20% of the total matter, the remaining 80–95% being made up of extracellular organic and mineral matter (Nielsen et al. 2004).

The adsorption of micropollutants onto the sludge did not show any particular trend, and weak correlations were found between the concentrations of organic micropollutants in the sludge and the log Kow value. This suggests that the organic carbon content of the biosolids may not be the only factor controlling micropollutant adsorption. Compounds with low water solubility and a low Kow value are generally expected to be more likely to be present in sludge than highly soluble micropollutants. The biological removal of micropollutants is complex. Several factors must be considered: the amount of organic matter entering the influencing stream (which generally varies over time and space), the volume of the influencing stream, the biosolids-to-water ratio and the sludge retention time all affect the micropollutant distribution in the different phases. It is also possible that there is a classification: biodegradable micropollutants are transformed into finished products (CO_2 , H_2O), micropollutants that are difficult to biodegrade depend on operating conditions, while refractory micropollutants are eliminated by adhesion to biomass. Observations at one treatment plant are not systematically reproduced at another, even if the plant is supplied with the same micropollutant load. The appearance of micropollutants in various streams of municipal wastewater treatment plants, as well as their fate and elimination processes, are here discussed. The fate of micropollutants in wastewater treatment plants depends largely on adsorption onto suspended particles, primary and secondary sludge and dissolved organic carbon, with removal occurring through coagulation–flocculation and biodegradation. The log Kow (> 2.5) and pKa are the dominant micropollutant properties, and the concentration, organic fraction and surface loading of suspended particles dictate the degree of micropollutant adsorption. Most conventional wastewater treatment plants do not effectively remove complex micropollutants through biodegradation or biotransformation ($k_{\text{bio}} \leq 0.0042 \text{ L/gss/h}$). Elimination varies considerably for different compounds, as well as for the same substance, due to operating conditions such as aerobic, anaerobic and anoxic conditions;

sludge retention time; pH; redox potential; and temperature. Membrane bioreactors perform better for moderately biodegradable compounds due to the diverse nature of microorganisms and greater adaptability resulting from a longer sludge retention time.

Filamentous bacteria contribute to the adhesion of small flocs to form a more robust floc macrostructure, but the flocculation process is essentially due to electrostatic interactions between microorganisms, exopolymers and metal ions. Bonds form between the negatively charged functional groups of exopolymers and divalent cations such as Ca^{2+} or Mg^{2+} . This bridging promotes aggregation and stabilizes the matrix formed by biopolymers and microorganisms.

Extracellular polymers (ECPs) play a key role in the adsorption of metallic micropollutants (Guibaud et al. 2009). Furthermore, humic acids (insoluble at $\text{pH} < 2$ and soluble at higher pH levels) and fulvic acids (soluble at all pH levels) are substances that play an important role in micropollutant interaction. This humic material is generally considered to be the constituent with the greatest influence on the transport and behavior of organic micropollutants present in organic matrices such as sludge (Pan et al. 2008).

Due to their physicochemical properties (molecular weight, solubility, water-octanol partition coefficient K_{ow} , partition coefficient with organic carbon K_{oc} , etc.), many micropollutants are likely to be degraded from the water stream during wastewater treatment, and byproducts end up in the effluent. Some of them are adsorbed onto bacterial flocs and then end up in the extracted sludge.

At low concentrations, the micropollutant biodegradation/biotransformation kinetics follow first-order reaction kinetics:

$$\text{Rate} = K_{\text{bio}} X C_o$$

where K_{bio} is the biodegradation rate constant, X is the suspended solid concentration in the reactor and C_o is the micropollutant concentration in the wastewater.

As a general rule, complex aromatic structures containing more than one benzene ring and/or chlorine and nitro groups are not efficiently biodegraded (Pomiès 2013). The aerobic biodegradation constants of 20 aromatic species using activated sludge were reported, and the kinetic constants were correlated with the molecular structure (Andreozzi et al. 2006). The normalized first-order rate K_{bio} constants ($\text{L.g}^{-1}\text{TSS.h}^{-1}$) using X (g.L^{-1}) were 0.003, 0.02 and 3.80, respectively, for 3,5-dinitrobenzoic acid, 2,6-dichlorophenol and benzoic acid.

Pomiès (2013) summarized a list of aerobic and anaerobic rate constants for 20 pharmaceuticals, including antibiotics and compounds such as bisphenol A and nonylphenol. The aerobic rate constants K_{bio} range from 0.0025 to 7.08 $L \cdot g^{-1} SS \cdot h^{-1}$. Carbamazepine had the lowest value, while galaxolide proved to be the most biodegradable compound. Log Kow was 2.45 for carbamazepine and 5.9 for galaxolide. Compounds with K_{bio} values $< 0.0042 L \cdot g^{-1} SS \cdot h^{-1}$ are not well removed ($< 20\%$), whereas compounds with $K_{bio} > 0.4 L \cdot g^{-1} SS \cdot h^{-1}$ are transformed at a rate of over 90%. Consequently, with existing biological treatment schemes in municipal wastewater, 90% of micropollutants are not removed or biotransformed. Much plant data do not distinguish between adsorption and biotransformation due to chemical analysis challenges. In most cases, overall elimination is estimated based on the concentrations of the influencer and the effluent, and information on intermediate steps is not systematic. Many questions remain unanswered, such as the fate of intermediate metabolites, pharmaceutical processing products and the complex chemistry involved in these compounds.

In fact, removal efficiency depends heavily on the physicochemical properties of a substance. The removal of hydrophobic compounds occurs first during the removal of suspended solids, in the primary treatment stage. Micropollutants can also be removed during secondary treatment, such as in activated sludge, by sorption onto biological sludge and biodegradation. Furthermore, removal efficiencies vary depending on the operating conditions of the wastewater treatment plant, such as sludge retention time, hydraulic retention time and temperature – although the influence of these parameters is not always clearly understood. For example, higher removal efficiencies were observed for most of the micropollutants studied when nitrification occurs due to a high sludge retention time (Choubert et al. 2011). Previous studies have shown that a higher sludge retention time increases the elimination efficiency of pharmaceutical compounds (Clara et al. 2004; Carucci et al. 2006). Indeed, a longer sludge retention time could promote the diversity of bacterial communities, as well as the presence of slower-growing species, thus increasing the biomass biodegradation potential (Kreuzinger et al. 2004; Suarez et al. 2010). Furthermore, a high sludge retention time combined with a reduced food/microorganism ratio appears to promote antibiotic biodegradation (Göbel et al. 2007). However, some authors have not observed any improvement in the elimination efficiency of pharmaceutical compounds for a sludge retention time between 10 and 80 days (Joss et al. 2005; Vieno et al. 2007). A longer hydraulic retention time implies a longer contact time between the wastewater and the sludge, which appears to promote the biodegradation reaction. Evaluating the degradability of micropollutants, in terms of half-lives, is an important task for discussing integrated solutions for mitigating pollutant entry into the water cycle. By linking the calculation of micropollutant removal rates to half-lives, while assuming that compound degradation follows pseudo-first-order kinetics, it is suggested that conventional wastewater treatment processes applied to wastewater treatment plants

are incapable of completely removing all pharmaceuticals. The long half-lives observed by many pharmaceuticals lead to the use of longer hydraulic retention times to improve their degradation.

Understanding the influence of operating conditions on the removal of micropollutants is key to better understanding and improving removal mechanisms. However, as shown above, the experimental results presented in the literature do not allow for definite conclusions.

In fixed-film processes, biofilms offer several advantages over free-culture processes for removing micropollutants: a high biomass retention time, high microbial diversity, the presence of microniches and redox potential conditions are biofilm characteristics that promote micropollutant removal.

The biofilm harbors a significant diversity of microorganisms that can be very beneficial in eliminating micropollutants.

Drug residues, pesticides and hormones are effectively eliminated and are quantified at levels often below $0.1 \mu\text{g.L}^{-1}$ or even at values below the limit of detection (LOD). However, polycyclic aromatic hydrocarbons (PAHs), detergents, plasticizers and certain chemicals are quantified in the sludge and at residual concentrations in the effluent (Ellis 2006; Kümmerer 2009; Besnault and Martin 2011).

The concentrations of micropollutants quantified in the sludge are shown in Figure 1.11.

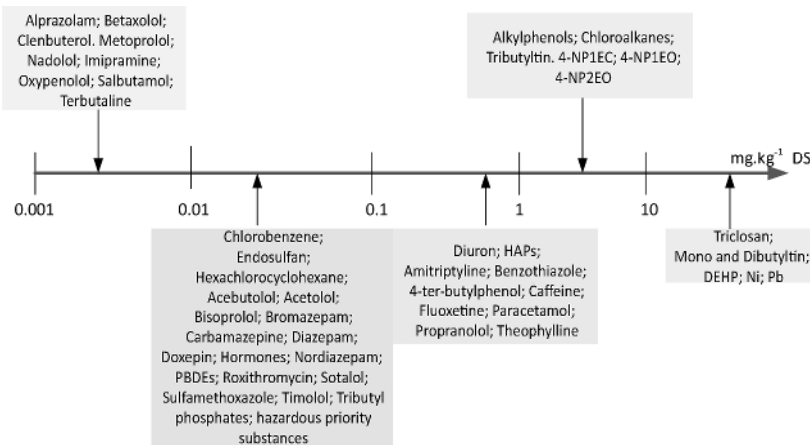


Figure 1.11. Average concentrations of various micropollutants quantified in the extracted sludge (Besnault et al. 2009; Besnault and Martin 2011)

Pesticides and insecticides are not frequently found in sludge, with the exception of diuron, alachlor and aldrin, which are found at average concentrations between 0.1 and 1 mg.kg⁻¹SS in French wastewater treatment plant sludge (Coquery et al. 2011).

With regard to medicines (or pharmaceutical compounds) in particular, the effectiveness of the wastewater treatment plants is variable and not absolute (Heberer 2002; Petrovic et al. 2003; Yu et al. 2006). Upon exiting the treatment plant, a fraction of the initial wastewater contamination remains, consisting of pharmaceutical compounds that can contaminate natural waters. Among these compounds, the removal of antibiotics is of particular interest because they can lead to antibiotic resistance (Stalder 2012).

Overall, drug residues are found in natural waters at concentrations of approximately 0.01–1 µg/L (Heberer 2002; Lindberg et al. 2005; Yu et al. 2006; Mullot et al. 2010).

Due to their physicochemical properties, drugs for human and veterinary use such as ciprofloxacin, erythromycin, ibuprofen, tetracyclines and fluoroquinolones are preferentially adsorbed onto bacterial flocs (Beausse 2004). Levels exceeding 0.1 mg.kg⁻¹SS traces of fluoxetine, paracetamol, propranolol or amitriptyline were found in the sludge (Soulier et al. 2011).

The 2009 EPA study measured more than 70 pharmaceutical products in the sludge and found low levels for most substances, such as trimethoprim (0.012–0.2 mg.kg⁻¹SS) or clarithromycin (0.0087–0.62 mg.kg⁻¹SS). Other substances were found at higher levels, such as carbamazepine (0.009–6.03 mg.kg⁻¹SS) or cimetidine (0.004–8.33 mg.kg⁻¹SS). For antibiotics, the most common groups found in the environment are macrolides, fluoroquinolones and sulfonamides, while tetracyclines and penicillins are generally found at lower concentrations (Golet et al. 2003; Beausse 2004; Lindberg et al. 2006).

In Switzerland, fluoroquinolones (in particular ciprofloxacin and norfloxacin) are molecules that have been detected in both wastewater and sludge (Golet et al. 2002). They were found there at levels in the order of ng.L⁻¹ in water and mg.kg⁻¹ in sewage sludge. The presence of fluoroquinolones (norfloxacin and ciprofloxacin) has been measured in wastewater and sludge from treatment plants in Sweden (Lindberg 2006). Concentrations of 66 ng/L of norfloxacin and 74 ng/L of ciprofloxacin were measured in filtered wastewater. In sludge, ciprofloxacin concentrations are higher: 2.4 mg.kg⁻¹SS (activated sludge) and 6.3 mg.kg⁻¹SS (digested sludge).

Table 1.6 lists the removal rates of pharmaceutical compounds in wastewater treatment plants.

Hormones	Process (min–max removal percentage)				
	Activated sludge	Trickling filters	Biofilters	MBR	MBBR
Estrone	55–79	58–63	84–95	94–95	–
17-β-estradiol	69–99	64–70	85–96	90–96	92–96
17-α-estradiol	69–98	72–76	81–94	91–94	90–94
Ethinylestradiol	74–94	60–83	34–58	64–78	–

Table 1.6. *Percentage of hormone elimination by biological processes*

Substances	BRM process		
	Inlet (ng.L ⁻¹)	Outlet (ng.L ⁻¹)	Effectiveness (%)
Paracetamol	28,370	356	98
Diclofenac	1,494	808	46
Fluoxetine	1,527	1,067	30
Carbamazepine	422	126	70
Bezafibrate	475	146	70
Gemfibrozil	888	161	81
Metoprolol	224	< LOQ	98
Propranolol	328	210	57
Sulfamerazine	52	< LOQ	LOD
Sulfamethoxazole	480	141	70
Trimethoprim	246	119	51
Clarithromycin	540	49	90
Erythromycin	66	13	> 80

Table 1.7. *BRM effectiveness (Biosep™ Veolia) in micropollutant removal (LOQ: limit of quantification, LOD: limit of detection)*

Thus, for many micropollutants, it appears extremely difficult to predict or generalize the removal efficiency values of activated sludge, which can vary greatly from one installation to another (Miège et al. 2009).

1.4.2. Activated sludge

Micropollutant		Plant inlet	Plant outlet	Reliability	Amperes
Organic micropollutants	Estradiol-alpha ng.L ⁻¹	12	< 0.4	LOQ	90–100%
	Estradiol-beta ng.L ⁻¹	40	0.7	–	80–100%
	Estrone ng.L ⁻¹	96	3.3	–	80–100%
	Ethinylestradiol ng.L ⁻¹	< 0.8	< 0.8	LOQ	–
Benzene compounds	Toluene µg.L ⁻¹	2.2	< 0.5	OK	–
Polycyclic aromatic hydrocarbons	Benzo (b) fluoranthene µg.L ⁻¹	0.033	< 0.005	LOQ	70–100%
	Benzo (k) fluoranthene	0.02	< 0.005	LOQ	80–90%
	Benzo (g, h, j) perylene µg.L ⁻¹	0.017	< 0.005	LOQ	–
	Indeno (1,2,3-c,d) pyrene µg.L ⁻¹	0.034	< 0.005	LOQ	80–90%
	Fluoranthene µg.L ⁻¹	0.1	< 0.01	LOQ	70–100%
	Benzo (a) pyrene (3,4) µg.L ⁻¹	0.03	< 0.01	LOQ	–
Urea-based pesticides	Diuron µg.L ⁻¹	0.04	0.3	LOD	0–50%
Non-ionic detergents	4-Nonylphenol-diethoxylate (NP2OE) µg.L ⁻¹	< 0.1	< 0.1	LOQ	–
	4-Nonylphenol-ethoxylate (NP1OE) µg.L ⁻¹	0.4	< 0.1	LOQ	–
	Octylphenol-ethoxylates (OP1OE) µg.L ⁻¹	< 0.1	< 0.1	LOQ	–
	Octylphenol-diethoxylates (OP2OE) µg.L ⁻¹	< 0.1	< 0.1	LOQ	–
	Octylphenols µg.L ⁻¹		0.043	LOD	70–100%
	Nonylphenols µg.L ⁻¹	0.2	1.757	LOD	60–100%
Analgesics/anti-inflammatories	Paracetamol ng.L ⁻¹	> 2,500	1,006	LOQ	90–100%
	Diclofenac ng.L ⁻¹	2,509	2,509	LOD	0–40%
	Phenazone ng.L ⁻¹	< 25	< 25	LOQ	–
Antidepressant	Fluoxetine ng.L ⁻¹	21	32	LOD	15–85%

Micropollutant		Plant inlet	Plant outlet	Reliability	Amperes
Anti-epileptic	Carbamazepine ng.L ⁻¹	395	390	OK	0%
	Primidone ng.L ⁻¹	461	462	LOD	–
Liporegulators	Bezafibrate ng.L ⁻¹	351	157	OK	–
	Gemfibrozil ng.L ⁻¹	422	153	OK	70–100%
Beta-blockers	Metoprolol ng.L ⁻¹	492	380	OK	30–100%
	Propranolol ng.L ⁻¹	1,092	1,060	OK	0–40%
Antibiotics	Sulfamerazine ng.L ⁻¹	< 25	< 25	LOQ	–
	Sulfamethoxazole ng.L ⁻¹	3,106	1,004	OK	30–70%
	Sulfachloropyridazine ng.L ⁻¹	< 25	< 25	LOQ	–
	Trimethoprim ng.L ⁻¹	282	215	OK	–
Macrolide antibiotics	Erythromycin + erythromycin-H ₂ O ng.L ⁻¹	143.6	118	OK	–
	Clarithromycin ng.L ⁻¹	54.2	74.1	LOD	–
	Roxithromycin ng.L ⁻¹	162.1	274.9	LOD	20–60%

Table 1.8. *Biological efficiency using activated sludge on the reduction of micropollutants (LOQ: limit of quantification; LOD: limit of detection)*

1.5. Biological removal of micropollutants using hybrid systems

Fixed-film processes result in longer sludge ages than free-film processes, as well as potentially higher biodiversity. Hybrid systems with a moving support (MBBRs) appear to be a promising alternative to conventional activated sludge for the removal of poorly biodegradable micropollutants such as diclofenac and contrast agents. Biofilm thickness can potentially impact biomass structure and activity. The diffusion of substrates, particularly oxygen, into the biofilm is the primary load-limiting process in a biomass bioreactor (MBBR), creating substrate gradients within the biofilm. Its thickness induces steeper concentration gradients and a stratification of metabolic processes, resulting in the formation of a more heterogeneous and diverse biofilm (Torresi et al. 2016; Edefell et al. 2021).

However, it is not yet known exactly how biofilm thickness influences biodiversity and functionality (e.g. micropollutant removal) in biofilm systems, partly due to the lack of technologies to control biofilm thickness.

In order to obtain biofilms of varying thicknesses, researchers at the company Veolia-AnoxKaldnes™ used Z-shaped polyethylene supports with a saddle-shaped grid surface, allowing biofilm to grow on the outside the support rather than inside it (see Figure 1.12). Because the supports continuously rub against each other during MBBR operation, the grid thickness corresponds to the maximum biofilm thickness.

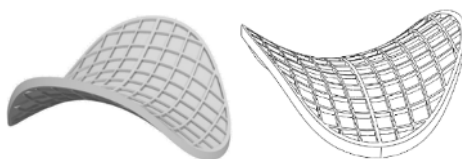


Figure 1.12. Z supports, AnoxKaldnes™

The principle of biological treatment using the Exeno™-M process is shown in Figure 1.13.

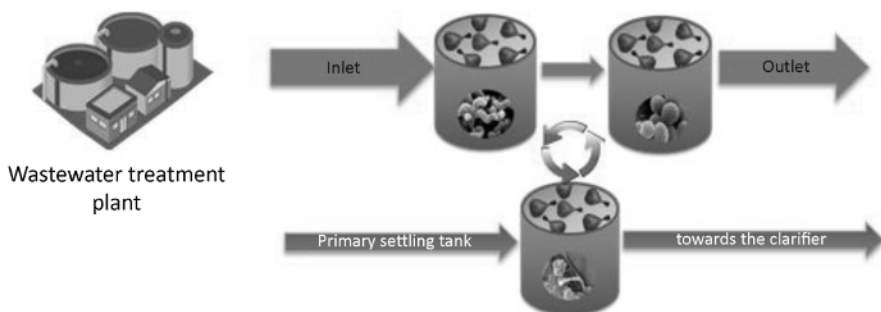


Figure 1.13. Principle of the Exeno™-M process (AnoxKaldnes™)

The biotransformation mechanism is governed by a pseudo-first-order equation. The rate constant k_{bio} ($L \cdot g^{-1} \text{ SS} \cdot d^{-1}$) is obtained by:

$$C_f = C_o e^{-Xt}$$

where C_f is the concentration of the micropollutant (ng L^{-1}) measured in the reactor and X is the biomass concentration on the Z support (g.L^{-1}). Sorption on the biofilm is represented by the coefficient K_d (L.g^{-1}). At equilibrium, we write:

$$\frac{dC}{dt} = -\frac{k_{\text{bio}} CX}{(1 + K_d X)}$$

Retransformation rates k_{dec} ($\text{L.g}^{-1} \text{SS.d}$) are estimated using the equation:

$$\frac{dC}{dt} = -\frac{k_{\text{bio}} CX}{(1 + K_d X)} + k_{\text{dec}} CX$$

$$k_{\text{bio}} = k/X$$

$$k_{\text{surf}} = k/A$$

where C is the dissolved concentration of the compound (ng.L^{-1}), k is the biodegradation rate constant (d^{-1}), t is time (d), X is the concentration of biomass on the support (g.L^{-1}), k_{bio} is the biodegradation rate constant relative to biomass ($\text{L.g}^{-1} \text{SS.d}^{-1}$) and k_{surf} is the degradation rate constant relative to the total surface area of the supporting materials ($\text{L.m}^{-2} \text{.d}^{-1}$).

Figure 1.14 shows the biodegradation rate constants per unit area for biofilms in MBBRs with high secondary and tertiary loading. The differences are more pronounced for sulfonamides (sulfamethoxazole and sulfamethiazole) and nonsteroidal anti-inflammatory drugs (ibuprofen and phenazone). As in activated sludge, an increase in sulfonamide biodegradation is observed when the MBBR is operating at a high loading (Majewsky et al. 2011; Achermann et al. 2018b). This is linked to strong bacterial growth and folic acid synthesis (Achermann et al. 2018a).

However, substantial differences are evident between secondary and tertiary treatments under high-load conditions. These results cannot be explained solely by the difference in biomass concentrations. The biodegradation capacity of several micropollutants, such as beta-blockers (atenolol and metoprolol), antibiotics (clarithromycin and erythromycin) and iodinated contrast agents (iohexol, iomeprol and iopromide), is more concentrated when comparing rate constants per unit biomass, k_{bio} . The tertiary stage results are more significant than those for the high-loading stage. Therefore, decreased substrate availability appears to have a positive effect on k_{bio} for several micropollutants, but at the cost of a decrease in biomass concentration.

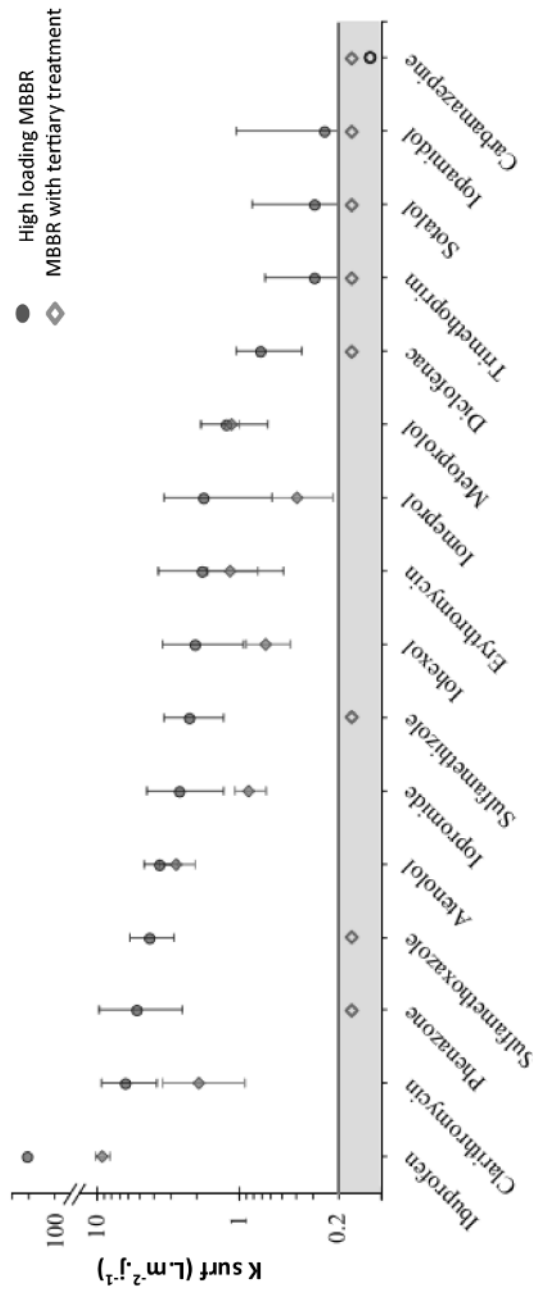


Figure 1.14. Biodegradation rate constant k_{surf} , relative to the total surface area of the supports (Edefell et al. 2021). For a color version of this figure, see www.iste.co.uk/gaid/wastewaterengineering4.zip

1.5.1. Impact of effluent recirculation

A systematic increase is observed in biomass concentration and $ksurf$ values during the operation of MBBRs with effluent recirculation.

$ksurf$ values increase significantly for iodinated contrast agents (iopromide, iohexol, iopamidol and iomeprol) when an additional substrate is added to the biofilm in a recirculation configuration.

This solution exhibits higher $ksurf$ values for atenolol and metoprolol compared to a conventional MBBR regime.

Figure 1.15 shows the results obtained in MBBRs operating under aerobic, anoxic and anaerobic conditions. Higher biodegradation rates are obtained under aerobic conditions compared to the other two operating modes.

Trimethoprim, sulfamethoxazole and antibiotics (clarithromycin, roxithromycin and erythromycin) are degraded under all conditions (see Figure 1.15). Sulfamethoxazole and trimethoprim are the only compounds with higher degradation rates under anaerobic conditions than under anoxic conditions (Edefell et al. 2021).

Iodinated contrast agents are more sensitive to redox than trimethoprim and antibiotics. Their degradation occurred at higher rates under aerobic conditions than under anoxic conditions and was very low under anaerobic conditions.

Beta-blockers exhibit variable sensitivity to redox conditions. This sensitivity is as pronounced for atenolol as it is for metoprolol and sotalol. Atenolol degrades under all conditions, likely due to hydrolysis of the primary amide (Helbling et al. 2010; Achermann et al. 2018b). Sotalol is degradable under aerobic conditions, but stable under anoxic and anaerobic conditions. The same is true for other micropollutants such as ibuprofen, diclofenac, valsartan and losartan.

The molecular oxygen requirements for the degradation of many micropollutants are particularly concerning in biofilm systems, where limiting diffusion within biofilm structures is crucial (Bishop et al. 1995), leading to a stratification of microbial layers (Almstrand et al. 2014; Suarez et al. 2019) and to changes in metabolic pathways (Alvarino et al. 2018b).

In a stratified biofilm, only the aerated outer layer would be likely to contribute to the aerobic biodegradation of micropollutants. This may justify the choice of considering the parameter $ksurf$ rather than k_{bio} .

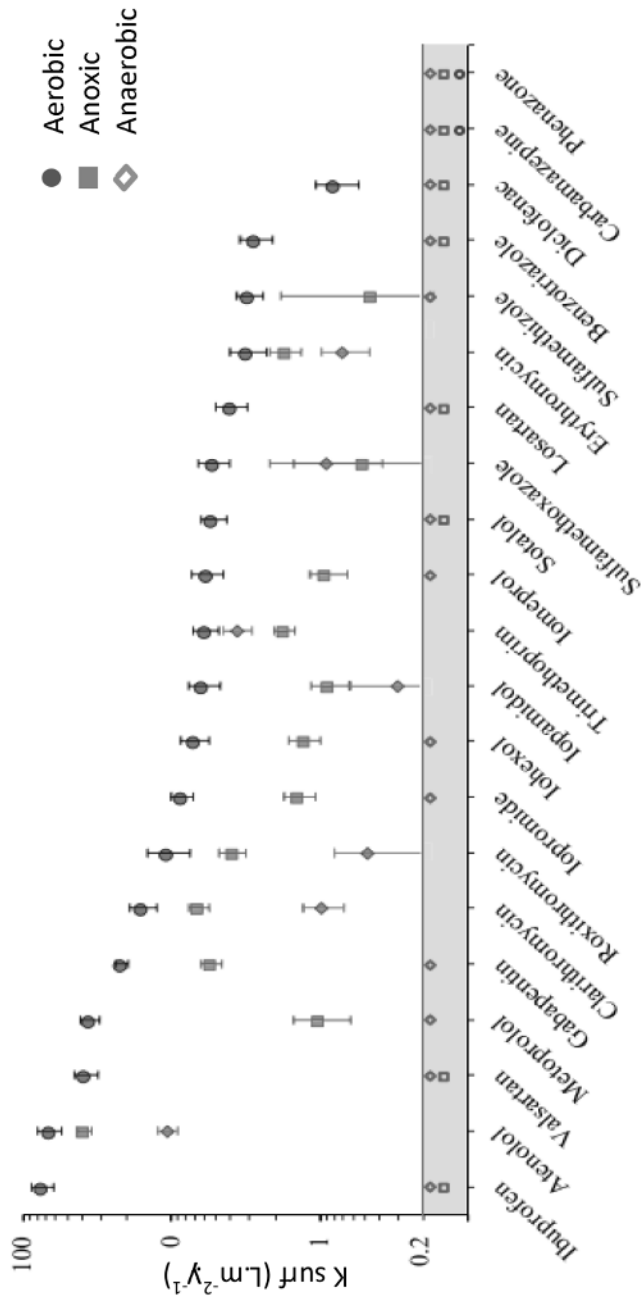


Figure 1.15. Biodegradation rate constant k_{surf} under different operating conditions (aerobic: presence of oxygen; anoxia: presence of nitrates; anaerobic: without oxygen or nitrates) (Edefell et al. 2021).
For a color version of this figure, see www.iste.co.uk/gaid/wastewaterengineering4.zip

A similar line of reasoning applies to nitrification in biofilms, as nitrification rates are negatively affected by oxygen depletion within the biofilm caused by high organic loads

It thus appears that the evaluation of the biodegradation rate constants of micropollutants as a function of the support surface area in a high-load MBBR and a tertiary MBBR shows that the organic load is an important parameter due to the high biomass that accumulates on the materials. This is confirmed if a portion of the raw wastewater supplies the tertiary MBBR and influences biomass growth. Aerobic conditions promote the biodegradation of many micropollutants, such as diclofenac, metoprolol and valsartan.

1.6. Conclusion

The current challenge is to remove micropollutants and microplastics from wastewater, especially if the wastewater is intended for reuse as drinking water (urban and industrial).

Therefore, additional treatment processes are necessary (Gaid 2025a, 2025b). Some factories are already operational with these additional treatments (known as quaternary treatments).

Micropollutants can be eliminated using various methods:

- adsorption onto activated charcoal (powder or granules);
- ozonation alone or combined with activated charcoal;
- advanced oxidation combining various chemical and physical oxidants;
- high-pressure membranes (nanofiltration and reverse osmosis).

These treatments are now well known because most have already been tested and used for drinking water treatment. They have proven their effectiveness in the field against organic matter (humic substances), as described in Gaid (2025a, 2025b).

As mechanisms for the removal of organic pollutants, the efficiency of transformation by biological units and the role of microbial communities are poorly understood, and the predictability of trace organic contaminant removal is very limited.

1.7. References

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