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(Review Article)

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Biological treatment of emerging organic micropollutants in wastewater: Recent advances and perspectives

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Abstract

The review paper comprehensively evaluates the progress and challenges in the biological removal of emerging organic micropollutants (EOMs) such as pharmaceuticals, personal care products, endocrine-disrupting compounds, industrial chemicals, and pesticides from wastewater. Traditional wastewater treatment plants, primarily designed for conventional pollutants, often fail to adequately remove these persistent and bioactive substances, raising concerns over ecological and human health impacts. This review explores the efficacy of various biological treatment processes, including conventional methods like activated sludge and trickling filters, and advanced technologies such as membrane bioreactors (MBRs), biofilm-based systems, and hybrid systems. While conventional methods offer moderate efficiency, advanced processes demonstrate significantly enhanced EOM removal due to their improved biodegradation and sorption capabilities. However, the long-term sustainability and practical applicability of these advanced methods remain areas of concern. The paper highlights the need for further research on the interaction effects of mixed EOMs, the formation of harmful transformation products, and the economic and operational feasibility of these technologies in real-world settings. The review proposes future research directions focused on developing novel hybrid treatment technologies and real-time monitoring systems to ensure more effective and sustainable EOM removal, ultimately aiming to safeguard water quality and public health.

Keywords: Biological Treatment Processes; Emerging Organic Micropollutants; Environmental Contamination; Membrane Bioreactors; Transformation Products; Wastewater Treatment

1. Introduction

Emerging organic micropollutants (EOMs), encompassing compounds such as personal care products (PPCPs), industrial chemicals, pharmaceuticals, endocrine-disrupting compounds (EDCs), and pesticides, pose a significant environmental and health challenge [1, 2]. These substances, often present in trace amounts, can have profound ecological and health effects due to their persistence, potential for bioaccumulation, and biological activity [3]. Wastewater treatment is essential for maintaining water quality and safeguarding public health. However, traditional wastewater treatment plants (WWTPs), designed primarily to remove conventional pollutants like organic matter,

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nutrients, and pathogens, are not specifically equipped to eliminate EOMs [4, 5]. Consequently, these micropollutants are discharged into receiving water bodies, raising concerns about the contamination of drinking water sources [1]. This inadequacy has spurred extensive research into advanced treatment processes that can more effectively remove EOMs from wastewater [6, 7].

Biological treatment processes, which utilize the metabolic capabilities of microorganisms to degrade and transform pollutants, play a crucial role in wastewater treatment [8]. These processes are generally categorized into conventional methods, such as activated sludge and trickling filters, and more advanced methods, including membrane bioreactors (MBRs), biofilm-based systems, and hybrid systems [9-11]. Each approach offers unique advantages and limitations in the context of EOM removal, highlighting the need for a comprehensive review of their efficacy and sustainability. While biological treatment processes are commonly employed in WWTPs, their efficiency in removing EOMs remains limited. Recent advancements in these processes have shown promise in enhancing EOM removal, yet there remains a need for further research to develop more effective and sustainable solutions [12-14].

Despite the progress made in biological treatment processes for EOM removal, several challenges persist. Current research has largely concentrated on the efficiency of various treatment technologies, with less attention given to their long-term sustainability and practical applicability in real-world settings [4, 15]. Additionally, there is a scarcity of data on the interaction effects of mixed EOMs in wastewater and the potential formation of harmful transformation products during treatment. This review seeks to address these knowledge gaps by providing a comprehensive evaluation of recent advancements, identifying potential drawbacks, and proposing future research directions. The aim is to develop more robust and sustainable solutions for EOM removal in WWTPs [16-18].

This review will critically assess the long-term sustainability of advanced biological treatments, including MBRs, biofilm-based systems, and granular sludge-based systems. It will investigate how mixed EOMs in wastewater interact and affect the performance of biological treatment processes, as well as the implications for treatment efficiency and effluent quality. The formation and fate of transformation products resulting from the biological treatment of EOMs will also be analyzed. Furthermore, the review will identify key factors influencing the practical applicability of these advanced biological treatment technologies in real-world WWTPs, considering economic, operational, and regulatory aspects. Finally, it will propose future research directions aimed at developing novel hybrid treatment technologies, integrating biological and physical-chemical processes, and implementing real-time monitoring and control systems to enhance the removal of EOMs.

2. Classification and Characteristics of Emerging Organic Micropollutants

EOMs are a diverse group of compounds that include PPCPs, EDCs, industrial chemicals, and pesticides. Pharmaceuticals and personal care products, such as antibiotics and analgesics, are widely used in human medicine and agriculture. Endocrine-disrupting compounds, such as bisphenol A and parabens, are commonly found in personal care products and plastics. Industrial chemicals, such as perfluorinated compounds (PFCs), are used in various industries, including textiles and firefighting. Pesticides, including herbicides and insecticides, are extensively used in agriculture to control pests and weeds [8, 19, 20].

The physical and chemical properties of EOMs significantly impact their removal in biological treatment processes. Hydrophobic EOMs tend to sorb onto biomass and biofilm, whereas hydrophilic EOMs are more readily biodegradable [21]. The complexity of EOMs, including their molecular structure and functional groups, also influences their interactions with microbial communities in WWTPs [21-23].

2.1. Pharmaceuticals and Personal Care Products (PPCPs)

Pharmaceuticals and Personal Care Products (PPCPs) encompass a wide range of chemicals used in human and veterinary medicine, as well as in personal hygiene products [24]. These compounds are often only partially metabolized by humans and animals, leading to their presence in wastewater. Due to their bioactive nature, PPCPs can adversely affect aquatic ecosystems and potentially human health when present in treated wastewater. The diverse nature of PPCPs, including antibiotics, hormones, and various personal care products like sunscreens and fragrances, poses significant challenges for water treatment processes [24, 25].

Emerging contaminants, such as PPCPs, are increasingly detected in water bodies around the globe due to advancements in analytical technologies. These compounds, often found in trace amounts, can have a wide range of adverse effects, from disrupting aquatic life to contributing to the development of antibiotic resistance. Research has shown that hormones such as estrogen and synthetic compounds like 17α-ethinylestradiol, commonly used in contraceptives, have been linked to endocrine disruption in aquatic species, including the feminization of male fish [26, 27]. Additionally, the widespread use of antibiotics in both medical and agricultural settings has led to the proliferation of antibiotic-resistant bacteria in the environment, raising significant public health concerns [26].

The persistence of PPCPs in the environment is exacerbated by their complex chemical structures, which often resist conventional wastewater treatment methods [25, 28]. As a result, these substances can accumulate in sediments and bioaccumulate in aquatic organisms, posing risks not only to ecosystems but also to human health through potential exposure via drinking water. The continuous discharge and bioaccumulation of PPCPs highlight the urgent need for improved wastewater treatment technologies and stricter regulations on the disposal of these compounds [29]. PPCPs have been detected in surface water and wastewater in concentrations ranging from nanograms to micrograms per liter worldwide. This widespread occurrence is partly due to the inability of current wastewater treatment processes to effectively remove all PPCPs, which can persist in the environment due to their unique physical and chemical properties [30].

According to Godoy & Kummrow [31], the mixtures of PPCPs can produce synergistic toxic effects, which may not be predictable based on the toxicity of individual compounds. This is particularly concerning given the variety of PPCPs present in the environment, including antibiotics, hormones, antidepressants, and nonsteroidal anti-inflammatory drugs (NSAIDs). For instance, antibiotics not only pose ecological risks but also contribute to the spread of antibiotic-resistant bacteria and genes, which is a growing public health concern. Hormones and certain pharmaceuticals have been linked to endocrine disruption in aquatic species, affecting reproduction and development [28, 31].

The environmental impact of PPCPs is further complicated by the fact that these substances can affect a wide range of organisms, including bacteria, algae, invertebrates, fish, and amphibians. The toxicity of PPCPs can manifest in various ways, such as changes in behaviour, inhibition of enzyme activity, and physiological alterations, even at low concentrations. For example, the psychiatric drug oxazepam has been observed to alter the behaviour of fish, increasing their feeding rate and activity levels, which could disrupt natural behaviours and ecological balance [25, 27-29]. The presence of PPCPs in the environment raises concerns about long-term sustainability. With growing populations and increasing water scarcity, the reuse of water is becoming more common, potentially leading to higher concentrations of PPCPs in water supplies. This scenario stresses the need for enhanced treatment technologies capable of removing these contaminants to ensure the safety and sustainability of water resources [24-26, 30].

2.2. Endocrine-Disrupting Compounds (EDCs)

EDCs are chemicals that can interfere with endocrine (hormonal) systems, potentially causing developmental, reproductive, neurological, and immune effects in both humans and wildlife [32, 33]. Common EDCs include bisphenol A (used in plastics) and various phthalates (used as plasticizers). EDCs can mimic or block natural hormones, leading to significant ecological and health impacts even at low concentrations. EDCs are also implicated in the impairment of reproductive functions. Various studies highlight that EDCs such as Bisphenol A (BPA) and phthalates have been associated with adverse effects on human fertility and fecundity. These substances are pervasive in everyday products like plastics and personal care items, making human exposure almost unavoidable. The compounds can leach into water sources, leading to widespread environmental contamination [32, 34].

In addition to BPA and phthalates, organochlorine pesticides (OCs) such as dichlorodiphenyltrichloroethane (DDT) and its metabolite, dichlorodiphenyldichloroethylene (DDE), have been extensively studied for their endocrine-disrupting properties [32]. These compounds can accumulate in the environment and biological tissues, posing long-term risks to wildlife and humans. The negative impacts of EDCs on fertility are more pronounced in males, affecting sperm quality and hormonal balance. Moreover, exposure to these chemicals during critical developmental windows, such as fetal development and puberty, can lead to lasting reproductive health issues, including decreased sperm count, altered hormone levels, and increased risk of reproductive diseases [35, 36].

According to Green et al. [35], EDCs can also disrupt other hormonal pathways, contributing to a range of health problems beyond reproductive health, such as metabolic disorders, immune dysfunction, and neurodevelopmental issues.

2.3. Industrial Chemicals

Industrial chemicals include a broad spectrum of substances used in manufacturing processes. Examples include perfluorinated compounds (PFCs), which are used for their resistance to heat, water, and oil. PFCs are particularly challenging to remove due to their stable chemical structure, which resists degradation [37, 38]. Margot et al. [37] noted that these chemicals can persist in the environment for extended periods and accumulate in living organisms, posing long-term ecological and health risks. Other industrial chemicals such as brominated flame retardants and various plastic additives, which are used widely in manufacturing, are also difficult to eliminate in wastewater treatment processes due to their chemical stability and hydrophobic nature [37].

These compounds often escape conventional wastewater treatment methods because they are resistant to biodegradation and do not readily adsorb onto sludge [37, 38]. This is particularly concerning given their potential toxic effects, which include endocrine disruption and other chronic health risks at low exposure levels [35]. The challenge is compounded by the fact that many of these chemicals are not removed efficiently in WWTPs and thus enter aquatic environments, where they can have detrimental effects on aquatic life and potentially enter the human food chain through bioaccumulation in fish and other wildlife [35, 36].

Advanced treatment processes, such as ozonation and activated carbon adsorption, have shown promise in enhancing the removal of these recalcitrant compounds [39]. These technologies work by breaking down the chemical structures of pollutants or trapping them in a medium from which they cannot be released back into the environment. However, the implementation of these advanced technologies is often limited by high costs and operational complexities [39, 40].

Pesticides: Pesticides, including herbicides, insecticides, and fungicides, are used extensively in agriculture to control pests and increase crop yields. These compounds can enter wastewater through agricultural runoff and improper disposal [41]. Pesticides are designed to be toxic to specific organisms, which can pose significant risks to non-target species in aquatic environments [41, 42]. The chemical diversity of pesticides, ranging from highly water-soluble compounds to those that are strongly hydrophobic, complicates their removal in WWTPs [37, 42].

3. Biological Treatment Processes for EOM Removal

3.1. Conventional Biological Treatment Processes

Conventional biological treatment processes, including activated sludge, trickling filters, and rotating biological contactors, are widely used in WWTPs [43]. However, these processes often struggle to remove EOMs efficiently (see Table 1).

3.2. Activated Sludge Process

The activated sludge process is one of the most widely used biological treatment methods in WWTPs. It involves aerating wastewater to promote the growth of a mixed microbial community that degrades organic pollutants [43-45]. While effective for removing many conventional contaminants, the activated sludge process often fails to completely remove EOMs [45]. This is due to the resistance of many EOMs to biodegradation, leading to their persistence in treated effluent. Despite its widespread use, the activated sludge process faces challenges in removing EOMs [43,45]. Factors such as the molecular structure and chemical properties of EOMs significantly influence their biodegradability. Compounds with complex aromatic structures or those containing halogen atoms, like chlorine, exhibit lower degradation rates [43]. This low biodegradability is attributed to the resistance of these compounds to microbial metabolism under standard activated sludge conditions [45]. According to Cesaro et al. [44], the adsorption of micropollutants onto sludge flocs, a secondary mechanism in the activated sludge process, often fails to achieve substantial removal for certain hydrophilic or low molecular weight compounds. Studies have shown that while hydrophobic compounds with higher octanol-water partition coefficients (log Kow) tend to adsorb more effectively onto sludge, this is not universally applicable, especially for compounds with specific functional groups that resist adsorption [43, 46-53]. This variability in removal efficiency stresses the need for tailored treatment strategies or supplementary processes to enhance the removal of EOMs in wastewater treatment plants [47].

Table 1 A Comparative Overview of Different Biological Treatment Methods

Treatment Method	EOM Removal Efficiency	Operational Complexity	Cost	Sustainability	Typical Application Scenarios	Strengths	Limitations
Activated Sludge	Moderate	High	Moderate	Moderate	Municipal and industrial wastewater	Well-established, cost-effective	Sludge production, energy-intensive
Trickling Filters	Moderate to High	Low to Moderate	Low	Moderate	Small to medium-sized WWTPs, rural areas	Simple operation, low maintenance	Limited to specific EOMs, space requirements
Membrane Bioreactors (MBRs)	High	High	High	High	Municipal and industrial wastewater with stringent discharge standards	High removal efficiency, compact footprint	High cost, membrane fouling
Biofilm-Based Systems	High	Moderate	Moderate	High	Industrial wastewater, decentralized systems	High EOM degradation, resilience to fluctuating loads	Biofilm control, potential clogging
Granular Sludge Systems	High	Moderate	High	High	High-load industrial wastewater, space-constrained WWTPs	High settling velocity, compact footprint	Granule stability, operational complexity

3.3. Trickling Filters and Rotating Biological Contactors

Trickling filters and rotating biological contactors (RBCs) are fixed-film systems where microorganisms form a biofilm on a stationary medium, with wastewater passing over this biofilm [54, 55]. These systems are advantageous due to their lower energy requirements and ease of operation compared to activated sludge systems [55]. However, their performance in removing EOMs is often limited. The primary challenge is the relatively short contact time between the wastewater and the biofilm, which may not be sufficient for the complete biodegradation of EOMs [55]. Additionally, the removal efficiency is influenced by the physical and chemical properties of the pollutants, such as hydrophobicity and molecular complexity [5]. Waqas et al. [55] noted that compounds with high hydrophobicity may adsorb more readily onto the biofilm, whereas hydrophilic compounds, especially those with complex molecular structures, may persist in the effluent. This can result in variable and often inadequate removal rates for different EOMs.

3.4. Limitations of Conventional Methods

The primary limitation of conventional biological treatment processes is their inability to consistently achieve high removal efficiencies for a wide range of EOMs. This limitation arises from several factors, including the composition of the microbial community, which may not possess the specific metabolic capabilities required to degrade certain micropollutants. Operational conditions, such as temperature, hydraulic retention time (HRT), and organic loading rates, also play critical roles in determining treatment outcomes [53-57]. Moreover, the chemical properties of EOMs, such as molecular size, polarity, and functional groups, significantly affect their biodegradability and adsorption behaviour [55]. Given these challenges, there is an increasing need for the development and implementation of advanced treatment technologies that can more effectively target and remove these persistent contaminants from wastewater [52].

3.5. Advanced Biological Treatment Processes

Recent advances in biological treatment processes have significantly improved EOM removal. Notable advancements include membrane bioreactors (MBRs), advanced oxidation processes (AOPs), biofilm-based systems, granular sludge-based systems and Hybrid Systems [58, 59].

3.6. Membrane Bioreactors (MBRs)

MBRs represent a hybrid technology that combines biological treatment with membrane filtration, significantly enhancing the removal of EOMs from wastewater (Figure 1). These systems utilize microfiltration or ultrafiltration membranes to retain biomass and suspended solids while allowing treated water to pass through [60, 61]. This dual mechanism of biodegradation and sorption onto biomass makes MBRs particularly effective for EOM removal, often outperforming conventional activated sludge processes [13, 40, 60, 61].

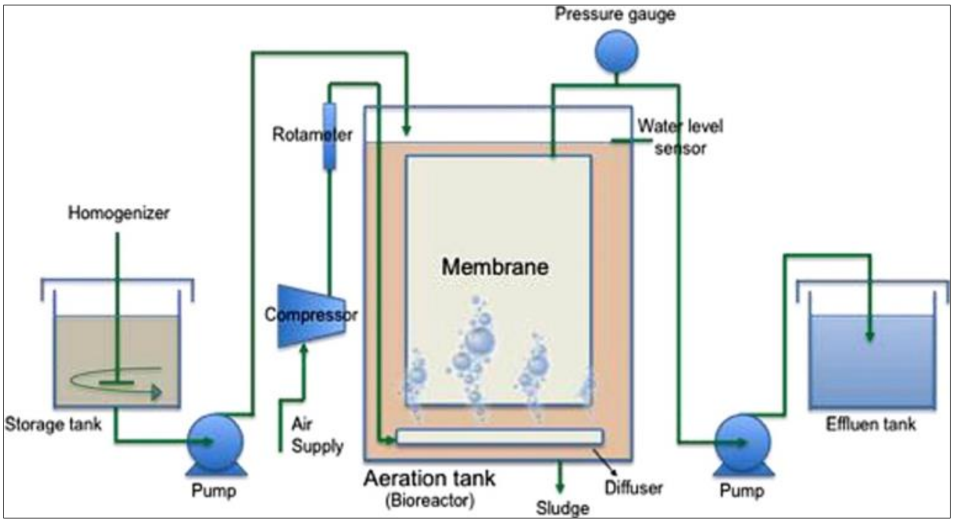

Figure 1 A Typical Membrane Bioreactor. Reproduced with permission from Fulazzaky et al. [65]

Recent applications have demonstrated the efficacy of MBRs in treating domestic wastewater, which contains a complex mixture of contaminants. These include nutrients like nitrogen and phosphorus, organic matter such as proteins and fats, and various household chemicals like detergents and disinfectants [62,63]. MBR systems are particularly effective in removing these contaminants, ensuring that treated water meets stringent regulatory standards for safe discharge or reuse [62, 63]. In a notable study by Caglak et al. [64], lab-scale anoxic/oxic membrane bioreactor (A/O-MBR) and oxic membrane bioreactor (O-MBR) systems were used to treat domestic wastewater. These systems utilized a submerged polysulfone hollow-fiber membrane module with a pore size of 0.01 µm. The study examined the impact of varying sludge retention times (SRT) on contaminant removal efficiencies. The findings revealed that the A/O-MBR system achieved a higher total nitrogen removal efficiency than the O-MBR system, particularly at an infinite SRT, with efficiencies of 72.3% and 33.1%, respectively. The A/O-MBR system also demonstrated COD removal efficiencies ranging from 82.4% to 91.5%, compared to the O-MBR system's range of 79.3% to 89.8%. Additionally, the A/O-MBR system significantly reduced the trihalomethane formation potential (THMFP) by 88.91%, while the O-MBR system achieved an 85.39% reduction. These findings suggest that the MBR process, especially the A/O-MBR system, is effective for treating wastewater, particularly in regions with emerging contamination issues. The study highlights the potential of MBR technology for improving water quality in developing countries [64].

In another study, Fulazzaky et al. [65] demonstrated the efficacy of MBRs in treating oilfield-produced water, which contains complex mixtures of EOMs, including hydrocarbons, surfactants, and heavy metals. The study showed that a lab-scale MBR system with a 0.4-micron membrane achieved a 95.9% reduction in oil content and a 96.4% removal of chemical oxygen demand (COD). These results emphasize the system's efficiency in degrading and removing contaminants that are challenging for conventional treatment methods. The treated effluent consistently met stringent regulatory standards for discharge, emphasizing the potential of MBR technology in environmental compliance [65].

Additionally, MBRs offer advantages such as a smaller footprint, higher biomass concentration, and better effluent quality, making them suitable for decentralized wastewater treatment applications. The integration of MBR systems in oilfield operations not only addresses the high pollutant load but also provides a sustainable solution for water reuse, which is increasingly critical in water-scarce regions. However, the high operational costs and membrane fouling challenges remain significant limitations [66,67]. Advances in membrane technology, fouling control strategies, and hybrid system integration are areas of ongoing research aimed at improving the cost-effectiveness and operational stability of MBRs [68,69].

According to Khan et al. [70], the incorporation of adsorption and advanced oxidation processes (AOPs) alongside MBRs has been shown to improve the removal of recalcitrant organic micropollutants. This integration addresses some limitations of standalone MBR systems, particularly in terms of membrane fouling and the degradation of persistent contaminants. Moreover, the review discusses the application of novel membrane materials, such as graphene oxide and nanocomposite membranes, which offer higher permeability and resistance to fouling. These advancements are pivotal in extending the operational lifespan of MBR systems and reducing maintenance costs. The review emphasizes the importance of ongoing research and development in membrane materials and hybrid process configurations to overcome existing challenges and optimize the performance of MBRs in wastewater treatment [70].

- **Immobilized Cell Bioreactors**: In these systems, microorganisms are immobilized on carriers or within a matrix, allowing for higher biomass concentration and increased stability. This setup improves the degradation efficiency of EOMs by maintaining an optimal microenvironment for microbial activity.
- **Two-Phase Partitioning Bioreactors (TPPBs)**: Two-phase partitioning bioreactors (TPPBs) enhance the removal of hydrophobic EOMs by using a combination of an aqueous phase and a non-aqueous phase. The non-aqueous phase acts as a reservoir for hydrophobic pollutants, gradually releasing them into the aqueous phase where microbial degradation occurs [57].

In TPPBs, the hydrophobic EOMs preferentially partition into the non-aqueous phase due to their solubility properties. This system ensures a consistent supply of EOMs to the microorganisms and prevents inhibitory concentrations of pollutants, thus optimizing the degradation process. The high-speed agitation in TPPBs creates small-sized droplets of the organic phase, increasing the surface area for mass transfer and enhancing the overall removal efficiency of hydrophobic micropollutants [71, 72].

- **Advanced Oxidation Processes (AOPs):** AOPs are integrated with biological treatment systems to enhance the removal of EOMs by generating highly reactive species, such as hydroxyl or sulfate radicals, that can non-selectively oxidize organic pollutants [30, 44]. When combined with biological processes, AOPs break down complex EOMs into simpler compounds more amenable to microbial degradation, resulting in higher removal efficiencies and reduced formation of toxic by-products. A study by Mir-Tutusaus et al., [73] showed combining activated sludge processes with advanced oxidation techniques like ozonation or UV/H2O2 treatment can significantly improve the degradation of recalcitrant micropollutants, ensuring a more comprehensive removal of EOMs from wastewater.

- **Biofilm-Based Systems:** Biofilm-based systems, such as biofilters and biotrickling filters, utilize microbial biofilms to degrade pollutants [74-76]. In these systems, microorganisms grow on a solid medium, forming a biofilm that provides a high surface area for EOMs to adsorb and subsequently degrade. These systems offer several advantages, including higher biomass retention and greater resistance to shock loads. Biofilm-based systems can effectively remove EOMs through mechanisms such as sorption onto the biofilm matrix and subsequent biodegradation [74,75]. The efficiency of these systems depends on factors such as biofilm thickness, hydraulic retention time, and the characteristics of the EOMs being treated [74].
- **Granular Sludge-Based Systems:** Granular sludge-based systems, including granular activated sludge and anaerobic granular sludge, leverage the compact structure and high microbial activity of granules for EOM removal [77]. These systems offer benefits such as improved settling characteristics, high biomass concentration, excellent settling properties, and the ability to support diverse microbial communities. Granular sludge systems can achieve high removal efficiencies for a range of EOMs through a combination of sorption and biodegradation processes. These characteristics contribute to enhanced EOM removal efficiency [77, 78].

3.7. Hybrid Systems

Hybrid systems that integrate biological and physicochemical processes are also being explored to improve the removal efficiency of EOMs [54-56, 79]. These systems leverage the strengths of both approaches, offering advantages such as reduced accumulation of toxic by-products and enhanced overall treatment performance. These systems can combine elements such as activated sludge and biofilm reactors, combining activated sludge processes with AOPs or integrating biofilm reactors with adsorption techniques to achieve higher treatment efficiencies [9, 48, 55]. Hybrid systems offer flexibility in operation and can be tailored to target specific EOMs, making them a versatile option for advanced wastewater treatment.

4. Mechanisms of EOM Removal in Biological Treatment Processes

The removal of EOMs in biological treatment processes primarily relies on the metabolic activities of microorganisms, including bacteria, fungi, and algae [6]. These microorganisms can biodegrade or transform micropollutants through several mechanisms:

4.1. Biodegradation (Microbial Degradation)

Microbial degradation is a fundamental process in the biological treatment of EOMs, relying on the metabolic activities of various microorganisms, including bacteria, fungi, and algae. These microorganisms utilize the micropollutants as a source of carbon and energy, breaking them down into simpler, less harmful compounds [44, 71].

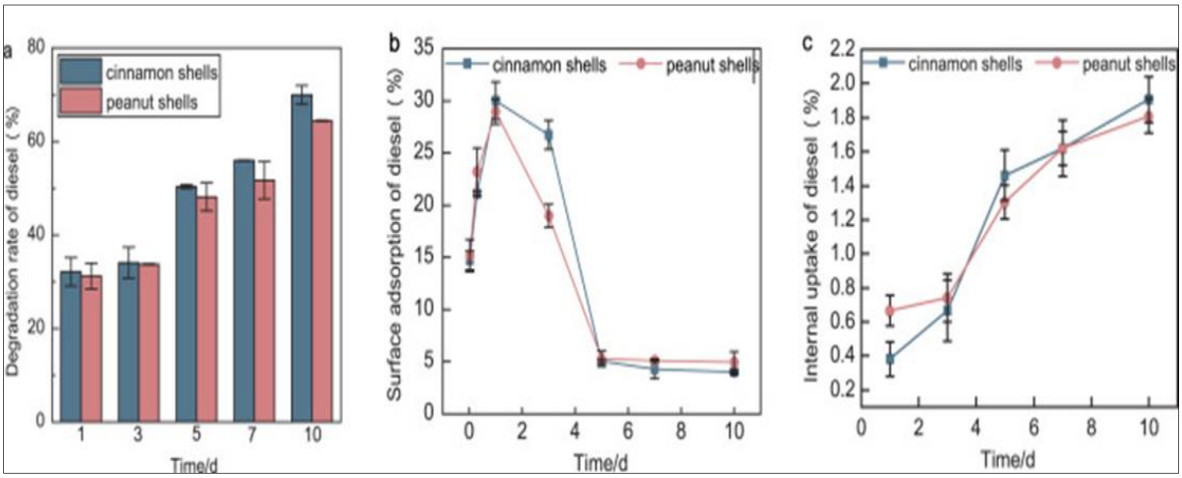

Figure 2 Investigation into the degradation behaviour of bacteria immobilized on various carriers: (a) The rate at which immobilized bacteria degrades diesel, varied with time. (b) The amount of diesel adsorbed onto the surface of the immobilized bacteria over time. (c) A comparison of the diesel internal uptake by various immobilized bacteria Reproduced with permission from Fu et al. [80]

Recent studies have demonstrated the potential of immobilized bacteria on natural materials, such as cinnamon and peanut shells, for the biodegradation of diesel in wastewater. According to Fu et al. [80], the degradation rate of diesel increased over time, with maximum degradation rates of 69.94% and 64.41% achieved by bacteria immobilized on cinnamon and peanut shells, respectively, within 10 days (Figure 2a). Interestingly, the surface adsorption of diesel by the immobilized bacteria initially increased, peaking at 30.01% and 28.97% on the first day, before decreasing and stabilizing at near-zero levels after 5 days. In contrast, the internal uptake of diesel by the immobilized bacteria increased steadily over time, albeit at a much lower rate, with a maximum internal uptake of only 2% (Figures 2b and c). These findings highlight the effectiveness of immobilized bacteria on natural materials for the biodegradation of organic micropollutants, such as diesel, and warrant further investigation into the mechanisms underlying this process.

4.2. Sorption by Microbial Flocs

Sorption by microbial flocs is a critical mechanism for the removal of hydrophobic EOMs (Figure 3). These compounds tend to adsorb onto the surface of microbial aggregates or flocs due to their hydrophobic nature. The adsorbed pollutants are then subjected to biodegradation by the microorganisms within the flocs [81]. This mechanism is particularly significant in activated sludge systems and biofilm-based reactors, where the large surface area of microbial flocs enhances the adsorption and subsequent degradation of EOMs [12, 81].

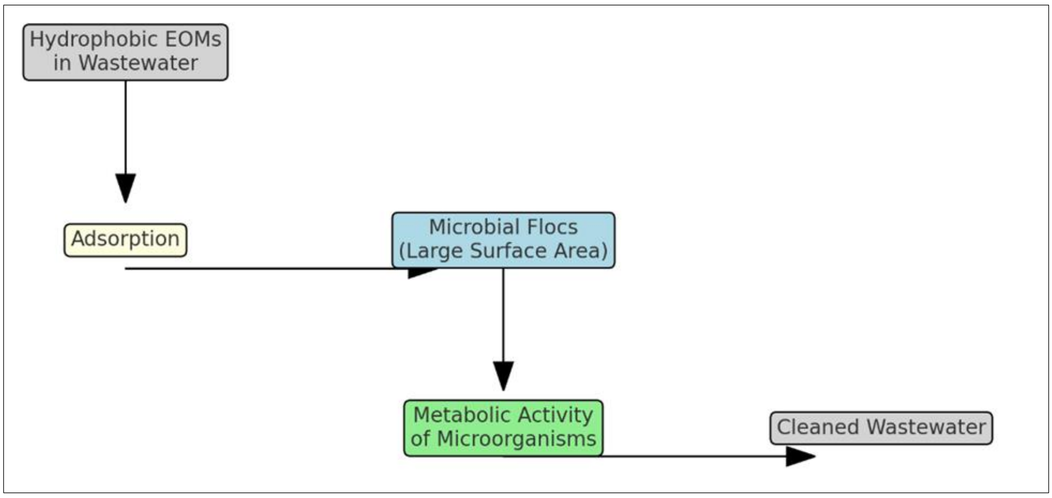

Figure 3 A schematic diagram illustrating the concept of sorption by microbial flocs

In activated sludge processes, the microbial flocs consist of a diverse community of microorganisms that work synergistically to degrade various pollutants [12]. The hydrophobic EOMs are initially captured by the flocs and then degraded through the metabolic activities of the microorganisms. This dual process of adsorption and biodegradation ensures the effective removal of a wide range of micropollutants from wastewater [12, 49, 81].

4.3. Co-Metabolism

Co-metabolism is a process where microorganisms degrade EOMs incidentally while metabolizing a primary substrate [82]. The micropollutants are not used as the primary source of carbon and energy but are transformed by enzymes produced during the metabolism of other compounds. For example, bacteria can co-metabolize pharmaceuticals in the presence of easily degradable organic matter, leading to the breakdown of these micropollutants even though they are not the primary target of microbial activity [82-84]. Some researchers highlighted the significant role of nitrifying and methanotrophic bacteria in co-metabolic biodegradation processes, particularly in rapid sand filters (RSF) used in groundwater treatment [82, 85, 86]. Nitrifying bacteria, such as *Nitrosomonas* and *Nitrospira*, were shown to enhance the removal of compounds like caffeine, 2,4-dichlorophenoxyacetic acid (2,4-D), and bentazone [85]. Similarly, methanotrophic bacteria, including genera like *Methylobacter*, *Methylomonas*, and *Methylotenera*, improved the degradation of caffeine, benzotriazole, and 2,4-D [85].

In co-metabolism, the presence of a readily degradable substrate is crucial as it induces the production of enzymes that can also act on EOMs. This process is particularly important for the degradation of micropollutants that are not readily biodegradable under normal conditions [86]. Nitrifying bacteria produce ammonia monooxygenase (AMO), which not only oxidizes ammonia but can also degrade hydrocarbons and other complex organic compounds. Methanotrophic bacteria use methane monooxygenase (MMO) for methane oxidation, which can co-metabolically degrade compounds such as sulfamethoxazole and benzotriazole [85]. Wang et al. [85] also suggested that bioaugmentation with enriched nitrifying and methanotrophic cultures could be a promising approach to improve micropollutant removal in RSF systems. This approach highlights the potential of leveraging specific microbial communities to optimize biodegradation processes in wastewater treatment, offering a more sustainable and cost-effective solution compared to traditional methods like activated carbon adsorption and advanced oxidation processes [85, 86].

4.4. Nitrification and Denitrification

Nitrification and denitrification are two linked biological processes that play a significant role in the removal of nitrogen-containing EOMs [85, 87]. Nitrification is an aerobic process where ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (AOB), followed by the oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB) [87-89]. This process not only reduces the concentration of ammonia in wastewater but can also contribute to the degradation of certain EOMs during the oxidation steps. The role of nitrifying bacteria in the biodegradation of micropollutants has been well-documented, with studies indicating that enzymes such as ammonia monooxygenase (AMO) and methane monooxygenase (MMO) present in these bacteria can hydroxylate hydrocarbons, facilitating the breakdown of compounds like caffeine, bentazone, and 2,4-dichlorophenoxyacetic acid (2,4-D) as shown in Figure 4 [85, 89].

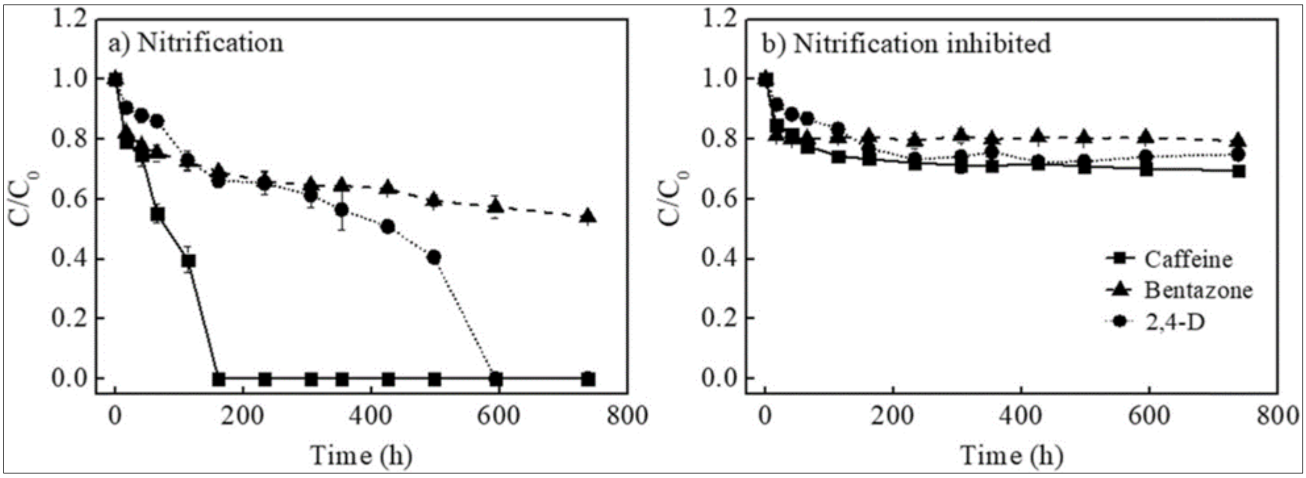

Figure 4 Impact of nitrification on the biodegradation of bentazone, 2,4-D, and caffeine, in experimental runs. (a) Nitrification: OMPs and NH_4^+-N added; (b) Inhibited Nitrification: Allylthiourea (ATU), OMPs and NH_4^+-N added. Reproduced with permission from Wang et al. [85] © Elsevier

Denitrification, on the other hand, is an anaerobic process where nitrate is reduced to nitrogen gas by denitrifying bacteria [85, 89]. This process can also lead to the transformation of EOMs, particularly those containing nitrogen groups [85]. The combined effect of nitrification and denitrification can result in the effective removal of nitrogenous micropollutants from wastewater, reducing their environmental impact. Moreover, the interaction between nitrification and methanotrophic bacteria has been explored, showing that these processes can occur synergistically, further enhancing the removal of certain micropollutants when present together [85-87].

4.5. Enzymatic Transformation

Certain microorganisms produce extracellular enzymes capable of degrading complex organic micropollutants [90]. These enzymes, such as laccases, peroxidases, and cytochrome P450 monooxygenases, can break down a wide range of EOMs, including pharmaceuticals, personal care products, and pesticides [90, 91]. Enzymatic transformation often leads to the formation of intermediate metabolites, which can be further degraded through microbial metabolic pathways [91].

4.6. Redox Reactions

Redox reactions, involving the transfer of electrons, are fundamental to the biodegradation of many micropollutants [92, 93]. Microorganisms can induce redox transformations of EOMs, converting them into less toxic forms [92]. For example, the reductive dehalogenation of halogenated compounds, a process facilitated by certain anaerobic bacteria, results in the removal of halogen atoms and the formation of simpler hydrocarbons [92,93].

5. Factors Affecting EOM Removal in Biological Treatment Processes

Several factors influence the efficiency of EOM removal in biological treatment processes.

5.1. Microbial Community and Diversity

The composition and diversity of microbial communities play a crucial role in EOM degradation. Specific microorganisms are responsible for breaking down particular pollutants, and the presence of a diverse microbial population can enhance the overall degradation efficiency [6, 94]. The ability of microbial consortia to adapt and develop enzymatic pathways specific to EOMs is crucial for effective removal. A study by Zhou et al., [7-9, 95] revealed bacteria such as Pseudomonas, Streptomyces, and Arthrobacter have shown effectiveness in degrading a wide range of micropollutants.

5.2. Environmental Conditions

Optimal environmental conditions such as temperature, pH, and oxygen levels significantly affect microbial activity and, consequently, the efficiency of EOM removal [2, 96]. For example, certain bacteria perform better at specific pH levels or temperature ranges, and deviations from these optimal conditions can reduce the degradation efficiency [95].

5.3. Hydraulic and Solid Retention Times (HRT and SRT)

The hydraulic retention time (HRT) and solid retention time (SRT) are critical operational parameters in biological treatment processes [1-3, 97]. Longer HRT and SRT generally allow for greater contact time between microorganisms and pollutants, enhancing the degradation process. However, extremely long retention times can lead to biomass decay and reduced microbial activity [5, 97].

5.4. Presence of Co-substrates

The presence of co-substrates or additional organic matter can enhance the biodegradation of certain EOMs through co-metabolism [3-5]. Microorganisms may use these co-substrates for growth and energy, simultaneously degrading EOMs [1, 2]. However, excessive co-substrate concentrations can also lead to competition for microbial enzymes, potentially reducing EOM degradation [97].

5.5. Reactor Configuration and Design

The design and configuration of bioreactors, such as activated sludge processes, biofilm reactors, and membrane bioreactors, significantly impact the efficiency of EOM removal [2, 7]. For example, membrane bioreactors can provide higher biomass concentrations and longer SRTs, improving the removal of recalcitrant pollutants compared to conventional activated sludge systems [97].

5.6. Inhibition by Toxic Substances

Certain EOMs or other pollutants present in wastewater can inhibit microbial activity and reduce the efficiency of biological treatment processes [98]. Toxic compounds can interfere with microbial metabolism or even cause cell death, leading to decreased pollutant removal rates [99].

5.7. Operating Conditions

Operational parameters such as aeration, mixing, and nutrient supply need to be carefully controlled to maintain optimal microbial activity [3, 96-98]. Insufficient aeration can lead to anaerobic conditions, while excessive aeration can cause biomass washout. Similarly, the availability of essential nutrients (e.g., nitrogen and phosphorus) is necessary for sustaining microbial growth and activity [85-87].

5.8. Characteristics of EOMs

The physical and chemical properties of EOMs, such as their molecular weight, polarity, solubility, and functional groups, significantly affect their biodegradability and sorption behavior [2, 100-102]. Hydrophobic compounds tend to adsorb more readily onto biomass, whereas hydrophilic compounds are generally more biodegradable. The presence of specific functional groups, such as hydroxyl or carboxyl groups, can also influence the susceptibility of EOMs to microbial degradation [100, 103].

5.9. Operational Conditions

Operational conditions, including temperature, pH, hydraulic retention time (HRT), and sludge retention time (SRT), play crucial roles in EOM removal [97, 100]. Optimal temperature and pH conditions enhance microbial activity and enzyme function, thereby improving biodegradation efficiency [100-102]. HRT and SRT are critical parameters that determine the contact time between EOMs and microbial communities. Longer HRT and SRT generally result in higher EOM removal efficiencies, as they provide sufficient time for microbial degradation and sorption processes [85, 97, 103].

5.10. Presence of Co-Contaminants

The presence of co-contaminants in wastewater can impact EOM removal. Co-contaminants, such as heavy metals and other organic pollutants, can inhibit microbial activity and reduce the efficiency of biodegradation processes [1-2, 85, 100]. Additionally, co-contaminants can compete with EOMs for adsorption sites on biomass, affecting sorption efficiency [104].

5.11. Nutrient Availability

Nutrient availability, particularly carbon, nitrogen, and phosphorus, is critical for the growth and activity of microbial communities [62, 63]. The presence of sufficient nutrients supports microbial metabolism and enhances the biodegradation of EOMs [105]. Conversely, nutrient limitations can hinder microbial activity and reduce EOM removal efficiencies. Balancing nutrient concentrations is essential for maintaining optimal microbial activity in biological treatment systems [1, 62, 63].

6. Challenges and Future Directions

The biological treatment of EOMs faces several inherent challenges. A significant issue is the recalcitrant nature of many EOMs, which resist biodegradation due to their complex molecular structures [26, 31]. These compounds often require specialized microbial consortia or genetically modified organisms capable of breaking down specific chemical bonds, which are not always feasible to maintain in conventional systems. The variability of EOMs in wastewater streams also complicates treatment efforts, as changes in industrial discharge, seasonal variations, and other factors lead to inconsistent removal efficiencies [41, 53]. Consequently, treatment facilities must be adaptable to these fluctuations without compromising performance. Additionally, toxic substances in wastewater can inhibit microbial activity, further complicating the degradation process and reducing overall efficiency [31, 41, 53]. Operational constraints, such as maintaining optimal environmental conditions (e.g., temperature, pH, oxygen levels), add to the complexity and cost of treatment processes.

To address these challenges, several future directions are being explored. Bioaugmentation strategies, where specific strains of microorganisms known for their EOM-degrading capabilities are introduced into treatment systems, can help establish robust microbial communities tailored to degrade particular pollutants. Advances in synthetic biology offer another promising direction by engineering microbial metabolic pathways to create "designer" microbes capable of efficiently breaking down a wide range of EOMs [26, 41]. These engineered microbes can target specific micropollutants, enhancing the versatility and effectiveness of biological treatments. The integration of advanced monitoring and control systems using AI and ML can optimize operational parameters by predicting changes in wastewater composition and adjusting treatment conditions in real-time, ensuring optimal performance. Exploring hybrid treatment systems that combine biological processes with technologies such as AOPs or membrane filtration can also offer synergistic benefits, enhancing the removal of recalcitrant EOMs. Lastly, decentralized treatment systems using small-scale, modular bioreactors deployed close to pollution sources can reduce the burden on centralized facilities and allow for targeted, efficient treatment of specific EOMs, facilitating easier adaptation to local conditions and pollutant profiles [60, 61, 67, 68].

7. Conclusion

The biological treatment of EOMPs has shown significant potential in mitigating the adverse impacts of these contaminants on the environment and public health. The integration of advanced biological processes with emerging technologies offers promising avenues for enhancing the efficiency and effectiveness of EOMP removal from wastewater. The challenges posed by the recalcitrant nature of many EOMPs and the variability in wastewater composition necessitate ongoing research and innovation. Bioaugmentation, synthetic biology, and the use of AI and ML for real-time monitoring and control are some of the future directions that hold considerable promise. Furthermore, hybrid treatment systems and decentralized bioreactors could provide flexible and robust solutions tailored to local conditions and specific pollutant profiles.

Although substantial progress has been made in the biological treatment of EOMPs, continued efforts in research, technological development, and regulatory frameworks are essential to address the evolving landscape of wastewater contaminants. The collaboration between researchers, industry stakeholders, and policymakers will be crucial in advancing these technologies and ensuring sustainable water management practices. The ongoing exploration of novel microbial consortia, engineered microbes, and integrated treatment systems will play a pivotal role in overcoming current limitations and achieving higher removal efficiencies for a broad spectrum of EOMPs.

Compliance with ethical standards

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The authors declare that they have no conflict of interest to be disclosed.

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