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Pharmaceuticals and personal care products in aquatic environments and their removal by algae-based systems

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Abstract: The consumption of pharmaceuticals and personal care products (PPCPs) has been widely increasing, yet up to 90-95% of PPCPs consumed by human are excreted unmetabolized. Moreover, most of PPCPs cannot be fully removed by wastewater treatment plants (WWTPs), which release PPCPs to natural water bodies, affecting aquatic ecosystems and potentially humans. This study sought to review the occurrence of PPCPs in natural water bodies globally, and assess the effects of important factors on the fluxes of pollutants into receiving waterways. The highest ibuprofen concentration (3738 ng/L) in tap water was reported in Nigeria, and the highest naproxen concentration (37700 ng/L) was reported in groundwater wells in Penn State, USA. Moreover, the PPCPs have affected aquatic organisms such as fish. For instance, up to 24.4×10^3 ng/g of atenolol was detected in *P. lineatus*. Amongst different technologies to eliminate PPCPs, algae-based systems are environmentally friendly and effective because of the photosynthetic ability of algae to absorb CO_2 and their flexibility to grow in different wastewater. Up to 99% of triclosan and less than 10% of trimethoprim were removed by *Nannochloris* sp., green algae. Moreover, variable concentrations of PPCPs might adversely affect the growth and production of algae. The exposure of algae to high concentrations of PPCPs can reduce the content of chlorophyll and protein due to producing reactive oxygen species (ROS), and affecting expression of some genes in chlorophyll (*rbcL*, *psbA*, *psaB and psbc*).

Keywords: Algae; Genes; Groundwater; Pharmaceuticals; Wastewater

1. Introduction

Water resources are increasingly becoming limited, and quality of water bodies has been seriously threatened by the presence of different contaminants that pose a risk to the human health and the aquatic environments (Balusamy et al., 2020, Wu et al., 2020). Of current major concern are emerging organic micropollutants such as pharmaceuticals and personal care products (PPCPs) (Mojiri et al., 2019a). PPCPs are designed to have the maximum impacts at low concentrations; consequently, they have a significant effect on environments and humans at trace concentrations (Patel et al., 2019). Thus, the increasing use of PPCPs has raised questions regarding their potential risks to human and ecosystems, especially by promoting the development of antibiotic resistance genes (Zhou et al., 2012). It is therefore important to critically review the concentrations and treatment of PPCPs in water bodies around the world, as the aim of this study.

PPCPs are employed for prevention or treatment of diseases in animals and humans, as well as to enhance the quality of daily life. PPCPs may easily dissolve in water and not evaporate easily in normal conditions. These properties allow PPCPs to reach water sources over several modes (Wang et al., 2019). Generally, PPCPs with the concentration varying from ng/L to μg/L have been found in water and wastewater samples. The occurrence of PPCPs in aquatic environments leads to the harmful toxicological consequences and different ecological impacts on the environment and human (Wang et al., 2020).

Most wastewater treatment plants (WWTPs) cannot fully eliminate the emerging micropollutants (MPs). Therefore, alternative methods have been sought with high performance in order to overcome this challenge. Several methods for the treatment of MPs have been investigated, physicochemical (such as advanced oxidation process, AOP) (Kudlek et al., 2018) and biological methods (such as membrane bioreactor-MBR, moving bed biofilm bioreactor-MBBR, algae-based methods) (Besha et al., 2017, Abtahi et al., 2018). One of the efficient methods in removing PPCPs from water bodies is bioremediation using algae/microalgae (Larsen et al., 2019). Each method used for the removal of PPCPs has some advantages and disadvantages (Table A.1 in supplementary file). For instance, while AOPs have a smaller footprint and a better performance in comparison with conventional methods, they consume a high amount of energy and produce secondary pollutions. Moreover, MBR involves a high operation cost, and contains less efficient oxygen transfer. However, MBR has advantages of enhanced biodegradability of hydrophobic organic micropollutants, and a smaller footprint in comparison with conventional treatment methods. Of special interest are algae-based systems with several advantages including generating biomass for producing biofuel or biochar, absorption of CO_2, low-cost, and high efficiency for the removal of PPCPs. Villar-Navarro et al. (2018) expressed that algae-based systems are considered as an efficient and eco-friendly technique to clean water and wastewater without threatening human health. Gentili and Fick (2017) removed 18 emerging micropollutants with removal efficiency between <10% to >90%, using the algae-based technique during 1 week. However, there is a demand for further research on the occurrence and removal of PPCPs in water environments (Al-Mashaqbeh et al., 2019). Therefore, this review paper attempts to present a detailed assessment of PPCP pollution and treatment in the aquatic systems.

2. Pharmaceuticals and personal cares products

PPCPs are a group of emerging micropollutants which contain “any product applied for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock” (US EPA). PPCPs comprise thousands of chemicals that make up cosmetics, fragrances, drugs (containing over-the-counter drugs), and veterinary medicines (Dhodapka and Gandh, 2019). Generally, several thousands of PPCPs are produced per year around the world, and the discharge and accumulation of PPCPs in the environments are considered as an unavoidable by-product of a modern lifestyle (Tran et al., 2015). PPCPs can be simple aromatic molecules (e.g. anesthetic propofol), simple aliphatic molecules (e.g. vasodilator and nitroglycerine), or more complex molecules with low molecular weight (e.g. statin and atorvastatin) and with heavy molecular weight biopharmaceuticals (e.g. hyaluronic acid) (Taylor and Senac, 2014).

2.1. Pharmaceuticals

Pharmaceuticals usually comprise over the counter (OTC) or prescription human/veterinary drugs and nutraceuticals applied for prophylaxis/therapeutic and health supplements reasons (Cizmas et al., 2015). Pharmaceuticals found in aquatic environments can be divided into five main groups (Table A.2 in the supplementary file) including antibiotics, analgesic and antipyretic (counting nonsteroidal anti-inflammatory), cardiovascular agents (blood lipid regulator (BLR) or antilipemic agents, β-blockers), central nervous drugs (e.g. antipsychotic and antidepressant), endocrinology treatment (Liu and Wong, 2013). These therapeutic agents are constantly discharged to the water bodies from point and non-point industrial including domestic sources (Zhou et al., 2012).

2.1.1. Antibiotics

There has been a worldwide request for antibiotics during the last decades due to effective treatment of infectious diseases induced by the fast urbanization and increasing population as well as for the growth promotion of animals (Bao et al., 2021). Antibiotic usage has increased by 65% during 2000-2015. Additionally, the total antibiotic consumption for livestock was 63,151 tons in 2015, which is expected to be increased by 15% in 2030. It is estimated that 30% to 90% of antibiotics used by an organism is excreted without metabolism (Mojiri et al., 2021b). Based on the chemical characteristics and mechanisms of action, antibiotics can be divided into seven classes as: penicillins/β-lactams, aminoglycosides, tetracyclines, quinolones, macrolides, and sulfonamides, lincosamides (Bhagat et al., 2020). Penicillins/β-lactams are the most consumed antibiotics (Carvalho and Santos, 2016).

Because antibiotics are employed to kill or prevent pathogenic bacteria at trace concentrations, their presence in natural environments may cause a critical risk for the aquatic communities comprising non-targeted organisms (Serra-Compte et al., 2021). Manzetti and Ghisi (2014) stated that maximum concentrations of antibiotics in aquatic environments are mostly detected in wastewater treatment plants.

2.1.2. Analgesic and antipyretic, and nonsteroidal anti-inflammatory drugs (NSAIDs),

Antipyretic analgesics are a type of diverse substances comprising acidic (nonsteroidal anti-inflammatory drugs, NSAIDs) and nonacidic (pyrazolone and paracetamol) drugs (Hinz and Burne, 2007). NSAIDs are mostly the derivatives of carboxylic acid that inhibit prostaglandin synthesis produced by cyclooxygenase enzymes (Derle et al., 2006). NSAIDS reduce the production of prostaglandins through the blockage of cyclooxygenase (COX) enzymes controlling inflammation, pain and fever. NSAIDs are the most common OTC medicines to ease the pain and fever, and control inflammation (Duan and Zhao, 2021; Márta et al., 2018). For instance, annual NSAIDs prescriptions in the US, Canada, and UK were estimated to be more than 100 million in 2015 (He et al., 2017). Ibuprofen, aspirin, diclofenac, acetaminophen, naproxen and ketoprofen are the most consumed NSAIDs (He et al., 2018). The exposure to NSAIDs causes severe toxicity in aquatic environments even at ng/L or μg/L concentrations (Thalla and Vannarath, 2020). One of the most widely used analgesic and antipyretic agents is paracetamol (Shakeel et al., 2013). Paracetamol contains a benzene substituted by a hydroxyl group and the nitrogen atom of an amide group at the (1,4) *para* positions (Żur et al., 2018), which can only be degraded by hydroxylation and cleavage of the aromatic ring. Hence, traces of paracetamol can remain untreated in sewage water of various concentrations (Al-Kaf et al., 2017).

2.1.3. Cardiovascular agents (Blood lipid regulator (BLR) or antilipemic agents, Blood Pressure, and β-blockers)

Cardiovascular disorders are the second most common cause of deaths around the world. Thus, consumption of cardiovascular drugs is significantly high. The presence of cardiovascular compounds in aquatic environments can have a long-term impact even at trace concentrations (Giebułtowicz et al., 2016).

Blood lipid regulators (BLRs) are highly consumed as a medicine not only for the treatment of unhealthy cholesterol levels but also for cardiovascular diseases and postmenopausal complications (Peña-Méndez et al., 2020). Among the prescribed medications around the world, the cardiovascular drugs and lipid regulating agents are two of the most consumed drugs. For instance, 24.5% of the most commonly prescribed drugs in the United States are classified as cardiovascular drugs and lipid regulating agents (Zhang et al., 2020). Most used BLRs are fenofibrate, bezafibrate, gemfibrozil and clofibrate, which are commonly reported in aquatic environments (Rosal et al., 2010). These are considered as the resistant drug to biodegradation with a strong persistence in the environment (Mourid et al., 2020). In Ontario (Canada), Patel et al. (2019) reported the high concentration (ng/L) of blood pressure drugs (7333600 of metoprolol, 116000 of diltiazem, 1200000 of furosemide, and 22900 of amlodipine) in water bodies, which has been resulted by discharges of five manufacturing facilities. Apart from that, β-blocker drugs stand as the third most common pharmaceuticals recorded in the aquatic environment (Rezka and Balcerzak, 2015). Rezka and Balcerzak (2015) stated that atenolol, metoprolol, nadolol, propranolol, sotalol, and timolol are the most common β-blockers detected in aquatic environments.

2.1.4. Central nervous system (CNS) drugs, and antipsychotic and antidepressant

Caffeine and diazepam are the most consumed CNS agents. Due to broad application of caffeine (presence in coffee, sodas, tea and chocolates as well as in medicaments and appetite modulators), caffeine has been reported in different water bodies around the world (Zarrelli et al., 2014). That is considered as a stable compound under different environmental conditions. Because of small pKa (0.7), high water solubility (21.7 g L^{-1}), low octanol/water partition coefficient (-0.07), along with insignificant volatility and molecular mass of 194.19 g, caffeine is considered as highly persistent in aquatic environments (Mizukawa et al., 2019). The presence of caffeine in water sources reveals that this compound is not completely eliminated from sewage treatment plants. Benzodiazepines (BDZ) is a group of psychiatric substances which affect the central nervous system, having anxiolytic, sedative and hypnotic impacts. Diazepam, alprazolam, oxazepam and lorazepam are the most important agents in this group (Calisto et al., 2011).

2.1.5. Endocrinology treatment (ET) drugs

Drugs consumed in endocrine therapy can be remarked as endocrine disruptors and therefore require consideration because of their specific hormonal or anti-hormonal properties (Besse et al., 2012). Research demonstrated that hormones are environmentally stable and potentially deleterious even at very low concentrations (Olatunji et al., 2017). For instance, 17α-ethynylestradiol has the potential to trigger numerous endocrine dysfunctions impacts at exposure levels as low as 1 ng/L (Wee et al., 2020). The most reported hormones are listed as: testosterone, estrone, progesterone, 17β-estradiol, and 17α-ethynylestradiol (Wee et al., 2020). Disruption of the endocrine system can lead to various developmental, neurological, reproductive, immune and metabolic disorders (Ingre-Khans et al., 2017).

2.2. Personal care products

Personal care products are various chemicals applied in soaps, lotions, fragrances, toothpaste, shampoos and sunscreens (Brausch and Rand, 2011). Liu et al. (2013) reported that the sunscreen UV filters (e.g. 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC), 4-methyl-benzilidine-camphor (4MBC)), antimicrobial agents (e.g. triclosan, triclocarban), insect repellants (e.g. N,N-diethyl-m-toluamide (DEET)), synthetic musks (e.g. nitro musks such as musk xylene, musk ketone, musk moskene, musk ambrette and musk tibetene) polycyclic musks (such as galaxolide and toxalide)], and preservatives (e.g. parabens) are the most widely used personal care products. The US, China and Japan are the top countries in the consumption of personal care products (Liu et al., 2013). Eriksson et al. (2003) stated that personal care products are one of the most frequently detected compounds in water bodies in the world.

Peck (2006) stated that sunscreen agents (UV filters) are broadly added to lotions and cosmetics as protection against harmful UV radiation. The hydrophobicity of these compounds (log K_{ow} 5–8) reveals the potential for bioaccumulation.

Triclocarban and triclosan are the most commonly reported antimicrobial agents, which have been added in many personal care products (such as hand disinfecting soaps, medical disinfectants, body wash products, kitchen detergents and toothpastes) (Tsai et al., 2008). Both have the hydrophobic nature, and are persistent in the environment whether aerobic or anaerobic (Zhao et al., 2010).

For a long time, DEET, a lipophilic organic compound, has been applied as an insect repellent, and can be frequently found in aquatic environments (Sun et al., 2016). DEET is mobile and persistent. In the central east coast of Australia, DEET was reported in 97% of surface-water samples collected from waterways (Costanzo et al., 2007).

Synthetic musk fragrances are widely added to several personal care products, such as shampoo, deodorant and detergents for scent enhancement (Peck, 2006). As mentioned above, two types of synthetic musk fragrances are nitro musk fragrances and polycyclic musk fragrances. The nitro substituents can be reduced to the amino metabolites of these compounds (Peck, 2006). Parabens are also employed as preservatives in products such as food and pharmaceutics. This group comprises propylparaben, methylparaben, butylparaben, ethyl paraben, and benzyl paraben (Peck, 2006).

3. Presence of PPCPs in water bodies

Several studies have reported that up to six million PPCPs are commercially available, and their consumption is increasing by 3-4% by weight per year (Delgado et al., 2020). PPCPs reach the environment as components of animal/human wastes, after incomplete absorption and excretion from the body, as well as emissions of medical, agricultural, industrial or household wastes (Taylor and Senac, 2014). Environmental pollution with PPCPs has become a major public concern since these compounds have been approved to have negative effects on aquatic organisms (Zhang et al., 2021), as well as having a role in increasing antibiotic-resistant bacteria (Oliveira et al., 2015). Bu et al. (2013) expressed that several PPCPs are persistent or pseudo-persistent in the environment and hazardous to non-target organisms. PPCPs may arrive water sources through direct release by wastes from hospitals, industries and households. (Molina et al., 2020). For emphasis, several studies (Xu et al., 2019; Liu et al., 2021) have revealed that the presence of PPCPs in aquatic environments has mostly derived from anthropogenic activities such as the treatment and discharge of different kinds of wastewater, aquaculture, livestock breeding, and landfill.

The physicochemical properties of PPCPs such as molecular weight, octanol-water partition coefficient (K_{OW}), octanol-water distribution coefficient (D_{OW}), organic carbon partition coefficient (K_{OC}), and ionization constant (pK_a) can affect the fate of PPCPs in aquatic environments (Delgado et al., 2020).

The K_{OW} (equation 1, Gutiérrez et al., 2021) is frequently applied to predict the adsorption of emerging microcontaminants on solids, with log $K_{OW}<2.5$ indicating low sorption potential, $2.5<\log K_{OW}<4$ indicating medium sorption potential, and log $K_{OW}>4$ showing high sorption potential (Lou et al., 2014). On the other hand, the K_{OW} specifies pollutant mobility, where the compounds with $K_{OW}<1.5$ tend to stay in the dissolved phase (more mobility) and are more likely to occur in water (Karnjanapiboonwong et al., 2011). Tijani et al. (2013) stated that most PPCPs are highly hydrophilic with low K_{OW} and partially soluble in aqueous media.

$$K_{OW} = \frac{\text{concentration in } n\text{−octanol}}{\text{concentration in water}} \qquad (1)$$

Wells (2007) expressed that D_{OW}, a pH-dependent coefficient, is a better measure of hydrophilicity. Dubey et al. (2021) stated that D_{OW} can be calculated (equations 2 to 4) based on the K_{OW} values with consideration the pH value.

Neutral compounds:

$$\log D_{OW} = \log K_{OW} \qquad (2)$$

Acidic compounds:

$$\log D_{OW} = \log K_{OW} + \log \frac{1}{1+10^{pH-pKa}} \qquad (3)$$

Basic compounds:

$$\log D_{OW} = \log K_{OW} + \log \frac{1}{1+10^{pKa-pH}} \qquad (4)$$

log Koc <1.0 often displays the low sorption potentials, log Koc <3.0 are more likely to show the medium sorption potentials, and log Koc >3.0 have high sorption potentials onto the particulate phase (Koumaki et al., 2021). Generally, as the log Kow increases, the log Koc would also be anticipated to increase (Crookes and Fisk, 2018).

The pK_a can affect the mobility, movement of pollutants from one phase to another (e.g., soil-water movement), of the PPCPs (Kim and Zoh, 2016). Several micropollutants, which enter wastewater treatment plants, comprise ionizable functional groups with pKa values within pH range of 6.2 to 8.1. For example, 40% of PPCPs with a dominant substance class in wastewater influents include at least one functional group with pKa in the range of 5-10 and cationic-neutral speciation, and 10% include at least one functional group with neutral-anionic speciation in the same pKa range. Hence, the degree of speciation of such ionizable micropollutants would vary across activated sludge systems with different operational pHs (Glude et al., 2014).

Usually, the pollution and fate of PPCPs in water bodies are investigated through the analysis of water samples, which is generally limited to monitoring parent compounds (Wilkinson et al., 2017). The reported concentration of PPCPs in water bodies worldwide is shown in Table 1, suggesting that the maximum PPCPs was reported for ibuprofen at 3738 ng/L in tap water in Nigeria. Moreover, ciprofloxacin was found at 10000 – 1100000 ng/L in Isakavagu-Nakkavagu rivers (India). Also, naproxen at 37700 ng/L was reported in a groundwater wells sample in Penn State (USA). The maximum PPCPs concentration in wastewater samples was reported for acetaminophen in Penn State's wastewater treatment plant (USA). Therefore, a significant amount of PPCPs has been reported in water sources worldwide.

Table 1: Reported PPCPs in water bodies around the world

PPCPs in water samples can be analyzed with different methods (Table 2), for example gas chromatography-mass spectrometry (GC-MS), although the most widely used technique currently is ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) (Zhou et al., 2012; Mojiri et al., 2019b; Hoi et al., 2021). Cao et al. (2020) and Wang et al. (2020) employed UHPLC for monitoring the PPCPs in water. The UHPLC applies smaller particle size chromatographic columns (<2.0 μm) and reaches higher pressure than traditional

LC. The application of UHPLC leads to observing the peaks in a shorter run time and consequently consumes less mobile phases (Oliveira et al., 2015).

Table 2: Techniques employed to analyze PPCPs in aqueous solutions

3.1. Effects of PPCPs on aquatic environments and microorganisms

Xu et al. (2019) expressed that although the PPCPs are found in water bodies at trace concentrations (ng/L to μg/L), evidences have suggested that PPCPs are potentially harmful to environments, organisms and human health, by inducing teratogenicity, mutagenicity, carcinogenicity, endocrine-disrupting effects as well as reproductive developmental toxicity (Ebele et al., 2017). Table 3 shows the accumulation of PPCPs in fishes around the world.

Besides bioaccumulation, chronic exposure to PPCPs can occur, which makes them more toxic to the organisms concerned (Pereira et al., 2015). For instance, Larsson et al. (2000) stated that the presence of PPCPs in the aquatic environment possibly impairs reproduction and elicits sexual anomalies in *Cyprinus carpio, Rutilus rutilus*, and *Oryzias latipes*. Moreover, Pereira et al. (2015) expressed that exposure to hormones, such as estrogens, may cause fish feminization through sexual differentiation. Bolong et al. (2009) listed some problems about exposure of aquatic organisms to PPCPs as follows:

(A) Reproductive and immune function interference in Baltic Sea fishes affecting population decline

(B) Eggshell thinning and transformed gonadal development in birds

(C) Changes in reproductive endocrine function in fishes

(D) Masculinization of marine gastropods

Table 3: Reported PPCPs in fishes

4. PPCPs removal via algae-based systems

Using algae in treating wastewater is a clean, environmentally friendly and effective way because of the photosynthetic capability of algae to absorb CO_2 and their adaptability to grow in different types of wastewater (Villar-Navarrow et al., 2018). Elrayies (2018) reported that each pound of algae biomass consumed 1.8 pounds of CO_2. Furthermore, algae produce 60% to 75% of the oxygen required for humans and animals even though they represent only 0.5% of total plant biomass. Moreover, its operation is simple, and diminishes sludge management issues since it produces algae biomass, which may be employed as biofuel (Bhatt et al., 2014). Apart from that, algae-based methods for treatment of water and wastewater can consume lower energy in comparison with several wastewater treatment approaches. For instance, Yadav et al. (2021) reported that microalgae use 0.2 kW-h/m^3, while conventional treatment methods could consume up to 2 kW-h/m^3. Craggs et al. (2013) expressed 50% energy reduction during treatment of water by using microalgae compared with conventional treatment methods. Algae include both macroalgae and microalgae, and microalgae are usually better in growth rate and high lipid content than macroalgae (Elrayies, 2018). Main algae-based systems, including stirred-tank photobioreactors (STPs), high rate algal ponds (HRAPs), rotating algal biofilm reactors (RABRs), and membrane photobioreactor (MPBRs) have been reported to treat water and wastewater, and remove emerging contaminants (Zimmo et al., 2003, Craggs et al., 2014, Mohammed et al., 2014, Fica and Sims, 2016, Praveen et al., 2016).

STPs have a simple design and are conventional reactors, and usually include a glass tank continuously stirred by impellers or baffles (Ismail et al., 2017). At the bottom of reactor, CO_2-enriched air is bubbled to supply a carbon source for algae growth (Mohan et al., 2014). STPs are suitable for shear sensitive microalgae cultivation (Verma et al., 2018). Main disadvantage of STPs is the low surface-area-to-volume ratio, which in turn decreases light-harvesting effectiveness (Mohan et al., 2014). Ismail et al. (2017) removed 95% of p-aminophenol (an intermediate for the manufacture of paracetamol and acetanilide) and COD by a stirred-tank photobioreactor using microalgal-bacterial consortium (*Chlorella* sp. was the main microalgal strain) with hydraulic retention time (HRT) of 4 days. Mojiri et al. (2021a) removed 35.4% of carbamazepine, 33.1% of sulfamethazine and 36.5% of tramadol with a STP containing *Chaetoceros muelleri*.

In comparison with conventional wastewater stabilization ponds (WSPs), HRAPs offer an enhanced wastewater treatment by overcoming several drawbacks of WSPs (such as limited nutrient and pathogen removal, and poor and highly variable effluent quality) (Park and Craggs, 2011). The resource recovery of algal biomass and water as effluent treated to a high standard are other advantages of HRAPs over WSPs (Sutherland et al., 2014). HRAPs are shallow (0.2–0.5 m), continuous raceways around which wastewater is gradually mixed by a paddlewheel (Mehrabadi et al., 2015). The photosynthesis of algae in HRAPs causes dissolved oxygen supersaturation (up to 20 g/L), which enhances bacterial oxidation of biodegradable dissolved and particulate organic matter (Craggs et al., 2012). Hom-Diaz et al. (2017) employed the HRAPs for the removal of ciprofloxacin. The outdoor batch assays during daytime showed 40.8% of ciprofloxacin removal at initial concentration (C_i) of ciprofloxacin 2.25 mg/L, during day time. However, the indoor light batch assays indicated 83.7% of ciprofloxacin removal at C_i of ciprofloxacin 1.11 mg/L. de Godos et al. (2012) removed up to 69% of tetracycline (C_i= 2 mg/L) by HRAPs. Lindberg et al. (2021) investigated the HRAPs (including Nordic microalgal strains) for removal of 14 Active pharmaceutical ingredients (APIs). 69% of APIs were removed during 6 days. Matamoros et al. (2014) removed less than 30% of carbamazepine and 2,4-D, 40-60% of diclofenac and celestolide, 60-90% of ketoprofen, galaxolide and tonalide, and more than 90% of caffeine, acetaminophen and ibuprofen.

RABRs provide a very good condition for algal biomass production (Hoh et al., 2016). In the RABR, a vertically material for the attachment of algae rotates through the water or wastewater for absorbing nutrients, then rotates out of the water to accelerate CO_2/O_2 exchanges and light exposure (Zhao et al., 2018). RABRs have several advantages such as simple installation, improving growth of biomass, a good gas exchange mechanism, and high nutrient removal efficiency (Woolsey, 2011). The maximum biomass production rate in a pilot-scale RABRs reached 19 g m^{-2} d^{-1} (Wang et al., 2018). Hassard et al (2015) reported a removal efficiency of 52%-95% for ciprofloxacin, tetracycline and trimethoprim during running a modified RBAR. Chen et al. (2021) removed 70-100% of five PPCPs (oxybenzone, ibuprofen, bisphenol A, triclosan, and N, N-diethyl-3-methylbenzamide-DEET), which the elimination of PPCPs was mostly attributed to the degradation by the algae.

MPBRs with a high potential in removal of nutrients from wastewater, have been considered as a system that couples the culture of microalgae with a continuous biomass separation using a membrane filtration system (Novoa et al., 2020). MPBRs enable the system to operate with a short HRT without the washout of microalgae (Honda et al., 2017). Application of MPBR in large-scale is limited, which can be considered as the main drawback of MPBRs, because of membrane fouling and consequent permeate flux reduction (Novoa et al., 2020). Thus, the application of MPBRs for the removal of emerging contaminants has not been widely reported. 84.3% of an emerging contaminant (atrazine) was removed by a microalgal-bactrial MPBR under a hydraulic retention time of 12 h and initial pollutant concentration of 0.01 mg/L (Derakhshan et al., 2019).

In general, several studies (Matamoros and Rodríguez, 2016) expressed that algae-based treatment methods can increase the removal of emerging contaminants from aquatic environments. For instance, 28% of levofloxacin was eliminated by *Chlorella vulgaris* (Xiong et al., 2017), while 50–64% of clarithromycin was eliminated by *Chlamydomonas* sp.

(Escudero et al., 2020). The removal efficiencies of PPCPs with different algae and microalgae species are shown in Table 4. Liu et al. (2021) stated that four main pathways (Figure 1) to remove PPCPs from water samples are the biodegradation, biosorption, photodegradation and volatilization. Matamoros et al. (2015) expressed that although the ability of algae-based wastewater treatment systems to eliminate nutrients and heavy metals has been studied well, the removal of PPCPs with the algae still needs more studies. Research (Matamoros et al., 2015; Gruchlik et al., 2018) stated that biodegradation and photodegradation are the main removal processes during the elimination of PPCPs by algae-based systems. In reality, most PPCPs can be eliminated by more than one pathway (R. Liu et al., 2021).

Figure 1: Mechanisms of PPCPs removal by algae-based technique

Table 4: Algae and microalgae to remove PPCPs

4.1. Biodegradation

Biodegradation is one of the main elimination mechanisms of PPCPs from aqueous solutions by algae-based systems (Hultberg and Bodi, 2018). Microbial biodegradation comprises varied and complementary mechanisms, from adsorption of contaminants onto biomass, to mineralization where final degradation products are inorganics (e.g., CO_2 and H_2O) and biomass (Garcia-Becerra and Ortiz, 2018). Papazi et al. (2017) stated that several factors (such as concentration of organic pollutants, temperature, pH, oxygen content, and light intensity) can affect the biodegradation. For instance, Papazi et al. (2017) stated that algal cells apply more energy for biodegradation at the highest concentrations of organic pollutants in comparison with the energy applied for lower concentrations. Furthermore, Hong et al. (2008) expressed that when two or more organic pollutants are present in influent, there will be competition for biodegradation by different compounds. Additionally, Al-Dahhan et al. (2018)

stated that both biodegradation rate and growth rate of microalgae can be enhanced with increasing light intensities and adding inorganic carbon sources (such as sodium bicarbonate and CO_2).

The main mechanisms of biodegradation can be categorized as metabolic degradation that PPCPs serve as the carbon sources or electron donors/acceptors for algae; and co-metabolism that additional organic substrates serve to both sustain biomass production, and act as an electron donor for the non-growth substrate (Xiong, 2021). Hena (2021) expressed that biodegradation depends on the cellular metabolism of microalgae that involves a series of complex enzymatic acts. Biodegradation quality rate of organic pollutants with algae can be calculated based on equation 5 (Zhang et al., 2010). In the equation, to exclude non-biodegradation, a blank is set with only a culture medium without algae.

$$DR = \left[\frac{I_q-(M_q+C_q+N_q)}{I_q} \times 100\right] \qquad (5)$$

where DR (%) indicates the biodegradation quality rate, the initial concentration of pollutant is shown by *Iq*, the cellular residual amount of pollutant is shown by *Cq*, *Mq* defines the medium residual quantity of contaminant, and the non-biodegradation amount of contaminant is shown by *Mq*.

Algae include enzymes that metabolize a range of xenobiotics in three phases (Wang Y et al., 2017):

Phase-I contains oxidation, reduction, or hydrolysis that converts lipophilic xenobiotics into more hydrophilic compounds to facilitate their excretion. Cytochrome P450s are microsomal heme-thiolate proteins anchored in the membrane, and usually catalyze the primary step of detoxification.

Phase-II is characterized by the addition of hydrophilic moieties to accelerate excretion. Xenobiotics with −COOH, −OH or $-NH_2$ and metabolites from phase-I might be conjugated with glutathione/glucuronic acid catalyzed by glutathione S-transferases/glucosyltransferases.

Phase-III comprises compartmentation of xenobiotics in vacuoles or cell walls. The capability of algae to detoxicate xenobiotics is similar to the mammalian liver and therefore algae are remarked as “green livers” for the detoxification of pollutants. 54% and 65% removal of malathion by *S. platensis* and *A. oryzae* were attributed to biodegradation (Mustafa et al., 2021).

4.2. Biosorption, and bioaccumulation and biodegradation

Biosorption, and bioaccumulation and biodegradation (Figure 2) are the interactions and concentration of organic contaminants in the biomass, either living (bioaccumulation) or non-living (biosorption) (Chojnacka, 2010). This could be divided into three stages: 1) a physicochemical reaction between the cell surface and contaminants, 2) a fairly slow transfer of molecules over the cell membrane, and 3) bioaccumulation and biodegradation (Xiong et al., 2021).

The biosorption of contaminants is a complex procedure containing integration of some active and passive mechanisms. These mechanisms vary based on the type of biomass, and culture conditions (Muñoz et al., 2006). Moreover, algae biosorption processes have generally been attributed to the structure of cell wall comprising functional groups (such as amino, carboxyl, hydroxyl and sulphate) that can have a role as binding sites for pollutants via electrostatic attraction, ion exchange and complexation (Tuzen et al., 2009). For instance, hydrogen bonds were reported as the key mechanism for the elimination of sulfamethoxazole and sulfacetamide by marine algae (Navarro et al., 2014). Aravindhan et al. (2009) expressed that hydrophobic and donor acceptor interactions have been remarked as important processes in biosorption of organic compounds.

Silva et al. (2019) stated that the progress of the biosorption procedure contains four phases: (I) mass transfer of the sorbate from the bulk liquid to the hydrodynamic boundary layer around the biosorbent particles;

(II) film diffusion through the boundary layer to the external surface of the biosorbent; (III) intraparticle diffusion toward the interior of the biosorbent particle; and (IV) energetic interaction between the sorbate molecules and the sorption sites.

The biosorption process is usually modeled by the equilibrium distribution via equation 6 (Aravindhan et al., 2009).

$$q_e = (C_0 - C_e)\frac{V}{W} \qquad (6)$$

where initial and equilibrium concentrations of pollutants in water are defined by C_0 and C_e, equilibrium concentration (mg/g) of pollutant in biosorbent is shown by q_e, and volume of the solution (L) and the mass of algae use (g) are shown by V and M, respectively.

Bioaccumulation is described as the intracellular accumulation of sorbate (Chojnacka, 2010). Although bioadsorption is the first step of bioaccumulation, not all contaminants adsorbed onto the surface of microalgae can reach into the cell (bioaccumulation) (Xiong et al., 2021). The bioaccumulation potential of a chemical in aquatic organisms plays an important role in the evaluation of environmental hazards. A high bioaccumulation potential of a chemical in biota indicates the possibility of toxic impact being encountered in aquatic organisms (Geyer et al., 2001).

Xiong et al. (2021) stated three main pathways for transporting PPCPs (such as antibiotics) through the algae cell membrane into the cell interiors: (I) PPCPs with low molecular weights and high lipid solubility can diffuse through the cell membrane from a region of high (external) to low (internal) concentration through passive diffusion. (II) Passive-facilitated diffusion transfer PPCPs across the cell membrane with transporter proteins. (III) Energy-dependent/active uptake, which is an active transport process using energy.

Li et al. (2009) removed BPA with *S. hantzschii*, and reported that higher amounts of BPA could accumulate in cells while increasing the initial concentration of BPA. After eight days, the accumulation of BPA was 11.53, 35.30 and 45.44 ng BPA/mg fw (fresh weight) at initial concentrations of 5.00, 7.00 and 9.00 mg/L BPA, respectively. Wang et al. (2019) stated that with increasing time, the intracellular absorption is greater than the extracellular adsorption during removal of nonylphenol by marine algae.

Figure 2: Bioaccumulation and biosorption of PPCPs in algae
(*ESP (extracellular polymeric substance); **Source: Xiong et al., 2021, the permission for re-using the figure received on 17 August 2021 from Elsevier)

4.3. Photodegradation

The photodegradation is a transformation process in which complex molecules are decomposed, and is categorized into indirect and direct photodegradation (Jiménez-Bambague et al., 2020). If the PPCPs can absorb light under the deployed irradiation condition, they would have a potential to undergo direct photolysis. However, if the PPCPs could not absorb the light, then indirect photodegradation possibly occurs in the presence of photosensitizers (Liu et al., 2021). Yang et al. (2018) stated that algae, with excretion biopolymers such as polysaccharides and proteins, can enhance the photodegradation of PPCPs. Additionally, Tian et al. (2018) expressed that chlorophyll can enhance the photodegradation of emerging contaminant (such as chlortetracycline). Wei et al. (2021) stated that chlorophyll in the intracellular organic matters may play a role as photosensitizers since substituted porphyrin ring is one of the important components of chlorophyll that has a vital role in absorbing energy from light sources. Norvil et al. (2016) expressed that these biopolymers can increase the photodegradation in several mechanisms, containing redox cycling, catabolic process, production of hydroxyl radicals, and inhibiting photo-oxidation by competitive reaction with radicals (Sutherland and Ralph, 2019). Overall, algae can facilitate photodegradation by enhancing the free radical yield (equations 7 and 8; Wang et al., 2017). Usually, photodegradation can be calculated by equation 9 (Matamoros et al., 2016).

$$O_2 + \text{cell organelles o algae} + hv \longrightarrow {}^1O_2/O_2^{\circ -} \qquad (7)$$

$$O_2 + \text{cell secretion of algae} + hv \longrightarrow {}^{\circ}OH \quad (8)$$

$$Photodegradation = \frac{(K1-K2)}{K3} \times 100 \quad (9)$$

K_1 defines the organic pollutants concentration in uncovered and aerated control reactor, K_2 shows the organic pollutants concentrations in covered and aerated control reactors, and K_3 indicates the concentration of organic pollutants in reactors fed with microalgae.

40-60% of diclofenac was removed by *Chlorella sorokiniana* which was mostly attributed to the photodegradation process (Wilt et al., 2016).

Propranolol, naproxen, ketoprofen, and gemfibrozil are reported to undergo photodegradation after reaching the aquatic environments. Moreover, paracetamol is remarked as biodegradable and photodegradable, whereas fenofibric acid is considered as a compound with rapid photodegradation potential (Jiménez-Bambague et al., 2020). The rapid direct photodegradation of ketoprofen (and other PPCPs with similar structure) might be justified by the point that carbonyl moiety is in conjugation with two aromatic rings. When the carbonyl is highly conjugated, the energy of the n-π* transition is reduced, causing a very reactive triplet state (Lin and Rienhard, 2009).

In algae-based system, Liu et al. (2021) reported the abatement efficiencies of > 80% for photodegradation of norfloxacin, ciprofloxacin and enrofloxacin, and abatement efficiencies 62–85% for cephalosporins photodegradation, and removal efficiency of > 90% for photodegradation of triclosan, metronidazole, chlortetracycline, paracetamol and anilines. The photodegradation products can be either less or more toxic than the parent compounds; for instance, photodegradation products from carbamazepine are more toxic (Patel et al., 2019). Apart from that, Jiménez-Bambague et al. (2020) stated that recalcitrant and highly hydrophilic PPCPs (such as carbamazepine) are very stable and resistant to biodegradation and photodegradation.

The physicochemical properties of the PPCPs, the intensity and wavelength of light, the physicochemical properties of the water and the algae species can affect the phytodegradation (Sutherland and Ralph, 2019). For instance, Norvill et al. (2016) expressed that the photodegradation of PPCPs by algae-based systems is increased in the presence of Fe^{3+} in water because of photosensitive organic molecules. Complex of carboxylic acids with iron further increases the hydroxyl radical production by photosensitive Fe^{3+}. Apart from that, Bai and Acharya (2019) reported that the presence of nitrate in the waterway could enhance the indirect photolysis of triclosan and hormone active substances in an algae-based system. Moreover, the presence of oxygen can affect the photodegradation. For instance, the presence of oxygen increased the photodecarboxylation of naproxen (Boscá et al., 2001).

5. Effects of PPCPs concentrations on algae

Several studies showed that PPCPs can affect the algae health (Mojiri et al., 2021a). In terms of studying the effects of PPCPs on algae, important factors which should be considered are growth rate, chlorophyll and carotenoid, and protein content (Mojiri et al., 2021b).

Xiong et al. (2020) expressed that low concentration (< 2 mg/L) of PPCPs does not have any significant effects on growth of tolerant species of algae (such as *Scenedesmus obliquus* and *Chlamydomonas*). However, Li et al. (2020) reported that roxithromycin (in concentration of 0.25 to 2 mg/L) had a significant effect on *Chlorella pyrenoidosa*. Additionally, they found that the roxithromycin (in low concentrations <0.2 mg/L) did not have a significant effect on growth rate of *Chlorella pyrenoidosa* during a short time (less than 14 days) exposure to roxithromycin, but it significantly decreased its growth rate after more than 14 days. In general, several studies (Li et al., 2020, Mojiri et al., 2021a) reported that low concentrations of PPCPs can improve the growth rate of algae because they can be used by algae as a carbon source, and they increased the chlorophyll content at the beginning. High concentrations of PPCPs are toxic to algae and can decrease their growth rate because they can damage cell structures and organelles by disturbing the homeostasis of reactive oxygen species (Xiong et al., 2019). Yang et al (2009) expressed that some antibiotic and antibacterial agents can inhibit the growth of algae even at environmentally relevant concentrations (μg/L). For instance, 17.5 μg/L of triclocarban decreased the growth rate of 50% of algae (Yang et al., 2009). Sulfamethazine and sulfamethoxazole reduced the growth rate of *S. obliquus* in concentrations of less than 0.05 mg/L (Xiong et al., 2019).

Concentration of chlorophyll is a rational assessment for the activity of algae in aquatic environments (Tretiach et al., 2007). Additionally, protein content of algae is a vital factor for algae, especially for using as feed (Chai et al., 2019). Several studies (Xin et al., 2017, Mojiri et al., 2021a and 2021b) confirmed that low concentrations of PPCPs in a short time can increase the concentration of chlorophyll and carotenoid, and protein because of two main reasons (Mojiri et al., 2021a): an increase in chlorophyll and protein content can support algae to decrease the accumulated reactive oxygen species in chloroplasts; low concentration of PPCP causes inductive impact of pharmaceutically active compounds on cells. Moreover, Chen et al. (2020b) expressed that increasing the content of protein during exposed to low concentrations of PPCPs can be justified by an increase in enzymes synthesis or other energy-producing fractions.

High concentrations of PPCPs can reduce the content of chlorophyll and protein. For instance, more than 50% of protein content and chlorophyll of microalgae was reduced by exposure to 50 mg/L of antibiotics (Mojiri et al., 2021b). High concentrations of PPCPs may inhibit the protein synthesis by binding to the 50S subunit of the ribosome. Moreover, oxidative damage resulted by PPCPs exposure may cause DNA damage (Li et al., 2020). Reducing the chlorophyll content can be explained with the reactive oxygen species (ROS)-mediated damage to the photosystem and chlorophyll biosynthesis. Chlorophyll in cells might be used as a protective way to reduce the ROS in chloroplasts (Mojiri et al., 2021b).

6. Effects of other abiotic factors on algae

Several abiotic factors such as HRT, temperature, and light intensity can affect the algae-based systems in terms of PPCPs removal (Miazek et al., 2015, Fang et al., 2015). HRT, as a key operating parameter in treatment of wastewater, is the time taken for which raw wastewater stays in a reactor before its discharge as effluent; thus, it determines the quantity of organic matter and volatile solids to be fed into the digester (Ogwueleka and Samson, 2020). Gao et al. (2016) stated that a long HRT is generally needed for nutrients uptake by algae. Valigire et al. (2012) reported that HRAPs are mostly operated at 2-8 days of HRTs, while longer HRTs have inhibited microalgal growth due to excess DO (Valigore et al., 2012). Kang and Kim (2021) stated that a short HRT combined with a long solids retention time (SRT), have provided a greatest productivity and settleability of algal–bacterial consortia.

Other important factors are the light intensity and temperature. The influence of light availability may affect the growth of microalgae as well as production of oxygen through the photosynthesis of the microalgae (Bazdar et al., 2018). Normally, an increase in light intensity promotes algal growth up to a photoinhibitory threshold; however, both the strength of this impact and the threshold differ among species (Nzayisenga et al., 2020). At full-scale outdoor conditions, current algae-based treatment systems suffer from low natural lighting for effective nutrient conversion due to the shortage of light during the rainy days. In addition, excessive light at noontime inhibits photosynthesis of algae (Yan et al., 2013). Xu et al. (2021) expressed that very low and high temperatures can considerably decrease the algal growth rate, and negatively affect wastewater treatment using algae. In high temperature serious inhibition occurs because of inactivation and denaturation of enzymes (Zhang et al., 2021).

7. Genes involved in microalgae system during exposure to PPCPs

Algae, bacteria, and fungi have catabolic genes for degrading several pollutants in water and soil (Subashchandrabose et al., 2013). Several studies (Zuo, 2019, Das and Roychoudhury, 2014) reported that reactive oxygen species (ROS) increase with increasing exposure to organic contaminants. Many genes are involved in defense mechanisms of oxidative stress, including glutaredoxin (GRX), ascorbate peroxidase (APX), and glutathione-S-transferase (GST) (Jamers and Coen, 2010).

In photosynthetic eukaryotes (such as algae), the range of glutaredoxin proteins is larger than other organisms, which may have vital roles regulating processes related to photosynthesis (Couturier et al., 2009). Chloroplast APXs are very sensitive to H_2O_2 at low ascorbate levels. During the stress, the thylakoid membrane-bound ascorbate peroxidase decreases H_2O_2 back into water with ascorbate as an electron donor (Maruta et al., 2016). A potential mechanism decreasing the toxic impacts involves GST, which catalyzes the conjugation of microcystin-leucine arginine (MC-LR) with glutathione; this procedure is generally remarked as the first step in the detoxification in various aquatic organisms (Lyu et al., 2016).

Chen et al. (2015) stated that inhibition of chlorophyll by PPCPs (such as antibiotics) was detected as an interruption of gene expression, which finally affected protein synthesis. The *rbcL* (RuBisCO large subunit) and *psbA* (PSII D1 protein) are photosynthetic genes. Expression of both genes decreases during the exposure of cyanobacteria to organic pollutants (Fernández-Pinos et al., 2017). Additionally, Wu et al. (2014) expressed that the transcript abundance of *psaB* gene increased with exposure to organic pollutants over a short time (6-12 h), then reduced with longer exposure. Furthermore, they expressed that organic pollutants could decrease the transcript abundance of *psbc* by up to 30%.

8. Conclusions

The occurrence of PPCPs has been widely reported in aquatic environments globally. Thus, the monitoring of PPCPs and their elimination using green techniques are of great importance. Algae-based treatment methods are fully reviewed in the removal of PPCPs, with key findings as follows:

- The most common pharmaceuticals are ibuprofen with the highest concentrations of 3738 ng/L in tap water in Nigeria, and caffeine at 3068 ng/L in Aegean Sea and Dardanelle in Turkey and Greece.
- The PPCPs can be found in aquatic organisms such as fish, with 24.4×10^3 ng/g of atenolol detected in *P. lineatus*.
- Algae-based systems could remove PPCPs from up to 99%.
- In comparison with STPBs, RABRs and HRAPs, algal bioreactors have demonstrated better performance in PPCPs removal.
- Short term exposure to low concentration of PPCPs can increase chlorophyll and protein contents in algae, which are however reduced by increasing PPCPs concentrations and exposure time.

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