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A review on environmental occurrence, toxicity and microbial degradation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

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ABSTRACT

In recent years, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have surfaced as a novel class of pollutants due to their incomplete degradation in wastewater treatment plants and their inherent ability to promote physiological predicaments in humans even at low doses. The occurrence of the most common NSAIDs (diclofenac, ibuprofen, naproxen, and ketoprofen) in river water, groundwater, finished water samples, WWTPs, and hospital wastewater effluents along with their toxicity effects were reviewed. The typical concentrations of NSAIDs in natural waters were mostly below 1 μg/L, the rivers receiving untreated wastewater discharge have often showed higher concentrations, highlighting the importance of effective wastewater treatment. The critical analysis of potential, pathways and mechanisms of microbial degradation of NSAIDs were also done. Although studies on algal and fungal strains were limited, several bacterial strains were known to degrade NSAIDs. This microbial ability is attributed to hydroxylation by cytochrome P450 because of the decrease in drug concentrations in fungal cultures of Phanerochaete sordida YK-624 on incubation with 1-aminobenzotriazole. Moreover, processes like decarboxylation, dehydrogenation, dechlorination, subsequent oxidation, demethylation, etc. also constitute the degradation pathways. A wide array of enzymes like dehydrogenase, oxidoreductase, dioxygenase, monooxygenase, decarboxylase, and many more are upregulated during the degradation process, which indicates the possibility of their involvement in microbial degradation. Specific hindrances in upscaling the process along with analytical research needs were also identified, and novel investigative approaches for future monitoring studies are proposed.

1. Introduction

Emerging contaminants (ECs) can be defined as newly recognized environmental contaminants causing adverse environmental and/or human health effects (Rasheed et al., 2019). The presence of emerging contaminants in the environment has received more attention over the last few decades. A broad class of these emerging contaminants is pharmaceuticals and personal care products (PPCPs), known to have the ability to stimulate physiological complications in humans at low doses. The widespread use of PPCPs in recent years has led to their accumulation in the ecosystem and their assimilation into living organisms through their involvement in food webs.

The presence of pharmaceuticals in the environment has a direct relation to their increasing global uses reflected from their market size. Among the various classes of pharmaceuticals, analgesics and NSAIDs had the largest market size in 2020, amounting to 46.69 billion US$ and 48.2 billion US$, respectively, while others like antidepressants (28.6 billion US$), antihypertensives (24.17 billion US$), antifungal (13.06 billion US$), and anaesthetics (2.0 billion US$) had significantly smaller market share (IMARC, 2021; Globe Newswire, 2020). NSAIDs are thus regarded as one of the most used pharmaceuticals in human and veterinary medicine and since most of these can be purchased off the counter, NSAIDs constitute one of the most significant groups of pharmacologically active substances from an environmental perspective.

NSAIDs can reach the environment through sewage or hospital wastewater treatment plants, solid waste management plants, leachate from solid waste landfills, or direct dumping by pharmaceutical industries (Paíga et al., 2016). NSAIDs are considered to be persistent and therefore, are found in the range of ng/L to μg/L in various aquatic environments including rivers, lakes as well as drinking waters all

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around the world (Sibeko et al., 2019; Shanmugam et al., 2014; Lindholm-Lehto et al., 2015; Brozinski et al., 2013; Vulliet and Cren-Olive, 2011; Rabiet et al., 2006). Due to their ubiquitous occurrence in the aquatic environment and chronic ecotoxic effects on the biotic components of the ecosystem, they are considered an emerging contaminant of concern.

NSAIDs have anti-inflammatory, antipyretic, and analgesic properties. The antipyretic effect is majorly attributed to the inhibition of production of prostaglandins induced by interleukin-1 (IL-1) and interleukin-6 (IL-6) in the hypothalamus and due to the reorganizing of the thermoregulatory system leading to vasodilation and increased heat loss (Osafo et al., 2017). The analgesic effect is thought to be related to the peripheral inhibition of prostaglandin generation (Cashman, 1996). The anti-inflammatory action of NSAIDs is due to the inhibition of COX or cyclooxygenase enzyme, which are responsible for the conversion of arachidonic acid to prostaglandins (Vane, 1971). Diclofenac, ibuprofen, salicylic acid, naproxen, celecoxib, mefenamic acid, and ketoprofen are some of the major NSAIDs available in the market. Table 1 highlights the major properties of some of the most popular NSAIDs.

Over the last decade, several NSAIDs have attracted extensive research on their occurrence and persistence in the aquatic environment, including rivers, lakes, groundwater as well as wastewater treatment plants. The harmful impacts of NSAIDs on the environment and humans along with the toxicity on many model organisms have also been studied. Although there are few reviews attempting to compile the presence and toxicity of some of the NSAIDs, either these have been limited to specific compound such as diclofenac (Lonappan et al., 2016), or NSAIDs have been given little attention while reviewing emerging contaminants or pharmaceuticals in general (Couto et al., 2019; Tran et al., 2017). Comprehensive reviews focusing on NSAIDs are rare and have been limited to studies like toxicity (Parolini, 2020) and metabolism by non-target wild-living organisms (Mulkiewicz et al., 2021). Reviews on mitigation and removal of NSAIDs are also scarce, as limited focus was given to NSAIDs in wider reviews on removal of pharmaceuticals or trace organic contaminants by conventional or advanced oxidation processes (Caban and Stepnowski, 2021; Tufail et al., 2020; Couto et al., 2019). Even though, the viable microbial degradation of xenobiotics is often regarded as a more sustainable approach for the remediation of such environmental contaminants, and research on microbial degradation of NSAIDs at lab as well as field conditions have received lot of attention in the last decade, no critical review is available on aqueous-phase biodegradation of NSAIDs.

To fill the void, this paper attempted a comprehensive and critical review of microbial degradation potential and pathways of major representative NSAIDs (Diclofenac, Naproxen, Ibuprofen, and Ketoprofen) along with a discussion regarding their environmental occurrence and toxicity. The study also reviewed the status of daughter-products and metabolites of these NSAIDs, and potential mechanism and pathways of the degradation. Special comments regarding the factors impacting scenarios of occurrence and treatment in wastewater treatment plants (WWTPs) has also been made along with an insight in limitations of implementing advanced bioremediation technologies in field scale WWTPs. Overall, the paper aimed at advancing critically reviewed knowledge on the environmental risk and mitigation of major NSAIDs.

2. Occurrence of NSAIDs in waters around the world

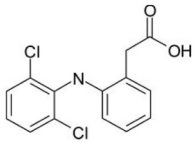
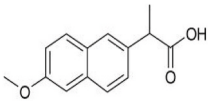
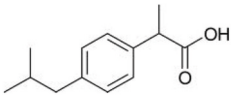
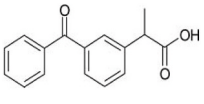
NSAIDs along with other PPCPs can reach the aquatic environment through several routes. NSAIDs are excreted from humans and animals mainly in urine, passing on to the environment directly or via sewage plants. These may be intact or metabolized into some other intermediates (Mulkiewicz et al., 2021). They may also reach the environment unused either via household water or via urban solid garbage handling, mainly through leachate from solid waste landfills with digested sludge (containing quantifiable amounts of pharmaceuticals) for agricultural purposes. Manufacturing plants producing the active pharmaceutical ingredients (API) may unintentionally discharge pharmaceuticals into the water bodies, which may lead to a high presence of parent compound in the nearby aquatic environment (Paíga et al., 2016).

2.1. Presence and Persistence of NSAIDs in rivers and lakes

The reported levels of the selected NSAIDs in the rivers and lakes around the world are summarized in Table S1 in Supplementary Materials. Since strict monitoring and discharge levels are generally not maintained for NSAIDs, they are found in water bodies and wastewater treatment plants globally in concentrations mainly ranging from ng/L to μg/L (Sibeko et al., 2019; Shanmugam et al., 2014; Lindholm-Lehto et al., 2015; Brozinski et al., 2013; Paíga et al., 2016). European Union considers NSAIDs like diclofenac, ibuprofen, and naproxen among the high-priority pharmaceutical substances based on factors such as toxicity, persistence, occurrence etc. (Sousa et al., 2018; de Voogt et al., 2009).

These NSAIDs are generally ranked as persistent, however, few have reported somewhat contradictory results. Varying results for the persistence of diclofenac and ibuprofen have been reported, where the half-life of diclofenac and ibuprofen in the field were in the range of <8 - < 30 and 4.6–32 days, respectively (Bu et al., 2016). Although a lower half-life has been stated for diclofenac in lake and raw river water under specific conditions, the same study reported much higher half-life of 63.6 days for ibuprofen under river water conditions (Araujo et al., 2014). A further prolonged photolysis half-life of 413 days was reported for ibuprofen by Yamamoto et al., 2009, while most studies reported ibuprofen with a half-life shorter than the persistence threshold (Yamamoto et al., 2009; Bu et al., 2016; Araujo et al., 2014). Naproxen had a half-life in the range of 9.6–18.5 days for lake and reservoir water,

Table 1
Properties of some of the popular NSAIDs (Data sourced from PubChem and Nishi et al., 2015).

Properties	Diclofenac	Naproxen	Ibuprofen	Ketoprofen
Chemical formula	$C_{14}H_{11}Cl_2NO_2$	$C_{14}H_{14}O_3$	$C_{13}H_{18}O_2$	$C_{16}H_{14}O_3$
Chemical name	2-[2-(2,6- dichloroanilino)phenyl] acetic acid	(2*S*)-2-(6-methoxynaphthalen-2-yl) propanoic acid	2-(4-(2-methylpropyl)phenyl) propanoic acid	2-(3-benzoylphenyl) propanoic acid
CAS No.	15307-86-5	22204-53-1	5687-27-1	22071-15-4
Chemical structure				
Molecular Weight (g/mol)	296.1	230.26	206.28	254.28
Log K_{ow}	4.02	3.10	3.79	3.0
pK_a	4.18	4.15	5.3	4.45

although a higher half-life of 27 days has been reported for rivers, essentially categorizing it under persistent APIs (Araujo et al., 2011). The half-life of ketoprofen is reported in hours rather than days, demonstrating its high biodegradability and low persistence due to which ketoprofen is not detected in many of the surface water sources tested (Araujo et al., 2014; Bu et al., 2016). Although another factor in case of ketoprofen was almost complete photodegradation of ketoprofen after only 10 min of UV-B irradiation (León et al., 2021), which could lead to a lower apparent concentration during detection than actual concentration present in water bodies. Although the half-life of diclofenac in soil was within the range of that in water (Lin and Gan, 2011), ibuprofen and naproxen had relatively higher persistence in soil than water with a maximum half-life of 49.9 days and 69.3 days, respectively (Lin and Gan, 2011). As the natural attenuation of NSAIDs or other such compounds depends on highly variable environmental conditions like pH, temperature, nutrients, and inherent microbial species , variable data regarding half-lives in water bodies is somewhat inevitable.

This variability in reported levels of NSAIDs can be attributed to several factors like demography, per capita consumption of NSAIDs in a given area, illegal dumping by pharmaceutical industries, processes and efficiency for wastewater treatment, retention times in WWTPs and water quality characteristics of the influent in WWTPs. The demographical considerations including age and incidence of diseases along with the per capita consumption of NSAIDs is a prerequisite to determine the daily intake of NSAIDs which eventually determines the daily load of NSAIDs in the influent wastewater. Further the treatment units determine the percent removal obtained by any WWTP. Treatment processes like activated sludge process (ASP), trickling filter and sewage lagoons have shown variable removal efficiencies which are further explained in Section 2.3.

In addition, NSAIDs levels in rivers and lakes also depend on geographic location of sampling, geographical terrain, type of wastewater effluent draining, environmental conditions, biological complexity of the water body including the indigenous microbes present, and seasonal variation (Wang et al., 2010; Paíga et al., 2016, Stepnowski et al., 2020). Further, these factors often work in combination, and its been extremely difficult to establish direct correlations. For example, no significant differences were observed between the dry season (December) and wet season (May) NSAIDs level in the Yellow River, while seasonal variations were observed in waters of the Hai and Liao River in China (Wang et al., 2010).

The WWTPs performance, especially in terms of NSAIDs removal, also governs their levels in receiving water bodies. The absence of sewerage system or WWTPs may lead to wastewaters either infiltrating into the soil or flowing to nearby steams, ponds of lakes, and contaminating the waterbodies, as reported for Portuguese Lis River and various other African rivers (Paíga et al., 2016; Sousa et al., 2011). Compared to typical NSAIDs levels detected in the rivers of Poland, unusually higher levels of diclofenac (9.0 μg/L), ibuprofen (5.3 μg/L) and naproxen (0.7 μg/L) were found six days after the malfunctioning of the sewage collection system in Warsaw at the end of August 2019 (Stepnowski et al., 2020; Baranowska and Kowalski, 2012; Kasprzyk-Hordern et al., 2007; Migowska et al., 2012).

Ibuprofen concentration of up to 30 μg/L has been reported in the Nairobi river whereas, an even higher concentration of diclofenac (193 μg/L) was detected in the Beberibe river, Brazil, attributed to the direct release of raw sewage into the rivers (Veras et al., 2019; Koreje et al., 2012). Although naproxen and ketoprofen mainly had concentrations below 1 μg/L, Malir river in Pakistan (11.4–32 μg/L) and Beylikcayi creek in Turkey 12.3 μg/L had inexplicably high levels of naproxen (Scheurell et al., 2009; Selke et al., 2010; Aydin and Talinli, 2013). The maximum concentration of ketoprofen (2.71 μg/L) was found in the Llobregat river, Spain (Ginebreda et al., 2010).

The occurrence of NSAIDs in specific lakes is not elaborately studied. Still, in comparison to the rivers, the concentrations of NSAIDs found in lakes were very low. The maximum concentration of NSAIDs was majorly found in Lake Päijänne, Finland (Lindholm-Lehto et al., 2015). In addition to NSAIDs and other pharmaceuticals, Päijänne Lake was found to have some metabolites like hydroxyibuprofen (Lindholm-Lehto et al., 2015).

The concentration of drugs found in the water bodies is thought to be directly proportional to the population densities and the yearly sale of prescription as well as non-prescription drugs in the country. However, this claim appears to be untrue, since the highest population densities of India and China do not translate to a pronounced NSAID concentration in their water bodies. Moreover, one of the most prominent NSAID, diclofenac is banned in veterinary medicine by the Indian government in 2006. The presence of significant concentrations of pharmaceuticals in countries like Uganda in Africa is mainly attributed to extensive use and medication without prescription along with incongruous disposal of used and expired pharmaceuticals in human medicine as well as agriculture (Nantaba et al., 2020).

In the regional context, highest number of NSAIDs reporting studies are from European countries, and most of them had observed significantly low concentrations of NSAIDs, presumably due to the stricter provision of tertiary treatment in their WWTPs. However, remarkably high level of NSAIDs have also been reported in the rivers of Spain, particularly in the Llobregat river (Table S1) attributed to the absence of sewage treatment processes as well as a multitude of pharmaceutical industrial discharges directly in the river (Ginebreda et al., 2010; Silva et al., 2010).

Although NSAIDs are present in surface waters around the world, the Predicted Environmental Concentrations (PEC) were often lower (Table S1) than the Predicted No-Effect Concentration (PNEC), leading to PEC/PNEC ratio (risk quotient) of <1 indicating no significant risk related with the environmental presence of the NSAID. The PNEC values for diclofenac, ibuprofen, naproxen and ketoprofen has been observed as 10, 7.1, 6.6 and 0.16 μg/L (Carlsson et al., 2006a, 2006b, Salvador Gamarra et al., 2015; Orias and Perrodin, 2013; Komori et al., 2013). However, the PNEC value for diclofenac prescribed in the EU Watch List 2015 was set much lower at 0.05 μg/L (EU Watch List EU COM, 2015/495).

2.2. Presence of NSAIDs in groundwater and finished drinking water

Groundwater contamination with PPCPs can have some of the most severe consequences since they may be more persistent and difficult to eliminate because of relatively reduced redox conditions and an absolute lack of photodegradation than in surface waters. Microbial degradation is also relatively much less efficient in groundwater systems (Peng et al., 2014). Table 2 incorporates the NSAIDs concentration in groundwater around the world.

While the concentration of NSAIDs is well below 100 ng/L in most cases, some countries have an alarming concentration of more than 1 μg/L. Ibuprofen was detected at a startling concentration of 2.25 μg/L and 0.988 μg/L in the groundwaters of Nigeria and Spain respectively (Ebele et al., 2020; López-Serna et al., 2019; Peng et al., 2014). Even higher concentrations of up to 3.11 μg/L was reported in groundwaters tested across 18 states of USA (Barnes et al., 2008). Further, a maximum ketoprofen concentration of 2.886 μg/L was reported in some European groundwaters (Loos et al., 2010; Peng et al., 2014). This was even more astonishing considering the generally lower persistence of ketoprofen.

The higher concentrations of NSAIDs in finished drinking water can be viewed as a reflection of the contamination of water bodies in their respective countries. A very few studies investigated the NSAID concentration in drinking water treatment plants and finished drinking water. Although diclofenac, naproxen, and ibuprofen were reported to be under 20 ng/L in finished drinking water samples (Simazaki et al., 2015; Padhye et al., 2014), a maximum concentration of 54 ng/L ibuprofen and 53 ng/L naproxen were detected in Spain and Switzerland respectively (Boleda et al., 2014; Morasch et al., 2010). Though there is no legislation monitoring the concentrations of PPCPs in drinking

Table 2
Concentrations of Diclofenac, Ibuprofen and Naproxen in groundwater (Ketoprofen has rarely been reported).

Location	Diclofenac (ng/L)	Ibuprofen (ng/L)	Naproxen (ng/L)	References
Lagos, Nigeria	<1–42	<4–2250	<3 - 17	Ebele et al. (2020)
Barcelona, Spain	0.184–380	0.16–988	BDL — 5.59	Peng et al. (2014), López-Serna et al. (2019)
France	9.7	0	1.2	Peng et al. (2014), Vulliet and Cren-Olive (2011)
USA		3110		Peng et al. (2014), Barnes et al. (2008)
Europe	24	3–395		Peng et al. (2014), Loos et al. (2010)
Rastatt, Germany	BDL – 129	BDL – 104		Sui et al. (2015), Wolf et al. (2012)
Guangzhou, China		BDL - 57.9	86.9	Sui et al. (2015), Peng et al. (2014)
Ottawa, Canada		10		Sui et al. (2015), Gottschall et al. (2012)
Wadi Shueib, Jordan		BDL – 65		Sui et al. (2015), Zemann et al. (2015)
Serbia		92	27.6	Sui et al. (2015), Petrovic et al. (2014)
Yverdon-les-Bains, Switzerland	BDL – 3		BDL - 12	Sui et al. (2015), Morasch (2013)
North Carolina, USA		0–2	0–12	McEachran et al. (2016)
Taiwan	2.1–33.2	7–836.7	128	Lin et al. (2015)

BDL- Below Detection Limit.

waters, where even minimal contamination can lead to severe consequences in the future.

2.3. Presence of NSAIDs in wastewater treatment plants influents and effluents

NSAIDs are partially metabolized in human bodies and excreted in urine and faeces, thereby reaching the sewer systems to be transported into the wastewater treatment plants. Along with this, some unused or expired drugs are also disposed unceremoniously into the toilets which further increases the pharmaceutical load on the WWTPs. NSAIDs are present in relatively high concentration in the influent of WWTPs when compared to the concentration of NSAIDs in river waters. Along with the partial removal of NSAIDs in the WWTPs, this can also be attributed to the dilution effect when treated or untreated sewage is discharged to rivers. Photodegradation and microbial degradation also play a major role in lower concentrations of NSAIDs in rivers and lakes. Table 3 enlists the presence of NSAIDs in influent and effluent wastewaters from WWTPs around the world.

The concentration of NSAIDs present in the wastewater influent depends on various factors, such as the number of inhabitants served by the WWTP, types of wastewater treated by the WWTP, the process of treatment employed, and seasonal variation. The concentrations of NSAIDs are often maximum in the pharmaceutical industry and hospital WWTPs followed by municipal WWTP (Sim et al., 2011). The concentration of NSAIDs follows the trend where higher concentrations were observed in the dry season as compared to the wet season, primarily because of the dilution of influent wastewater with rainwater in places of combined sewers.

In addition to NSAIDs, a few also investigated the presence of their metabolites in the wastewaters. Metabolites of diclofenac like 4′-hydroxydiclofenac, 5-hydroxydiclofenac and acyl glucuronides were detected in WWTPs effluents (Stülten et al., 2008; Scheurell et al., 2009; Kallio et al., 2010), whereas ibuprofen metabolites like 1-hydroxyibuprofen, 2-hydroxyibuprofen, carboxyibuprofen and a few others were detected in rivers and/or WWTP influents and even rivers receiving the discharge (Chopra and Kumar, 2020; Ferrando-Climent et al., 2012)). O-Desmethylnaproxen, a metabolite for naproxen was also detected in surface water and effluents from Germany and Pakistan (Selke et al., 2010).

Though major NSAIDs are eliminated in WWTPs with variable removal efficiency, sometimes the effluent concentration of the drug was reported higher than that of the influent concentration (Radjenovic et al., 2007). This could be due to input conjugate compounds present in wastewaters getting transformed into original compounds during treatment (Radjenovic et al., 2007). Obviously, the removal efficiencies of different NSAIDs in WWTPs vary as per treatment scheme (Table 3). The diclofenac removal efficiencies from WWTPs lie in the range of 20–40% (Kummerová et al., 2016), though at some places, removal efficiencies up to 80% have also been reported (Aissaoui et al., 2017b). Naproxen is also barely removed (<15%) in WWTPs (Madikizela et al., 2017). Compared to diclofenac and naproxen, the removal of ibuprofen is highly efficient, mostly exceeding 90% (Rutere et al., 2020); whereas, ketoprofen removal varies largely ranging from 38% to almost 100% (Lindqvist et al., 2005; Santos et al., 2007).

Fig. 1 depicts NSAIDs removal efficiencies by typically reported wastewater treatment methods. Most of the studies featured conventional ASPs and conventional ASPs with sand filters, whereas relatively limited data is available for other treatment methods shown in Figure 1. Note that, usually none of these WWTPs are designed with specifications to remove PPCPs. Though conventional ASPs are the most widely used treatment methods, it was not found to be the most efficient in the removal of NSAIDs. However, the addition of sand filters to the conventional ASP setup increases the removal efficiency by a considerable margin. This might be possible because of adsorption of NSAIDs on suspended solids and their subsequent trapping in the sand bed (Rizzo et al., 2015). Another reason can be the formation of biofilms leading to biosorption and biotransformation of NSAIDs. Although limited data was available for treatment systems other than conventional ASPs, a single factor ANOVA showed statistically significant (at 95% confidence interval) differences with a *p-value* of 0.04 in the removal efficiencies of ibuprofen across different treatment systems reported, whereas the difference weren't significant for naproxen (*p-value* = 0.37) due to high variations (≈22–92%) in the reported naproxen removal efficiencies with the conventional ASPs. There wasn't enough data to carry out statistical significance test for diclofenac and ketoprofen removal efficiencies across different treatment systems. The *t-test* showed average diclofenac removal efficiency under conventional ASP was significantly smaller than that in TF with a *p-value* of 0.07.

The main process for ibuprofen removal in conventional ASPs was biodegradation (nitrification and carbon biodegradation) while no adsorption was observed (Peng et al., 2019). A high removal rate was observed in the aerobic reactor as compared to the anoxic system (Abegglen et al., 2009; Min et al., 2018; Suarez et al., 2010; Zwiener et al., 2002). On the other hand, diclofenac was found persistent in the aerobic process (Kruglova et al., 2014; Tauxe-Wuersch et al., 2005), however, significant removal of diclofenac was observed in anoxic conditions (Jewell et al., 2016; Zwiener and Frimmel, 2003).

Treatment by trickling filters followed by UV disinfection and chlorination also resulted in comparatively higher removal efficiencies for diclofenac. Biological filtration of ozonated effluent in trickling filter showed an improved pharmaceutical degradation because ozonated effluent better sustained the growth of microbes due to elevated oxygen levels, which ultimately leads to enhanced biological drug degradation (Ingabire, 2013). Sewage lagoons were observed to have the highest removal efficiency in the case of diclofenac, naproxen, and ketoprofen mainly because of an extremely high retention time of >150 days (Metcalfe et al., 2003). This was especially worth emphasizing for

Table 3
Presence of NSAIDs in influent and effluent wastewaters from WWTPs around the world.

Location	Wastewater type	WWTP type	Influent concentration (ng/L)	Effluent concentration (ng/L)	Reference
Lis, Portugal	Domestic wastewater, hospital and piggeries effluents, landfill leachate	Conventional ASP	Diclofenac: BDL-972 Ibuprofen: 3877–24,505 Naproxen: BDL-3245 Ketoprofen: BDL-147	Diclofenac: BDL-724 Ibuprofen: BDL-3304 Naproxen: BDL-270 Ketoprofen: BDL-233	Paíga et al. (2016)
Duoro, Portugal	NA	NA	Diclofenac: 431–1597 Ibuprofen: 1992–3588 Naproxen: 2583–3474 Ketoprofen: 122-135	Diclofenac: 1107–1429 Ibuprofen: 1088–1527 Naproxen: 461–2748 Ketoprofen: 446-562	Sousa et al. (2011)
South Korea	NA	Preliminary clarification + final clarification + aeration tank + sand filtration + UV disinfection	Diclofenac: BDL- 9870 Ibuprofen: BDL-580	Diclofenac: BDL- 10960 Ibuprofen: BDL-310	Han et al. (2006)
Tehran	NA	NA	Diclofenac: 44-230 Ibuprofen: 233-1051 Naproxen: 88-430	Diclofenac: 22-33 Ibuprofen: 31-45 Naproxen: 33-54	Eslami et al. (2015)
Spain	Municipal sewage	Conventional ASP + final clarification	Diclofenac: 200-3600 Ibuprofen: 34000-168000	Diclofenac: 140-2200 Ibuprofen: 240-28000	Go'mez et al. (2007)
Montreal	Raw sewage	Physico-chemical process	Diclofenac: 20-216 Ibuprofen: 827-1171 Naproxen: 349-3934 Ketoprofen: ND	Diclofenac: ND Ibuprofen: 609-1060 Naproxen: 217-2579 Ketoprofen: ND	Lajeunesse and Gagnon (2007)
Sweden	Municipal sewage	Conventional ASP + chemical treatment + sand filter	Diclofenac: 230 Ibuprofen: 6900 Naproxen: 4900	Diclofenac: 490 Ibuprofen: 47.5 Naproxen: 290	Zorita et al. (2009)
Barcelona, Spain	Urban, domestic and industrial	NA	Diclofenac: 50-540 Ibuprofen: BDL-900 Naproxen: 190 Ketoprofen: 160-970	Diclofenac: 390 Ibuprofen: 40-800 Naproxen: 160 Ketoprofen: 130-620	Gros et al. (2006)
Baltimore	Municipal wastewater	Biological nutrient removal + bleach disinfection	Diclofenac: 110 Ibuprofen: 1900 Naproxen: 3200 Ketoprofen: 1200	Diclofenac: 90 Ibuprofen: 250 Naproxen: 380 Ketoprofen: 280	Yu et al. (2006)
Taiwan	Domestic, industrial, hospital, and livestock wastewaters	ASP/Trickling filter/deep shaft and step aeration + UV/ chlorination	Diclofenac: 3-437 Ibuprofen: 711-17933	Diclofenac: 4-101 Ibuprofen: 313-3777	Lin et al. (2009)
Bangkok, Thailand	NA	Various forms of ASP	Diclofenac: 58-367 Ibuprofen: 385-1260 Naproxen: 39-933	Diclofenac: 25-182 Ibuprofen: 22-149 Naproxen: 1.3–159	Tewari et al. (2013)
Xiamen, China	Domestic and industrial wastewater	W1 = biological aerated filters + UV disinfection. W2= Orbal oxidation ditches + UV disinfection W3 = anaerobic/anoxic/oxic (A2/O) + chemical disinfection	Diclofenac: 14.8–71.8 Ibuprofen: 34.8–406 Naproxen: 30.6 Ketoprofen: BDL-158	Diclofenac: 17.7–69.2 Ibuprofen: BDL-99.4 Naproxen: BDL-13.8 Ketoprofen: BDL-183	Sun et al. (2016)
Rome, Italy	Domestic and industrial wastewater	Conventional ASP	Diclofenac: 514-952 Ibuprofen: 77-409 Naproxen: 20-49 Ketoprofen: ND	Diclofenac: 321-691 Ibuprofen: 60-133 Naproxen: 13-38 Ketoprofen: ND	Patrolecco et al. (2015)
Greece	Municipal wastewater	Conventional ASP + chemical treatment + sand filter + disinfection	Diclofenac: BDL-3900 Ibuprofen: 2800-25400 Naproxen: BDL-2000	Diclofenac: BDL-2600 Ibuprofen: 500-2600 Naproxen: 700	Kosma et al. (2010)
Tricity, Poland	Municipal and industrial (food, chemical, and shipbuilding) wastewater	Conventional mechanical–biological treatment	Diclofenac: 2138 Ibuprofen: 6586.1 Ketoprofen: 2700	Diclofenac: 3018.4 Ibuprofen: 141.7 Ketoprofen: 159.3	Kot-Wasik et al. (2016)
Algiers, Algeria	Domestic and industrial wastewater	Mechanical and biological treatments	Diclofenac: 2318.5 Ibuprofen: 8612.9 Naproxen: 9584.8 Ketoprofen: 565.2	Diclofenac: 2710.7 Ibuprofen: 431.3 Naproxen: 333.7 Ketoprofen: 1034.5	Kermia et al. (2016)
Korea	NA	NA	Ibuprofen: 9494 Naproxen: 5938	Ibuprofen: 15 Naproxen: 120	Kim et al. (2012)
Canada	Residential and industrial (Textile meat, chemical, steel, food, brewery, paper, metal, dairy, plastics, auto, animal slaughter, tannery, poultry, wood) wastewater	Sewage lagoons + UV/chlorine disinfection/no disinfection	Diclofenac: 1300 Ibuprofen: 75800 Naproxen: 611000[a] Ketoprofen: 5700	Diclofenac: ND Ibuprofen: 24600 Naproxen: 33900 Ketoprofen: ND	Metcalfe et al. (2003)
Kanagawa, Japan	Domestic and industrial wastewaters	Conventional ASP + UV/chlorine disinfection	Ibuprofen: 69-1090 Naproxen: 179-305 Ketoprofen: 160-1060	Ibuprofen: ND Naproxen: 74-166 Ketoprofen: 64-107	Nakada et al. (2005)
Seville, Spain	Domestic wastewaters	Conventional ASP	Diclofenac: 720 Ibuprofen: 12900-50600 Naproxen: 2540-4090 Ketoprofen: 1690-2110	Diclofenac: 90-740 Ibuprofen: 1050-8000 Naproxen: 990-2580 Ketoprofen: 880-940	Martin et al. (2012)
Catalonia, Spain	Municipal and industrial wastewater	Conventional ASP	Diclofenac: 1250-1709 Naproxen: 5545-10150 Ketoprofen: 1720-6007	Diclofenac: 743-1100 Naproxen: 307-2624 Ketoprofen: 80-948	Jelic et al. (2010)

(*continued on next page*)

Table 3 (*continued*)

Location	Wastewater type	WWTP type	Influent concentration (ng/L)	Effluent concentration (ng/L)	Reference
Mangalore, India	Household and hospital sewage, run off	Upflow anaerobic sludge blanket reactor (UASBR)	Diclofenac: 49180- 721370 Ibuprofen: 43510- 2109880 Naproxen: 115380-2132480 Ketoprofen: 559560-2747290	Diclofenac: BDL-131150 Ibuprofen: BDL- 22700 Naproxen: BDL- 173080 Ketoprofen: BDL-270760	Thalla and Vannarath (2020)
Korea	Municipal wastewater	NA	Diclofenac: 94-523 Ibuprofen: ND Naproxen: 480-12500	Diclofenac: 52-1760 Ibuprofen: ND Naproxen: 21-740	Sim et al. (2011)
	Hospital wastewater		Diclofenac: 28-6880 Ibuprofen: ND Naproxen: 306	Diclofenac: 46-221 Ibuprofen: ND Naproxen: 6040	
	Livestock waste		Diclofenac: ND Ibuprofen: ND Naproxen: ND	Diclofenac: ND Ibuprofen: ND Naproxen: ND	
	Pharmaceutical manufacture wastewater		Diclofenac: 160000-203000 Ibuprofen: ND Naproxen: 410-206000	Diclofenac: 457-19200 Ibuprofen: ND Naproxen: 361-39300	

[a] = analytes were quantified after dilution of the final sample volume by 1:10; BDL- Below Detection Limit; ND- Not Detected.

diclofenac because sewage lagoons offered the highest ever reported removal efficiency for diclofenac. Even after showing the most promising results for the removal efficiencies, sewage lagoons would not be used as the preferred treatment method because of the elevated hydraulic retention times needed for treatment.

2.4. Presence of NSAIDs in hospital and pharmaceutical industry effluents

The complexity of hospital and pharmaceutical industry effluents makes them a foremost source of PPCPs contamination of receiving water bodies. The concentration of NSAIDs present in the hospital and pharmaceutical industry effluents far exceeds than that of municipal WWTPs (Sim et al., 2011). However, contradictions have also been reported as effluents from two hospitals of Tehran found to have lower concentrations of ibuprofen (0.141 and 0.29 μg/L), naproxen (0.092 and 0.084 μg/L), and diclofenac (0.027 and 0.077 μg/L) than their corresponding concentrations observed in the four WWTPs investigated in the same study (Eslami et al., 2015). Seasonal or temporal variations have also shown to produce significant variations in NSAIDs levels in such effluents (Kosma et al., 2010, 2014). Overall, diclofenac, ibuprofen, naproxen, and ketoprofen have been detected in hospital and pharma industry effluents up to concentrations of 228.5, 1500, 1.05 and 0.23 μg/L, respectively (Lin and Tsai, 2009; Eslami et al., 2015; Kosma et al., 2010; Zorita et al., 2009).

The sampling procedure and framework is also important to obtain a truly representative contaminants level. A 24-h time-integrated sample of hospital wastewater did show different levels of diclofenac (0.38 μg/L), ibuprofen (8.8 μg/L), and naproxen (9.3 μg/L) when compared with that recorded with a grab sample from the same source resulting in diclofenac, ibuprofen, and naproxen as 0.21, 10.8, and 6.6 μg/L, respectively (Zorita et al., 2009). At times, the concentration of NSAIDs and other drugs in the hospital and pharmaceutical industry effluents are found to be of the similar order of the concentrations present in the WWTPs receiving sewage from densely populated areas. However, the complexity and toxicity owing to higher antibiotic resistance, pathogen concentration and variety of drugs present still makes the hospital and pharmaceutical industry effluents far more challenging to deal with. Furthermore, even if the concentrations of commonly used drugs like NSAIDs, antibiotics and analgesics might be similar for both cases, the concentration of less common drugs like anitdepressants or beta blockers will usually be higher in the case of hospital effluents. However, these concentrations might be reduced due to dilution, if hospital effluents are drained into a general WWTP.

3. Environmental toxicity and health hazards of NSAIDs and metabolites

NSAIDs are ubiquitously present in our surface waters as well as groundwater. Due to such a widespread presence, the surface water quality and aquatic life are highly affected, which ultimately leads to a decline in the number of aquatic organisms. Bioaccumulation of NSAIDs along the food chain is also a possibility that is rapidly gaining ground since NSAIDs like diclofenac are reported to accumulate in bile or liver of rainbow trout up to a concentration factor of about 2700. (Schwaiger et al., 2004). Ibuprofen and diclofenac were also detected in the bile of wild fish caught downstream of a wastewater treatment plant in Finland, and the concentrations ranged from 15 to 34 ng/mL, 6–103 ng/mL, and 6–148 ng/mL for ibuprofen, naproxen, and diclofenac respectively (Brozinski et al., 2013). This also poses a threat to the health of humans consuming fish.

The total bioconcentration factor for diclofenac and its metabolites in bile of rainbow trout was estimated from 320 to 950 which suggested the accumulation of diclofenac and its metabolites in bile of rainbow trout living downstream of the WWTP due to chronic exposure (Kallio et al., 2010). No change in lipid peroxidation, reduced glutathione, glutathione-S-transferase, superoxide dismutase, and catalase was observed for diclofenac metabolites suggesting that these metabolites did not exhibit any toxic effect to the mice (Aissaoui et al., 2017b). However, hydroxylated ibuprofen metabolites such as 1-hydroxyibuprofen, 2-hydroxyibuprofen, 1,2-dihydroxyibuprofen was suggested to have higher toxicity than the parent ibuprofen (Makuch et al., 2021).

Although NSAIDs doses intentionally consumed by several humans for medi-care purpose are regarded safe, and no visible effects of NSAIDs are detected in humans yet, concerns for future health problems related to the chronic exposure issue can't be overruled. These concerns were also valid because of the studies demonstrating the uptake of NSAIDs and their metabolites in edible plants. Klampfl (2019) reviewed the uptake and metabolization of NSAIDs in several edible plants such as cress, barley, tomato, cucumber, beet, horseradish, lettuce etc (Klampfl, 2019). The presence of metabolites such as 4′-hydroxydiclofenac and 4′-hydroxydiclofenac glucopyranoside in barley and horseradish was also reported by Huber et al. (2012). Ibuprofen metabolites were detected in duckweed and reed (He et al., 2017; Di Baccio et al., 2017) whereas naproxen metabolites were observed in plants like cress (Emhofer et al., 2017).

The toxic effects of NSAIDs on the environment were studied on several model species from different taxa. Some of the chronic and acute toxicity effects for diclofenac, ibuprofen, naproxen, and ketoprofen are listed in Table 4. Only limited information is available on the toxicity of metabolites, as enlisted in Table S2 of Supplementary Material.

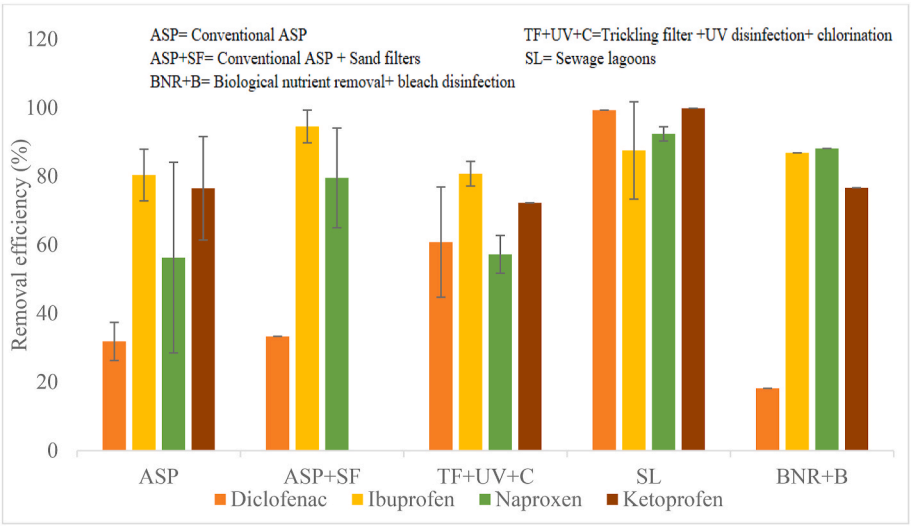


Fig. 1. Removal efficiencies for NSAIDs by different wastewater treatment methods. [Data sourced from. ASP = Paíga et al. (2016); Go'mez et al. (2007); Patrolecco et al. (2015); Martin et al. (2012); Jelic et al. (2010); Nakada et al. (2005) (Average of data points). ASP + SF = Zorita et al. (2009); Kosma et al. (2010) (Average of data points) BNR + B= Yu et al. (2006). TF + UV + C= Lin et al. (2009); Camacho--Muñoz et al. (2012); Kasprzyk-Hordern et al. (2009). SL = Metcalfe et al. (2003); Hoque et al. (2014); Camacho-Muñoz et al. (2012)]

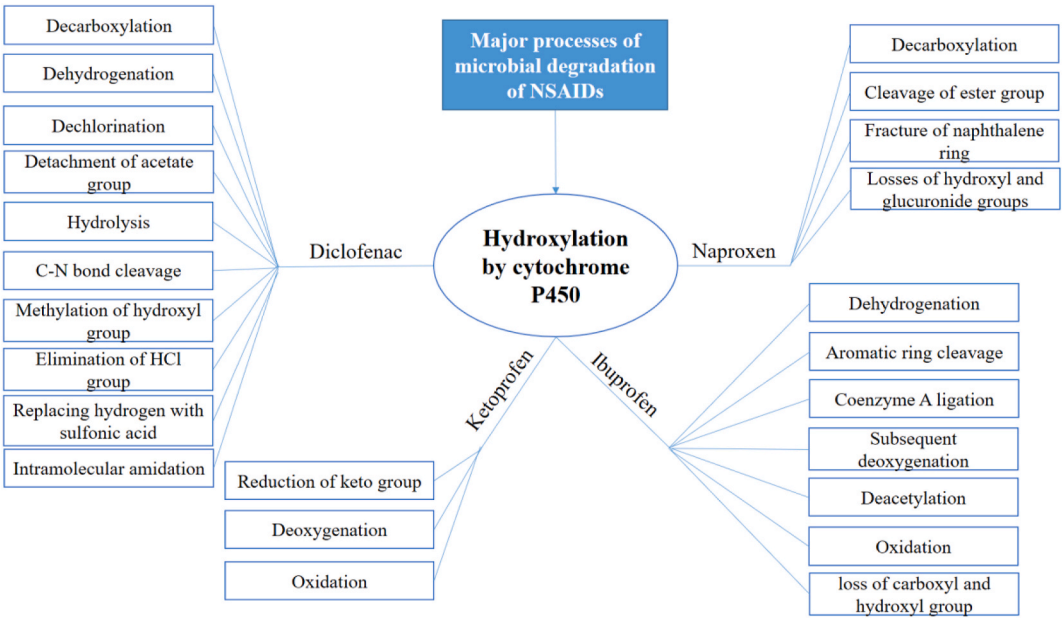


Fig. 2. Major processes employed in microbial degradation pathways.

Enzymatic irregularities, histopathological alterations in liver, gill or kidneys, modification of proteins, genetic and cellular damage along with reproduction abnormalities have been observed as a result of NSAIDs toxicity in aquatic organisms. In addition, lower productivity of agricultural land, altering agricultural infrastructure and massive death of livestock and fishes have also been attributed to NSAIDs contamination. Future human health effects may include genotoxic effects like damage of DNA, respiratory problems, stomach ulcers, chronic depression, congenital problems including mental retardation, and physical abnormalities.

Cleuvers (2004) investigated the ecotoxicity of four NSAIDs, (diclofenac, ibuprofen naproxen, and paracetamol) individually as well as in a mixture on *Daphnia magna* using acute *Daphnia* and algal test. Toxicities of single NSAIDs were relatively low, with half-maximal EC50 values in *D. magna* ranging from 68 to 166 mg/L. The toxicity of the mixture composed of the four NSAIDs at various concentrations was substantial, even at concentrations that caused no or minor effects on exposure to the single substances (Cleuvers, 2004). In another study, Parolini et al. (2011) examined the sub-lethal effects induced by a mixture of diclofenac, ibuprofen, and paracetamol, on *Dreissena polymorpha* (Parolini et al., 2011). Parolini (2020) also recently reviewed studies on the toxicity effects of NSAIDs.

Exposure to diclofenac leading to renal failure and visceral gout is said to be one of the main reasons for the population decline of three species of Indian subcontinent vultures, *Gyps bengalensis, Gyps indicus, Gyps tenuirostris* which were severely affected, reduced by 98%, and was included in "critically endangered" species list of IUCN (Oaks et al., 2004). As it was known to act by damaging renal and gastrointestinal tissues of several vertebrate taxa and was also responsible for the near-extinction of the Indian subcontinent vulture population, India became the first country to ban the manufacture and veterinary use of diclofenac in 2006 and additional limitations were placed for animal use in 2008. Nepal and Pakistan also banned diclofenac for veterinary use in 2008 followed by Bangladesh in 2010.

Diclofenac was also highlighted by the European Environmental Agency and included in the Watch List of EU Decision 2015/495 considering it as a "contaminant of emerging concern". Due to this inclusion in the watch list, diclofenac is obligatorily monitored and the

reported data is collated for the determination of risk reduction measures. According to this proposed EQS document, the maximum allowable concentrations for diclofenac are 0.1 μg/L in freshwaters and 0.01 μg/L in marine waters. The United Kingdom has also placed diclofenac in the 'list of priority substances' which forced the water industries to search for technologies to remove diclofenac from wastewater. No such legislation has been passed for ibuprofen, naproxen, or ketoprofen due to less obvious harmful effects on the environment.

4. Removal of NSAIDs from waters and wastewaters

Due to the pervasive presence of NSAIDs in waters and wastewaters in several parts of the world, and the risk posed with the chronic exposure of such compounds, the treatment or removal of NSAIDs becomes critically vital. At present, highly selective and rapid reactions, such as advanced oxidation processes (AOPs), or adsorbents, such as activated carbon, are seen as efficient ways to remove micropollutants like NSAIDs. AOPs use combinations of reactive oxidants including ozonation, photocatalysis, and ultrasound oxidation and are characterized by the generation of extremely reactive species, such as hydroxyl radicals, which can degrade recalcitrant molecules into possible biodegradable intermediate compounds or completely mineralize them into CO_2, H_2O, and inorganic ions. Wang et al. (2019) reported the degradation of ibuprofen using UV-LED/catalytic advanced oxidation process (Wang et al., 2019).

Other recent methods include ion exchange, membrane filtration, Fenton oxidation, electrochemical oxidation, and soil aquifer treatment. Membrane technologies are often utilized in a combination of physical, chemical, and biological processes to improve removal efficiency. Seifollahi and Rahbar-Kelishami demonstrated the extraction of diclofenac from aqueous solution by emulsion liquid membrane prepared by using tetrabutylammonium bromide (TBAB) as the carrier (Seifollahi and Rahbar-Kelishami, 2017). Fenton oxidation refers to the processes during which highly reactive oxygen species are formed. ZVI (Zero-Valent Iron)-Fenton was found to be highly effective in the degradation of ibuprofen (Minella et al., 2019). Soil aquifer treatment has a good efficiency in sewage effluents for several pharmaceuticals after the secondary treatment of wastewater. Soil aquifer treatment has been known to effectively degrade diclofenac, naproxen, and ketoprofen (Chiron and Duwig, 2016; He et al., 2016).

Several other studies evaluating the degradation efficiency of these methods are widely reported for all the NSAIDs but are not mentioned here since they are outside the point of discussion of this paper. However, these methods may lead to the formation of many potentially toxic by-products, and such oxidation technologies are characterized by a large ecological footprint due to high energy use (Schwarzenbach et al., 2006). Due to these reasons, microbial degradation is seen as a potential method to eliminate these micropollutants. Several pharmaceuticals have been successfully degraded using different microbes or microbial consortia in a laboratory setup. They demonstrate varying degrees of removal efficiencies based on the pharmaceutical and the microbes used.

5. Microbial degradation of NSAIDs

The microbial degradation of NSAIDs has garnered an incredible interest from the research community all over the world because of their advantages over other treatment methods. While assessing the percentage of biological degradation and sorption in a conventional activated-sludge wastewater treatment plant from Murcia, Spain, Martínez-Alcalá et al. (2017) concluded that microbial degradation was very important for ketoprofen and ibuprofen, since biodegradation contributed 54.3% and 99.7% of the total removal for ibuprofen and ketoprofen, respectively (Martínez-Alcalá et al., 2017). This establishes the incredibly important role microbial degradation plays in NSAID removal in WWTPs. Numerous studies investigating the degradation potential of several bacterial and fungal pure cultures were found. Table 5 mentions the microbial degradation of NSAIDs using pure cultures. Although the removal efficiencies and time taken by all the microbes vary significantly, a common result of decreased toxicity in the end-products was obtained.

Several fungi like *Trametes versicolor* (white-rot fungus) and *Phanerochaete sordida* YK-624 have shown successful degradation as well as mineralization of diclofenac, ibuprofen, and naproxen, while *Trametes versicolor* has also been reported to degrade ketoprofen (Hata et al., 2010; Li et al., 2015; Marco-Urrea et al, 2009, 2010a, 2010b, 2010c, 2009, 2010c). Marco-Urrea et al. have worked extensively on the degradation of all selected NSAIDs using *Trametes versicolor* along with the elucidation of pathways and metabolites produced during degradation (Marco-Urrea et al, 2009, 2010a, 2010b, 2010c, 2009, 2010c). Compared to the fungal degradation of diclofenac, more studies have been carried out regarding bacterial degradation of NSAIDs. In this pursuit, mass spectrometric and ^{1}H nuclear magnetic resonance analyses have proved an indispensable tool for the revelation of the metabolites produced.

Although the environmentally relevant concentration of NSAIDs ranges mainly between ng/L and μg/L, several studies employ the initial concentrations well beyond that range. These strains can particularly be used for the bioremediation of pharmaceutical production facilities or hospital wastewater effluents, where the concentrations will be in accordance with these studies (Lin and Tsai, 2009).

5.1. Microbial degradation of diclofenac

A few microbial strains have shown the ability to degrade diclofenac both as a primary substrate as well as a secondary substrate. In few studies, the addition of a carbon substrate, such as acetate or toluene enhanced the removal efficiency due to co-metabolism. On the contrary, the addition of a carbon source has also resulted in a decrease in removal efficiency presumably due to competitive inhibition from secondary substrates. Different metabolites were also obtained during degradation using the same strain under different conditions (mono-substrate and co-metabolic conditions). In mono-substrate and co-metabolic conditions, 4′-hydroxydiclofenac and diclofenac lactam were observed whereas phthalate acid was detected only for co-metabolic conditions in *Pseudomonas moorei* KB4 (Zur et al., 2020).

Because of the presence of mono- and di-hydroxylated metabolites during the degradation of diclofenac by the fungal strains, the major process involved was suggested to be hydroxylation catalyzed by cytochrome P450 (CYP). This was further supported by the fact that smaller decreases in diclofenac were observed in cultures of *P. sordida* YK-624 incubated with 1-aminobenzotriazole which is a known inhibitor of CYP (Hata et al., 2010). Purified laccase was also able to catalyze the transformation of diclofenac to 4-(2,6-dichlorophenylamino)-1,3 benzenedimethanol but it did not seem to be the enzymatic system accountable for diclofenac degradation in *T. versicolor* pellets (Marco-Urrea et al., 2010a).

Hydroxylation is supposed to be the most common process observed even in the bacterial degradation of diclofenac. Apart from hydroxylation, other major mechanisms for degradation of diclofenac include decarboxylation, dehydrogenation, de-chlorination, detachment of acetate group, and cleavage of the non-chlorinated aromatic ring (Stylianou et al., 2018) in *Klebsiella* sp. KSC whereas oxidation, hydrolysis and C–N bond cleavage are the mechanisms of degradation in the case of *Rhodococcus ruber* strain IEGM 346. Degradation proceeded via additional processes like methylation of the hydroxyl group, decarboxylation, elimination of HCl group, and replacement of a hydrogen atom with a sulfonic acid group in *Labrys portucalensis* F11 (Moreira et al., 2018). This replacement of a hydrogen atom with a sulfonic acid group was a novel step suggested by Moreira et al. (2018) during diclofenac degradation. Intramolecular amidation and sulphate conjugation of the hydroxyl group was also reported to be the processes involved during

Table 4
Harmful effects of NSAIDs on living organisms.

NSAID	Organism	Harmful effects	Concentration	Additional information	References
Diclofenac	*Oryzias latipes* (Japanese medaka)	64.0-fold, 13.0-fold and 60.3-fold increase in CYP1A gene in the liver, gills, and intestines, respectively.	8 mg/L		Hong et al. (2007)
		132.2-fold, 12.7-fold and 58.0-fold increase in p53 gene in liver, gills, and intestines, respectively.			
		179.5-fold, 16.5-fold, and 179.8-fold increase in vitellogenin (VTG) gene in liver, gills, and intestines, respectively.			
	Rainbow trout	Kidney lesions, high gill damage accumulation in liver, kidney, gills and muscle tissue	>5 μg/L	BCF in 12–2732 in liver, 5–971 in kidney, 3–763 in gills, and 0.3–69 in muscle	Schwaiger et al. (2004); Triebskorn et al. (2004)
				NOEC = 320 μg/L	
	Brown trout	Decrease in packed erythrocyte volume	>0.5 μg/L		Hoeger et al. (2005)
		Histopathological alterations in liver	5 μg/L		
		Increased secondary lamellar clubbing in gills	50 μg/L		
		Histopathological alterations in kidney	>0.5 μg/L		
	Gyps bengalensis, Gyps indicus, Gyps tenuirostris	renal failure, visceral gout leading to population decline	0.25 mg/kg and 2.5 mg/kg		Oaks et al. (2004)
	Daphnia magna	modulation of heat shock protein 70 (hsp 70)	>40 mg/L		Haap et al. (2008)
		induction of high mortality rate at acute concentrations			Ferrari et al., 2004
	Dreissena polymorpha (zebra mussel)	destabilization of the lysosomal membranes primary genetic lesions and fixed damage to DNA	250 μg/L		Parolini et al. (2009)
	Danio reria (zebrafish)	increase in glutathione-S-transferase, catalase and malondialdehyde levels indicative of oxidative stress	1 mg/L	NOEC = 3 mg/L	Diniz et al. (2015); de Carvalho Penha et al. (2021)
Ibuprofen	*Dreissena polymorpha*	Induction of moderate genetic and cellular damage along with transitory antioxidant defense responses and destabilization of lysosomal membranes.	9 nM and 35 nM		Parolini et al. (2011);
	Mytilus galloprovincialis	imbalance in catalase, glutathione peroxidase, superoxide dismutase and glutathione S-transferase activity	250 ng/L		Gonzales-Rey and Bebianno (2012)
	Daphnia magna	decrease in total amount of eggs	0.4 mg/L	NOEC = 33.3 mg/L	Du et al. (2016)
		decrease in body length	2.51 mg/L		Wang et al. (2016);
		decrease in total number of brood per female,	>0.5 μg/L		
		increased activities of glutathione S-transferase, superoxide dismutase and catalase	>0.5 μg/L		
		inhibition at low concentration; induction of CYP360A gene expression level at high concentration	>0.5 μg/L		
		mortality on exposure after 24 h.	200 mg/L		Han et al. (2010)
	Cirrhinus mrigala	Altered levels of hemoglobin, leukocytes, hematocrit, mean cellular volume, mean cellular hemoglobin, plasma glucose and alanine transaminase enzyme activity	14.2 ppm	LC50 = 142 ppm for 24 h	Saravanan et al. (2012)
	Oryzias latipes	Increased 17β-estradiol production and aromatase activity in H295R cells, delay in hatching of eggs	2 mg/L	NOEC = 0.0001 mg/L	Han et al. (2010)
			0.1 μg/L		
	Ruditapes philippinarum	Reduced health status, alterations of blood parameters and hemocytes	10 μg/L		Aguirre-Martínez et al. (2013)
	Psammechinus miliaris	Significant decrease of fertilization success	≥1 μg/L		Zanuri et al. (2017)
Naproxen	*Danio reria*	lower heart rate	100 mg/L and 125 mg/L	LC50 = 115.2 mg/L for embryos and 147.6 mg/L for larvae.	Li et al. (2016)
		pericardial oedema and teratogenic effects;	20 mg/L	BCF values of 1684 in 0.1 μg/L	
		reduction in survival rates, body length and weight	10 μg/L		Xu et al. (2019)
		thyroid disruption and decrease in thyroid hormone levels	>10 μg/L		

(*continued on next page*)

Table 4 (*continued*)

NSAID	Organism	Harmful effects	Concentration	Additional information	References
	Hydra magnipapillata	contraction of body column and tentacles	40 mg/L	LC50 = 51.99 mg/L	Yamindago et al. (2019)
	Elliptio complanata	Phagocytosis,	35 μM		Gagné et al. (2006)
		adherence to microplate wells, intracellular esterase activity,	4 μM		
		lipid peroxidation	152 μM 35 μM		
	Hyalella Azteca	increased superoxide dismutase and catalase activity; oxidative stress damage of the genetic material	76.6 and 339.2 mg/kg		Garcia-Medina et al. (2015)
	Bacillus thuringiensis	B1-significant increase in the value of the ratio of saturated and unsaturated fatty acids	4.8 g/L and 5.2 g/L	EC50 = 4.69 g/L	Górny et al. (2019b)
	Ceriodaphnia dubia	inhibition of growth after 7 days of exposure at low concentration	0.33 mg/L	EC50 = 0.33 mg/L	Li et al. (2016)
	Jordanella floridae	decrease in egg fertilization	>0.1 μg/L		Nesbitt (2011)
Ketoprofen	*Daphnia magna*	Inhibition of swimming speed, reduction in thoracic limb activity	>0.005 mg/L		Bownik et al. (2020)
		Inhibition of hopping frequency, inhibition of mandible movements	5 and 50 mg/L		
		Biochemical alterations in enzymatic activities	0.05 and 5 mg/L >0.24 μg/L		Alkimin et al. (2020)
	Lemna minor	alterations in enzyme activities of catalase, GST and carbonic anhydrase	>0.24 μg/L		Alkimin et al. (2020)
	Cyprinus carpio	delay in hatching and development	2.1 mg/L		Prášková et al. (2013)

NOEC = No Observed Effective Concentration BCF = Bioconcentration Factor.
EC50 = Half-maximal Effective Concentration.
LC50 = Lethal Concentration 50.

diclofenac degradation by *Pseudoxanthomonas* sp. DIN-3 along with the mechanisms already mentioned in earlier studies (Lu et al., 2019). Major processes employed in the microbial degradation pathways are further shown in Fig. 2.

The hydroxylation of diclofenac to 4′-hydroxydiclofenac was identified as the limiting step for biodegradation in *Brevibacterium* sp. D4 (Bessa et al., 2017). Apart from mono- and di-hydroxylated metabolites, phenylacetic acid, acetoacetic acid, fumarylacetoacetic acid, and fumaric acid were also reported to be the metabolites in the case of *Rhodococcus ruber* strain IEGM 346 (Ivshina et al., 2019). Benzoquinone imine was a noteworthy metabolite of diclofenac degradation by *Labrys portucalensis* F11 (Moreira et al., 2018). Table 6 enlists some of the important metabolites generated during microbial degradation of NSAIDs.

Total of 337 enzymes were found that were possibly involved in degradation, which included dehydrogenase, hydrolase, oxidoreductase, oxidase, dioxygenase, monooxygenase, and decarboxylase along with the possible involvement of hydroxylase and dehalogenase (Lu et al., 2019). Aissaoui et al. (2017b) further analysed the effects of metabolites obtained after degradation by *Enterobacter cloacae* (D16) on biomarkers such as reduced glutathione, glutathione-S-transferase, catalase, lipid peroxidation, and superoxide dismutase in mice which exhibited no toxic effects in mice (Aissaoui et al., 2017b). Though maximum research has been carried out in aerobic conditions, diclofenac did not readily biodegrade under anaerobic conditions. Only 25–40% of diclofenac was bio-transformed under methanogenic/fermentative conditions (Zur et al., 2020).

5.2. Microbial degradation of ibuprofen

As in the case of diclofenac, hydroxylation seems to the major process involved in the degradation of ibuprofen. Some other pathways for ibuprofen degradation have also been explored in recent times. Murdoch and Hay (2005, 2015) identified two bacterial species capable of degrading ibuprofen. *Variovorax* Ibu-1 and *Sphingomonas* sp. Ibu-2 degraded ibuprofen with varying efficiencies through different degradation pathways. Trihydroxyibuprofen bearing all three hydroxyl groups was only detected in *Variovorax* Ibu-1 after the addition of 3-fluorocatechol (a meta ring-fission inhibitor), which indicates the possibility of ibuprofen metabolism advancing via a trihydroxyibuprofen meta ring-fission pathway (Murdoch and Hay., 2015). This aromatic ring cleavage followed by the formation of catechols was also seen during degradation by *Patulibacter medicamentivorans*. While *Variovorax* Ibu-1 directly trihydroxylated the aromatic ring as the sole prerequisite to ring-cleavage, *Sphingomonas* sp. Ibu-2 demonstrated almost complete removal via a degradation pathway that employs coenzyme A ligation with subsequent dioxygenation and deacetylation to isobutylcatechol. Catechols were found to be the key metabolites in ibuprofen degradation and were cleaved by extradiol dioxygenase (Murdoch and Hay., 2005). Hydroxylation of both aromatic ring and aliphatic chain of ibuprofen was also indicated in *Bacillus thuringiensis* B1 (2015b) (Marchlewicz et al., 2016).

Two biodegradation pathways were proposed in *Patulibacter medicamentivorans,* where in one pathway, the metabolites generated via hydroxylation, dehydrogenation, and oxidation correspond to isobutylbenzene and 3-isobutylphenol and have higher toxicity compared to the more biodegradable and less toxic products formed in the second pathway by hydroxylation and subsequent oxidation (Salgado et al., 2018). Ibuprofen degradation proceeded via hydroxylation, loss of carboxyl and hydroxyl group, and subsequent oxidation in *Pseudoxanthomonas* sp. DIN-3 (Lu et al., 2019); whereas, oxidation of isopropyl chain of ibuprofen was suggested as the major mechanism of degradation in *T. versicolor*.

The formation of 2-hydroxy ibuprofen through monooxygenase activity was found to be the rate-limiting step in *Bacillus thuringiensis* B1 (2015b). Other metabolites like 2-(4-hydroxyphenyl-) propionic acid, 1,4-hydroquinone, and 2-hydroxyquinol were also detected in this study. High activity of aliphatic monooxygenases and phenol and

Table 5
Microbial degradation of NSAIDs using pure cultures.

S. No	Microorganism used	Initial concentration of NSAID	Removal %	Time taken	References
Diclofenac					
1.	*Trametes versicolor*	10 mg/L	94%	1 h	Marco-Urrea et al. (2010a)
		45 μg/L	100%	0.5 h	
2.	*Phanerochaete sordida* YK-624	29.615 μg/L[a]	90%	3 days	Hata et al. (2010)
			100%	6 days	
3.	*Klebsiella* sp. KSC	70 mg/L	100%	72 h	Stylianou et al. (2018)
4.	*Rhodococcus ruber* strain IEGM 346	50 μg/L	100%	6 days	Ivshina et al. (2019)
		50 mg/L	50%	60 days	
5.	*Labrys portucalensis* F11	503.455–10069.1 g/L[a]	70%	30 days	Moreira et al. (2018)
		503.455 g/L and 10069.1 g/L with supplementary feeding with acetate[a]	100%	6 and 25 days	
6.	*Brevibacterium* sp. D4	10 mg/L	35%	30 days	Bessa et al. (2017)
		10 mg/L with periodic supplementary feeding with acetate	90%	30 days	
7.	*Enterobacter cloacae* D16	10 mg/L	67.57%	48 h	Aissaoui et al. (2017b)
8.	*Pseudoxanthomonas* sp. DIN-3	50 μg/L	23%	14 days	Lu et al. (2019)
9.	*Ganoderma applanatum* and *Laetiporus sulphurous*	15 mg/L	80% and 87% respectively	72 h	Bankole et al. (2020)
10.	*Pseudomonas moorei* KB4	1 mg/L	51%	12 days	Zur et al. (2020)
		1 mg/L with supplementary glucose	100%	11 days	
		1 mg/L with supplementary glucose and acetate	100%	12 days	
Ibuprofen					
1.	*Variovorax* Ibu-1	Activated sludge +500 mg/L ibuprofen	50%	7 days	Murdoch and Hay (2015)
2.	*Trametes versicolor, Irpex lacteus, Ganoderma lucidum* and *Phanerochaete chrysosporium*	10 mg/L	70–88%	7 days	Marco-Urrea et al. (2009)
3.	*Bacillus thuringiensis* B1 (2015b)	25 mg/L	46.56%	20 days	Marchlewicz et al. (2016)
4.	*Patulibacter* sp. strain I11	50 μg/L supplemented with yeast extract	62% on M9, and 92% on OD2-medium	90 h	Almeida et al. (2013b)
5.	*Sphingobium yanoikuyae*	50 mg/L	68%	130 days	Balciunas et al. (2020)
6.	*Pseudoxanthomonas* sp. DIN-3	50 μg/L	41%	14 days	Lu et al. (2019)
7.	*Ganoderma applanatum* and *Laetiporus sulphurous*	15 mg/L	66% and 79% respectively	72 h	Bankole et al. (2020)
Naproxen					
1.	*Stenotrophomonas maltophilia* KB2	6 mg/L	28%	35 days	Wojcieszynska et al. (2014)
		6 mg/L supplementary feeding with glucose	78%		
		6 mg/L supplementary feeding with phenol	40%		
2.	*Bacillus thuringiensis* B1 (2015)	6 mg/L	100%	35 days	Marchlewicz et al. (2016)
3.	*Phanerochaete chrysosporium*	20 mg/L	86–90%	7 days	Li et al. (2015)
4.	*A. niger*	50 mg/L	98%	48 h	Aracagök et al. (2017)
5.	*Cymbella* sp. and *Scenedesmus Quadricauda*	1 mg/L	97.1% and 58.8% respectively	30 days	Ding et al. (2017)
6.	*Planococcus* sp. S5	6 mg/L	30% as sole substrate; 75–86% with supplementary carbon sources	35 days	Domaradzka et al. (2015)
7.	*Pseudoxanthomonas* sp. DIN-3	50 μg/L	39%	14 days	Lu et al. (2019)
8.	*Trametes versicolor*	10 mg/L	100%	6 h	Marco-Urrea et al. (2010c)
		55 μg/L	95%	5 h	
Ketoprofen					
1.	*Trametes versicolor*	10 mg/L	100%	24 h	Marco-Urrea et al. (2010b)
		40 μg/L	100%	5 h	
2.	*Pleurotus ostreatus*	10 mg/L	90% in batch, while 70–85% in continuous stage	7–15 days	Palli et al. (2017)
3.	*Pleurotus djamor*	10 mg/L	83%	72 h	Cruz-Ornelas et al. (2019)

[a] = Concentration reported in M in literature.

hydroquinone monooxygenases was also reported in *Bacillus thuringiensis* B1 (2015b) (Marchlewicz et al., 2017). Marco-Urrea et al. (2009) detected three metabolites namely 1-hydroxy ibuprofen; 2-hydroxy ibuprofen and 1,2-dihydroxy ibuprofen on studying the degradation of ibuprofen by four fungal species. It was suggested that an alternate enzyme system different from laccases, MnPs, and Cyt P450 monooxygenases is involved in the initial step of ibuprofen degradation in *T.* versicolor (Marco-Urrea et al., 2009). 1-hydroxy ibuprofen along with ibuprofen carboxylic acid were also reported as the metabolites for ibuprofen degradation by *Pseudoxanthomonas* sp. DIN-3 (Lu et al., 2019).

Bankole et al. (2020) demonstrated that *Ganoderma applanatum* and *Laetiporus sulphureus* individually were capable of degrading a mixture of NSAIDs containing celecoxib, diclofenac, and ibuprofen with a removal efficiency of 61% and 73% for diclofenac and ibuprofen, respectively. A higher removal efficiency was obtained when NSAIDs were degraded individually as well as when the consortium of both fungal mycelia was added to the mixture of NSAIDs (Bankole et al., 2020).

5.3. Microbial degradation of naproxen

The research on microbial degradation of naproxen was also pioneered by Marco-Urrea et al. (2010c), who reported minor and almost complete naproxen degradation in experiments with purified laccase and purified laccase plus mediator 1-hydroxybenzotriazol, respectively. A significant inhibition on naproxen degradation in *T. versicolor* and *A. niger* on the addition of cytochrome P450 inhibitor 1-aminobenzotriazole suggests that both enzymatic systems could play a role in naproxen degradation (Aracagök et al., 2017; Marco-Urrea et al., 2010c). Different degradation pathways were observed during degradation using the same strain under different co-metabolic conditions for naproxen, since different supplemental carbon sources activated different sets of enzymes. When glucose was used as a supplemental carbon source, gentisate 1,2-dioxygenase was activated leading to the oxygenolytic cleavage of its product, whereas catechol 2,3-dioxygenase activity was observed with phenol as a growth substrate in both *Stenotrophomonas maltophilia* KB2 and *Planococcus* sp. strain S5. (Wojcieszynska et al., 2014; Domaradzka et al., 2015).

Hydroxylation followed by the intradiol cleavage of the ring catalyzed by hydroxyquinol 1,2-dioxygenase is reported to be the degradation process in *Stenotrophomonas maltophilia* KB2 and *Planococcus* sp. strain S5 (Domaradzka et al., 2015; Wojcieszynska et al., 2014). Some other major processes in naproxen degradation by *Cymbella* sp. and *Scenedesmus quadricauda* include demethylation, decarboxylation, cleavage of the ester group, losses of hydroxyl and glucuronide groups, and fracture of naphthalene ring (Ding et al., 2017). Similar processes were observed for naproxen degradation by *Pseudoxanthomonas* sp. DIN-3 (Lu et al., 2019).

1-(6-methoxynaphthalen-2-yl) ethenone and 2-(6-hydroxynaphthalen-2-yl) propanoic acid (commonly known as 6-desmethylnaproxen) were reported to be the metabolites present as a result of fungal degradation of naproxen in *T. versicolor* (Marco-Urrea et al., 2010c). 6-O-desmethylnaproxen was a key metabolite reported in *Bacillus thuringiensis* B1 (2015b), *A. niger* and algal cultures of *Cymbella* sp. and *Scenedesmus quadricauda* (Aracagök et al., 2017; Górny et al., 2019a; Ding et al., 2017). A series of tyrosine conjugated metabolites such as 6-(2-amino-1-hydroxy-3-(4-hydroxyphenyl)propoxy) naphthalene-2, 3-diol; 6-hydroxy-7-methoxynaphthalen-2-yl-2-amino-3-(4-hydroxyphenyl) propanoate, and 6-hydroxy-7-methoxynaphthalen-2-yl-2-amino-3-(4-hydroxyphenyl)-3-(4-hydroxyphenyl)propanoate were also detected as a result of conjugation of 6-O-desmethylnaproxen with amino acid in the algal cultures (Ding et al., 2017). Apart from 6-O-desmethylnaproxen, 2-formyl-5-hydroxyphenylacetic acid and salicylic acid were found to be the major metabolites of naproxen degradation in *Bacillus thuringiensis B1* (2015b).

The activity of tetrahydrofolate-dependent O-demethylase and catechol 1,2-dioxygenase was related to the presence of O-desmethylnaproxen and cleavage of catechol to cis,cis-muconic acid respectively in *Bacillus thuringiensis B1* (2015b). Salicylate 1,2-dioxygease and gentisate 1,2-dioxygenase were also observed, which suggested the transformation of salicylate to maleyl puryvate or 2-oxo-3,5-heptadienedioic acid by gentisic acid (Górny et al., 2019b). The importance of enzymatic activity like phenol monooxygenase, naphthalene dioxygenase, and hydroxyquinol 1,2- dioxygenase was also reported in *Stenotrophomonas maltophilia* KB2 and *Planococcus* sp. strain S5. (Domaradzka et al., 2015; Wojcieszynska et al., 2014).

Li et al. (2015) also reported that with an initial concentration at 10 mg/L, more than 90% of naproxen was removed by the crude enzyme in the first two days which was higher than the removal performance in whole-cell cultivation since 90% naproxen removal was not reached until Day 7 in whole-cell studies. This indicates that the extracellular enzymes produced by *P. chrysosporium* played an important role in naproxen removal (Li et al., 2015).

5.4. Microbial degradation of ketoprofen

Fungal degradation of ketoprofen via pure cultures has been explored in three white rot fungi namely, *Trametes versicolor*, *Pleurotus ostreatus,* and *Pleurotus djamor*. No bacterial pure strain has been known to degrade ketoprofen as per our knowledge, though bacterial mixed cultures have been reported to degrade ketoprofen, which will be discussed in Sections 5.5.

Ketoprofen degradation was inhibited after the addition of cytochrome P450 inhibitor 1-aminobenzotriazole in both *Trametes versicolor* and *Pleurotus ostreatus,* which indicated cytochrome P450 to be involved in the first oxidation step of ketoprofen degradation. Reduction of the keto group was also indicated as a major process in both these biotransformations. Hydroxylation of an aromatic ring was observed in *T. versicolor* and *P. ostreatus*; whereas, deoxygenation was reported only in *T. versicolor* (Palli et al., 2017; Marco-Urrea et al., 2010c).

(2-[(3-hydroxy (phenyl)methyl)phenyl]-propanoic acid) was formed as a major metabolite in both *T. versicolor* and *P. ostreatus*. Metabolites like (2-(3-benzoyl-4-hydroxyphenyl)-propanoic acid) and 1 (2-[3-(4-hydroxybenzoyl) phenyl]-propanoic acid) were generated as minor metabolites in *T. versicolor* (Marco-Urrea et al., 2010c); whereas, 2-[3-(4-hydroxybenzoyl)phenyl] propanoic acid was formed in *P. ostreatus* (Palli et al., 2017).

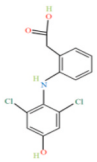
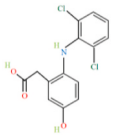
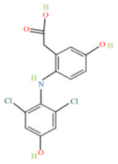
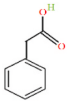
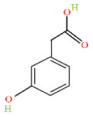
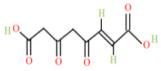
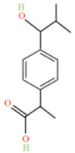
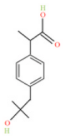
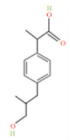
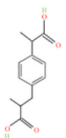
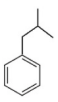
Cruz-Ornelas et al. (2019) reported the degradation of ketoprofen even in presence of diclofenac and naproxen by *Pleurotus djamor*. After 6 and 48 h, 68% and 83% removal for ketoprofen were obtained, respectively, when it was added along with diclofenac and naproxen. Additionally, 99% of diclofenac and 87% of naproxen degradations were reported in a mixture of NSAID after 48 h. Individually, 93% of diclofenac and 90% of naproxen were degraded by *P. djamor* in 6 and 72 h, respectively (Cruz-Ornelas et al., 2019).

5.5. Microbial degradation of NSAIDs using mixed cultures

While studies on the microbial degradation of NSAIDs using fungal, algal, or bacterial pure cultures yielded some promising results in lab-scale experiments, maintenance of pure culture on a field scale of treatment is extremely tedious. Therefore, several studies explored the use of mixed consortia for individual as well as a mixture of NSAIDs. Only a few characterized the components of the mixed consortia as enlisted in Table 7. Other studies mainly comprised of NSAID degradation using activated sludge and mixed cultures (Abu Hasan et al., 2016; Nguyen et al., 2019; Martínez-Alcalá et al., 2017). Several genera like *Nitratireductor*, *Asticcacaulis*, *Pseudacidovorax* and *Bacillus pseudomycoides, Rhodococcus ruber*, *Vibrio mediterranei* were observed to gain competitive advantages or had a higher toxicity resistance towards diclofenac, ibuprofen, and ketoprofen, which was suggested as an indication that they might contribute to their respective biodegradation (Nguyen et al., 2019). Biodegradation pathways for NSAIDs with mixed cultures have been scantily reported.

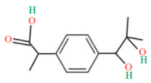
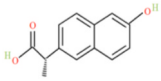
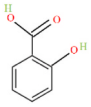
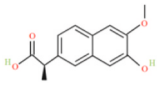
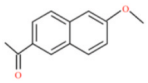
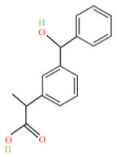
Quintana et al. (2005) studied the biodegradation of ibuprofen, naproxen, diclofenac, and ketoprofen as sole carbon substrates using activated sludge, and observed that only 20 mg/L ketoprofen was degraded within 28 days following the biphenyl pathway; whereas, all others remained unaltered. However, in the same study, O-desmethylnaproxen along with 2-hydroxylated ibuprofen and 1-hydroxylated ibuprofen were also reported as a result of demethylation of naproxen and hydroxylation of ibuprofen by mixed cultures (Quintana et al., 2005). Ketoprofen degradation yielded 3-(hydroxy-carboxymethyl) hydratopic acid and 3-(keto-carboxymethyl)-hydratopic acid due to dioxygenation, reduction of keto group, oxidative ring-opening of catechol by meta-cleavage, subsequent hydrolysis and loss of CO_2, water, and a CO group (Quintana et al., 2005). Meanwhile, ketoprofen degradation using K_2 bacterial consortium generated three new metabolites namely, (3-ethylphenyl) (phenyl)methanone; (3-hydroxyphenyl) (phenyl)methanone, and (3-hydroxyphenyl) (oxo)acetic acid

Table 6
Various metabolites formed during microbial degradation of NSAIDs.

S. No	Microbes	Metabolites	Metabolites structure*	Generation	References
Diclofenac					
1.	*Phanerochaete sordida* YK-624, *T. versicolor, L. portucalensis* F11, *Brevibacterium* sp. D4, *Pseudoxanthomonas* sp. DIN-3, *Pseudomonas moorei* KB4	4′-hydroxydiclofenac		I	Marco-Urrea et al. (2010a); Hata et al. (2010); Moreira et al. (2018); Bessa et al. (2017); Lu et al. (2019); Zur et al. (2020)
2.	*Phanerochaete sordida* YK-624, *T. versicolor, L. portucalensis* F11, *Pseudoxanthomonas* sp. DIN-3	5-hydroxydiclofenac		I	Marco-Urrea et al. (2010a) Hata et al. (2010) Moreira et al. (2018) Lu et al. (2019)
3.	*Phanerochaete sordida* YK-624	4′,5-dihydroxydiclofenac		Unknown	Hata et al. (2010)
4.	*R. ruber* IEGM 346	phenylacetic acid		II	Ivshina et al. (2019)
5.	*R. ruber* IEGM 346	3-hydroxyphenylacetic acid		III	Ivshina et al. (2019)
6.	*R. ruber* IEGM 346	2-(*p*-benzoquinone-2)acetic acid		IV	Ivshina et al. (2019)
7.	*R. ruber* IEGM 346	fumarylacetoacetic acid		V	Ivshina et al. (2019)
8.	*L. portucalensis* F11	Benzoquinone imine		II	Moreira et al. (2018)
Ibuprofen					
1.	*Pseudoxanthomonas* sp. DIN-3, *Trametes versicolor*	1-hydroxyibuprofen		I	Lu et al. (2019); Marco-Urrea et al. (2009)
2.	*Bacillus thuringiensis* B1 (2015b), *Trametes versicolor*	2-hydroxyibuprofen		I	Marchlewicz et al. (2016); Marco-Urrea et al. (2009)
3.	*Variovorax* Ibu-1	3-hydroxyibuprofen		Unknown	Murdoch and Hay. (2015)
4.	*Pseudoxanthomonas* sp. DIN-3	Ibuprofen carboxylic acid		I	Lu et al. (2019)
5.	*Patulibacter medicamentivorans*	Isobutylbenzene		I	Salgado et al. (2018)

(*continued on next page*)

Table 6 (*continued*)

S. No	Microbes	Metabolites	Metabolites structure*	Generation	References
6.	*Trametes versicolor*	1,2-dihydroxy ibuprofen		II	Marco-Urrea et al. (2009)
Naproxen					
1.	*Bacillus thuringiensis* B1 (2015b), *Cymbella* sp. and *Scenedesmus quadricauda, A. niger, T. versicolor*	O-desmethylnaproxen		I	Górny et al. (2019a); Ding et al. (2017); Aracagök et al. (2017); Marco-Urrea et al., (2010c)
2.	*Bacillus thuringiensis* B1 (2015b)	Salicylic acid		III	Górny et al. (2019a)
3.	*A. niger*	7-hydroxynaproxen		Unknown	Aracagök et al. (2017)
4.	*T. versicolor*	1-(6-methoxynaphthalen-2-yl) ethanone		Unknown	Marco-Urrea et al. (2010c)
Ketoprofen					
1.	*T. versicolor, P. ostreatus*	(2-[(3-hydroxy (phenyl) methyl)phenyl]-propanoic acid)		Unknown	Marco-Urrea et al. (2010b)

were identified, which were not detected in studies with pure cultures (Ismail et al., 2016).

Degradation of ibuprofen and naproxen was also carried out by an algal-bacterial approach, where 2 photobioreactors, anoxic-aerobic photobioreactor (phase A), and anaerobic-anoxic-aerobic photobioreactor (phase B) were used. Though the bacterial component was not discussed, the microalgae population in the photobioreactors was mainly composed of *Chlorella vulgaris* and *Phormidium* sp in phase A and *Chlorella vulgaris, Pseudonabaena acicularis* and *Scenedesmus acuminatus* in phase B, respectively (López-Serna et al., 2019).

NSAIDs were also known to be degraded by microbes in environmental conditions, but not many studies delved into the composition of the microbial community. Ibuprofen microbial degradation in the hyporheic zone sediments of a river was investigated where 1-, 2-, 3-hydroxyibuprofen and carboxyibuprofen were the observed ibuprofen biotransformation products. Strains belonging to taxas of Acidobacteria, Gemmatimonadetes, Actinobacteria, Proteobacteria, Bacteroidetes and Latescibacteria along with two strains from genera *Pseudomonas* and *Novosphingobium* were isolated from the river sediment which were suggested to be involved in ibuprofen degradation (Rutere et al., 2020). Actinobacteria and Bacteroidetes were also enriched in the presence of diclofenac and naproxen indicating their ability to adapt in the presence of NSAIDs and successfully degrade them (Jiang et al., 2017). Bacterial community of a river sediment associated with its capacity for degrading diclofenac was studied and concentrations of *Ralstonia, Pseudomonas, Hyphomicrobium* and *Novosphingobium* were significantly elevated in the sediment incubations where fast removal was detected (Coll et al., 2020).

Studies involving the analysis of degradation based on different conditions were also carried out using mixed cultures. Illumination, nitrification, concentration of ammonium and dissolved organic matter were some of the criteria studied. A K_2 bacterial consortium was reported to degrade ketoprofen with a higher biodegradation rate under dark conditions with complete biodegradation within 48 h compared to the biodegradation rate of ketoprofen in light. Additionally, the effect of a diurnal cycle was also reported (Ismail et al., 2016). Lower ibuprofen biotransformation rates were observed at high ammonium concentrations (Dawas-Massalha et al., 2014); whereas contrastingly, an increase in diclofenac degradation efficiency was reported in another study (Tran et al., 2009).

Ibuprofen and naproxen (1 mg/L) were completely removed in 35 days, whereas diclofenac was found to be persistent in presence of nitrification (He et al., 2018). Ammonia oxidizing bacteria containing ammonia monooxygenase enzyme can easily hydroxylate linear alkyl carbons specifically for the secondary and tertiary carbons in the chain, which are found in naproxen and ibuprofen, because of its low specificity and broad substrate spectrum (Fernandez Fontaina et al., 2016). It was noted that the addition of dissolved organic matter enhanced the biodegradation of ibuprofen and naproxen, which could be attributed to higher biomass production and microbial respiration that indicates a more active microbial community capable of increasing drug biodegradation capacity (He et al., 2018).

5.6. Applications and limitations of bioremediation strategies in removal of NSAIDs

Though there are a plethora of laboratory studies for the biodegradation of NSAIDs, applications at pilot scale or large wastewater treatment plants are limited. While scaling up to a WWTP, the major point of focus needs to be on the type of microbes employed for biodegradation. This needs to be decided based on the type of wastewater treated by the WWTP. In the case of hospital or pharmaceutical industry effluent, where NSAIDs are present in relatively high concentration, the microbes used need to have a very high Minimum Inhibitory Concentration (MIC) for their effective working. The type of microbes used also depends on the pH of the wastewater, since maximum microbes degrade NSAIDs at low pH in laboratory experiments (Vieno and Sillanpää, 2014).

Another consideration about scaling up needs to be regarding the location of the tertiary biological treatment reactor for removal of pharmaceuticals in the WWTP. Since a pure culture or a mixed microbial culture needs to be maintained, relevant sterility of the incoming wastewater will be preferred. Due to this, the appropriate site for the reactor will be after an initial round of disinfection. After the treatment, a second round of disinfection will be preferred to eliminate the residual

Table 7
Microbial degradation of NSAIDs using mixed cultures.

S. No	Microorganism Used	Initial Concentration	Removal %	Time taken	References
1	*Gordonia amicalis, Acinetobacter bouvetii, Paracoccus aminophilus* and *Patulibacter americanus*	1000 μg/L or 500 μg/L ibuprofen	100%	2–6 h	Almeida et al. (2013a)
	Methylobacterium populi, Gordonia hydrophobica, Tsukamurella spumae, Paracoccus aminovorans, Rhodococcus qingshengii, Gordonia terrae, Rhodococcus zopfii and *Bosea thiooxidans*	250 μg/L or 100 μg/L ketoprofen	100%	10–25 h	
2	*Arthrobacter nicotianae. Pseudomonas* sp., *Enterobacter hormaechei and Citrobacter youngae*	3 mg/L of each NSAIDs	23.08% for ibuprofen and 9.12% for diclofenac	48 h	Aissaoui et al. (2017a)
		3 mg/L of each NSAIDs with supplementary feeding with glucose	100% for ibuprofen and 56% for diclofenac	48 h	
3	K_2 bacteria consortium (*Raoultella ornithinolytica* B6, *Pseudomonas aeruginosa* strain JPP, *Pseudomonas* sp. P16, *Stenotrophomonas* sp. 5LF 19TDLC)	1.27 g/L[a] of ketoprofen under dark conditions	100%	48 h	Ismail et al. (2016)
	K_2 bacteria consortium + microalga *Chlorella* sp. Iso 4	508.5 mg/L[a] of ketoprofen under diurnal conditions	100%	10 days	
4	Stable nitrifying enrichment culture	25–100 μg/L of ibuprofen	100%	24 h	Dawas-Massalha et al. (2014)
		25–100 μg/L of Ketoprofen	100%	~150 h	
5	Pentane enrichment culture	2.9 mg/L of diclofenac, 2 mg/L ibuprofen and 2.3 mg/L naproxen respectively[a]	0%, 100% and 64% respectively	96 h	Bragança et al. (2016)

[a] Concentration reported in M in literature.

microbial community. Additionally, if the wastewater treatment plant uses chlorine disinfection, a supplementary step comprising of inspecting the wastewater for any residual chlorine before entering the reactor needs to be carried out, since any residual chlorine will prove detrimental to the microbes in the reactor. This assessment along with secondary disinfection will significantly increase the cost of the treatment. Due to this, an alternative position for the reactor can be just before disinfection, given that the relative purity of the mixed cultures can be maintained and tested regularly. Although this location will probably yield a lower efficiency for the removal of NSAIDs, it will prove to be a cost-effective process. The reactor can be placed after an initial round of disinfection in case of UV or ozone disinfection. Along with the location, the hydraulic retention time (HRT) for the treatment will also affect the biodegradation process. Since the reduction of many pharmaceuticals will be preferred in the same reactor, the HRT needs to be decided carefully for optimal results.

Very few microbes are capable of utilizing NSAIDs as a sole carbon source. Since maximum microbial degradation of NSAIDs is carried out in co-metabolic conditions using a supplementary carbon source, the wastewater needs to be analysed for the presence of the optimum supplementary carbon source. If not present in enough concentration, it is not feasible to add the said source externally; therefore, an alternative supplementary carbon source present in an abundant concentration in the wastewater needs to be explored for the microbes.

Several limitations can be observed while upscaling the lab experiments of degradation to full application scale by a specific strain or microbial consortium. These include impractical treatment conditions for full-scale applications and unlikely maintenance of pure culture conditions or even a defined consortium because of transient conditions in WWTPs. It is crucial for the survival of microbes to remain in optimum conditions (such as pH and temperature), which can differ for each microbe factoring in the number of microbes necessary to degrade all major pharmaceuticals present in the hospital or even regular WWTPs.

In addition, the biodegradation pathways of one microorganism cannot be easily generalized to the biodegradation capabilities of microbial consortia, which makes the prediction of degradation metabolites hectic for the entire WWTPs. Furthermore, though lab studies involving degradation of multiple pharmaceutical compounds using a pure culture or microbial consortium are present, the same microbes when introduced in an operational WWTPs are less effective or completely futile because of the highly heterogeneous mixtures of micropollutants and diverse microbial consortia which may interfere or even inhibit the degradation. This may also result in complete failure of the enzyme systems responsible for degradation in microbes. This can be remedied by exploring the synergistic effect of different pharmaceuticals in determining the biodegradation capabilities of microorganisms in real-time WWTPs.

Another major limitation is the dearth of studies regarding degradation of NSAIDs in presence of metabolites, since degradation metabolites and parent compound exists simultaneously in real-time WWTPs. The role of feedback inhibition cannot be overruled in these conditions. Moreover, these metabolites might have a higher toxicity in the reactor which might lead to severe consequences for the environment and aquatic ecosystems. Although some research is done regarding the toxicity of metabolites in laboratory conditions, further research is needed to assess and monitor the toxicity in the reactor during treatment process.

Though several studies have been performed for the degradation of pharmaceuticals using fungi strains like *T. versicolor* and *P. chrysosporium,* fungal as well as algal strains are comparatively less explored in this field. Although some studies utilizing a fungal-algal consortium have been known to degrade NSAIDs, more research in this field can open new avenues concerning the degradation of these micropollutants.

6. Conclusion

NSAIDs were viewed as a future potential environmental threat due to its ubiquitous occurrence in water bodies around the world and the resultant environmental implications in aquatic ecosystems. Following are the key conclusions drawn through the critical assessment of the state-of-the-art knowledge available on NSAIDs:

- Typical concentrations of individual NSAIDs in surface water bodies is well below 1 μg/L. Only 17.24% of surface water sources of the 29 reports showed a higher concentration of diclofenac, while percentage of sources exhibiting higher than 1 μg/L concentration for ibuprofen, naproxen, and ketoprofen were 33.33%, 15.38%, and 10.53%, respectively. The concentrations in the groundwater samples were even lower. The higher concentrations in waters were primarily attributed to untreated wastewater disposal.

- NSAIDs have been reported in 0.003–2747.29 μg/L concentration ranges in WWTPs influent, and removal efficiencies in WWTPs were usually limited for diclofenac (44.4 ± 26.5%), while naproxen (70.2 ± 24.8%), ketoprofen (77.6 ± 16.3%), and ibuprofen (84.8 ± 10.2%) had significantly higher removal efficiencies. A comparative assessment of different wastewater treatment techniques used indicated sewage lagoons to be more effective than ASP and TF based system, but required high hydraulic retention time limiting its application. The addition of sand filters to ASP also improved NSAIDs removal efficiency.
- The toxicity of the metabolites were typically lower than that of the parent compounds. NSAIDs and its metabolites were also found in some edible crops as well as bile and liver of fishes downstream of WWTPs, which could be potentially detrimental to future generations.
- Considering the toxicity at environmentally relevant concentrations, diclofenac and ibuprofen poses bigger risk than naproxen and ketoprofen. The toxicity of NSAIDs metabolites were lower than that of the parent compounds.
- Several pure and mixed microbial strains were found capable of degrading NSAIDs in laboratory as well as environmental conditions. The major step in degradation was hydroxylation by Cytochrome P450. Other mechanisms like decarboxylation, dehydrogenation, dechlorination, deacetylation, oxidation, hydrolysis, demethylation etc. were responsible for subsequent steps of degradation. Enzymes-specific variable degradation pathways leading to different degradation mechanisms can be activated using different supplementary carbon sources and specific microbial consortia.
- Field-scale implementation of microbially-enriched bioremediation methods are challenging owing to vulnerability to additional implicit and explicit factors and variations in the parameters. Further research for full-scale development of the bioremediation process commissioning would be vital to ensure technology transfer from lab to field.

Declaration of competing interest

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

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Appendix A. Supplementary data

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