Review Paper:

Dynamics of Bacterial and Fungal Degradation of Ibuprofen, a Potential Environmental Contaminant

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Abstract

Ibuprofen is a nonsteroidal anti-inflammatory medication with analgesic and antipyretic properties used in the pharmaceutical sector. The rising usage of this medicine has hurt the environment, particularly aquatic life. This necessitates the use of various procedures for degrading this chemical. As aggressive chemical treatment procedures, particularly advanced oxidation processes (AOP) are marked by severe reaction conditions. Bioremediation technologies based on microbial strains appear to be a potential alternative.

Bacterial biodegradation, the most common kind of biodegradation, often happens via side chain hydroxylation, whereas, various fungal extracellular enzymes rely on oxidation of ibuprofen and subsequent breakdown into less toxic metabolites. This review provides an extensive overview of different microbial degradation techniques for a sustainable way of Ibuprofen degradation.

Keywords: Ibuprofen, bacterial degradation, fungal degradation, laccase.

Introduction

One of the significant issues faced by humankind in this era is the contamination of the environment by wastes generated by anthropogenic activities. Biodegradation refers to the breakdown and removal of organic wastes by microbial sources like bacteria, algae, fungi, protozoans etc.[18,36,66] This can help to transform hazardous and toxic waste materials into simpler compounds that organisms can reuse. Biodegradation is a process that involves biological, chemical and mechanical actions that can lead to changes in the chemical structure of molecules that help in their degradation[33]. Besides the microbe type, many physical factors influence the biodegradation process. This includes pH, temperature, oxygen, sunlight, humidity, rainfall etc.[36]

Ibuprofen is a drug that is a derivative of propionic acid (2-(4-isobutylphenyl) propanoic acid) in which one of the hydrogens at position two is substituted by a 4-(2-methyl propyl) phenyl group. It has a molecular formula of $C_{13}H_{18}O_2$[30]. It belongs to the NSAID (nonsteroidal anti-inflammatory drugs), having anti-inflammatory, analgesic and antipyretic effects[23,52]. The discovery of ibuprofen was propelled by the need to find alternative non-corticosteroid pharmacotherapeutic drugs for rheumatoid arthritis Ibuprofen is an FDA-approved painkiller which can also be used for fever reduction in adults and children.

Applications: Ibuprofen is approved as a non-prescription over-the-counter (OTC) drug used for the treatment of migraines, muscle pain, fever, acute lower back pain and toothaches. Due to the antipyretic property of Ibuprofen, it was administered in the early stages of COVID-19. Ibuprofen also acts as an effective remedy to reduce inflammatory rheumatic disease and pain relief in treating chronic wounds[50,65]. Recent studies show that ibuprofen users who take it frequently and for a long time, have lower risks of developing cancer[64]. Among the aspirin and paracetamol group of analgesics, ibuprofen is the least toxic, because it is rarely associated with deaths from accidental or intentional intake. For paediatric use, ibuprofen is more effective than paracetamol as an antipyretic.

Higher doses (1,800–2,400 mg day-1) are used long-term to treat rheumatic and severe musculoskeletal conditions[57]. Ibuprofen was made in response to complications associated with the use of corticosteroids in the treatment of rheumatoid arthritis, as well as the general intolerance of the established NSAIDs and their gastrointestinal adverse effects [67]. The use of ibuprofen is associated with a decreased future risk of Parkinson's Disease (PD). Compared to nonusers, ibuprofen users have 30% lower PD risk. The drug reduces the risk of PD in the carrier of a disease-causing mutation in the LRRK2 gene[58]. Ibuprofen has neuroprotective properties which aspirin and other regularly used analgesics do not possess[20].

Ibuprofen is commonly used by young adult women to treat menstruation pain. The drug's therapeutic effects are inhibiting prostaglandin PGF2a and PGE2, which cause local inflammation and spasms of the uterine smooth muscle[70]. Ibuprofen is also frequently used to treat dental pain such as the discomfort caused by impacted third molars and the surgical removal of these teeth. Numerous research on children and young adults have shown that ibuprofen is also useful in treating headaches and migraines. Ibuprofen is frequently used in sports and other acute minor injuries because it is more effective than other NSAIDs and non-narcotic analgesics[5].

Toxicity: Ibuprofen, which has been metabolised, enters the environment through various ways of which wastewater effluents are the major ones, including hospitals and drug production factories. Ibuprofen by-products also enter

municipal wastewater via consumer faeces or urine. In humans, ibuprofen is rapidly transported throughout the body, with the majority (99%) linked to plasma albumin. 15% of orally taken Ibuprofen is excreted unchanged, conjugated, or hydroxylated (2-, 3- and 1-hydroxyibuprofen) or as carboxyibuprofen or carboxyhydratropic acid[40]. Glucuronide-conjugated Ibuprofen can be degraded in the environment, releasing free Ibuprofen[40,47]. Expired drugs are also regularly dumped in municipal garbage bins, where they develop the risk of contaminating the soil[26].

It has been shown that isobutylbenzene and 3-isobutylphenol, two of the reported ibuprofen metabolites, are harmful both in the short- and long-term and none of the other ibuprofen metabolites will have a short or long-term harmful effect on aquatic organisms[63]. Aquatic animals subjected to ibuprofen for an extended period of time may experience chronic, severe side effects. Studies found that drugs can cause genetic and cellular damage as well as cellular oxidative stress in zebra mussels (*Dreissena polymorpha*) and zebrafish (*Danio rerio*).

Moreover, it affected the kidneys of freshwater rainbow trout, *Oncorhynchus mykiss*, causing hyalinosis, increased oxidative stress and modifications in the expression of heat shock protein[76]. Several biological freshwater species models (*Oryzias latipes*, *Daphnia magna* and *Moina macrocopa*) have shown chronic ibuprofen toxicity, which is accompanied by changes in hormonal balance.

The growth and development of tadpoles were severely influenced by Ibuprofen at ambient concentrations, which has resulted in delayed metamorphosis[75]. Ibuprofen has also been shown to have negative effects on plants; in cowpea (*Vigna unguiculata*), ibuprofen decreased the levels of the enzyme glutathione reductase, shoot and root length, fresh and dry weights, chlorophyll a and b, carotenoid, total chlorophyll and mineral (K and Mg)[63]. Very little data is available on the amount of ibuprofen collected in soil and water in India. According to a 2019 study, the concentration of Ibuprofen in surface water in India ranged from 0.001 to 0.029 micrograms per litre (ug/L)[53].

Another study discovered that ibuprofen concentrations in soil in India varied from 0.001 to 0.012 mg/kg. The highest levels were discovered in soils surrounding industrial locations[26]. According to these investigations, ibuprofen is accumulating in the environment in India, but the amounts are still modest. However, it is critical to monitor ibuprofen levels in the environment, as they may rise in the future due to increased production and consumption of this medicine.

Biodegradation: The transformation of organic pollutants in soils into metabolites, microbial biomass, mineralization products and non-extractable residue (NER) is known as biodegradation[32]. However, simple humic acid-contaminant models were used to illustrate these interactions that contributed to the creation of the residues[9]. In soil biodegradation experiments using 14C-labelled chemicals, they were quantified by quantifying $^{14}CO_2$ emitted from soil samples after earlier extraction of contaminant residues from soil[7]. This method is quick and accurate, but it does not reveal information about the chemical structure of the residues[32]. Compounds immobilised in soil inorganic matter are often considered a toxicological hazard to living beings after being freed from soil inorganic matter due to a lack of this understanding [11,34]**.**

Importance of Ibuprofen degradation: The biodegradation of pharmaceutical drugs by microorganisms is an environmentally beneficial method. Due to increased usage, ibuprofen's toxicity and concentration in wastewater treatment plants and water bodies are increasing daily[55]. It has been observed in numerous toxicological studies that the intermediates produced during advanced chemical treatment are more hazardous than the parent molecules. Therefore, ibuprofen's biodegradation is a potential substitute for its removal from water bodies[10].

Table 1
Common bacterial strains causing biodegradation of Ibuprofen

Bacterial strain	**Mechanism of action**
Gram-positive bacteria	
Bacillus thuringiensis	Phenol, hydroquinone and aliphatic monooxygenases hydroxylase aromatic ring[39]
Patulibacter medicamentivorens	Action with coenzyme A followed by deacetylation and ring cleavage[46]
Nocardia sp.	Degrades into Ibuprofenol and Ibuprofenol acetate[13]
Micrococcus lysodeikticus	Homoprotocatechuate pathway[46]
Streptococcus rimosus	2-(p-hydroxyphenyl) propionic acid is created from the p-hydroxylation of 2-phenylpropionic acid (2PPA)[46]
Gram-negative bacteria	
Sphingomonas sp.	Catechol formation followed by hydroxylation[46]
Variovorax sp.	Catechol formation followed by hydroxylation[46]
Pseudomonas putida	Cumate (p-isopropylbenzoate) molecule meta-cleavage[46]
Pseudomonas cepacia	2PPA and tropic acid are decarboxylated to produce phenylacetaldehyde, which is then oxidised to produce phenylacetic acid[46]

Table 2
Common fungal strains causing biodegradation of Ibuprofen

Fungal strain	Mechanism of action
Aspergillus niger	Lignin modifying enzymes - laccase, lignin peroxidase and manganese peroxidase[45]
Aspergillus sydowii	Adsorption by mycelium[71]
Ganoderma applanatum	Ligninolytic enzyme degradation[6]
Ganoderma lucidum	Adsorption[41]
Laetiporus sulphureus	Ligninolytic enzyme degradation[6]
Mucor circinelloides	Lignolytic enzyme degradation[54]
Phanerochaete chrysosporium	Extracellular enzyme degradation[61]
Trametes polyzona	Laccase, lignin peroxidase and manganese peroxidase[61,68]
Trametes versicolor	Extracellular enzyme degradation[41]
Rhizopus oryzae	Lignin peroxidase and manganese peroxidase enzyme degradation[35]

Factors which increase the removal efficiency of ibuprofen include ibuprofen concentration, microbial community, the methods employed (activated sludge biodegradation and biosorption), the pathway of degradation and the intermediates produced during degradation [15]. Since aggressive chemical treatment methods, mainly advanced oxidation processes (AOP), are characterised by harsh reaction conditions and are not highly adapted to remove analgesics, bioremediation processes utilising bacterial strains with increased abilities to degrade xenobiotics seem to be a promising alternative.

It is also known that microbial degradation of Ibuprofen may proceed via ligation with coenzyme A and the formation of isobutyl catechol or by direct tri-hydroxylation of the aromatic ring, which is a pre-requisite for further ring cleavage. Several bacteria were shown to use ibuprofen as a sole carbon and energy source; however, metabolic pathways of biodegradation, for ibuprofen, remain poorly characterised[76].

Environmental hazards: Pharmaceuticals and personal care products (PPCPs) remain the most important among the developing organic pollutants. Ibuprofen is the world's third most often used medication. Human pharmaceutical use introduces this chemical into our water system. Environmentalists are drawn to it because of its risks and its existence and transformation in the environment [15]. Although NSAID concentrations in surface waters are minimal, the significant biological activity of these compounds may make them harmful to non-target aquatic creatures. As invertebrates play an important role in ecosystem functioning, the impacts of NSAIDs may have serious ramifications for the entire freshwater trophic chain.

Acute NSAID toxicity occurs only in large, impractical quantities, but sub-lethal effects occur at low, environmentally plausible concentrations[52] and hence, there should be more concrete efforts for the detection and elimination. In this review, we have made an attempt to describe the available biological approaches for ibuprofen elimination.

Bacterial biodegradation of Ibuprofen: Of all the bioremediation strategies for removing ibuprofen from the ecosystem, the most promising approach is bacterial biodegradation. Bacterial degradation is defined as the breakdown of compounds by bacteria using their intracellular enzymes, resulting in much smaller and less toxic compounds than the parent compound, which can either be utilised by the bacteria or released into the environment[37]. This process of bacterial biodegradation can either be aerobic or anaerobic[59]. Ibuprofen is biodegraded by anaerobic bacteria through side-chain hydroxylation, which produces ibuprofenol and carboxyhydratropic acid [76]. The high branch structure of Ibuprofen, along with the existence of substitutions at the aromatic ring's para position and their spatial arrangement, all point to the drug's strong biodegradation resistance[39]. Both Gram-positive and Gram-negative bacteria participate in the biodegradation of Ibuprofen.

Mechanism of action of bacteria on Ibuprofen: The biodegradation of ibuprofen by bacteria usually takes place through two different mechanisms. The first mechanism includes ibuprofen reacting with coenzyme A, followed by deacetylation, bioxygenation and ring cleavage to produce isobutyl catechol. Under aerobic conditions, the molecule can go through ring cleavage in this pathway, synthesising catechol intermediates by dioxygenation of the aromatic ring. Two enzymes that may contribute to catechol formation are thiolase and dehydrogenase. The aromatic ring is quickly cleaved by enzyme activity, resulting in molecules that are highly biodegradable and less poisonous. In the first mechanism of the ibuprofen biodegradation route, isopentene is lost and 2-phenylpropionic acid is produced[63].

The Rieske (2Fe-2S) iron-sulphur domain protein may cause this change. This enzyme, which is a member of the oxidoreductase family, uses the CH-CH group of donors as

a source of energy for the bacterium while using either nicotinamide adenine dinucleotide phosphate (NADP+) or nicotinamide adenine dinucleotide (NAD+,oxidised) as an electron acceptor. The activities of the enzymes ibuprofen-CoA ligase and ibuprofen-CoA-1,2-dioxygenase can be used for the breaking of the link in the two starting molecules in this process [62] (Figure 1).

In metabolic mechanism two, the ibuprofen molecule is hydroxylated, the methyl groups are converted to alcohols by adding -OH and the -CH_2OH group is subsequently converted to -CHO and finally -COOH. Enoyl-CoA hydratase, which was found to be up-regulated during the biodegradation of Ibuprofen, can oxidise the ibuprofen molecule by adding hydroxyl groups. The dehydrogenase enzyme oxidises Ibuprofen by reducing an electron acceptor such as flavin coenzyme (flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) or NAD+ (oxidised)/NADH (reduced).

The oxidation of alcohols to aldehydes and the conversion of aldehydes to carboxylic acids, which correspond to the transit of metabolites, are catalysed by the dehydrogenase enzyme[63] (Figure 1). The primary processes in metabolic mechanism number one were hydroxylation and decarboxylation, whereas, in mechanism number two, the primary processes were hydroxylation, methyl group oxidation to alcohols, aldehydes and carboxylic acids, as well as esterification of the acidic groups.

Metabolites produced during bacterial degradation of Ibuprofen: While some metabolites can be detected using GC-MS, most ibuprofen metabolites can be found using LC-MS/MS mass spectra analysis. For each metabolite, structures were suggested based on the precursor m/z values and MS fragmentation patterns. Compared to the parent molecule, the water solubility of the ibuprofen metabolites tends to rise in both pathways. Compared to mechanism two, the Ibuprofen metabolites in mechanism one (such as 3-isobutylphenol and isobutyl benzene) have a stronger propensity to adsorb to the biomass. In contrast to mechanism one, the metabolic transformation of ibuprofen under mechanism two produced less acute toxicity. Because of the synthesis of intermediate catechols, some of the intermediate metabolites in mechanism one were also shown to be toxic to living beings. Although the transformation products produced by mechanism two are less toxic than Ibuprofen, they do not significantly alter the chemical structure's fundamental elements compared to ibuprofen, which is consistent with a detoxifying mechanism[62].

Factors affecting bacterial biodegradation of Ibuprofen: The temperature and pH of the environment are the fundamental factors affecting environmental degradation processes. The pH of sewage depends on the compounds discharged from the sewage treatment plants. Due to its impact on bacterial physiology and the rate of enzymatic activities, temperature is a critical factor in the degradation of materials. The majority of xenobiotic biodegradation reactions are known to take place between 30 to 40 °C. The protonation or deprotonation of the active site of degradation enzymes, which alters the enzyme's affinity for the substrate, is, in turn, influenced by the pH value[48].

Additionally, the pH has an impact on the charge that bacteria have on their surfaces, which has an impact on how effectively sorption processes work. Because mesophilic strains were present in the composition, the bacterial consortium under development performed best in the 20–35 °C temperature range[2].

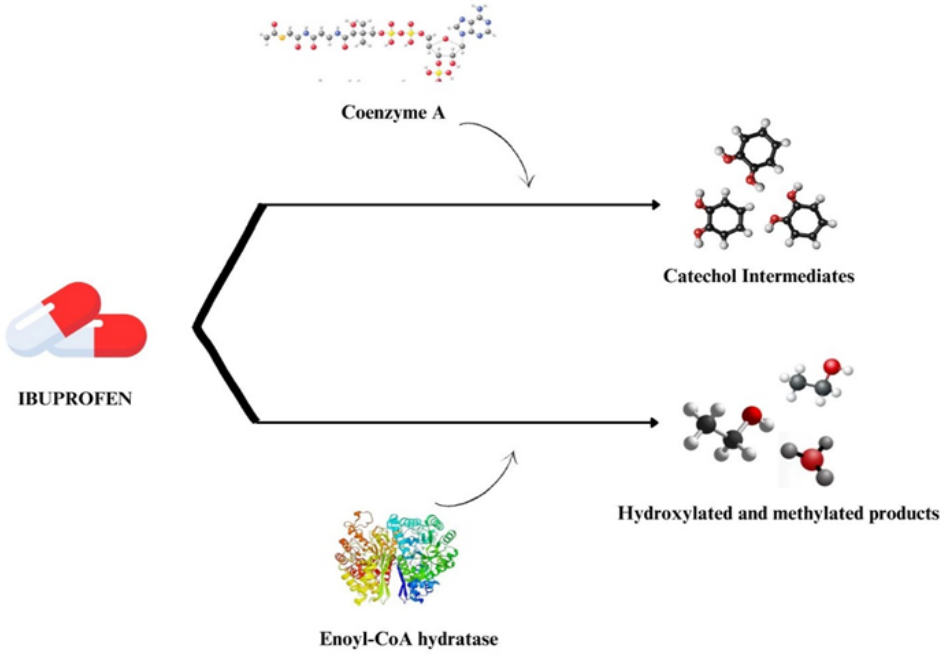

Figure 1: Bacterial degradation of Ibuprofen through two distinct mechanisms

Ibuprofen can be significantly digested by the bacterial consortium at 5 mg/L and only in a limited pH range of 6 to 8.5. Over 90% of the breakdown of Ibuprofen may be inhibited by nitrophenol concentrations above the permitted range in the environment. Two other inhibitors of bacterial biodegradation of Ibuprofen include ethanol and copper ions[39].

Ibuprofen-mineralizing bacterial consortium: Continuous enrichment can be used to produce a very effective Ibuprofen-mineralizing consortium from activated sludge and this consortium has a different metabolic pathway and degradation efficiency than the original consortium. This may identify bacteria that can degrade Ibuprofen very effectively[2]. According to studies, ibuprofen adsorbed in sewage sludge was eliminated up to 90% in 16 days and degraded five times as quickly. After ibuprofen degradation, the consortium's bacterial community can become significantly more abundant in *Sphingomonas wittichii, Bordetella petrii, Pseudomonas stutzeri and Bosea genosp*, with a particular increase in *S. wittichii*'s abundance, which is likely the most important potential bacterial species involved in ibuprofen degradation[42].

Biodegradation of Ibuprofen by fungi: Using fungi to treat wastewater containing harmful substances appears to be a more cost-effective alternative to current approaches. Fungal strains with tolerance levels above 70% are considered effective for the microbial degradation of PAHs (polyaromatic hydrocarbons) and organic xenobiotic compounds in wastewater. The extracellular lignin-modifying enzymes capable of degrading lignin structure in basidiomycete fungus, particularly white rot fungi such as *Trametes versicolor* aid in the degradation of Ibuprofen into smaller metabolites. Moreover, enzymes found in ascomycete fungi like *Aspergillus niger* and zygomycete fungi such as *Rhizopus oryzae* have similar qualities[31].

Ibuprofen has been reported to be well removed (80-100%) by white rot fungi such as *Trametes versicolor, Irpex lacteus, Ganoderma lucidum and Phanerochaete chrysosporium* following 7 days of incubation at baseline. It is worth noting that ibuprofen breakdown by these *Trametes versicolor* began practically immediately after their addition. Ibuprofen was fully depleted after 3 hours of incubation, indeed an interesting time period for bioremediation applications. *M. circinelloides* eliminated 98% of ibuprofen after two days[16]. In 72 hours, the fungal consortium (*Ganoderma applanatum* and *Laetiporus sulphureus*) was found to remove 95% of the ibuprofen. Within 72 hours, *Laetiporus sulphureus* had a high degrading efficiency of 79%, followed by *Ganoderma applanatum*, at 66% on ibuprofen[6]. Some of the fungal species are efficient in degrading ibuprofen. Arbuscular mycorrhizal fungi (AMF) are natural microorganisms that can create a mutualistic relationship with plants[8]. The interaction of AMF with wetland plants is important in constructed wetlands (CW). AMF colonisation could be a viable technique for improving ibuprofen removal in CW systems, with ibuprofen removal efficiencies increasing by 5.82-13.88%. At the same time, it can potentially lower the amounts of their metabolites in the effluent.

Furthermore, AMF may reroute ibuprofen transformation in plant tissues, with higher accumulation in the roots and lesser transportation to the shoots. Furthermore, the presence of AMF aided in the accumulation of ibuprofen and its metabolites in rhizosphere soils[24].

Pathway of fungal degradation of Ibuprofen: Much of the Ibuprofen transformation starts with the hydroxylation [an oxidation reaction in which carbon–hydrogen (CH) bond oxidised into carbon–hydroxyl (COH) bond] of Ibuprofen. The oxidation of the isopropyl chain resulted in 1-hydroxy and 2-hydroxy ibuprofen which was then decomposed to 1,2-dihydroxy ibuprofen. Removal mechanisms during white rot fungi whole-culture treatment include sorption onto fungal biomass followed by degradation by extracellular enzymes and mycelium-bound or intracellular enzymes[73]. Figure 2 depicts a pictorial representation of the mechanisms by which different fungal strains degrade ibuprofen. Among the ligninolytic enzyme classes, laccases and peroxidases, specifically lignin peroxidase (LiP) and manganese peroxidase (MnP), were found to be aiding in the degradation. These fungal peroxidases are all heme-containing glycoprotein enzymes needed for oxidation[22].

Depending on the strain of *T. versicolor*, the predominant enzymes could be laccase, LiP and MnP. The extracellular fungal enzyme systems did not appear to have a part in the initial degradation step, according to *in vitro* investigations using manganese peroxidase and a laccase-mediator system. However, the *in vivo* investigations with the cytochrome P450 inhibitors 1-aminobenzotriazole and piperonyl butoxide, revealed that the cytochrome P450 system may be involved in the initial phase of *T. versicolor*'s oxidation step[73]. Notably, the pollutant degradation by white rot fungi is a co-metabolic process i.e. occurs in an easily degradable substrate. The NSAIDs were completely removed in this investigation at a lower pH[6].

Factors affecting fungal biodegradation: It has been reported that the aerobic condition strongly links ibuprofen elimination in constructed wetlands (CW) systems[49]. Similarly, it was proved that the dissolved oxygen in the substrate pores of CWs can play a key role in eliminating medications such as ibuprofen [4,74]. The hydrophobicity (log D) of the substrate is also an important feature that affects biosorption onto fungal biomass and may aid in removing certain chemicals[73]. This can result in considerable variations in elimination by whole-cell white rot fungi and harvested enzymes[3]. Ibuprofen was entirely removed in liquid media but only slightly (47%) eliminated in solid sludge treatment. A commercial laccase solution removed Ibuprofen at 37.5 - 50% levels whereas with crude fungal enzymes (laccase and MnP), removal was only 12.5-15%.

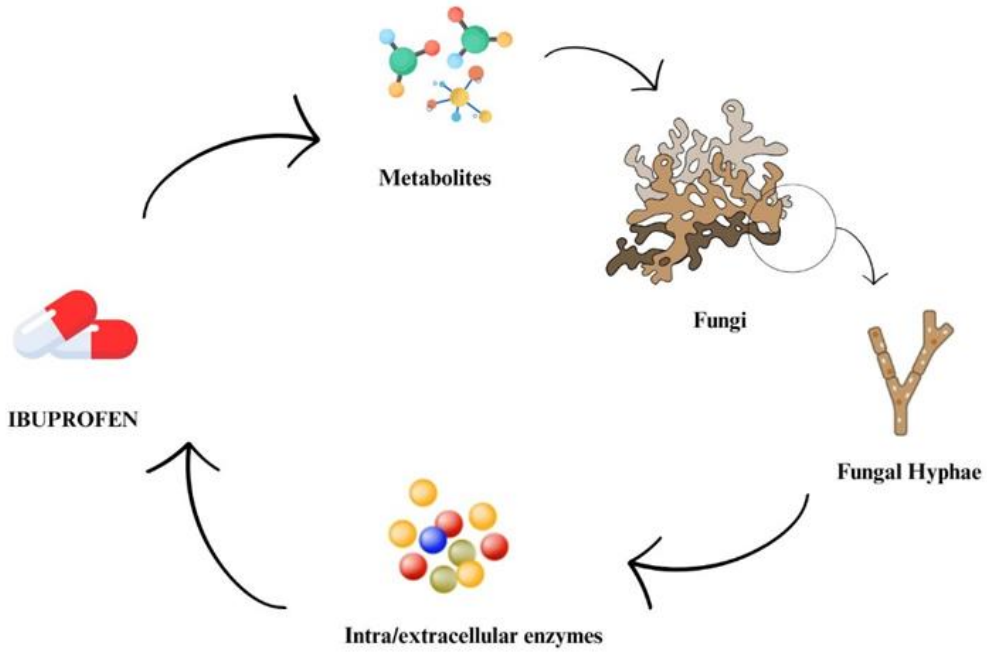

Figure 2: Mechanism of fungal degradation of Ibuprofen

As Ibuprofen is a hydrophilic molecule, which contains not only a methyl group (electron donating group) but also a carboxyl group, the role of biosorption in its removal is limited. The nearly total elimination of these chemicals in whole-cell white rot fungi, on the other hand, supports the role of mycelium-bound and/or intracellular enzymes[4]. As a result, the high clearance in whole-cell white rot fungi treatment can be attributed to the synergistic effects of extracellular, intercellular, and/or mycelium-bound enzymes.

The optimal conditions for fungal enzymatic activity are found to be pH 4.3, temperature 37 ± 15 °C and incubation time of 6 days [31]. Extracellular enzyme activities confirmed the presence of a large proportion of laccases and manganese peroxidases, which are responsible for the NSAID's quick removal rate. Additionally, isotherm experiments (Langmuir) indicated the role of the mycelia dissipated by the fungal consortia. GCMS and HPLC investigations revealed the diverse fates of the metabolites after NSAID breakdown[6,31]. It is also important to consider that several oxidative treatments of Ibuprofen produce a number of compounds, including 1-[4-isobutylphenyl]-1- ethanol and 4-isobutylacetophenone also, which have exhibited *in vitro* and *in vivo* harmful effects respectively[41].

Biodegradation of Ibuprofen by algae: Biosorption, photodegradation and biodegradation have been identified as the three primary removal processes of pharmaceutical drugs in an algal system. The primary removal mechanism in microalgae reactors is aerobic biodegradation, particularly in an extracellular manner, because of the release of organic exudates. The total removal efficiency of Ibuprofen by *Parachlorella kessleri* was found to be 51.3% by biosorption. The removal efficiency is influenced by microalgae by the process of bioaccumulation/adsorption (23.3%) and other processes such as biodegradation and photodegradation (40.6%). The last method in the removal of Ibuprofen is direct photolysis with an efficacy of 15.1%. This is because of the presence of microalgae on the surface which decreases light penetration.

As the concentration of Ibuprofen increases, the removal efficiency of the algae decreases. When the initial concentration of Ibuprofen was 1, 10, or 50 mg L^{-1}, the diatom *Navicula* sp. removed up to 60.0%, 27.2% and 19.7% of Ibuprofen throughout the period of the 15-day incubation period respectively[39]. Studies have also found that the half-life of Ibuprofen in a Swedish lake at low concentration was found to be around 10 days. The presence of *Navicula* sp. may extend the stress time of Ibuprofen in the aquatic environment since it significantly reduced Ibuprofen degradation at 1 mg^{L-1} (p 0.05), with an estimated $t^{1/2}$ of 12.0 ± 1.5 days when compared to 9.6 ± 1.8 days in the control with no algae. It is likely that *Navicula* sp. ingested Ibuprofen before releasing it into the water, making *Navicula* sp. appear to be a "post house" for Ibuprofen.

As a result, the existence of Ibuprofen was subsequently prolonged in aquatic environments. There was also a significant reduction in diatom growth rate that resulted from this treatment, which reduces degradation[17]. Algal growth was found to be inhibited by the cellular accumulation of parent Ibuprofen which leads to ROS accumulation and the disruption of algal physiological or biochemical processes. The removal efficiencies of microalgal-based wastewater systems such as HRAP (High-rate algal ponds) and closed PBRs (photobioreactors) studied under real conditions, are

expected to be higher, especially when other organisms such as bacteria are present. Additional studies have shown that microalgae/bacteria association in algal systems can remove organic and inorganic contaminants more effectively than individual organisms. Ibuprofen removal from 83.3% to 99% was observed in bioreactors like HRAP and tubular PBR[29].

Conclusion

The biological degradation mechanisms of Ibuprofen and their genetic basis are still poorly known despite its widespread usage and accidental presence in the environment. Ibuprofen is also often found in effluents and domestic water due to the ineffective removal process in water treatment plants. Ibuprofen is hazardous to aquatic organisms as well as other animals and plants. Additionally, when it degrades, more harmful compounds than the initial molecule are created, thus contributing to the issue of environmental contamination. Isolating microorganisms with a higher potential for environmental adaptation and high biodegradation of Ibuprofen are required. To effectively understand and manage Ibuprofen as an emerging pollutant, more confirming investigations on its metabolic biodegradation pathways are required.

Research in the field of biodegradation should continue to concentrate on the creation of highly efficient treatment methods that combine high removal rates with cost-effective alternatives[38,69]. The genetic basis of degradation abilities and the control of their expression are urgent issues with degradation[43]. The most popular methods for achieving those goals include obtaining mutants, metagenomics research, sequencing complete bacterial genomes and comparative genomics[76].

Ibuprofen's highly branched structure, occurrence of substitutions at the aromatic ring's para position and their spatial arrangement all point to the drug's excellent biodegradation resistance. Ibuprofen has an aromatic ring with branching replacements in the para position which confers significant resistance to biodegradation. Typically, aliphatic molecules are less resistant to degradation than aromatic ones. As a result, due to its structural makeup, Ibuprofen is susceptible to microbial oxidation and is a highly mobile substance in aquatic environments[26]. Experimental evidence also suggests that it has less persistence than other drugs[43]. It is well known that cyclic compounds are less prone to biodegradation than aliphatic ones; similarly, polycyclic aromatic compounds are more resistant to degradation than monocyclic ones; this is mainly influenced by molecular size[51].

Although the bioremediation approach shows promise, it still needs to be implemented at the wastewater treatment plant level[69]. Ibuprofen biodegradation assays have been developed in the lab, scaling up is difficult because microorganisms need specific environmental conditions such as ideal temperature and pH, a second carbon source and sufficient levels of xenobiotics to induce the right enzymes for degradation. These conditions are infrequently present in the environment[12,44].

The isolation of native microorganisms with the ability to degrade Ibuprofen from the contaminated area, since they would be adapted to environmental conditions, would be an alternative in achieving the adaptation of Ibuprofen degrading microorganisms, despite the difficulty of controlling environmental conditions[14,37]. Many water bodies in cities were found to harbour endophytic microbes with a lot of bioactive and bioremediation potential[19,28,56]. Such microbes can be screened for ibuprofen degrading potential to identify fast degraders. The depletion of Ibuprofen in water systems is frequently caused by the accidental creation of hydroxylated derivatives[21,27], which may later undergo polymerization to bonded residues and evade physico-chemical studies[21]. This is in addition to actual mineralization (full destruction).

Other than biodegradation, effective ways to degrade Ibuprofen include co-doped carbon matrix generated from peat[60], UV/chlorine advanced oxidation process (AOP) and heterogeneous activation of persulfate by carbon nanofiber supported Fe_3O_4 carbon composites[72]. The application of the enzymes involved in the breakdown of Ibuprofen is another approach, as it is genetically altering the native microorganisms. *Ex situ* degradation, which involves removing large samples from the contaminated area and transferring them to a treatment plant where the ideal circumstances for microorganisms are regulated, is a final unworkable method [26].

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