

SUSTAINABLE BIOREMEDIATION OF ACETAMINOPHEN USING BACTERIA: A CRITICAL REVIEW

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Abstract: Acetaminophen is a common antipyretic and analgesic pharmaceutical contaminant and has become one of the most emerging contaminants in the environment. Bioremediation is the suitable method to degrade acetaminophen as it is sustainable, mimics nature and is low-cost. The bioremediation of acetaminophen is performed by identifying the bacterial characteristics and the source of the bacteria involved in the degradation of acetaminophen. Based on this review study, gram-negative bacteria showed the highest efficiency in degrading acetaminophen by *Pseudomonas aeruginosa* strain HJ1012 and *Cupriavidus necator* with an efficiency of 99% and 100%, respectively. In addition, synergistic or antagonistic interaction among bacteria in mixed culture is a gap of study. The findings from the previous study showed that the optimum conditions are pH 7.0, temperature ranges between 30–40°C, and a poor culture medium of minimal salt solution. Furthermore, the study sheds light on enzyme involvement and characterisation of acetaminophen degradation pathways toward less harmful intermediates are addressed in order to achieve sustainable development goals through environmental security and health safety. These identified research gaps offer fundamental knowledge and shed insight into upcoming mechanism and application studies.

Keywords: Acetaminophen, bacteria, interaction, medium, pathway.

Introduction

Nowadays, pharmaceutical compounds have been utilised for various medicinal purposes, especially for humans and animals. Pharmaceutical compounds reach the environment through treated wastewater discharge, animal waste runoff and sewer lines (Patneedi *et al.*, 2015). Due to incomplete removal or improper disposal, the presence of pharmaceutical compounds in the aquatic system adversely influences the aquatic ecosystem and thus raises significant concerns.

Acetaminophen is a common antipyretic and analgesic pharmaceutical contaminant that has become one of the emerging contaminants in the environment. The concentrations of acetaminophen are usually in the range of ng/L to mg/L levels (Žur *et al.*, 2018a). Wu

et al. (2012) stated that acetaminophen had caused severe ecological consequences, although it was not highly persistent in the environment. Acetaminophen transforms into several metabolites such as benzoquinone and hydroquinone. These metabolites are toxic to the aquatic ecosystem. Thus, acetaminophen should be removed to protect the environment (Žur *et al.*, 2018b).

The conventional methods used for removing pharmaceutical compounds are physical and chemical. However, these methods are not the desirable option for removing pharmaceutical compounds due to the high operational cost and the generation of secondary pollutants. Biological methods are chosen to remove pharmaceutical compounds because the methods are sustainable mimic nature

and have low operation costs (Edrees *et al.*, 2017). Bioremediation is a biological method that converts contaminants into less harmful forms. *In-situ* and *ex-situ* bioremediation are the two types of bioremediation processes. *In-situ* bioremediation is the treatment of contaminated substances at the same location whereas *ex-situ* bioremediation involves the removal of contaminated substances at different places from the original contaminated site (Kumar *et al.*, 2018). Biosparging, biostimulation, bioventing and bioaugmentation are examples of *in-situ* bioremediation, meanwhile, land farming and composting are examples of *ex-situ* bioremediation. In bioremediation, bacteria, fungi and algae degrade toxic compounds (Vidali, 2001).

Bioremediation for Acetaminophen

Bioremediation degrades or transforms contaminants into less toxic forms by using microorganisms and their enzymes (Abatenh *et al.*, 2017). In bioremediation, microorganisms, include algae, fungi and bacteria have been utilised to degrade pharmaceutical contaminants (Shabani *et al.*, 2021). This is because microorganisms excrete enzymes that facilitate the utilisation of environmental contaminants and removal of contaminants (Abatenh *et al.*, 2017).

Table 1 shows the advantages and disadvantages of using microorganisms in bioremediation. Algae is an autotroph that can grow in harsh environments, including severe temperatures and pH. In contrast to heterotrophic microorganisms, algae development is unaffected by nutrient depletion (Fu *et al.*, 2016). However, the drawback of algae for bioremediation is low survival rates when drastic changes occur (Silva *et al.*, 2019).

Fungi have been known for the ability to use intracellular and extracellular oxidative enzymes to convert a wide spectrum of recalcitrant compounds, which is resistant to microbial attack (Silva *et al.*, 2019). According to Table 1, mycelial fungi have a physiology and colonisation strategy that enable them to resist unexpected changes in pH. It also efficiently breaks down complex organic molecules, despite their long growth cycle and low survival rate (Anastasi *et al.*, 2013).

Bacteria have an amazing ability to multiply, contributing to their fast growth rate (Table 1). For example, *E. coli*, under ideal conditions can double its population every 20 minutes (Allen & Waclaw, 2018). The ability to perceive its environment and respond to external stimuli is crucial for bacterial development and survival. The availability of nutrients for development,

Table 1: Type of microorganisms used in bioremediation for acetaminophen

Microorganisms	Advantages	Disadvantages	References
Algae • <i>Chlorella vulgaris</i> • <i>Lessonia nigrescens</i> Bory • <i>Macrocystis integrifolia</i> Bory	• Can grow in very harsh environmental conditions	• Low survival rate • Degradation of contaminants with limited coverage	Silva <i>et al.</i> (2019)
Fungi • <i>Trametes versicolor</i> • <i>Aspergillus niger</i> • <i>Mucor hiemalis</i>	• Degrade complex organic compounds efficiently • Tolerate a wide pH range	• Low survival rate • Long growth cycle	Silva <i>et al.</i> (2019)
Bacteria	• High survival rate • Widely diverse organisms • Faster growth	• The scope of the application is limited	Coelho <i>et al.</i> (2015)

secondary metabolites, and other microbes are examples of stimuli (Waters *et al.*, 2005). Coelho *et al.* (2015) mentioned that bacteria have a higher survival rate than algae and fungi when exposed to high contaminant levels, resulting in high degradation performance. This suggests bacteria is a potential degrader for pharmaceutical contaminants.

Bacteria for Bioremediation of Acetaminophen

The structure of bacteria cell membranes plays a role in their ability to degrade organic micro-pollutants and serves as selective barriers between the cell interior and the outer side (Fisher & Mobashery, 2020). Organic contaminants can get through the cytoplasmic membrane and impair the physiological activities of the membrane. To mitigate the negative effects and accumulation in cells, bacteria had to evolve adaption mechanisms (Murínová & Dercová, 2014). The fluidity of the membrane and the ratio of the bilayer to non-bilayer phospholipids, as well as toxic chemical efflux, protein repair processes, and contaminant degradation are all maintained through cell adaptation.

Furthermore, bacteria develop biofilms that are embedded with extracellular polymeric substances. Such condition increases their protection against harsh environmental conditions, which increase their access to hydrophobic impurities or specialised contaminants degrading enzymes (Edrees *et al.*, 2017). The efficiency of gram-positive and gram-negative bacteria in degrading acetaminophen is shown in Table 2.

Generally, gram-negative bacteria showed higher efficiency in degrading acetaminophen at 79.40–100% compared to gram-positive bacteria 86.90–97% (Table 2). This may be due to gram-negative cell walls made up of thin layers of peptidoglycan that can absorb many foreign substances and convert them into less toxic products (Žur *et al.*, 2018a).

Additionally, most studies only focus on utilising one gram-negative or gram-positive bacteria to degrade acetaminophen. To improve the understanding and efficiency

of acetaminophen degradation, comparing the performance of gram-positive and gram-negative bacteria degrade acetaminophen in a proposed study is critical.

Many studies on acetaminophen biodegradation have been reported using single pure cultures of acetaminophen-degrading bacteria, as shown in Table 2. However, relatively few experimental studies have been reported on acetaminophen biodegradation using mixed microbial cultures (Dionisi, 2014). Findings from Zhang *et al.* (2013), *Stenotrophomonas* sp. strain f1 and *Pseudomonas* sp. strains f2 and fg-2 were grown and completely degraded acetaminophen at concentrations of 400 mg/L in mixed cultures with acetaminophen as the sole carbon, nitrogen, and energy source. Thus, a synergistic interaction of the consortium derived from the acetaminophen-degrading mixed culture boosts acetaminophen degradation significantly. In addition, mixed microbial cultures are widely perceived as having the potential to be effective at biodegrading recalcitrant substances (Kim *et al.*, 2009). However, there is a gap in the study to further insight into the synergistic and antagonistic interaction among bacteria in mixed culture.

Table 2 shows that the sources of the gram-positive and gram-negative bacteria obtained were from sludge samples. The aromatic compounds are abundant in wastewater treatment plant influents, and the bacteria in the sludge samples are adapted to decompose aromatic compounds. As a result, microorganisms from this source are expected to degrade acetaminophen efficiently.

Factors Affect Bioremediation of Acetaminophen Using Bacteria

Bioremediation is affected by several factors, including the presence of a microbial population capable of degrading acetaminophen and environmental conditions such as temperature, pH and medium (Vidali, 2011). As stated by Žur *et al.* (2018a), the value of pH influences

Table 2: Gram-positive and gram-negative bacteria efficiency in degrading acetaminophen

Bacteria Type	Bacteria Name	Source of Bacteria	Concentration of Acetaminophen (mg/L)	Efficiency (%)	References
Gram-positive bacteria	<i>Bacillus drentensis</i> strain s1	Sludge sample	300	95.10%	Chopra and Kumar (2020)
	<i>Enterococcus faecium</i>	Sludge sample	200	86.90%	Palma <i>et al.</i> (2021)
	<i>Corynebacterium nuruki</i>	Sludge sample	200	97.00%	Palma <i>et al.</i> (2021)
Gram-negative bacteria	<i>Pseudomonas</i> sp. STB2	Sludge sample	3000	79.40%	Abdullah (2018)
	<i>Pseudomonas aeruginosa</i> strain HJ1012	Sludge sample	2200	99.00%	Hu <i>et al.</i> (2013)
	<i>Cupriavidus necator</i>	Sludge sample	400	100%	Wei <i>et al.</i> (2011)

acetaminophen degradation because it affects microbial cell shape, activity, and membrane characteristics. Meanwhile, temperature affects the rate of most biochemical reactions in the bioremediation process (Das & Chandran, 2011). Medium plays an important role in stimulating the growth of certain bacteria that utilise acetaminophen as a source of energy and food thus bioremediation takes place (Kram & Finkel, 2015).

The value of pH affects the biosorption and toxicity of contaminants by changing the surface cells ionisation (Žur *et al.*, 2018a). According to Wolski *et al.* (2006), the surface of bacterial cells becomes negative at higher pH values, resulting in alterations in the electrostatic interaction between pharmaceutical contaminants and biomass surface, thus, influencing biodegradation performance. According to Xagoraki *et al.* (2008), the production of the protonated form (ROH) of acetaminophen has been found at lower pH, whereas acetaminophen occurs as phenolate (RO⁻) form in an alkaline environment. In a laboratory experiment, a lower degradation of acetaminophen was observed at pH values greater than 8.0 or lower than 6.0. It was determined that the suggested optimum

conditions for pH were at 7.0, where the highest acetaminophen degradation occurred.

Temperature affects bacterial physiology and enzymatic reaction and thus, plays a crucial role in degradation processes (Žur *et al.*, 2018a). The rate of most biochemical reactions is affected by temperature, and the rate of reactions doubles with an increase of every 10°C. Bacterial membranes became more rigid at lower temperatures, resulting in greater viscosity of membrane phospholipids, meanwhile, membrane transport was usually inhibited at higher temperatures by the denaturation of membrane-associated proteins (Žur *et al.*, 2018a). Although bacteria can survive in these extreme conditions, the ideal temperature should be maintained for the best growth and reproduction (Žur *et al.*, 2018a). Das and Chandran (2011) stated that the optimum temperature for the degradation of acetaminophen was between 30-40°C.

Rich and poor culture mediums are two different types of medium. Bushnell Hass Medium and Trypticase Soy Broth are examples of the rich medium. Meanwhile, the mineral salt solution is a poor medium lacking essential

nutrients (Kram & Finkel, 2015). As shown in Table 3, the Bushnell Haas Medium showed higher efficiency of 95.10% in the degradation of acetaminophen than Trypticase Soy Broth with an efficiency of 89% in degrading acetaminophen. A rich culture medium contains all essential nutrients and enables the bacteria to grow rapidly (Akay & Tezel, 2020). As for the poor culture medium study, bacteria in mineral salt solution such as *Pseudomonas aeruginosa* strain HJ1012, *Pseudomonas moorei* KB4 strain and *Delftia tsuruhanensis* degraded acetaminophen with the efficiency of 99%, 100%, 97%, respectively. Kram and Finkel (2015) state that a poor medium lacks nutrients. The bacteria in the Mineral Salt Solution break down the complex molecules to become the source of carbon and energy for growth and survival. There are limited studies comparing the types of mediums used for degrading acetaminophen.

Acetaminophen Degradation Pathway by Bacteria

To provide a realistic design of the biodegradation system for acetaminophen, the metabolic pathway must be investigated to understand harmful intermediates, their accumulation, and their final products. There are several pathways and different intermediates involved in the degradation of acetaminophen. Figure 1 illustrates the metabolic degradation pathway of acetaminophen by *Bacillus*

drentensis strain s1. According to Chopra and Kumar (2020), the conversion of 4-aminophenol to hydroquinone is expected to be the primary degradation pathway for acetaminophen biodegradation by bacteria. Acetaminophen was transformed to 1,4-benzenediol or hydroquinone via the hydroquinone pathway. During catalysis by amidohydrolase, the amino group of acetaminophens was substituted by a hydroxyl group, resulting in hydroquinone. Hydrolytic enzymes catalysed the hydroxylation of acetaminophen during degradation, releasing acetamide to generate hydroquinone. It was then converted into 2-isopropyl-5-methylcyclohexanone and oxalic acid (Chopra & Kumar, 2020).

Another pathway of acetaminophen degradation was proposed by Zhang *et al.* (2012). Figure 2 shows the acetaminophen degradation pathway. In the first stage, an amidohydrolase catalyses the acetate release from acetaminophen and converts to 4-aminophenol, which is subsequently ring fissioned and the amino group replaced by a hydroxyl group to yield hydroquinone. Following that, a hydrolytic enzyme catalyses the initial hydroxylation of acetaminophen to form hydroquinone with the release of acetamide, which is then converted into oxamic acid. Some strains converted aminophenol to the equivalent 1,4-benzenediol or hydroquinone molecules. Attempts to isolate and identify acetamide and oxamic acid, on the other hand were unsuccessful.

Table 3: Medium used in degrading acetaminophen

Parameters	Bacteria	Medium	Efficiency	References
Rich medium	<i>Bacillus drementensis</i> strain s1	Bushnell Haas Medium	95.10%	Chopra and Kumar, 2020
	<i>Pseudomonas</i> sp. STB3	Tryptic Soy Broth	89%	Edrees <i>et al.</i> , 2018
Poor medium	<i>Pseudomonas aeruginosa</i> strain HJ1012	Minimal Salt Solution	99%	Hu <i>et al.</i> , 2013
	<i>Pseudomonas moorei</i> KB4 strain	Minimal Salt Solution	100%	Žur <i>et al.</i> , 2018b
	<i>Delftia tsuruhanensis</i>	Minimal Salt Solution	97%	De Gussemme <i>et al.</i> , 2011

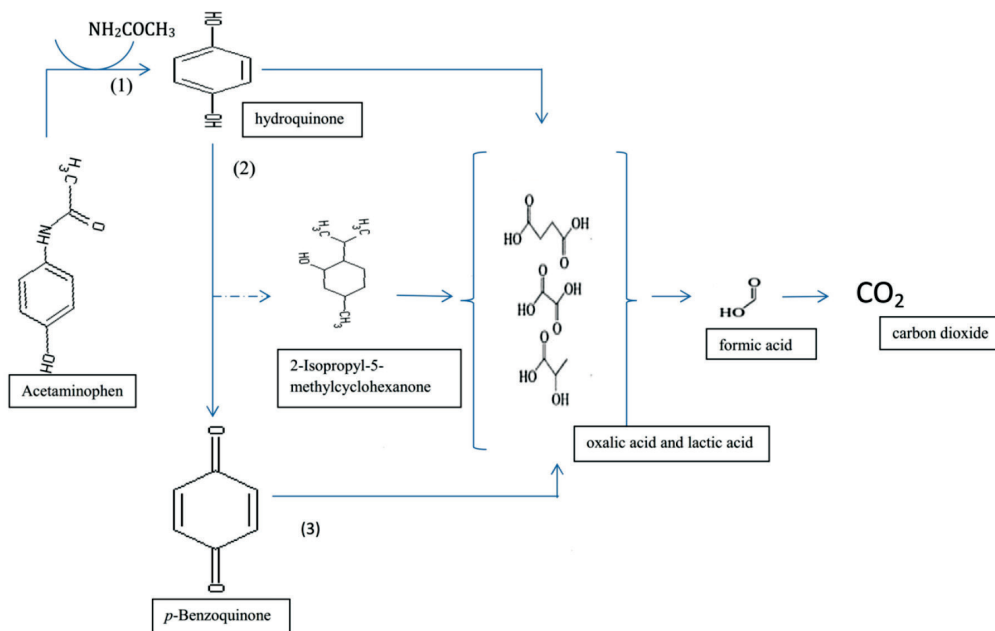


Figure 1: *Bacillus drentensis* strain s1 metabolic pathway for acetaminophen degradation (Chopra & Kumar, 2020)

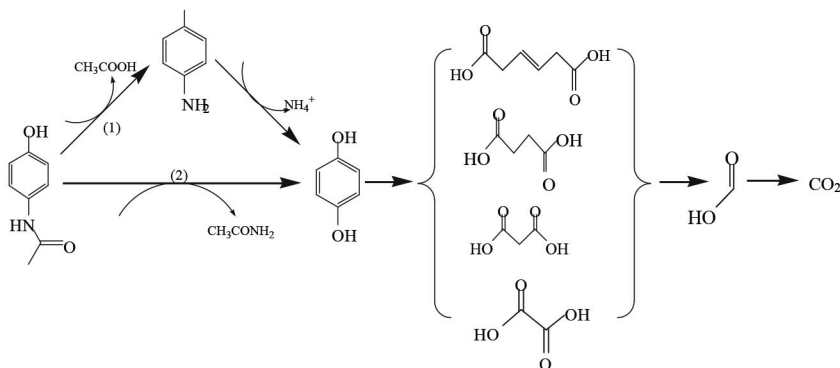


Figure 2: Acetaminophen degradation pathway (Zhang *et al.*, 2012)

Both proposed pathways are similar in identifying the two primary acetaminophen degradation intermediates, 4-aminophenol and hydroquinone. Moreover, both pathways share similar final products: formic acid and carbon dioxide. The differences between these two pathways are, in the first pathway, suggested by Chopra and Kumar (2020), 2-isopropyl-5-methylcyclohexanone and oxalic acid were identified, meanwhile, in the second pathway, suggested by Zhang *et al.* (2012), attempts at isolating and identifying oxamic acid and acetamide failed. Besides quinones, oxalic acid

also is a toxic compound when the concentration is high (Schuler *et al.*, 2021). There has been limited research on the metabolic pathways of acetaminophen biodegradation, and they are still poorly characterised. Furthermore, there is a lack of research on acetaminophen degradation, including a study of the specific activity of enzymes involved in the pathways of acetaminophen degradation. Aside from that, research needs to focus on initiating a pathway that produces less harmful intermediates to overcome toxicity issues.

Enzyme for Degradation of Pharmaceutical Contaminants

In general, the performance of degradation is evaluated through removal percentage. There is a lack of studies on the degradation rate of pharmaceutical compounds due to the involvement of many factors and complex processes. The degradation rate of pharmaceutical contaminants is usually influenced by the concentration of contaminants and the amount of catalyst present. In this context, the amount of catalyst refers to the number of organisms capable of metabolising the contaminants and the number of enzymes produced by each bacterium (Abatenh *et al.*, 2017). In addition, environmental factors also may contribute to a variation in the rate of degradation.

Enzymes increase the speed of a reaction by lowering the activation energy of molecules (Ruggaber & Talley, 2006). Enzymes like oxidoreductase (laccase and peroxidase) and hydrolases (lipase and haloalkane dehalogenase) are involved in bioremediation. The list of enzymes involved in bioremediation, producing microorganisms and their functions are shown in Table 4.

Oxidoreductases are a class of enzymes that absorb electrons from molecular oxygen and catalyse the oxidation/reduction reaction. Due to the ability to oxidise resistant pollutants, several researchers have widely investigated oxidoreductase enzymes in recent years. Table 5 shows the examples of oxidoreductase enzymes involved in the bioremediation of acetaminophen.

Oxidoreductases are enzymes produced by bacteria, fungi, and plants capable of eliminating natural and artificial pollutants (Okino-Delgado *et al.*, 2019). Based on Table 5, the bacteria that produce laccase, oxygen oxidoreductase, aldehyde oxidoreductase and manganese peroxidase are *Pseudomonas*, *Pseudomonas aeruginosa*, *Desulfovibrio desulfuricans* ATCC 27774, and *Bacillus velezensis*, respectively. Each enzyme has its reaction for the bioremediation of acetaminophen. Laccases, aldehyde oxidoreductase and manganese peroxidase are widely used for the bioremediation of a wide range of industrial pollutants (Chandra & Chowdhary, 2015; Saikia *et al.*, 2022). While research on enzymatic activities of oxygen oxidoreductase is rarely discussed.

Table 4: Enzymes involved in bioremediation, producing microorganisms and their functions

Enzyme Classification	Example	Producing Microorganism	Functions
Oxidoreductase	Laccase	<i>Bacillus</i>	Aromatic compounds have a cleave ring that reduces one molecule of oxygen in water, resulting in the formation of free radicals (Arregui <i>et al.</i> , 2019)
	Peroxidase	<i>Pseudomonas</i> sp.	After the oxidation of organic molecules, catalyse reduction reactions in the presence of peroxides like hydrogen peroxide (H ₂ O ₂) and generate reactive free radicals (Bansal & Kanwar, 2013)
Hydrolases	Lipase	<i>Burkholderia</i>	Triglycerol is broken down into glycerol and fatty acid, useful in polyaromatic hydrocarbon degradation and wastewater treatment (Sharma <i>et al.</i> , 2019)
	Haloalkane dehalogenases	<i>Pseudomonas pavonaceae</i>	It breaks the carbon-halogen bonds in halogenated compounds and converts them into non-toxic forms (Satpathy, 2021)

Table 5: Example of oxidoreductase enzyme produced by bacteria

Examples of Enzyme	Producing Microorganism	Reaction	References
Laccase	<i>Pseudomonas</i>		Chandra and Chowdhary (2015)
Oxygen oxidoreductase	<i>Pseudomonas aeruginosa</i>		Entsch and Ballou (1989)
Aldehyde oxidoreductase	<i>Desulfovibrio desulfuricans</i> ATCC 27774		Kozono et al. (2020)
Manganese peroxidase	<i>Bacillus velezensis</i>		Al-Dhabi et al. (2021)

Laccases are multi-copper oxidoreductases that can oxidise various aromatic compounds (Varga *et al.*, 2019). Laccases use molecular oxygen as an electron acceptor to catalyse the oxidation of different aromatic compounds, especially compounds with electron-donating groups of phenols (OH) into free radicals (Chandra & Chowdhary, 2015; Silva *et al.*, 2019). Laccase is a “green catalyst” because it only produces water as a by-product (Kumar & Chandra, 2020). In addition, laccases are suitable catalysts for the bioremediation of acetaminophen due to the stability of bacterial laccases under varied pH, temperature, organic solvents, and salt concentrations (Arregui *et al.*, 2019).

Oxygen oxidoreductase, also known as alcohol oxidase (Veteikytė *et al.*, 2013). Oxygen oxidoreductase uses molecular oxygen to oxidise primary aromatic alcohol into aromatic aldehyde, producing hydrogen peroxide as a by-product (*KEGG enzyme: 1.1.3.7*). By providing insight into the pathways and mechanisms, this enzyme has the potential to bioremediate aromatic compounds of acetaminophen. However, a minimal study has been conducted on oxygen oxidoreductase.

Aldehyde oxidoreductases are members of the xanthine oxidase family (Fourmond, 2018). According to Koehler *et al.* (1996), a wide variety of aliphatic and aromatic aldehydes are specific substrates for aldehyde oxidoreductase. Aldehyde oxidoreductase uses water molecules to oxidise aldehyde to carboxylic acid, which then enters the Krebs cycle (Fetzner, 2000). Fourmond (2018) mentioned that aldehyde oxidoreductase oxidises a variety of aldehydes. However, their physiological substrates and functions are commonly unspecified.

Manganese peroxidase, also known as Mn (II): Hydrogen-peroxide oxidoreductase is ubiquitous (Zdarta *et al.*, 2018). Manganese peroxidase uses hydrogen peroxide to oxidise Mn²⁺ into Mn³⁺ and produce water, thus, completing the catalytic cycle of MnP. These ions then combine with anions of dicarboxylic acids to form chelate complexes and enter the Krebs cycle (Khatoon *et al.*, 2017). According to Hofrichter (2002), the capability of MnP enzymes is proved by their products; the chelated Mn³⁺ ions, which act as charge-transfer mediators, attack phenolic compounds. Manganese peroxidase is an eco-friendly enzymatic activity

that bioremediates pharmaceutical compounds into non-toxic forms and cleans the environment (Saikia *et al.*, 2022).

In conclusion, enzymes play an important role in the bioremediation of acetaminophen. The demand for these enzymes in the biotechnological sector has increased in recent years due to their being environmentally friendly. Therefore, these enzymes are recommended as catalysts for the bioremediation of acetaminophen.

Conclusion

This review highlights the bioremediation of acetaminophen by using bacteria. It is found that gram-negative bacteria have higher efficiency than gram-positive bacteria in degrading acetaminophen. However, only a few studies compare the effectiveness of gram-positive and gram-negative bacteria as well as bacteria interactions in mixed culture in the degradation of acetaminophen. The source of bacteria was a significant factor in improving the efficiency of degrading acetaminophen. Most of the bacteria used in the review study were isolated from sludge samples in wastewater treatment plants as bacteria evolved and adapted to environmental changes. The findings of this review show that the optimised conditions for acetaminophen degradation are pH 7.0, temperature 30–40°C, and the poor culture medium minimal salt solution. Bacteria degrade complex molecules to provide carbon and energy for bacteria growth and survival. This review also addresses gaps of study on the characterisation and enzyme in the degradation pathway that produce less toxic intermediates and achieve a sustainable environment and human health safety.

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