Review

Microbial Degradation of Paracetamol in Pharmaceutical Wastewater: A Review

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Abstract: Paracetamol (4′-hydroxyacetanilide or N-acetyl-p-aminophenol or Acetaminophen) is an analgesic and antipyretic over-the-counter commonly used drug. Paracetamol has been detected in, surface waters, wastewater, and drinking water globally because of its significant utilization and unregulated release into the surroundings which have been a great concern and require an urgent approach. Microbial degradation of paracetamol is considered a desirable choice because of its lenient reaction conditions, low-cost operation, and eco-friendly process. This review focuses on summarizing the current processes for the biodegradation of paracetamol. The review includes characteristics and prevalent pharmaceutical drugs in wastewater, toxicity, degrading microorganisms, enzymes, and possible intermediates. Factors affecting the microbial degradation process of paracetamol such as growth pH, microbial cell concentration, temperature, and glucose have also been reported. The wide knowledge of biotransformation sequence and enzymatic processes engaged in the usage of paracetamol will help enable the optimization and simple design of microbial degradation techniques, which are expected to be more efficient in the treatment of paracetamol-contaminated wastewater.

Keywords: Paracetamol, Wastewater, Microbial, Biodegradation Pathway

Introduction

Pharmaceutical compounds are biologically active products that are continuously being used for various forms of prevention, cure, or treatment of diseases and one of the major worrying classes of emergent contaminants are these pharmaceutical contaminants from various pharmaceutical industries (Tiwari et al., 2017; Samal and Trivedi, 2020) and these organic pollutants have been identified worldwide in wastewater and surface water (Phillips et al., 2010). These pharmaceutical contaminants are consistently introduced into the environment via various emissions stemming from agricultural practices, manufacturing processes, consumption and disposal by consumers, and hospital waste discharge (Mahmood et al., 2022). Pharmaceutical compounds are molecules premeditated to be biologically active which thus pose a great effect on aquatic organisms and humans when leached into the environment, even though the contaminants are normally detected at low levels, which range from ng/L (nano-gram per liter) to μg/L micrograms per liter (de Oliveira et al., 2020). Varying levels of possible adverse impacts, such effects encompass chronic toxicity and acute damage, endocrine destruction, sexual reproductive damage, and alteration of behavior are detected at these minimal concentrations (Tiwari et al., 2017; Samal et al., 2022).

It occurs that most common pharmaceuticals detected in wastewater and the environment are usually those that are often obtainable either by prescription or non-prescription and over-the-counter purchase, including a range of substituted acetanilides like paracetamol and its intermediates. Paracetamol (or Acetaminophen) is a non-opioid analgesic, an antipyretic and Non-Steroidal Anti-Inflammatory Drug (NSAID), and a popular over-the-counter pain relief medication (Chiam et al., 2015) for headaches, fever, etc. Additionally, during COVID-19 disease outbreak in 2019, the major component of the therapeutic plans was this molecule which was consumed globally for the treatment of symptoms like cough, pain, flu, cold, and sleep disorders (Tony et al. 2020). Paracetamol with IUPAC N-(4-hydroxyphenyl) ethanamide, having a benzene ring core with a hydroxyl group and an amide group's nitrogen atom substituted in the para (1, 4) position (C₈H₈N₂O₂) (Maes et al., 2016).
Paracetamol has become an issue of concern that couldn’t be overlooked due to its prevalent occurrence in drinking water and the environment's continuous documentation of its possible effects on human and environmental wellness. The intake of paracetamol has risen even more following the COVID-19 crisis, it has become the most recommended drug by various healthcare authorities across the world (Leal et al., 2021). In America, about 50 million adults utilize products with paracetamol on a weekly basis (Mund et al., 2015), and one of the most widely taken pain relief medications in Europe (Varrassi et al., 2010). According to previous research, the occurrence of paracetamol concentration was observed in surface water, drinking water, and underground waters (Wadhah Hassan, 2017). Studies have proved that microorganisms play crucial and effective contributions to the degradation of paracetamol in the environment across various conditions, as stated by Rios-Miguel et al. (2022). This review aims to consolidate previous and recent studies on microbial degradation of paracetamol, focusing on the occurrence of paracetamol, biodegrading microorganisms, environmental toxicity and health risks of paracetamol in wastewater, factors affecting biodegradation of paracetamol and the proposed metabolic/biodegrading pathways by microorganisms.

The approach of this review stems from an examination of data from both previous and recent investigations into the microbial degradation of paracetamol and other pharmaceutical wastewater pollutants. However, a few articles (4 articles) published from 1975-1996 were considered when describing the microbial degradation and transformation pathway of paracetamol. Also, a few articles published between 2006-2010 were considered, although the majority of the utilized articles were published between 2011-2023 since they presented significant information. The principal database source for these articles was Scopus, accessed with search terms including "paracetamol"; "pharmaceuticals"; "microbial"; "pollutants"; "wastewater"; "biodegradation pathway" and "review". To analyze the range of available studies and highlight literature gaps concerning this topic, a database search was carried out, revealing studies spanning over 10 years between 2014-May 2024 with approximately 1,344 papers on biodegradation of paracetamol comprising 428 review articles and 610 research articles. 340 review articles and 292 research articles publications on the use of bacteria, 191 review articles and 162 research articles on the use of microalgae, and 104 review articles and 82 research articles on the use of fungi were published (Fig. 1). The quest to identify the most efficient microbial degradation method for wastewater polluted with paracetamol has garnered major attention, resulting in an increase in publications over recent years. Several comprehensive reviews exist; however, most of them lack a comprehensive critical analysis of both traditional and high-level techniques, in conjunction with optimizing operational details to enhance the efficiency of microbial processes in the complete removal of paracetamol.

![Figure 1: Quantity of published articles on paracetamol biodegradation in wastewater from the Scopus database between 2014-2024](image)

**Characteristics of Pharmaceuticals in Industrial Wastewater**

There is a need to characterize and discard effluent from the pharmaceutical industry so that safety standards are maintained before pharmaceutical wastewater discharge. Wastewater contains potent chemicals that are mutagenic, teratogenic, carcinogenic, and have other serious detrimental effects, so it is important to classify the elements and their forms before treatment. It is mainly composed of organic elements which pose greater harm in contrast to their inorganic variants (Kumar et al., 2018). It is certain that pharmaceutical companies' generated wastewater streams are not uniform and are always found to contain substances such as active biomass, polycyclic aromatic hydrocarbons, antibiotics, and phenols. (Dixit and Parmar, 2013; Kumar et al., 2019). The wastewater could incorporate the following: heavy metals, non-biodegradable organic, biodegradable organic materials, and inorganic materials, and viable inhibitors that may be leached into the groundwater or ultimately flow into a water body. The presence and concentration of this contaminant can be assessed through water analysis by examining a water quality index employing several physicochemical parameters such as pH, turbidity, conductivity, Total Suspended Solids (TSS), Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) amongst others. A comprehensive interpretation of wastewater composition and properties (Table 1) is crucial to the implementation of a particular method and certification of its operation in a wastewater treatment plant (Mhlanga and Brouckaert, 2013).

To devise an efficient method for wastewater treatment, it is crucial to identify the properties of water and its contaminants (Deegan et al., 2011). The Pharmaceutical Industries' wastewater characteristic feature shows that it contains several intermediates, catalysts, solvents, and additional raw materials used during the synthesis and development of a certain drug in
its required dosage formula and not just the Active Pharmaceutical Ingredients (APIs) and these materials are quite harmful to the water bodies and environment been discharged in Zaman et al. (2014). Previous studies have evaluated the occurrence of the most prevalent drugs in pharmaceutical industrial wastewater and they have been classified into different therapeutic groups. (a) NSAIDs are largely found in Pharmaceutical Industrial Wastewater, such as Paracetamol: Ibuprofen, diclofenac, and Indomethacin, and the likes in their average mean and highest influent concentrations were detected. (b) Antibiotics; another group of prevalent drugs that are found largely consist of Antibiotics such as Ofloxacin, Ciprofloxacin, Trimethoprim, Sulpham ethaxazole, Chloramphenicol and Penicillin (c) Additionally, β-blockers like Propranolol, Metoprolol and Atenolol were also be discovered over the years. (d) Anticonvulsant and psychiatric drugs, for example, Carbamazepine have also been detected as prevalent drug contaminants found in pharmaceutical wastewaters (Petrovic et al., 2009; Shah and Shah, 2020). An overview of prevalent drugs in pharmaceutical wastewater is enumerated below in Table 2.

### Table 1: Physicochemical parameters of pharmaceutical wastewater adapted (Rana et al., 2017)

<table>
<thead>
<tr>
<th>Parameters/Items</th>
<th>Ranges</th>
<th>Heavy metals and toxic compounds</th>
<th>Ranges (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>5.8-8.5</td>
<td>Lead</td>
<td>0.03-6.53</td>
</tr>
<tr>
<td>Biological oxygen demand</td>
<td>20-1800 mg per liter</td>
<td>Iron</td>
<td>8.5-10.8</td>
</tr>
<tr>
<td>Chemical oxygen demand</td>
<td>128-28,640 mg per liter</td>
<td>Selenium</td>
<td>0.428-0.67</td>
</tr>
<tr>
<td>TDS</td>
<td>600-20,000 mg per liter</td>
<td>Cadmium</td>
<td>0.036-0.56</td>
</tr>
<tr>
<td>TSS</td>
<td>48-7500 mg per liter</td>
<td>Nickel</td>
<td>0.02-2.35</td>
</tr>
<tr>
<td>Total phosphate</td>
<td>18-47 mg per liter</td>
<td>Manganese</td>
<td>6.41-8.47</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>775.0 mg per liter</td>
<td>Chromium</td>
<td>0.01-1.11</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>80-164 mg per liter</td>
<td>Chloride</td>
<td>200-2800</td>
</tr>
<tr>
<td>Temperature</td>
<td>31-46°C (degree Celsius)</td>
<td>Sulphate</td>
<td>82-360</td>
</tr>
<tr>
<td>Phenol</td>
<td>95-125 mg per liter</td>
<td>Arsenic</td>
<td>0.0049-0.0076</td>
</tr>
<tr>
<td>Conductivity</td>
<td>157±115.8-1673±119 (μS/cm)</td>
<td>Sulphide</td>
<td>42-100</td>
</tr>
<tr>
<td>Turbidity</td>
<td>2.2-138 Nephelometric turbidity units</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>45.0 mg per liter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkalinity</td>
<td>50-2500 mg per liter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total acidity</td>
<td>300.0 mg per liter</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Prevalent drugs in pharmaceutical industrial wastewater (Petrovic et al., 2009; Shah and Shah, 2020)

<table>
<thead>
<tr>
<th>Pharmacological class</th>
<th>Drugs</th>
<th>Chemical class</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSAIDs</td>
<td>Paracetamol</td>
<td>Para-aminophenol derivative</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>Propionic acid derivative</td>
</tr>
<tr>
<td></td>
<td>ketoprofen</td>
<td>Propionic acid derivative</td>
</tr>
<tr>
<td></td>
<td>Diclofenac</td>
<td>Acetic acid derivative</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Sulphamethaxozole</td>
<td>Sulphonamide</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>Penicillin</td>
</tr>
<tr>
<td></td>
<td>Sulfadiazine</td>
<td>Sulphonamide</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>Fluoroquinolone</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>Fluoroquinolone</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>Fluoroquinolone</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>Amphenicol-class antibacterial</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
<td>Aminopyrimidine</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>Atenolol</td>
<td>Beta blocker</td>
</tr>
<tr>
<td></td>
<td>Metoprolol</td>
<td>Beta blocker</td>
</tr>
<tr>
<td></td>
<td>Propranolol</td>
<td>Beta blocker</td>
</tr>
<tr>
<td></td>
<td>Sotalo</td>
<td>Beta blocker</td>
</tr>
<tr>
<td>Anticonvulsant/Antiepileptic drugs</td>
<td>Carbamazepine</td>
<td>Tricyclic Anti-depressant</td>
</tr>
<tr>
<td>Lipid and</td>
<td>Clofibric Acid</td>
<td>Clofibrate metabolite</td>
</tr>
<tr>
<td>Cholesterol regulating</td>
<td>Gemfibrozil</td>
<td>Fibric acid derivative</td>
</tr>
</tbody>
</table>
Occurrence of Paracetamol in Industrial Wastewater

Paracetamol was found to be one of the most abundant pharmaceuticals identified with widely varying concentrations in wastewater across the globe (Gracia-Lor et al., 2012; Thomas et al., 2007). A record has shown a range between 1.75-43.22 μg/L in inflow samples of wastewater purification plants and about 83% of wastewater treatment plant effluent has shown a range between 0.025-4.319 μg/L. Also, from Ulleval University effluent samples between the range 13.87-177.67 μg/L were observed, and 5.42-1368.5 μg/L from wastewater, Norway (Thomas et al., 2007).

In Saudi Arabia, paracetamol concentrations of 12 and 0.073 micrograms per liter were observed in influent and effluent in wastewater purification facilities (Al Qarni et al., 2016). Also, paracetamol was found with a maximum concentration of 2.086 and 0.0521 μg/L in wastewater treatment plant inflow samples and effluent samples sequentially in the year 2011 in Kuwait (Alajmi, 2014).

In Canada, concentrations between 57.5-77.4 μg/L were found in influent samples of treatment plants and about 90.2 micrograms per liter in inflow samples from the hospital (Ba et al., 2014).

Furthermore, in Italy, 246 μg/L paracetamol concentration was found in the raw influent wastewater treatment plant sample (Verlicchi et al., 2012a) and in influents from two hospital wastewater varying from 1.4-5.9 μg/L as well as 1.2 and 0.058 μg/L concentration in influent and effluent of wastewater treatment systems accordingly (Verlicchi et al., 2012b). However, in Switzerland, a paracetamol concentration of 107 μg/L was recorded in hospital wastewater influent (Kovalova et al., 2012). In Taiwan, it was recorded that influents from hospital wastewater samples contain up to 186.5 μg/L and influents from drug production facilities contain about 417.5 μg/L (Lin and Tsai, 2009) and from 1.80-30.967 μg/L in effluents from six wastewater treatment plants (Lin et al., 2010), about 2.69 μg/L in inflow and 0.33 μg/L in outflow of sewage water treatment facility (Dutta et al., 2014) and 150 μg/L in hospital wastewater sample in China (Wu et al., 2012). In North Korea, 41.90 and 6.760 μg/L (influent and effluent samples) of paracetamol were subsequently detected in a hospital wastewater treatment facility. Also, 6.80 μg/L was reported in influent from municipal wastewater purification facility (Sim et al., 2010) then 10.234 μg/L in wastewater processing system influent was found in Ulsan (Behera et al., 2011).

In the USA, a paracetamol concentration level of 1.06 μg/L was found in the effluent of a wastewater purification system (Glassmeyer and Shoemaker, 2005). A concentration of 61 and 0.86 μg/L in influent and effluent of a wastewater purification facility in New York City (Benotti and Brownawell, 2007), another influent contains 140 μg/L concentration in San Marcos, Texas, from a hospital wastewater purification system (Foster, 2007), in the Back River, a concentration of 0.96 μg/L found inflow of a wastewater treatment facility in Baltimore (Yu et al., 2006), Influent concentrations of 182-233 μg/L at five wastewater management plants in the Northwest Pacific (Lubliner et al., 2010) and in effluents from fifty wastewater treatment plants, about 150.079 μg/L was recorded (Kostich et al., 2014) and in influent of a wastewater purification system in Wisconsin, up to 1000 μg/L was found (Wilcox et al., 2009).

In the United Kingdom, the concentration of paracetamol observed ranges between 5.53 to 69.57 μg/L as found in Howdon wastewater treatment plant influent (Roberts and Thomas, 2006), 0.129-0.555 μg/L was also detected from wastewater treatment plant effluent in England (Bound and Voulvoulis, 2006) and between 211.4 and 11.73 μg/L was found in inflow and outflow wastewater treatment plant in Cilfynydd, likewise 178.12 and 0.35 μg/L were found in a treatment facility of inflow and outflow wastewater in South Wales (Kusprzyk-Hordern et al., 2009).

In Spain, a concentration of 0.5-29 μg/L was found in wastewater from the hospital, in Almería (Gómez et al., 2006), a level of 0.123 μg/L concentration was found in the influent of the wastewater treatment facility (Radjenovic et al., 2007), influent level of 16.72 μg/L and effluent level of 0.34 μg/L in the wastewater treatment system, from Barcelona and Catalonia was noticed (Gros et al., 2012). In Croatian, 0.130-26.10 and 5.990 μg/L inflow and outflow of a wastewater purification system (Gros et al., 2006), between 1.13-201 μg/L in the inflow of a wastewater purification system detected in Castellon (Gracia-Lor et al., 2012). In Girona hospital wastewater between the range of 109.3-114.4 μg/L concentration (Cruz-Morató et al., 2014) and 58.857 μg/L from hospital wastewater influent, concentration of 9.29 and 0.11 μg/L from influent wastewater treatment plant and 0.106 μg/L effluent in Portugal (Santos et al., 2013).

Microbial Degradation

Biodegradation or microbial degradation is a biological process that offers an environmentally friendly means of breaking down various compounds found in the environment. This process converts pollutants into carbon dioxide (CO₂) and water (H₂O), which are then released as the final by-products of degradation (Chopra and Kumar, 2020a). Presently, wastewater containing pharmaceuticals is primarily treated using Advanced Oxidation Processes (AOPs), like Fenton and photo-Fenton reactions, photocatalysis employing titanium dioxide, ozonation combined with hydrogen peroxide (H₂O₂), or UV photolysis. However, understanding of the subsequent paracetamol characteristics in the environment is still limited. Studies have summarized the efficiency of chemical, and biological treatment systems.
and hybrid approaches in wastewater treatment, reporting that a hybrid system employing zonation and biological activated carbon proved highly efficient in pesticide removal, pharmaceuticals, and beta-blockers (Ahmed et al., 2017). However, despite their high efficacy, the significant operational expenses, stringent operational conditions, and the production of secondary compounds with elevated toxic effects often made these procedures undesirable. Hence, the microbial degradation of pharmaceuticals, including NSAIDs, employing microbial strains with high degradation capabilities, portrays a sustainable and promising tool in both the environmental and economic aspects of wastewater treatment. The potential of any microbes to degrade xenobiotics is driven by several environmental conditions (Żur et al., 2018a), which impact the degradation process. It is widely understood that temperature, pH, and other environmental factors also contribute significantly to this degradation process, influencing microbial physiology and modulating the enzymatic reaction rate.

Factors Affecting Microbial Degradation Processes

There are substantial numbers of previous studies that have found that pharmaceutical compound degradation is highly dependent on operating factors including pH, temperature, shaking speed, carbon, and energy sources, etc. (Chopra and Kumar, 2020b; Sharma et al., 2020).

Effects of Temperature on Paracetamol Biodegradation

The significant impact of temperature on xenobiotic degradation is widely recognized, as it impacts bacterial functioning and the efficiency of enzymatic-driven activities. The optimum xenobiotics biodegradation rate is observed within the temperature range of 30-40°C. In lower temperatures, the rigidity of bacterial membranes increased, leading to heightened viscosity of membrane phospholipids. Conversely, higher temperatures often impede membrane transport due to the alteration of proteins associated with membranes (Żur et al., 2018a). However, for biodegradation of paracetamol, a wide range temperature of 25-35°C can still be considered to show a moderately high degradation, although, optimization studies as shown an efficient temperature of 25, 28, and 30°C (Edrees et al., 2018; Palma et al., 2022; Wadhah, 2018). As shown in Fig. 2, the study observed an optimal temperature of 25°C and the reducing effects of microbial degradation of paracetamol was recorded at temperatures below 20°C and above 30°C.

Effect of pH on Biodegradation of Paracetamol

The pH stands as another critical factor regulating the xenobiotic degradation of paracetamol, impacting membrane properties and microbial cell structure. For paracetamol, in a basic environment, it exists as a phenolate (RO−) form, whereas, at lower pH, it has been observed that there is a formation of a protonated form (ROH). Given that the acid dissociation constant (pKa) of paracetamol is 9.5, under mildly basic conditions, paracetamol exists predominantly in its non-ionic state (Xagoraraki et al., 2008). It can be inferred from these findings that the optimal breakdown rate of paracetamol could be observed at a neutral pH level. This assumption was validated for Pseudomonas aeruginosa strain DSM 50071, Pseudomonas aeruginosa strain NBRC 12689 (Wadhah, 2018), and Pseudomonas moorei strain KB4, with an efficient paracetamol biodegradation at an optimal pH of 7.0 (Żur et al., 2018b). However, in Fig. 3, a study on fungi degradation efficiency has shown a relatively higher degradation within a 5.5-6.5 pH range and the breakdown rate greatly reduced at a pH >7.0 (greater than 7.0) or 5.0<5.0 (smaller than 5.0) (Edrees et al., 2018).

Effects of Cell Concentration on Paracetamol Biodegradation

Several studies have observed that the cell concentration has a high influence on the degradation rate of paracetamol which plays a substantial role in transport elements. Some reports have proven this finding, as observed in Pseudomonas aeruginosa strain DSM 50071 and P. aeruginosa strain NBRC 12689 (Wadhah, 2018) and Rhodococcus erythropolis (Akay and Tezel, 2016) which demonstrated the effect of varying cell concentrations on paracetamol degradation. Microorganisms are supplied with energy and cell-building materials through the degradation of organic substrate and are used up for cell maintenance, regeneration of cells, and co-metabolization of non or less degradable or other materials (Cornelissen and Sijm, 1996). Another optimization study has confirmed that cell concentrations affect the biodegradation rate of paracetamol (Edrees et al., 2018). For example, at a higher bacterial concentration of 10^6 CFU/mL an optimal breakdown was achieved within 48 h (Fig. 4). That is, a higher cell concentration led to an increased rate of paracetamol breakdown by microbes.

Effects of Glucose Level on Paracetamol Biodegradation

The role of glucose in paracetamol breakdown, serving as a carbon and energy source for microbial processes. Several studies have proven that the biodegradation of paracetamol glucose medium increased with higher glucose levels. It revealed that glucose acts as a facilitator, providing extra energy to bacteria for paracetamol breakdown. In a similar study, the result was
also the same for the effect of glucose, an optimal degradation was achieved at concentration of 5 g/L within 72 h (Fig. 5). The availability of glucose supplies microbes with energy which subsequently boosts their ability to use up the tolerant aromatic amines (Edrees et al., 2018; Palma et al., 2021; Wadhah, 2018).

**Paracetamol Biodegrading Microorganisms**

Microorganisms have evolved efficient biodegradation mechanisms, employing unique enzymatic mechanisms and metabolic routes to metabolize paracetamol for carbon and energy utilization. As a result, these microorganisms are efficient in breaking down paracetamol and transforming it into readily metabolizable materials (Hasan et al., 2011).

*Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlorella sorokiniana* are frequently utilized microalgae strains in wastewater treatment and have been identified to be efficient in the breakdown of 17-67% of Paracetamol in a study (Escapa et al., 2019). Other research has revealed a rapid removal of paracetamol using *Chlorella sorokiniana* strains (Escapa et al., 2015; 2017).

The wastewater environment consists of highly variable fungal communities, *Penicillium*, *Geotrichum*, and *Candida* species are the most highly represented then the next are *Trichoderma*, *Acremonium*, *Aspergillus*, *Trichosporon* and *Rhodotorula* (Buratti et al., 2022). A study designating fungi as F1 and F2 recognized as *Aspergillus niger* and *Fusarium oxysporum* degraded 26.1 and 35.7% of 2000 and 1000 mg/L of paracetamol concentration respectively at optimum temperature 25°C and pH 6.0 (Sharma et al., 2020). *Trichoderma harzianum* and *Pseudomonas* (Shabani et al., 2021) and *Scedosporium dehoogii* (Pontié et al., 2019) where a combination of bacterial and fungal biofilm having redox-active enzymes capable of electrogenic activity was used as a microbial fuel source and have shown to be effective in elimination of paracetamol contaminated wastewater.

Numerous bacteria with the ability to metabolize paracetamol as a carbon and energy source have been identified, with authors also proposing metabolic pathways for its degradation (Chopra and Kumar, 2020a). The isolation of *Cupriavidus necator* F1 from activated sludge has shown a complete breakdown of paracetamol at a starting concentration of 400.0 mg/L within 2 days. The results indicated that the strain possesses high capability for paracetamol mineralization as reported (Wei et al., 2011). A report of 97% degradation by *Delftia tsuruhatensis* was also observed by De Gusseme et al. (2011), within 48 h. In addition, the isolation of *Stenotrophomonas* sp. F1,
**Microbial Degradation and Transformation Pathway for Paracetamol**

Several researchers were focused on summarizing paracetamol biodegradation studies in aspects such as bacteria degrading paracetamol, their enzymatic mechanisms, proposed biodegradation pathways/metabolic routes in microorganisms, and their possible intermediates. Isolated *Penicillium* sp. was observed to have the ability to degrade paracetamol into 4-aminophenol and acetate, possibly employing arylacilamidase. 4-aminophenol is a non-metabolizable end product (Hart and Orr, 1975) (Fig. 6). Additionally, findings indicate that *Rhodococcus* strains have the capability to decompose paracetamol, resulting in the production of three (3) identifiable metabolites: 4-aminophenol, hydroquinone, and catechol (Ivshina et al., 2006).

![Fig. 6: Microbial transformation of paracetamol pathways (Hart and Orr, 1975; Kolvenbach et al., 2011; Takenaka et al., 2003; Li et al., 2014; Zhang et al., 2013)](image)

The procedure involved in the continual breakdown of 1,4-hydroxybenzene could progress in two directions. First, direct cleaving of Hydroquinone through hydroquinone 1,2-dioxygenase along with 4-hydroxymuconic semialdehyde, similar to an aliphatic material (Daubaras et al., 1996). As documented in the biotransformation of paracetamol by *Pseudomonas aeruginosa* and *Delftia tsuruhatensis* bacterial strains, the methylation of hydroquinone might yield the mono and di O-methylated intermediates "4-methoxyphenol and 1,4-dimethoxybenzene." (De Gussemse et al., 2011). Through an amidohydrolase reaction paracetamol possibly metabolizes and yields 4-aminophenol from the carbonyl group through the cleavage between nitrogen-carbon bond, in which the formation of hydroquinone would result from the nitrogen being removed followed by hydroxylation (Takenaka et al., 2003). However, the transformation of 4-aminophenol to 1,4-hydroxybenzene by *Burkholderia* sp. strain AK-4 and then progress to 1,2,4-trihydroxybenzene was detailed. Progressively, hydroxy-hydroquinone 1,2-dioxygenase gradually cleaved 1,2,4-trihydroxybenzene to produce maleylacetic acid, incorporated into the core metabolism process (Kolvenbach et al., 2011; Moonen et al., 2008) (Fig. 6).

In more detail, the biotransformation of paracetamol to hydroquinone was followed by the conversion to the aliphatic product hexa-3-enedioic acid, this could stem from aromatic ring fission or might suggest the bypassing of intermediate metabolites between aliphatic and aromatic compounds. The Hexa-3-enedioic acid is related to muconic acid, which resulted from ortho-ring cleavage of catechol. A primary pathway of paracetamol biodegradation could be suggested based on reported intermediates. The mechanism may involve the elimination of two carbon atoms as formic acid (Zhang et al., 2013) (Fig. 6). Additionally, a sequence of hydroxylation reactions resulted in the transformation of paracetamol to phenols and organic acids by using *Rhodococcus erythropolis* reported (Akay and Tezel, 2016). In the biotransformation course, paracetamol undergoes initial conversion to 4-aminophenol, then was later changed to hydroquinone by replacing the amino group with a hydroxyl and hydroquinone subsequently underwent ring fusion.

Moreover, filamentous fungi that produce glucoside conjugates with paracetamol by O and N linkages in soil were defined (Huang et al., 2006). Phase-II xenobiotic detoxication routes for humans are absolutely related to this process (Halling-Sørensen et al., 1998). Also, a proposed pathway for paracetamol breakdown by soil microorganisms was explained in depth. The first step indicated that Paracetamol's aromatic ring is hydroxylated to form 3-hydroxyparacetamol, which is followed by methylation to form p-acetanisidide or oxygenation to form N-acetyl-p-benzoquinone imine. Which is a more
stable and significant toxic metabolite i.e., the 1,4-benzoquinone, of the N-acetyl-p-benzoquinone imine, was produced next. Furthermore, the subsequent step transforms p-acetansidide into 4-methoxyphenol and 1,4-dimethoxybenzene. The occurrence of 2-hexenoic acid in the soil extract is demonstrated by the breakdown of paracetamol’s aromatic ring (Li et al., 2014). In soil, flavin-containing hydroxylases, and monoxygenases are generally allocated among the microorganisms and as well catalyze several oxidative processes as found in the hydroxylation reaction of phenols to catechols (Sariaslani and Dalton, 1989).

Environmental Toxicity and Health Risk of Paracetamol

Paracetamol is widely prescribed around the world for its analgesic antipyretic properties. However, at high doses, it becomes highly toxic (Nunes et al., 2014). The rising levels of paracetamol and other emerging contaminants create the potential for toxic effects on unintended species in aquatic habitats. Lately, the England and Wales Environment Agency recognizing the possible threat of these contaminants to aquatic ecosystems, suggested a ranking scheme identifying the top 10 compounds of significance, with paracetamol listed as the 5th (Ebele et al., 2017). The pervasive presence of paracetamol together with its primary product of degradation, 4-aminophenol, in the environment is somewhat linked to their application in the manufacture of azo dyes and photographic materials (Zhang et al., 2013). In 2017, the consumption habits of paracetamol by consumers were assessed, and it was found that paracetamol is majorly consumed for fever, headaches, and general pain relief (Chong et al., 2017). Even though paracetamol is generally regarded as safe, it is among the top causes of toxicity and liver impairment. Following administration, paracetamol is predominantly and rapidly metabolized in the liver by sulfotransferases and urine 5′-diphosphoglucuronosyltransferase (conjugating enzymes), which convert the drug into non-toxic molecules, biliary and renal excretion then followed. The residual paracetamol undergoes oxidation to form N-acetyl-p-benzoquinone-imine, a reactive electrophilic metabolite during Phase I cytochrome P450 isoenzymes. Glutathione detoxifies N-acetyl-p-benzoquinone-imine, forming conjugates of paracetamol with cysteine and mercapturate. A paracetamol overdose causes glutathione reserves to be depleted, resulting in the accumulation of N-acetyl-p-benzoquinone-imine. This buildup leads to covalent alteration of protein thiol groups, damage to genetic material, cell necrosis, oxidative damage to membrane lipids, and cell lysis (Zur et al., 2018a).

Effects on Aquatic Bodies

Several studies as observed the harmful effects connected to countless pharmaceutical contaminants. For example, A study on male fish (Rhamdia quelen), has shown that exposure to paracetamol caused an increase in thrombocytes and Leukocytes, and hemoglobin and hematocrit were reduced upon exposure to paracetamol concentration of 0.25 μg/L, reduced testosterone levels, increased dopamine and serotonin exposure level to 0.25 μg/L, estradiol levels increased at higher concentration and at 0.25 μg/L concentration hepatic genotoxicity arose; leucocytes infiltration and mild blood congestion in hepatic tissue (Guiloski et al., 2017). Also, several studies have confirmed the endocrine disruption and hepatotoxicity effect of Paracetamol in zebrafish (Danio rerio) (Ayobahan et al., 2020; Moreira et al., 2023).

Effects on Human Health

Paracetamol, as a non-opioid analgesic, operates through a unique mechanism different from that of other NSAIDs. The action mechanism is not fully comprehended, yet it seems to selectively target cyclooxygenase in the brain to alleviate pain or fever and possibly suppress prostaglandin production in the central nervous system. Paracetamol mode of action generates an antipyretic response by targeting the hypothalamus directly (Ghanem et al., 2016). Harmful effects such as histopathological and biochemical alterations in rat livers at 66 mg per kg body weight and 15mg per kg body weight have been attached to exposure to paracetamol, exposure to paracetamol in the primary stages of growth has been linked to the medulla oblongata shown to affect the neurotransmission (Blecharz-Klin et al., 2015a) or significantly influence effect on the spinal cord (Blecharz-Klin et al., 2015b).

In the past, pregnancy was considered to be safe with the usage of paracetamol but now, it has become debatable that its usage may generate future adverse impacts on the offspring if consumed during pregnancy. A recent report on various epidemiological studies linked to offspring having behavioral syndromes such as attention deficit hyperactivity disorder and Autism spectrum disorder has been connected to exposure to paracetamol despite the limited evidence linking paracetamol use during pregnancy to brain function (Bührer et al., 2021).

Current Techniques for Paracetamol Degradation

Several treatment methods have been developed in response to the threat caused by paracetamol as an organic contaminant found in wastewater. Different techniques are used, depending on the structure and properties of the organic component. Four of these methods; adsorption, membrane processes, advanced oxidation processes, and...
Biodegradation are particularly popular for wastewater treatment. Each method uses a very distinct process to break down paracetamol, even though they are all capable of degrading it. For example, oxidation mechanisms break down a variety of organic contaminants by causing the compounds to undergo radical splitting by in situ formation via oxidation. The chief benefit of oxidation processes is their capability to completely break down pollutants without transitioning them into another phase or producing secondary waste (Lee et al., 2020). The adsorption process operates through solutes and adsorbent interaction. Pollutants are attracted to the adsorbent by hydrophobic and electrostatic interactions and are subsequently removed by water. The primary benefit of adsorption is its non-toxic nature. Nevertheless, it has a short lifespan, is somewhat costly, and is ineffective against some pollutants. Regenerating the adsorbent is feasible, however, it frequently results in considerable mass loss, making it unfeasible from an economic standpoint (Cabrita et al., 2010; Reungoat et al., 2010). Membrane processes use a semi-permeable membrane's charge repulsion, solute adsorption, pressure, and size exclusion to separate solutes from water (Lee et al., 2020). The benefit of membrane techniques lies in their capability to effectively eliminate nearly all types of pollutants. However, their operational costs are high due to significant energy consumption and the need for membrane replacement resulting from fouling (Babu et al., 2019; Hua et al. 2020). Biodegradation is a broader term that encompasses the breakdown of organic compounds by biological processes, including microbial degradation. While microbial degradation is a type of biodegradation, not all biodegradation processes necessarily involve microorganisms. Biodegradation employs either an anaerobic or aerobic microbial process to effectively break down contaminants. Broadly, the resultant products are less harmful and more resilient than the original compound (Rana et al., 2017). The flexibility of microbes in targeting a wide array of substrate media is a key advantage of biodegradation. However, microbial growth may be hindered in high-salinity effluents. In addition, biodegradation is a lengthy process and may result in non-biodegradable soluble or cellular residues. The effectiveness of the method is also contingent on the compound’s biodegradability.

A closer examination is undertaken to explore the efficiency of microbial degradation of different pharmaceuticals (NSAIDs), sourced from multiple research findings. The focus lies on understanding the efficacy of different microbes in breaking down various pharmaceutical compounds. Table 3 presents a compilation of data sourced from multiple research papers, detailing the origins of these microbes, the pharmaceutical products of the target, and the rates and conditions of biodegradation.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Habitat/location of isolation</th>
<th>Pharmaceutical products (drugs)</th>
<th>Biodegradation rate (%)</th>
<th>Biodegradation conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>Marseille, France</td>
<td>Diclofenac</td>
<td>Greater than 99%</td>
<td>Speed: 100 rpm, temp: 20°C, within 17 h</td>
<td>Grandclément et al. (2020)</td>
</tr>
<tr>
<td>Brevibacterium sp. D4</td>
<td>Wastewater Treatment Plant (WWTP) in, Portugal</td>
<td>Diclofenac</td>
<td>About 90% breakdown</td>
<td>A temp: 25°C, speed: 150 rpm within 30 days</td>
<td>Bessa et al. (2017)</td>
</tr>
<tr>
<td>Klebsiella sp. KSC</td>
<td>Sourced from livestock soil</td>
<td>Diclofenac</td>
<td>90% breakdown</td>
<td>pH 7, 30°C, at a speed: 100 rpm within 72 h</td>
<td>Stylianou et al. (2018)</td>
</tr>
<tr>
<td>Microbacterium paraoxydans</td>
<td>East India pharmaceutical wastewater</td>
<td>Ibuprofen</td>
<td>92.01% breakdown</td>
<td>pH 7, 30°C, speed: 150 rpm, 0.3% yeast extract</td>
<td>Show et al. (2023)</td>
</tr>
<tr>
<td>Patulibacter sp. Strain L11</td>
<td>Lisbon, Portugal, From WWTP activated sludge</td>
<td>Ibuprofen</td>
<td>92% breakdown</td>
<td>A speed: 110 rpm, temp: 28°C, within 90 h</td>
<td>Almeida et al. (2013)</td>
</tr>
<tr>
<td>Sphingopyxis granuli RW412</td>
<td>Downstream of the Hamburg harbor on the Elbe River, Germany</td>
<td>Ibuprofen</td>
<td>80% breakdown</td>
<td>Speed of 200 rpm, temp: 30°C, within 72 h</td>
<td>Aguilar-Romero et al. (2021)</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>Delft, Netherlands Hospital WWTP Pharma filter, sludge</td>
<td>Paracetamol</td>
<td>&gt;99% breakdown</td>
<td>A pH 7, 500 rpm, temp: 20±1°C, airflow 30 mL/min, within 10 days</td>
<td>Rios-Miguel et al. (2022)</td>
</tr>
<tr>
<td>Pseudomonas moorei KB4</td>
<td>Poland. Activated sludge from Klizmowiec</td>
<td>Paracetamol</td>
<td>99% breakdown within 1.5 h</td>
<td>pH 7, temp: 30°C</td>
<td>Żur et al. (2018b)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>Obtained from a</td>
<td>Paracetamol</td>
<td>About 71.4%</td>
<td>A pH 7, 30°C, temp: 30°C</td>
<td>Hu et al. (2013)</td>
</tr>
</tbody>
</table>
Research GAP and Future Directions

In spite of the fact that microbial degradation is a well-established process widely used in the treatment of wastewater, the removal of paracetamol and pharmaceuticals, in general, still requires further exploration in both theoretical and practical aspects related to risk assessment and ecological footprint. Key areas needing additional study include (a) Optimizing operational conditions to improve the microbial efficacy of processes in the complete removal of paracetamol; (b) Evaluating the risks associated with paracetamol and its metabolites in order to address legislative gaps; and lastly (c) Conducting direct measurement-based ecological footprint assessments of wastewater treatment systems, taking into consideration all emissions before, during and after treatment.

Conclusion

This review delves into the critical issue of paracetamol contamination in pharmaceutical wastewater and explores microbial degradation as a potential solution. Paracetamol, extensively produced and consumed worldwide, poses a significant environmental threat as it enters water bodies through various sources, including pharmaceutical production, consumer use, and improper disposal. Despite efforts by wastewater treatment plants, current methods fail to efficiently remove paracetamol, leading to its persistence in water sources.

Microbial degradation emerges as a promising approach, leveraging the natural abilities of microorganisms to break down paracetamol into less harmful compounds. Enzymes secreted by these microorganisms are essential in this process, highlighting the potential of biodegradation as an economically viable and environmentally friendly solution.

This review consolidates findings from a wide range of studies, emphasizing the importance of optimizing operational conditions to enhance microbial efficiency in wastewater treatment systems. This review will contribute to the advancement of wastewater treatment practices, safeguarding water quality, and protecting environmental health by addressing these research gaps aspects and focusing on future research directions.

Acknowledgment

Thank you to the publisher for their support in the publication of this research article. We are grateful for the resources and platform provided by the publisher, which
have enabled us to share our findings with a wider audience. We appreciate the efforts of the editorial team in reviewing and editing our work, and we are thankful for the opportunity to contribute to the field of research through this publication.

**Funding Information**

The authors have not received any financial support or funding to report.

**Author’s Contributions**

**Yahya Seun Yisau:** Conceptualization data curation, written original drafted.

**Naeif Hamoud Al-Makishah:** Visualization, supervision, written, reviewed and edited.

**Mohamed Abou El-Fetouh Barakat:** Investigation, validation, written, reviewed and edited.

**Ethics**

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

**Conflict of Interest**

The authors affirm no conflicts of interest.

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