



Metabolic conversion of phenol to polyhydroxyalkanoate (PHA) for addressing dual environmental challenges: A review

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ABSTRACT

A sustainable approach to microbial polyhydroxyalkanoate (PHA) production involves utilizing waste as a substrate, which can include toxic pollutants like phenol as a carbon feedstock. Phenol-contaminated effluents offer cost-effective and readily available resources for PHA production, while simultaneously addressing phenol contamination issues. Understanding the metabolic conversion of phenol to PHA is crucial to enhance its efficiency, especially considering phenol's toxicity to microbial cells and the substrate-dependent nature of microbial PHA production. In this review, the mechanisms of phenol biodegradation and PHA biosynthesis are first independently elucidated to comprehend the role of bacteria in these processes. Phenol can be metabolized aerobically via various pathways, including catechol *meta*-cleavage I and II, catechol *ortho*-cleavage, protocatechuate *ortho*-cleavage, and protocatechuate *meta*-cleavage, as well as anaerobically via 4-hydroxybenzoate and/or *n*-caproate formation. Meanwhile, PHA can be synthesized through the acetoacetyl-CoA (pathway I), *de novo* fatty acids synthesis (pathway II), β -oxidation (pathway III), and the tricarboxylic acid (TCA) cycle, with the induction of these pathways are highly dependent on the substrate. Given that the link between these two mechanisms was not comprehensively reported before, the second part of the review delve into understanding phenol conversion into PHA, specifically polyhydroxybutyrate (PHB). While phenol toxicity can inhibit bacterial performance, it can be alleviated through the utilization of microbial mixed culture (MMC), which offers a wider range of metabolic capabilities. Utilizing phenol as a carbon feedstock for PHB accumulation could offer a viable approach to boost PHA's commercialization while addressing the issue of phenol pollution.

1. Introduction

Petroleum-derived plastic is extensively produced due to its high demand and wide applicability. Over the past century, the global production of petroleum-derived plastic has reached 320 million tons (Mt) annually (Ragusa et al., 2021). However, this high production rate is alarming due to the extremely low degradability of plastics, posing a serious environmental threat. Until today, recycling rates for plastic waste remain discouraging, with a significant portion ending up in landfills. Plastics can take up to two thousand years to degrade, and while in landfills, they can contaminate the groundwater sources

through the leaching of toxic additives. Furthermore, the use of fossil fuels as raw materials for plastic production has raised serious concerns due to the emission of greenhouse gases into the atmosphere, contributing to climate change and global warming (Naser et al., 2021).

As a result, much attention has shifted towards bioplastic as an alternative to the issues associated with petroleum-derived plastics. Atiwesh et al. (2021) defined bioplastic as an environmentally sustainable polymeric substance with similar functionality to petroleum-derived plastics, meanwhile Park et al. (2024) added that bioplastics are synthesized from renewable resources and can biodegrade. However, it's important to note that not all bioplastics

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synthesized from renewable resources are biodegradable, and conversely, not all biodegradable bioplastics are produced from renewable resources, as shown in Fig. 1. The ideal bioplastics should be both biodegradable and derived from renewable resources, such as polylactic acid (PLA), polyhydroxyalkanoate (PHA), and polyvinyl alcohol (PVA). Only this type of plastic can be regarded as “environmentally friendly bioplastics”.

Polyhydroxyalkanoate (PHA) is a microbial bioplastic produced by various species of microorganisms as intracellular inclusion bodies for carbon and energy storage under stressful environments (McAdam et al., 2020). It is considered an environmentally friendly bioplastic as it can be degraded and synthesized from renewable feedstock (Liao et al., 2018). PHA can easily be degraded by microbial enzymatic activity, generating carbon dioxide (CO₂), water, and microbial biomass as the final products (Sirohi et al., 2020). In addition, PHA is biocompatible with humans, and its physical properties are generally similar to those of petroleum-derived plastics (Khamkong et al., 2022). For example, the tensile strength, melting temperature, Young's modulus, and crystallinity degree of polyhydroxybutyrate (PHB), a type of PHA, are comparable to polypropylene (PP) (Abate et al., 2024).

However, PHA is expensive, primarily due to the cost of its raw materials, which account for 40 – 48 % of the total production costs (Sirohi et al., 2020). Common feedstocks used for PHA production include sugars and fatty acids extracted from crops such as corn starch, sugarcane, and vegetable oil, constituting more than 50 % of the total production costs (Zytner et al., 2023). The price of PHA could be up to 16 times higher than that of petroleum-derived plastics (Alvarez Chavez et al., 2022). Despite being the most effective way to reduce plastic pollution in the environment, the high production costs of PHA limit its commercialization. Therefore, the utilization of a cheaper carbon substrate is anticipated to reduce overall production costs and enhance its applicability in everyday use.

The field of PHA research is expanding into exploring various waste resources as potential raw materials. Utilizing waste for PHA synthesis is considered a viable option as it meets the ideal raw material requirements of being abundant, affordable, renewable, and carbon-rich. Furthermore, converting waste materials into PHA will help mitigate their negative effects on living organisms and reduce the emission of hazardous compounds into the environment. Previous studies have documented PHA production from various waste streams such as animal waste (Shahzad et al., 2017), cheese whey (Pais et al., 2016), olive mill wastewater (Bacha et al., 2023), waste cooking oil (Ruiz et al., 2019),

municipal wastewater (Bengtsson et al., 2017), paper mill wastewater (Munir et al., 2015), food waste (Colombo et al., 2017), and crude glycerol (Luo et al., 2016). Shah and Kumar (2021) supported the idea that using cheap, abundant, and renewable waste materials as substrates might be the solution to reduce the price of PHA. Furthermore, according to Liao et al., 2018, using renewable waste materials as substrates is expected to halve PHA production costs.

Industrial effluents rich in toxic compounds are constantly generated and continuously discarded at very high volumes, making them a potentially remarkable source of feedstock for PHA production. The presence of toxic contaminants will exert stress on microorganisms, diverting their metabolic responses toward PHA accumulation (Saharan et al., 2014). However, research on PHA production from toxic compounds has been limited to very few studies (Zhang et al., 2018), possibly due to the toxic nature of the compounds, which can hamper bacterial growth. One of the most prevalent toxic contaminants, phenol, is typically found in the environment through the discharge of industrial effluents. Since its discovery in 1834, phenol has been widely used to synthesize many other chemical compounds such as acetylsalicylic acid, phenolic resins, bisphenols, polycarbonates, aniline, alkylphenols, diphenols, and salicylic acid (Weber et al., 2020). Phenol can exert mutagenic, teratogenic, and carcinogenic effects on living organisms (Reddy et al., 2015a; Saputera et al., 2021). Concentrations of phenol ranging between 9 – 25 mg/L are fatal to fish, and between 10–24 mg/L are hazardous to humans (Hamad, 2021). Hence, the United States Environmental Protection Agency (US EPA) and the National Pollutant Release Inventory (NPRI) of Canada have designated phenol as a priority pollutant, ranked 11th out of 126 harmful chemicals (Liu et al., 2020; Naguib and Badawy, 2020; Villegas et al., 2016). The US EPA has also set a limit for phenol concentration at 0.001 mg/L in surface waters (Mohd, 2020). Therefore, removing phenol from industrial effluent before discharge is crucial to mitigate its harmful effects on the environment and living organisms.

The integration of bioremediation technology with the production of value-added products is advantageous in addressing two environmental issues at once and can simultaneously offer an economically viable solution, specifically by coupling phenol bioremediation with PHA production. The utilization of phenol for PHA production has been documented previously (Chen et al., 2018; Kanavaki et al., 2021; Mas-kow and Babel, 2000; Nair et al., 2009; Reddy et al., 2015a). Additionally, the interaction between different species of bacteria in microbial mixed culture (MMC) can exert a synergistic effect in

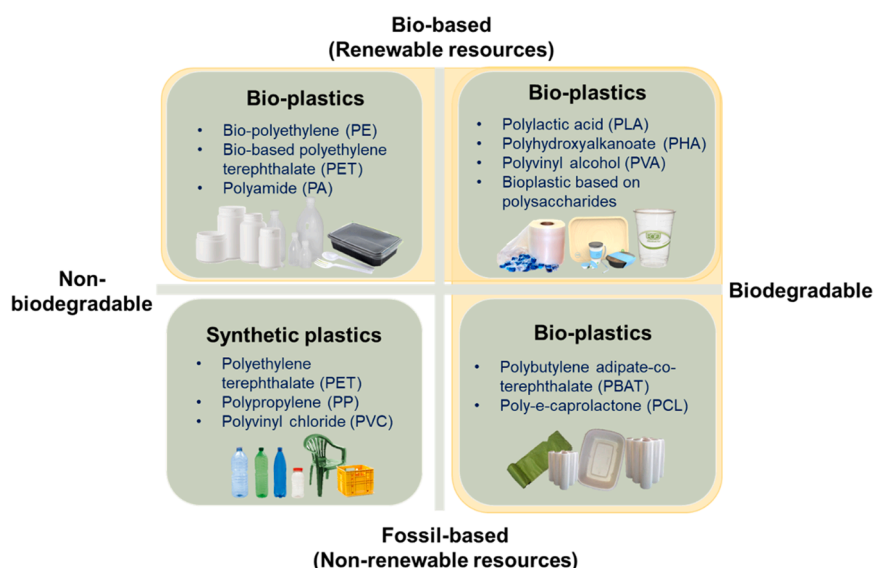


Fig. 1. Classification of polymers based on their raw materials and their degradation capability.

enhancing these processes. Nevertheless, to the best of our knowledge, comprehensive studies that discuss the mechanism of phenol conversion into PHA are very limited. It is important to deeply understand the mechanisms of both processes, as various improvements can be made possible, such as identifying the limiting factor and re-engineering the metabolic pathways for enhanced efficiency of phenol degradation and PHA synthesis. Therefore, this review aims to define the underlying mechanisms of phenol degradation and its transformation into PHA, while assessing the enhancement of these processes using MMC.

2. Polyhydroxyalkanoate (PHA)

PHA is a family of microbial polymers composed of repeating units of monomers linked by ester bonds (Yang et al., 2022). These polymers accumulate as carbon and energy storage or serve as electron sinks for redundant reducing power in response to nutrient deprivation. Once the availability of the limiting nutrient is restored, accumulated PHA is degraded by intracellular depolymerase (PhaZ) and utilized as a carbon and energy source (Cappelletti et al., 2020). PHA is regarded as a biodegradable polymer as it can be depolymerized and utilized by the bacteria that produce it (Marjadi and Dharaia, 2018).

PHA polymers usually comprise 600 to 35,000 of (R)-hydroxy fatty acid monomeric units (Tan et al., 2014). The common structure of a PHA monomer is shown in Table 1, where “x” is the number of repeating monomeric units and “R” is the functional group that varies depending on the type of PHA (McAdam et al., 2020; Pagliano et al., 2017). PHA can be categorized based on the total number of carbon atoms in the monomer (Table 1). Monomers consisting of 3 – 5 carbon atoms are classified as short-chain length PHA (scl-PHA), 6 – 14 carbon atoms as medium-chain length PHA (mcl-PHA), and more than 14 carbon atoms as long-chain length PHA (lcl-PHA) (Kuddus and Roohi, 2021; Li and Wilkins, 2020). Scl- and mcl-PHAs are well-known, while lcl-PHA is the least studied (Bhat et al., 2024) due to its production difficulty. This is attributed to its water-insoluble substrates, such as alkanes and alkanolic acids, and other substrates for lcl-PHA production being toxic to the bacteria at low concentrations (Zhila et al., 2022).

Scl-PHA has thermoplastic properties similar to PP. Some examples of monomeric units of scl-PHA are 3-hydroxybutyrate (3-HB), 4-hydroxybutyrate (4-HB), and 3-hydroxyvalerate (3-HV). Polyhydroxybutyrate (PHB), composed of repeating units of 3-HB, is the most recognized and

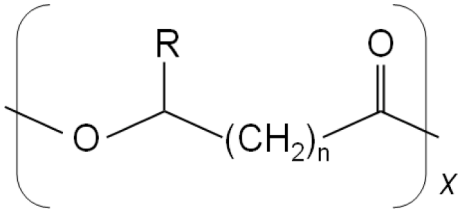
widely researched biopolymer since its discovery in 1926. It contains a methyl group in its structure, making it the most prominent representative of the scl-PHA group (Kuddus and Roohi, 2021). Approximately 60 – 80 % of the microbial dry cell weight (DCW) has been reported to consist of PHB granules (Sharma, 2019). Various bacterial species have been reported to synthesize scl-PHA, such as *Ralstonia eutropha*, *Alcaligenes latus* (Khosravi-Darani et al., 2013), *Bacillus cereus*, *Bacillus subtilis* (Saratale et al., 2021), and *Cupriavidus necator* H16 (Prasad et al., 2019). In addition to single culture, MMC also has demonstrated the ability to accumulate PHA. For example, enriched MMC from activated sludge of wastewater treatment plant, dominated by two species of *Thaurea* genus, has been reported to produce scl-PHA (Bhalerao et al., 2020).

Mcl-PHA has a higher carbon chain length, which results in reduced crystallinity and increased flexibility, similar to the properties of elastomers and latex-like materials. They have a low glass transition temperature and lower molecular mass compared to scl-PHA (McAdam et al., 2020). Examples of monomers belonging to the mcl-PHA class include 3-hydroxyhexanoate (3-HHx) and 3-hydroxydecanoate (3-HD). *Pseudomonas* species have a unique metabolism for diverse compounds and are well-known for producing mcl-PHA from various types of carbon feedstock. *Pseudomonas putida* has been shown to accumulate intracellular mcl-PHA (Khosravi-Darani et al., 2013; Ramírez-Morales et al., 2021), and *P. putida* KT2440 is widely exploited for the production of this type of PHA (Borrero-de Acuña et al., 2021b; Liu et al., 2017; Oliveira et al., 2020; Yang et al., 2019). Additionally, *Thermus thermophilus* (Pantazaki et al., 2003), *Cupriavidus basilensis* B-8 (Si et al., 2018), and *Bacillus thermoamylovorans* strain PHA005 (Choonut et al., 2020) have also been documented to accumulate mcl-PHA. Additionally, enriched MMC from activated sludge, mainly composed of *Pseudomonas aeruginosa*, has been reported to produce mcl-PHA utilizing nonanoic acid as the substrate (Lee et al., 2011).

PHA can also be categorized based on the types of monomer(s) that make up the polymeric structure. Biopolymers can be formed from one, two, or three types of repeating units of monomer(s), and are known as the homopolymers, copolymers, or terpolymers, respectively. PHB is a homopolymer containing repeating 3-HB monomeric units in its polymeric structure. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is an example of a copolymer, made up of 3-HB and 3-HV fractions, while poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) (P-3HB-co-3HV-co-3HHx) is classified as a terpolymer, comprising 3-HB,

Table 1

Typical structure of the monomer unit of PHA and the classification of PHA based on the number of carbon atoms in the PHA polymer.

					
Type of PHA	n	R	Monomer unit	No. of carbon atoms	Type of polymer
Short-chain PHA (scl-PHA) 3–5 carbons	1	Hydrogen	3-hydroxypropionate	3	Poly-(3-hydroxypropionate) (PHP)
		Methyl	3-hydroxybutyrate	4	Poly-(3-hydroxybutyrate) (PHB)
		Ethyl	3-hydroxyvalerate	5	Poly-(3-hydroxyvalerate) (PHV)
	2	Hydrogen	4-hydroxybutyrate	4	Poly-(4-hydroxybutyrate) (P4HB)
		Methyl	4-hydroxyvalerate	5	Poly-(4-hydroxyvalerate) (P4HV)
Medium-chain PHA (mcl-PHA) 6–14 carbons	3	Hydrogen	5-hydroxyvalerate	5	Poly-(5-hydroxyvalerate) (P5HV)
		Propyl	3-hydroxyhexanoate	6	Poly-(3-hydroxyhexanoate) (PHHx)
		Pentyl	3-hydroxyoctanoate	8	Poly-(3-hydroxyoctanoate) (PHO)
	3	Nonyl	3-hydroxydodecanoate	12	Poly-(3-hydroxydodecanoate) (PHDD)
		Methyl	5-hydroxyhexanoate	6	Poly-(5-hydroxyhexanoate) (P5HHx)
		Hexyl	6-hydroxydodecanoate	11	Poly-(6-hydroxydodecanoate) (P6HDD)
		Dodecyl	3-hydroxypentadecanoate	15	Poly-(3-hydroxypentadecanoate)
Long-chain PHA (lcl-PHA) >14 carbons	1	Tridecyl	3-hydroxyhexadecanoate	16	Poly-(3-hydroxyhexadecanoate)

3-HV and 3-HHx fractions (Cappelletti et al., 2020). Microbes can produce various types of PHAs based on the carbon feedstocks and their conversion mechanisms (Thapa et al., 2019). It has been reported that *Pandoraea* sp. ISTKB can synthesize the PHBV co-polymer (Kumar et al., 2017), while the MMC of *Aeromonas hydrophila* and *Thiococcus pfennigii* can produce copolymers from scl- and mcl- PHAs (Khosravi-Darani et al., 2013). Moreover, polymers with scl- and mcl- PHAs properties have also been reported to be produced by enriched MMC from activated sludge of a municipal wastewater treatment plant dominated by *Xanthobacter*, *Leadbetterella*, *Aequorivita*, *Achromobacter*, *Mesorhizobium*, *Enterococcus*, and *Pseudomonas* (Tamang and Nogueira, 2021).

The value of PHA as a sustainable alternative to petroleum-based polymers has been established over the past decades. PHA has been proposed for use in a wide variety of contexts, including single-use consumer goods, packaging, agriculture, and waste management. Notably, the most successful applications of PHA are in the medical field, such as sutures, implants, surgical meshes, scaffolds, and time-release medication delivery devices. In particular, surgical meshes and sutures have been marketed, manufactured, and used in recent years (Lu et al., 2016). PHAs can be applied in various contexts, making them suitable replacement for petroleum-derived plastics, especially in the manufacturing of single-use plastics, which pose a serious threat to the environment.

2.1. PHA biosynthesis pathway

PHA is synthesized intracellularly through a series of microbial enzymatic reactions. Its properties depend on the carbon feedstocks, metabolic pathways, enzyme activities, and substrate specificities of the involved enzymes. PHA biosynthesis can be divided into two phases; which are the catabolism of the carbon source and the anabolic pathway of the PHA polymer. PHA can be produced from diverse carbon sources such as CO₂, sugars, methane (CH₄), methanol, fatty acids, oils, and amino acids, as shown in Fig. 2.

PHA synthesis is closely linked to bacterial central metabolism, including glycolysis, the TCA cycle, β -oxidation, *de novo* fatty acids synthesis, amino acid catabolism, the Calvin cycle, and the serine pathway, which metabolize the supplied carbon source into intermediates. These pathways share many common intermediates with the PHA anabolic pathways, primarily acetyl-coenzyme A (acetyl-CoA) (Tan et al., 2014). PHA synthesis pathways that involve acetyl-CoA as the intermediate include the acetoacetyl-CoA pathway (Pathway I), *de novo* fatty acid synthesis (Pathway II), and/or the TCA cycle.

PHA synthesis can be regarded as a versatile mechanism, as various

types of carbon sources, ranging from simple C1 molecules like CO₂ and CH₄ to higher carbon compounds such as glucose, can be channeled into PHA production mechanisms. Acetyl-CoA is the key intermediate in the synthesis of PHAs, serving as a link between the catabolism of the feedstocks and the anabolic mechanism of PHA. Therefore, any carbon feedstock that can be converted to acetyl-CoA could serve as potential raw materials for the production of intracellular PHA. This includes toxic compounds such as phenol, from which acetyl-CoA is formed as a result of phenol degradation. In addition, the key enzyme for PHA production is the PHA synthase (PhaC), which polymerizes the hydroxyacyl monomers into PHA polymers (Lu et al., 2009). PhaC enzyme can be classified into four classes based on their substrate specificity and preferences in producing scl- or mcl-PHA. Class I, III, and IV PhaC enzymes produce scl-PHA, while Class II PhaC enzymes produce mcl-PHA (Kanavaki et al., 2021). It can be concluded that the production of different classifications of PHA can be influenced by both the carbon source and the types of PhaC enzyme harbored by the microbial cell.

In addition, PHA production from various carbon sources can be improved through genetic engineering strategies. These approaches include overexpressing the PHA synthesis operon or eliminating the ability to degrade accumulated PHA (Drakonaki et al., 2023). For example, Song et al. (2023) reported a 53.8 % increase in PHA yield in *Pseudomonas* sp. 4502 by enhancing the expression of the PhaC2 enzyme through the insertion of a *tac* enhancer. Additionally, accumulated PHA molecules can be depolymerized by PHA depolymerase (PhaZ), which is often co-expressed with PhaC (Zhou et al., 2023). The deletion of PhaZ gene has been shown to significantly enhance mcl-PHA accumulation in *P. putida* KT2440, with an approximately 100 % increase compared to the wild-type strain when lignin was used as the carbon source (Salvachúa et al., 2020).

3. Phenol

Phenol is a prevalent organic compound commonly used as a precursor in various industries. Despite its usefulness in synthesizing other chemicals, it poses a significant threat to living organisms and the environment due to its toxicity. The discharge of industrial effluents containing a high concentration of phenol contamination without proper treatment can lead to serious health complications to living organisms and environmental crisis. Table 2 summarizes the concentration of phenol detected in various industrial wastewater. A phenol concentration exceeding 50 mg/L in industrial effluent can inhibit the rate of biodegradation (Kietkwanboot et al., 2020; Saputera et al.,

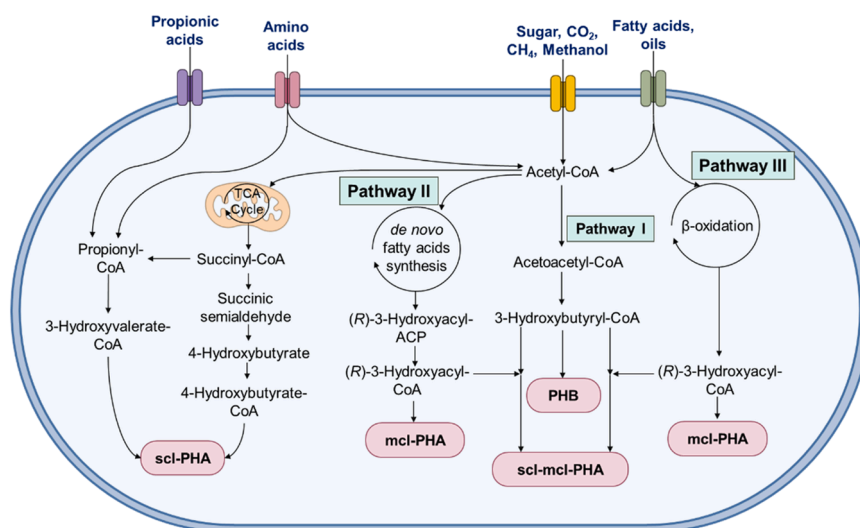


Fig. 2. PHA biosynthesis pathways from various carbon sources. This image was created using Microsoft PowerPoint.

Table 2

The concentration of phenol in industrial effluents.

Industrial effluents	Phenol concentration (mg/L)	Reference(s)
Petrochemical	100 – 1220	(Liu et al., 2016; Mohd, 2020)
Chemical plant	6000	(Jiang et al., 2003)
Paper mill	685	(Sun et al., 2015)
H-coal liquefaction	4900	(Veeresh et al., 2005)
Coke	333 – 1200	(Vázquez et al., 2007)
Phenolic resin	440	(Akyol et al., 2020)
Paint	1.1	(Naguib and Badawy, 2020)
Fiberglass manufacturing	40 – 2564	(Naguib and Badawy, 2020)
Biomass-based gasification	772 – 4630	(Saputera et al., 2021)
Palm oil mill	5800	(Iskandar et al., 2018)
Rubber	3 – 10	(Mohd, 2020)
Pharmaceutical	0.51 – 295.79	(Mareai et al., 2020)
Wood preserving	50–953	(Mohd, 2020)

2021). In addition, phenol is soluble in water and organic solvents such as alcohol and petroleum glycerol. Its water solubility makes degrading phenol to reach safety levels of 0.1–1 mg/L challenging (Hussain et al., 2015), rendering it a recalcitrant compound that can persist longer in the environment. Therefore, it is of utmost importance to remove toxic contaminants before effluent discharge.

Phenol is a common water pollutant in the environment and usually persists in aquatic environments at high concentrations (Duan et al., 2018). Phenol and its derivatives can alter the taste and odor of water (Sachan et al., 2019), prompting international regulatory authorities to impose strict discharge limit for phenols in water. For example, the US EPA has set a limit of phenol concentration below 0.001 mg/L in surface water (Villegas et al., 2016). Other than that, phenol can be present in non-aquatic environments. Compared to aquatic environments, the movement of phenol is restricted in terrestrial environments, making its removal more challenging (Liu et al., 2020).

In humans, the primary route of phenol exposure is through inhalation (Saputera et al., 2021) and respiration (Othmani et al., 2022). The Health Protection Agency (HPA) has reported that 60–88 % of phenol exposure occurs through inhalation, followed by exposure via oral ingestion and skin contact (Saputera et al., 2021). Phenol exposure has been associated with skin burns, tissue damage, liver damage, blurred eyesight, and diarrhea (Liu et al., 2020). Exposure to high concentrations of phenol can lead to necrosis and systemic poisoning by causing protein coagulation and cell inactivation (Zhang et al., 2021). Acute toxicity of phenol can result in unconsciousness, tremors, muscle weakness, and respiratory problems, while chronic exposure can lead to anorexia, weight loss, diarrhea, vertigo, salivation, and dark urine (Villegas et al., 2016). In addition, phenol can cause protein destruction (Weber et al., 2020), leading to corrosion of the skin, eyes, and mucous membranes. Phenol in human blood at a concentration of 1500 mg/L can result in mortality (Sachan et al., 2019; Saputera et al., 2021).

Phenol contamination in the environment has also been reported to impact animals, causing symptoms such as abnormal body temperature, bradypnea, dyspnea, tremors, seizures, lethargy, and coma. Inhalation of phenol can lead to nose and eye irritation, loss of coordination, and muscle spasms in animals. Mortality in animals has been observed within 5–10 min of exposure to a high oral dose (Sachan et al., 2019). A study has reported that the freshwater fish *Cirrhinus mrigala* and the marine opossum shrimp *Archaeomysis kokuboi* are the most sensitive species to phenol in their respective habitats (Duan et al., 2018).

3.1. Mechanisms of microbial phenol degradation

The high stability of phenol is contributed by the presence of a benzene ring in its structure, making it persistent and resistant to natural

degradation. However, certain microbes can tolerate and metabolize phenol as their carbon and energy source (Al-Khalid and El-Naas, 2012). Phenol can be biologically degraded via two processes, which are aerobic and anaerobic processes. Aerobic phenol degradation is more efficient and preferable compared to anaerobic degradation. The rate of phenol degradation in the presence of oxygen is 1.5 times higher than in its absence (Huang et al., 2022). This is attributed to the rapid growth of aerobic bacteria and the efficiency of the aerobic process in converting phenol into inorganic compounds such as CO₂ and water (Al-Khalid and El-Naas, 2012).

The mechanisms of phenol degradation in both oxic and anoxic environments have been extensively studied previously. However, the majority of research has focused only on the conversion of phenol through either *ortho*- or *meta*- cleavage pathways (Bai et al., 2021; Banerjee and Ghoshal, 2010; Bera et al., 2017; Emelyanova and Solyanikova, 2020; Suhaila et al., 2019), which are the most common pathways for the aerobic bacterial degradation of phenol. It is important to establish comprehensive and complete phenol degradation pathways, including all intermediate metabolites, to determine which intermediates and at what point during the degradation process phenol will be transformed into PHA. The following sections describe comprehensive aerobic and anaerobic microbial degradation mechanisms of phenol, adapted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2023; Kanehisa and Goto, 2000) and the MetaCyc Metabolic Pathway Database (Caspi et al., 2018).

3.1.1. Aerobic degradation of phenol

Aerobic phenol degradation is initiated by the phenol hydroxylase enzyme. This enzyme utilizes molecular oxygen to incorporate one hydroxyl (-OH) group at the *ortho*-position of the phenol ring, forming catechol. Then, catechol can be degraded via two main routes: the catechol *ortho*-cleavage (β -ketoadipate) pathway and the catechol *meta*-cleavage pathway. In the catechol *ortho*-cleavage pathway, catechol is cleaved by the enzyme catechol 1,2-dioxygenase between the two hydroxyl groups (intradiol fission), forming *cis,cis*-muconate. On the other hand, the catechol *meta*-cleavage pathway involves the action of the catechol 2,3-dioxygenase enzyme, which cleaves the catechol ring adjacent to the two -OH groups (extradiol fission), forming 2-hydroxymuconate semialdehyde (Schie and Young, 2000). Alternatively, catechol can be carboxylated to protocatechuate by the enzyme protocatechuate decarboxylase (Gu et al., 2018). Protocatechuate is then further metabolized to either β -carboxymuconate by protocatechuate 3,4-dioxygenase or to 4-carboxy-2-hydroxymuconate semialdehyde by protocatechuate 4,5-dioxygenase enzymes. Ultimately, acetyl-CoA and pyruvate are produced, entering the TCA cycle for the generation of energy and CO₂. The aerobic phenol degradation enzymes and pathways are illustrated in Fig. 3 and summarized in Table 3.

Microorganisms have a versatile metabolic capacity, allowing them to degrade phenol through various degradation pathways as illustrated in Fig. 3. Phenol degradation can occur via both the catechol-*ortho* and catechol-*meta* cleavage pathways. However, degradation through the catechol *ortho*-cleavage pathway is more common (Mahiuddin et al., 2012). *Arthrobacter* sp. has been reported to likely degrade phenol via the catechol *ortho*-cleavage pathway. This is supported by the detection of the muconolactone isomerase enzyme (Li et al., 2016) responsible for converting (+)-muconolactone, a product of the catechol 1,2-dioxygenase and muconate cycloisomerase enzymes, into 3-oxoadipate-enol-lactone. In addition, *Kocuria* sp. strain TIBETAN4 (Wu et al., 2018), *Serratia plymuthica* strain GC (Pradhan and Ingle, 2007), and *Acinetobacter tandoii* (Van Dexter and Boopathy, 2019) have been reported to degrade phenol via the catechol *ortho*-cleavage pathway.

Nevertheless, the catechol *meta*-cleavage pathway has been reported to exhibit higher phenol degradation efficiency (Chen et al., 2003). *Arhodomonas* sp. strain Seminole can degrade phenol through the catechol *meta*-cleavage pathway, catalyzed by catechol 2,3-dioxygenase (Dalvi et al., 2012). Additionally, *Pseudomonas fluorescens* PU1

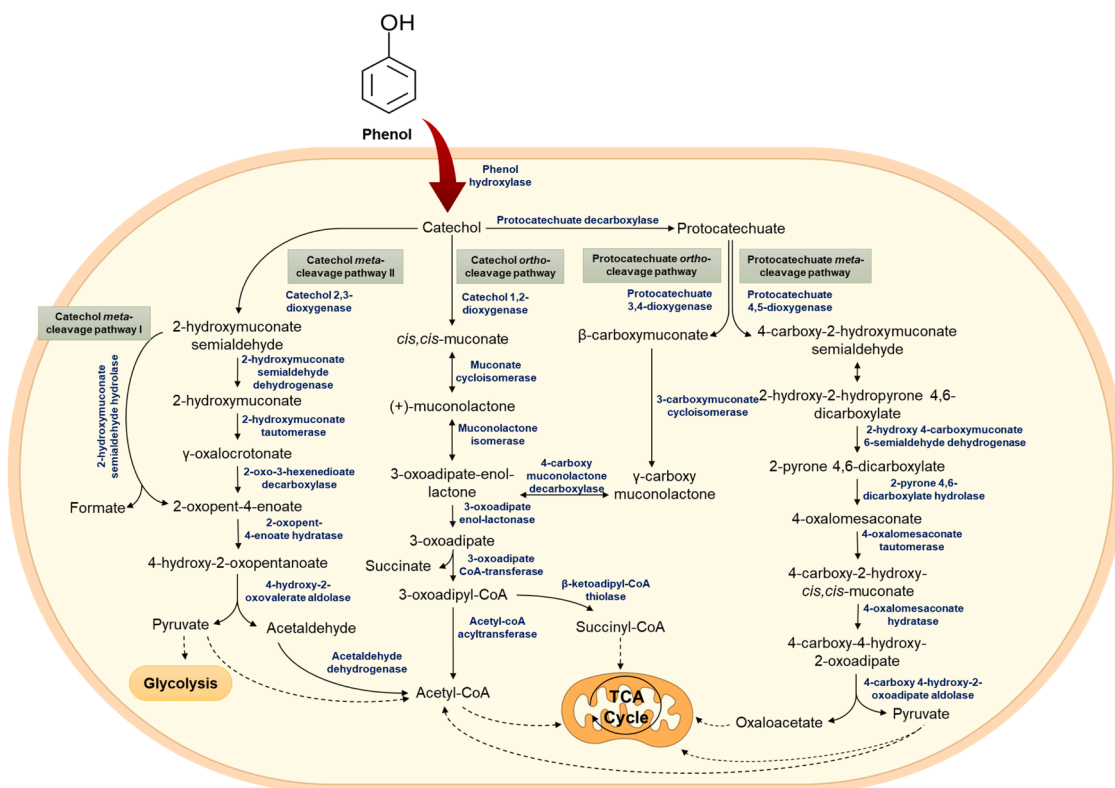


Fig. 3. The mechanisms of aerobic microbial phenol degradation. The information used in designing this figure was retrieved from the MetaCyc Metabolic Pathway Database (Caspi et al., 2018) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2023; Kanehisa and Goto, 2000). This image was created using Microsoft PowerPoint.

metabolizes phenol via the catechol-*meta* cleavage pathway, as it exhibits high catechol 2,3-dioxygenase specific activity when grown in a high concentration of phenol (1000 mg/L) (Mahiuddin et al., 2012). Other bacteria that catabolize phenol via the catechol *meta*-cleavage pathway include *Acinetobacter* sp. strain AQ5NOL 1 (Ahmad et al., 2017), *Pseudomonas* sp. SA01 (Shourian et al., 2009) and *Pseudomonas* sp. strain pHDV1 (Kanaevski et al., 2021).

The β -ketoadipate pathway comprises both the catechol *ortho*- and protocatechuate *ortho*- cleavage pathways, which include catechol and protocatechuate as intermediates. These intermediates are further degraded by catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase, respectively. The catechol *ortho*- and protocatechuate *ortho*-cleavage pathways converge, producing 3-oxoadipate-enol-lactone and 4-carboxymuconolactone decarboxylase, respectively. *Acinetobacter* sp. DW-1 (Gu et al., 2021) and *Rhodococcus* sp. CS-1 (Gu et al., 2018) can metabolize phenol via this reaction mechanism. To date, limited information is available on the degradation of phenol via the protocatechuate 4,5-dioxygenase (protocatechuate *meta*-cleavage pathway) in bacteria. However, substantial protocatechuate 4,5-dioxygenase enzyme activity was detected in *Thalassiosira* sp. HP9101, a marine diatom, when exposed to phenol (Lovell et al., 2002). Therefore, it cannot be ruled out that the protocatechuate *meta*-cleavage pathway also could degrade phenol. Thus, the protocatechuate *meta*-cleavage pathway is included as one of the phenol degradation mechanisms.

3.1.2. Anaerobic degradation of phenol

Phenol degradation in the absence of oxygen can be achieved via the formation of 4-hydroxybenzoate or n-caproate. Anaerobic degradation of phenol via 4-hydroxybenzoate proceeds via two mechanisms; (i) the phosphorylation of phenol to phenylphosphate by phenylphosphate synthase, followed by the formation of 4-hydroxybenzoate catalyzed by phenylphosphate carboxylase, and (ii) the direct conversion of phenol to

4-hydroxybenzoate via 4-hydroxybenzoate decarboxylase. Then, 4-hydroxybenzoate-CoA ligase catalyzes the formation of 4-hydroxybenzoyl-CoA from 4-hydroxybenzoate, and 4-hydroxybenzoyl-CoA reductase catalyzes the formation of benzoyl-CoA. Eventually, the two pathways will form the central intermediate, benzoyl-CoA, which enters the anaerobic benzoyl-CoA degradation pathway.

Benzoyl-CoA reductase initiates the anaerobic benzoyl-CoA degradation pathway by catalyzing the reductive dearomatization of benzoyl-CoA to form cyclohexa-1,5-diene-1-carbonyl-CoA (Kuntze et al., 2008). Cyclohexa-1,5-diene-1-carbonyl-CoA is cleaved via two routes; the first route involves the hydration of cyclohexa-1,5-diene-1-carbonyl-CoA to form 6-hydroxycyclohex-1-ene-1-carbonyl-CoA, followed by the formation of 6-oxocyclohex-1-ene-1-carbonyl-CoA through a reduction reaction. Further catabolism of this metabolite is achieved by a hydrolase enzyme that opens its ring structure, forming 3-hydroxypimeloyl-CoA. In the second route, cyclohexa-1,5-diene-1-carbonyl-CoA is converted to cyclohex-1-ene-1-carbonyl-CoA, followed by a reduction reaction that produces 2-oxocyclohexane-1-carbonyl-CoA. Then, a hydrolysis reaction forms pimeloyl-CoA, which is further reduced and hydrated to form 3-hydroxypimeloyl-CoA. The two pathways then converge and proceed with the formation of acetyl-CoA (Tomei et al., 2021).

Anaerobic phenol degradation via 4-hydroxybenzoate in the first route, which involves the formation of 6-hydroxycyclohex-1-ene-1-carbonyl-CoA, has been well-studied in *Thauera aromatica*, a denitrifying bacterium. In addition, *Geobacter metallireducens* GS-15, an iron-reducing bacterium, and *Desulfatiglans anilini*, a sulfate-reducing bacterium, also share the same mechanism of anaerobic phenol degradation as *T. aromatica* (Xie and Müller, 2018). Furthermore, *Sedimentibacter hydroxybenzoicus* has been reported to degrade phenol via the formation of 4-hydroxybenzoate by the 4-hydroxybenzoate decarboxylase enzyme (Zhang and Wiegel, 1994).

The second pathway of anaerobic phenol degradation is via n-caproate and usually occurs in a thermophilic environment. In this pathway,

Table 3

Enzymes involved in the aerobic mechanism of phenol degradation. The information was compiled from the MetaCyc Metabolic Pathway Database (Caspi et al., 2018) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2023; Kanehisa and Goto, 2000).

Name(s)	Symbol	Enzyme Commission (EC) number	BioCyc Pathway ID	KEGG Orthology (KO) ID
Phenol hydroxylase;	dmpLMNOPK	1.14.13.244	PWY-5418	K16242, K16243, K16244,
Phenol 2-monooxygenase				K16245, K16246, K16249
Catechol 2,3-dioxygenase	dmpB, xylE, catE	1.13.11.2	PWY-5415, PWY-5420, P183-PWY, PWY-5419	K00446, K07104
2-hydroxymuconate semialdehyde hydrolase	dmpD, xylF	3.7.1.9	PWY-5415, P183-PWY	K0216
2-oxopent-4-enoate hydratase;	mhpD,	4.2.1.80	PWY-5415, PWY-5420,	K02554, K18364
2-keto-4-pentenoate hydratase	bphH, xylJ, tesE		PWY-5162	
4-hydroxy-2-oxovalerate aldolase	mhpE, bphI, xylK, nahM, tesG	4.1.3.39	PWY-5415, PWY-5420, PWY-5162	K01666, K18365
Acetaldehyde dehydrogenase	mhpF, bphJ, xylQ, nahO, tesF	1.2.1.10	PWY-5415, PWY-5420, PWY-5162	K04073, K18366
2-hydroxymuconate semialdehyde dehydrogenase	dmpC, xylG, praB,	1.2.1.85	PWY-5420, PWY-6336, PWY-5419	K10217
2-hydroxymuconate tautomerase;	praC, xylH	5.3.2.6	PWY-5420, PWY-6336, PWY-5419	K01821
4-oxalocrotonate tautomerase				
2-oxo-3-hexenedioate decarboxylase	dmpH, xylI, nahK	4.1.1.77	PWY-5420, PWY-6336, PWY-5419	K01617
Catechol 1,2-dioxygenase	catA	1.13.11.1	PWY-5417	K03381
Muconate cycloisomerase	catB	5.5.1.1	PWY-5417	K01856
Muconolactone isomerase	catC	5.3.3.4	PWY-5417	K03464
β -ketoadipate enol-lactone hydrolase;	pcaD, pcaL	3.1.1.24	PWY-5417, PROTOCATECHUATE-ORTHO—CLEAVAGE-PWY	K01055, K14727
3-oxoadipate enol-lactonase				
3-oxoadipate CoA-transferase	pcaI, pcaJ	2.8.3.6	PWY-5417, PWY-2361	K01031, K01032
β -ketoadipyl CoA thiolase;	pcaF	2.3.1.174	PWY-5417, PWY-2361	K07823
3-oxoadipyl-CoA thiolase				
Protocatechuate decarboxylase;	NA	4.1.1.63	NA	NA
3,4-dihydrobenzoate decarboxylase				
Protocatechuate 4,5-dioxygenase	ligA, ligB	1.13.11.8	P184-PWY	K04100, K04101
2-hydroxy 4-carboxymuconate 6-semialdehyde dehydrogenase;	ligC	1.1.1.312	P184-PWY	K10219
2-hydroxy-4-carboxymuconate semialdehyde hemiacetal dehydrogenase				
2-pyrone 4,6-dicarboxylate hydrolase;	ligI	3.1.1.57	P184-PWY	K10221
2-pyrone-4,6-dicarboxylate lactonase				
4-oxalomesaconate tautomerase	galD	5.3.2.8	P184-PWY	K16514
4-oxalomesaconate hydratase	ligJ, galB	4.2.1.83	P184-PWY	K10220, K16515
4-carboxy 4-hydroxy-2-oxoadipate aldolase;	ligK, galC	4.1.3.17	P184-PWY	K10218
4-hydroxy-4-methyl-2-oxoglutarate aldolase				
Protocatechuate 3,4-dioxygenase	pcaG, pcaH	1.13.11.3	PROTOCATECHUATE-ORTHO—CLEAVAGE-PWY	K00448, K00449
3-carboxymuconate cycloisomerase;	pcaB	5.5.1.2	PROTOCATECHUATE-ORTHO—CLEAVAGE-PWY	K01857
3-carboxy-cis,cis-muconate cycloisomerase				
4-carboxymuconolactone decarboxylase	pcaC, pcaL	4.1.1.44	PROTOCATECHUATE-ORTHO—CLEAVAGE-PWY	K01607, K14727
Acetyl-coA acyltransferase	fadA, fadI	2.3.1.16	NA	K00632

phenol is first reduced to cyclohexanone and n-caproate, which then undergo β -oxidation to form fatty acids (Tomei et al., 2021). However, knowledge about anaerobic phenol degradation via n-caproate is still lacking due to the difficulty in accumulating degradation intermediates. This pathway has a high degradation rate, which impedes further investigation (Hoyos-Hernandez et al., 2014). The pathways and enzymes involved in the anaerobic degradation of phenol via 4-hydroxybenzoate and n-caproate are shown in Fig. 4 and Table 4, respectively.

4. Phenol conversion to PHB

Previously, several studies have demonstrated the potential of phenol as the substrate for PHA accumulation (Table 5). Phenol, as the substrate, can exclusively produce PHB homo-polymer as the product. However, despite variations in microbial culture types, microbial species, the amount of supplied phenol, and fermentation strategies, only around 50 % of PHB could be maximally accumulated in the biomass. This limitation arises from the complex phenol degradation process, which involves only acetyl-CoA in PHB formation. The production of other products, such as oxaloacetate and succinyl-CoA, does not serve as substrates for PHB biosynthesis. While phenol as a substrate achieves approximately 50 % PHB of DCW, its utilization for PHB accumulation is

considered superior compared to other toxic compounds. For example, *Bacillus* sp. CYR1 accumulated 51 % of PHB content when phenol was used as the substrate (Table 5). This strain was also tested with other toxic compounds, such as naphthalene, 4-chlorophenol, and 4-nonylphenol, resulting in PHB contents of 42 %, 32 %, and 29 %, respectively (Reddy et al., 2015a). Similarly, the accumulation of PHB in *Cupriavidus* sp. CY-1 was highest when phenol was used (48 %) (Table 5), followed by naphthalene (42 %), 4-tertiary-butylphenol (23 %), 4-chlorophenol (13 %), and 4-tertiary-octylphenol (11 %) (Reddy et al., 2015b). These findings highlight phenol as a highly desirable carbon source for PHA accumulation compared to other toxic compounds.

To further understand the conversion of phenol into PHB, stoichiometric equations for phenol degradation were constructed individually, and the link between phenol degradation and PHB production was established (Table 6). Each mole of phenol generates one mole of acetyl-CoA. However, PHB formation requires two moles of acetyl-CoA. Therefore, the production of one mole of PHB necessitates the degradation of two moles of phenol. A limited supply of acetyl-CoA can lead to low production of PHB (Parveez et al., 2012). In this case, phenol acts as the limiting factor for PHB production, as only half of the supplied phenol can be converted into PHB, assuming that all acetyl-CoA is

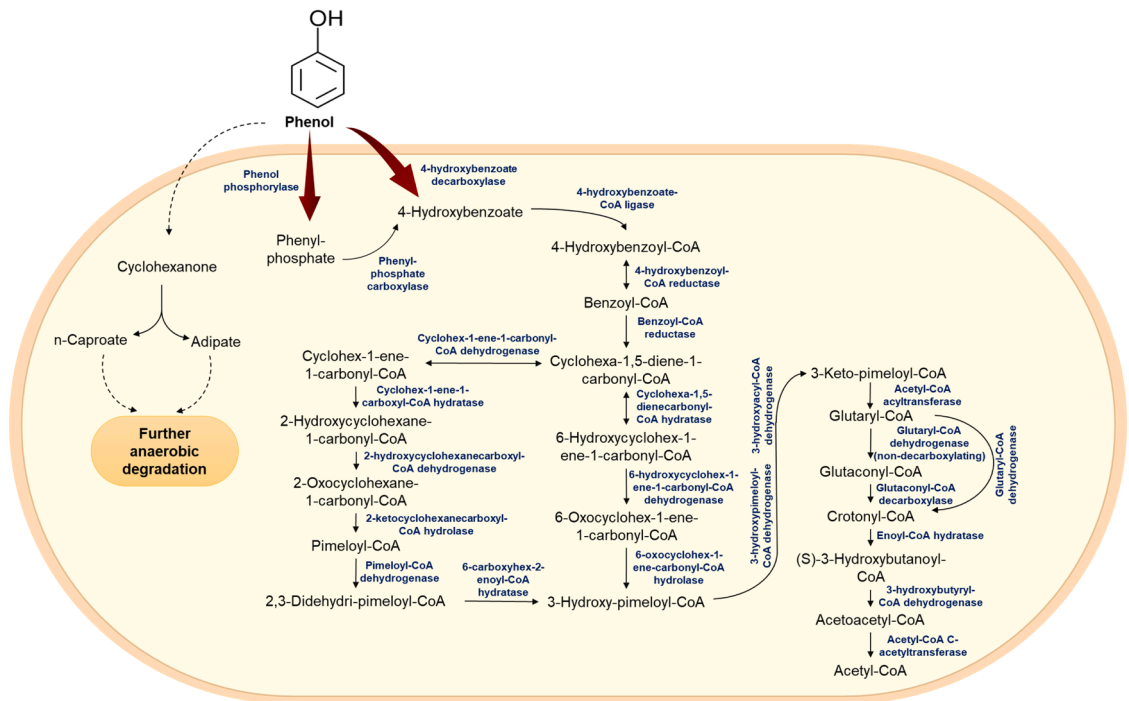


Fig. 4. The mechanism of anaerobic microbial phenol degradation. The information used in designing this figure was retrieved from the MetaCyc Metabolic Pathway Database (Caspi et al., 2018) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2023; Kanehisa and Goto, 2000), as well as from the study by Tomei et al. (2021). This image was created using Microsoft PowerPoint.

Table 4

Enzymes involved in the anaerobic degradation of phenol. The information was compiled from the MetaCyc Metabolic Pathway Database (Caspi et al., 2018) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2023; Kanehisa and Goto, 2000), as well as from the study by Tomei and co-workers (Tomei et al., 2021).

Name(s)	Symbol	Enzyme Commission (EC) number	MetaCyc Pathway ID	KEGG Orthology (KO) ID
Phenol phosphorylase; Phenylphosphate synthase	ppsA, ppsB, ppsC	2.7.1.238	PHENOLDEG-PWY	K25936, K25937, K25938
Phenyl-phosphate carboxylase	ppcA, ppcB, ppcC, ppcD	4.1.1.123	PHENOLDEG-PWY	K25932, K25933, K25934, K25935
4-hydroxybenzoate decarboxylase	bsdC, bsdD	4.1.1.61	N/A	K16239, K01612, K21759
4-hydroxybenzoate-CoA ligase	hbaA, hcl	6.2.1.27	PHENOLDEG-PWY	K04105, K20458
4-hydroxybenzoyl-CoA reductase	hcrC, hbaB, hcrA, hbaC, hcrB, hbaD	1.1.7.1	PHENOLDEG-PWY	K04107, K04108, K04109
Benzoyl-CoA reductase	bcrC, badD, bcrB, bade, bcrA, badF, bcrD, badG	1.3.7.8	CENTBENZCOA-PWY, P321-PWY	K04112, K04113, K04114, K04115
Cyclohexa-1,5-dienecarboxyl-CoA hydratase	dch	4.2.1.100	CENTBENZCOA-PWY	K07537
6-hydroxycyclohex-1-ene-1-carboxyl-CoA dehydrogenase	had	1.1.1.368	CENTBENZCOA-PWY	K07538
6-oxocyclohex-1-ene-carboxyl-CoA hydrolase	oah	3.7.1.21	CENTBENZCOA-PWY	K07539
3-hydroxypimeloyl-CoA dehydrogenase	N/A	1.1.1.259	CENTBENZCOA-PWY	N/A
3-hydroxyacetyl-CoA dehydrogenase	fadJ, fadB, fadN	1.1.1.35	PWY-5177	K01782, K01825, K07516
Acetyl-CoA acyltransferase	fadA, fadI	2.3.1.16	CENTBENZCOA-PWY, P321-PWY	K00632
Glutaryl-CoA dehydrogenase	GCDH, gcdH	1.3.8.6	N/A	K00252
Glutaryl-CoA dehydrogenase (non-decarboxylating)	acd	1.3.99.32	PWY-5177	K16173
Glutaconyl-CoA decarboxylase	gcdA	7.2.4.5	PWY-5177	K01615
Enoyl-CoA hydratase	paaF, echA, fadJ, fadB	4.2.1.17	PWY-5177	K01692, K01782, K01825, K13767
3-hydroxybutyryl-CoA dehydrogenase	paaH, hbd, fadB, mmgB	1.1.1.157	PWY-5177	K00074
Acetyl-CoA C-acetyltransferase	ACAT, atoB	2.3.1.9	PWY-5177	K00626
Cyclohex-1-ene-1-carboxyl-CoA dehydrogenase	N/A	1.3.8.10	P321-PWY	K19066
cyclohex-1-ene-1-carboxyl-CoA hydratase	badK	4.2.1.-	P321-PWY	K07534
2-hydroxycyclohexanecarboxyl-CoA dehydrogenase	badH	1.1.1.-	P321-PWY	K07535
2-ketocyclohexanecarboxyl-CoA hydrolase	badI	3.1.2.-	P321-PWY	K07536
Pimeloyl-CoA dehydrogenase	N/A	1.3.1.62	P321-PWY	K04118
6-carboxyhex-2-enoyl-CoA hydratase	N/A	4.2.1.-	P321-PWY	N/A

Table 5

The PHB production synthesized using phenol as the carbon feedstock by various types of microbes and different cultivation modes.

Types of culture	Total concentration of the supplied phenol (mg/L)	Microorganisms	Phenol degradation (%)	PHB production (mg/g of biomass)	Dy cell weight (DCW) (g/L)	PHB content (%)	Incubation time (h)	Cultivation mode	Reference
Single culture	100	<i>Bacillus</i> sp. CYR1	91 ± 5	510	1.01	51	72	Shake flask	(Reddy et al., 2015a)
	1200	<i>Pseudomonas</i> sp. phDV1	N/A	4.64 ± 0.06*	N/A	N/A	72	Shake flask	
	2000	<i>Pseudomonas</i> sp. phDV1	N/A	4.42 ± 0.91*	N/A	N/A	72	Shake flask	(Kanavaki et al., 2021)
	2400	<i>Pseudomonas</i> sp. phDV1	N/A	6.52 ± 0.21*	N/A	N/A	72	Shake flask	(Nair et al., 2009)
	150	<i>Alcaligenes</i> sp. d2	N/A	25	N/A	N/A	24	Shake flask	
	100	<i>Cupriavidus</i> sp. CY-1	N/A	196.8	0.41	48 ± 6	72	Shake flask	(Reddy et al., 2015b)
	1000	<i>Ralstonia eutropha</i> DMSZ 4058	N/A	N/A	N/A	12	N/A	Isothermal heat flux calorimeter	(Maskow and Babel, 2000)
	500	<i>Cupriavidus taiwanesis</i> 187	100	0.06	0.27	23.25	≈35	5L-fermenter	(Chen et al., 2018)
Mixed microbial culture (MMC)	423.50	<i>Pseudomonas</i> sp. phDV1 (phaZ knockout mutant)	N/A	0.31	N/A	N/A	72	Shake flask	(Drakonaki et al., 2023)
	580	Microbial mixed culture from activated sludge of domestic wastewater treatment plant	100	N/A	N/A	>50	864	Sequencing batch reactor (SBR)	(Wosman et al., 2016)
	2000	Microbial mixed culture from activated sludge of municipal wastewater treatment plant	N/A	1277	N/A	N/A	114	Sequencing batch reactor (SBR)	(Zhang et al., 2018)

N/A: The data are not available;

* The data was expressed in terms of wet cells.

Table 6

Stoichiometric equations for the generation of acetyl-CoA from phenol via different degradation mechanisms and its conversion into PHB.

Stoichiometric equations for phenol-to-PHB conversion	
Degradation of phenol to acetyl-CoA	Catechol meta-cleavage pathway I: $C_6H_6O + 2O_2 + H_2O + SCoA \rightarrow C_2H_3O-SCoA + C_3H_4O_3 + CHO_2$ Catechol meta-cleavage pathway II: $C_6H_6O + 2O_2 + H_2O + SCoA \rightarrow C_2H_3O-SCoA + C_3H_4O_3 + H^+ + CO_2$ Catechol ortho-cleavage pathway: $C_6H_6O + O_2 + 2SCoA + H_2O \rightarrow C_2H_3O-SCoA + C_4H_5O_3-SCoA$ Protocatechuate ortho-cleavage pathway: $C_6H_6O + O_2 + 2SCoA + H_2O \rightarrow C_2H_3O-SCoA + C_4H_5O_3-SCoA$
Conversion of acetyl-CoA to PHB	PHB biosynthesis (Pathway I): $2C_2H_3O-SCoA \rightarrow C_4H_6O_2 + 2SCoA$
Overall theoretical stoichiometry equation	$2 \text{ Phenol} \rightarrow \text{PHB}$

directed toward PHB formation. Nonetheless, PHB synthesis is a complex process, and the carbon resulting from phenol degradation could also be utilized for other cellular requirements, such as the production of cellular components and the generation of energy (Zhang et al., 2018), resulting in much lower intracellular accumulation of PHB.

The mechanism of phenol conversion into PHB, as depicted in Fig. 5, involves the conversion of two molecules of phenol into two molecules of acetyl-CoA. These acetyl-CoA molecules then enter the PHB biosynthesis pathways. Initially, the PhaA enzyme catalyzes the condensation of acetyl-CoA molecules to form acetoacetyl-CoA. Subsequently, the PhaB enzyme reduces acetoacetyl-CoA into (R)-3-hydroxybutanoyl-CoA. Finally, the PhaC enzyme catalyzes the esterification of several (R)-3-hydroxybutanoyl-CoA molecules to form the elongated polymer of PHB (Koch and Forchhammer, 2021).

PHB biosynthesis and the TCA cycle share a common precursor, which is acetyl-CoA. Normally, acetyl-CoA favors the TCA cycle over PHB synthesis. However, the presence of phenol can redirect the carbon

flow towards PHB synthesis mechanisms instead of entering the TCA cycle. Phenol has been reported to severely inhibit the activity of citrate synthase, an enzyme responsible for channeling acetyl-CoA into the TCA cycle. This inhibition leads to inefficient energy production and a reduction in the bacteria's growth rate and biomass yield. This demonstrates how phenol affects the carbon flow of the central carbon metabolism. Previous studies have demonstrated that the presence of phenol disrupts the flow of acetyl-CoA into the TCA cycle in *E. coli* (Kitamura et al., 2019).

As previously reported, the synthesis of intracellular PHB can be regulated by acetyl-CoA, CoA, reduced nicotinamide adenine dinucleotide (NADH), and/or reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Leonard and Lindley, 1998). A high level of NADH/NAD+ ratio in a nutrient-deficient environment will hinder the activity of citrate synthase, causing interference with the flux of acetyl-CoA into the TCA cycle. This occurs due to the failure of citrate synthase to convert acetyl-CoA to citrate and CoA. Reduction in the CoA level will activate the PhaA enzyme, as it is negatively regulated by CoA. The inactivation of citrate synthase, coupled with the activation of PhaA, results in a high level of intracellular acetyl-CoA, enabling its entry into the PHB biosynthesis mechanism (Shrivastav et al., 2013). Therefore, phenol, along with the levels of NADH and/or NADPH, can detrimentally affect citrate synthase activity, thus directing acetyl-CoA into the PHB production pathway.

PHA can also be synthesized in the absence of oxygen. Analysis of the anaerobic phenol degradation mechanisms in Fig. 4 reveals that acetyl-CoA is formed as a product of anaerobic phenol degradation. Thus, hypothetically, PHA can be produced from phenol in an oxygen-deficient environment. However, phenol degradation and PHB accumulation were found to be inhibited under low oxygen availability (Zhang et al., 2018). Conversely, rapid conversion of phenol to PHB was observed under high and medium saturation of dissolved oxygen. Although PHA synthesis under anaerobic conditions using other carbon sources has been documented before (Samal et al., 2023; Zhang et al., 2022), utilizing phenol as the substrate to produce PHA is more efficient in the presence of oxygen.

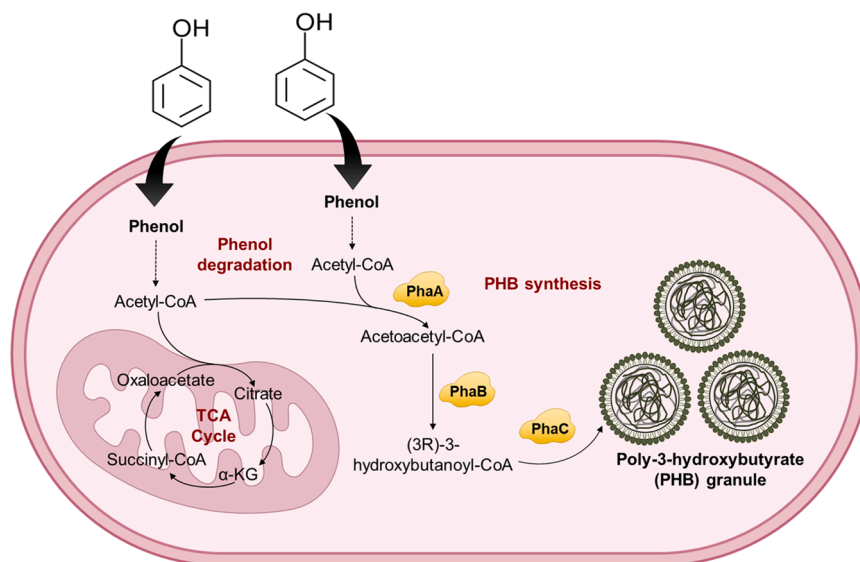


Fig. 5. The aerobic conversion of phenol molecules into polyhydroxybutyrate (PHB). This image was created using Microsoft PowerPoint.

By understanding the underlying mechanisms involved in the conversion of toxic compounds, specifically phenol into PHB, various advanced approaches can be adopted to enhance these processes. Genetic engineering has shown significant improvements in catechol degradation and PHA synthesis in *P. putida* H. This strain employs two distinct pathways for the degradation of catechol derived from benzoate: the *ortho*-cleavage and *meta*-cleavage routes. The *ortho* pathway is encoded by two catechol 1,2-dioxygenase genes, *catA* and *catA2*, located in separate operons, while the *meta*-cleavage pathway is facilitated by a plasmid-based gene *phlH*, encoding catechol 2,3-dioxygenase. Deletion of the *catA2* gene balanced the activation of both pathways, resulting in a 30 % improvement in catechol degradation compared to the wild-type strain. Additionally, the efficient conversion of catechol into acetyl-CoA in the *catA2* knockout mutant led to a twofold increase in PHA production compared to the wild-type strain (Borrero-de Acuña et al., 2021a). Similarly, knocking out the *PhaZ* gene in *Pseudomonas* sp. phDV1 resulted in a higher PHB yield after 72 h of growth on phenol compared to its wild-type strain (Drakonaki et al., 2023).

5. Microbial mixed culture (MMC) for enhanced bioconversion of phenol into PHA

Phenol toxicity poses a significant challenge, limiting its application in PHA production through bacterial fermentation. The efficiency of PHA synthesis using phenol is largely influenced by the bacterial cell population and the availability of carbon feedstock. Elevated phenol concentrations can cause substrate inhibition (Lob and Tar, 2000), hinder bacterial growth (Pishgar et al., 2012), and consequently reduce phenol degradation efficiency. Nevertheless, an adequate supply of carbon is crucial to optimize PHA productivity. Enhancing bacterial tolerance and phenol degradation capabilities is pivotal for effective PHA production, which can be achieved through MMC that offers a broader metabolic spectrum (Monteiro et al., 2000).

The use of MMC for phenol degradation has been well-documented in previous studies (Bera et al., 2017; Chakraborty et al., 2015; Kılıç and Dönmez, 2013; Sivasubramanian and Namasivayam, 2015; Wosman et al., 2016). Additionally, these studies highlight the superior efficiency of MMC in phenol removal compared to pure bacterial cultures (Senthilvelan et al., 2014; Viggor et al., 2020). The synergistic action of four *Pseudomonas* strains, each possessing different key phenol degradation enzyme(s) (*Pseudomonas* sp. PH11: catechol 1,2-dioxygenase; *Pseudomonas* sp. PH7: catechol 1,2-dioxygenase and catechol 2,3-dioxygenase; *Pseudomonas* sp. PH10: catechol 1,2-dioxygenase and

protocatechuate 3,4-dioxygenase; *Pseudomonas* sp. PH8: protocatechuate 3,4-dioxygenase), resulted in a significantly high removal rate, achieving complete degradation of 500 mg/L of phenol within 42 h compared to the performance of individual strains – *Pseudomonas* sp. PH7 (99.7 %), *Pseudomonas* sp. PH11 (93.4 %), *Pseudomonas* sp. PH10 (92.1 %), and *Pseudomonas* sp. PH8 (86.3 %) within 48 h (Tian et al., 2017).

The variety of metabolic enzymes in MMC results in a range of response mechanisms to toxic compounds. This diversity helps to reduce the inhibitory effect of intermediate metabolites on enzyme activity, thereby minimizing feedback repression (Tian et al., 2017). In addition, the buildup of degradation intermediates may stimulate the growth of other species capable of using these metabolites as substrates. The symbiotic relationships between different microbial species can also enhance pollutant degradation (Viggor et al., 2020). The presence of different species in MMC could improve phenol degradation by synergistically metabolizing phenol and accumulated intermediates, alleviating inhibitory effects, and ultimately enhancing PHA production.

Improvements in PHB production have been observed when using MMC compared to single cultures. For instance, a study reported that *Saccharophagus degradans* 2–40 accumulated 22.7 % PHB content when grown on xylan. Co-culturing this strain with *B. cereus* resulted in a significant increase, with 34.5 % PHB accumulation from the same carbon source (Sawant et al., 2017). Nevertheless, studies directly comparing single cultures and MMC in the dual processes of phenol degradation and PHB synthesis remain limited. The first report documenting the use of MMC for PHA production from phenol was published in 2016. It showed that a phenol-acclimatized MMC, consisting of alpha- and beta- proteobacteria populations, accumulated more than 50 % of the DCW as PHA, as summarized in Table 5 (Wosman et al., 2016). Another study documented the production of 1277 mg PHA from 2000 mg/L of phenol using 2.4 – 2.7 g/L biomass of phenol-utilizing MMC (Zhang et al., 2018), achieving higher PHB production compared to other single strains listed in Table 5. However, the bacterial composition in the phenol-acclimated activated sludge from municipal wastewater treatment plants, which was involved in both processes, was not assessed. Additionally, the performance of single culture and MMC in the conversion of phenol-to-PHB has not been comprehensively evaluated.

Utilizing MMC presents a promising strategy for mitigating phenol toxicity through the interaction of diverse microbial species, thereby enhancing carbon conversion into PHA. To date, the specific roles of various bacterial species in phenol-to-PHB conversion remain unclear,

presenting opportunities for new research avenues, particularly with advancements in multi-omics techniques such as metatranscriptomics and metagenomics.

6. Conclusion and future outlooks

Comprehending the metabolic conversion of phenol to PHA unlocks the potential for developing microbes with tailored functionality and improved efficiency. This has been demonstrated through the utilization of MMC, where interactions between different species enhance phenol degradation and improve PHA accumulation. By employing toxic substances like phenol for PHA formation, two environmental issues – phenol pollution and the high substrate cost for PHA production – can be addressed, promoting a circular economy and enhancing environmental sustainability. While this review primarily focuses on the conversion mechanism of phenol into PHA, phenol, as one of the simplest aromatic compounds, can also serve as a model for degrading other aromatic compounds and converting them into PHA. Given the variety of toxic substances present in industrial wastewater, other contaminants capable of generating acetyl-CoA could also serve as potential carbon sources for PHA formation, paving the way for the bioconversion of numerous aromatic compounds into PHA.

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Ethics statement

None required.

CRedit authorship contribution statement

Izzati Sabri: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Mohd Zulkhairi Mohd Yusoff:** Writing – review & editing, Supervision. **Nor Azlan Nor Muhammad:** Writing – review & editing, Supervision. **Li Sim Ho:** Writing – review & editing, Supervision. **Norhayati Ramli:** Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision.

Declaration of competing interest

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

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Data availability

No data was used for the research described in the article.

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