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Review

Enhancing polyhydroxalkanoate (PHA) production from phenol through fermentation strategies: A review

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Abstract. Microbial conversion offers a promising solution to two environmental challenges, phenol and plastic pollutions, via the transformation of phenol into bioplastics, specifically polyhydroxyalkanoate (PHA). Synthetic plastics are widely used across various sectors; however, their non-biodegradable nature and extensive daily use significantly contribute to environmental deterioration. Similarly, phenol, an important industrial material, is often released into the environment through inadequately treated effluents. Phenol is toxic even at low concentrations and may lead to severe environmental and health problems if not properly managed. Microorganisms not only degrade phenol into non-harmful compounds, facilitating its removal from the environment, but they also accumulate intracellular PHA, providing a biodegradable alternative to synthetic plastics. However, phenol's toxicity at high concentrations can inhibit this process, leading to cell death. This review explores various fermentation strategies aimed at enhancing PHA production while addressing phenol toxicity. These strategies include the use of mixed microbial community (MMC), acclimatization to increasing phenol concentrations, feast-and-famine strategies, co-substrate supplementation, and substrate feeding strategies. An integrated approach would be more effective in overcoming phenol toxicity, leading to complete phenol degradation and improved PHA accumulation. However, these strategies must be tailored to the capabilities of microorganisms in adapting to and utilizing phenol as feedstock. Overall, these fermentation strategies have the potential to improve the management of plastic waste and phenol-contaminated wastewater, contributing to a more sustainable future.

Keywords: bioconversion, bioplastics, fermentation, phenol, polyhydroxyalkanoate

INTRODUCTION

Synthetic plastics are widely used across various industries and in everyday life, leading to an ever-increasing demand for their production (Zheng *et al.*, 2023). Global production of synthetic plastics is projected to reach 540 million metric tonnes by 2040. However, due to their non-biodegradable nature, a significant portion of these plastics accumulates in the environment, taking centuries to decompose, with only 14% effectively recycled each year (Zytner *et al.*, 2023). The incomplete degradation of plastics results in the formation of plastic debris, which negatively impacts aquatic life through entanglement, ingestion, debilitation, and suffocation of marine species (Yi *et al.*, 2020).

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Additionally, microplastics have been detected in seafood, drinking water, human intestines, and even the human placenta (Ragusa *et al.*, 2021). Given the persistent use of synthetic plastics and their substantial environmental impact, there is an urgent need for biodegradable and environmentally friendly alternatives.

On the other hand, phenol is a major environmental pollutant that can adversely affect environmental quality and human health. Its high water solubility (Mahgoub *et al.*, 2023) poses risks to aquatic organisms and can alter the taste and odor of drinking water (Aljbour *et al.*, 2021). Phenol concentrations in drinking water have been reported to range from 0.21 to 1,130 mg/L (Gu *et al.*, 2016). Despite this, phenol usage remains essential, serving as a key material in the production of phenolic resins, pharmaceuticals, textiles, and coal conversion industries (Zhang *et al.*, 2022). Its frequent presence in the environment is often due to the release of improperly treated industrial effluents (Aisami *et al.*, 2021), with concentrations ranging from 1 mg/L to 7,000 mg/L (Mohd, 2020). Recognizing its hazard, phenol has been classified as a priority pollutant by the United States Environmental Protection Agency (EPA), which has set discharge limits for phenolic compounds at 0.01 mg/L for surface water and 0.0001 mg/L for drinking water (Chen and Sun, 2023).

Various treatment approaches have been adopted for phenol removal from wastewater, which can be categorized into physical, chemical, and biological strategies. However, physical and chemical methods often generate secondary pollutants and are costly. In contrast, biological methods utilizing microorganisms capable of achieving complete mineralization of phenol have emerged as an economical and promising approach for phenol removal (Zhang *et al.*, 2022). Additionally, this approach is highly specific and accessible, with no generation of harmful secondary pollutants (Bibi *et al.*, 2023). Notably, microbial phenol degradation has been linked to polyhydroxyalkanoate (PHA) production, as observed in *Pseudomonas* sp. phDV1 (Kanavaki *et al.*, 2021), *Cupriavidus taiwanesis* 187 (Chen *et al.*, 2018), and in an acclimatized microbial community from activated sludge of a municipal wastewater treatment plant (WWTP) in China (Zhang *et al.*, 2018).

PHA is a family of biodegradable polyesters synthesized by microorganisms (Zhou *et al.*, 2023), with diverse compositions and physicochemical properties. They are classified into short-chain length (scl) with three to five carbon atoms, medium-chain length (mcl) comprises of 6 to 14 carbon atoms, and long-chain length (lcl) made up of 17 to 18 carbon atoms types (Zhila *et al.*, 2022). PHA serves as an energy reserve material in microbes under unfavorable growth conditions (Acharjee *et al.*, 2024) and is considered a potential alternative to synthetic plastics (Zhou *et al.*, 2023). It shares similar properties with polypropylene, with the added advantage of being biodegradable through enzymatic reactions by organisms such as bacteria, yeast, and fungi (Sirohi *et al.*, 2020). PHA has applications in various sectors, including packaging, food containers, textiles, animal feeds, medical implants, drug delivery, biofuels, and agriculture (Park *et al.*, 2024). However, the commercialization of bioplastics is currently hindered by the high cost of raw materials, which account for more than half of the total production costs (Sirohi *et al.*, 2020), making it less competitive than synthetic plastics.

Using waste as a carbon feedstock for PHA production is expected to reduce overall PHA production costs. Industrial wastewater, often containing high levels of phenols, serves as an abundant carbon source for PHA biosynthesis. The microbial conversion of phenol into PHA not only facilitates its removal from wastewater, ensuring compliance with the discharge regulations, but also produces bioplastics with the potential to replace synthetic plastics. However, PHA production requires an excess of carbon, posing a significant challenge when using phenol due to its toxicity at high concentrations. Therefore, this mini-review explores various fermentation strategies to enhance PHA production from phenol, with a focus on addressing phenol toxicity toward microbial cells to promote the use of phenol-laden wastewater as a feedstock for PHA production.

Fermentation strategies to enhance phenol conversion into polyhydroxyalkanoate (PHA)

PHA synthesis via phenol occurs in two major steps, initiated with the degradation of phenol into acetyl-CoA, followed by the conversion of acetyl-

CoA into polyhydroxybutyrate (PHB) (Figure 1) (Tao *et al.*, 2024). When phenol is utilized as the sole carbon source, only PHB, a scl-PHA is produced. Kanavaki *et al.* (2021) reported that PHA production in *Pseudomonas* sp. phDV1 was 32.21% higher in the presence of 600 mg/L of phenol compared to 200 mg/L, as the increased carbon availability supports cell growth and PHA synthesis. Tao *et al.* (2024) reported a similar finding, where the phenol-acclimatized consortia of the activated sludge of a WWTP produced 28.57% more PHA when exposed to 1,000 mg/L of phenol compared to 500 mg/L in batch cultivation mode. However, previous studies have reported that high phenol concentrations inversely influence cell growth (Zhang *et al.*, 2022) and the rate of degradation (Barik *et al.*, 2021; Dankaka *et al.*, 2023).

Phenol is well-known for its toxicity and can impose bactericidal effects on microorganisms (Huang *et al.*, 2022). Phenol toxicity is considered as the main challenge that limits its application in PHA production through bacterial fermentation. Certain bacteria are capable of tolerating and utilizing phenol as a carbon source at specific concentrations. Nevertheless, biotoxicity will increase with the increment of phenol levels, resulting in growth inhibition and a reduction in degradation performance (Li *et al.*, 2019; Pishgar *et al.*, 2012). Elevated levels of phenol lead to the disruption of cell membrane integrity, hindering the cell's ability to adapt to phenol stress (Shahryari *et al.*, 2018). Eventually, cellular functions can be suppressed which may lead to cell death (Zhang *et al.*, 2018). In addition, high phenol concentrations can impose a substrate inhibition effect on the microbe. According to Lob & Tar (2000), substrate inhibition hinders bacterial growth at high phenol concentrations, thereby impeding phenol degradation activities.

Nevertheless, a surplus of carbon is required to produce a large amount of PHA. The number of bacterial cells and the amount of the supplied carbon feedstock are limiting factors for PHA production using phenol. More PHA can be produced when there is an abundance of bacterial cells capable of utilizing and converting the supplied feedstock into PHA. Since phenol is bactericidal, a high concentration of phenol can induce an inhibitory effect on bacterial growth. When utilizing phenol as the carbon feedstock, overcoming substrate inhibition is necessary for enhancing PHA production. Hence, effective PHA production can be achieved by enhancing the tolerance and phenol degradation activities of bacteria. This section outlines several strategies to improve phenol tolerance, as efficient phenol degradation is believed to enhance PHA accumulation. A high initial phenol concentration results in a high percentage of PHA in the biomass. Additionally, for the PHA content to reach a high level, phenol must be completely degraded by the microorganisms (Zhang *et al.*, 2018). The advantages, disadvantages, and challenges of various fermentation strategies utilizing phenol as the carbon feedstock for PHA production are summarized in Table 1.

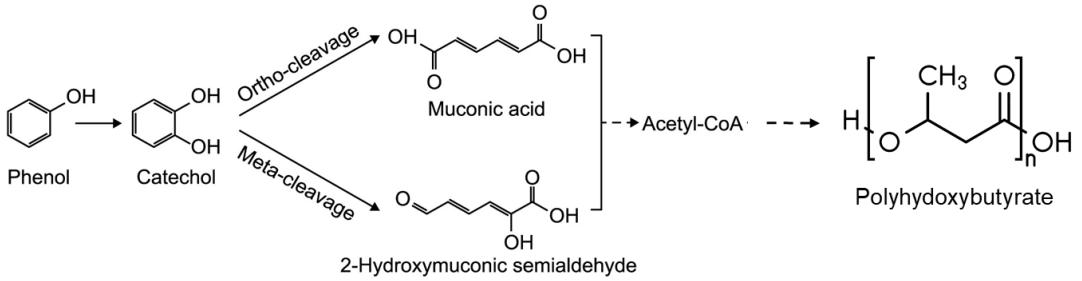

Figure 1. Simplified pathways of phenol conversion into polyhydroxybutyrate (PHB), a type of polyhydroxyalkanoate (PHA).

Table 1. Advantages, disadvantages, and challenges of each fermentation strategy for the conversion of phenol into polyhydroxyalkanoate (PHA).

Fermentation strategies	Advantages	Disadvantages	Challenges	References
Mixed microbial community (MMC)	• The synergistic interaction between different microbial species can enhance phenol conversion into PHA • Presence of a wide metabolic capacity • Low cost due to the use of open MMC under non-sterile conditions	• Potential antagonistic interactions between different species in the community	• Sometimes, MMC accumulate lower PHA content than pure cultures • Varied compositional distribution of the produced PHA	(Ntaikou *et al.*, 2019; Vicente *et al.*, 2023; Zytner *et al.*, 2023)
Acclimatization to phenol	• Improve tolerance to higher phenol concentrations • Enrichment of a phenol-resistant bacterial community	• Time consuming	• An overly dramatic increase in phenol could cause severe inhibitory effects or potentially result in total reactor failure	(Gu *et al.*, 2016; Wosman *et al.*, 2016)
Feast-and-famine regime	• Enrich bacteria with PHA-producing capabilities • Improve PHA productivity	• Time consuming • Requires constant monitoring	• Requires further optimization • Potential formation of by-products	(Anburajan *et al.*, 2019; Huang *et al.*, 2020)
Co-substrate supplementation	• Allows bacteria cells to flourish and adapt to phenol toxicity	• Increases operational cost • The bacteria might metabolize the co-substrate only, which could reduce phenol conversion into PHA • Prolong lag phase	• Generate different types of PHA due to the presence of different substrates	(Mohammad and Bhukya, 2022; Pishgar *et al.*, 2012)
Substrate feeding strategies	• Alleviate substrate toxicity by gradually feeding phenol into the system • Could simulate real-world application	• Increase operational cost	• Risk of contamination • Requires further optimization of the feeding strategy	(Tao *et al.*, 2024)

The use of mixed microbial community

A mixed microbial community (MMC) comprises of either characterized or uncharacterized microbial consortia that can work synergistically or antagonistically with each other. This MMC can include bacterial co-cultures, enrichment cultures, consortia, or environmental samples. The use of MMC is more practical than pure culture as it can reduce the cost and time required for sterilization. MMCs are robust, have a wide metabolic capacity, and can simulate real environmental conditions, making them more suitable for real-world applications. The utilization of MMC for phenol degradation is not new and has been documented before (Bera *et al.*, 2017; Chakraborty *et al.*, 2015; Kılıç and Dönmez, 2013; Sivasubramanian and Namasivayam, 2015; Wosman *et al.*, 2016). MMCs have been found to

have a better effect on phenol removal as compared to the pure cultures (Senthilvelan *et al.*, 2014; Viggor *et al.*, 2020).

Phenol degradation in the MMC system often benefits from the presence of diverse metabolic pathways. For instance, *Acinetobacter venetianus* ICP1 and *Pseudomonas oleovorans* ICTN13, which contain *ortho-* and *meta-* cleavage pathways respectively, accumulated intermediates of catechol degradation that are toxic to cells when grown individually. However, the use of a mixed culture of ICP1 and ICTN13 achieved complete phenol removal while minimizing the accumulation of toxic intermediates (Viggor *et al.*, 2020). Another study reported that the co-culture of *Paenibacillus thiaminolyticus* (DQ435022) and *Bacillus cereus* (DQ435023) degraded 84.72% of 700 mg/L of phenol more efficiently than their single culture system (Chandra *et al.*, 2011). MMC has a wider spectrum of metabolic properties (Monteiro *et al.*, 2000). Hence, it can be postulated that MMC exhibits better tolerance to phenol than a single strain.

PHA production by MMC is considered one of the cost-effective approaches and is expected to improve PHA production from phenol. The undefined MMC from the activated sludge of a WWTP was continuously acclimatized in increasing phenol concentrations (50, 100, 180, 250 mg/L), with more than 99% phenol removal observed during this process. Simultaneously, more than 50% of the dry cell weight (DCW) of PHA was accumulated intracellularly. Zhang *et al.* (2018) optimized operational conditions for PHA production using phenol-utilizing MMC as the microbial source which generated 1,277 mg of PHA from 2,000 mg/L of phenol. The use of MMC offers the advantages of alleviating phenol toxicity and enhancing PHA accumulation.

Acclimatization to higher substrate concentrations

The common method for improving microbial phenol tolerance is through repeated exposure to the same or increasing concentrations of phenol. This process, known as acclimatization or enrichment, is widely used to enhance microbial resistance towards toxic compounds, as depicted in Figure 2. According to Madigou *et al.* (2016) and Mohammad & Bhukya (2022), acclimatization can be defined as a step-wise adaptation process that allows the bacteria to synthesize the required enzymes for metabolic activities, switch to appropriate metabolic pathways, and build up biomass in response to introduced stress. Exposure to phenol will cause an alteration in the fatty acid composition of the cell membrane, thereby improving the cell's resistance to phenol (Kitamura *et al.*, 2019). Esteban *et al.* (2022) observed the formation of biofilm by *Acinetobacter* EMY as an adaptive response to phenol toxicity.

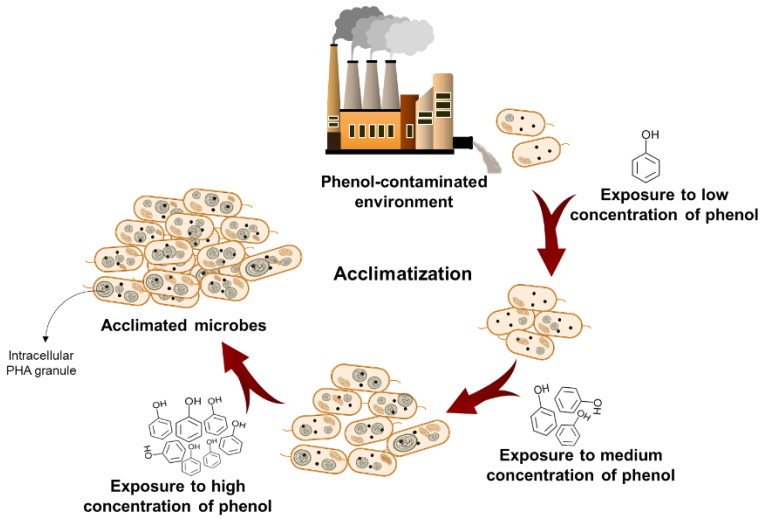

Figure 2. Bacterial acclimatization to elevated phenol concentrations resulting in increased cell biomass, improved tolerance towards high phenol concentrations, and high accumulation of intracellular polyhydroxybutyrate (PHB).

Microorganisms have a versatile metabolic capacity and adaptation mechanism that allow them to survive in the presence of toxic contaminants, such as phenol. Acclimatization causes a shift in the microbial community composition, allowing phenol-degrading bacteria to dominate. As a result, the adapted community exhibits improved phenol utilization, which can be exploited for bioremediation and PHA production applications. Following successful acclimatization, the adapted community can be directly utilized for its intended application. Alternatively, a single strain with high phenol tolerance could be isolated for further in-depth research.

Previous researchers have adopted several acclimatization strategies to improve microbial resistance to phenol. For example, Gu *et al.* (2016) performed continuous acclimatization of microorganisms from drinking water biofilters with increasing phenol concentration from 50 to 300 mg/L over 50 days. The final acclimated community was able to degrade over 80% of 300 mg/L of phenol within 3 days. Kamali *et al.* (2019) observed a significant improvement in phenol degradation by the acclimated sludge, which almost completely degraded 500 mg/L of phenol within 180 minutes. In another study, Jusoh and Razali (2008) demonstrated that, although the acclimated and unacclimated microbial communities showed comparable phenol degradation performance, the acclimated community had a significantly higher affinity for phenol and a better survival rate, making it more robust during prolonged degradation. In addition, acclimatization of *Pseudomonas putida* has been shown to reduce the time required for phenol degradation from approximately 350 hours to 250 hours for a phenol concentration of 1,000 mg/L (González *et al.*, 2001). Hence, acclimatization can help microbial cells adapt to high concentrations of toxic compounds, increase cell numbers, and eventually reduce the time for the degradation of toxic compounds, while improving degradation efficiency.

The utilization of high biomass with improved phenol tolerance can subsequently enhance PHA accumulation. Wosman *et al.* (2016) reported a significant improvement in PHA content following the acclimatization of a mixed culture from the activated sludge of a domestic WWTP. The acclimated mixed culture in their study demonstrated the ability to endure and completely degrade high concentrations of phenol, while sustaining a high PHA level for an extended period of operation. Another study showed that more than 50% of PHA was recovered after 6 weeks of acclimatizing activated sludge with a gradual increase in phenol concentration (Zhang *et al.*, 2018). Acclimatized MMC from activated sludge of the municipal wastewater has better PHA productivity compared to PHA production without acclimatization (Estévez-Alonso *et al.*, 2021). It can be safely said that enhanced PHA production can be achieved through an acclimatization process that allows cells to prepare themselves to adapt to the presence of toxic contaminants and subsequently improve their substrate utilization for PHA production. Therefore, in addition to improving phenol endurance for bioremediation purposes, an acclimatization strategy can be adopted to enhance PHA accumulation using toxic compounds as substrates, particularly phenol.

Feast-and-famine regime

PHA serves as an energy reserve stored by microorganisms as part of their stress regulation mechanism. In the presence of excess nutrients, the carbon source is metabolized for energy and growth. Deprivation of nutrients will shift the conversion of carbon into PHA storage. Therefore, this stress-coping mechanism of microbes can be exploited to enhance PHA production through a feast-and-famine strategy. A feast-and-famine regime is a dynamic feeding pattern consisting of alternating surplus (feast) and limited (famine) carbon sources and/or nutrients. This regime induces a physiological adaptation process in microorganisms, leading to the storage of carbon as intracellular lipid granules (Çığgın *et al.*, 2012).

The feast stage allows the microbes to metabolize the supplied carbon for growth and PHA accumulation, while the famine stage promotes the utilization of the stored biopolymer for cell maintenance and growth (Huang *et al.*, 2018; Wosman *et al.*, 2016). During carbon starvation, cells compete for the substrate uptake, with PHA-producers dominating as they can utilize accumulated PHA to sustain growth (Tyagi *et al.*, 2019), while microbes unable to store PHA

are eliminated (Corsino *et al*., 2022). Feast-and-famine strategy is beneficial in enriching the PHA-producer community by alternating the availability of the carbon source (Huang *et al*., 2017) and is usually applied in the sequencing batch reactor (SBR) to enrich the PHA-producing culture (Zhou *et al*., 2023). When phenol is utilized as the carbon source, exposure to this compound is believed to enhance cell endurance and adaptation to its toxicity. Consequently, the dominant microbial community serves a dual function as both a phenol-degrader and a PHA-producer. After successful enrichment, the population consisting of the phenol-degrading PHA-producing community is used in the subsequent production step, typically with nutrient limitation to maximize PHA accumulation (Korkakaki *et al*., 2017).

The feast-and-famine regime is not only restricted to alternating the availability of the carbon source. Other studies have varied the feast-and-famine strategy to suit the abilities of their microbes, aiming to enhance PHA accumulation. As reported previously, nitrogen limitation is well-known to improve the intracellular PHA content (Ntaikou *et al*., 2019; Ramírez-Morales *et al*., 2021; Zhang *et al*., 2022). Therefore, an alternate supply of nitrogen with surplus carbon can enhance PHA production. Nitrogen feast-and-famine conditions have successfully directed biopolymer accumulation in *P. putida* KT2440 with an excess supply of carbon sources (Mohammad and Bhukya, 2022). However, this is not always the case. Some studies have found that nitrogen limitation does not significantly improve cellular PHA content (Chen *et al*., 2019; Zhang *et al*., 2018). This is because certain microbes can accumulate PHA during active growth and do not require nutrient limitation (Khanna and Srivastava, 2005; Mohapatra *et al*., 2017). Therefore, the choice of the alternating feast-and-famine strategy must be tailored to the specific abilities of the microbes.

Co-substrates supplementation

The addition of co-substrates can improve bacterial tolerance to phenol toxicity. Bajaj *et al.* (2008) mentioned that supplementing co-substrates as extra carbon sources can positively affect the rate of biodegradation. Glucose and acetate are common carbon sources for bacterial growth (Wang *et al*., 2022); therefore, the presence of additional carbon sources is expected to increase cell biomass for phenol degradation. In the presence of glucose as a co-substrate, faster phenol degradation was observed by MMC derived from coke oven effluent. When phenol was used as a single substrate, the degradation for 600, 800, and 1000 mg/L of phenol were 83.7, 34.3, and 4.4%, respectively. However, the addition of glucose as a co-substrate enhanced phenol removal to 100%, 48%, and 8.9%, respectively for the same concentrations (Pishgar *et al*., 2012). This was further supported by Shen *et al.* (2020), where the presence of acetate as a co-substrate improved the degradation of 500 mg/L of phenol to 78.8%, compared to 45.8% when using phenol alone by the undefined MMC from activated sludge of the coking wastewater.

Nonetheless, a high concentration of co-substrate can lead to a complete dependence of the bacteria on the co-substrate as their preferred source for growth, causing them to lose their ability to utilize the intended toxic compounds (Bajaj *et al*., 2008). To avoid carbon catabolite repression and complete dependence of organisms on simple carbon sources, a low concentration of co-substrate should be incorporated, just enough to support the cells during the adaptation phase to toxic compounds. As the incorporation of additional carbon provides support for microbial growth and improves its tolerance towards toxic compounds, phenol can be efficiently degraded by bacteria for PHA production. The addition of a small amount of co-substrate can help bacteria cope with the toxicity of phenol, reduce the lag phase, and contribute to the reduction in degradation time.

Since complete phenol metabolization leads to high PHA accumulation (Zhang *et al*., 2018), the incorporation of co-substrate is expected to improve PHA production at high phenol concentrations. The addition of co-substrate can also promote the development of a robust microbial community capable of withstanding disturbances during operation. Wosman *et al.* (2016) mentioned that a microbial community supplied with phenol and sodium acetate was more stable, robust, and resistant to disturbances compared to the mixed culture exposed to phenol alone, with a similar capacity for PHA accumulation (>50% of DCW).

Research on the supplementation of co-substrates for PHA production using phenol as the carbon feedstock is currently limited. A study reported that adding 0.02% of Tween-80 as a co-substrate to support the growth of *Cupriavidus* sp. CY-1 during phenol degradation yielded 0.41 g/L of dry biomass, with 48 ± 6 % of PHB accumulation per DCW (Reddy *et al*., 2015). This is a complex process, as the co-substrate can also be metabolized and converted into different types of PHA fractions. Mohammad & Bhukya (2022) reported that the addition of 0.5% of glucose as a co-substrate with phenol as the main carbon source produced the scl-co-mcl-co-lcl type of PHA, comprised of poly(3-hydroxyhexanoate-co-3-hydroxydecanoate) (P(3HHxD)), poly(3-hydroxyvalerate) (P(3HV)), poly(3-hydroxyoctanoate-co-3-hydroxydecanoate) (P(3HODE)), poly(3-hydroxyhexanoate-co-3-hydroxydecenoate) (P(3HHxDE)), and poly(3-hydroxydodecanoate) (P(3HDD)) by *P. putida* KT2440.

Substrate feeding strategy

The substrate feeding strategy is a critical aspect of optimizing microbial bioprocesses. While Table 2 provides a comparative overview of various feeding strategies, this section focuses on the underlying principles that guide the choice of feeding methods. Effective strategies ensure a steady supply of substrate to maintain microbial activity without causing inhibitory effects. Understanding the interaction between feeding strategy and microbial metabolism allows for better control over the bioconversion process, leading to improved yields and process efficiency.

Table 2. Repetitive and gradual feeding strategies of phenol as a carbon source for polyhydroxyalkanoate (PHA) production.

Feeding strategy	Microbes	Description	Total phenol supplied (mg/L)	Phenol degradation (%)	Phenol degradation time (h)	PHA concentration (g/L)	PHA concentration (g PHA/g biomass)	References
Repetitive feeding	Phenol-acclimatized consortia from activated sludge of municipal WWTP	4 × 250 mg/L	1,000	N/A	4.75	1.21	0.65*	(Zhang *et al*., 2018)
		2 × 500 mg/L	1,000	N/A	2.53	0.66	0.55*	
	Cupriavidus taiwanesis 187	3 × 500 mg/L	1,500	100	>30	0.213	0.25*	(Chen *et al*., 2018)
	Pseudomonas sp. phDV1	7 × 200 mg/L	1,400	N/A	72	N/A	0.0044**	(Kanavaki *et al*., 2021)
		5 × 400 mg/L	2,000	N/A	72	N/A	0.0046**	
		4 × 600 mg/L	2,400	N/A	72	N/A	0.0065**	
Gradual feeding	Phenol-acclimatized consortia from activated sludge of municipal WWTP	Phenol was pumped at a flow rate of 9 mL/min until total volume in the flask reached 500 mL	1,000	100	2	0.71	0.42*	(Tao *et al*., 2024)
		Phenol was pumped at a flow rate of 18 mL/min until total volume in the flask reached 500 mL	1,000	100	1.75	0.88	0.52*	

Notes: N/A: Information is not available; *Biomass was expressed in terms of dry cell weight; **Biomass was expressed in terms of wet cells

In a fed-batch system, phenol can be supplied as a carbon source through either repetitive or gradual feeding. Repetitive feeding involves the multiple additions of substrate, in this case, phenol, to the culture media once the previously supplied phenol has been depleted. In contrast, gradual feeding refers to supplying phenol at a constant flow rate. Both techniques can alleviate substrate inhibition caused by the toxicity of high phenol concentrations, thereby enhancing and increasing PHA accumulation in bacterial cells.

A previous study reported that the accumulation of PHA by *C. taiwanesis* 187 increased by 66.20% after three rounds of repetitive feeding (Chen *et al.*, 2018). A similar improvement was also observed in other studies utilizing different carbon source as the feedstock. For example, high PHA accumulation has been achieved when acetate was supplied repetitively in three pulses, instead of one pulse in the pulse-wise feeding mode (Serafim *et al.*, 2004). In addition, acclimation steps with a pulse-wise feeding mode of acetate have improved PHA productivity in the microbial community of waste activated sludge in municipal WWTP (Estévez-Alonso *et al.*, 2022). To avoid substrate inhibition caused by high concentrations of substrates such as phenol, it can be supplied in multiple doses that remain below the toxic threshold level, facilitating the accumulation of more intracellular PHA.

A recent study by Tao *et al.* (2024) demonstrated a gradual feeding strategy for PHA production from phenol by the phenol-acclimatized consortia derived from the sludge of a municipal WWTP. This feeding strategy was more efficient in reducing phenol degradation time and increasing both cell biomass and PHA concentration (g/L) compared to batch mode, which required an additional 30-45 minutes for complete degradation.

Integrated approaches and future perspectives

The fermentation step is crucial in determining high PHA recovery. Proper fermentation technology needs to be designed to utilize phenol-contaminated wastewater for PHA production. MMC from phenol-contaminated environments or isolated pure culture can be acclimatized to increasing phenol concentrations to promote biomass growth and enhance their endurance towards high concentrations of phenol toxicity. Phenol can be supplied through a feast-and-famine regime to enhance the dominance of the phenol-degrading PHA-producing microbial community. Once cells with desired capability are obtained, phenol can be supplied repetitively below its respective toxic threshold to provide a surplus carbon source for enhancing PHA accumulation. Additionally, a nitrogen feast-and-famine regime can be introduced to further direct carbon conversion into PHA. A low concentration of co-substrate can be supplemented with phenol to promote biomass generation and enhanced PHA accumulation.

Integrated fermentation strategies for enhancing PHA production using phenol as a carbon feedstock are illustrated in Figure 3. These strategies, which integrate multiple approaches to enhance phenol degradation and PHA accumulation, have been demonstrated in several studies, as summarized in Table 3. However, these strategies must be specifically tailored to the capabilities of the microorganisms and the characteristics of the toxic substrates used as feedstock.

CONCLUSION

Phenol-contaminated effluents serve as a potential source of feedstock for PHA production, as microbes can metabolize phenol while accumulating intracellular PHA. This strategy offers a promising approach to both waste management and sustainable bioplastic production. Although this review primarily focuses on fermentation strategies to enhance the conversion of phenol into PHA, the discussed approaches can also be applied to other pollutants that pose significant toxicity to microbial cells. This strategy is expected to improve waste management while simultaneously producing biodegradable bioplastics, which is essential in advancing effective waste management technologies and promoting a circular green bioeconomy.

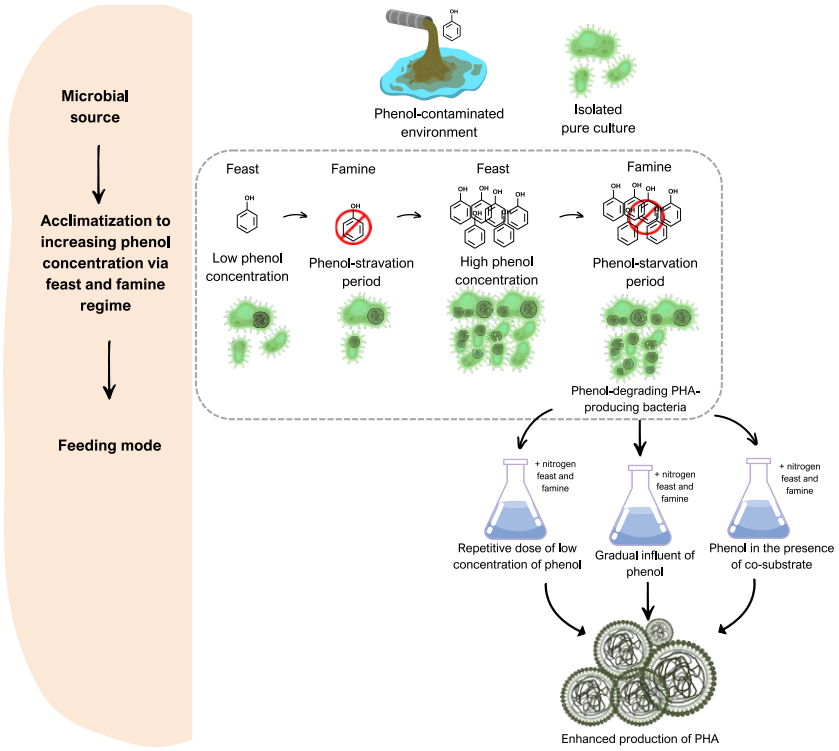

Figure 3. Integrated fermentation strategies for enhancing PHA production using phenol as the carbon feedstock

Table 3. Examples of phenol conversion into polyhydroxyalkanoate (PHA) by various types of microorganisms under integrated fermentation strategies.

Microorganisms	Integrated fermentation strategies	Phenol degradation (%)	PHA content (%)	References
Mixed microbial community of activated sludge from WWTP	Mixed microbial community, acclimatization to phenol, repetitive feeding	>99% of phenol removal during most of the operation time (90 days)	Highest PHA content (70%) on day 26	(Wosman *et al.*, 2016)
Mixed microbial community of activated sludge from WWTP	Mixed microbial community, acclimatization to phenol, co-substrate supplementation, repetitive feeding	>99% of phenol removal during most of the operation time (90 days)	Highest PHA content (60%) on day 31	(Wosman *et al.*, 2016)
Mixed microbial community of activated sludge from municipal WWTP	Mixed microbial community, acclimatization to phenol, repetitive feeding strategy	N/A	65% of PHA content after 4 times addition of 250 mg/L of phenol	(Zhang *et al.*, 2018)
		N/A	55% of PHA content after 2 times addition of 500 mg/L of phenol	
Pseudomonas putida KT2440	Acclimatization to phenol, co-substrate supplementation, nitrogen feast-and-famine regime	N/A	30.33% of PHA content in 96 hours	(Mohammad and Bhukya, 2022)

Note: N/A; Information is not available.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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