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Microbial biodegradation of nitrophenols and their derivatives: A Review

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KEYWORDS

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Bioremediation

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Recalcitrant

Xenobiotic

ABSTRACT

Today, nitrophenols (NPs) represent chemicals highly in demand not only due to their function in synthetic chemistry but also due to their huge applications in several industries. Such diverse requirements and applications has resulted in a widespread abundance of these chemicals. Improper application and waste disposal practice results in the continuous discharge of these compounds into the environment and causes pollution threat to soil, groundwater, river water, etc. These xenobiotic chemicals are hazardous, toxic, carcinogenic, and mutagenic which results in serious health problems. The Nitro group present in the phenol makes them recalcitrant which causes the persistence of these chemicals in the environment. Although several chemicals, electrochemical, physical, and physicochemical methods have been proposed, bioremediation approaches mainly involving bacteria are considered best. To date, very few successful attempts (related to microbe-assisted bioremediation) have been carried out with environmental habitats for the removal of NPs (both *in-situ* and *ex-situ* attempts). So, as far as the effectiveness of the bioremediation process for NP decontamination is concerned, we are far away. More explorative studies using efficient aerobic-anaerobic NP degrading bacterial consortium (or combination of microbes- plant systems) and advanced techniques including omics approaches and nanotechnologies may help towards developing better practicable bioremediation approaches, in the future. This review article focuses on the list of nitrophenol degrading microorganisms, biodegradation pathways of NPs, bioremediation by immobilized cell technique, and the advantages and disadvantages of bioremediation. This article will increase our knowledge of the biodegradation of NPs.

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1 Introduction

NPs are aromatic compounds that contain the nitro group attached to phenol rings. These are used as raw materials in industries for the manufacturing of pesticides, fungicides, paints, explosives, drugs, dyes, and cosmetics (Ju and Parales 2010; Xu et al. 2021). Many of these are considered pollutants of significant importance due to their toxicity to the living world, high mobility, and long persistence in most natural ecosystems (Kulkarni and Chaudhari 2007). The release of these organic compounds from industries, agriculture uses, domestic activities, and medical applications not only pose threat to living systems and the environment (Spain 1995) but are also responsible for contamination of almost all ecosystems on earth (representing soil, air, and water) (Kulkarni and Chaudhari 2007).

Degradation of toxic compounds by microbes has been established as one of the best possible approaches toward decontamination of xenobiotic, hazardous, toxic compounds (Chen et al. 2012). A survey of the literature revealed that reports from the bacterial domain outnumber other representative groups. The reason might be due to their higher abundance, better adaptability due to their long evolutionary ancestry, rapid growth rate, small generation time, ability to utilize diverse compounds (as electron donors and acceptors), and availability of more versatile metabolic resources to utilize newly added xenobiotic compounds (Schleifer 2004). This review focuses on the current understanding of the biodegradation of various nitrophenols over the last two decades. Comprehensive research has been done on the isolation of nitrophenol degrading bacteria in the last two to three decades. But reports documenting catabolic utilization of NPs are very rare. Microorganisms-mediated biodegradation of nitrophenols are summarized in Table 1.

Table 1 List of microorganisms involved in biodegrading of nitrophenols

Name of microorganism	Type of NPs & (conc. of degradation)	Nature of metabolism	Isolation site	Reference
		Bacteria		
Achromobacter xylosoxidans Ns strain	PNP, 1.8mM	Catabolic	Mai Po Nature Reserve in Hong Kong, China	Wan et al. 2007
Alcaligenes eutrophus JMP 134; *Alcaligenes eutrophus* JMP 222	2,6-dinitrophenol; 0.05mM	cometabolic	NR	Ecker et al. 1992
Alcaligenes sp. strain NyZ215	2NP	cometabolic	Activated sludge, China.	Xiao et al. 2007
Arthrobacter aurescens TW17 *Nocardia* sp. Strain TW2	PNP, 0.1mM	cometabolic	The soil of Chico, California	Hanne et al. 1993
Arthrobacter protophormae RKJ100	PNP and 4-Nitrocatechol (NC); 0.3-0.5mM	Catabolic (C, N source)	Agricultural soil of Chandigarh, India	Chauhan et al. 2000
Arthrobacter protophormiae RKJ100	PNP; 0.5mM	cometabolic	An agricultural field containing pesticide	Labana et al. 2005
Arthrobacter sp. HY2	PNP, 100mg/L	cometabolic	The soil of pesticide factory, Anyang, Henan Province, China	Qiu et al. 2009
Arthrobacter sp. JS443	PNP; 100mg/Liter	cometabolic	Soil of Florida	Jain et al. 1994
Arthrobacter sp. SPG	PNP, 0.3mM	Catabolic (C, N source)	Pesticide contaminated site, Hyderabad, India;	Arora 2012b
Arthrobacter sp. Y1	PNP, 100mg/liter	cometabolic	Activated sludge, China	Li et al. 2008
Arthrobacter sp. CN2	PNP, 0.05mM	Catabolic (C source)	Activated sludge, China;	Wang et al. 2016
Bacillus sp. *Pseudomonas* sp.	PNP	Catabolic (C source)	Parathion amended alluvial soil of Orissa, India	Siddaramappa et al. 1973
Bacillus sphaericus JS905	PNP; 0.15mM	cometabolic	Agricultural soil of Chandigarh, India	Kadiyala and Spain 1998
Bacillus subtilis RKJ700	4-Chloro-2-nitrophenol; 1.5mM	cometabolic	The soil of Pesticide contaminated site at Bathinda, Panjab, India	Arora 2012b
Brevibacterium linens	PNP; 100mg/L	cometabolic	Garden soil of Imphal, India	Ningthoujam 2005
Citriococcus nitrophenolicus sp. PNP1	PNP, 0.7mM	Catabolic (C, N source)	The wastewater treatment plant, pesticide factory at cheminova A/S, Denmark	Nielsen et al. 2011

Name of microorganism	Type of NPs & (conc. of degradation)	Nature of metabolism	Isolation site	Reference
Flavobacterium sp.	PNP	cometabolic	Paddy water of Philippines;	Sethunathan and Yoshida 1973
Janthinobacterium sp.	2,4-DNP	Catabolic	Forest soil and Freshwater stream respectively	Hess et al. 1990
Mixed culture: *Enterbacter cloacae, Alcaligens* sp. TK2	4-Chloro-2-nitrophenol	cometabolic	Sludge of sewage plant, Munster	Beunink and Rehm 1990
Moraxella sp.	PNP, 150mg/L	Catabolic (C source)	Activated sludge of Florida	Spain and Gibson 1991
Nocardioides simplex FJ2-1A	2,4,6-trinitrophenol; 2,4,6-trinitrotoluene (0.35mM); 2,4-DNP; (0.35mM)	cometabolic	Picric acid waste water	Ebert et al. 2001
Nocardioides sp. NSP41	PNP	cometabolic	Industrial wastewater	Cho et al. 2000
Nocardioides sp. Strain CB 22-2	2,4,6-trinitrophenol (0.44-2.2mM); 2,4- DNT	cometabolic	Soil samples from Nitroaromatic compound production sites, German	Behrend and Heesche-Wagner 1999
Ochrobactrum sp. B2	PNP (100mg/liter), methyl parathion	cometabolic	Soil, China	Qiu et al. 2007
Pseudomonas cepacia strain RKJ200	PNP, 0.5mM	cometabolic	Assam agricultural field	Prakash et al. 1996
Pseudomonas psudomallai ENB-10	PNP; 50mg/liter	cometabolic	Pharmaceutical industry wastewater, Pakistan	Rehman et al. 2007
Pseudomonas putida	PNP	cometabolic	Effluent sediment of the pesticide industry, Jalgaon, India	Kulkarni and Chaudhari 2006
Pseudomonas putida 2NP8	3-NP,	cometabolic	NR	Zhao and Ward 2001
Pseudomonas putida B2	3-Nitrophenol, 1mM	cometabolic	NR	Meulenberg et al. 1996
Pseudomonas putida DLL-E4	PNP and 4-nitrocatechol (0.5mM)	Catabolic (C, N source)	NR	Shen et al. 2010
Pseudomonas putida JS444	PNP	Catabolic	Activated sludge of California	Lei et al. 2005
Pseudomonas putida PNP1	PNP	cometabolic	El-Harrach River near Algiers	Löser et al. 1998
Pseudomonas sp. JHN	4-Chloro-3-nitrophenol	Catabolic	Wastewater, India	Arora et al. 2014a
Pseudomonas sp. PNP1	PNP; 100mg/liter	Catabolic	Municipal sludge, America	Heitkamp et al. 1990
Pseudomonas sp. *Bacillus* sp.	PNP, 15g/L	Catabolic	Parathion amended flooded soil.	Sudhakar-barik et al. 1976
Pseudomonas sp. BUR11	PNP, 200ppm	Catabolic	Agricultural soil, India,	Pailan and Saha 2015
Pseudomonas sp. WBC-3	PNP	Catabolic (C, N source)	NR	Zhang et al. 2009a
Pseudomonas sp. strain N26-8	2,4-Dinitrophenol 2,5-Dinitrophenol 2,6-Dinitrophenol (all utilize 0.5mM)	cometabolic	Mixed soil sample, Gottingen, The Federal Republic of Germany,	Bruhn et al. 1987
Ralstonia eutropha JMP 134 (DSMZ 4058)	3NP 0.5mM;2,4-dichloroPhenoxy-acetate, 2mM.	cometabolic	NR	Schenzle et al. 1997

Name of microorganism	Type of NPs & (conc. of degradation)	Nature of metabolism	Isolation site	Reference
Ralstonia eutropha JMP 134	2-chloro-5-nitrophenol; 0.46mM	Catabolic	NR	Schenzle et al. 1999
Rhodobacter capsulatus ElFI	2NP,3NP, PNP, 2,4DNP	cometabolic	NR	Blasco and Castillo 1992
Rhodococcus erythropolis strain HL PM-1	2,4,6-trinitrophenol, 2,4-dinitrophenol as sole source of Nitrogen	cometabolic	NR	Heiss et al. 2003
Rhodococcus erythropolis HL 24-2	2, 4-Dinitrophenol; Picric Acid; 0.5mM	cometabolic	Water from river Rhine, Germany	Lenke and Knackmuss 1992
Rhodococcus imtechensis RKJ300	PNP, 2,4-dinitrophenol	Catabolic (C source)	Pesticide contaminated soil of Punjab, India	Ghosh et al. 2010
Rhodococcus opacus SA0101	PNP	Catabolic	Soil of Japan	Kitagawa et al. 2004
Rhodococcus sp. PN1	PNP	Catabolic	Contaminated soil of Japan	Takeo et al. 2003
Rhodococcus opacus strain RB1	2,4-Dinitrophenol (0.5mM)	cometabolic	activated sludge, waste water plant in Alicante, Spain	Blasco et al. 1999
Rhodococcus wratislaviensis	PNP; 0.72mM	Catabolic	River sediment in Buenos Aires, Argentina	Gemini et al. 2005
Serratia sp. DS001	PNP, 0.3mM	Catabolic (C source)	Agricultural district of Anantapur district, Andhra Pradesh, India	Pakala et al. 2007
Sphingomonas sp. UG30	PNP and Pentachlorophenol	cometabolic	Agricultural site in Cambridge, Ontario, Canada	Alber et al. 2000
Sphingomonas sp. UG30; *Sphingomonas chlorophenolica* strain R2A; *Sphingomonas chlorophenolica* strain ATCC 39723	PNP; (0.31-1.10)mM	cometabolic	Fresh water sediment of Canada; contaminated soil of Canada	Leung et al. 1997
Burkholderia sp. KU-46.	2,4-dinitrophenol, 0.5mM	cometabolic	Agricultural soil contaminated pesticide in Japan	Iwaki et al. 2007
Arthrobacter sp. SJCon	2-Chloro-4-nitrophenol; 0.2mM	Catabolic	Pesticide contaminated soil of Punjab, India.	Arora and Jain 2011
Exiguobacterium sp. PMA (JQ182409)	4-chloro -2-nitrophenol; 0.5mM	Catabolic	soil from chemically conta - minated site- Gajraula, Uttar Pradesh, India	Arora et al. 2012
Bacillus sp. MW-1	4-chloro -2-nitrophenol; 0.3mM	cometabolic	Bay of Bengal, India	Arora and Jain 2011
Cupriavidus sp. strain CNP-8 (CCTCC M 2017546.)	2-chloro-4-nitrophenol; 0.3mM	cometabolic	soil of Yantai, Shandong, China	Min et al. 2018
Sphingomonas strain UG30	2,4-dinitrophenol, 150µM	cometabolic	PCP contaminated soil, Canada	Zablotowicz et al. 1999
Pseudomonas sp. JHN	4-chloro-2-nitrophenol; 0.2-0.6mM	cometabolic	Waste water	Arora and Bae 2014b
Burkholderia sp. SJ98	3-methyl-4-nitrophenol; 0.5mM	cometabolic	NR	Min et al. 2016
Burkholderia sp. strain SJ98	2-chloro-4-nitrophenol; PNP; 0.3mM	cometabolic	NR	Min et al. 2014
Burkholderia sp. strain RKJ 800	2-chloro-4-nitrophenol; 3-methyl-4-nitrophenol; PNP; 0.3mM	Catabolic	Pesticide contaminated soil, India	Arora and Jain 2012

Name of microorganism	Type of NPs & (conc. of degradation)	Nature of metabolism	Isolation site	Reference
Spirodela polyrrhiza	3-nitrophenol, 0.5mM	Catabolic	Water sample of Nigori river, Fuefuki River, Kamanashi River; Japan	Kristanti et al. 2012
Rhodococcus sp. CN6	PNP; 50mg/L	cometabolic	Effluentsediment of industry in Shandong province, China,	Zhang et al. 2009b
Pseudomonas sp. JHN	4-Chloro-3-nitrophenol; 0.4mM	Catabolic	Waste water, India	Arora et al. 2014a
Sphingobacterium sp. RB	PNP, 5mM	cometabolic	Rhizosphere soil of palm tree, Maharashtra, India	Samson et al. 2019
Fungi				
Phanerochaete chrysosporium (ATCC 34541)	PNP, 0.25mM	Catabolic	NR	Teramoto et al. 2004
Aspergillus niger VKM F-1119	3-methyl-4-nitrophenol; 25mg/L	cometabolic	NR	Kanaly et al. 2005
Algae				
Chlorella vulgaris; Chenochloris pyrenoidosa	PNP; 10mg/L	Catabolic	NR	Lima et al., 2003

Abbreviations: Conc., Concentration; NR, Data not reported.

2 Nitrophenols: Types, applications, and adverse effects

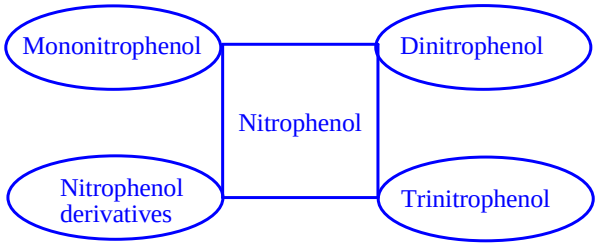
When one or more hydrogen atom(s) from the ring of phenol is replaced by one or more nitro groups, this structure is called NP. These compounds are generally water-soluble and moderately acidic. NPs are broadly classified into four subtypes, depending upon the number of nitro groups substituted (Figure 1a). These types are Mononitrophenol - (Example- PNP, 2NP, 3NP), Dinitrophenol - (Example- 2,4-DNP; 2,6-DNP; 2,5-DNP), Trinitrophenol - (Example- 2,4,6-trinitrophenol) and NP derivatives - (Example- 2-cl-5NP; 5-cl-2NP; 4-cl-2NP; 2-cl-4NP, 4,6-dinitro-2-methyl phenol; 2-cl-4,6-dinitrophenol; 2-amino-4-nitrophenol; 2-amino-4,6-dinitrophenol; 3-methyl-4-nitrophenol; 2-methyl-4-nitrophenol). Their structures are provided in Figure 1b.

NPs are commonly used organic xenobiotic compounds, having a variety of applications. Due to the occurrence of the highly active NO_2 (nitro) group, they are highly desirable chemicals in synthetic chemistry (Ju and Parales 2010) and their applications are listed in Table 2. However, extensive, widespread application of NP is of huge public concern due to its persistent, recalcitrant, hazardous nature and toxicity to non-target organisms. NPs like- 2,4-DNP, and PNP act as an uncoupler of mitochondrial oxidative phosphorylation and reduce ATP production. Again, some of them are water-soluble and mobile, contaminating drinking water sources (Samuel et al., 2014; Kuang et al., 2020). Available reports suggest that some NPs (e.g. PNP) have high toxicity and can threaten human health, through dysfunction of the liver, kidney, and other important physiological life processes, as has been documented for animal models (Wang et al. 2018; Kuang et al. 2020). Direct or indirect contact with these NPs either by inhalations or accidental ingestion may cause chronic toxicity (Wyman et al. 1992; Ju and parales 2010; Przybyla et al. 2021). Although some NPs were used in the health sector (di-nitrophenols were used in cataracts and among athletes for weight loss), due to their negative impact on health, these are no longer prescribed. Moreover, many NPs (like PNP and 2NP) are considered carcinogenic (Karim and Gupta 2001).

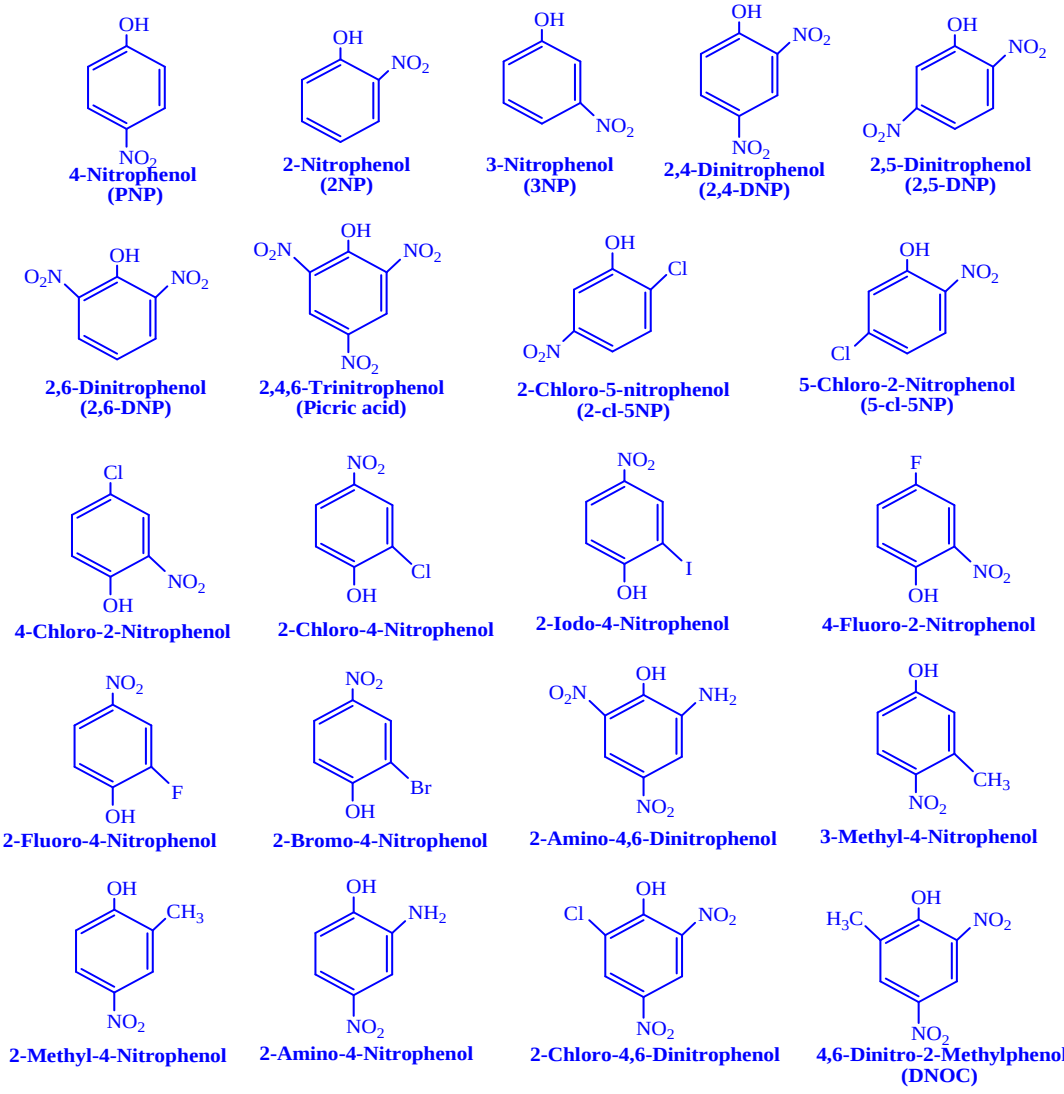
The Environmental Protection Agency (USEPA) has enlisted PNP, 2-NP, and 2, 4-dinitrophenol as priority pollutants because of their high toxicity and wide environmental distribution in the ecosystem (Karim and Gupta 2001; Zhang et al. 2022). USEPA recommended the restriction of these compound concentrations in natural water below <10ng/liter (Karim and Gupta 2001; She et al. 2005; Gemini et al. 2005). Some NPs may contaminate the environment during industrial or agricultural uses and their improper application and/ or storage practices by users (having no technical knowledge, especially in economically poor countries) have resulted in their pollution of the environment, especially soil and groundwater. PNP is toxic, and due to its extensive use and widespread abundance, it is believed to may have accumulated in the food chain (Herrera-Melián et al. 2012).

Although the ultimate impact and long-time fate of these toxic NPs released into the ecosystems remain known, studies indicated these inhibit the growth of microorganisms and are reported to destabilize ecosystems (for example, the sewage treatment plant as reported by Bruhn et al. 1987). NPs are toxic, and many are suspected to be mutagenic and carcinogenic (Wan et al. 2007).

These are harmful to the health of animals including humans and fauna. The LD_{50} (50% of lethal density) value and the half-life of some nitrophenols are provided in Table 3. From this information, it is deducible that most of the NPs may be considered as a potential threat to public health as well as ecosystems. Their presence in non-permissible limits in most ecosystems is of huge public concern and therefore suitable measures must be taken either to remove them completely or ensure their concentration below toxic levels. For microbial bioremediation, it is desirable to know how these NPs are degraded or hydrolyzed by microorganisms. The biodegradation pathways of NPs are discussed in the coming headings.



(a)



(b)

Figure 1a and 1b Classification of nitrophenol and structure of representative types

Table 2 Applications of different types of nitrophenol

Type of NP	Applications	References
Mononitrophenol	Paracetamol, a well-known analgesic and antipyretic, is prepared from PNP. Pesticides like parathion; methyl parathion is prepared by PNP. 2NP and 3NP are used for synthesis of fungicides, dyes, chemicals etc.	Arora et al. 2014b; Ebert et al., 2001
Dinitrophenol	Several herbicides, pesticides (like- 4,6-dinitro-o-cresol, Fluorodifen, Binapacryl) fungicides are prepared from dinitrophenols. 2,5-DNP is used as pH indicator. Dinoseb, DNOC are also used as herbicides. Azo dyes, pesticides, explosives are prepared. 2,4-DNP is used for wood preservatives, herbicides, pesticides.	Arora et al. 2014b; Bruhn et al. 1987
Trinitrophenols	2,4,6-trinitrophenol (picric acid) used as explosive during world war I and II; used for colouring for silk, leather, wool; synthesis of nitrofungin, dicapthon.	Arora et al. 2014a
Nitrophenol derivatives	2-cl-4NP is used for preparation of several pesticides like nitrofungin, dicapthon; Previously used for seed protection and leather conservation 2-A-4NP is used for acid dye like leather, nylon, silk, wool; used as mordant.	Arora et al. 2014b; Lang et al. 2001

Table3 LD_{50} and half-life of some nitrophenols

Compounds	LD_{50}	Half-life	References
PNP	21900µg/liter for daphnids; 8280µg/liter for bluegills; 7170µg/liter for mysid shrimp and 27100µg/liter for sheepshead minnow	In the top soil 1-3 days (aerobic); 14 days (anaerobic); In the sub soil 40 days (aerobic)	Epa 1980
2,4-Dinitrophenol	4090 µg/liter for daphnids; 620µg/liter for bluegills; 48505500 µg/liter for mysid shrimps and 5500µg/liter for herring embryo	28 days	Epa 1980; Przybyla et al. 2021
2,4,6-Trinitrophenol	84700µg/liter for daphnids; 167mg/liter for bluegills; 19.7mg/liter for mysid shrimp	13.4hour	Epa 1980; Wyman et al. 1992
Dinitrocresol/4,6-Dinitro-o-cresol (DNOC)	25-40 mg/kg for mice	153.6 hour	Brown and Chessin 1995

NR- data not reported.

3 Biodegradation of mono-nitrophenols and their responsible genes and proteins

3.1 PNP degradation

There were mainly two pathways of bacterial degradation of PNP viz. the first one is Hydroquinone pathway which was initially reported from Gram-negative bacteria, subsequently, many Gram-positive bacteria were also informed to follow this pathway, and the second pathway is nitrocatechol pathway which was initially known for Gram-positive bacteria, several Gram-negative bacteria also adopt this pathway.

Several bacteria like *Pseudomonas putida* JS444; *P. cepacia* strain RKJ200; *Arthrobacter protophormiae* strain RKJ100; *Rhodococcus opacus* SA0101; *Moraxella* sp.; *Arthrobacter aurescens* TW17 (Spain and Gibson 1991; Hanne et al. 1993; Prakash et al. 1996; Pandey et al. 2003; Kitagawa et al. 2004; Lei et al. 2005), etc. could degrade PNP via hydroquinone pathway. In this pathway, PNP was first transformed to 1,4 benzoquinone/ para benzoquinone via the enzyme monooxygenases, with the requirement of one mole of NADPH. In the following step, para benzoquinone was converted to hydroquinone by the enzyme reductase, and one mole of NADPH was required in this second step. In the subsequent step, the dioxygenase enzyme was responsible for the cleavage of hydroquinone to 4-hydroxymuconic semialdehyde (HMS). Next maleylacetate was produced from HMS by the enzyme Dehydrogenase, which was again degraded through β-ketoadipic acid and TCA cycle intermediates (Figure 2a)

Gram-positive, as well as Gram-negative bacteria, degraded PNP via the nitrocatechol pathway. These include- *Bacillus sphaericus* JS905; *Nocardia* sp. TW2 (Hanne et al. 1993; Kadiyala and Spain 1998). Few bacteria like *Pseudomonas* sp. 1-7, *Burkholderia* sp. SJ98; (Bhushan et al. 2000; Zhang et al. 2012) degraded PNP via both hydroquinone and nitro catechol pathways (figure 2a).

Jain et al. (1994) first time elaborated on the entire nitro catechol pathway of PNP degradation. In this pathway, PNP was first converted to 4-nitrocatechol (4NC) by the enzyme monooxygenase. In the next step, 4NC was converted to 1,2,4 benzenetriol by the enzyme monooxygenase. Here Oxygen was used and nitrite was released. In the next step maleyl acetate (MA) was produced by the enzyme dioxygenase. MA was further degraded via TCA cycle intermediates.

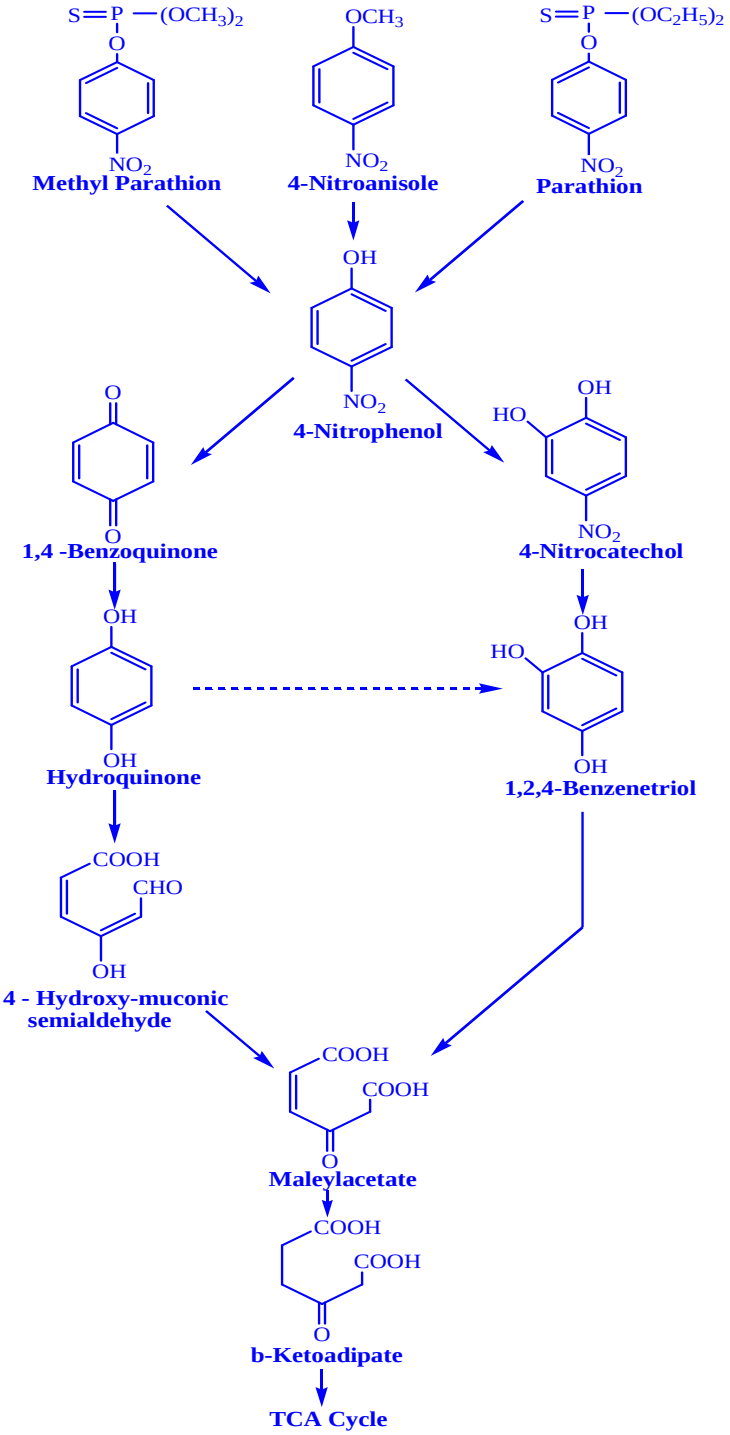


Figure 2a Biodegradation of PNP (from three different compounds) by hydroquinone pathway and nitrocatechol pathway

Prakash et al. (1996) demonstrated that approximately 50Kb transmissible plasmid was responsible for PNP degradation in *P. cepacia* strain RKJ 200. Further, Zhang et al. (2009a) characterized the enzymes i.e., monooxygenase, p-benzoquinone reductase, hydroquinone dioxygenase, 4-hydroxymuconic semialdehyde dehydrogenase, maleylacetate reductase, which catalyzed the transformation of PNP to β-ketoadipate in *Pseudomonas.* sp. strain WBC-3. Guo et al. (2021) demonstrated that a single component and two component monooxygenases were responsible for Gram negative and Gram positive bacterial PNP degradation, respectively.

Approximately 70Kb plasmid of the strain WBC-3, designated as pZWL0 was responsible for the degradation of parathion and PNP (Liu et al. 2005). Vikram et al. (2012) reported that a 41kb DNA fragment of *Burkholderia* sp. strain SJ98 contains multiple ORFs like *pnp*C, *pnp*D, *pnp*F, *pnp*E1, and *pnp*E2. Heterotetrameric protein complex *pnp*E1 and *pnp*E2 can transform hydroquinone to 4-hydroxymuconic semialdehyde. The Gram-positive bacteria *Rhodococcus opacus* SAO101 contain *npc*A, *npc*B, and *npc*C gene clusters for mineralization of PNP. Among these monooxygenases were encoded by the genes *npcA* and *npcB* while hydroxyquinole 1,2-dioxygenase was encoded by the *npcC* gene. When *npcA* and *npcC* genes were mutated, inhibition of growth was recorded under catabolic growth conditions (Kitagawa et al. 2004). A multicomponent enzyme, benzenetriol dioxygenase (*btd*) played a significant role in the PNP biodegradation of soil bacteria. This dioxygenase catalyzed the conversion of benzenetriol to maleylacetate (Paul et al. 2008). In the strain, *B. cepacia* AC1100, *tftH*, and *tftE* genes encoded the enzyme dioxygenase and maleylacetate reductase respectively responsible for the conversion of 1,2,4-Benzenetriol to 3-ketoadipae (Daubaras et al. 1996). The bacterium *Ralstonia pickettii* strain DTP0602 was reported to have the gene *hadC* encoded the enzyme 1,2-dioxygenase that transformed hydroxyquinol to maleylacetate (Hatta et al. 1999). Chauhan et al. (2010) demonstrated that within the genomic DNA of *Burkholderia* sp. SJ98, the *pnpC* gene-encoded Benzenetriol dioxygenase, and *pnpD* gene-encoded maleylacetate reductase. These pnpC, and pnpD genes converted Benzenetriol to beta-ketoadipate.

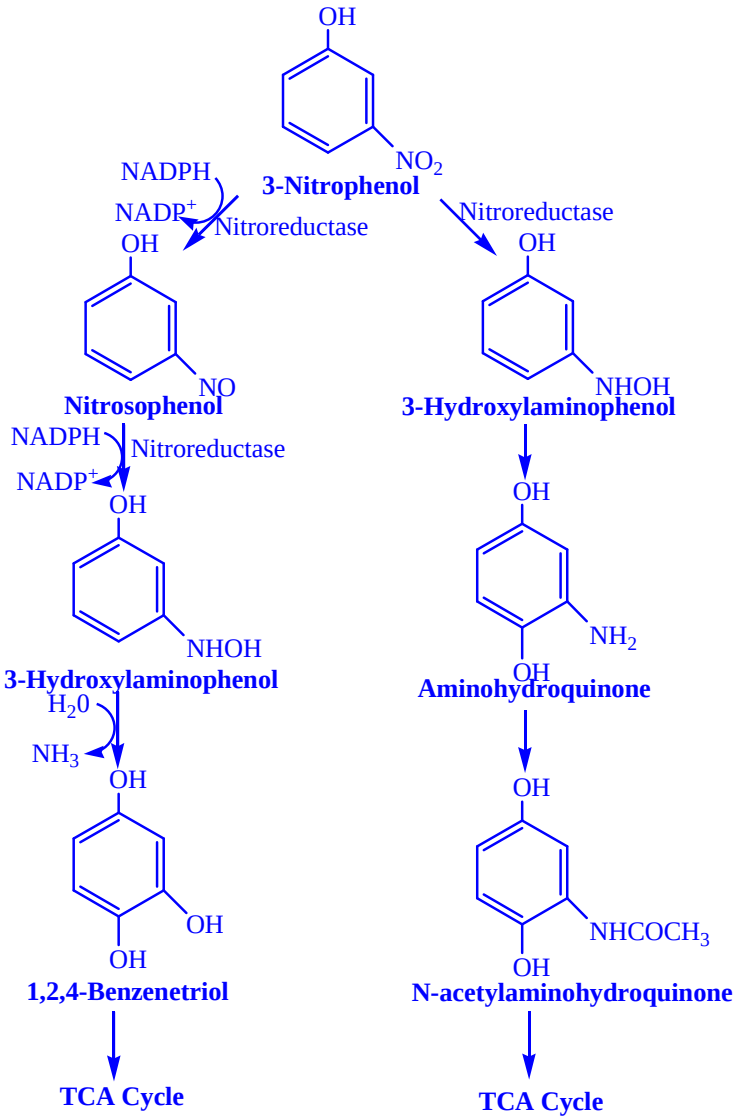


Figure 2b Biodegradation of 3-nitrophenol by the strain *Pseudomonas putida* strain B2 (left side) and *Ralstonia eutropha* strain JMP134 (right side)

Schäfer et al. (1996) reported that *Rhodococcus opacus* AS2 and *R. erythropolis* AS3 can demethylate 4-nitroanisole to PNP with the help of the enzyme oxygenase. After that, PNP entered into the TCA cycle via the Nitrocatechol pathway.

Liu et al. (2007) reported that the *Stenotrophomonas* sp. strain LZ-1 could utilize 4-chlorophenol and PNP co-metabolically and degrade those compounds via the hydroquinone pathway.

3.2 3NP degradation

Capability to degrade 3NP was reported for a few bacteria, in the literature, these includes *Spirodela polyrrhiza* (Kristanti et al. 2012), *P. putida* B2 (Meulenberg et al. 1996); *Cupriavidus nector* strain JMP 134 (Schenzle et al. 1997; 1999) (Figure 2b). Schenzle et al. (1997) first to report biodegradation of 3NP by *R. eutropha* JMP 134. Here, 3NP was first converted to 3-Hydroxylaminophenol by the enzyme nitro-reductase (MnpA) which was then transformed to amino hydroquinone by the enzyme mutase. It was then entered into the TCA cycle via N-acetylamino hydroxyquinone. A different pathway of 3NP biodegradation was reported in the case of *P. putida* strain B2. Whereas 3NP was first converted by the enzyme nitroreductase to nitrosophenol which was then cleaved to 3-Hydroxylamino phenol. Then it was converted to 1,2,4- Benzenetriol and ultimately entered into the TCA cycle.

The 3NP removal by anaerobic treatment is an effective method. She et al. (2005) reported that 60-80% of 3NP could be removed by anaerobic treatment co-metabolically. However, the detailed mechanism of anaerobic biodegradation is in scarcity.

Yin et al. (2010) proposed that in *Rhodococcus* JMP 134, mnp gene clusters played a significant role in the biodegradation of 3NP. MnpA encoded the nitroreductase enzyme to which flavin mononucleotide (FMN) was bound tightly, catalyzing the conversion of 3NP to 3-hydroxylaminophenol via nitrosophenol.

3.3 2NP degradation

Alcaligenes sp. NyZ215, *P. putida* B2 (Zeyer and Kearney 1984; Xiao et al. 2007) were reported to metabolize 2NP. In *Alcaligenes* sp. strain NyZ215, the ortho nitrophenol was first converted to 1,2-benzoquinone/ortho-benzoquinone (1,2-BQ) with the help of the enzyme monooxygenase. Next, 1,2-BQ was converted to catechol by the enzyme reductase, which was subsequently reduced to hexa-2,4-dienedioic acid/ muconic acid by the enzyme dioxygenase which was again degraded to β-Keto adipic acid. Then it was converted to succinic acid and acetyl CoA by TCA cycle intermediates (Figure 2c). In the case of *P. putida* strain B2 monooxygenase enzyme performed both steps.

Xiao et al. (2007) proposed that three genes were present in *Alcaligenes* sp. strain NyZ215, viz; *onpA*, *onp*B, and *onp*C which encoded important enzymes for the catabolism of 2NP. OnpA was encoded for the monooxygenase enzyme that catalyzed the alteration of 2NP to catechol; onpB was encoded for the reductase enzyme that converted benzoquinone to catechol; onpC was encoded for the dioxygenase enzyme that catalyzed the transformation of catechol to hexa-2,4-dienedioic acid. All three genes were transcribed from a single operon from genomic DNA.

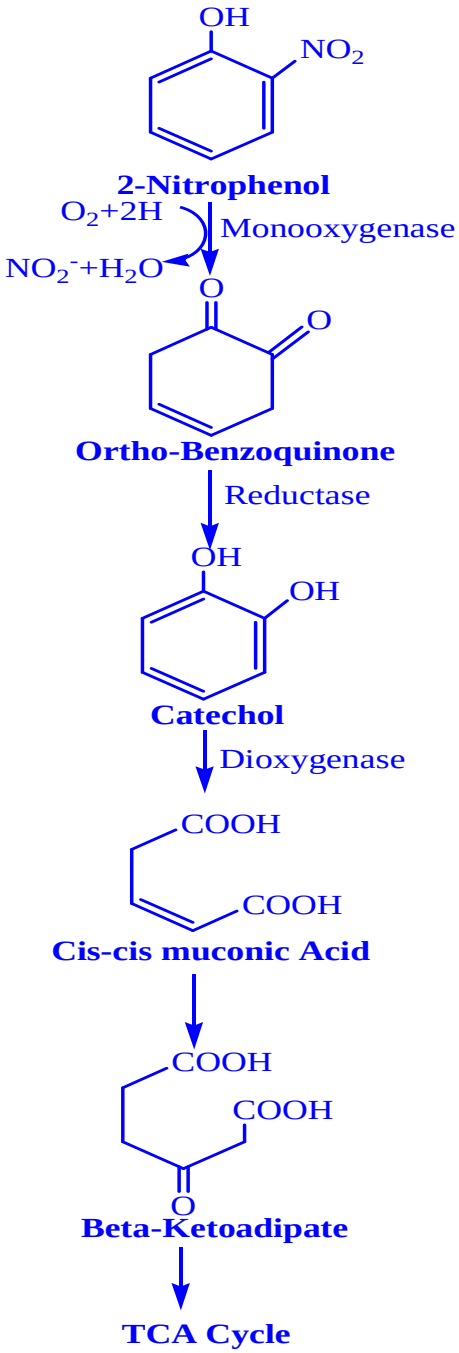


Figure 2c Biodegradation pathway of 2-Nitrophenol

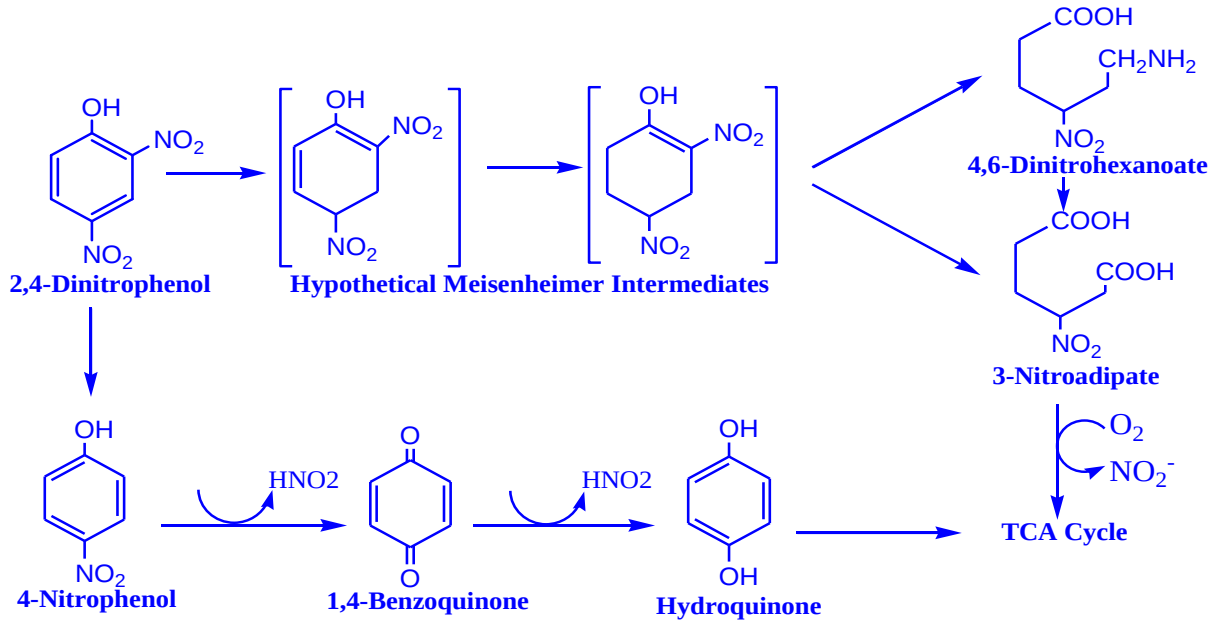


Figure 3a Biodegradation of 2,4-dinitrophenol

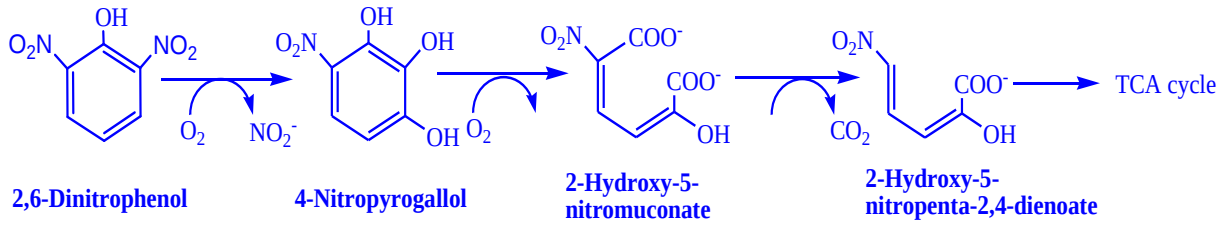


Figure 3b Biodegradation of 2,6-dinitrophenol

4 Biodegradation of dinitrophenol and their responsible genes and proteins

4.1 2,4-dinitrophenol degradation

Iwaki et al., (2007) first time reported that a soil-borne bacterium *Burkholderia* sp. strain KU-46 was capable of degrading 2,4-dinitrophenol cometabolically. This strain degraded 2,4-dinitrophenol via the intermediates of PNP, 1,4-BQ, and HQ. Two successive nitro groups were released during these biodegradation steps (Figure 3a).

Further, Blasco et al. (1999) reported that *R. opacus* strain RB1 mineralized 2,4 dinitrophenol cometabolically. During the mineralization process, this strain released two nitro groups as nitrite. The first phase was aromatic ring reduction by two successive hydride transfers; the second phase was the nitro group of ortho position released as nitrite and formation 3-nitroadipate and hydration of double bond may convert this intermediate to 4,6-Dinitrohexanoate. Ultimately 3-nitroadipate was metabolized with the simultaneous release of the nitro group in the form of nitrite ion (Figure 3a). Lenke et al. (1992) demonstrated that 2,4-dinitrophenol was cometabolically utilized by *R. erythropolis* strain HL 24-1 and HL-2. Here 4,6-Dinitrohexanoate acts as a minor dead-end product and nitrite were released in a significant amount. Heiss et al. (2003) proposed that *npdI* and npdG genes were responsible for the biodegradation of dinitrophenol in *Rhodococcus* sp.

4.2 2,6-dinitrophenol degradation

Bruhn et al. (1987) informed that the soil bacterium *Pseudomonas* sp. N-26-8, cometabolically utilizes 2,6-dinitrophenol. The strain degraded 2,6-dinitrophenol only under nitrogen limitation conditions in the presence of another carbon source. Though nitrite was released, no other intermediates could be recorded during this xenobiotic compound degradation. Ecker et al., (1992) reported that *Alcaligenes eutrophus* strain JMP134 could exploit 2,6-dinitrophenol catabolically. Concomitant nitrite release was recorded during biodegradation of 2,6-dinitrophenol. The parent compound was degraded by the enzyme dioxygenase to form 4-nitropyrogallol, which was converted to produce 2-hydroxy-5-nitromuconate. After that decarboxylation reaction proceeded to form 2-hydroxy-5-nitropenta-2,4-dienoate (Figure 3b).

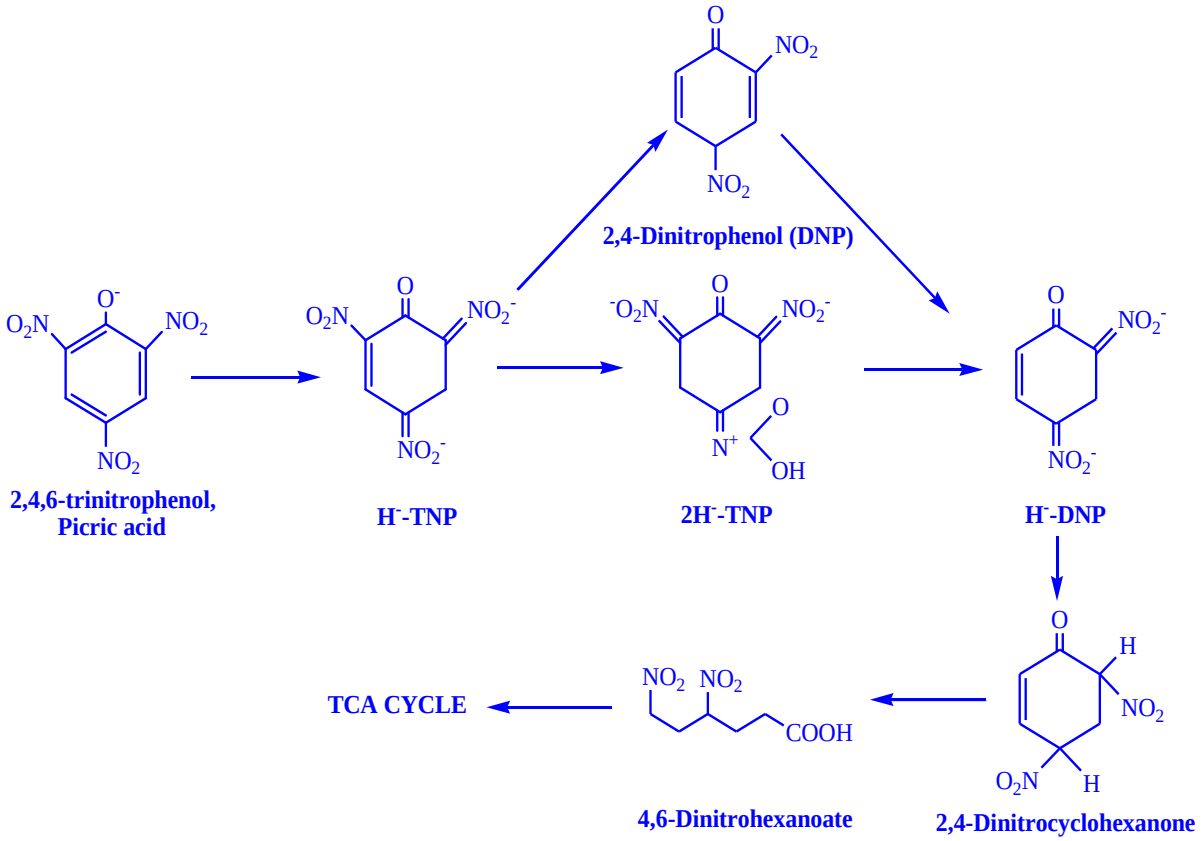


Figure 4 Biodegradation pathway of picric acid

5 Biodegradation of Trinitrophenol and their responsible genes and proteins

5.1 Picric acid degradation

Several microorganisms such as *Rhodococcus erythropolis* strain HL 24-1; *R. erythropolis* HL PM-1; *Rhodococcus* sp. NJUST16; *Nocardioiodes simplex* Nb; *N. simplex* FJ2-1A (Lenke and Knackmuss 1992; Rajan et al. 1996; Ebert et al. 1999; Heiss et al. 2003; Shen et al. 2009) utilized picric acid (2,4,6-Trinitrophenol or TNP) as a sole source of carbon. Picric acid biodegradation was investigated in the strain *Rhodococcus* sp. NJUST16. Initially, two hydrogenations occurred simultaneously in the TNP, converting it to the 2H-TNP (Figure 4). Nitrite was eliminated from the later compound to form hydride Meisenheimer complex, H^--DNP. Further hydrogenation of H^--DNP led to the form of 2,4-dinitrocyclehexanone, which then undergoes ring fission to form 4,6-dinitrohexanoate. The product 1,3,5-trinitriopentane, an analogue of 4,6-dinitrohexanoate, was obtained after acid treatment of culture fluid in the strain HL-2 (Lenke and Knackmuss 1992). Heiss et al. (2003) reported that reductase and hydride transferase II enzyme of strain HL PM-1, played a pivotal role in the conversion of 2,4,6-trinitrophenol and 2,4-dinitrophenol to their respective hydride Meisenheimer complexes.

6 Biodegradation of NP derivatives and their responsible genes and proteins

6.1 2-chloro-4-nitrophenol degradation

Multiple pathways were reported in the case of the biodegradation of 2-chloro-4-nitrophenol. This chlorinated nitroaromatic compound was degraded through the same metabolic pathway by *Burkholderia* sp. RKJ 800 and *R. imtechensis* RKJ 300 (Arora and Jain 2012; Ghosh et al. 2010). Here the nitro group was released with the help of the enzyme monooxygenase followed by cholo group which was released by the enzyme dehalogenase to form HQ. Ultimately dioxygenase enzyme converted it to 4-hydroxymuconic semialdehyde which entered into the TCA cycle (Figure 5a). Pandey et al. (2011) demonstrated the utilization of 2-cl-4NP by the strain *Burkholderia* sp. strain SJ98 which converted 2-cl-4NP to PNP with the help of the enzyme dehalogenase; after that PNP was degraded via the nitrocatechol pathway to enter into the TCA cycle. Arora and Jain (2011) demonstrated that *Arthrobacter nitrophenolicus* SjCon was able to degrade 2-cl-4NP via chlorohydroquinone and MA. CHQ dioxygenase enzyme was involved in the conversion of chlorohydroquinone to maleylacetate (Arora and Jain 2011).

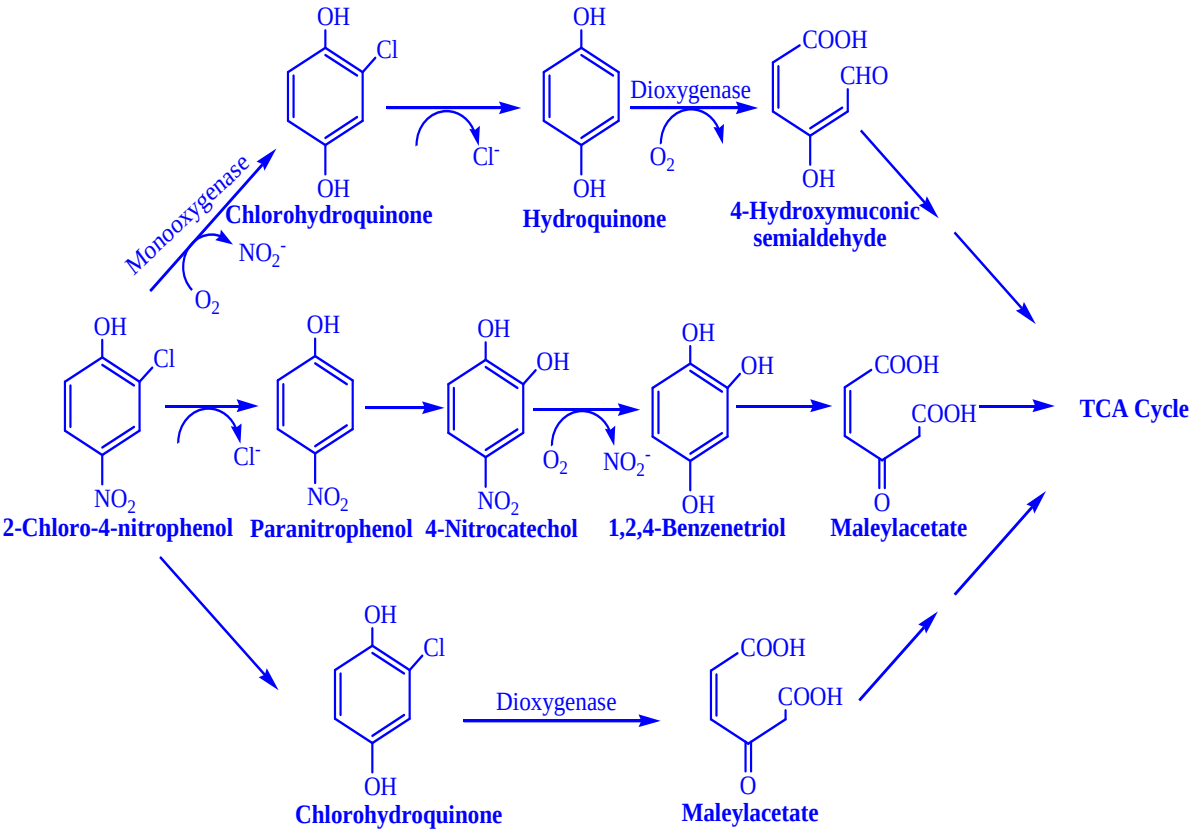


Figure 5a Biodegradation of 2-chloro-4-nitrophenol by *Rhodococcus imtechensis* strain RKJ 300 and *Burkholderia* sp. strain RKJ 800 (upper row); *Burkholderia* sp. strain SJ98 (middle row); *Arthrobacter nitrophenolicus* SiCon (lower row)

6.2 4-chloro-2-nitrophenol degradation

Arora et al. (2012) demonstrated that the strain *Exiguobacterium* sp. PMA was able to degrade 0.5mM (4-chloro-2-nitrophenol) 4-cl-2NP catabolically. Two important metabolites obtained in this degradation were 4-chloro-2-aminophenol and 2-aminophenol. At first reductase enzyme converted it into 4-chloro-2-aminophenol and then the dehalogenase enzyme released the chloro group from the para position of it and converted it into 2-aminophenol (2AP) (Figure 5b). Bruhn et al. (1988) prepared a transconjugant strain by transferring plasmid from either *Pseudomonas* sp. B13 or the bacterium *Alcaligenes eutrophus* JMP134 to the strain *Pseudomonas* sp. N31. The genetically modified bacterium *Pseudomonas* sp N31 was able to mineralize 0.5mM 4-chloro-2-nitrophenol co-metabolically. The parent compound was completely mineralized via the intermediate of 4-chlorocatechol. The soil and marine bacterium *Bacillus subtilis* RKJ 700 and *Bacillus* sp. strain MW-1 (Arora and Jain 2011; Arora 2012a) respectively decolorized and bio-transformed the highly toxic nitroaromatic compound into less poisonous 5-chloro-2-methylbenzoxazole via a same metabolic pathway. The chlorinated nitrophenol was biotransformation to 5-chloro-2-methylbenzoxazole through 4-chloro-2-aminophenol and 4-chloro-2-acetaminophenol. Beunink and Rehm (1990) investigated a mixed culture of bacteria *Alcaligenes* sp. TK-2 and *Enterbacter cloaceae* degraded the chlorinated nitrophenol to 4-chloro-2-aminophenol and then it was entered into the TCA cycle pathway. Arora et al. (2016) reported that *B. aryabhattai* strain PC-7 was capable of degrading 2mM 4-chloro-2-nitrophenol cometabolically. The strain bio transformed the parent compound into 5-chloro-2-methylbenzoxazole.

6.3 2-chloro-5-nitrophenol degradation

Schenzle et al. (1999) reported that the bacterium *Ralstonia eutropha* JMP 134 could exploit 2-chloro-5-nitrophenol (2-cl-5NP) and the parent compound was first converted to 2-chloro-5-hydroxylaminophenol by the enzyme reductase. The enzyme mutase in the next step converted it to 2-amino-5-chlorohydroquinone. In the third step, reductive dehalogenation of 2-Amino-5-chlorohydroquinone to amino hydroquinone was noticed (Figure 5c). Such a type of dehalogenation by aerobic bacteria is rarely observed in the aromatic ring. Schenzle et al. (1997) proposed that amino hydroquinone was further converted to N-acetyl-amino hydroquinone under anaerobic conditions.

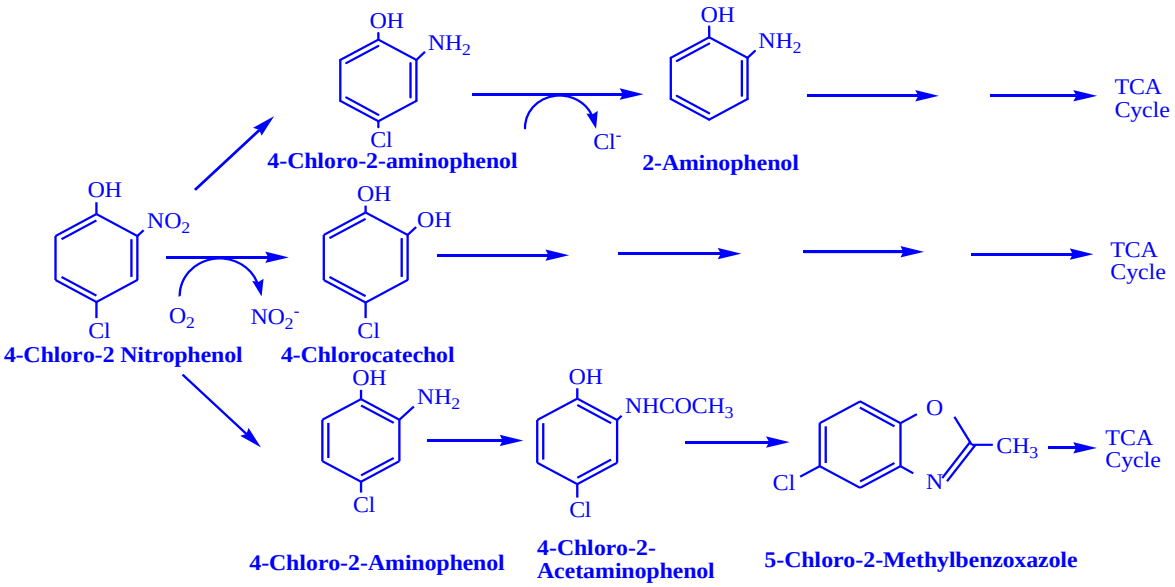


Figure 5b Biodegradation pathways of 4-chloro-2-nitrophenol by *Exiguobacterium* sp PMA (upper pathway); *Pseudomonas* sp. N31 (middle pathway); *Bacillus subtilis* strain RKJ 700 and *Bacillus* sp. strain MW-1 (lower pathway)

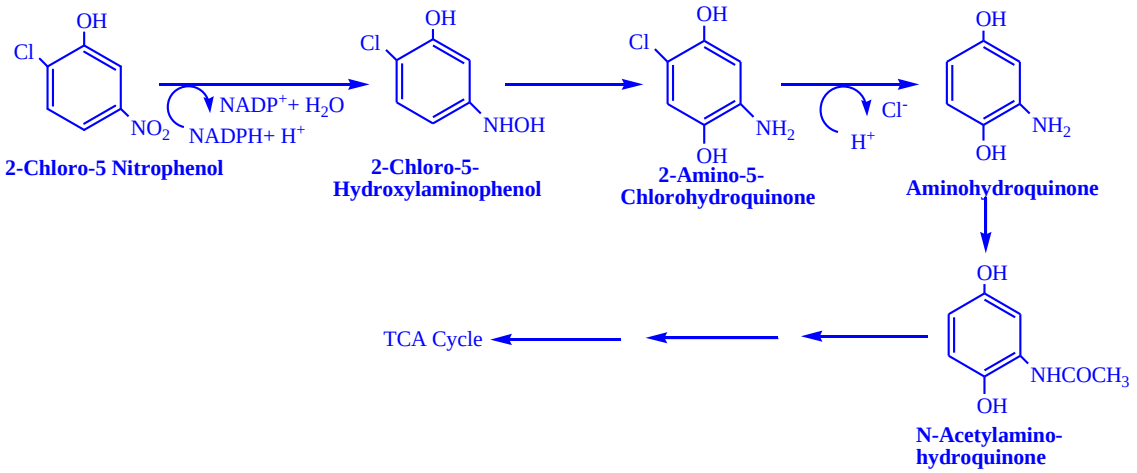


Figure 5c Biodegradation pathway of 2-chloro-5-nitrophenol

6.4 3-methyl-4-nitrophenol degradation

A survey of literature suggested that 3-methyl-4-nitrophenol (3M4NP) was reported to degrade by several bacterial strains like *Ralstonia* sp. SJ98; *Burkholderia* sp. FDS-1; *B.* sp. NF100 (Hayatsu et al. 2000; Bhushan et al. 2000; Zhang et al. 2006; Min et al. 2016) and fungal strain like *Aspergillus niger* VKM F-1119 (Kanaly et al. 2005). Bhushan et al. (2000) reported that the Gram-negative, chemotactic bacteria *Ralstonia* sp. SJ98 utilized 3M4NP as a sole source of carbon, energy, and nitrogen sources. The strain converted the methylated non-polar nitroaromatic compound to a highly polar compound catechol. Bacterial strain *Burkholderia* sp. NF100, *Burkholderia* sp. FDS-1 degraded the methylated nitroaromatic compound via methyl hydroquinone. Kanaly et al. (2005) reported that the soil fungi *Aspergillus niger* strain VKM F-1119 bio transformed 3M4NP to hydroxylated derivative like 2-methyl-1,4-benzenediol which would increase the water solubility of the target compound. Again, methylated nitroaromatic compounds may be transformed into carcinogenic compounds like 4-amino-3-methyl phenol which was further dimerized itself (Figure 5d). Min et al., (2016) demonstrated that the *pnpABA1CDEF* gene cluster was responsible for this methylated nitroaromatic compound degradation. In *Bacillus* sp. SJ98 (Min et al. 2016) pnp A gene-encoded monooxygenase enzyme, that catalized the conversion of 3M4NP to methyl-1,4-benzoquinone and nitrite was released during this conversion. The *Pnp B and PnpCD* genes were responsible for further transformation to 2-methyl-4-hydroxymuconic semi-aldehyde (Figure 5e).

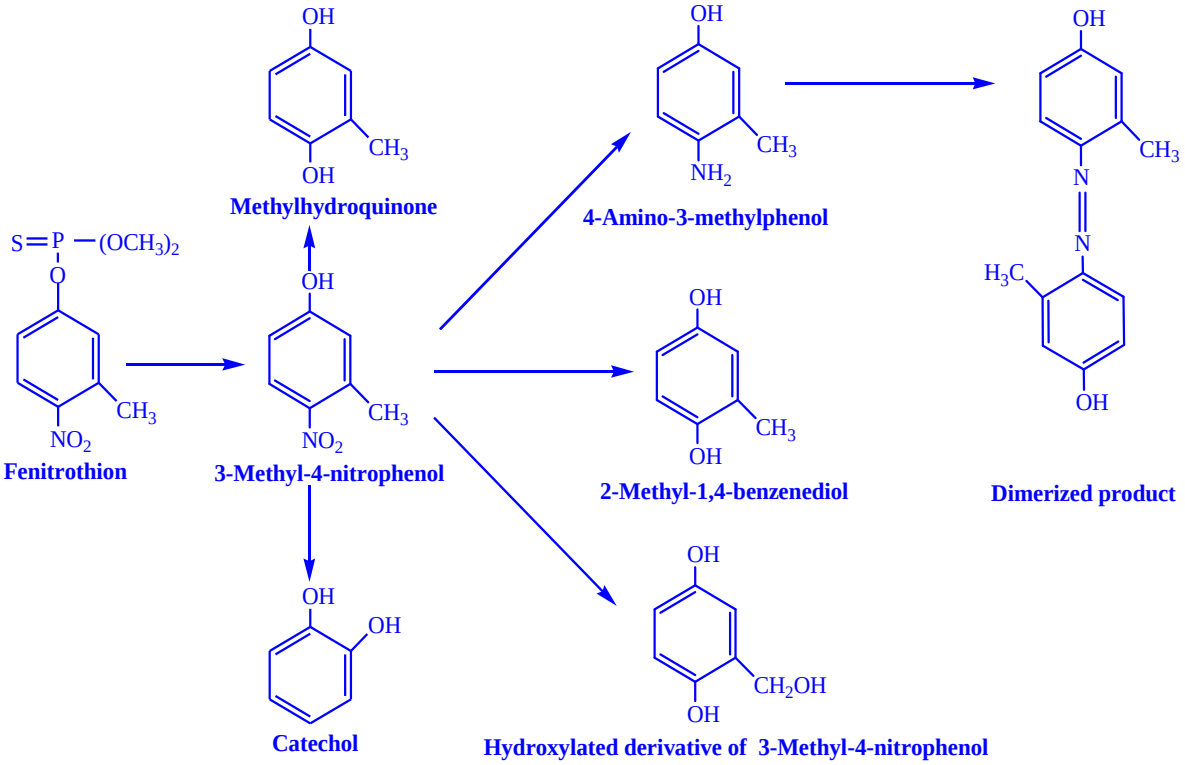


Figure 5d Biodegradation pathways of 3-methyl-4-nitrophenol

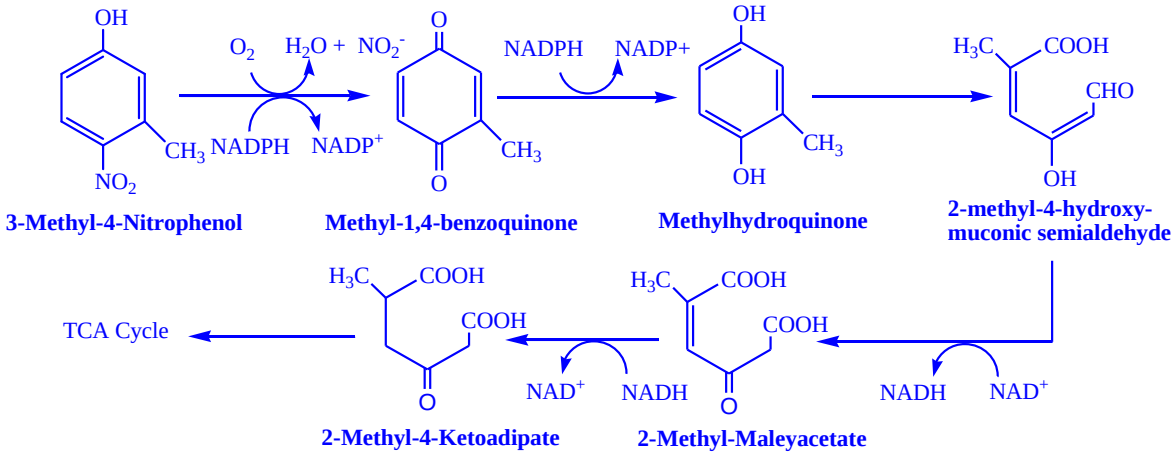


Figure 5e Biodegradation of 3-methy-4-nitrophenol

Hayatsu et al. (2000) reported that *Burkholderia* sp. NF100 harbored two plasmids pNF1 and pNF2; which were exclusively important for fenitrothion degradation. Loss of pNF1 and pNF2 from the strain NF100 destroyed methyl hydroquinone and fenitrothion hydrolyzing abilities respectively.

6.5 DNOC degradation

Several bacteria like *Rhizobium leguminosarum, R. trifolii, R. meliloti, Rhodococcus erythropolis* HL 24-1 (Hamdi and Tewfik 1970; Lenke and Knackmuss 1996), *Azotobacter* sp. and *Rhizobium* sp. (Wallnoefer et al. 1978) were reported to degrade 4,6-Dinitro-o-cresol (DNOC) (Figure 5f). In the strain *R. erythropolis* HL 24-1, DNOC was protonated by 2 hydrogen ions and subsequent protonation converted it to 4,6-Dinitro-2-methylhexanamine. After hydrolysis the later was converted to 4,6-Dinitro-2-methylhexanoate (Lenke and Knackmuss 1996). Wallnoefer et al. (1978) proposed that different *Azotobacter* and *Rhizobium* strains bio-transformed DNOC to 6-acetamido-2-methyl-4-nitrophenol.

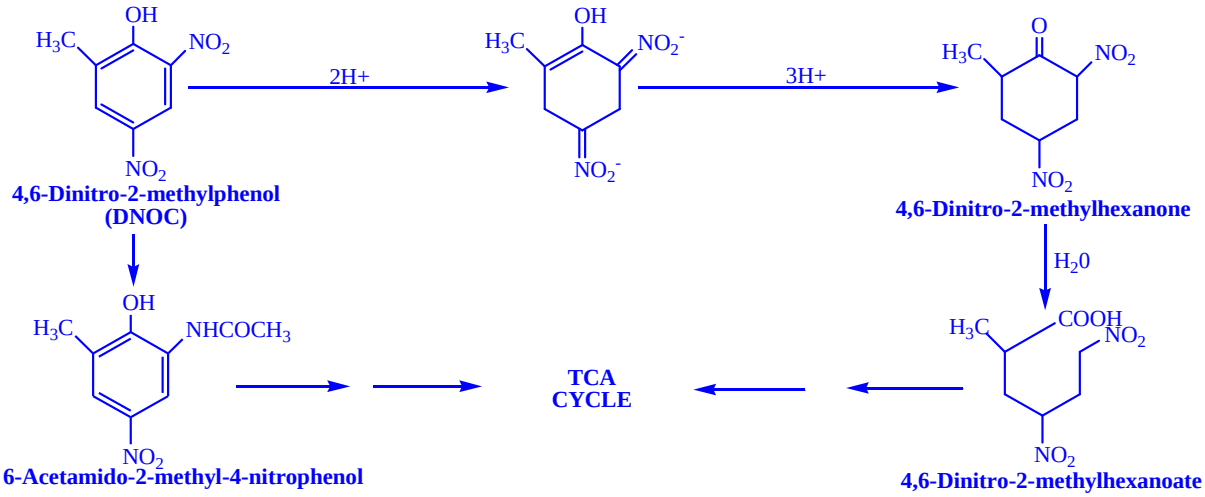


Figure 5f Biodegradation pathways of DNOC

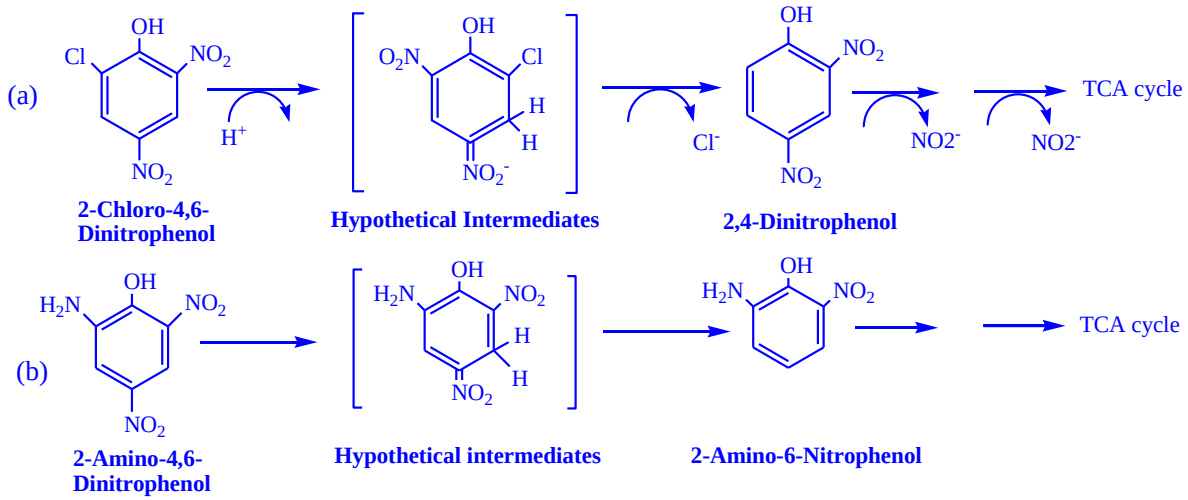


Figure 5g Degradation pathway of (a) 2-chloro-4,6-dinitrophenol and (b) 2-amino-4,6-dinitrophenol

6.6 2-amino-4,6-dinitrophenol and 2-chloro-4,6-dinitrophenol degradation

Lenke and Knackmuss (1996) proposed that the bacterium *R. erythropolis* strain HL 24-1 cometabolically transformed 2-amino-4,6-dinitrophenol to 2-amino-6-nitrophenol with the release of nitrite ion. Ammonia was absent in the culture fluid. They also proposed that during aerobic conversion of 2-Chloro-4,6-dinitrophenol, nitrite and chloride were liberated. This halo nitroaromatic compound was converted to 2,4-dinitrophenol and no other metabolite was obtained during conversion (Figure 5g).

7 Enzymes involved in biodegradation of nitrophenols

Nitrophenols are one of the xenobiotic compounds. NPs are generally recalcitrant due to reasons like substitution of the H group by other groups such as amino, carboxyl, nitro, and methoxy groups resulting in low electron density around the aromatic ring. As the nitro group has the electron-withdrawing capacity, so, it prevents oxidative degradation and electrophilic attack of oxygenase, that's why nitroaromatic compounds become resistant to aerobic degradation (Carbon-Halogen bond is more stable than the Carbon-Hydrogen bond and more energy is required for cleavage of such bonds). If the benzene ring contains more substituent groups, then this compound becomes more recalcitrance. Compounds that have a nitro group at the para position are supposed to be more recalcitrant than the other two positions (ortho or para) (Arora et al. 2012). Decreasing order of recalcitrant capacity of the NPs is Meta>Ortho>Para. Aliphatic hydrocarbons are more easily degraded than aromatic hydrocarbons. Aliphatic unsaturated hydrocarbons and branched aliphatic hydrocarbons show decreased biodegradation rate, and resist biodegradation. A list of enzymes reported to be involved in the biodegradation of nitrophenols are summarized in Table 4.

Table 4 Enzymes involved in biodegradation of Nitrophenols

Enzyme	Removal of	Insertion of	Role/ function	Reference
Monooxygenase	nitro group as nitrite ion	one oxygen atom added	PNP to Hydroquinone conversion	Kitagawa et al. 2004
Dioxygenase	nitro group as nitrite ion	insertion of two hydroxyl group	4-nitrocatechol to 1,2,4-benzenetriol conversion	Kitagawa et al. 2004
Meisenheimer Complex forming enzyme	nitro group as nitrite ion	hydride ion addition to the aromatic ring	Picric acid degradation	Rieger et al. 1999
Halogenase	halogen atom remove from halo nitrophenol	nothing	In the 2-cl-4NP degradation, hydroquinone to 4-hydroxy-muconic semialdehyde conversion	Pandey et al. 2011
Mutase	nothing	nothing	2-chloro-5-nitrophenol degradation;	Schenzle et al. 1999
Hydroxylaminolyase	nitro group as ammonia	nothing	3-nitrophenol degradation	Meulenberg et al. 1996; Haigler and Spain 1993

Table 5 Nitrophenol biodegradation by microorganism based whole cell immobilization technique

Immobilization substrate	Microorganism involved in biodegradation	Biodegradation	References
Ca-alginate	*Enterobacter cloacae; Alcaligenes* sp. TK-2	4-Chloro-2-nitrophenol	Beunik and Rehm 1990
k-carrageenan, alginate	*Moraxella* sp. G21	PNP	Errampalli et al. 1999
Diatomaceous earth	*Pseudomonas* sp. PNP1, PNP2, PNP3	PNP	Heitkamp et al. 1990
Zeolites	*Burkholderia cenocepacia* (JTLT00000000)	Methyl parathion, PNP	Fernandez-lopez et al. 2017
Agar	*Pseudomonas putida* C-11, BA-11	PNP	Ignatov et al. 2002
Agar	*Rhodococcus erythropolis* HL PM1	2,4 Dinitrophenol	Kitova et al. 2004
Polystyrene microplates	*Sphingomonas* sp. JK1	Methyl parathion	Kumar and D'Souza 2010
k-carrageenan	*Sphingomonas* sp. UG30	PNP	Alber et al. 2000

8 Bioremediation by immobilized cell technique

By the introduction of an immobilized cell system, impetuous bioremediation of NP compounds may be accomplished. Biological durability of cells together with plasmids increases in this technique. Such a technique protects the inoculated microorganisms from unfavorable conditions like minimization of conflict with indigenous microflora, the appearance of toxic substances, unsuitable pH, salt concentration, etc. Again, immobilized cells can degrade and withstand higher concentrations of xenobiotic compounds compared to the free cell system (Fernández-López et al. 2017). Various naturals (agarose, agar, clay, diatomaceous earth, alginate, k-carrageenan, dextran, zeolites, aubasidan, chitosan) and synthetic (polyvinyl alcohol, polyacrylamide) substrates are used to bewilder the limitations faced during bioremediation with free cell system (Mrozik and Piotrowska-Seget 2010; Fernández-López et al. 2017; Van Elsas and Heijnen 1990). One of the important drawbacks of the immobilized cell system technique is that oxygen transfer is restricted in the substrate matrix by molecular diffusion (Beunink and Rehm 1990). A reported list of microorganisms used for bioremediation by immobilized cell technique is summarized in Table 5.

9 Advantages and disadvantages of bioremediation

Bioremediation is a biological process wherein the controlled condition of organic pollutants is biologically degraded from contaminated sites. Nowadays, it has wide acceptance due to its low cost, eco-friendly, pollution-free, effective technique for the treatment of pollutant environment. Here microorganisms enzymatically utilize the pollutants and alter them into innocuous products. The purpose of bioremediation is not only to exclude environmental contaminants but also to regain environmental quality. In the soil environment, organic pollutants are mainly dissipated biologically. Various factors influence the complex phenomenon of bioremediation viz. pH, presence of oxygen, temperature, salt, nutrients; the presence of microbial population (inoculum size) to the pollutant site, activity, and density of introduced microorganism, nutrients, co-substrate, soil properties, microenvironment, etc. There are various bioremediation techniques such as land farming, phytoremediation, bioreactor, composting, enzyme-catalyzed bioremediation, and wastewater treatment. Again biostimulation, adaptation bioaugmentation, bacterial chemotaxis, and bioavailability of pollutants to the microorganism might enhance bioremediation rates (Mohan et al., 2006).

There are several reports available on the bioremediation of NP by single bacterial strains (Labana et al. 2005; Hong et al. 2007, Ghosh et al. 2010) and engineered strains (Xu et al. 2021), alongside reports are also available on bioremediation by bacterial consortia.

10 Bioremediation of nitrophenols

The *in-situ* and *ex-situ* represent two bioremediation processes for the bioremediation practice. The *in-situ* technique is the spoiling of pollutants at their original contaminated location. Kristanti et al. (2012) proposed that *Spirodela polyrrhiza* has the potential for rhizoremediation of 3-nitrophenol contaminated soil. Labana et al. (2005) investigated that *Arthrobacter protophormiae* RKJ100 can degrade PNP co-metabolically in soil microcosms. They reported that when cells were pre-exposed to PNP, it can degrade PNP more quickly than the uninduced cells; higher concentrations of pollutants act as poison for microorganisms; a rise in inoculum density significantly improves the mineralization efficiency of the pollutant. It was demonstrated by Labana et al. (2005) that the PNP bioremediation rate is inversely proportional to the increasing depth of soil. Barles et al. (1979) demonstrated that *P. stutzeri* is capable of decontaminating parathion-containing soil under *in situ* field condition. The bacterium *R. imtechensis* strain RKJ300 could degrade multiple NPs compounds such as PNP, 2,4-dinitrophenol, and 2-cl-4NP (Ghosh et al. 2010). They proved that the bioremediation rate is quicker in non-sterile soil as compared to sterile soil, thereby indicating the probable role of aboriginal microflora of the soil in NP biodegradation.

Ex-situ technique is the spoiling of pollutants elsewhere other than the original contaminated location like a bioreactor under controlled conditions. Donlon et al. (1996) observed that in the USAB reactor anaerobic granular sludge was able to transform PNP and 2,4-dinitrophenol to 4-aminophenol and 2,4-diaminophenol respectively; 2NP was completely degraded to methane via 2AP. Some notable demerits of bioremediation practices such as – the *ex-situ* technique is that, these are more expensive requiring the intervention of sophisticated equipment, than *in-situ* technique. Slow and low degradation rates were recorded in the case of the *in-situ* techniques, too long a time might be required to attain the desired reduction of the pollutant in the environment. Moreover, the strain survivability in the bio remedial site is another question, which needs to be addressed systematically using.

11 Future perspective black box of NP biodegradation study

Although, there are plenty of reports on the biodegradation of NPs by microorganisms, the search for novel microorganism continue from better catalytic perspectives. This is especially true because a major portion of microbial biodiversity is still unknown. Currently, the wealth of knowledge for biodegradation of NPs is based on aerobic microorganisms; ironically, the anaerobic microorganisms (especially prokaryotic ones) are dominants in the subsurface habitats of Earth. Technological barriers in the cultivation of obligate anaerobes and fewer exploration studies with anaerobic NPs degrading bacteria are other reasons. The study of anaerobic NPs degrading bacteria will lead to its better understanding in the future. As omics approaches have started to intervene in most the modern biological sciences, in the biodegradation study of NPs also omics approaches will help to decipher the molecular mechanism of adaptation of bacteria towards these toxic compounds and a better understanding of their fate and movement in the cell through integrated cell imaging approaches. There is a huge void as far as cell signaling mechanisms are concerned concerning NPs biodegradation activities. Future research will also aim to understand this signaling process which will help to design *in situ* bioremediation strategies in better ways. Another future aspect is cell imaging techniques. With current innovations and advancements, cell imaging techniques will evolve in the future that will benefit to realize the process of mineralization of NPs and track the movement of its hydrolytic metabolites in a living cellular milieu. Moreover, with integrated advanced computational systems, quantitative aspects of different cellular components and their probable interaction may also be anticipated/ speculated, in the future.

In the future, more promising strains will be targeted directly from the environmental samples through single cell genomics approaches followed by their omics studies to understand their potentiality. Synthetic biology-dependent metabolic engineering will be carried out to fish out the desirable genes and explore their expression possibilities in heterologous systems for better in situ application purposes. To have better effects on this, nanotechnology may also be incorporated.

Conclusion

The NPs are one of the most common chemical compounds in synthetic chemistry and are widely used in almost all industries, today. Due to their comprehensive use and applications, their presence in soil, water, and air at non-recommended levels has been considered as threat to ecosystems and all their living components (both microscopic and macroscopic, including humans). Therefore effective, eco-friendly, cost-effective approaches were considered for the decontamination of various habitats contaminated with NPs. With these aims, the initial focus was given to isolation, characterization, and identification of NPs degrading microorganisms. The latter were targeted because their enzymes have huge catalytic diversity as well as specificities. This was followed by the study of hydrolysis intermediates, and metabolites using analytical equipment. This added precision to the study and knowledge on pathways of biodegradation of NPs were

excavated. With these, the enzymology and genetic studies came that lead to the identification of specific genes encoding enzymes responsible for catalyzing individual steps of multi-step pathways of biodegradation. To develop bioprocess and engineering; these genetic elements were next fished out from the source organism and through recombinant DNA technology and genetic engineering were expressed in more efficient heterologous expression systems for better enzyme function leading to catalysis of the target NP compound and thus biodegradation. Very recently the five genes encoding PNP degradation function were chemically synthesized and through metabolic engineering was expressed in *E. coli* host. The latter was demonstrated to degrade PNP very efficiently under laboratory culture conditions. With the advent of NGS-based DNA sequencing technologies, many NPs degrading bacterial genome sequence were sequenced, to understand their overall genomic potential. These were followed by transcriptome and proteome studies to reveal expression products for understanding molecular mechanisms behind their adaptation during the biodegradation process. But these were all carried out at laboratory levels. Microcosm based studies were also conducted to understand the applicability of microbes mediated bioremediation process in the environmental habitats, both with free cells, immobilized cells and genetically engineered strains. Unfortunately, very few successful attempts have been carried out with environmental habitats for the removal of NPs (both in situ & ex-situ attempts). So, as far as the effectiveness of the bioremediation process for NP decontamination is concerned, we are far away. More explorative studies using efficient aerobic-anaerobic NP degrading bacterial consortium (or combination of microbes- plant systems) and advanced techniques including omics approaches and nanotechnologies may help towards developing better practicable bioremediation approaches, in the futures.

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Conflict of interest

Both the authors declare no conflict of interest in this study.

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