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A Review on Microbial Degradation of 2,4-Dinitrophenol

Sufiah Md. Zin, Syahir Habib, Nur Adeela Yasid, Siti Aqlima Ahmad*

Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Dr Siti Aqlima Ahmad
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 Serdang, Selangor,
Malaysia
Email: aqlima@upm.edu.my

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ABSTRACT

Nitrophenols (NPs) is one the most widely used chemical compounds due to their mass usage in agricultural industry, medical applications and domestic activities. However, due to their intensive application in these industries, many reports have been arising in regard to the toxicity effects towards human health and the environments. It has been acknowledged that various microbial species were able to utilize the NPs as their sustenance are being exploited for their mechanistic approach. Current research has been focused on the isolation of those strains and the study of microbial species of potential environmental and biological impact. This review highlights on one of the examples of polynitrophenols - 2,4-dinitrophenol (2,4-DNP), their usage in recent industry, implications of 2,4-DNP to the human health and environmental niche, and role of 2,4-DNP-utilising microbes in current bioremediation.

INTRODUCTION

2,4-dinitrophenol (2,4-DNP) is one of the phenolic compound derivatives in which, two of the hydrogen atoms of the benzene molecule is replaced with the nitrite group (NO_2). Also known as Solfo Black and Aldifen, this yellow crystalline solid with sugary and musty odour is synthesized by the alkaline hydrolysis reaction of 1-chloro-2,4-dinitrobenzene [1] or by the nitration reaction of benzene, phenol, or mononitrophenol [2]. This compound can undergo sublimation, volatile with the presence of steam, and soluble in both organic solvents and aqueous alkaline solutions [3]. **Fig. 1** shows the structure of 2,4-DNP.

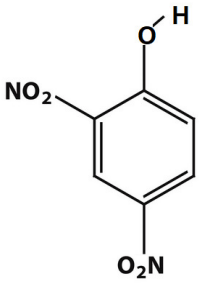

Fig. 1. Structure of 2,4-DNP. Two nitro side-chains (-NO_2) occupy the second and fourth position of the 2,4-dinitrophenol molecule.

Practical usage of 2,4-DNP in industry

DNP was initially commercialized during the 1930s by Maurice Tainter, who realized that intake of 2,4-DNP by human-led to significant weight loss and greatly increased the metabolic rate of the consumer [4, 5]. The mechanism of action of 2,4-DNP involves the role of DNP as a proton ionophore that enables the seapage of protons throughout the inner mitochondrial membrane and therefore sidestep the ATP synthase. For this reason, production of ATP is less powerful. At the same time, portion of the energy that is normally generated by cellular respiration is lost in the form of heat. As more DNP is consumed the energy production is becomes more ineffective, and results int the metabolic process is raised and more fat within the body muscle is used up in an effort to balance back the ineffectiveness that took place and also to satisfy energy requirements [6].

However, due to the increasing reports on the side effects of 2,4-DNP consumption such as rapidly developing cataract, organ failure and death, the production of DNP was stopped in the United States in 1938 [6, 7]. Commercialized 2,4-DNP is primarily utilized as raw materials or intermediates in the production of dyes, explosives, wood preservers, and rubber chemicals [3, 8–10]. This compound can also be used for the detection of ions of ammonium and potassium [11]. Furthermore, 2,4-DNP is used widely in the pharmaceutical and agricultural industry as antiseptic and as a pesticide,

respectively [12]. Dinitrophenol herbicides such as dinitro-ortho-cresol (DNOC), 2,4-DNP, dinseb and dinoterba are phenolic compounds with the dual nitro group that are somewhat water-soluble, extremely harmful to animals and may even trigger developmental toxicity. Among the known herbicides, dinoseb, is recognized to bring about teratogenic outcomes in a number of animal species. Rats that is maternally force-fed with this compound results in a decrease body weight of the foetus and elevated numbers of extra ribs was noticed. Additionally, in rabbits, a number of health consequences including eye defects, neural malformations and the accompanied maternal toxicity was noticed after exposure through the dermal route [13].

However, recent years have shown a rising production of 2,4-DNP (outside of U.S) in different names such Chemox, Dinosan and Dnoc as due to the extensive physiological study on the substances [14]. One of the examples of an extensive study of 2,4-DNP is in thyroid metabolism. From an article reviewed by Weiss and Refetoff [15], 2,4-DNP raises the basal metabolic rate (BMR), lowers the serum thyroxine (T4) concentration, speed up the peripheral metabolism of T4 and reduce the thyroidal radioactive iodine uptake (RAIU) and secretion. Although the actions of 2,4-DNP in the thyroidal system may be intricate, specifically 2,4-DNP acts as the uncoupler compound that uncouples the oxidative phosphorylation in mitochondria, thus stimulating the thyroid metabolism [15].

Several other functions of 2,4-DNP in thyroid metabolism are to displace hormones secreted by thyroid from T4-binding serum proteins, improves both the biliary and faecal excretion of T4 and to raise the deiodination of T4. Although part of 2,4-DNP is to impersonate the action of T4, the compound does not share certain aspects of T4 such as the initiation of tadpole metamorphosis or offers substitution therapy in myxedema [15]. Khan et al. [16] also evaluated the ability of a novel mitochondrial uncoupler prodrug of 2,4-DNP, MP201 based on the ability to prevent the neuronal damage and maintain the visual function in an experimental autoimmune encephalomyelitis (EAE) model for optic neuritis. Based on the study, the pronounced mild mitochondrial uncoupling properties of MP201 together with the measured removal of DNP may promote to the neuroprotective effect by directly modulating the entire mitochondria's physiology thus can be recommended as the prospective novel treatment for optic neuritis.

NPs are widely used as intermediates in many industrial manufactures (**Fig. 2**). Consequently, it is not shocking that these substances appear as significant pollutants in water bodies including river-groundwater, wastewaters, and in the atmosphere. Several studies have demonstrated that phenols and phenolic compounds can be mutagens and organic carcinogens [17]. Certain soluble reactive DNPs, that are currently employed in large quantities have been shown to be hydrolyzed during application and can spell more troubles due to the unknown toxicity of the hydrolyzed products. This indirectly will result in an even larger proportion of these compounds being released to the environment.

The difficulties in developing a sensitive analytical method for the determination of these substances lie in their polar and hydrophilic properties beside they can be present in natural water in the free phenolic form, ester form or phenolate form [18]. These problems lead from the pre-concentration and derivatization steps.

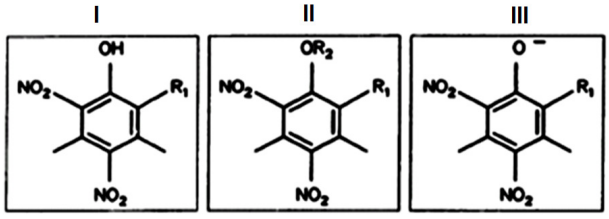

Fig. 2. 2,4-DNP presence in natural water in the free phenolic form (1), in the ester form (II), or in the phenolate form (III).

Toxicity of 2,4-DNP

2,4-DNP toxicity is related to its mechanism as an uncoupler substance [19]. In its protonated form, it can cross the membrane and operates as a carrier of H^+, resulting in the dissipation of the electrochemical gradient. The result in an uncoupling of the oxidative phosphorylation pathway but without the blockage of any consumption of oxygen. Sue to this, DNP is listed by the U.S Environmental Protection Agency in its "priority pollutants" list and has advocated in the restriction of DNP concentration to below 0.01 μg/L in natural waters [20]. Japan had set a standard for phenol (precursor of 2,4-DNP) in industrial effluents or wastewater that had undergone treatment at 5 mg/L according to Hirooka et al. [21]. Having said that, nitrophenols happen to be stated to be poisonous to marine life at concentrations below the emission standard. The toxic effects of 2,4-NDP are summarized in **Table 1**.

Table 1. Toxic effects of 2,4-DNPs.

Toxicity	Comments	Reference(s)
Cataracts	Causes clouding of the eye lenses and irritation	[22]
Hyperthermia	Body temperature rising to as high as 44°c (111°F)	[6]
Toxic blood	High concentration of DNPs in blood stream	[23]
Fever	The increase of body temperature within DNP consumption	[24]
Respiratory failure	Causes of bronchitis to develop with a cough, phlegm, and shortness of breath	[24]
Diaphoresis	Excessive sweating due to a side effect of DNPs	[6]
Multiorgan failure	Renal failure, disturbance of hormone secretion	[25]
Extreme weight loss	Uncontrollable loss of weight within a short period of time	[25]
Skeletal malformations	By oral and dermal administration in the rat	[13]
Abortions in sows	Tested orally in sows	[13]
Tachycardia	Irregular heartbeat	[7]
Tachypnoea	Rapid and irregular breathing	[6]
Disturbance to kidney	Caused by potassium accretion in the renal tissue of rabbit	[26]

Exposure to NPs in the environment can occur through a number of exposure routes – through the contaminated air, food and drinks consisted of DNP and from direct contact (skin) with the pollutants [10]. The nitrophenols can then be absorbed into the blood stream and reach organs and body tissues such as liver, kidney, eyes and brain where these nitrophenols can be transformed into more toxic metabolites through the redox reactions and conjugation with acids in the body.

Even though considered as too precarious for clinical use, the mechanistic fashion of 2,4-DNP keeps on studied as a potential methodology for obesity treatment [14, 27, 28]. Currently, research shows an increase in the study on uncoupling proteins naturally found in humans [16, 29, 30].

Degradation of 2,4-DNP by microbial species

The discharge of 2,4-DNP as xenobiotic effluents is hazardous to the environment. Furthermore, this substance is also recalcitrant because the nitro substituents of the aromatic ring provide further stability to the compounds. This ultimately slows down the physicochemical and biodegradation process for these compounds [31]. Reports also have shown that at elevated concentrations, this compound is not easily biodegraded in anaerobic studies and can inhibit the activity of methanogenic microorganisms [8, 32, 33]. In addition, NPs can be detoxified by methanogenic consortia forming aminophenols that can be toxic several orders of magnitude compared to the parent compounds [34–36]. 2,4-DNP may exist in the gaseous or particulate form in the atmosphere and cab be removed by action of photolysis, by washout or settling during precipitation. In addition, it can also reacts with photochemically-produced hydroxyl radical in the gas phase [37].

In general, DNPs effluents are removed via physical, chemical or biological treatments. Although the physicochemical means of remediation are widely effective, nevertheless they have been acknowledged to suffer from certain drawbacks such as high cost of implementation, low efficiency, introduction and formation of hazardous by-products and high energy requirements [38]. Therefore, there is a need to find alternative treatments that are effective and more economical in the removal of 2,4-DNP from the environment.

Biological approach is the most environmentally friendly method as it does not require a large amount of energy, lower operational expenses, and does not generate toxic substances and hazardous by-products [39–42]. Bacterial remediation may be used as an efficient technology to eliminate 2,4-DNP from the environment given that several bacteria have the ability to use this compounds as their sole carbon and energy sources [43–46]. Nonetheless, the effectiveness of biological treatment highly dependent on the survival, adaptability and activities of the selected microorganisms.

The exploitation of enzymatic mechanism possessed by microbial life for crucial catabolic steps in xenobiotic degradation has been the subject of interest in many research works around the globe. Efforts to isolate bacterial cultures capable of degrading 2,4-DNP started since the acknowledgements of microbial adaptability towards this xenobiotic compound. Commonly, in microbial remediation, studies were conducted on the basis of the potential of microorganisms through growth adaptation and ability to transform the said toxic compounds.

A number of microbial species are capable of the biotransformation of these phenolic compounds in a biogeochemical cycle. Arora et al. [38] have reviewed the microbial species that have been reported to degrade several groups of NPs which are highly skewed towards the bacterial communities. **Table 2** shows the list of bacterial species that are able to mineralise 2,4-DNPs.

Table 2. List of bacterial species that capable to degrade 2,4-DNP.

Bacteria	Comments	Reference(s)
Anabaena variabilis and *Anabaena cylindrica*	Photoautotrophic bacteria that able to remove 5- 150 μm 2,4-DNP from flask culture	[21]
Burkhorderia KU-46	Isolated from pesticide-contaminated agricultural soil	[47]
Haloanaerobium praevalens DSM 2228 and *Sporohalobacter marismortui* ATCC 35420	2,4-DNPs was completely transformed at concentrations of 50 to 100 mg/l while growth was gradually inhibited at the higher concentrations.	[33]
Janthinobacterium sp. and *Rhodococcus* sp.	Used as inoculum for activated sludge in bench-scale sequencing batch reactors (SBRS). The inoculum was able to utilize 10 mg/l of 2,4-DNP to varying degrees with the addition of 50, 100 and 500 mg/l glucose	[43]
Methanococcus sp. B	0.5 mm 2,4-DNPs were transformed completely after incubation for seven days with no growth inhibition occurred	[48]
Rhodobacter capsulatus E1F1	DNP was photoreduced	[49]
Rhodococcus erythropolis HL 24-1 and HL 24-2	Able to utilize 0.5 mm 2,4-dnp as sole carbon and nitrogen source under concomitant liberation of stoichiometric amounts of nitrite and 4,6-dinitrohexanoate as a minor dead-end metabolite.	[44]
Rhodococcus erythropolis HL-PM1	Used as the receptor of cell-based sensor for 2,4-DNP detection	[17, 39, 50]
Rhodococcus imtechensis RKJ300	Isolated from pesticide-contaminated soil of Punjab	[40]
Rhodococcus koreensis DNP505	Isolated from industrial effluents	[51]
Rhodococcus opacus	Able for a complete utilization of 0.27 mM and 0.54 mM 2,4-DNP within 22 and 28 h respectively	[52]
Rhodococcus sp. PN1	Able to grow on 2,4-DNP and 4-nitrophenol as a sole nitrogen source	[53]
Rhodococcus sp. RB1	Involves in two-step degradation of 2,4-DNP with 3-nitroadipate as a metabolic intermediate	[45]
Sphingomonas UG30	The strain was able to transform 90% of 103 μm 2,4-DNP into secondary metabolites with glucose-glutamate induced culture in ^{14}C-labelled study	[54]
Thiobacillus ferrooxidans nasf-1	The iron-oxidizing bacteria show promising growth on media supplemented with 2,4-DNP (10 μm) as a carbon source	[19]

Although the mechanistic approach of bacteria in degrading DNP are commonly predicted under aerobic degradation as it offers more rapid and complete degradation, the anaerobic approach also has found success in degrading this compound. Donlon et al. [35] have reported on a positive degradation of several nitrophenols including 2,4-DNP by using the upflow anaerobic sludge blankets (UASB) reactors through continuous detoxification, transformation and eventual degradation. The UASB reactors were regularly fed with a volatile fatty acid (VFA) mixture as the primary substrate. Transformation of several dinitroaromatic compounds at 50 to 300 μM in the anaerobic state by methanogenic bacteria was also described [34]. She et al. [8] also studied the biodegradability kinetics of 2,4-DNP in an anaerobic toxicity assay (ATA), with supplementation of glucose and fatty acids as co-substrates.

Mechanism of 2,4-DNP degradation

The type of metabolic pathway is depended on the culture (inoculum) and the starting substrate [55]. Due to the attachment of nitro groups in the aromatic ring, the structure of the compound is harder to deactivate. Therefore, DNP and in similar cases of trinitrophenol (TNPs), their degradation occur via the use of a successive pathway, which usually commences with the initial process of reduction of the nitro group into amino or hydroxylamino derivatives [17] and then prior to fission, an oxidative pathway occurs in which the nitro group replacement by the hydroxyl group occur before coupling into the assimilative TCA cycle [56].

In a number of bacterial strains including *Rhodococcus opacus*, it has been shown that the degradation process needs a tautomerase, which catalyses a proton shift between the act-nitro and the nitro from the 2,4,6-TNP dehydrate Meisenheimer complex. The nitrite released product is hydride Meisenheimer complex of 2,4-DNP that is then converted to the dihydride form by a hydrogenation process catalyzes by the hydride transferase I and the NADPH-dependent F_{420} reductase [57].

In the aerobic degradation of 2,4-DNP, a number of bacterial degradation pathways are examined including the creation of the equivalent amino derivatives [21]. In a consortium of cyanobacterial, biodegradation commences via the reduction of 2,4-DNP to 2-amino-4-nitrophenol by the organism *A. variabilis*. The other cyanobacterium *A. cylindrica* in the consortia then degrades the aminonitrophenol. The liberation of the nitro group from the *ortho*-position of 2,4-DNP was observed by Blasco et al. [45] who also noticed the formation of 3-nitroadipic acid in the bacterium *Rhodococcus* sp. B1. The produced metabolite was then subsequently further degraded through the removal of the second nitro group.

The utilization of 2,4-DNP as the sole carbon, nitrogen and energy sources was observed in two strains of *Rhodococcus erythropolis* (HL 24-1 and HL 24-2) isolated from soil and river water which reflects the fluidic capability of bacteria from this genus to biodegrade complicated substances. The catabolic mechanism in the two bacteria proceeds with the mineralization of 2,4-DNP via the release of nitrite ions with the formation of a minor dead-end product—4,6-dinitrohexanoate (DNH) [44]. *Burkholderia* sp. strain KU-46 utilizes 2,4-DNP as both carbon and nitrogen sources, and was isolated by Iwaki et al. [47] who found that biodegradation occur through the formation of 4-nitrophenol and benzoquinone. A degradation pathway of 2,4-DNP via the hydride Meisenheimer complex of 2,4-DNP, 2,4-dinitrocyclohexane was reported by Ghosh et al. [40], and DNH in the bacterium *Rhodococcus imtechensis* RKJ300. **Fig. 3** shows the simplified schematic scheme of 2,4-DNP degradation. While the anaerobic bacterial degradation of 2,4-DNP is still not well understood, studies on the identification and characterization of anaerobic degradation by bacterial species was reported [8,33]. Similar to aerobic degradation, anaerobic transformation involves the reduction of DNP into corresponding aminophenols but with the use of methanogenic and photosynthetic anoxygenic bacteria.

CONCLUSIONS AND PERSPECTIVES

Microbial flora and fauna are a crucial part of natural ecosystems. Due to their ability to survive in restrictive conditions, they are widely discovered in industrially contaminated soils and waters. From the view of environmental aspect, ability of microorganisms to swiftly and effectively decontaminate the environment from NPs contamination is significant and crucial, with the major purpose to shield the living environment and human health from these contaminants.

In addressing this topic theoretically, the study of mineralization mechanism involved in the catabolism of 2,4-DNP is substantial. Furthermore, from a technological point of view, it can be aimed at the progressive means by recent modern and effective treatment.

First of all, current study and analysis based on the application of molecular approaches and application of the strains that have the capacity to transform this hazardous chemical contaminant to a lesser degree could lead to a new scheme that will optimize the technologies that will be used in the future. Besides, studies on specificity of 2,4-DNP biodegradation by different microbial strains can be profound for the comparison of one strain to another. This might eventually guide proper and effective remediation technologies for industrial wastes where the NPs substrates are a common occurrence. The increased interest in the current research works is apparent by the gradual growth of studies and publications on the identification, characterization, and analysis of the isolated microbial strains that encompass the metabolic potential to degrade and transform this compound.

Secondly, although the genetic and biochemical characterization of several 2,4-DNPs degradation pathways have been extensively studied in bacteria, a number of fundamental elements regarding the chemotaxis, field bioremediation and biodegradation haven't yet been investigated. Even though current works and endeavors have demonstrated an extraordinary and fast developments in understanding the bioremediation possibilities of 2,4-DNP-degrading bacterial strains, a number of queries have been brought up in the direction of this issue that should not be ignored and overlooked. Such questions for example what are going to occur to the indigenous microbial flora and fauna when NP-degrading bacteria was enhanced and whether or not NPs-degrading bacteria are able to break down NPs at NP-contaminated sites under uncontrolled environmental aspects have invariably been questioned regarding this subject matter. These kinds of queries and concerns demonstrate that more work is still essential to totally know the amalgamated process of bioremediation of NPs by bacteria.

One critical aspect of DNPs microbial remediation is the bacterial chemotactic responses toward NPs. To date, the data and information on bacterial chemotaxis toward NPs is very limited. While few bacteria reported to exhibit chemotaxis in

regards of NPs and possess the genes responsible for chemotactic responses, yet no present data that shows a proper identification of this subject. Along with appropriate identification and characterization of chemotaxis genes in NPs-degrading bacteria, an understanding into exactly how NPs are being degraded by bacteria will yield important clues and knowledge for the complete bioremediation of these toxic compounds. The knowledge of the biodegradation processes at the molecular level, which also involves bacterial chemotaxis will be the central elements for the success of bioremediation of these compounds.

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