Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Review

Recent advances in nitroaromatic pollutants bioreduction by electroactive bacteria

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ARTICLE INFO

Keywords:
Nitroaromatic
Nitrobenzene
Bioreduction
Electroactive bacteria
Electron transfer

ABSTRACT

Nitroaromatic compounds (NACs) are widely used in modern industry and are considered as harmful pollutants due to their high toxicity and mutagenesis. Moreover, as the NACs contain one or more electron-withdrawing nitro-groups, they are resistant to biodegradation and are classified as recalcitrant pollutants. Thus, reduction of the nitro groups in NACs to form more biodegradable derivatives was considered as the most important step for NACs removal. Interestingly, it was found that some bacteria are able to reduce the nitro groups in NACs, and bioreduction was proved as a cost-effective way for NACs reduction. Particularly, the electroactive bacteria (EAB) with high NACs reduction efficiency have been identified recently. In this review, the transformation pathways for NACs bioreduction and the identified EAB species are summarized. Furthermore, the electron transfer pathways and strategies for augmentation on NACs bioreduction with EAB are highlighted. The research directions and perspectives for NACs bioreduction by EABs are also discussed.

1. Introduction

Nitroaromatic compounds (NACs) are widely used chemicals with various applications such as synthesis of pesticides, explosives, dyes, polymers and other intermediates [1–3]. With the development of the NACs related industries, their emissions into the environment increased year by year. In recent years, it was found that NACs were prevalent in surface water, groundwater, and soils all over the world [2,3]. More seriously, some NACs were even detected in some common foods, which dramatically threaten the human health [3]. Most NACs are proved as mutagenic and carcinogenic agents towards human beings and could cause serious health problems. For example, nitrobenzene (NB), one of the simplest model NACs, may cause several health problems such as methemoglobinemia, anemia, hepatic toxicity and liver damage [4]. It was found that the NB treatment dramatically enhanced the tumor rates at concentration of 5 ppm [5]. Nitropyrenes (NP) including mononitropyrenes and dinitropyrenes are also widespread carcinogenic and mutagenic agents [6]. Similarly, 2,4,6-trinitrotoluene (TNT), which was widely used as explosive, has also been reported to cause cytotoxic effects on the liver and spleen [7]. It was also found that TNT and its derivatives have the possibility to form hemoglobin adducts, which results in genotoxicity and potential carcinogenicity [6]. In addition, the toxicity of NACs is also associated with their transformed intermediates or products generated during nitro group reduction. Some of the intermediates are highly toxic (e.g., the hydroxylamino derivatives) which would readily interact with DNA and result in mutagenesis [8].

So, it is essential to develop strategies that can efficiently remove NACs from the environment. Several physical methods such as sonolysis [9], radiolysis [10], and adsorption [11], have been developed for NACs removal. Among these strategies, adsorption of NACs with granular activated carbon (GAC) is considered as one of the efficient, cost-effective, and practical methods. It has been successfully applied for removal of NACs from explosives manufacturing plants [12], however the adsorption with GAC encountered the risk of secondary pollution as the adsorbed NACs were not mineralized. Meanwhile, chemical methods including advanced oxidation processes [13] and photolysis [14] have also been developed for NACs degradation in aerobic or anaerobic environment. However, as the NACs contain one or more electron-withdrawing nitro-groups, they showed high

Abbreviations: NACs, nitroaromatic compounds; EAB, electroactive bacteria; NB, nitrobenzene; NP, nitropyrenes; TNT, 2,4,6-trinitrotoluene; GAC, granular activated carbon; 2,6-DNT, 2,6-dinitrotoluene; CNTs, carbon nanotubes; AQS-PUF, anthraquinone-2-sulfonate-modified polyurethane; BC/biochar, foam black carbon and biochar; GO, graphene oxide; DIET, direct interspecies electron transfer; BES, bioelectrochemical system; CAP, chloramphenicol; ET, electron transport; NAD(P)H, nicotinamide adenine dinucleotide phosphate; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide

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https://doi.org/10.1016/j.procbio.2018.04.019
Received 28 March 2018; Received in revised form 27 April 2018; Accepted 28 April 2018
1359-5113/

resistance to directly oxidative degradation. Nevertheless, once the nitro-groups are reduced to amino-groups at anaerobic condition, the electron-withdrawing effect could be eliminated, and the degradation of the amino-derivatives is much more feasible to various aerobic or anaerobic biological methods.

Therefore, the bioreduction of nitro-groups at anaerobic condition was considered to be an efficient way to overcome the recalcitrance of NACs, including chemical, electrochemical, and biological reduction, which became the first consideration and indispensible step in NACs treatment [15]. Thus, different chemical methods have been developed for NACs reduction. For example, different reductive reagents with the noble metal catalysts such as palladium have been used for nitro-groups reduction [16]. However, this chemical reduction process required expensive noble metal catalysts and excessive reductive reagents, limits the practical application in wastewater treatment. It was also found that the nitro-groups of NACs could be electrochemically reduced by cathode electrode, which offered another simple alternative to replace the conventional chemical reduction [15,17]. More impressively, it was found that some bacteria could efficiently reduce the nitro-groups of NACs [18–21]. As the NACs bioreduction usually occurred at the anaerobic digestion process which required minimum energy and cost input compared with chemical or electrochemical reduction methods. Thus it is considered as the most efficient method for NACs reduction [22,23]. Particularly during recent decades, it was found that electro-active bacteria (EAB) with unique electrons rewiring mechanisms had great potential for efficient NACs bioreduction. In this review, recent progresses on NACs bioreduction with EABs including the bioreduction pathways, bacterial species and electron transfer pathways are presented and discussed.

2. Bioreduction pathways for NACs

Nitrobenzene (NB) is the most commonly used model compound for NAC bioreduction research. The main pathways for NB reduction by bacteria are illustrated in Fig. 1. The NB is firstly reduced to nitrosobenzene by nitroreductase. It was found that there were two types of bacterial nitroreductase (type I and type II) that can reduce the NB to nitrosobenzene with two electrons pathway (pathway I) or single electron reduction pathway (pathway II). Nitrosobenzene is further reduced to hydroxylaminobenzene with a much faster reaction rate than the nitrosobenzene formation step. Thus, hydroxylaminobenzene is the common intermediate compound that can be detected during most of the bacterial reduction processes [8]. Further, there are three main pathways for hydroxylaminobenzene transformation (Fig. 1). The most common pathway is the transformation of hydroxylaminobenzene to aniline, which is less toxic and more biodegradable than NB [22,24]. Similarly, it was found that several bacteria prefer to transform hydroxylaminobenzene to 2-aminophenol that other than aniline. For example, *Pseudomonas pseudoalcaligenes* JS45 could reduce the NB to nitrosobenzene, hydroxylaminobenzene, and 2-aminophenol by hydroxylaminobenzene mutase [25]. It is interesting as it is difficult to prepare hydroxylated aromatics chemically. Another interesting pathway is the transformation of hydroxylaminobenzene to catechol [25]. It was found that 3, 4-dihyroxybenzoate could be identified as the 4-nitrobenzoate bio-reduction product for *Comamonas acidovorans* NBA-10. The result suggested that the hydroxylamino derivate could be biotransformed to dihydroxyl derivate, which could be considered as another new pathway for NAC bioreduction although the enzyme responsible for this reaction is still unclear [26,27].

For other polynitroaromatics, the reduction pathways are similar to the NB bioreduction [8]. The nitro groups were initially reduced to their hydroxylamino derivatives by nitroreductase with broad substrate specificity, and then transformed into its amino derivates as the end products. However, more nitro groups on the aromatic ring resulted in higher stability of the NACs and decreased the bioreduction efficiency. According to previous reports [1,8], it is believed that the NACs

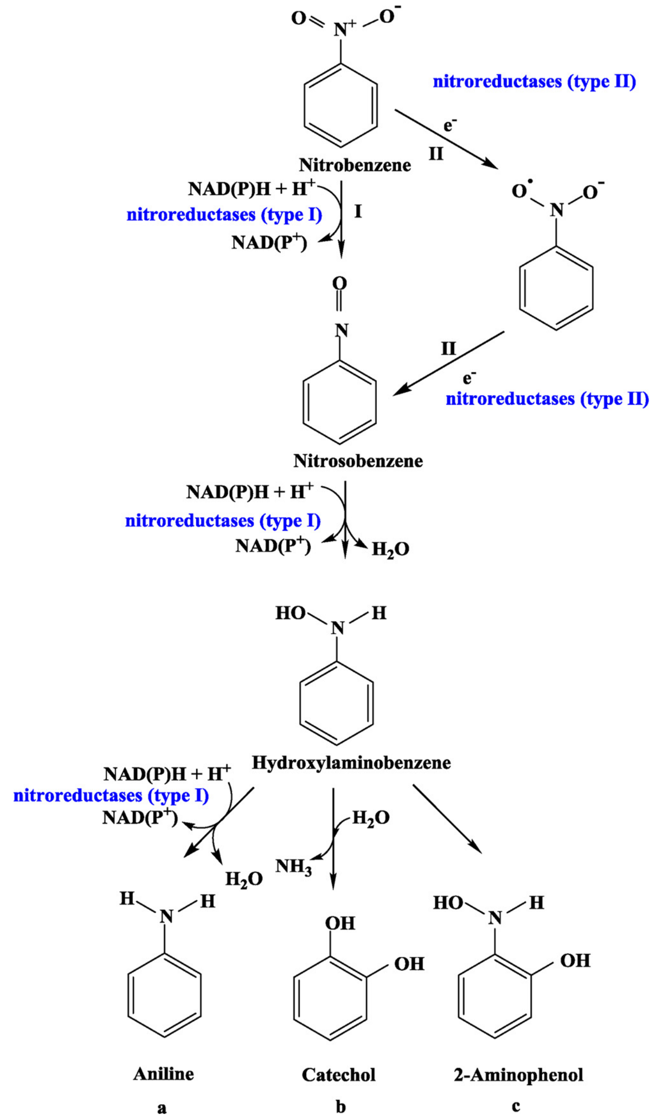

Fig. 1. Proposed transformation pathway for microbial reduction of nitrobenzene.

reduction by EABs is carried out with the pathways similar to that presented in Fig. 1.

Nitroreductase was considered as the key enzyme for NACs bioreduction, which could reduce a lot of nitro-group compounds with cofactor of NAD(P)H, FAD or FMN. It was found that there were two types of bacterial nitroreductases that can reduce NB to nitrosobenzene, including type I (oxygen-insensitive) and type II (oxygen-sensitive) [28,69]. A lot of type I nitroreductase genes, such as nfsA and nfsB in *Escherichia coli*, have been successfully cloned and characterized [28]. In *Shewanella* species, the oxygen-insensitive NAD(P)H nitroreductase NfnB has the ability to reduce 2, 6-DNT [44]. The type II nitroreductase is difficult to be purified and characterized, because it is sensitive to oxygen and to be inactive under aerobic condition. Therefore, the nitroreductase in NACs reduction has not been fully characterized. The detailed NACs transformation pathways and key enzymes/genes for NACs reduction are still unclear even in the model EABs such as *Shewanella oneidensis*, which deserved more detailed investigation.

3. NACs bioreduction by EAB

EAB is a kind of unique bacterial group that can exchange the intracellular electrons with the extracellular compounds or interfaces that