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Anaerobic Metabolism of Nitroaromatic Compounds by Sulfate-Reducing and Methanogenic Bacteria

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ABSTRACT

Ecological observations suggest that sulfate-reducing and methanogenic bacteria might metabolize nitroaromatic compounds under anaerobic conditions if appropriate electron donors and electron acceptors are present in the environment, but this ability had not been demonstrated until recently. Most studies on the microbial metabolism of nitroaromatic compounds used aerobic microorganisms. In most cases no mineralization of nitroaromatics occurs, and only superficial modifications of the structures are reported. However, under anaerobic sulfate-reducing conditions, the nitroaromatic compounds reportedly undergo a series of reductions with the formation of amino compounds. For example, trinitrotoluene under sulfate-reducing conditions is reduced to triaminotoluene by the enzyme nitrite reductase, which is commonly found in many Desulfovibrio spp. The removal of ammonia from triaminotoluene is achieved by reductive deamination catalyzed by the enzyme reductive deaminase, with the production of ammonia and toluene. Some sulfate reducers can metabolize toluene to CO_2. Similar metabolic processes could be applied to other nitroaromatic compounds like nitrobenzene, nitrobenzoic acids, nitrophenols, and aniline. Many methanogenic bacteria can reduce nitroaromatic compounds to amino compounds. In this paper we review the anaerobic

metabolic processes of nitroaromatic compounds under sulfate-reducing and methanogenic conditions.

INTRODUCTION

Many xenobiotic chemicals introduced into the environment for agricultural and industrial use are nitro-substituted aromatics. Nitro groups in the aromatic ring are often implicated as the cause of the persistence and toxicity of such compounds. Nitroaromatic compounds enter soil, water, and food by several routes such as use of pesticides, plastics, pharmaceuticals, landfill dumping of industrial wastes, and the military use of explosives. The nitroaromatic compound trinitrotoluene (TNT) is introduced into soil and water ecosystems mainly by military activities like the manufacture, loading, and disposal of explosives and propellants. This contamination problem may increase in future because of the demilitarization and disposal of unwanted weapons systems. The disposal of obsolete explosives is a problem for the military and the associated industries because of the polluting effect of explosives in the environment (80). Concerns about the environmental fate of TNT residues have intensified because the recent vegetation of contaminated plots could allow TNT, TNT metabolites, and plant-produced TNT intermediates to be introduced into the food chain (33).

The toxicity of TNT is well documented (39,79). Past methods for disposing of munitions wastes have included dumping in the deep sea, dumping at specified landfill areas (36), and incineration when quantities were small. All of these methods may cause serious harm to ecosystems. For example, incineration causes air pollution, and disposal on land may lead to soil and groundwater contamination that will affect aquatic life forms, humans, and animals. The alternative may be to remove TNT through biological process. Biotransformation of TNT by aerobic bacteria in the laboratory has been reported frequently (11,12,18,40,46,51,55,67,78). Biodegradation of 2,4-dinitrotoluene by a Pseudomonas sp. has been reported to occur via 4-methyl-5-nitrocatechol in a dioxygenase-mediated reaction (62). White rot fungus has been shown to mineralize radiolabeled TNT (24). The work of Spiker et al. (63) showed that Phanerochaete chrysosporium is not a good candidate for bioremediation of TNT contaminated sites containing high concentration of explosives because of its high sensitivity to contaminants. Michels and Gottschalk (48) showed that the lignin peroxidase activity of P. chrysosporium is inhibited by the TNT intermediate hydroxylamino-dinitrotoluene. Valli et al. (72) found that 2,4-dinitrotoluene is degraded completely by the white rot fungus. Under anaerobic conditions, the sulfate-reducing bacterium, Desulfovibrio sp. (B strain) transformed TNT to toluene (7,8,53) by reduction followed by reductive

deamination. Gorontzy et al. (28) reported that under anaerobic conditions, methanogenic bacteria reduced nitrophenols and nitrobenzoic acids. Duque et al, (20) successfully constructed a Pseudomonas hybrid strain that mineralized TNT. However, the degree of mineralization was not significant. The bioremediation of TNT-contaminated soil by composting (76) has many limitations because large amounts of additives are needed restrict the volume of soil treated. Funk et al. (26) showed potential removal of TNT under anaerobic conditions with potato starch as carbon source. The bacterial metabolism of various other nitroaromatic compounds has been reported by many workers (17,19,21,29,30,31,32,35,38,50,54,60,61,62,82). Recently Williams et al. (77) showed a novel pathway for the catabolism of 4-nitrotoluene by Pseudomonas with the formation of protocatechuate and ß-carboxy-cis,cis-muconate.

Most of the aerobic studies described above showed only superficial modification of the TNT molecule and not decomposition. The initial steps in the metabolism of TNT appear to involve a stepwise reduction of nitro groups, through nitroso and hydroxylamino groups to amino groups (66). Anaerobic metabolism of nitroaromatics may provide a treatment solution for contamination with nitro compounds. Recently Boopathy et al. (8,13) described the ability of sulfate-reducing and methanogenic bacteria to metabolize TNT under different growth conditions. In this paper, we review

progress in the study of anaerobic metabolism of TNT and its usefulness in treating nitroaromatic compounds.

Anaerobic Transformation of Nitroaromatics

Mono- and dioxygenase enzymes present in aerobic bacteria can insert either one or two oxygen atoms into a substrate and can degrade aromatic molecules. Anaerobes clearly unable to use oxygen with oxygenases overcome this metabolic problem by combining a dehydrogenation reaction with hydration of the substrate. For example, benzoic acid is converted to pimelic acid by Rhodopseudomonas palustris by the series of dehydrogenation and hydration reactions as shown in Fig. 1 (34,37).

The anaerobic bacterial metabolism of nitroaromatics has not been studied as extensively as of aerobic pathways, perhaps because of the difficulty in working with anaerobic cultures and perhaps the slow growth of anaerobes. Earlier studies on anaerobic metabolism of nitroaromatic compounds by McCormick et al. (46) laid the foundations for such study and established the usefulness of anaerobic organisms. Successful demonstration of degradation of hexahydro-1,2,3-trinitro-1,3,5-triazine (RDX) by sewage sludge (47) under anaerobic conditions further demonstrated the usefulness of anaerobes in waste treatment. RDX was reduced sequentially by the anaerobes to the nitroso derivatives, which were

further converted to formaldehyde and methanol. Hallas and Alexander (32) showed successful transformation of nitrobenzene, nitrobenzoic acid, nitrotoluene, and nitroaniline by sewage sludge under anaerobic conditions.

The studies described above did not use specific anaerobic conditions. Boopathy et al. (9) showed that TNT can be transformed under anaerobic conditions by using different electron acceptors. A soil sample collected from the Joliet Army Ammunition Plant was incubated under sulfate-reducing, nitrate reducing and methanogenic conditions. The results showed that TNT was transformed under all three conditions. However, when no electron acceptor was supplied no TNT was transformed (Table 1). The intermediates observed during the study were 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene. This study showed that if the appropriate electron acceptor is present in the system, anaerobic bacteria will reduce TNT to amino compounds.

Sulfate-Reducing Bacteria

The most common types of anaerobic respiration are summarized in Fig. 2. Although oxygen is the most widely used electron acceptor in energy metabolism, a number of different kinds of bacteria are able to reduce other compounds and hence use them as electron acceptors. This process of anaerobic respiration is less energy efficient,

but it allows these bacteria to live in environments where oxygen is absent.

Sulfate-reducing bacteria are obligate anaerobes that are conveniently considered together because of their shared ability to perform dissimilatory sulfate reduction, a process analogous to aerobic respiration in that the sulfate ion acts as an electron acceptor, like oxygen in the aerobic process. The genera of sulfate reducers are defined on the basis of morphology rather than physiology. All sulfate reducers are gram positive, except Desulfotomaculum. The most frequently encountered genus is Desulfovibrio.

The use of various non-fermentable aromatic compounds in the absence of oxygen or nitrate is apparently of the natural roles of sulfate-reducing bacteria. Aromatic compounds with more than two hydroxyl groups are readily degraded by fermenting bacteria (75). Several new types of sulfate-reducing bacteria have been isolated directly with aromatic compounds (2,57,74). Most of these isolates are extremely versatile sulfate reducers that use many aliphatic compounds. Aromatic compounds oxidized by sulfate-reducing bacteria include benzoate, phenol, p-cresol, aniline, and the n-heterocyclic compounds like nicotinate, indole, and quinoline. All the known degraders of aromatic compounds are complete oxidizers. The sulfate reducers employ reactions like those detected in denitrifying bacteria, phototrophic

bacteria, and methanogenic co-cultures using aromatic compounds (5,23,71). The principal reactions recognized in non sulfate-reducing bacteria are activation of benzoate to benzoyl CoA (27), caroboxylation of phenol to p-hydroxybenzoate (42,70) or the reductive removal of hydroxyl groups (68).

Metabolism of TNT by Sulfate-Reducing Bacteria

Boopathy and coworkers (7,8) recently showed that a sulfate-reducing bacterium, Desulfovibrio sp. (B strain) can convert TNT to toluene. This organism isolated from an anaerobic digester treating furfural-containing waste water (6), used nitrate as electron acceptor apart from using sulfate as electron acceptor. It also used nitrate as a nitrogen source. Further experiments showed that this bacterium could use the nitrite group present in TNT molecules either as an electron acceptor or as a nitrogen source.

Some sulfate-reducing bacteria can use nitrate in addition to sulfate as their terminal electron acceptor (41). The reaction is coupled to electron transfer phosphorylation (64) and is catalyzed by a respiratory nitrite reductase that has a molecular mass of 65 KDa and contains six c-type hemes. This nitrite reductase known as the hexaheme cytochrome C3 is widely distributed in strict and facultative aerobes

(44,45). This nitrite reductase is unrelated to the regulated nitrite reductase (nonheme iron siroheme containing) found in many plants and bacteria (73), where its function is nitrogen assimilation. As shown in Fig.3, oxidation of H_2 by nitrite is coupled to scalar and vectorial proton transport. According to Steenkamp and Peck (64), nitrite reductase is closely associated with a hydrogenase and is probably a transmembrane protein. This conclusion is based on the presence of proton-releasing and nitrite-binding sites on the periplasmic aspect of the cytoplasmic membrane and a benzyl viologen-binding site on the cytoplasmic side of the membrane.

As Fig.4 shows, added TNT (100 mg/L) was metabolized by Desulfovibrio sp. (B strain) within 10 days (8), with pyruvate as the main substrate, sulfate as the electron acceptor and TNT as the sole nitrogen source. Boopathy et al. (8) showed that under different growth conditions that this bacterium used TNT as its sole source of nitrogen. This result indicates that the isolate has the necessary enzymes to use the nitrite groups present in TNT molecules as a nitrogen source.

Apart from pyruvate, lactate served as the best substrate for TNT metabolism, followed by $H_2 + CO_2$, ethanol, and formate. Comparison of the rate of TNT biotransformation by Desulfovibrio sp. with that of other sulfate-reducing

bacteria showed that this new isolate has a unique metabolic ability to degrade TNT. As shown in Table 2, Desulfovibrio sp. transformed 100% of TNT present in a relatively short period of time (7 days). Other Desulfovibrio spp. (ATCC cultures) converted 59-72% TNT within 21-23 days, whereas Desulfobacterium indolicum transformed 82% of TNT in 36 days of incubation.

Mass spectral analyses showed that various intermediates were produced depending upon the culture conditions of the isolate (Table 3). When ammonium was the main nitrogen source, 2,4-diamino-6-nitrotoluene was the major intermediate. When TNT was the sole source of carbon and energy, it was first reduced to 4-amino-2,6-dinitrotoluene and then to 2,4-diamino-6-nitrotoluene. When TNT was the sole source of nitrogen, all the TNT in the medium was converted to 2,4-diamino-6-nitrotoluene within 10 days of incubation and traces of 2- and 4-amino compounds were identified. Later these intermediates were converted to toluene. The quantitative analysis of the aqueous and gas phases of the culture bottle by GC (equipped with a purge and trap unit) showed a good mass balance of TNT. From the initial 100 mg/L of TNT, 30 mg/L toluene was found in the gas phase and 68 mg/L toluene was identified in the liquid phase (8).

Nitroaromatic compounds are considered resistant to microbial attack (25,30), partly because the reduction of

electron density in the aromatic ring by the nitro groups can hinder electrophilic attack by oxygenases and thus prevent aerobic degradation of nitroaromatic compounds (17). Under anaerobic conditions, the sulfate-reducing bacteria metabolized TNT. Of all the metabolites produced, the formation of toluene from TNT seems to be very significant in the biotreatment of soil and water contaminated with TNT. The mass balance of toluene further indicates that TNT conversion was nearly stoichiometric.

TNT was reduced to diamino-nitrotoluene by the isolate through the 2-amino- and 4-amino-dinitrotoluenes when pyruvate served as the main substrate in the presence of sulfate and ammonia (Fig. 5), in a simple reduction process carried out by the enzyme nitrite reductase. Most Desulfovibrio spp. have nitrite reductase enzymes that reduce nitrate to ammonia (74). This isolate reduced the nitrite groups present in TNT to amino groups. When TNT served as the sole source of nitrogen, toluene was formed from the TNT. Figure 6 elaborates a general pathway for the transformation of TNT, involving the initial reduction of aromatic nitro groups to aromatic amines. The presence of diamino-nitrotoluene as a major intermediate in the initial period of incubation suggests that two nitro groups of TNT are reduced to amino groups by nitrite reductase via the formation of 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene. This diamino-nitrotoluene is further reduced to

triaminotoluene. McCormick et al. (46) showed that TNT was reduced by H_2 in the presence of enzyme preparations of Veillonella alkalescens to triaminotoluene: 3 mol H_2 is required to reduce each nitro group to the amino group. Recently Preuss et al. (53) observed the formation of triaminotoluene from TNT by a sulfate reducing bacterium isolated from sewage sludge.

Boopathy et al. (8) speculated the formation of triaminotoluene by reduction. This was reductively deaminated to toluene. In the process, the isolate used the ammonium released from the original TNT molecule as a nitrogen source for growth. A toluene concentration of 98 mg/L was observed in the culture sample, and virtually no nitrite ions were detected during TNT metabolism. The aromatic ring structure was not cleaved, and no metabolites other than toluene appeared even after six months of incubation. Reductive deamination is catalyzed by a deaminase enzyme in Pseudomonas sp. (49). Reductive deamination reactions were postulated first for 2-aminobenzoate degradation by methanogenic enrichment cultures (69). Reductive dehydroxylation of gentisate to benzoate and acetate was demonstrated in the fermenting bacterium HQGO1 (65).

According to Preuss et al. (53), the nitro groups in positions 2 and 4 are easily reduced by the sulfate-reducing

bacteria in a reaction that appears to be rather unspecific and is probably mediated by enzymes that reduce low-potential electron carriers like ferredoxin. The rate-limiting step is the reduction of the nitro group in position 6 of the intermediate 2,4-diamino-6-nitrotoluene. The first reduction step, leading to the formation of 2,4-diamino-6-hydroxyl-aminotoluene is also catalyzed by ferredoxin-reducing enzymes like hydrogenase and carbon monoxide dehydrogenase. This reaction is considered to be rather unspecific because it also occurs with reduced ferredoxin or methyl viologen in the absence of enzyme. The rate of reduction in the presence of the enzyme is higher by order of magnitude than in its absence, indicating an additional involvement of the enzyme in the reaction. Preuss et al. (53) suggested that converting 2,4-diamino-6-nitrotoluene to triaminotoluene involves two reduction steps. The first is catalyzed by hydrogenase or carbon monoxide dehydrogenase, and the second is mediated by an enzyme especially active in the sulfate-reducing bacteria. The formation of the intermediate diamino-hydroxyl-toluene in the presence of carbon monoxide led to the conclusion that the enzyme was inhibited or inactivated by carbon monoxide. An enzyme especially active in sulfate-reducing bacteria and known to react with carbon monoxide is the dissimilatory sulfite reductase (43). The work of Boopathy et al. (8) suggested that some sulfate-reducing bacteria have the enzyme reductive deaminase, which is responsible for converting triaminotoluene to toluene by

deaminating all the amino substituents in the aromatic ring. Beller et al. (4) and Edwards et al. (22) demonstrated the complete mineralization of toluene under sulfate-reducing conditions. These toluene degrading sulfate reducers could be used in combination with the Desulfovibrio sp. described by Boopathy et al (8) to degrade TNT completely to CO_2.

The very limited literature available on the metabolism of TNT by sulfate-reducing bacteria suggests that under anaerobic conditions the best candidates for bioremediation of TNT are sulfate reducers. Much basic work remains to be done in elucidating the enzyme mechanisms and studying the continuous and semi-continuous modes of reactor operation for treating nitroaromatic compounds with sulfate reducers.

Anaerobic Removal of Other Nitroaromatics by Sulfate-Reducing Bacteria

Test of its ability to metabolize various nitroaromatics showed that the Desulfovibrio sp. (B strain) can metabolize 2,4-dinitrophenol (2,4-DNP), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), and aniline. As shown in Fig. 7 the Desulfovibrio sp. used all the nitroaromatics studied as a sole source of nitrogen. It also used 2,4-DNT, 2,6-DNT and 2,4-DNP as electron acceptors in the absence of sulfate (10). The GC/MS analyses of the culture samples showed the presence of phenol from 2,4-DNP and benzene from aniline as

intermediates. The quantitative analyses of the gas and aqueous phases showed nearly stoichiometric conversion of these intermediates. As described earlier where TNT was reduced and reductively deaminated to toluene, a similar mechanism may be used by the bacterium to metabolize 2,4-DNP. 2,4-DNP might have been first reduced to diaminophenol and this diaminophenol reductively deaminated to phenol. Similarly the amino group in the aniline was reductively deaminated to yield benzene and the ammonia released from the aromatic ring was used by the bacterium as a nitrogen source. Gorontzy et al. (28) showed transformation of nitrophenols and nitrobenzoic acids by the sulfate reducers Desulfovibrio desulfuricans, D. gigas, Desulfococcus multivorans, and Desulfotomaculum orientis. All of the nitroaromatics were transformed to corresponding amino compounds.

Schnell et al. (57) isolated a new sulfate-reducing bacterium, Desulfobacter anilini, which degraded aniline completely to carbon dioxide and ammonia with stoichiometric reduction of sulfate to sulfide. This is the first obligate anaerobic bacterium observed to grow in pure culture with aniline as its sole electron donor and carbon source. The organism oxidizes aniline completely to carbon dioxide and releases the amino nitrogen quantitatively as ammonia. Two metabolic pathways were suggested. First, aniline could be carboxylated to 2-aminobenzoate or 4-aminobenzoate, with the aminobenzoate then reductively deaminated to benzoate and

metabolized further (81). Alternatively, aniline could be deaminated hydrolytically to phenol, which is subsequently degraded either by carboxylation to 4-hydroxybenzoate or by reductive transformation to cyclohexanol or cyclohexanone. Both pathway appear possible, because the bacterial strain used each of these intermediates as a sole source of carbon.

Schnell and Schink (58) reported that Desulfobacterium anilini degraded aniline via reductive deamination of 4-aminobenzoyl CoA (Fig. 8). The first step, the carboxylation of aniline to 4-aminobenzoate, is followed by activation of 4-aminobenzoate to 4-aminobenzoyl CoA, which is reductively deaminated to benzoyl CoA. This product enters the normal benzoate pathway leading to three acetyl CoA. Carbon monoxide dehydrogenase and formate dehydrogenase are present in Desulfobacterium anilini indicating that acetyl residues are oxidized via the carbon monoxide dehydrogenase pathway (56).

Schnell and Schink (59) isolated a sulfate-reducing bacterium that oxidized 3-aminobenzoate to carbon dioxide with concomitant reduction of sulfate to sulfide and release of ammonium. High activity of carbon monoxide dehydrogenase indicated that acetyl CoA is oxidized via the carbon monoxide dehydrogenase pathway, although 2-oxoglutarate synthase activity was found as well. Similar activity was found with pyruvate as substrate. Perhaps both synthase activities can

be attributed to an enzyme needed in assimilatory metabolism. Carbon monoxide dehydrogenase and pyruvate synthase are probably also key enzymes during autotrophic growth with hydrogen and sulfate. The complete oxidation of 3-aminobenzoate yields -186 kJ per mole according to the following equation:

$$2\ C_7H_6NO_2^- + 7\ SO_4^{2-} + 11\ H^+ \text{-------}> 14\ CO_2 + 2\ NH_4^+ + 7\ HS^- + 4\ H_2O \quad \Delta G_o' = -180\ kJ$$

The first step in degradation of 3-aminobenzoate by this new sulfate-reducing bacterium was found to be activation to 3-aminobenzoyl CoA (59). Further reduction of 3-aminobenzoyl CoA did not yield benzoyl CoA, but rather a product tentatively described as a reduced CoA-ester. The activation of benzoyl CoA depends on the presence of the cofactors, ATP and Mg^{2+}. Acyl-CoA synthetase reactions were identified as the initial step in the degradation of benzoate by anaerobic bacteria.

Methanogenic Bacteria

Methanogens are obligate anaerobes that grow in an environment with an oxidation-reduction potential of less than -300 mV. They transform various substrates to C1 products such as CH_4 and HCOOH. The role of some novel

compounds and the mechanism of single carbon flow in these bacteria remain to be formally proved, along with the arrangement of the electron transport chain. Because of the limited substrate capabilities, the metabolism of more complex molecules to methane depends on the activity of non methanogens in association with the methanogens. Under pure culture conditions, methanogens have not been reported to degrade aromatic compounds.

Biotransformation of Nitroaromatics by Methanogenic Bacteria

The studies of Gorontzy et al. (28) on microbial transformation of nitroaromatic compounds by methanogenic bacteria revealed that methanogens can transform nitroaromatic compounds to corresponding amino compounds. Boopathy and Kulpa (13) isolated a methanogen Methanococcus sp. from a lake sediment which transformed TNT to 2,4-diaminonitrotoluene. Its action on various nitroaromatic compounds (14) is summarized in Table 4. All the compounds tested were transformed by the methanogen. The intermediates observed were amino derivatives of the parent compounds. Comparison of the metabolic activities of the Methanococcus sp. with those other methanogenic bacteria suggest that the range of species for which transformation of nitroaromatics has been demonstrated extends to a new Methanococcus sp. According to some reports, the reductive transformation of

nitroaromatic compounds leads to detoxification of the substance (3,15). The specific enzymes responsible for the reduction process in methanogens is not yet characterized. Angermeier and Simon (1) suggested that the reduction of aromatic compounds may be catalyzed by hydrogenase and ferredoxin. The amino intermediates produced by the Methanococcus sp. did not undergo further degradation, even after 60 days of incubation. This result suggests that the reduction of nitro substituents by an unspecific detoxification reaction mediated by certain enzymes or cofactors present in the methanogens.

This review indicates that, under anaerobic conditions, the combining of sulfate reducers and methanogens with other toluene-degrading sulfate reducers will enhance the complete metabolism of nitroaromatic compounds. A co-culture study of all these organisms together or the sequential removal of the compounds under separate specific conditions will confirm the feasibility of using specific anaerobes to remediate contaminated sites.

Conclusions and Implications

The observation of sulfate reducers and methanogenic bacteria by many workers (7,8,13,14,28,53) suggests that these organisms could be exploited for bioremediation under

anaerobic conditions by supplying proper electron donors and electron acceptors.

The first step in the metabolism of nitroaromatics is reduction. This step is followed by reductive deamination, which removes all the nitro groups present in the ring, leaving the ring intact and forming toluene and ammonia as end products. The toluene can be further degraded by toluene degrading denitrifiers or toluene degrading sulfate-reducing bacteria.

Several reports on the aerobic transformation of TNT have shown the production of dead-end products like amino derivatives or azoxy compounds. Therefore, the applicability of aerobes in bioremediation of nitroaromatics-contaminated sites is doubtful at present. However, the use of anaerobes like sulfate-reducing bacteria may prove useful in decontaminating environmental sites polluted with nitro compounds.

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Table 1 Biotransformation of TNT under anaerobic conditions[a]

Growth Conditions	Bacterial Growth Protein (mg/L)	% TNT Removal	Intermediates Produced
Nitrate reducing	85	82	2- and 4- amino dinitrotoluenes
Sulfate reducing	18	30	2- and 4- amino dinitrotoluenes
Methanogenic	19	35	2- and 4- amino dinitrotoluenes
No specific electron acceptors	0	0	None

[a] Data from Ref. 9

Table 2 Comparison of TNT metabolism among different sulfate reducers[a]

Bacteria	% TNT transformed and days in paranthesis	Metabolites Produced
Desulfovibrio vulgaris	72 (23)	2,4-Diamino-6-nitrotoluene
D. gigas	59 (22)	2,4-Diamino-6-nitrotoluene
D. desulfuricans	66 (21)	2,4-Diamino-6-nitrotoluene
Desulfobacterium indolicum	82 (36)	2,4-Diamino-6-nitrotoluene
Desulfovibrio sp. (B strain)	100 (7)	4-Amino-2,6-dinitrotoluene 2-Amino-4,6-dinitrotoluene 2,4-Diamino-6-nitrotoluene Toluene

[a] Data from Ref. 8

Table 3 Metabolites of TNT under different growth conditions for *Desulfovibrio* sp. (B strain)[a]

Growth Condition	Metabolites Produced
Pyruvate as electron donor, Sulfate as electron acceptor, ammonium as nitrogen source in the presence of TNT	2-Amino-4,6-dinitrotoluene 4-Amino-2,6-dinitrotoluene 2,4-Diamino-6-nitrotoluene
Pyruvate as electron donor, TNT as electron acceptor, ammonium as nitrogen source	2,4-Diamino-6-nitrotoluene
TNT as sole source of carbon and energy, sulfate as electron acceptor, ammonium as nitrogen source	4-Amino-2,6-dinitrotoluene 2,4-Diamino-6-nitrotoluene
Pyruvate as electron donor, Sulfate as electron acceptor, TNT as nitrogen source	2-Amino-4,6-dinitrotoluene 4-Amino-2,6-dinitrotoluene 2,4-Diamino-6-nitrotoluene Toluene

[a] Data from Ref. 8

TABLE 4 Transformation of nitroaromatic compounds by methanogenic bacteria*[a]

Organism	Transformation of Nitroaromatic Compounds (%)#					
	2,4-DNT	2,6-DNT	DNP	TNB	DNB	TNT
Methanococcus strain B	98	95	85	100	98	100
Methanococcus deltae	75	65	50	70	55	20
Methanococcus thermolithotrophicus	50	55	40	65	40	10
Methanosarcina barkeri	0	0	0	0	0	0
Methanobacterium thermoautotrophicum	0	0	0	0	0	0
Methanobrevibacter ruminantium	0	0	0	0	0	0

[a] Data from Ref. 14

* Cultures were incubated for 30 days.

Concentration of nitroaromatics used was 0.5 mM.

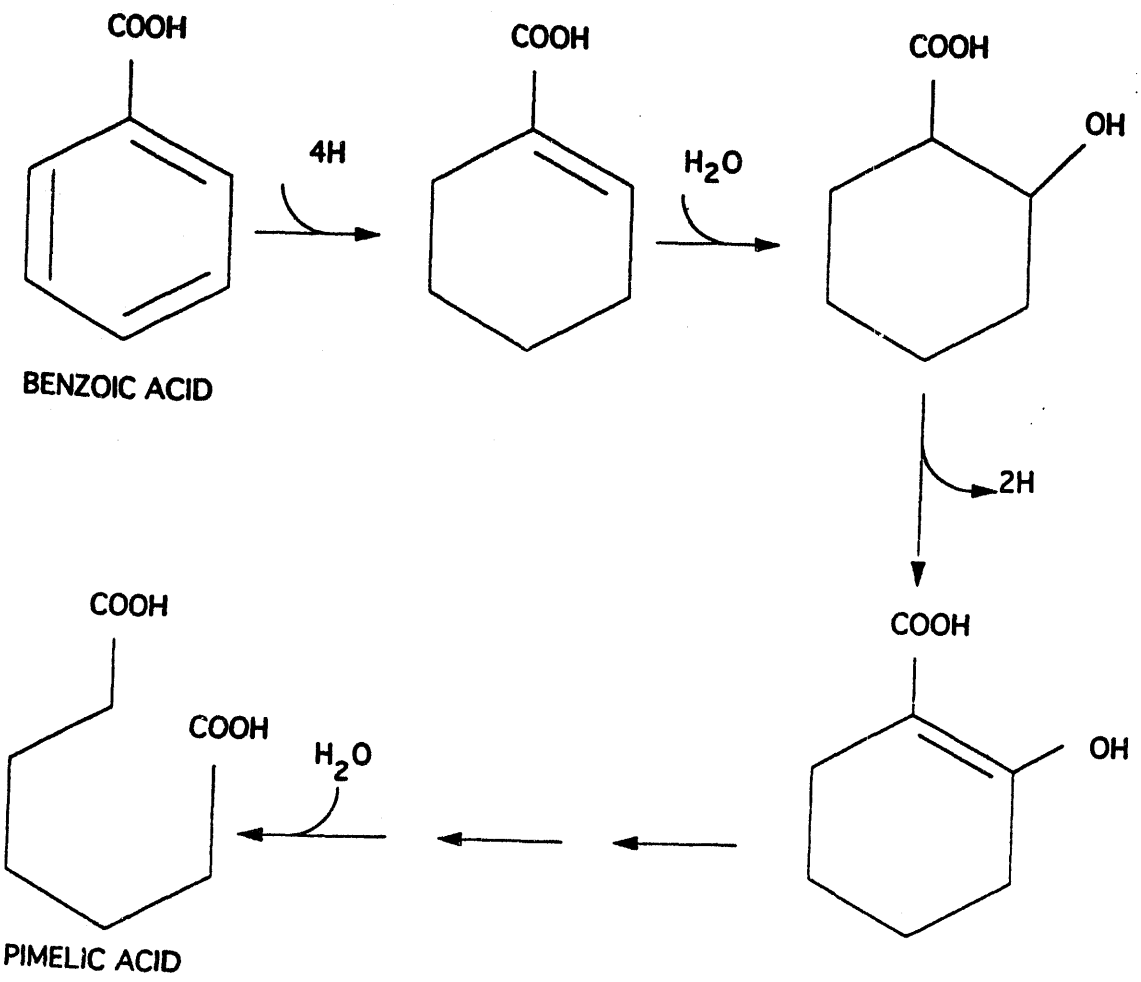

Figure 1. Degradation of benzoic acid by R. palustris. Adapted from Ref. 37.

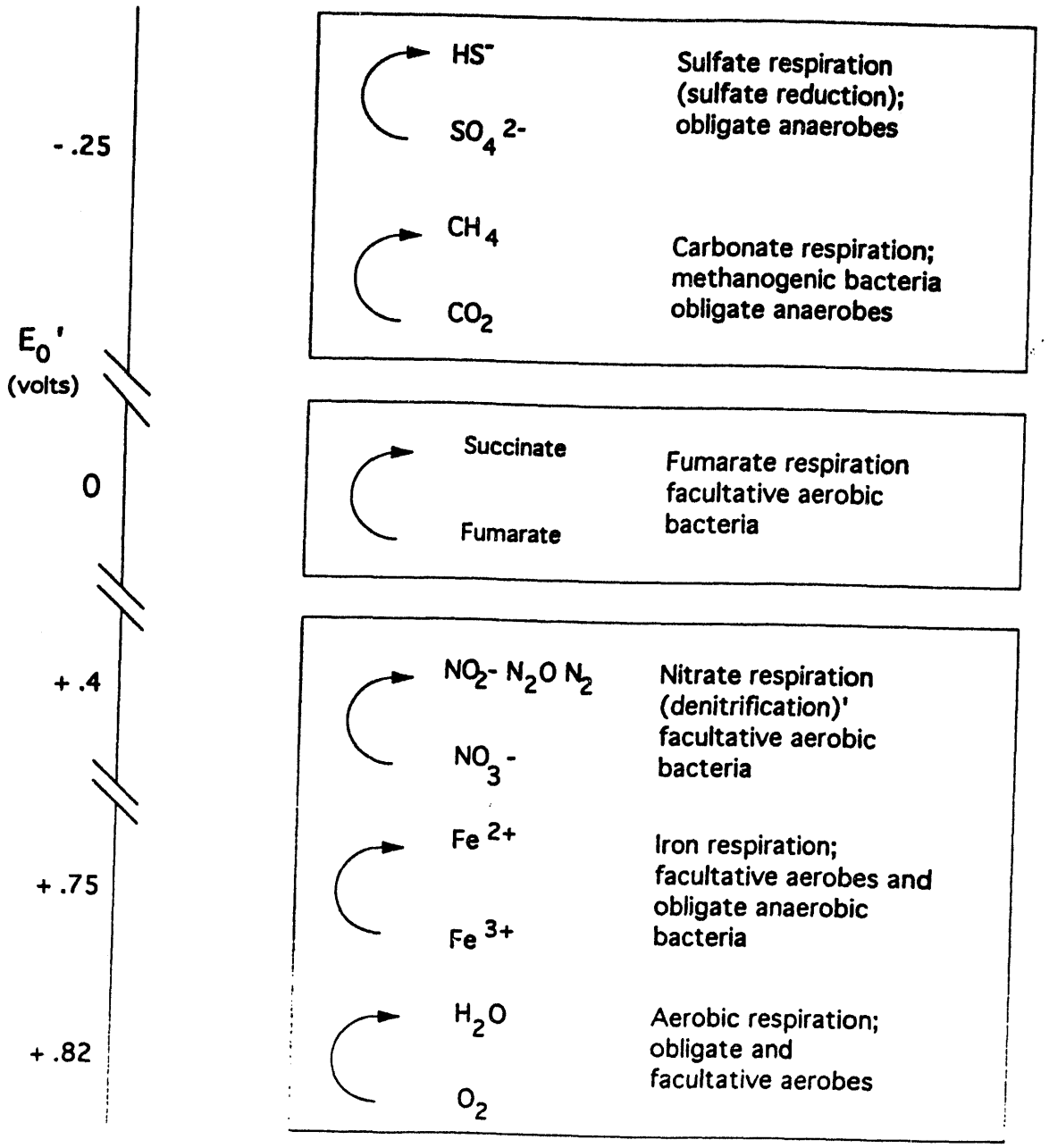

Figure 2. Various anaerobic respiration processes. Adapted from Ref. 16

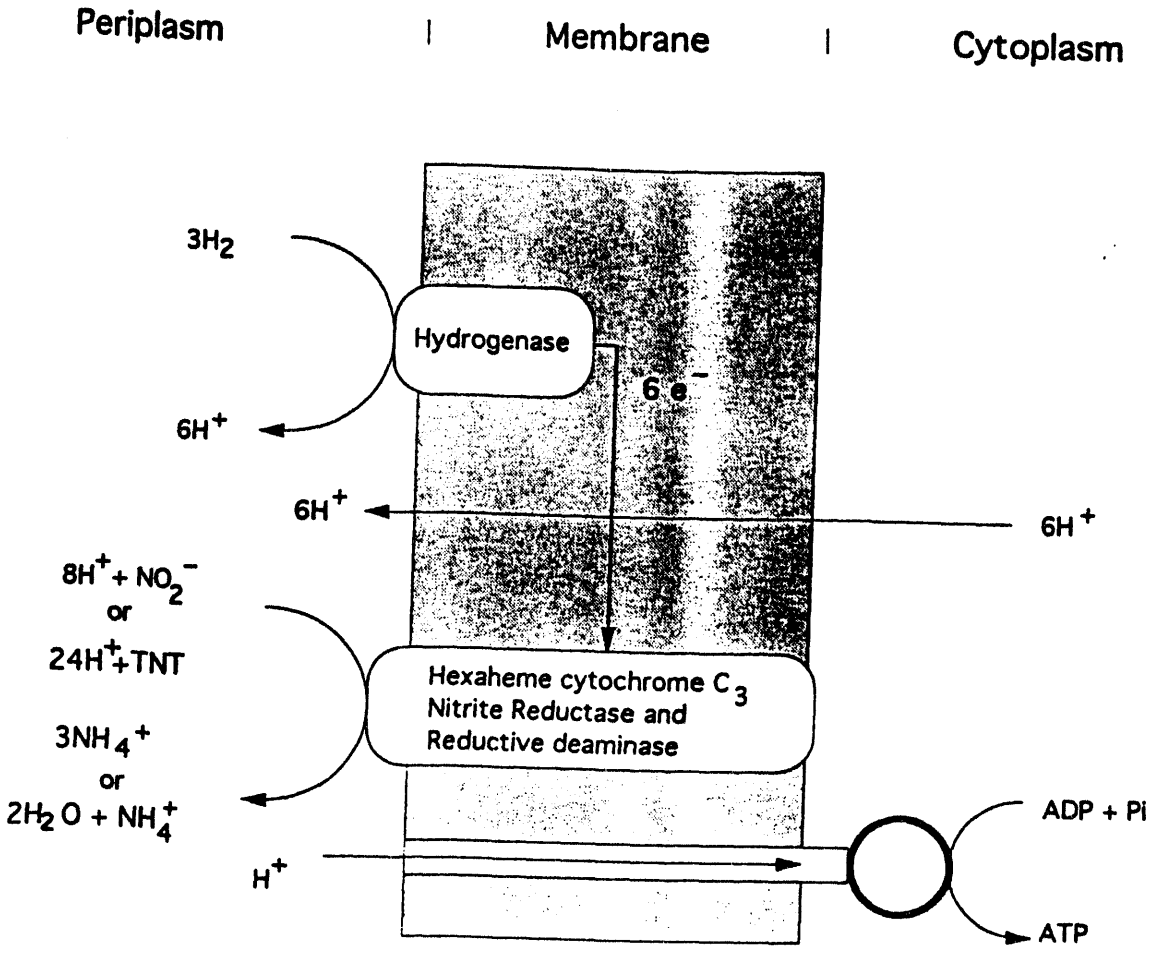

Figure 3. Scalar and vectorial electron transport coupled to oxidation of hydrogen with nitrite or TNT. Adapted from Ref. 52

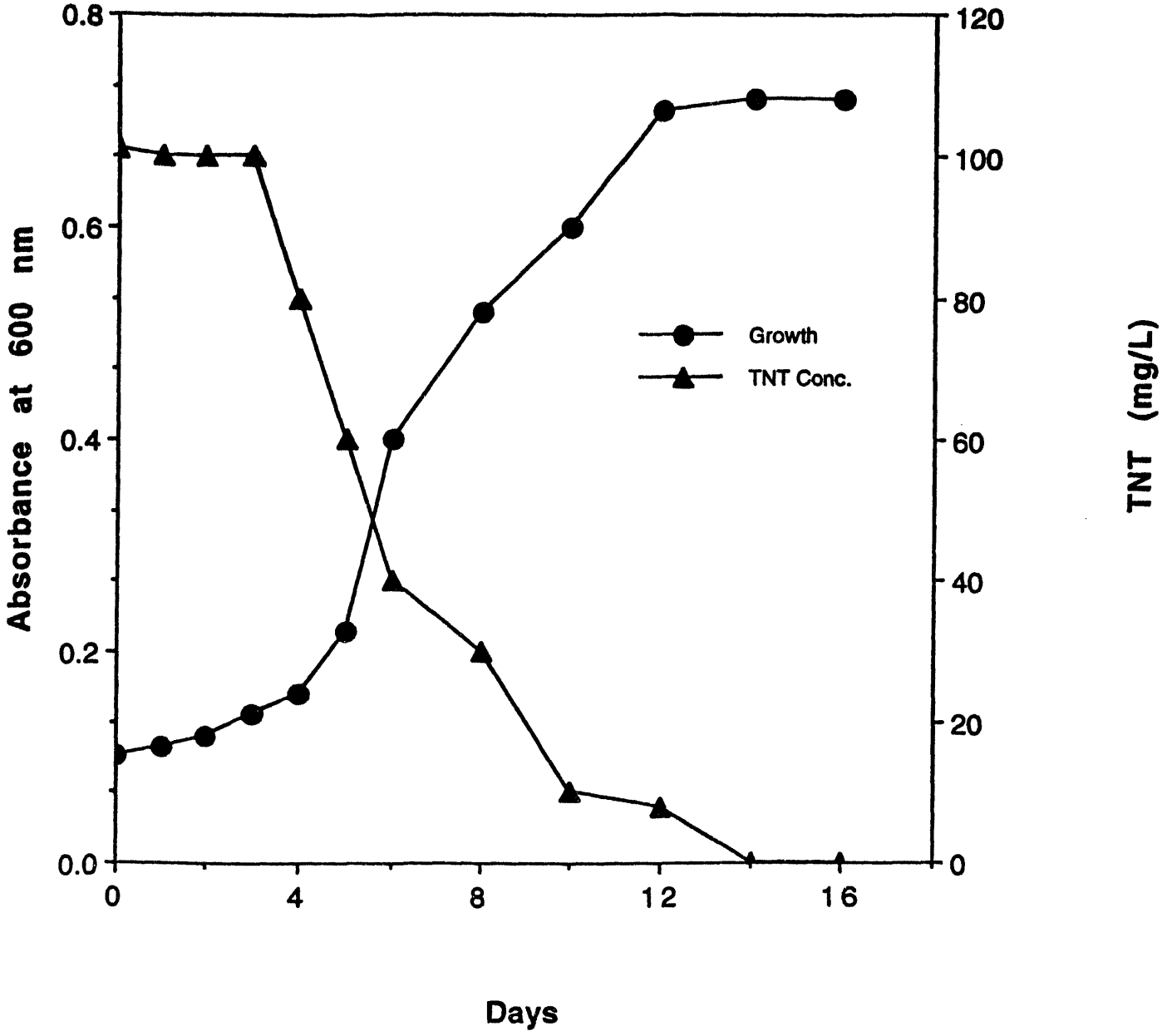

Figure 4. Metabolism of TNT by Desulfovibrio sp. (B strain), with TNT as the sole source of nitrogen.

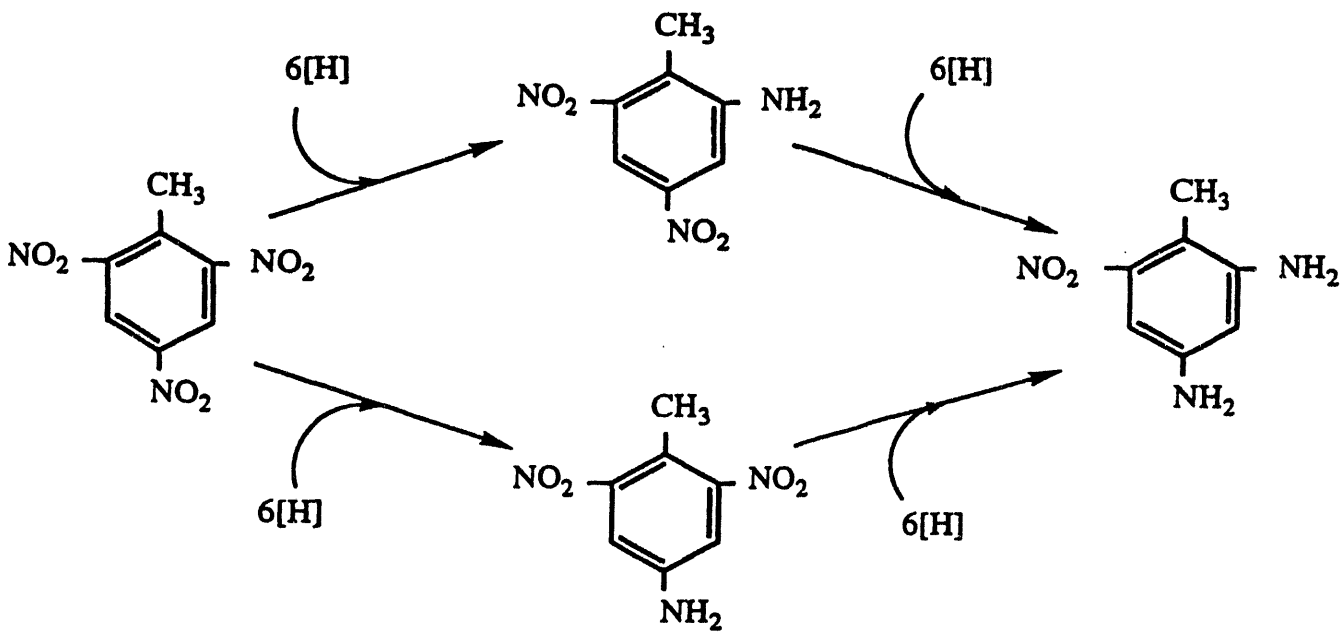

Figure 5. Transformation of TNT to 2,4-diamino-6-nitrotoluene by *Desulfovibrio* sp. (B strain), with ammonium as the nitrogen source. Adapted from Ref. 8.

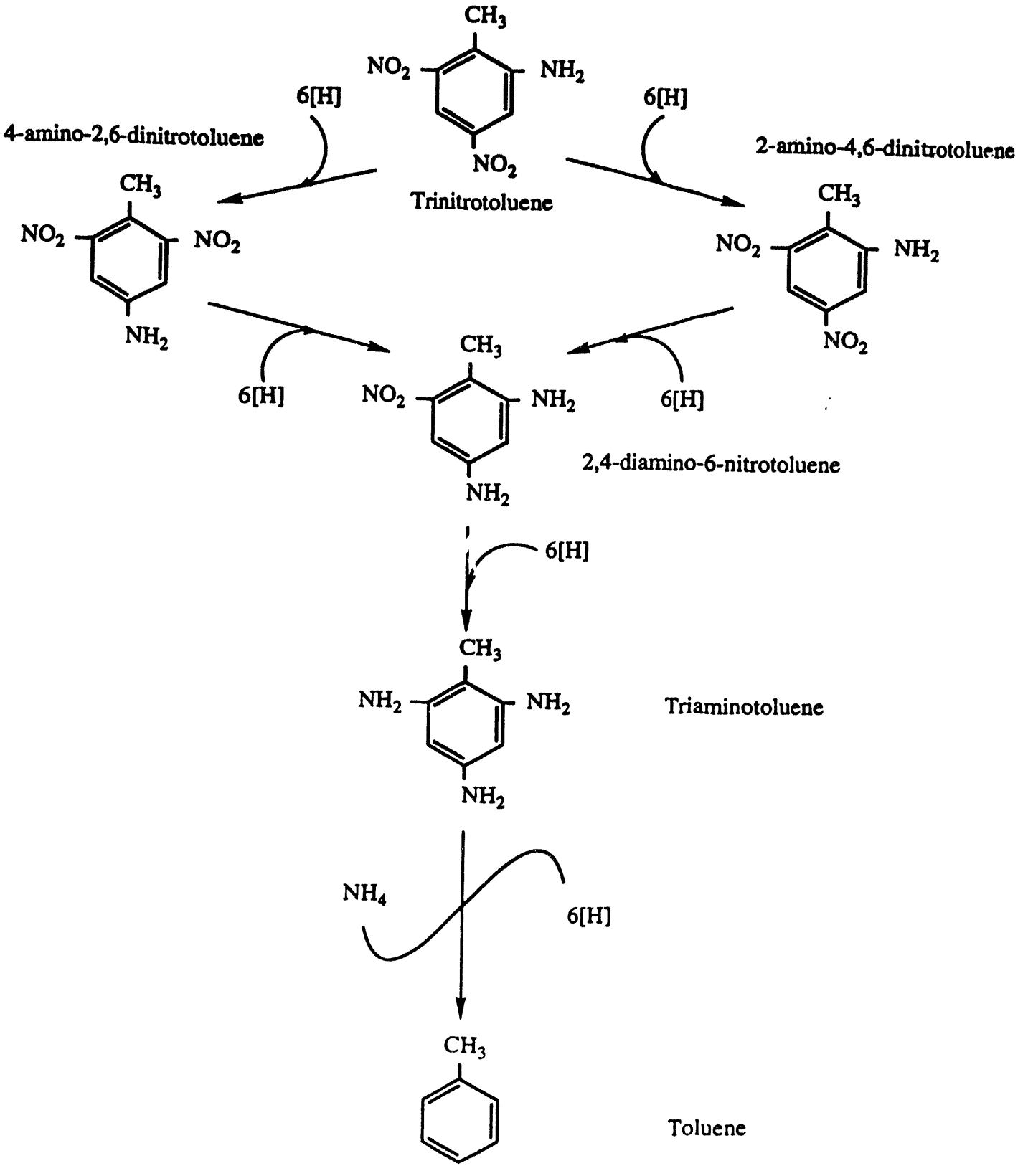

Figure 6. Metabolism of TNT by *Desulfovibrio* sp. (B strain), with TNT as the sole nitrogen source. Adapted from Ref. 8.

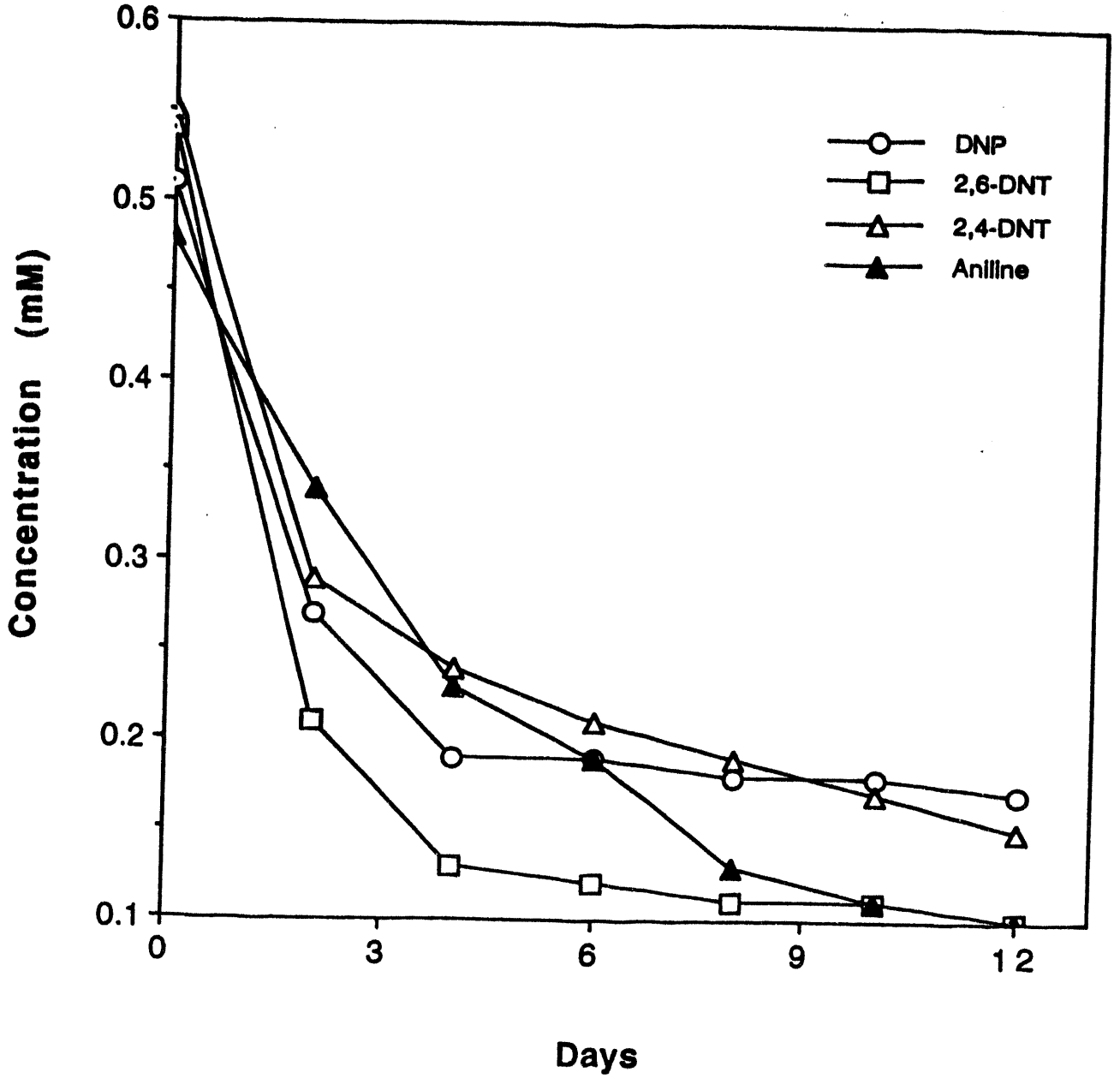

Figure 7. Metabolism of nitroaromatics by Desulfovibrio sp. (B strain) with nitroaromatics served as the sole nitrogen source.

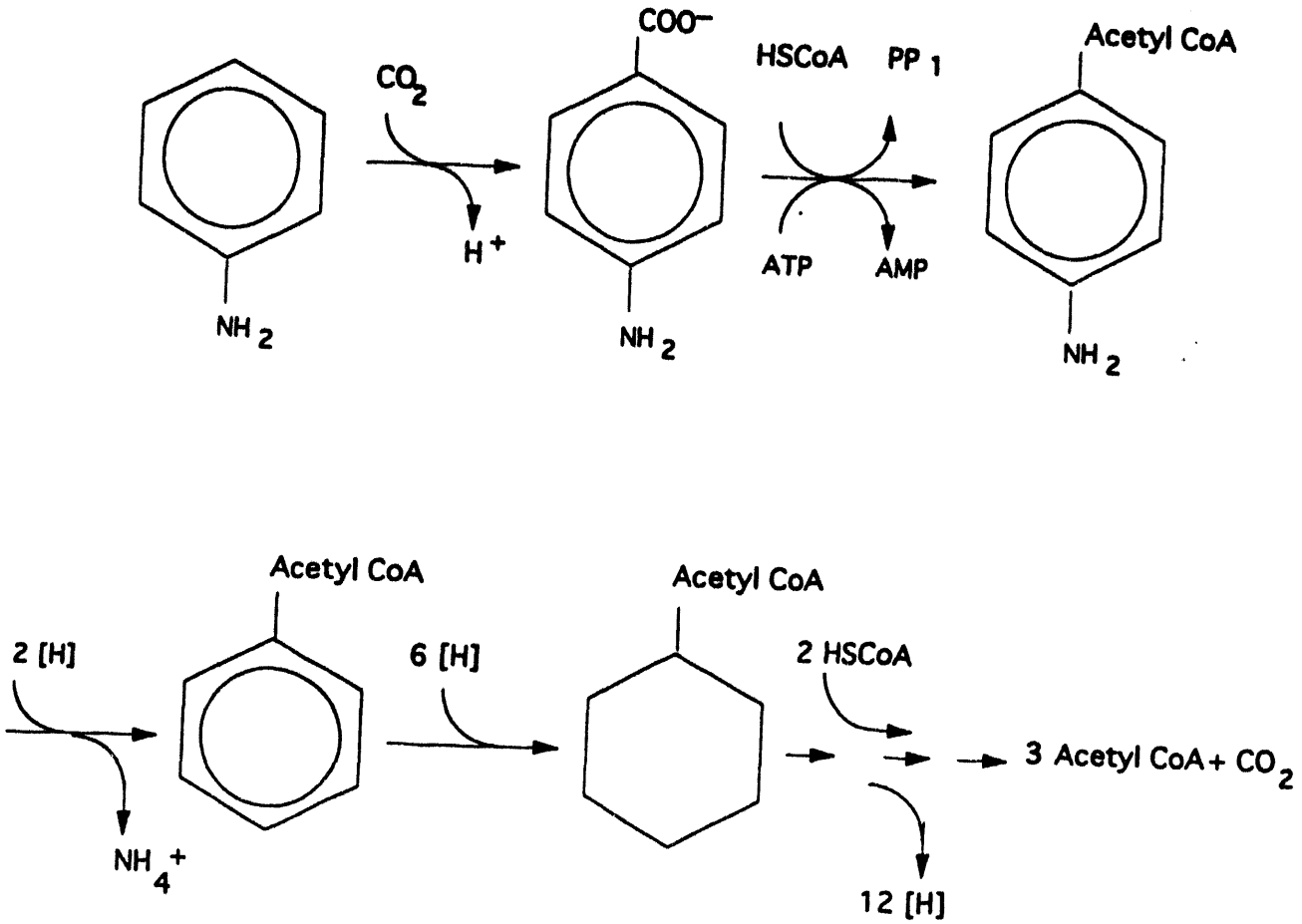

Figure 8. Pathway of anaerobic aniline degradation by *Desulfobacterium anilini*. Adapted from Ref. 58

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