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微生物修复含能材料污染场地的研究进展

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摘　要: 微生物修复技术是通过高耐受且人工驯化微生物的代谢作用将污染场地中的有害污染物降解的修复技术。提高微生物降解效率、探究代谢途径与中间代谢产物是含能材料污染场地的微生物修复的关键,为此,研究简述了含能材料的污染现状,介绍了修复含能材料污染场地的常见方法,探讨了微生物修复含能材料污染场地的优势,总结了微生物修复含能材料污染场地的常见菌株、外部营养源和实际应用,同时梳理了各含能材料在微生物降解过程中产生的中间代谢产物,归纳了典型含能材料降解过程中的代谢途径,最后展望了微生物在修复含能材料污染场地的发展方向:研究具有优良生物刺激效果的生物制剂,分析降解含能材料微生物菌株的宏基因组学、宏转录组学、宏蛋白质组学和宏代谢组学,加强DNA、RNA、蛋白质和代谢物等方面的研究,通过转基因技术来提升微生物对含能材料的降解效果,以更好地激发微生物修复含能材料污染场地的潜力。

关键词: 微生物修复;含能材料;污染场地;代谢途径;生物刺激
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0　引 言

含能材料是一种重要的化学能源材料,广泛应用于工农业建设生产和军事等领域[1]。在生产、运输、储存和销毁等环节,含能材料都有可能释放到环境中,并在土壤-水体-生物体系中进行一系列的迁移转化,污染周围地区的土壤和水体[2-3]。目前,应用较多的含能材料主要有硝基芳香族含能材料梯恩梯(TNT),和环状硝铵类含能材料黑索今(RDX)、奥克托今(HMX)等[4]。

RDX、TNT是对人类有潜在致癌性的C类致癌物,HMX是D类致癌物,即对人类无致癌性,但吸入体内会损害肝脏和肾脏等,自然条件下难降解[5-7]。人体意外吸入TNT会引发贫血,影响生殖系统,而人体接触RDX和HMX等环状硝铵类含能材料后会心律失常,引发癫痫,甚至会影响生命[8-12]。因此,RDX、TNT和HMX的环境效应倍受关注。含能材料污染场地的常用修复方法有物理法(吸附法)、化学法(光解法、电解法、超临界水氧化法)和生物修复法(植物修复法和微生物修复法)等[13-15]。微生物修复法在成本和安全性等方面有着较大的优势,热度不断提升。

为此,本研究在简述含能材料污染现状的基础上,介绍了修复含能材料污染场地的常见方法,说明了微生物修复含能材料污染场地的优势,概述了微生物在含能材料污染场地修复中的实际应用和最新进展,归纳了微生物降解含能材料过程中产生的中间代谢产物和代谢途径,指出了现存问题并对未来发展进行了展望。

1　含能材料污染现状

TNT、RDX和HMX等常见含能材料的化学性质如表1所示,它们的污染现状已有不少相关报道[16-17]。国际上,Boopathy等[18]检测到美国乔利特陆军弹药厂周围土壤的TNT浓度最高可达12000 mg·kg^{-1},RDX浓度达1100~4000 mg·kg^{-1}。爱荷华州伯灵顿的爱荷华陆军弹药厂的土壤也已经完全被RDX等含能材料

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表1 常见含能材料的化学性质[3,31]

Table 1 Chemical properties of common energetic compounds[3,31]

energetic compounds	chemical properties	chemical formulae	CAS number	melting point / ℃	boiling point / ℃	aqueous solubility / mg·L^{-1} at 25 ℃
nitroaromatics	TNT	$C_7H_5N_3O_6$	118-96-7	80.3	240.0	130.0
nitramines	RDX	$C_3H_6N_6O_6$	121-82-4	205.5	234.0	59.8
nitramines	HMX	$C_4H_8N_8O_8$	2691-41-0	276.0	906.1	5.0

污染，土壤中RDX的平均浓度达到了7000 mg·kg^{-1}[19]。到十九世纪末，美国军队造成含能材料污染的土壤已经超过了120万吨，在路易斯安那州明登的路易斯安那陆军弹药厂的土壤中，TNT、RDX和HMX浓度分别高达10000，1900 mg·kg^{-1}和900 mg·kg^{-1}[20]。1945年，美国和德国成为最大的TNT生产国，每条生产线的规模可达50吨，每年大量的土壤被TNT污染[21]。此外，相关研究表明在军事训练基地周围的表层土壤和地下水中也可以检测到大量的含能材料污染。1993年，美国国防部公布了1000多个被含能材料污染的场所，其中95%以上的场地污染物为TNT，87%的污染场地周围的地下水已经超过了正常的地下水污染水平[22]。1997年，加拿大和加利福尼亚州奥德堡等地区的反坦克靶场周围检测出大量HMX和TNT等[23]。2000年，加拿大103个国防训练场地被TNT污染，TNT在部分地点土壤和水中的浓度分别高达200 g·kg^{-1}和100 mg·L^{-1}[21]。2001年，美国11000个场所被列为硝基芳香族含能材料污染场地[24]。2003年，美国对爱德华兹营地军事靶场进行了一次彻底的污染物调查，发现军事靶场周围的地下水和土壤当中有着大量的HMX、TNT等含能材料及其转化产物[25]。2008年，加拿大国防部和国家生态环境科学研究所从可能被含能材料污染的场所当中收集了土壤、植被、地下水和地表水样本，发现大部分土壤样本被TNT、RDX和HMX等含能材料污染[26]。再有，未及时处理的废旧弹药同样对周围环境有着很大的影响，一枚破裂的M72火箭附近提取的土壤中HMX、TNT和RDX浓度分别高达10400，358 mg·kg^{-1}和46 mg·kg^{-1}[27]。截至2016年，加拿大的TNT污染场地超过1000个，美国的TNT污染场地超过2000个，占地面积超过9000万亩，污染场地中约87%的地下水遭到污染，30个污染场地被美国环境保护署列入优先控制名单[21-28]。相比之下，国内军事基地周围地区污染的报道较少，目前仅有山西太原、吉林敦化和中国北方某些地区的含能材料销毁地能够检测到TNT、RDX和HMX等，其中TNT的污染程度尤为严重，浓度最高达34083 mg·kg^{-1}[29-30]。

世界各国对含能材料的污染程度有不同的标准，美国环境保护署土壤（住宅）风险浓度对TNT、RDX和HMX等含能材料的标准限值分别为21，5.8 mg·kg^{-1}和3900 mg·kg^{-1}，加拿大环境初步土壤质量指南对TNT、RDX和HMX等含能材料的标准限值分别为3.7，4.7 mg·kg^{-1}和32 mg·kg^{-1}，国内参照美国环保署土壤（住宅）风险浓度对各含能材料污染设立了标准限值[30]。表2列出了各国受军事影响土壤的地表和地下土壤中含能材料的浓度，由表2可知，各国都有不少的含能材料污染场地，部分场地的污染浓度甚至达到了标准限值的百倍，国内外可查含能材料污染的研究记录，TNT、RDX和HMX的最大污染浓度高达87000，74000 mg·kg^{-1}和5700 mg·kg^{-1}[31]。

HMX，RDX，TNT有较高的水溶性，依次为5.0，59.8，130 mg·L^{-1}，TNT和RDX易渗透到土壤表层；HMX和其他污染物以复合污染累积在表层土壤，再通过渗流带迁移到地下水[38]，它们的污染迁移途径具体如图1所示。由图1可以看到，人类通过不同途径接触这些含能材料污染场地的污染物，包括摄入、吸入和皮肤直接接触，以及食用来自污染场地的动物或植物的间接接触。HMX，RDX，TNT等含能材料的化学性质较为稳定，降解缓慢，即使生产基地停产50年，其周围环境中仍有高浓度的污染，并向邻近社区的供水系统迁移[39]。含能材料及其同源物的毒性、诱变性和致癌潜力，使得迫切需要修复被污染场地。

2 微生物修复方法优势、应用及其基因组学研究

2.1 微生物修复含能材料污染场地的优势

目前，对于TNT、RDX和HMX等含能材料污染场地的修复，通常采用吸附法[27,40]、光解法[41]、电解法[42-43]、超临界水氧化法[44]、植物修复法和微生物修复法等（图2），这些方法现已有不少的应用实例。吸

表 2 既有研究中受军事影响土壤的地表和地下土壤中含能材料的浓度

Table 2 Concentrations of energetic compounds in surface and subsurface soils of military affected soils in reviewed studies

country	activity	c(TNT) / mg·kg^{-1}	c(RDX) / mg·kg^{-1}	c(HMX) / mg·kg^{-1}	reference
Canada	military base area (surface soil samples)	79,000	ND	ND	[32]
Canada	military base area (surface soil samples)	40–500,000	1.4–6000	20–1470	
U.S., Canada	military base area (surface soil samples)	0.78–36	5.6–51	0.53–9.1	[33]
U.S.	military base area (surface soil samples)	4000–10,000	800–1900	600–900	[34]
U.S.	military base area (surface soil samples)	2–6	0.5–1	489–874	[35]
U.S.	military base area (surface soil samples)	1.5–15,100	4.4–7.5	1.0–1.8	[36]
U.S.	military base area (surface soil samples)	130	340	40	[37]
China	military base area (surface soil samples)	10,700–26,100	18.43–37.80	0.16–9.01	[28]
China	military base area (surface soil samples)	120–167,000	20–72,000	180–238,000	[30]

Note: ND means not detected.

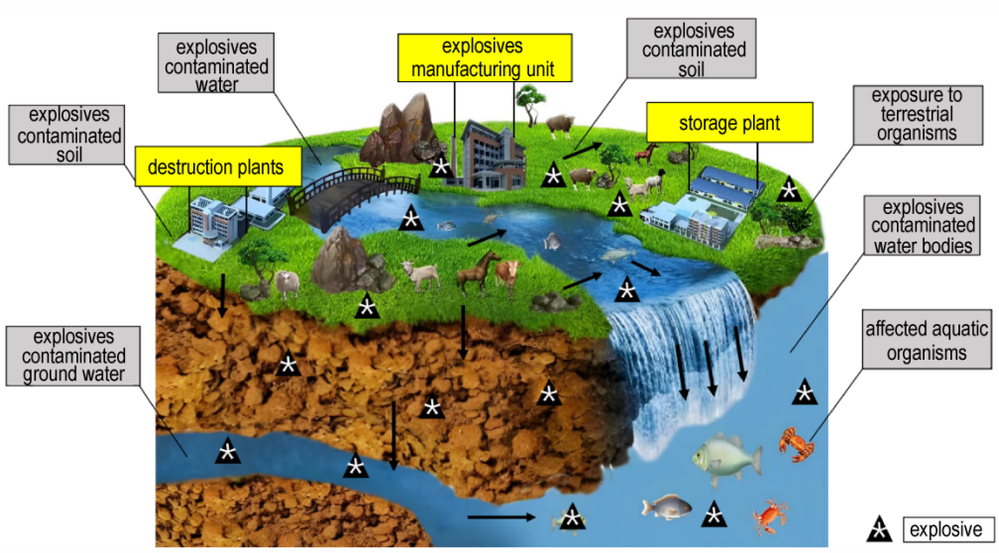


图 1 含能材料的污染迁移

Fig.1 Pollution migration of energetic compounds

附法处理RDX、TNT等废水成本低，但是无法将含能材料矿化，只能将其从一个相转移到另一个相[27,40]。光解法是指反应物吸收光能后发生直接转化，伴随着光电离和化学键断裂，形成新化合物的方法。该法用紫外灯直接照射含能材料废水，处理时间比其他方法短，但基于光的氧化作用，对进水透光率较为敏感，且紫外光的使用成本较高[41]。电解法是指物质通电后失去或得到电子（即发生氧化或还原反应）的化学方法，该法常以Ti/MMO为阳极来降解含能材料废水，去除效率高，运行成本也较高，且产生沉渣的综合利用问题没有良好的解决办法[42-43]。超临界水氧化法是指在超高温和超高压的情况下，通过氧化作用将含能材料完全氧化成H_2O和CO_2等小分子物质的过程，其优势是降解效率高，能在短时间内将含能材料完全降解，前期投入较大，高温高压的操作条件对设备要求较高，较难实现大规模的工业化应用[44]。植物修复法是

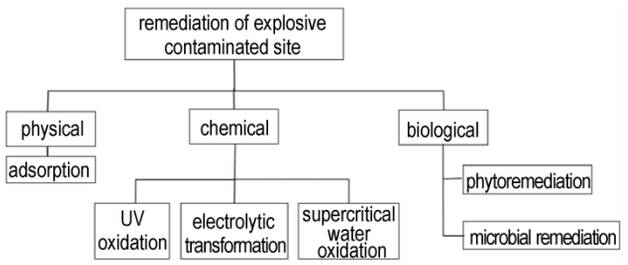


图2 修复含能材料污染场地的常见方法

Fig. 2 Common methods for repairing energetic compounds contaminated sites

利用绿色植物来原位去除和降解土壤、污泥、沉积物、地表水和地下水中的污染物。除了传统的植物修复法以外,转基因植物修复法近些年开始得到大规模研究,对RDX和TNT等常见含能材料有着更佳的吸收速率[45-48]。植物修复法有着较高的吸收率,但植物本身对于含能材料的降解能力却很低。相比之下,微生物修复法在处理含能材料污染过程有着更佳的成本效益和安全性,不会产生二次污染,在修复含能材料污染场地中有很大的优势。

微生物修复法是在适宜的环境里,利用对污染物有高耐受性且经过人工驯化微生物的自身代谢作用,将污染场地中的有害污染物降解成无害物质的修复技术。目前,微生物修复法已成为治理含能材料污染场地的一种新兴方法,其优势在于在好氧条件和厌氧条件下,微生物均可降解TNT、RDX和HMX等含能材料。微生物修复法按照是否改变污染土壤、水体位置,分为原位修复和异位修复。在治理含能材料污染场地时,通常从污染场地中筛选出对含能材料有较高耐受度的土著微生物,也可以人工添加外源微生物,如添加其他污染地点的微生物降解含能材料[49-50]。一般来说,土著微生物通常应用在原位修复含能材料污染场地当中,而筛选优势菌株和人工添加外源微生物等微生物修复方法通常应用在异位修复含能材料污染场地当中。微生物通常将含能材料中的C或者N作为生长的直接营养源,或者利用其他成分作为营养源,降解含能材料[51]。在采用微生物修复含能材料污染场地时,同时需要优化pH值和外部营养源等影响因素,通过微生物将污染场地中含能材料矿化,使其发生聚合、共价结合及络合反应,达到降解含能材料的目的。不少研究人员还会通过添加共基质作为外部氮源或碳源进行生物刺激,进一步促进微生物的生长,提高微生物对含能材料的降解能力[52]。

2.2 微生物修复含能材料污染场地的预处理与应用

2.2.1 微生物修复的预处理

微生物修复法是最具成本效益的方法,但微生物耐受含能材料的浓度有限,采用微生物修复法直接处理部分高浓度含能材料污染场地的修复时间长,难以彻底降解含能材料。因此,工作中一般还需要对污染场地进行预处理[1]。目前,针对实际生产过程中产生的COD可达105 mg·L^{-1}高浓度TNT红水,一般会采用络合萃取、高级氧化等工艺进行预处理后,再进行微生物法处理。王倩等[53]以三辛胺为络合萃取剂,用络合萃取法预处理TNT红水,通过改变萃取相比可控制出水COD,最多可降解TNT红水中99.4%的COD。Bhanot等[54]采用TiO_2-纳米管(NT)/SnO_2-Sb作为阳极对RDX废水进行预处理,可降解39.2%的COD,将生物降解指数从0.18提高到了0.51,促进微生物修复;在随后的中试中采用Fe-Ni CME(Fe-Ni催化微电解)电催化预处理TNT废水,成功降解了75.63%的COD。Radtke等[55]采用微生物法修复TNT浓度高达3000 mg·kg^{-1}的污染场地土壤时,加入丙酮进行预处理后,由未预处理的24 d降解90% TNT提高为6 d降解99.39% TNT。

2.2.2 微生物修复硝基芳香族含能材料污染场地的应用

国内外大量研究表明,多种微生物在好氧或厌氧条件下都能对硝基芳香族含能材料(如TNT)污染场地进行修复。部分微生物修复硝基芳香族含能材料污染场地的应用研究如表3所示。从表3中可看出,目前筛选优势菌株对TNT污染场地进行修复的研究很多,并有不少研究为混合菌群修复TNT污染场地的探索。例如,Xu等[56]采用混合菌群降解TNT红水污染土壤中的主要污染物DNTS(二甲基甲苯磺酸盐),在144 h内将TNT红水模拟污染土壤中浓度为50 mg·kg^{-1}的DNTS完全降解,可见未来可针对性地组合优化混合菌群,进行TNT污染场地修复。表3显示大部分研究是实验室模拟TNT废水或模拟TNT污染土壤,仅有小部分是TNT实际废水,因此,未来的研究应该更注重TNT实际污染场地的修复,挖掘与提升微生物修复技术的实际应用能力。

芳香族化合物不易被降解,因此在TNT的降解过程中,不少研究者通过添加外部营养源刺激原生微生物的生长,增强污染场地中原生微生物的修复力,这些外部营养源也被称为生物刺激剂,包括限制性营养物质和氮、磷或碳等电子受体。表4列举了部分采用生

物刺激剂对硝基芳香族含能材料污染场地进行微生物修复的应用研究。与筛选优势菌株进行修复不同，添加外源生物刺激剂是利用污染场地的原生微生物对污染场地进行原位修复，实用性强，更符合污染场地未来修复的趋势。从表4可以看出，实践中多添加葡萄糖、糖蜜、乳清、柠檬酸等碳源或氮源较为丰富的物质，刺激土著微生物的生长。综合考虑材料处理的便利性、成本和生物刺激剂的现场寿命等因素，以上物质可作为优先考虑使用的外源生物刺激剂，广泛应用于大规模的原位修复场地中。目前，微生物修复硝基芳香族

表3 微生物修复硝基芳香族含能材料污染场地的应用

Table 3 Application of microbial remediation of nitroaromatic energetic compounds contaminated sites

microbial type	microbial genus	microbial species	energetic compound	test phase	degradation time / h	degradation efficiency / %	reference
bacterium	*Pseudomonas*	*Pseudomonas mosselii*	TNT	simulated wastewater	28	100	[57]
		Pseudomonas stutzeri	TNT	actual wastewater	48	100	
		Pseudomonas X5	TNT	simulated soil	168	88	[58]
		Slight Halophilic Bacteria N33	TNT	simulated wastewater	48	100	[59]
		Slight Halophilic Bacteria N2	TNT	simulated wastewater	60	100	
		Pseudomonas sp. TNT3	TNT	simulated wastewater	48	100	[60]
	Cupriavidus	*Cupriavidus metallidurans DNT*	TNT	simulated wastewater	336	100	[61]
	Pseudarthrobacter	*Pseudarthrobacter chlorophenolicus*	TNT	simulated wastewater	360	100	[62]
	Klebsiella	*Klebsiella variicola T5*	TNT	simulated wastewater	30	100	[63]
	Cladosporium	*Cladosporium resinae*	TNT	simulated wastewater	72	99	[64]
fungus	*Tirchoderma*	*Trichoderma viride*	TNT	simulated wastewater	192	100	[65]
bacterial mixture		*Pseudomonas*, *Cyanobacteria*, *Bacillus*, *Acidimicrobiales*, *Amycolatopsis*, *Truepera*, *Mitochondria*, *Lactococcus*	DNTS（main components of TNT red water polluted soil）	simulated soil	144	100	[56]

表4 微生物采用生物刺激剂修复硝基芳香族含能材料污染场地的应用

Table 4 Application of microorganism in remediation of nitroaromatic energetic compounds contaminated sites with biological stimulants

energetic compound	test phase	exogenous biological irritant	degradation time / h	degradation efficiency / %	reference
TNT	simulated wastewater	glucose / citric acid	24	100	[66]
TNT	simulated wastewater	glucose / citric acid	72	100	
TNT	in situ repair	molasses	7320	100	[67]
TNT	in situ repair	emulsified oil	192	99	[68]
TNT	in situ repair	whey	2088	92	[69]
TNT	simulated soil	glucose	60	95	[70]
TNT	simulated wastewater	eggshells and cocopeat	840	99	[71]
TNT	simulated wastewater	citrate	120	88	[72]
TNT	simulated wastewater	lucose / KNO_3	16	100	[73]
TNT	simulated wastewater	cane molasses	48	100	[74]

含能材料TNT污染场地已进行过中试实验，接种量对TNT的转化程度几乎未产生影响，而生物刺激剂反应的最低值（ORP值）在转化过程中起着更重要的作用[52]。未来，在原位修复含能材料污染场地时，可根据现场实际情况来调整ORP值，选择能够适应污染场地ORP值的生物刺激剂，提高生物刺激剂修复污染场地的效果。

2.2.3 微生物修复环状硝铵类含能材料污染场地的应用

与TNT等硝基芳香族含能材料相比，环状硝胺类含能材料的稳定性较差，任何对硝胺或硝胺亚甲基的亲核攻击都会导致环断裂分解，这也是其容易降解的原因[75]。近年，环状硝铵类含能材料RDX污染场地的微生物修复研究广泛。表5列出了部分微生物修复环

表5 微生物修复环状硝铵类含能材料污染场地的应用

Table 5 Application of microbial remediation of cyclic ammonium nitrate energetic compounds contaminated sites

microbial type	microbial genus	microbial species	energetic compound	test phase	degradation time / h	degradation efficiency / %	reference
bacterium	*Stenotrophomonas*	*Stenotrophomonas maltophilia PB1*	RDX	simulated wastewater	165	100	[78]
	Rhodococcus	*Rhodococcus sp. Strain DN22*	RDX	simulated wastewater	14	100	[79]
	Clostridium	bacterial mixture (*Clostridium HAW-1*, *HAW-G3*, *HAW-G4*, *HAW-E3*, *HAW-HC1*)	RDX	simulated wastewater	192	99	[80]
	Williamsia	*Williamsia sp. KTR4*	RDX	simulated wastewater	48	100	[81]
	Gordonia	*Gordonia sp.KTR9*	RDX	simulated wastewater	72	100	
	Janibacter	*Janibacter cremeus*	RDX	simulated wastewater	720	88	[82]
	Bacillus	*Bacillus toyonensis WS4-TSB-3*	RDX	simulated wastewater	360	82	[83]
	Paenibacillus	*Aenibacillus S10-TSA-3*	RDX	simulated wastewater	360	85	
	Pseudomanas	*P. fluorescens I-C*	RDX	simulated wastewater	100	30	[84]
		P. putida II-B	RDX	simulated wastewater	400	46	
	Shewanella	*Shewanella halifaxensis HAW-EB4*	RDX	simulated wastewater	25	100	[85]
	Desulfovribrio	*D. desulfuricans A*, *D. desulfuricans B*, *D. gigas*, *and D. vulgaris.*	RDX	simulated wastewater	288	100	[86]
		D. desulfuricans A, *D. desulfuricans B*, *D. gigas*, *and D. vulgaris.*	HMX	simulated wastewater	432	100	
	Planomicrobium	*Planomicrobium flavidum S5-TSA-19*	HMX	simulated wastewater	480	70	[87]
	Morganella	*M. morganii B2*	HMX	simulated wastewater	1080	60	[76]
	Bacillas	*Bacillus aryabhattai H1*	HMX	simulated wastewater	24	91	[88]
	Rhodococcus	*Rhodobacter sphaeroides H*	HMX	simulated wastewater	96	89	[89]
fungus	*Klebsiella*	*Klebsiella pneumoniae Strain SCZ-1*	RDX	simulated wastewater	60	100	[90]
bacterial mixture		mixed culture	RDX	simulated wastewater	3744	>98	[81]

状硝铵类含能材料污染场地的应用,不难发现大部分研究都采用异位修复,通过筛选优势菌株降解含能材料污染。原位修复实际污染场地一般很少采用筛选优势菌株的方法,因为筛选优势菌株处理含能材料污染虽然有着较高的效率,但大部分都处理模拟污染场地,实际污染场地的污染成分复杂且影响因素较多,污染场地的氧化条件、共存污染物和生物刺激剂的适用性等因素都会影响微生物对污染场地的修复效果,所以筛选优势菌株修复实际污染场地的效率并不高,根据实际污染场地现状,有针对性地引入外源菌株更有利于污染场地的修复。与硝基芳香族化合物TNT不同,混合菌群对环状硝铵类含能材料RDX的降解效果并不如纯菌株,因此,纯菌株和混合菌群降解效果的优异性还有待商榷。同为环状硝铵类含能材料的HMX虽然是RDX的更高同系物,对化学和生物降解的抗性更强。因此,关于微生物对HMX的降解研究并不多。由表5可知,菌株降解HMX普遍要比RDX时间长,且降解率远不如RDX高,因此HMX在环境中有着更持久的危害性。正因如此,未来应该更加注重对HMX的实际污染场地研究。

考虑到实际场地的污染错综复杂,存在不少混合污染的情况,为此研究者们开始采用微生物处理混合含能材料污染。Kitts等[76]从硝铵类含能材料污染土壤中分离出了摩根氏菌,用该菌株对RDX和HMX等含能材料进行降解和转化,结果表明,RDX存在的条件下,摩根氏菌仍然能够对HMX进行转化。Yang等[49]的利用土著微生物修复TNT、RDX和HMX浓度均为100 mg·kg^{-1}的混合含能材料污染土壤,40 d内分别对TNT、RDX和HMX降解了12.43%,6.73%和5.20%。

与硝基类含能材料相似,微生物修复环状硝铵类含能材料污染场地的过程中,同样能够通过添加生物刺激剂来提升微生物对含能材料的降解效果,但不合适的生物刺激剂不仅不会提升降解效果,甚至会适得其反。例如,Jugnia等[77]采用木质素磺酸铵对微生物进行生物刺激,在厌氧条件下对RDX污染土壤进行修复,降解效果不佳,且高浓度木质素磺酸铵对RDX的降解还有一定的抑制作用。

表6为部分采用生物刺激剂对环状硝铵类含能材料污染场地进行微生物修复的应用。由表6可知,大部分环状硝铵类含能材料如RDX和HMX的生物刺激修复,还是通过筛选优势菌株,但可以看到实际污染场地中,该方法的修复效率并不高,分析认为这是因为微生物在修复实际污染场地时,污染场地的氧化还原条件、生物刺激剂适用性、其他共存污染物等,都会影响目标污染物的转化水平。因此,未来研究的重点是由模拟污染场地修复的高效率转换为原位修复实际污染场地的高效率,并在修复实际污染场地前,建立严格的评估体系与标准。

2.3 微生物修复含能材料污染场地的基因组学研究

微生物降解含能材料的过程中可能会因为添加的有机基质不同,产生的还原条件不同。由于这方面可了解到的信息有限,所以不少学者开始通过基因组学和微生物生态学来进一步分析降解含能材料的微生物。Khan等[31]对降解RDX的微生物进行了宏基因组学研究,并通过数据库比对识别了XplA和XplB等对RDX有高效降解效果的基因。Fuller等[94]则利用基因组学和蛋白质组学分析了地下水中RDX的自然降解过程。最初,地下水中RDX降解的微生物生态学研究仅限于实验室的柱状实验和富集培养方法。为适应实际需要,原位修复逐步成为主要研究趋势。Livermore等[95]提供了一个基于现场的独立微生物生态环境,研究在生物刺激剂刺激下的RDX转化效果,并分析实际污染场地中微生物群落DNA,总结出代谢RDX的优

表6 微生物采用生物刺激剂修复环状硝铵类含能材料污染场地的应用

Table 6 Application of microorganism in remediation of cyclic ammonium nitrate energetic compounds contaminated sites with biological stimulants

energetic compound	test phase	exogenous biological irritant	degradation time / h	degradation efficiency / %	reference
RDX	in situ repair	corn syrup	192	82	[68]
RDX	in situ repair	waste glycerol	27000	100	[52]
RDX	simulated contaminated soil	sucrose	720	78	[91]
RDX	simulated contaminated soil	wheat straw	720	66	
RDX	simulated wastewater	talcum powder and alginic acid	720	70	[92]
HMX	simulated contaminated soil	cocopeat	840	88	[93]

势菌群，为采用现代测序技术的RDX原位生物修复提供了现场规模的微生物生态学第一手资料，同时为未来的生物刺激原位修复应用提供了研究基础。

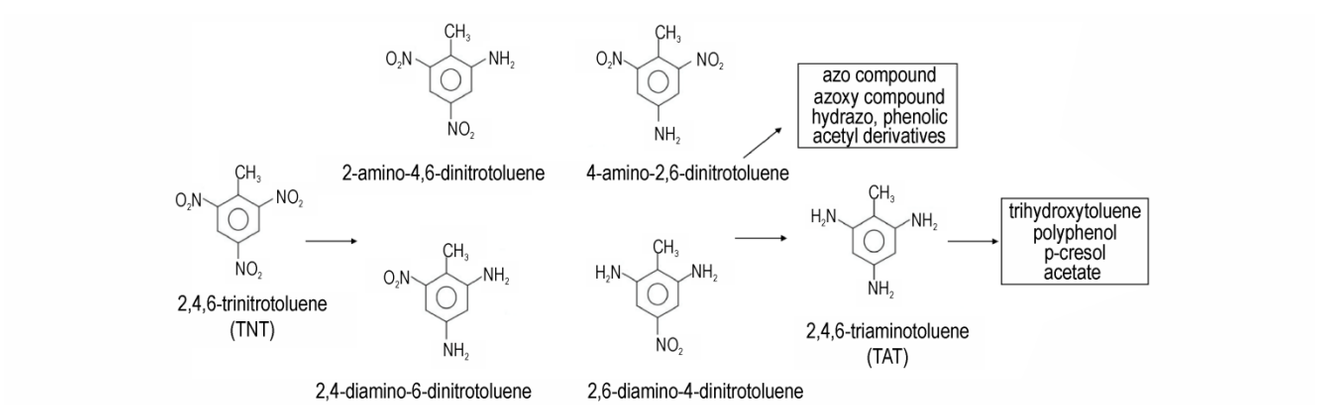
宏基因组学能够识别一些微生物体内对含能材料有着良好降解效果的特定基因，并将其进行克隆和强化表达，以期对含能材料产生更好的降解效果。目前，细胞色素P450酶催化系统是微生物降解RDX较常用的转基因系统，该系统由多辛还原酶XplB和融合黄多辛细胞色素P450 XplA组成，被克隆后的良好降解效果已被多位研究者验证。Jackson等[96]采用了表达细胞色素P450酶催化系统XplA和XplB的转基因拟南芥植物对RDX进行降解，该植物对RDX的降解效果显著提升，证明了细胞色素P450酶催化系统修复RDX污染场地的高效性。Seth-Smith等[97]从含能材料污染土壤中分离出了对RDX有着高效降解效果的红球菌株11Y，其基因与细胞色素P450具有同源性，采用表达其基因的菌株CW25对RDX进行降解，同样有着良好效果。Fuller等[86]采用恶臭假单胞菌II-B和荧光假单胞菌I-C的XenA酶和XenB酶对RDX和HMX等含能材料进行降解，结果表明，两种酶对RDX和HMX都有着较高的降解效率，可以考虑应用到工程改造菌中处理实际污染场地。但需要注意的是，对RDX和HMX有着较高降解水平的酶基因修复含能材料场地大都处于实验室阶段，因其生物安全未被评估，所以还未得到实际应用。

3 微生物降解含能材料的中间产物及代谢途径

3.1 微生物降解硝基芳香族含能材料的中间产物及代谢途径

在好氧和厌氧条件下，微生物代谢TNT的途径基本相同：微生物中的非特异性硝基还原酶将硝基还原为相应的单硝基、单羟基胺和胺类衍生物等，经过酰化反应生成乙酰基衍生物和偶氮化合物等[3]。Lamba等[98]验证了这一过程，他们采用爆炸性隐球菌S5-TSA-19对TNT进行降解，形成了二硝基甲苯（DNT）、2，4-二氨基-6-硝基甲苯（2，4-DANT）和2-氨基-4，6-二硝基甲苯（2-ADNT）等中间代谢产物。好氧条件下，微生物也通过氧化TNT进行脱硝，产生羟基、氨基和胺类衍生物等中间产物的同时，还形成了邻苯二酚或NO_2（与初始氧化的位置有关）等物质。Rodgers等[22]采用假单胞菌308C降解TNT，产生了羟基、氨基以及胺类衍生物（2-ADNT、4-ADNT、2，4-DANT和2，6-DANT）等中间产物，最终生成了偶氮化合物等，这证明TNT在降解过程中是首先经过脱硝，再形成NO_2等物质的。相比之下，真菌降解TNT的研究较少。Claus等[99]综述了经过测试的91个真菌菌株对TNT的代谢和矿化能力，几乎所有真菌代谢TNT的途径都与细菌相同，即TNT进行硝基还原后生成4-ADNT和2-ADNT等胺类衍生物，随后发生各种酰化反应，生成甲酰化产物和乙酰化产物。由此可见，真菌的代谢途径和细菌几乎没有差别，真菌的非特异性细胞外酶将硝基还原为羟基、氨基和各种胺类衍生物等，随后再生成各种酰化产物[3]。

综上所述，无论是细菌还是真菌，其代谢途径都是对TNT中甲基的邻位和对位硝基进行还原，进而生成2-ADNT、4ADNT、2，4-DANT和2，6-DANT等胺类衍生物，随后发生酰化反应生成偶氮化合物和乙酰基衍生物等物质，具体的TNT代谢途径见Scheme 1[3]。TNT的降解途径虽然已经有了比较清晰的研究，但是在硝基还原时，仍无法确定甲基邻位和对位硝基的反应优先顺序。未来，应进一步研究TNT代谢途径中甲基邻位和对位进行硝基还原的次序，进而对TNT代谢



Scheme 1 Microbial metabolic pathway of TNT[3]

途径有更清晰的认识。

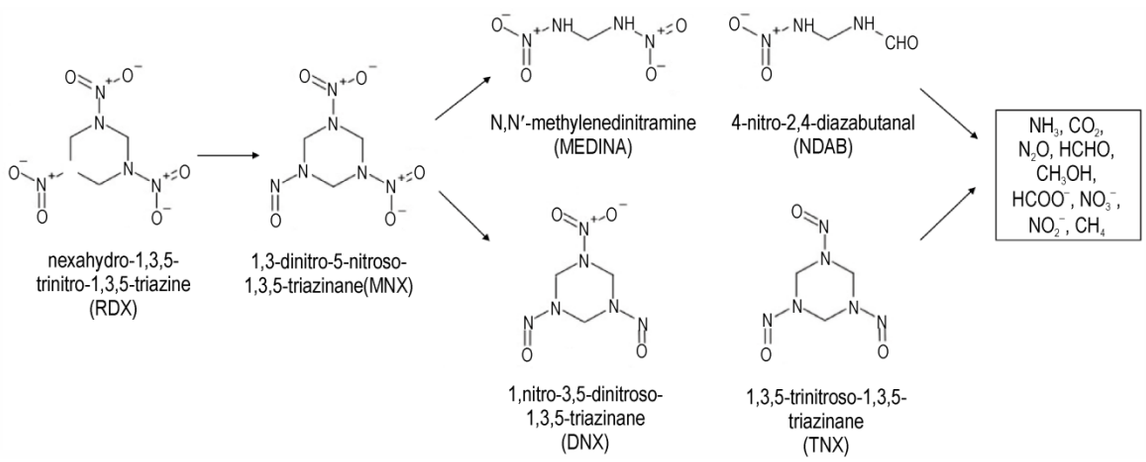
3.2 微生物降解环状硝铵类含能材料的中间产物及代谢途径

3.2.1 微生物降解RDX的中间产物及代谢途径

最初，人们认为RDX的微生物降解只能在厌氧条件下进行，并对其代谢途径进行了一系列研究。Zhao等[80,90]采用克雷白氏肺炎杆菌SCZ-1厌氧降解RDX时发现，SCZ-1产生的硝基还原酶可将RDX的硝基还原为相对应的亚硝基衍生物六氢-1-亚硝基-3，5-二硝基-1，3，5-三嗪（MNX），随后再发生环裂解，进而产生4-硝基-2，4-二氮杂-丁醛（NDAB）等中间代谢产物，并在最终生成HCHO、CH_3OH、CO_2和N_2O等小分子物质。同时，降解过程中检测到少量六氢1，3-二亚硝基-5-硝基-1，3，5-三嗪（DNX），据此判断，RDX厌氧降解的另一条途径是：RDX首先在微生物所产生的硝基还原酶作用下脱硝形成亚硝基衍生物MNX，再通过将MNX上的—NO_2基团还原为—NO基团，生成不稳定的中间产物DNX，最终彻底矿化成NH_3、CH_4等小分子物质[31]。Bhushan等[100]采用黑曲霉细胞在厌氧条件下对RDX进行降解，同样发现MNX和NDAB是微生物降解RDX过程中的主要中间代谢产物，并证明中间代谢产物不能持续存在，会随着HCHO和N_2O等小分子物质的生成而逐渐消失，同时验证了克雷白氏肺炎杆菌SCZ-1厌氧降解RDX初始反应中涉及的硝基还原假设。其化学计量和质量平衡测量结果也表明，代谢过程主要是RDX通过双电子还原机制向MNX进行初始转化，随后MNX被黑曲酶细胞还原，快速裂解生成NDAB，最终在水中分解，定量生成HCHO和N_2O等物质。Michalsen等[101]采用菌株KTR9和荧光假单胞菌I-C细胞对RDX进行厌氧降解，也证明了RDX的微生物厌氧降解途径为先脱硝，再环裂解，之后逐步生成小分子物质。

近年，RDX的好氧降解也被很多菌株证明，并且对其代谢途径有了进一步的认识。Khan等[2]采用水曲霉细胞对RDX进行好氧降解，并在降解过程中检测到4-硝基-2，4-二氮杂-丁醛（NDAB）和次甲基二硝铵（MEDINA）等中间代谢产物，这是因为微生物会在RDX诱导下产生硝基还原酶，将RDX进行脱硝形成MNX，环裂解后生成MEDINA和NDAB等中间产物，随后逐步生成小分子物质。Bhatt等[102]采用4种真菌对RDX进行好氧降解，在不同真菌降解RDX的过程中分别检测到了MEDINA和DNX、TNX等物质，验证了微生物在好氧降解RDX时至少通过2种途径，均涉及初始的硝基还原，一条途径是RDX脱硝后形成MNX，环裂解后生成MEDINA等中间产物，最后分解成HCHO等小分子物质；另一条途径则是在环裂解前还原成MNX，MNX再经过硝基还原分解成微量的DNX及TNX等，最终在水解、开环后彻底矿化为N_2O和HCHO等物质。

综上所述，微生物降解RDX时，微生物通常会在RDX的诱导下分泌硝基还原酶，硝基还原后产生MNX，此后经由两条代谢：一条是经过环裂解生成中间代谢产物MEDINA、NDAB等中间产物，随后彻底矿化为CO_2、N_2O和CH_4等小分子物质；另一条是MNX上的硝基再次还原，形成DNX、TNX等中间代谢产物，最终进一步矿化为HCHO、CH_3OH和N_2O等更简单、危害更小的小分子物质。具体的RDX代谢途径见Schem 2[3,31]。不过，目前关于RDX的降解和代谢途径研究还是以模拟实验为主，未来应该将研究重心放在原位修复污染场地时微生物降解RDX的代谢途径

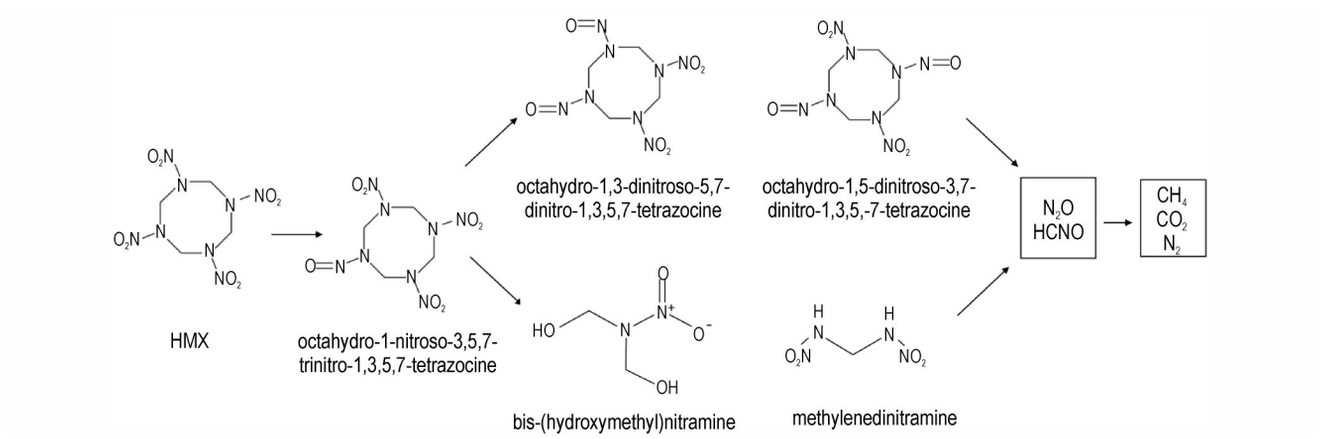


Scheme 2 Microbial metabolic pathway of RDX[3,31]

与中间产物中。

3.2.2 微生物降解HMX的中间产物及代谢途径

同为环状硝铵化合物，HMX的代谢途径和RDX相差不大。Nagar等[87]在菌株S5-TSA-19对HMX的降解过程中先后检测到了亚硝酸盐离子和亚甲基二硝铵等代谢产物，并提出HMX的降解途径为首先形成亚硝酸盐离子，随后逐步形成亚甲基二硝铵等代谢产物，再环裂解为N_2O和HCHO等无害的物质，最终通过反硝化或甲烷化作用转化为N_2、CO_2和CH_4等小分子物质，HMX的这一降解途径也是单一的亚硝酸盐消除途径。Nagar[51]在天然细菌WS2-R2A-65对HMX的降解过程中检测到了亚甲基二硝铵，探索出新的代谢途径，表明微生物产生的硝基还原酶将HMX还原为亚甲基中间体后，能再次对亚甲基中间体进行硝基还原，随后环裂解生成N_2O、HCHO和HCOOH等无害的物质，最终通过反硝化或甲烷化作用转化为N_2和CH_4等物质。总体来看，HMX通常有2种代谢途径，一种是涉及单一亚硝酸根离子提取下的硝铵环裂解，HMX经过硝基还原后生成亚甲基二硝铵等中间产物，环裂解形成N_2O、HCHO和CH_3OH等无害的代谢产物，最终彻底转化为N_2、CO_2和CH_4等小分子物质；另一种则是先将HMX还原为亚硝基、羟基、氨基衍生物、肼基衍生物及其甲基化形式，随后对亚硝基中间体等物质再次进行硝基还原，环裂解后生成N_2O、HCHO和CH_3OH等无害的代谢产物，最终通过反硝化或甲烷化作用转化为N_2、CO_2和CH_4等物质，具体如Scheme 3所示[3,31]。目前已经对HMX的代谢途径进行了一些研究，但其具体的脱硝过程尚未清楚，且邻位和对位硝基的还原顺序受共存污染物影响，在不同的反应体系中有所不同，这个问题还有待进一步研究。



Scheme 3 Microbial metabolic pathway of HMX[3,31]

4 存在问题与展望

含能材料的污染不可避免，其危害和污染场地的修复方法逐渐成为研究热点。与其他修复方法相比，微生物修复法更具成本效益、可持续性和安全性。

在目前的研究中，对含能材料污染场地的模拟实验修复已取得良好效果，但原位修复实际污染场地仍是一个挑战。现阶段研究中微生物修复模拟含能材料污染场地的效率普遍较高，大部分研究对硝基芳香族和环状硝铵类含能材料的降解率都能够达到100%，且降解时间不超过100 h。但是原位修复实际污染场地的效率通常较低，现有研究中微生物将含能材料完全降解至少需要上千小时，同时在模拟污染场地中具有良好降解效果的菌株，应用至实际污染场地时并未达到预期效果，即使部分微生物菌种在原位修复时对含能材料具有较高的降解率，其修复时间也较长。因此，未来的研究重心是菌株由实验室高效率修复向实际污染场地高效率修复的转换，将微生物修复大规模应用到实际的含能材料污染场地。与实验室模拟的含能材料污染相比，实际污染场地的影响因素较多，污染成分复杂，共存的重金属污染物会影响含能材料降解过程中的供电子和吸电子能力，而污染场地的氧化还原条件、生物刺激剂的适用性和污染场地其他共存污染物等都会影响微生物对污染场地的修复效果，这也正是原位修复和异位修复的差异所在。

其次，微生物修复含能材料污染场地虽然具有一定的优势，但是操作周期较长。在接下来的研究里，重点应探索更高效的含能材料降解菌株，以及更强刺激效果的生物制剂。在选择生物刺激剂时，首先要根据已有的实验案例，尽可能地选择碳源和氮源较为丰富，

已在模拟实验中应用成功且具有较高效率的生物刺激剂；其次，由于ORP值对厌氧修复过程有着较为明显的影响，所以厌氧修复还要适当根据所采用的生物刺激剂来调整污染场地的ORP值，以提升生物刺激剂的效果。因此，在修复之前应该先对污染场地进行评估，选择最合适的ORP值、外源生物刺激剂种类与剂量，以及其他影响条件，实现高效原位修复含能材料污染场地。

最后，随着生物技术的不断发展，未来还应该着重分析降解含能材料的微生物菌株的宏基因组学、宏转录组学、宏蛋白质组学和宏代谢组学，加强对DNA、RNA、蛋白质和代谢产物等方面的研究，通过宏基因组学识别出微生物菌株降解含能材料的关键基因和蛋白等，强化表达后，提升降解含能材料的效率。目前，工程改造菌修复模拟含能材料污染场地已经取得了较大的进展，但因可能涉及生物安全问题，目前还未见工程改造菌原位修复实际污染场地的研究，因而将工程改造菌应用在实际污染场地修复中需要建立在对生物安全问题有着充分评估的基础上，这也正是下一阶段的重点研究目标。同时，还需要建立完整的微生物修复含能材料菌株基因库，利用大数据分析含能材料污染场地，设计专门的算法，根据实地污染情况来针对性地使用工程改造菌株及微生物制剂完成对污染场地的修复。

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Research Process of Microbial Remediation of Energetic Compound Contaminated Sites

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Abstract: Microbial remediation technology refers to the remediation technology that uses artificially domesticated microorganisms with specific functions to degrade harmful pollutants in the contaminated site into harmless substances through their own metabolism in the appropriate environment. In the process of microbial remediation of energetic compounds contaminated sites, improving microbial degradation efficiency and exploring metabolic pathways and intermediate metabolites are key issues. This paper briefly described the pollution status of energetic compounds, introduces common methods for remediation of energetic compounds contaminated sites. The advantages of microbial remediation of energetic compound contaminated sites were focused on, and the common strains, external nutrition sources and practical applications of microbial remediation of energetic compound contaminated sites were summarized. Besides, the intermediate metabolites produced by various energetic compounds in the process of microbial degradation were sorted out, and the metabolic pathways in the degradation process of various energetic compounds were also summarized. The development trend of microorganism application in remediation of energetic compound contaminated sites was prospected. The prerequisite for improving the efficiency of existing microorganisms in degrading energetic compounds is the research of microbial agents with better biological stimulation effect. The analysis of metagenomics, macronenenebb transcriptome, macronenenebc proteomics and metabolomics of microbial strains that degrade energetic compounds should be emphasized as a way to strengthen the research on DNA, RNA, proteins and metabolites, and also to improve the degradation effect through transgene to better stimulate the potential of microbial remediation of energetic compound contaminated sites.

Key words: microbial remediation; energetic compounds; contaminated site; metabolic pathway; biological stimulation

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