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猪粪堆肥过程中四环素类抗生素的生物转化及降解研究进展

冯栋梁, 封林玉, 张倚剑, 梁天柱, 梁明振*

广西大学动物科学技术学院, 南宁 530004

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摘要: 四环素类抗生素(TCs)是畜禽养殖业中一类重要的兽药。随着畜禽养殖集约化,越来越多的抗生素经畜禽机体代谢进入环境,导致了对人类健康和生物安全等的危害。堆肥是有害化去除畜禽粪便中TCs的常见方法,能最大程度削减畜禽粪便中TCs的残留。笔者根据国内堆肥处理猪粪中抗生素残留的现状,结合国内外猪粪堆肥化技术的最新研究进展,从TCs生物转化、生物和非生物降解途径,阐述了猪粪堆肥过程中环境因子的调控、微生物群落结构与组成的变化和TCs的生物降解机制,旨在为未来畜禽粪便无害化、肥料资源化利用提供理论依据和技术参考。

关键词: 四环素类抗生素; 畜禽粪便; 生物转化; 降解机制; 堆肥; 微生物群落

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Advances in Biotransformation and Degradation of Tetracycline Antibiotics During Composting of Pig Manure

Feng Dongliang, Feng Linyu, Zhang Yijian, Liang Tianzhu, Liang Mingzhen*

College of Animal Science and Technology, Guangxi University, Nanning 530004, China

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Abstract: Tetracycline antibiotics (TCs) are an important class of veterinary drugs in the livestock and poultry industry. With the intensification of livestock and poultry farming, more and more antibiotics are metabolized into the environment through the livestock and poultry organisms, increasing the harm to human health and biosecurity. Composting is a common method for harmlessly removing TCs from livestock manure, which can minimize the residue of TCs in livestock manure. Based on the domestic status of antibiotic residues in pig manure treated by composting, combined with the latest research progress of domestic and foreign pig manure composting technology, this paper expounds the regulation of environmental factors, the change of microbial community structure and composition, and the biodegradation mechanism of TCs in the process of pig manure composting from the aspects of TCs biotransformation, biological and non-biological degradation. This paper aims to provide theoretical basis and technical reference for the harmless livestock manure utilization in the future.

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第一作者: 冯栋梁(1991—), 男, 硕士研究生, 研究方向为动物营养与饲料添加剂, E-mail: fengdongliang37370@126.com

* 通讯作者 (Corresponding author), E-mail: lmzhen62@163.com

Keywords: tetracycline antibiotics; livestock manure; biotransformation; degradation mechanism; composting; microbial community

兽药抗生素的使用大大降低了畜禽感染疾病的死亡率。随着畜禽的集约化养殖,抗生素的大量甚至过量且持续的使用,导致致病性细菌的抗药性也在稳步增加。据统计,2013年我国抗生素使用量16.2万t,兽用抗生素占52%^[1]。其中,四环素类抗生素(TCs)的用量占所有抗生素饲料添加剂的57%^[2-3]。有资料显示,2018年我国生猪养殖量占世界生猪总养殖量的56.6%,远高于美国等国家以及欧盟。猪粪在畜禽粪污排放量中所占比例最大,占畜禽粪便总量的36.71%^[4]。整体而言,国内外猪粪中抗生素残留量呈现以下规律:四环素类>氟喹诺酮类>磺胺类>大环内酯类^[5]。TCs是从链霉菌培养液中提取或半合成的一类抗生素,常见的有金霉素(chlortetracycline, CTC)、土霉素(oxytetracycline, OTC)、四环素(tetracycline, TC)和多西环素(doxycycline, DXC)等。它们在猪体内不能被完全吸收,一部分在猪组织内累积残留;另一部分随猪粪排出体外。据报道,TCs能通过吸附、迁移和降解在土壤、水、植物和沉积物等环境介质中进行转移,使TCs抗性基因在自然环境中呈不同程度的分布^[6]。

另外,长时间大量使用TCs,不仅使猪肠道产生耐药细菌,猪粪中抗生素抗性基因(antibiotic resistance gene, ARGs)增加,还使猪组织中TCs残留,给生态平衡和人类健康构成威胁^[7-11]。近年来,猪粪中抗生素的转归、降解及生态学效应日益受到关注。加快猪粪无害化处理和资源化利用,推进猪粪提质增效在农业生态领域已成为一个重要的研究课题。因此,笔者就猪粪堆肥过程中堆体环境控制、TCs生物转化过程、微生物群落动态变化和ARGs控制等方面的研究进展进行综述,旨在为猪粪中TCs的降解及猪粪无害化还田提供理论依据和技术参考。

1 猪粪中TCs的分布现状及堆肥中理化参数调控 (Distribution status of TCs in pig manure and regulation of physical and chemical parameters in compost)

1.1 猪粪中TCs的分布现状

自20世纪50年代美国食品与药物管理局(FAD)将抗生素用作饲料添加剂以来,在降低动物特别是幼龄畜禽死亡率方面成效显著,因此,抗生素在畜禽养殖领域得到迅速推广^[12]。大部分抗生素属于广谱抗菌药物,长期使用会导致ARGs在一个更大范围内转移和传播^[13-14]。最新研究表明,人类自身对抗生素的不恰当使用比率为25%,而在动物中此比率则高达50%。TCs作为一种被大量使用的抗生素,当前环境中的污染源主要来自畜牧业和水产养殖业粪污的排放、农药和医院及药厂废弃物的处理^[15],而农业土壤中TCs的分布主要源自畜禽粪便的施用^[16]。

TCs其化学结构属于氢化并四苯的衍生物,有4个环组成,属于酸碱两性化合物。其中,TC和OTC都是极性分子,有多种官能团。它们在猪体内吸收率低,在环境中能以不同方式与多种介质发生吸附作用,从而不易被降解^[17-19]。常见的TCs分子结构式如图1所示。TCs在猪体内经代谢后胃肠道吸收很少,少部分以无活性产物、大部分以母体的形式排出体外,新鲜猪粪中TCs含量高达69%~86%^[20-21]。这些随猪粪排泄进入环境的TCs,对自然环境和生物安全具有潜在安全隐患。我国不同地区猪粪中TCs的种类及检出率如表1所示。

1.2 猪粪堆肥中理化参数的调控

堆肥处理是猪粪中TCs降解的常用方法,而实际生产中,猪粪堆肥中TCs的降解又是一复杂过程,

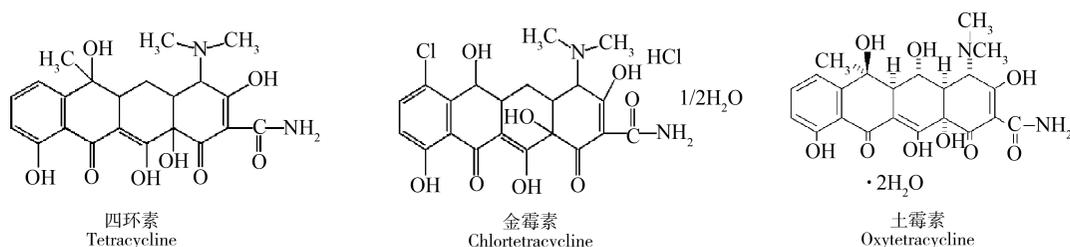


图1 常见的四环素类抗生素(TCs)分子结构式

Fig. 1 The molecular structures of common tetracycline antibiotics (TCs)

受多重理化参数的影响^[27]。Mitchell 等^[28]研究发现,堆肥中 TCs 的降解程度与堆体湿度、温度、总氮、总磷和 C/N 等理化参数间存有很大的相关性。当猪粪堆体含水率>70%时,堆体孔隙率降低,氧气透气量下降,不利于好氧发酵;当堆体含水率<40%时,好氧微生物的生理活性将会受到影响^[29]。常会庆等^[30]在不同温度下对脱水污泥堆肥,发现堆体环境温度对微生物的活性、有害物质的降解等具有重要影响,25℃比10℃更有利于有机质降解和污泥堆体的腐熟。相反,Huang 等^[31]通过定量 PCR 和 16s RNA 高通量测序发现,高温条件下猪粪中适度的抗生素残留,对猪粪微生物可产生短暂的抑制作用,但是在堆粪过程中如果不能有效地抑制病原体和抗性基因转移,高温并不一定能更好地去除粪中抗生素的 ARGs。此外,储意轩等^[32]调查发现,在堆肥过程中,粪中微生物代谢有机酸的产生或挥发、有机氮的分解和氨气的释放均可使堆体 pH 发生变化,对猪粪中 TCs 的降解产生不同程度的影响。有研究表明,猪粪堆肥中添加不同比例的生物炭,可使堆体提前 3 d 进入高温期,堆体生物炭总氮含量增加。高温条件下,当堆体中 C/N 含量降低时,猪粪中的氮会以氨气的形式挥发;当堆体 C/N 超过 35 时,粪中微生物需进行多次代谢循环,导致降解速度下降^[33-34]。周思等^[35]在猪粪中添加锯末和秸秆调控

猪粪中 C/N 时,发现初始 C/N 为(23~27):1 时,堆肥效果最好。因此,猪粪堆肥中理化参数的调控对猪粪中抗生素的降解至关重要。猪粪堆肥过程中堆体常见的理化参数控制值如表 2 所示。

2 猪粪中 ARGs 的水平传播 (Horizontal transmission of ARGs in pig manure)

猪粪不经堆肥处理直接作为有机肥在农田中使用,给猪粪中抗生素 ARGs 的传播、后续环境中重金属含量的增加埋下了潜在的风险^[46-47]。猪粪作为 TCs 的有机载体,含有多种病原微生物和营养养分,也是四环素类抗生素 ARGs 重要的富集位点。TCs 的污染及 ARGs 水平转移对致病菌耐药性的快速传播起着重要作用。抗生素进入环境后,会发生吸附、水解、光解和微生物降解等过程,将直接影响抗生素的生态毒性^[48]。Huang 等^[49]研究发现,雨季时,畜牧区地表水和地下水中存在严重的 ARGs 污染,可能会构成生态风险并引发食品安全问题。其次,猪粪中 ARGs 不仅可借助细菌的特异性同源重组,还可通过基因传播元件(质粒、转座子、整合子、插入序列和基因组岛)等实现菌种间的水平转移^[50-51]。同时,TCs 是疏水性有机物,可与碳基材料、纳米材料、高分子聚合材料、分子印迹材料和矿物质等诸多物质发生吸附作用^[52-54]。楼晨露^[55]对我国 4 个长期施用

表 1 国内不同地区猪粪中检测到的 TCs 种类及检出率

Table 1 The detected types and detection rate of TCs in the domestic pig feces from different areas

国内地区 /年份 Domestic area /year	环境介质 Environmental medium	TCs 的种类 Types of TCs	TCs 的含量范围 ($\mu\text{g}\cdot\text{kg}^{-1}$) TCs content range ($\mu\text{g}\cdot\text{kg}^{-1}$)	TCs 的含量均值 ($\mu\text{g}\cdot\text{kg}^{-1}$) Mean value of TCs ($\mu\text{g}\cdot\text{kg}^{-1}$)	检出率/% The detection rate/%
海南/2017 ^[22] Hainan/2017 ^[22]	猪粪 Pig manure	土霉素 Oxytetracycline	0 ~ 1 012	204	57.1
		金霉素 Chlortetracycline	0 ~ 1 827.3	95.1	63.3
浙江/2012 ^[23] Zhejiang/2012 ^[23]	猪粪 Pig manure	四环素 Tetracycline	0 ~ 16.75	2.01	61.29
		土霉素 Oxytetracycline	0 ~ 29.6	5.1	72.9
		金霉素 Chlortetracycline	0 ~ 11.63	2.17	69.03
广州/2011 ^[24] Guangzhou/2011 ^[24]	猪粪 Pig manure	四环素 Tetracycline	28.31 ~ 326.15	115.21	100
		土霉素 Oxytetracycline	ND ~ 63.96	9.09	94.1
辽宁/2016 ^[25] Liaoning/2016 ^[25]	猪粪 Pig manure	四环素 Tetracycline	4.5 ~ 41.4	25.12	94.1
		土霉素 Oxytetracycline	0 ~ 992.2	344.28	76.5
		金霉素 Chlortetracycline	0 ~ 850.6	570.06	82.4
山东/2018 ^[26] Shandong/2018 ^[26]	猪粪 Pig manure	四环素 Tetracycline	1 990 ~ 6 990	3 120	100
		土霉素 Oxytetracycline	65.2 ~ 1 680	613	100

注:ND 表示未检出。

Note: ND means not detected.

猪粪和不施用猪粪的定位试验点的表层和剖面土壤进行采样,发现 TCs 在长期施用猪粪稻田土壤表层有累积效应。与不施用猪粪的土壤相比,施用猪粪的土壤 ARGs 丰度明显提高,TCs 污染可从表层土壤向深层土壤迁移。Guo 等^[56]通过色谱质谱及实时定量 PCR 这 2 种方法抽检长期施用有机肥的表层土壤,发现土壤中存有锌、铜重金属污染,且 ARGs 含量显著增加。当前受堆肥原料及环境条件的限制,猪粪还不能达到无害化利用的要求。分析猪粪中 TCs 的来源及 ARGs 传播途径,是减少猪粪中 TCs 的重要方法。猪粪中 TCs 的来源及其潜在的转移途径如图 2 所示。

3 猪粪中 TCs 的生物转化 (Biotransformation of TCs in pig manure)

3.1 猪粪堆肥中微生物与 TCs 的相互作用

微生物降解是猪粪中 TCs 削减的一种重要方式,同时堆肥过程中微生物群落的变化也是猪粪中

TCs 抗性基因变异的主要驱动因素。张凯煜^[57]研究发现,堆肥过程中 TCs 能改变猪粪中细菌、真菌的多样性,使升温期内微生物的活性增加,TCs 的降解加速。时红蕾^[58]研究发现,随着猪粪堆肥中 TCs 浓度的增加、堆体温度的改变,粪中微生物的代谢活性将受到抑制,微生物群落的多样性指数逐渐下降。马骏^[59]利用添加 TCs 的猪粪和小麦秸秆进行堆肥,发现猪粪中微生物的活性和碳源呈先下降后升高的趋势,TCs 的降解率达 97.9%。可见,猪粪堆肥中微生物与抗生素可产生互作效应,对粪中微生物的代谢、抗生素的削减可产生不同程度的影响。

3.2 猪粪堆肥中四环素类抗生素 ARGs 的降解

猪粪中残留的抗生素进入环境后可产生 ARGs,ARGs 可通过猪粪转移,水平传播。王晓慧等^[60]对不同阶段的猪粪进行堆肥,发现猪粪中四环素类抗生素 ARGs 检出率大小顺序依次为成年猪粪>幼猪粪>堆肥猪粪>土壤。李海超^[61]在堆料中添加不同比例的生物炭,发现生物炭对四环素类抗生素

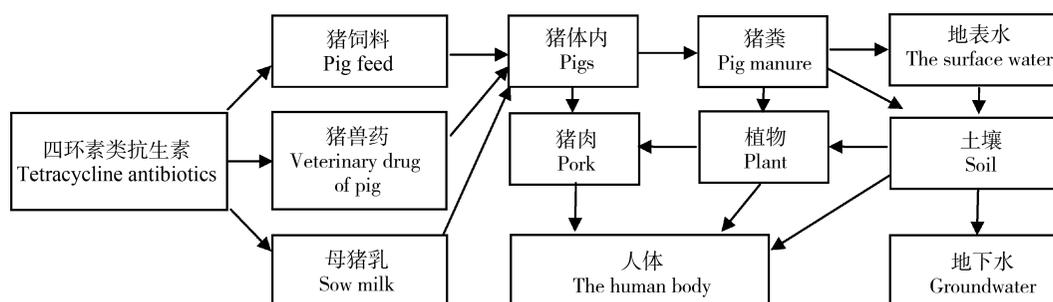


图 2 猪粪中 TCs 的来源及其潜在的转移途径

Fig. 2 Sources of TCs in pig manure and their potential transfer pathways

表 2 常见猪粪堆肥时的理化参数控制值

Table 2 The values of physical and chemical parameters in common pig manure composting

材料 Material	特点 Characteristics				参考文献 References
	N/(g·kg ⁻¹)	pH	C/N	含水率/% Moisture/%	
猪粪 Pig manure	29.63	5.11	-	70.36	[36]
猪粪 Pig manure	30.36	7.68	9.01	66.3	[37]
猪粪 Pig manure	23.25	7.11	17.8	68.3	[38]
猪粪 Pig manure	30.9	-	14.7	66.9	[39]
猪粪 Pig manure	27.4	-	13.2	71.2	[40]
猪粪 Pig manure	27.4	-	12.9	74.12	[41]
猪粪 Pig manure	21.28	-	18.3	50	[42]
猪粪 Pig manure	29.82	8.37	-	78.89	[43]
猪粪 Pig manure	26.8	-	13.8	76.8	[44]
猪粪 Pig manure	28.3	7.77	12.4	68.5	[45]

注:-表示数据不可获取。

Note:- means data is not available.

ARGs 中的 *tet C*、*tet G*、*tet W* 和 *tet X* 丰度有显著影响。Kang 等^[62]研究发现,猪粪经过 4 d 的短期高温堆肥处理,猪粪中四环素类抗生素 ARGs 不能被完全去除,但短暂的高温对猪粪中残留的四环素类抗生素 ARGs 在土壤中的积累和扩散具有控制作用。Zhang 等^[63]在堆料中加入木屑、稻壳和蘑菇残渣,在同温条件下进行堆肥试验,发现这些物质能通过改变微生物菌群,增强抗生素和 ARGs 去除的潜力,其去除率为 14.9% ~ 33.4%。也有研究表明,猪粪在堆肥过程中,粪中的磺胺类药物也会对粪中 TCs 及相关 ARGs 的降解产生影响^[64]。Mao 等^[65]用竹炭和 2 种菌粉加入猪粪进行堆肥实验,发现温度和有机碳含量可对细菌降解 TCs 产生影响。可见,堆肥可在很大程度上降低粪肥中四环素类抗生素 ARGs。粪中优势菌群的筛选、最适温度调控和外来环境因子的影响对抗生素的降解也非常关键。

3.3 猪粪堆肥中微生物菌群的变化

猪粪堆肥中抗生素削减的同时,伴随着微生物的变化。而微生物不仅是 ARGs 的携带者,也是粪中抗生素降解的参与者。据报道,猪粪堆肥中微生物菌群对堆肥过程中温度的调控起主导作用,猪粪中微生物及其产生的酶也是有机物降解的关键^[66]。黄雅楠等^[67]研究发现,猪粪堆肥过程中微生物与理化因子相互作用,优势菌梭菌属菌丰度从最初的 6.94% 增加至高温阶段的 18.56%,腐熟期后降低至 0.62%。曹云等^[68]研究发现,堆肥前期微生物数量变化趋势基本相同,嗜温菌丰度呈现先升高后下降,嗜热菌丰度随堆肥温度逐渐上升;同时,嗜热性纤维分解菌对堆体持续高温、加速有机质降解有促进作用。张海滨等^[69]研究发现,堆肥中微生物种类繁多,不同的堆肥原料可产生菌群差异。堆体纤维素的降解速度直接影响腐熟进程,优势菌可减少堆肥中的氮素流失。肖礼等^[39]在猪粪中添加白腐真菌及其混合外源菌剂,单一白腐真菌处理下的猪粪中铵态氮的降解最小;四环素和土霉素的含量随堆肥时间显著下降,经过 42 d 后 2 种抗生素降解率可达 90% 以上(OTC、TC 浓度 < 5 mg·kg⁻¹)。可见,猪粪堆肥中微生物菌群的变化可影响猪粪中抗生素的削减。

4 猪粪堆肥中 TCs 的降解 (Degradation of TCs in pig manure compost)

4.1 堆肥方式对 TCs 降解的影响

畜禽污染已成为农业面源污染的主要来源和重要成因。抗生素一旦经猪体代谢进入环境后,便会

随土壤、水和沉积物重新分布。目前,畜禽粪污处理和资源化利用主要有 3 种模式:资源化利用、肥料资源化利用和工业化处理。当前农业产业结构调整、猪粪集中排放和规模化处理已逐渐成为一种趋势^[70]。四环素类抗生素在生物转化过程中会产生代谢及降解产物,与母体相比,虽然活性降低,但毒性却增强^[71]。Winckler 和 Grafe^[72]给猪口服四环素 5 d,定期收集排泄物,发现实验结束 2 d 后的排泄物中,含 72% 四环素的活性成分。盐酸四环素在猪粪中表现出较强的稳定性,半衰期在 55 ~ 105 d 之间。猪粪堆肥化处理中,主要有好氧堆肥和厌氧堆肥,好氧堆肥是当前较为常见的技术,堆肥效果也更好^[73]。研究表明,不同堆肥方式对 TCs 的降解去除效果可产生不同程度的影响(表 3)。

4.2 堆肥中 TCs 的生物降解

猪粪中 TCs 的生物降解主要依赖于粪中微生物的活动。猪粪中抗生素的结构和药理学作用在微生物的作用下可发生一系列复杂的改变,不仅会产生有毒次生代谢产物,还有碳水化合物^[79]。猪粪堆肥中 TCs 的高效降解,优势耐药细菌菌株在其中发挥了很大的作用。成洁等^[80]在畜禽粪便池底部采集样品,进行平板涂布实验,得到木糖氧化无色杆菌对 CTC、TC 和 OTC 的降解率分别为 65.5%、63.9% 和 58.3%。吴学玲等^[81]从猪场粪便沉积池污泥中分离出的拉乌尔菌属的菌株,在代谢过程中能产生表面活性剂,可对 TCs 进行高效降解,降解率 > 70.68%。其次,Liu 等^[82]采用无土栽培技术,发现生姜生长能削减环境中的 TCs,其中生姜中根、茎根、茎和叶 TCs 残留量依次为:28.1、15.3、2.4 和 0.9 mg·kg⁻¹。因此,猪粪中降解 TCs 的微生物来自多个方面,经过生物降解能在很大程度上降低猪粪中 TCs 的比例,促进堆肥腐化进程,提高堆肥产品养分含量和堆肥品质。

4.3 堆肥中 TCs 的非生物降解

TCs 在堆肥中可发生不同的吸附过程,且可在光、水及与化学物质作用中发生非生物降解。王攀攀等^[83]对猪粪沼液中 TCs 进行光降解处理,发现不同波长光源中 TCs 的降解效果为:高压汞灯 > 紫外消毒灯 > 长弧氙灯 > 无光。用高压汞灯照射 2 h 后,粪中 TC、OTC 和 CTC 的降解率可达 91.68%、85.58% 和 81.18%。Wu 等^[84]研究发现,在堆肥过程中添加酸性磷酸盐,可控制氮损失和抑制有机物转化,因此,预期会优化堆肥效果。生物炭是一种良好

表3 不同堆肥方式对猪粪中TCs的降解效果

Table 3 Degradation of TCs in pig manure by different composting methods

材料 Material	TCs的种类 Types of TCs	半衰期/d Half-life/d	堆肥方式 Composting process	去除效率% Removal efficiency%	堆肥时间/d Composting time/d	参考文献 References
猪粪 Pig manure	土霉素 Oxytetracycline	-	好氧堆肥 Aerobic composting	92.8	28	[74]
	金霉素 Chlortetracycline	-	好氧堆肥 Aerobic composting	93.36	28	
猪粪 Pig manure	土霉素 Oxytetracycline	1.14	好氧堆肥 Aerobic composting	92	-	[75]
	金霉素 Chlortetracycline	8.25	好氧堆肥 Aerobic composting	74	-	
	四环素 Tetracycline	10.2	好氧堆肥 Aerobic composting	70	-	
猪粪 Pig manure	土霉素 Oxytetracycline	5.5	好氧堆肥 Aerobic composting	64.7	49	[76]
	金霉素 Chlortetracycline	2.4	好氧堆肥 Aerobic composting	73.3	49	
	四环素 Tetracycline	5.2	好氧堆肥 Aerobic composting	66.7	49	
猪粪 Pig manure	四环素 Tetracycline	14~18	厌氧堆肥 Anaerobic composting	88.6~91.6	45	[77]
	金霉素 Chlortetracycline	10	厌氧堆肥 Anaerobic composting	97.7~98.2	45	
猪粪 Pig manure	土霉素 Oxytetracycline	4.1~9.8	厌氧堆肥 Anaerobic composting	68.54	14	[78]
	四环素 Tetracycline	4.4~14	厌氧堆肥 Anaerobic composting	95.50	14	

的土壤改良剂,具有较强的氧化还原活性,对环境中的污染物有很好的吸附作用。Wang 等^[85]研究发现,稻草生物炭的吸附能力高于猪粪生物炭,且随着热解温度的升高而增大。另外,生物炭在好氧堆肥过程中能提高堆体温度,激活纤维素酶、脲酶活性,降低抗性基因 *tet C*、*tet G*、*tet W* 和 *tet X* 丰度^[20]。臭氧氧化也是一种降解 TCs 的有效方法。Khan 等^[86]发现 TCs 经臭氧氧化 4~6 min 后水溶液中 TCs 可完全去除。其中,臭氧可对 TCs 双键、芳香环和氨基进行修饰,使产物的 *m/z* 值发生变化。自由基可与 TC 的臭氧化产物发生非选择性反应。猪粪堆肥中 TCs 的非生物降解,也是促使猪粪中 TCs 降解的一种方法。

5 结论与展望 (Conclusion and prospect)

近年来,畜禽粪便堆肥化处理已逐渐成为一种趋势。在高浓度抗生素抗性选择压力条件下,猪粪妥善处理、抗生素的高效降解显得尤为重要。如今养猪生产上用药名录繁多,且粪尿干湿、雨污未能有效分流,这给猪粪堆肥处理增加了难度。基于近年来猪粪堆肥最新的研究进展,对当前我国猪粪堆肥中存在的突出问题,可在以下几个方面寻找突破:(1)猪粪中 TCs 的降解是个动态过程,主导 TCs 降解的微生物在堆肥中起很大作用,能抑制四环素类抗生素 ARGs 的水平转移和扩散。因此,在猪粪堆肥过程中筛选 TCs 降解的优异菌株,同时堆肥中结合

生物与非生物降解技术,可进一步提高猪粪堆肥中 TCs 的降解效果。(2)猪粪堆肥后,猪粪中残留的抗生素尚不能得到全部降解,残留的四环素类抗生素代谢的中间产物和微生物有毒代谢产物需得到进一步降解,才能实现粪肥在土壤中的无害化利用。(3)我国每年养猪生产上抗生素的用量和种类不断变化。对猪群不同饲养阶段饲用抗生素的用量及猪群 TCs 的代谢情况进行调查,探究猪粪便、污水及土壤中 TCs 的生态毒性,指导猪粪堆肥效果的进一步优化。

通讯作者简介:梁明振(1962—),男,农学博士,教授,主要研究方向为动物营养与饲料科学。

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