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基于原位控制技术的粪污除臭菌剂研发现状

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摘　要 恶臭污染已被列入世界七大公害之一，其危害日益受到各国重视，人畜粪污堆积与不科学处理加剧了恶臭污染. 对恶臭气体的形成、微生物除臭的原位控制机理、菌剂的研制与应用等方面进行综述. 粪污相关的恶臭气体包含氨和挥发性胺、挥发性硫化物、吲哚和酚、挥发性低级脂肪酸等4类，主要由肠道土著微生物分解粪污中残留的营养物质形成. 现有研究利用乳酸菌、酵母菌、放线菌等进行除臭，这些微生物生长迅速、占位效应强，成为优势群落后发挥益生特性协同抑制产臭微生物的生命活动. 据此原位除臭技术原理，选育有益菌种进行组配，固定于载体上制成除臭菌剂并研发出微生物除臭菌剂，应用于粪污堆肥除臭等多个领域. 然而，复合除臭菌剂的研发存在作用机制不明、适应范围狭窄、除臭能力不足等缺陷，针对这些问题，结合组学与合成生物学技术，提出深入研究菌剂除臭分子机理、代谢网络关系和细胞间通信的方案，绘制合成微生物群落指导菌剂构建的蓝图. 微生物除臭的应用前景广阔，对粪污无害化处理、集约化养殖场的健康运营及生态环境的改善具有重大意义，未来应继续重视除臭菌剂的研制和发展. （图1 表4 参102）
关键词 粪污；恶臭；除臭；微生物菌剂；原位控制

Research and development status of fecal microbial deodorant based on *in-situ* control technology

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Abstract Odor pollution is listed as one of the seven major public hazards in the world and has attracted increasing attention worldwide. Inappropriate disposal of accumulated feces from animals and humans exacerbate the formation of malodorous gases. This paper reviews the current literature on the original sources of malodorous gases, the mechanism underlying *in situ* management, and the formulation and application of microbial deodorants in odor gas control. Previous studies have revealed that malodor originating from feces primarily contains four kinds of chemicals: ammonia and volatile amines, volatile sulfur compounds, indoles and phenols, and volatile fatty acids. These malodorous substances are mainly produced by indigenous gut bacteria that degrade the remaining nutrients in feces. However, researchers have utilized specific microbial strains to suppress odors, including lactic acid bacteria, yeasts, and actinomycetes. These microorganisms reproduce rapidly and inhibit the growth and metabolism of odor-producing microbes, resulting in communities dominated by probiotic strains. Based on this *in situ* odor management theory, microbes with deodorizing features are screened and formulated to construct microbial deodorants and then applied in various disciplines, such as the deodorization of fecal compost. Although microbial deodorants have been achieved, the precise mechanism of deodorization remains unclear. Moreover, the narrow spectrum and insufficient ability of deodorization limit the further application of microbial deodorants. Thus, this review discusses potential avenues to address these issues and provides ideas for investigating the molecular mechanisms of deodorants, the relationship between metabolic networks, and cell-to-cell communication among deodorizing strains based on omics and synthetic biology methods. In addition, we propose a blueprint for the design of a microbial deodorant. We believe that the appropriate design and development of effective microbial deodorants will be beneficial for improving the living

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conditions of intensive livestock farms and the ecological environment.

Keywords feces; odor; deodorize; microbial deodorant; *in-situ* control

随着全球人口的不断增长和人们饮食结构的改善，肉类食品的需求量也持续攀升，其中猪肉的消耗量最大. 据美国农业部（USDA）2021年《畜禽：世界市场与贸易》最新报告，中国已成为第一大猪肉进口国与消费国. 在我国，单凭个体养殖户供应的猪肉生产远不能满足需求，因此，国家大力推进畜禽饲养的工厂化、集约化发展，依靠规模化养殖提升畜禽养殖效率. 然而，大量堆积的畜禽排泄物以及养殖废液会对畜牧集中地区造成负面影响，粪污肆意横流、臭气熏天，导致水体、土壤、大气受到不同程度的污染[1-2]. 除了养殖废弃物以外，人类每天产生的粪尿体量巨大，如何处置粪污并治理其产生的恶臭污染成为各国社会面临的难题. 截至2015年，全球仍有9.46亿人口露天排便，超过20亿人还在使用直接与化粪池相连的厕所[3]，如厕环境充满恶臭气味，滋生蚊蝇和病原微生物. 化粪池中未经无害化处理的污物，也是污染生态环境的潜在风险物质.

粪污中隐藏着威胁人畜健康的多种致病菌和寄生虫[4]，SARS冠状病毒、中东呼吸综合征冠状病毒（MERS-CoV）等致病源能在粪污中存活，继而通过粪便-口腔途径传播[5-6]. 据报道，2019年底暴发的新冠肺炎疫情中，新型冠状病毒（2019-nCoV）也会经粪口途径传播并扩散[5]，给人类健康带来威胁. 此外，粪污分解后产生的刺激性臭味不仅严重影响人体的情绪状态，而且恶臭气体中的有害化合物毒性极大，对养殖厂从业人员、周边居民的身体健康和畜禽的生长都有干扰，长期暴露在高浓度臭气环境里的人和动物会遭受不可逆的身体损害[7-9]，例如造成急性或慢性呼吸系统疾病，肺功能下降，刺激眼睛和呼吸道黏膜等形成炎症. 因此，粪污引起的恶臭问题亟待解决. 目前，国内外相关科研机构采取了各种措施应对此问题，并且研发出相应的除臭方法，包括物理法、化学法、生物法. 这3类方法都具有一定的脱臭效果，其中用微生物治理恶臭的方法具有成本低廉、操作方便、无二次污染等优点[10-11]，是研究的热点方向. 根据恶臭气体在脱除过程中的位置关系，微生物除臭技术可分为异位除臭和原位除臭，与异位控制技术相比，用微生物制剂进行恶臭原位治理一次性投入成本低、无需特殊设备[10-12]. 以原位除臭技术为基点，本文从恶臭气体的形成原理、除臭菌剂的作用机制以及微生物菌剂的制备与应用等方面对近年来微生物除臭菌剂的研发现状进行了综述.

1 微生物除臭菌剂的恶臭治理过程

1.1 恶臭的产生原理

恶臭污染被列为七大公害之一，国家标准《恶臭污染环境监测技术规范》将恶臭（odor）定义为一切刺激嗅觉器官引起人们不愉快感觉及损害生活环境的异味气体[13]. 恶臭气体一般是由土著细菌、外源细菌等腐生微生物分解粪便、尿液所致[12]，许多未被机体消化的营养物质会残留在大肠中，与粪便、尿液一起排出体外，随后由各种土著细菌和外源细菌分解，产生大量挥发性有机物（volatile organic compounds，VOCs）[14]，这些挥发性有机物多数具有刺激性气味.

根据化学性质，恶臭气体的主要成分可大致分为4类：氨和挥发性胺类、挥发性硫化物、吲哚类和酚类、挥发性低级脂肪酸[12, 15-16]. 尿液、尿酸分解后会挥发产生大量氨气（NH_3），氨基酸在脱氨过程中会产生腐胺、尸胺和氨等[17]，链球菌、消化链球菌和拟杆菌等参与此过程形成第一类恶臭物质[18]；第二类恶臭物质包括二甲硫和甲基、乙基硫醇等，在硫酸盐还原反应和含硫氨基酸代谢中产生[19]，参与此过程的有硫杆菌属、脱硫弧菌属等[20]；第三类恶臭物质含有吲哚、甲酚和4-乙基苯酚等，酚类化合物是通过动物肠道菌群分解氨基酸（例如酪氨酸和苯丙氨酸）以及粪污无氧发酵产生的，乙酸磷酸酯在色氨酸代谢过程中生成，后通过细菌基团将其转化为粪臭素（3-甲基吲哚），参与该反应的微生物主要有埃希氏杆菌属、丙酸杆菌属和梭状芽孢杆菌属等[21-22]；第四类恶臭物质如乙酸、丙酸、丁酸、已丁酸等，这些挥发性脂肪酸来源于蛋白质和碳水化合物的分解，由拟杆菌属、丙酸杆菌属、消化链球菌属、链球菌属、巨型球菌属、埃希氏杆菌属、乳酸杆菌属和梭菌属的代谢作用形成[18, 23].

虽然恶臭的产生及消除受外界条件（如湿度、温度、pH、通气量、尿液混入量等[24]）的影响很大，但最重要的影响因素还是产臭菌与除臭菌的群落结构及其丰度变化. 现有研究表明，参与形成恶臭气体的微生物类型主要是细菌，多数归类为厚壁菌门和拟杆菌门，表1概括了粪污中的关键产臭微生物菌属以及所形成臭气的主要成分[18, 25]，不同的微生物类型、处于动态变化的群落数量都会导致粪污释放的恶臭物质不完全相同. 因此，只有明确了微生物降解粪污时起主导作用的产臭物种，才能有针对性地选育抑菌活性强的功能菌株，实现粪污恶臭的高效脱除.

1.2 微生物除臭方式

除臭技术的原理，一般是通过物理、化学、生物或几种作用相结合，改变恶臭气体的理化性质以减弱其强度，或者从源头减少恶臭气体的产生，以实现臭气脱除. 现有的恶臭控制方法分为3大类，一是以活性炭或沸石吸附、芳香剂遮蔽为代表的物理法；二是以热氧化、化学试剂吸收为代表的化学法；三是以生物过滤、活性污泥净化为代表的生物法[10-11]. 其中，物理和化学除臭起效迅速、处理时间短且积累了大量的设计运行经验，但是这两类方法的投入成本较高、容易造成二次污染；生物除臭法由于具有运维成本低、除臭效率高、操作方便、安全性好等优点，成为主流的除臭方式[10]，其中最常用的是微生物除臭.

从除臭过程来看，微生物除臭技术又可分为异位控制技术和原位控制技术. 异位除臭是通过除臭滤池（塔）装置收集已释放的恶臭气体，采用微生物滤膜或填料对臭气进行集中脱除. 异位生物反应器主要有生物滤池、生物滴滤塔、生物洗涤器等，气体流经这些设备后，由固定于其中的微生物降解脱臭[10-11, 32].

原位除臭是通过除臭菌剂代谢降解恶臭物质作为自身的营养来源，或抑制产臭微生物的生长繁殖活动而进行除臭的技术. 一般除臭菌剂由功能微生物经过发酵复配制成，由于微生物组成、新陈代谢类型、营养方式及作用等多种多样，与恶臭异位治理技术相比，施用微生物制剂原位进行恶臭治理的优点在于适应环境能力强、除臭效果较持久、无需研制复杂

表1 粪污中产生的主要恶臭物质及相关微生物菌属
Table 1 Malodorous substances and the indigenous bacteria in manure

细菌 Bacteria	代谢类型 Metabolic type	恶臭物质 Malodorous substance
链球菌属[17, 26] *Streptococcus*	兼性厌氧或专性厌氧化能异养型 Facultatively or obligately anaerobic chemoheterotrophy	甲酸，乙酸，丙酸，丁酸，氨和挥发性胺 Formic acid, acetic acid, propanoic acid, butyric acid, ammonia and volatile amine
消化链球菌属[17, 26-27] *Peptostreptococcus*	厌氧化能异养型 Anaerobic chemoheterotrophy	甲酸，乙酸，丙酸，丁酸，异丁酸，戊酸，己酸，异戊酸，异己酸，氨和挥发性胺，吲哚和酚类 Formic acid, acetic acid, propanoic acid, butyric acid, isobutyric acid, valeric acid, caproic acid, isovaleric acid, isocaproic acid, ammonia and volatile amine, indole and phenols
埃希氏菌属[26, 28] *Escherichia*	需氧或兼性厌氧化能异养型 Aerobic or facultatively anaerobic chemoheterotrophy	甲酸，乙酸，丙酸和丁酸，吲哚和酚类 Formic acid, acetic acid, propanoic acid, butyric acid, indole and phenols
乳杆菌属[26-27] *Lactobacillus*	耐氧或厌氧化能异养型 Aero-tolerant or anaerobic chemoheterotrophy	甲酸，乙酸，丙酸和丁酸，吲哚和酚类 Formic acid, acetic acid, propanoic acid, butyric acid, indole and phenols
梭菌属[26, 29-30] *Clostridium*	厌氧化能自养型 Anaerobic chemoautotrophy	甲酸，乙酸，丙酸，丁酸，异丁酸，戊酸，己酸，异戊酸，异己酸，吲|哚和酚类，挥发性含硫化合物 Formic acid, acetic acid, propanoic acid, butyric acid, isobutyric acid, valeric acid, caproic acid, isovaleric acid, isocaproic acid, indole and phenols, volatile sulfur-containing compounds
丙酸杆菌属[26-27] *Propionibacterium*	专性厌氧或耐氧厌氧化能异养型 Obligately anaerobic or aero-tolerant chemoheterotrophy	甲酸，乙酸，丙酸，丁酸，异丁酸，戊酸，己酸，异戊酸，异己酸，吲哚和酚类 Formic acid, acetic acid, propanoic acid, butyric acid, isobutyric acid, valeric acid, caproic acid, isovaleric acid, isocaproic acid, indole and phenols
拟杆菌属[26, 31] *Bacteroides*	专性厌氧化能异养型 Obligately anaerobic chemoheterotrophy	甲酸，乙酸，丙酸，丁酸，异丁酸，戊酸，己酸，异戊酸，异己酸，氨和挥发性胺 Formic acid, acetic acid, propanoic acid, butyric acid, isobutyric acid, valeric acid, caproic acid, isovaleric acid, isocaproic acid, ammonia and volatile amine
巨型球菌属[26] *Megasphaera*	厌氧化能异养型 Anaerobic chemoheterotrophy	甲酸，乙酸，丙酸，丁酸，异丁酸，戊酸，己酸，异戊酸，异己酸，挥发性含硫化合物 Formic acid, acetic acid, propanoic acid, butyric acid, isobutyric acid, valeric acid, caproic acid, isovaleric acid, isocaproic acid, volatile sulfur-containing compounds

设备等. 因此，除臭菌剂多在中小型畜禽养殖场中使用[12, 33].

1.3 原位除臭微生物的类别

自然界中微生物的形态结构和生理功能千差万别，不同的菌种拥有的除臭功能不尽相同，不同菌株相互配伍、复合而成的菌剂作用也不相同. 已有研究表明，能够有效进行脱臭的主流功能菌群有以下几类[34-36]:

乳酸菌类，能够分泌脂肪酶、α-半乳糖苷酶、淀粉酶、脲酶和蛋白酶等[37]，在厌氧条件下将营养物质发酵为有机酸、酸菌素；周围环境的pH降低后，有害微生物的生长繁殖会受到限制；一些乳酸菌种类分泌的细菌素具有强大的杀菌能力[38]，可抑制粪污中产臭微生物对有机质的降解活动. 同时，乳酸杆菌代谢时产生的芳香物质，如乙醛、双乙酰等还能够掩盖粪臭味[39]. Liu等研究评估了短乳杆菌微生态制剂饲喂断奶仔猪的情况，发现短乳杆菌ZLBoo4对调节断奶仔猪的肠道菌群平衡、免疫力与生长性能有益[40].

酵母菌类，具有兼性厌氧的生理特性，对其适应粪污环境极其重要. 在粪污表面的有氧状态下，酵母菌会迅速增殖成为优势种群，利用粪污中的糖类、蛋白质、脂质等进行发酵，争夺产臭微生物的营养从而抑制其生长. 酵母菌分泌的生理活性物质还能作为其他益生菌的营养基质，促进有益微生物的生长繁殖，减少恶臭气体的释放量. 而粪污内部的酵母菌在缺氧状态下会发酵产生乙醇，可以有效地毒杀腐败性降解细菌. 此外，酵母菌分解氨基酸生成多种酯类芳香物质，其酒香味也能够对恶臭起到一定的掩盖作用[41].

光合细菌类，可以在光照厌氧的条件下通过光合自养作用或在黑暗好氧的状态下通过化能异养作用对各种含硫、含氮及含碳的化合物等进行利用. 因此，光合细菌能够对粪污中的多种恶臭物质进行分解转化，合成其他有益微生物生长繁殖所需要的养分. Elkahlout等通过琼脂固定的荚膜红杆菌从乙酸中提取生物氢[42]，光合细菌有望用于畜禽排泄废物的能源发酵与转化. 据国内外研究报道，在厌氧光照条件下，光合细菌几乎都具备卤代化合物的降解功能[43-44]，而在厌氧、微好氧和好氧条件下，光合细菌都能降解芳香族化合物[45-47]，这对恶臭气体的减少具有深远意义.

放线菌类，能够获取外界的营养并产生大量抗菌物质、酶及维生素，可以直接抑制有害细菌和产毒素霉菌，此外还能获取争夺病原微生物生长发育所需的基质，从而抑制其增殖. Shivaveerakumar等使用焦磷酸测序技术研究了堆肥中放线菌的多样性及其在7-578 d之间的群落结构变化，发现作为指示菌的大肠杆菌和肠球菌的数量与放线菌数量成反比[48]，致病菌因生长受到抑制而死亡.

芽孢杆菌类，这类微生物中严格需氧的代谢类型会消耗周围的氧气，创造厌氧环境，促进厌氧型除臭微生物迅速生长定植、占据生态位[49]. 它还能分泌蛋白酶、过氧化氢酶和氧化酶等多种生物活性物质，与畜禽消化道内的酶发挥协同作用，提高动物对蛋白质的分解吸收率，促进粪污的降解，减少恶臭的产生[50-51]. 有些芽孢杆菌还能分泌有机酸，使环境呈酸性，以此抑制杂菌生长.

霉菌类，主要为酿酒所用的曲霉菌属，能够将粪污中难以分解的淀粉降解为小分子物质以供养其它脱臭菌，保证其共生共存. 曲霉发酵产乙醇的能力尤其强大，可以杀灭产臭微生物，同时防止害虫和蛆生长[41]. 俞洁雅等筛选出一株烟曲霉菌，具有极强的纤维素降解活力，在猪粪发酵过程中促腐熟效果良好，能够显著抑制氨气的产生[52].

1.4 原位除臭菌剂的作用机制

由原位除臭微生物制备的除臭菌剂利用原位除臭机理进行恶臭控制，根据使用方式可分为体内调控剂和体外除臭剂[12]. 用于体内控制的微生态制剂，原理是将益生微生物加入动物的饮水或饲料中，动物进食完毕后使益生菌占据动物消化道内微生物群落的生态位，从而平衡肠道微生物的种群数

量，抑制产臭菌的生命活动；益生菌分泌消化酶、维生素、促生长因子等，提高动物对营养物质的消化和吸收效率，从而改变畜禽粪尿的理化性质，显著降低腐败物质的生成率和臭气的排放率[53-54]. Lei等用解淀粉芽孢杆菌直接饲喂生长后期的肉鸡，发现与抗生素相比，益生菌补剂可以通过提高养分的消化率和改善禽类的肠道健康，进而提高肉鸡的品质[55]. Wu等在家禽饲料里添加枯草芽孢杆菌后，能够增加肠道中乳酸杆菌的数量，同时减少大肠杆菌的丰度，从而改变家禽的机体健康状况[56].

用于体外控制的微生物菌剂，原理是向粪污中添加具有高效除臭能力的菌种，除臭菌株大量增殖后发挥“领头羊效应”，直接抑制腐败性分解菌的代谢，同时降解粪污中的恶臭物质，进而将土著微生物的发酵方向扭转到有益微生物的发酵方向，从源头上抑制臭气的产生. Mao等研究了竹炭与两种微生物菌粉制成的复合菌剂对猪粪堆肥过程中气体排放和细菌群落变化的影响，发现CH_4、N_2O、NH_3等气体的排放量都大幅降低，细菌群落丰富度和多样性明显增加[57]. Wang等通过添加不同浓度的嗜热脂肪芽孢杆菌进行粪便堆肥，试验证明施用该细菌可以加快堆肥升温，增加乳酸杆菌和硝化细菌的数量，从而改变土著微生物群落的结构，同时还能降低肥料pH值，促进硝化作用，并减少NH_3的排放[58]. 外源投加除臭菌剂简便易行，又可兼顾除臭、保氮两个要求，所以原位除臭作为粪污堆肥除臭的发展方向之一，是脱除含氮臭气较优选择.

2　微生物除臭菌剂研发与应用

2.1　微生物除臭菌种研究及菌剂制备

日本人很早以前就将家庭餐厨垃圾、庭院垃圾或牲畜粪便进行堆肥处理，获得的有机肥用于改良土壤，但随之带来的臭气问题经常遭到投诉[59]. 自20世纪70年代开始，日本、美国、德国、荷兰等发达国家即对恶臭污染展开了一系列立法和治理[60]，并相继研发出许多微生物菌剂，其中一些已经作为商品进行大规模生产，而我国在恶臭污染物治理与控制方面的理论和应用研究从20世纪90年代初期才开始进行[61].

粪污中残留着未利用的营养物质，生存着大量微生物，尽管所有臭源地都会产生异味，但恶臭环境中也会孕育出可以去除恶臭物质的微生物，因此，可以将这类微生物分离鉴定出来，作为生物除臭的潜在菌种. 1978年，日本的Ohta和Ikeda发现广岛市福山县的一位农民十多年来一直使用微生物进行除臭，他将来源于猪粪的微生物培养物接种于新鲜粪便进行脱臭，然后再将无臭的干燥粪便作为种子培养物循环利用. Ohta和Ikeda从粪便中分离出放线菌，验证了其有效消除挥发性脂肪酸的作用，为粪污恶臭的治理提供了研究基础[62].

微生物除臭菌剂是将选育出的高效除臭菌株固定于填料上，制成特殊的剂型，将其投加到粪污中，于适宜的条件（酸碱度、温度、湿度、通气量等）下发酵便可达到除臭的目的，除臭微生物的种类和效率决定了恶臭气体的去除效果. 除臭菌种的来源主要有3种[63]：自然筛选、诱导驯化、基因工程改造. 由于后两种方式耗时耗力、操作困难，目前大部分菌剂都是依靠传统的自然筛选方法来获取除臭菌种，即直接从恶臭污染场所选育高效除臭的功能菌株. Bourque等以挥发性脂肪酸和芳香族化合物作为培养基的唯一碳源，从猪粪中筛选出各种类型的细菌，包括乙酸钙不动杆菌、粪产碱杆菌、谷氨酸棒杆菌和微球菌等，它们对所有挥发性脂肪酸均具有降解活性[64]. Kazutaka等从动物粪便堆肥中分离出一株嗜热性芽孢杆菌TAT105，并制成菌剂，可以有效减少猪粪堆肥中NH_3的排放[65]. 除了从自然臭源地筛选，还可以通过驯化获得更具有环境适应性的除臭菌株，Zhao等通过高温驯化培养，从堆肥样品中富集获得耐热硝化细菌（TNB），接种到污泥堆肥中，发现与对照处理相比，氨排放减少29.7%，并通过TNB将更多的NH_4^+-N转化为NO_3^--N，减少了氮损失[66].

一种微生物一般只能针对性降解一种恶臭气体成分，而混菌体系可同时脱除多种恶臭气体，实践证明，单一菌种制成的菌剂效果远不如复合菌剂[67-68]，因此利用多种微生物制备复合菌剂更受青睐. 针对景区游客粪污的恶臭，唐微微等研制了由乳杆菌、东方伊萨酵母、链霉菌等6株菌合成的生态厕所除臭剂，该复合菌剂均能去除粪污产生的TVOC、H_2S、NH_3，且比起市售的EM菌液效果更佳，在缺水景区的免水生物降解型厕所中具有应用潜力[69]. Zhou等采用嗜热芽孢杆菌、产假丝酵母和枯草芽孢杆菌制成5种不同比例的复合微生物菌剂，用于鸡粪堆肥除臭，3种菌株以1:2:1、1:1:1复配时，堆体的总氨排放量明显低于空白对照组，分别减少了53.11%和46.23% [70].

除菌种外，菌剂除臭效果的优劣还与添加的载体有一定关系，载体为微生物提供依附的基质并对恶臭物质起到物理吸附作用与固着作用，优质的载体会显著提升除臭效果. Borowski等测试了微生物矿物质制剂对家禽粪污的除臭效果，其中喷雾干燥微胶囊与珍珠岩-膨润土作为混合载体形式的微生物菌剂对于NH_3和H_2S的去除特别有效，且该制剂在4 ℃或22 ℃下存放5个月后仍显示出很高的稳定性与除臭性能[71]. Kalus等评估了微生物矿物处理剂应用于蛋鸡鲜粪的除臭作用，该菌剂由6株异养菌复配而成，珍珠岩-膨润土矿物吸附剂作为载体，测得第4天挥发性有机化合物的减少水平在9%到96%之间，而单独使用矿物吸附剂处理则在31%到83%之间[72].

2.2　微生物除臭菌剂的应用

从单一菌种的利用到多个菌种的混合应用，国内外有关微生物除臭菌剂的研究皆取得了一些阶段性的突破和成果. Guo等人在猪粪好氧堆肥过程中添加巨大芽孢杆菌的菌剂和膨润土，研究了氮转化、氮功能基因和微生物群落动态的变化，发现菌剂处理组使NH_3排放减少了31.34%，N_2O排放减少了53.16% [73]. Kim和Ahmed等以不同浓度的解淀粉芽孢杆菌培养液作为添加剂处理猪粪，发现解淀粉芽孢杆菌具有降低粪污中NH_3、H_2S和SO_2排放量的强大能力[74].

环保领域也逐渐出现了关于复合菌剂研制的报道，最著名的菌剂当属琉球大学农学系比嘉照夫教授于1982年培养而成的EM菌群（effective microorganism），成分包含10属80余种微生物，有乳酸菌、酵母菌、光合细菌和放线菌等. EM菌剂不仅在生物肥料、有机废弃物处理中效益显著[75-76]，而且在养殖业、种植业以及污水净化方面也有广泛应用. 一些发达国家对微生物菌剂的研究和推广较早，相关的生物除臭技术也较成熟，相比之下，我国在这方面的发展稍显滞后，大部分菌剂都依靠从国外进口[63]. 近年来，国内相关科研机构和公司才陆续开展除臭菌剂的研究，研发出一系列的微生物菌剂产品，并申请了发明专利，如表2、表3所示.

此外，研究学者不断分离和筛选高效除臭菌种，寻找和优化安全高效的除臭菌剂组合——不仅适用于粪污除臭，还能处理其他场所污染源的恶臭. Qiu等利用氨化菌、硝化菌、亚硝酸盐氧化菌、固氮菌这几类菌属的12株细菌复合制成的保

表2 商品化的除臭菌剂产品
Table 2 Commercial products of microbial deodorant

研发单位 Research and development company	除臭菌剂产品 Deodorant product	功能 Function
潍坊某生物科技有限公司 A biological technology limited company in Weifang city	猪粪发酵菌剂 Fermentation starter of swine manure	具有特效分解能力的好氧微生物，在堆肥过程中分解粪便并除臭 It contains aerobic microorganisms with high-efficient ability of decomposition, degrading manure and deodorize during composting
河南某生物科技有限公司 A biological technology limited company in Henan Province	牛羊粪发酵菌剂 Microbial agent of cow and sheep manure fermentation	有效去除原料和牛羊粪好氧发酵中产生的恶臭气味 It effectively removes the odor produced from the aerobic fermentation of cow and sheep manure
河南某生物制品有限公司 A biological product limited company in Henan Province	益母康、益多菌 Yi Mu Kang, Yi Duo Jun	作为微生物饲料添加剂，提高营养物质消化率，降低臭气排放量 As a microbial feed additive, it improves the digesting rate of nutrients and reduces the emission of odor
北京某科技有限公司 A technology limited company in Beijing city	干撒式发酵床养鹅菌剂 Dry spreading microbial agent for raising geese in fermentation bed	含有天然复合益生菌群，促进养鹅脱臭发酵床稳定运行 It contains natural compound probiotics, promoting the stable operation of fermentation bed and deodorizing in the goose house
济南某化工有限公司 A chemical limited company in Jinan city	蓝净除臭除味剂 Lanjing Deodorant	快速消除养鸡场粪污中大量病菌和各种臭气分子，除臭率90%以上 It rapidly eliminates germs and odor molecules in chicken farms with deodorizing rate more than 90%

表3 除臭菌剂的发明专利
Table 3 Invention patents of microbial deodorants

研发单位 Research and development institution	专利 Patent	主要成分 Main composition
湖南省微生物研究院 Hunan Institute of Microbiology	一种针对城市生活垃圾填埋场的微生物菌株、除臭剂及其制备方法[77] A microbial deodorant for landfills of municipal solid waste and its preparation method	酿酒酵母 *Saccharomyces cerevisiae* 干酪乳杆菌 *Lactobacillus casei*
宜宾市万华生物环保有限公司 Yibin Wanhua Biological Limited Company of Environmental Protection	微生物环保剂[78] Microbial deodorant for environmental protection	枯草芽孢杆菌 *Bacillus subtilis* 沼泽红假单胞菌 *Rhodopseudmonas palustris* 德氏乳杆菌 *Lactobacillus delbrueckii* 中草药提取液 Chinese herbal extract
中国科学院成都生物研究所 Chengdu Institute of Biology, Chinese Academy of Sciences	一种用于公厕除臭的复合生物制剂制备方法及其应用[79] Preparation method and application of a composite biological deodorant for public toilets	乳酸杆菌 *Lactobacillus* sp. 嗜热链球菌 *Streptococcus thermophilus* 橘皮提取液 Citrus peel extract
上海莱泰生物科技有限公司 Shanghai Laitai Limited Company of Biological Technology	一种除臭微生物菌剂的制备方法[80] A preparation method of a microbial deodorant	恶臭假单胞菌 *Pseudomonas putida* 凝结芽孢杆菌 *Bacillus coagulans* 蜡状芽孢杆菌 *Bacillus cereus*
浙江省农业科学院 Zhejiang Academy of Agricultural Sciences	一种微生物除臭菌剂的用途[81] The application of a microbial deodorant	弯曲芽孢杆菌Z2 *Bacillus flexus* Z2 巨大芽孢杆菌Z3 *Bacillus megaterium* Z3

氮微生物制剂（NRMA），进行稻壳与鸡粪堆肥实验，堆肥结束后发现实验组的NH_3总排放量（4.9 g/kg）显著低于对照组（11.9 g/kg）[82]. Nakasaki等从厨余垃圾中分离出一种细菌和两种酵母，通过实验发现其中的克鲁斯假丝酵母变种产生的香味能够掩盖垃圾储存期间散发的恶臭[83]. Chen等采用限制性培养法从牛粪中富集了一个遗传稳定的微生物群落CC-E，能够有效去除牛粪堆肥中的氨，CC-E处理（151.11 mg/kg）产生的氨量比对照组（447.87 mg/kg）低63%，测序表明其中主要包含鞘氨醇单胞菌属、沙雷氏菌属和产碱菌属[84]，可作为微生物除臭的菌剂资源. Jiang等分别从成熟的牛粪、鸡粪和猪粪堆肥中分离出氨化细菌、硝化细菌和固氮细菌，用以组成氮转换菌剂（NTB），将其用于猪粪和麦草堆肥后，发现NTB菌剂可以延长嗜热阶段，促进堆肥成熟，并且减少氮素损失和NH_3排放[85]. 目前，除臭菌剂已经被广泛应用于多个领域的恶臭治理和减排[86]（表4）.

综上所述，无论是将除臭微生物应用于景区厕所、化粪池、垃圾填埋或有机固废堆肥等环境治理过程，还是将其作为牲畜的饲料添加剂，除臭菌剂的研发都具有积极的社会意义和应用价值.

3 微生物除臭菌剂研发过程问题与展望

生物除臭技术在处理效率和费用成本方面都比其他控制技术更加优越，在环保领域得到了广泛的利用，其中微生物除臭更成为研发应用的焦点，给社会带来了生态、经济和环境效益. 从经济性和实用性的角度来看，除臭菌剂不需要专业人员来操作，也不需要特殊的气味处理装置，简单易用，能够针对不同场景和用途进行定制.

微生物除臭菌剂虽然有许多优点，却也存在一定的局限性和问题，也有许多问题和细节需要继续研究探索[33, 41, 63]. 例如在除臭机理解析方面，为克服单一菌种应用面狭窄、适应性差的缺点，除臭菌剂多采用微生物复合制备，但是微生物之间的协同作用机制不明确，其群落结构越复杂，作用机理就越难分清，所以菌剂的应用效果只处于表观阐述的阶段，未涉及分子机理层面的解释. 后续在除臭过程中对微生物代谢恶臭物质的原理、有机质的降解动力学及菌群结构的时空演变规律进行深入研究，阐明各类型微生物间的互作关系和影响. 此外，复合菌剂内是否存在毒害同伴的菌株分泌物或代谢物质，是否存在微生物间共代谢难于分解的化合物，均无定论. 可以结合宏代谢组学、宏基因组学技术等，进一步研究有机质转化、微生物群落变化与异味气体演替的规律.

表4　除臭菌剂的应用

Table 4　Application of microbial deodorants for deodorization

应用领域 Application field	主要成分 Main component	应用效果 The effect of application
猪粪和麦麸堆肥 Compost of pig manure and wheat bran	副干酪乳杆菌 *Lactobacillus paracasei*	氨气的挥发和总氮的损失减少，保证猪粪经家蝇幼虫培养后的蛆产量，以替代畜禽粪便作为水产养殖业的蛋白质饲料[87] The volatilization of ammonia and the loss of nitrogen were reduced. The yield of maggots was ensured via housefly larvae cultivation, which could replace livestock manure as protein feed for aquaculture
猪粪和锯末堆肥 Compost of pig manure and dry sawdust	乳酸杆菌 *Lactobacillus* sp. 假丝酵母菌 *Candida* sp. 芽孢杆菌 *Bacillus* sp. 嗜冷杆菌 *Psychrobacter* sp.	在生物炭和微生物接种处理中，有机物的降解和堆肥的解毒能力得到增强，凯氮总含量提高了59% [88] In the biochar/microbial inoculation treatment, the degradation of organic matter and the detoxification of the compost were enhanced, and the content of total Kjeldahl nitrogen was increased by 59%
鸡粪和稻壳堆肥 Compost of chicken manure and fresh rice husks	斯氏假单胞菌 *Pseudomonas stutzeri* 鞘氨醇杆菌 *Sphingobacterium* sp. 八叠球菌 *Sporosarcina* sp.	氨转化为总氮，氮的损失显著减少，堆肥周期缩短了15 d [82] Nitrogen loss was significantly reduced by converting more ammonia to total nitrogen, and the compost cycle was shortened by 15 days
鸡粪和蘑菇渣堆肥 Compost of chicken manure and mushroom residues	排硫硫杆菌 *Thiobacillus thioparus*	排硫硫杆菌和硫的结合使用能够同时有效控制氨气和挥发性硫化合物[89] Combined use of sulphur and *Thiobacillus thioparus* effectively reduced both ammonia and VSCs
牛粪污水除臭 Deodorization of cow liquid dung	甲基营养型芽孢杆菌 *Bacillus methylotrophicus* 布氏乳杆菌 *Lactobacillus buchneri* 苍白杆菌 *Ochrobactrum*	纤维素分解菌群、有机物降解菌群、固氮菌群、H_2S/NH_3去除菌群的种类和丰度明显高于对照组，而H_2S/NH_3产生菌低于对照组，病原菌和土著菌株含量明显降低或消失[90] The diversity and abundance were greater than control treatments in terms of cellulose decomposition, organic degradation, nitrogen fixation, and H_2S/NH_3 dislodge while H_2S/NH_3 producing bacteria were less than the control group. The pathogenic bacteria and indigenous strains were significantly reduced or disappeared
畜禽饲料添加剂 Additive of animal feed	枯草芽孢杆菌 *Bacillus subtilis* 地衣芽孢杆菌 *Bacillus licheniformis*	极大地提高了营养物质的表观消化率，促进猪的生长，使粪便中乳酸菌的数量呈线性增加，NH_3和H_2S的排放率降低[91] The apparent digestibility of nutrients was greatly improved, and the growth of pigs was promoted. The number of lactic acid bacteria in the feces linearly increased, and the emission rate of NH_3 and H_2S was significantly decreased
生活垃圾除臭 Domestic waste transfer	恶臭假单胞菌 *Pseudomonas putida* 凝结芽孢杆菌 *Bacillus coagulans*	垃圾转运站集装箱内甲硫醇、甲硫醚等恶臭物质的浓度大幅降低[92] The concentration of methanethiol, methyl sulfide, and other malodorous substances was significantly decreased

在除臭菌种选育方面，需要考虑低温严寒、高原缺氧等不利于微生物生长的条件下，菌株的除臭能力会减弱的情况，应当大力挖掘在各种特殊环境条件下发挥作用的高效脱臭微生物资源，并研制开发出相应的超级菌剂．同时，可以借助高速发展的基因编辑技术，发展菌株驯化和基因改造技术，以培育出更为高效、更有针对性的除臭微生物．

在除臭菌剂产品开发和应用方面，国际公认的微生态制剂的质量标准要求菌剂无毒无害，而大部分菌剂产品在安全方面做工不足；需要建立更为细致的标准，对研发上市的菌剂进行严格的毒力检测、杂菌检验、杂菌病原性鉴定等工作．其次，微生物个体易受到群体组成和不利环境因素的影响，导致除臭菌株的稳定性下降，使复合菌剂的除臭效果降低，保质期变短；所以应当加强对复合除臭菌剂制备工艺的改进和创新，例如调整复合比例、筛选更优化的载体填料、联合物理化学方法、改变保藏剂型等，以实现最佳除臭效果．另外，微生物受环境影响，容易发生变异，所以在使用菌剂除臭时，应当定期检测菌剂有无致病性突变或者是否结合了有害物质，避免出现二次污染或生物风险．

近年来，突飞猛进的合成生物学技术为环境微生物的应用研究打开了新世界的大门．从微生物组学到合成微生物群落，合成生物学作为新兴的学科迅速发展到科技前沿并应用于生产生活领域，源于人工合成功能菌群所具有的自然微生物群落无法媲美的优点[93]．例如，合成功能菌群的物种组成清楚明了，对于菌株间互作机制的研究就更加简便、可控，不会轻易出现有害的变异；微生物群落以数学模型为学习基础由人工设计构建而来，混菌体系的结构与功能可根据研究者的意愿进一步改造，从而实现更强大的目标功能，完成更加复杂艰巨的任务．

国外已有学者开发出新型计算机软件作为工具，进行自动化设计合成微生物群落，以达到最优化的合成菌群目标[94-95]．此外，Pipat和Orit等团队研究发现通过操控益生菌的群感效应系统可以更改有害菌的信号传导途径，阻断其细胞间的通讯，达到抑制有害菌生长的目的[96-97]，这为粪污除臭菌剂研发过程中的问题提供了解决思路．根据最新的报道，本文围绕“设计—构建—测试—学习”的合成生物学理念[98]，结合日新月异的分子生物学技术、高通量测序技术、宏组学分析技术、基因工程等手段[99]描绘了利用人工合成微生物组的研究方法指导高效除臭菌剂构建的蓝图（图1），国内研究学者可以尝试运用新的技术开发更高效可控的除臭菌剂．

为科学有效地研发除臭菌剂，除臭菌剂的构建理论与应用需要继续突破现有问题与难点，加大力度开发新的野生菌株、构建新的重组菌株来补充菌种资源库，运用人工合成微生物组等新技术、新方法进行复合除臭菌剂科学配伍，加强环境因素对除臭菌剂效能的影响研究，开展菌种定植跟踪监测研究，建立除臭菌剂应用技术体系．未来，畜禽养殖业会更加注重安全、经济和可持续发展的绿色生产方式，养殖户应选择更加环保的除臭菌剂产品，实现从源头削减恶臭气体，改善畜禽圈舍的条件．因此，开发更高效的除臭菌剂具有广阔的应用前景，在实现经济利益的同时，也对保护生态环境、遏制空气污染具有重要意义．

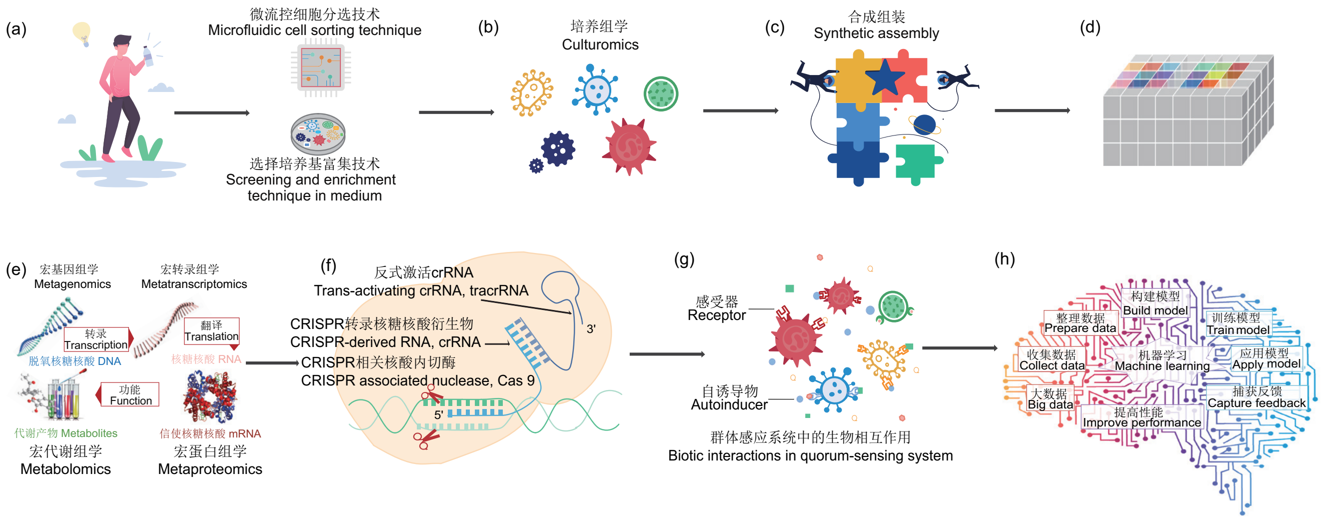

图1　除臭菌剂的构建蓝图. （a）在典型的臭源生境中采样，或者从功能菌种资源库选取目的菌株；（b）微流控细胞分选技术和选择培养基富集技术：对功能菌株团体进行分离纯化，再对单个物种进行生理生化分析；（c）生物反应器：改变培养条件，给予生物刺激，人工合成并组装微生物组；（d）微流控或自动微反应器平台：高通量筛选除臭微生物并测试分析其性能，收集除臭性能高的菌株数据；（e）多组学技术[100]代谢通量分析技术：获取目标除臭菌株的基因组信息，重建代谢网络，创造微生物组生态功能模型；（f）CRISPR/Cas9技术：对基因进行原位编辑，以优化除臭菌群的特定功能表型，提高互作稳定性及其与土著菌的竞争力；（g）群体感应系统构建和营养依赖型改造：协调益生菌种之间的互作关系，使其共生共荣，形成平衡的稳态；（h）数据驱动建模技术[101]和机器学习[102]据，检测菌剂生态系统的物理化学性质与微生物组的功能，评估除臭方案的可行性，自动化提升系统性能.

Fig. 1　Construction blueprint of microbial deodorant. (a) Target strains are collected from typical malodorous biotopes, or selected from a functional microbe reservoir. (b) Ideal strain is isolated and purified by microfluidic cell sorting and selective medium enrichment techniques, followed by physiological and biochemical analyses. (c) Bioreactor. The microbiome is artificially synthesized and assembled after altering incubation parameters and adding biostimulation. (d) Microfluidic platform or automatic microreactor. Deodorization effect is assessed by high-throughput analyzing, screening and testing, and the strain which exhibits superior performance is collected. (e) Multi-omics metabolic flux analysis techniques. To reconstruct the metabolic network and create an ecological functional model of microbiome, the genomic information of targeted deodorizing strain is obtained. (f) CRISPR/Cas9 technique. Genes are edited *in situ*, aiming to optimize the specific functions and phenotypes of deodorizing microbial community, improve the stability of interaction among deodorizing microbes and enhance their competitiveness with indigenous microbes. (g) Construction of quorum sensing system and nutrient-dependent transformation. The interaction among probiotic strains is coordinated, enabling them to constitute a symbiotic and prosperous system and form a balanced steady state. (h) Data-driven modeling technology and machine learning. Organizing experimental data, the physicochemical properties of ecosystem in microbial deodorant and the function of microbiome are tested, then the feasibility of deodorizing scheme is evaluated, thus automatically improving the performance of deodorizing system.

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