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·综　述·

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典型有机固废厌氧消化微生物研究现状与发展方向

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摘　要: 经过人工富集和驯化的兼性和严格厌氧微生物是厌氧消化工艺的核心。不同厌氧消化体系中存在的问题大多可以通过改变微生物群落的代谢活性来得到有效改善。得益于微生物组学检测技术的快速发展，对厌氧消化系统中微生物多样性的认识获得了极大的拓展，同时在微生物类群间、微生物与环境的互作关系研究方面也取得了一系列新的进展。然而，有机固废厌氧消化系统中，各种微生物以及微生物和物质的相互作用构成了更为复杂的代谢网络，所以目前对这些互作关系的解析尚不完善。本文重点关注了厌氧消化过程中的典型菌群互作关系，阐述了典型有机固废厌氧消化系统中存在的问题及微生物在其中发挥的作用，最后，立足于现有组学技术推动的微生物组研究进展，对未来有机固废厌氧消化系统微生物组的研究提出展望。

关键词: 微生物组，厌氧消化，有机固废，互营代谢，互作

Microorganisms in the typical anaerobic digestion system of organic solid wastes: a review

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Abstract: The facultative anaerobic and strict anaerobic microorganisms enriched and acclimated during the anaerobic digestion process are crucial for the efficiency of the anaerobic digestion system. Most of the problems encountered during running anaerobic digestion processes could be effectively improved via stimulation of microbial metabolic activity. Benefited from the rapid development of microbiome techniques, deeper insights into the microbial diversity in anaerobic digestion systems, e.g. the microbe-microbe interactions and microbe-environment interactions, have been gained. A complex and intricate metabolic network exists in the anaerobic digestion system of solid organic wastes. However, little is known about these interactions and the underlying mechanisms. This review briefly summarized the representative interactions between microbial communities during anaerobic digestion process discovered to date. In addition, typical issues encountered during the anaerobic digestion of solid organic wastes and how microbes can tackle and alleviate these issues were discussed. Finally, future priorities on microbiome research were proposed based on present contribution of microbiome analysis in anaerobic digestion system.

Keywords: microbiome, anaerobic digestion, organic solid wastes, syntrophic metabolism, interaction

生物处理是目前实现有机固废资源化、能源化、减量化和无害化的重要手段。厌氧消化 (Anaerobic digestion) 更是其中的研究热点，已被广泛用于多种有机固废的消减和资源化，如市政污泥、餐厨垃圾、园林和农业废弃物、畜禽粪便等[1-2]。厌氧消化系统的宏观调控较易实现，通常是在遵循微生物组代谢规律的前提下，优化工艺条件以实现特定产物的富集和能源物质的回收[3-5]。然而，以微生物代谢为主体的微观过程却难以实现定向调控。根本原因是有机固废成分的

复杂性、环境影响因素的多变性以及它们与菌群交互作用的多样性，限制了对有机固废厌氧消化过程的深入理解。近年来化学分析技术 (例如高分辨质谱的应用)、微生物检测技术 (例如多组学技术的应用) 以及生物信息技术 (例如模型的构建) 的快速发展[6-7]，开创了微生物组研究的新方向[8-9]，也为更全面地解析有机固废厌氧消化的微观机制提供了新的契机。

1 厌氧消化系统中的微生物以及典型菌群的互作关系

厌氧消化的工程调控依然处于“黑箱”操作，随着组学技术的发展，对厌氧消化系统相关微生物分类和功能的认识更加深入，且对其参与的不同阶段的认识也在与时俱进[10-12]。厌氧消化的不同阶段 (水解、产酸、产乙酸和产甲烷) 是基于物质流确立的，传统观念认为厌氧消化过程每个阶段的微生物类群具有专一性，然而最新宏基因组学证据表明，微生物类群演替在整个厌氧消化过程具有连续性、无明显的阶梯性变化，例如随着初始底物的变化沿着物质流传递到整个厌氧消化过程时，包括产甲烷菌在内的菌群均会发生演替[13]。参与不同物质转化的微生物被称为相应的功能菌，这种功能菌群的界定是基于微生物的代谢功能而非系统发育信号，所以同一类微生物可能在厌氧消化过程中发挥多重作用。

1.1 参与厌氧消化的微生物类群

微生物作为各种物质转化的主要承担者在整个厌氧消化过程中发挥了不可替代的作用。事实上，厌氧消化系统中能够明确鉴定谱系的微生物非常有限，超过 90%的微生物无法在属或种的水平进行分类[14]。厌氧消化过程中已知的细菌主要属于厚壁菌门 Firmicutes、拟杆菌门 Bacteroidetes、变形菌门 Proteobacteria[14-15]，承担着将复杂有机大分子转化为简单有机物以及 CO_2、H_2 等气体成分的一系列任务。相比细菌的高谱系多样性，厌氧消化工程系统中的古菌较为单一，来源于广古菌门 Euryarchaeota 的产甲烷菌[6,12]。虽然最新的宏基因组学证据表明深古菌 Bathyarchaeota[16] 和佛斯特拉古菌 Verstraetearchaeota[17-18]等也具有甲烷代谢功能，从而将产甲烷菌谱系扩展到 Euryarchaeota 之外[19]，但目前没有任何非 Euryarchaeota 的产甲烷菌在厌氧消化系统中被发现。除了细菌和古菌，真菌同样是厌氧消化系统中的重要微生物，参与固体物质的分解和有机大分子的降解与转化过程，然而很少有报道关注真菌在整个系统中的功能和命运[3,20-21]。正常运行的厌氧消化系统，各类微生物的物种数可能高达上千个[22]，这些微生物通常具有高度的功能冗余从而增强了系统应对复杂环境和抵抗环境扰动的能力[23-24]。Yu 等认为当功能冗余和群落多样性降低时，菌群间互作网络简单化，系统可能会崩溃[23]。

1.2 厌氧消化系统中典型菌群的互作关系

厌氧消化系统中不同菌群在代谢功能上是相互依存的，在维持系统稳定的同时，遵循着物料守恒和能量守恒规律[25]。相互关联的复杂酶系为厌氧消化系统中菌群执行互补或可替代的代谢活动提供了支持[26]。通过鉴定编码关键酶基因的存在和表达是解析系统代谢路径和解构互营关系的重要方法。譬如 Hao 等结合宏基因组和宏转录组，预测了互营脂肪酸氧化菌互营代谢途径并证实了不同环境下代谢功能存在选择性表达[27]。Zhu 等根据参与 Wood-Ljungdahl 途径和甘氨酸裂解途径关键基因的表达差异，重构了发酵细菌以甘氨酸裂解替代乙酸代谢并耦合氢营养产甲烷的互营模型[24]。厌氧消化前段的发酵细菌与后段的产甲烷菌通过产物交叉喂养构成互营关系，缓解了产物积累造成的压力，并使整个反应在热力学上是有利的。一方面发酵细菌产生的乙酸、H_2、CO_2、甲基化合物为产甲烷菌提供底物，同时这些细菌

的代谢活动消耗了氧、硝酸盐、Fe(Ⅲ) 等氧化性物质，为产甲烷创造适宜的氧化还原条件；另一方面产甲烷菌通过消耗乙酸、H_2 和 CO_2，为脂肪酸的氧化解除了产物的抑制作用，提供了能量，同时维持了体系的 pH 稳定[19]。这种互营依赖于细胞间的电子传递，分为依靠 H_2、甲酸等小分子进行的种间电子传递 (Interspecies electron transfer, IET) 和依靠细胞上的微生物纳米导线或多血红素细胞色素的直接电子传递 (Direct interspecies electron transfer，DIET)[28-29]。一直以来，IET 被认为是互营菌的主要电子传递方式[30]，而 Rotaru 等证实了 DIET 在厌氧消化工程系统中的存在，并提出 DIET 可能在全球产甲烷过程中起重要作用[31]。最新研究显示，电子传递和能量获取策略是构建互营关系的关键决定因素[25]。

厌氧消化各阶段产物汇聚于胞外空间形成一个共享的物质池。除了互营，不同菌群对物质的竞争也广泛存在于整个厌氧消化系统中。例如，互营乙酸氧化菌 (Syntrophic acetate oxidation bacteria，SAOB) 和乙酸营养型产甲烷菌构成了对乙酸的竞争，而氢营养产甲烷菌和同型产乙酸菌均以 H_2 和 CO_2 为底物[32]。同型产乙酸菌/乙酸营养产甲烷菌以及互营乙酸氧化菌/氢营养产甲烷菌分别通过产物交叉喂养成为互营伙伴，并由于近似的互营代谢作用而产生生态位的竞争[33] (主要代谢途径见图 1)。微生物的生态位竞争受到其环境适应性的影响，而整个互营代谢的存在往往受限于互营伙伴中更为脆弱的一方。研究表明，互营乙酸氧化 (图 1C) 作为 Wood-Ljungdahl 途径产乙酸 (图 1B) 的逆反应在热力学上极为不利，所以 SAOB 在与乙酸营养型产甲烷菌的竞争中常处于劣势，从而造成整个互营代谢处于不利地位[3,34]。但温度升高时，乙酸氧化的热力学壁垒会相对降低，从而提升 SAOB 的竞争能力[3,35]。此外，相比于发酵细菌，产甲烷古菌一般冗余度较低，具有更低的生态位宽度，更容易受到恶劣条件的影响，进而影响到互营伙伴的代谢乃至危及整个系统的稳态。尤其是乙酸营养型产甲烷菌，相比其他两种类型的产甲烷菌其生态位更窄[36-38]，在极端环境如高游离氨、低 pH 和高盐条件下难以有效存活，造成的生态空位会由耐受性更好的互营乙酸氧化菌/氢营养产甲烷菌填补[36-38]。

共享物质池中的某些物质在细胞间频繁作为产物和底物传递，如甲酸、乙醇、CO_2、H_2 等，其浓度会直接影响到相应代谢反应的平衡，从而对菌群演替施加选择压力，其中 H_2 的作用尤为显著[25,39]。厌氧消化过程中存在能够应对高 H_2 浓度的 H_2 耐受型代谢和在极低 H_2 浓度下就会受到抑制的 H_2 敏感型代谢。葡萄糖降解作为 H_2 耐受型代谢可耐受 1 020 Pa 的 H_2 分压，而丁酸降解作为 H_2 敏感型代谢仅耐受 2.8 Pa[37]。H_2 敏感型代谢会受到由其他反应生成的 H_2 的抑制，因此厌氧消化系统需要通过耦合消耗 H_2 的互营代谢方式降低 H_2 的积累。而 H_2 的浓度降低不利于细胞间的电子传递，系统需要采取其他途径转移并处置电子，如通过甲酸、细胞色素和纳米导线进行电子传递以及耦合低浓度的氧气代谢作为辅助的电子汇[37]。因此，互营关系的构建以及不同代谢方式对于物质流的竞争受到反应热力学条件的调控，从而使整个系统实现对物质和能量的“精打细算”和“系统最优”[24,36,40]。

正是由于厌氧消化系统中存在独特的物质-环境-微生物互作关系，前人研究未能在该类系统中发现普遍存在的“核心功能菌”[14]。因此，深入解析菌群间的互作关系以及菌群-物质的代谢耦合关系，有助于发现优化构效关系的关键环境因子及微生物类群，对于生物转化定向调控策略的制定及转化效率的提高至关重要。

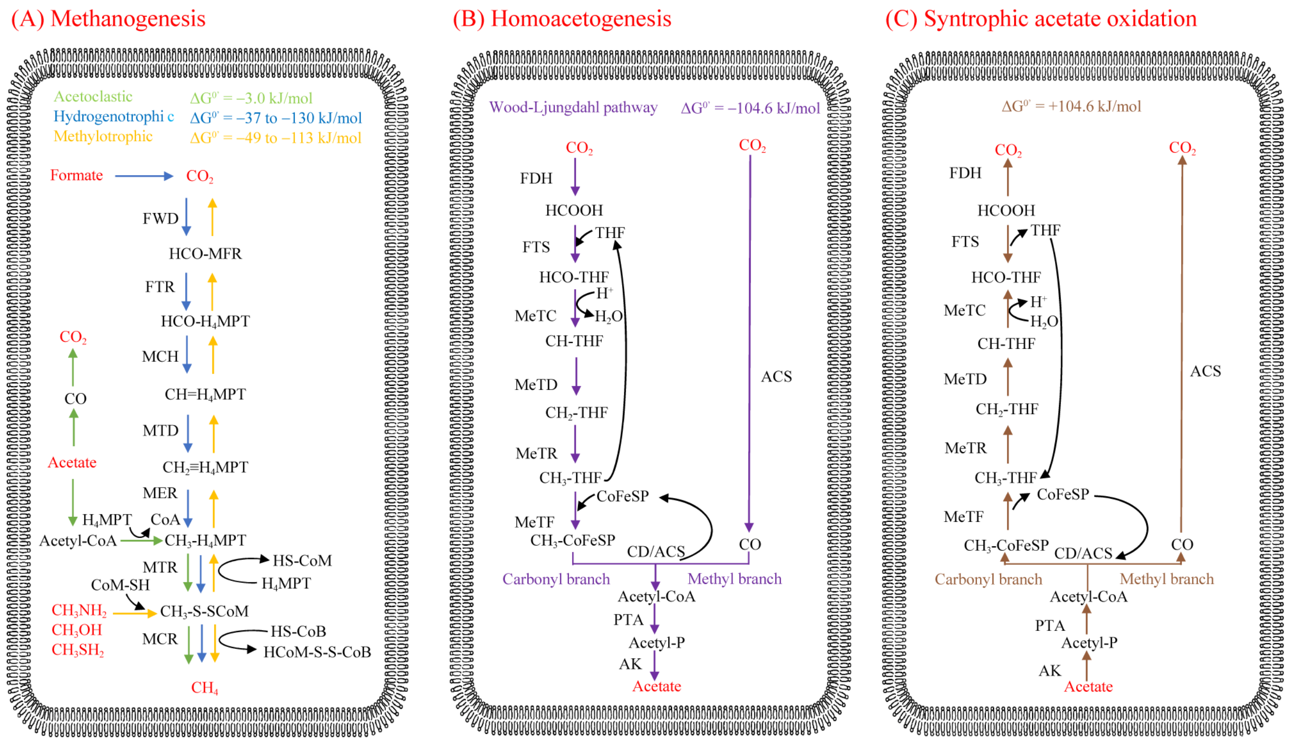


图 1 经典甲烷代谢途径、同型产乙酸途径和互营乙酸氧化途径[5,19,34,41] **(FWD：甲酰甲烷呋喃脱氢酶；FTR：四氢甲烷蝶呤转移酶；MCH：次甲基四氢甲烷蝶呤环水解酶；MTD：亚甲基四氢甲烷蝶呤脱氢酶；MER：亚甲基四氢甲烷蝶呤还原酶；MTR：四氢甲烷蝶呤甲基转移酶；MCR：甲基辅酶 M 还原酶；H_4MPT：四氢甲烷蝶呤；THF：四氢叶酸；CoFeSP：可啉铁硫蛋白；FDH：甲酸脱氢酶；FTS：甲酸四氢叶酸合成酶；MeTC：亚甲基四氢叶酸环水解酶；MeTD：亚甲基四氢叶酸脱氢酶；MeTR：亚甲基四氢叶酸还原酶；MeTF：乙酰转移酶；ACS：乙酰辅酶 A 合成酶；CD：CO 氧化酶；PTA：磷酸转乙酰酶；AK：乙酸激酶)**

Fig. 1 Classical metabolic pathway of methanogenesis, homoacetogenesis and SAO[5,19,34,41]. FWD: formyl-methanofuran dehydrogenase; FTR: formylmeth-anofuran-H_4MPT formyltransferase; MCH: methenyl-H_4MPT cyclohydrolase; MTD: F_{420}-dependent methylene H_4MPT dehydrogenase; MER: F_{420}-dependent methylene-H_4MPT reductase; MTR: Na^+-translocating methyl-H_4MPT-coenzyme-M-methyltransferase; MCR: methyl-coenzyme M reductase; H_4MPT: tetrahydromethanopterin; THF: tetrahydrofolate; CoFeSP: corrinoid iron-sulphur protein; FDH: formate dehydrogenase; FTS: formate-THF synthetase; MeTC: methylene-THF cyclohydrolase; MeTD: methylene-THF dehydrogenase; MeTR: methylene-THF reductase; MeTF: methyltransferase; ACS: acetyl-CoA synthase; CD: CO dehydrogenase; PTA: phosphotransacetylase; AK: acetate kinase.

2 典型有机固废厌氧消化存在的问题以及微生物在其中发挥的作用

有机固废同时具备污染属性和资源属性，其组成复杂，富含多糖、蛋白质、脂质等大分子成分，可以转化生成生物沼气、脂肪酸、羟基酸、氨基酸、醇类、植物化合物等多种产物[42-44]。丰富的有机成分为微生物提供了多样化的代谢底物和物质环境，相应的微生物群落结构和多样性与特定基质的组成也表现出高度相关[14]。例如有报道称，纤维素的降解和梭菌属解纤维梭菌 *Clostridium cellulolyticum* 的水平呈正相关[45]，脂类的降解和互营单胞菌属 *Syntrophomonas* 水平呈正相关[46]，梭菌纲 Clostridia 和软壁菌门 Tenericutes 特异性地出现在粪便的厌氧消化池[47]，高蛋白的食物废物容易造成氨氮的大量积累，使 SAOB 在生态位上占优势[3,48]。同时某些成分或中间产物也可能对部分微生物有胁迫或者毒害作用[3,49]。下

面针对 3 种典型有机固废厌氧消化过程中存在的问题及微生物的作用进行简述。

2.1 市政污泥

市政污泥是市政污水处理的副产物，具有含水率高、有机质含量低、产量大、水解难度大等特点。剩余活性污泥中包含大量的微生物细胞，其中约 80%是活细胞[7]。实际运行过程中有机物和微生物细胞形成稳定的絮状污泥限制了有机成分的释放，而大量存在的腐殖质也难以被生物利用[7]。因此，常采用预处理手段来提高有机质的可生化性[50-51]。Liu 等[52]通过热水解提高了污泥的厌氧消化降解能力，发现 Firmicutes 和 Bacteroidetes 等发酵细菌丰度显著增加，而产甲烷菌组成变化不大，这可能是由于产甲烷菌以细菌代谢的中间产物为营养源而不受水解过程初始底物的影响[53]。除了预处理，向消化池投加酶和化学试剂也可以通过生物和化学效应改善水解条件[54-56]。外源酶的投加直接降低了系统水解菌群的产酶需求，人为破除了水解阶段对整个厌氧消化过程的速率限制[54-55]。化学试剂 CaO_2 的投加可以提供羟基自由基从而有效去除难降解的有机污染物，并在生物效应上表现为水解和产酸酶系的活性相对提高[57]。最新的研究发现，市政污泥厌氧消化中 CaO_2 的投加能够富集 10 余种脂肪酸生物合成的关键基因并促进溶菌酶的作用和多种单糖的代谢[58]。

市政污泥中存在的大量活细胞是病原微生物和抗生素抗性基因持续输入的来源[59-60]。温度被认为是影响厌氧消化对病原体和抗性基因去除能力的重要因素，通过调节营养物质和温度，选择性地恶化目标微生物的生活环境，能够在厌氧消化系统中实现对它们的有效去除[60-61]。对不同温度厌氧消化系统的宏基因组分析表明，高温 (55 ℃) 能够有效降低抗生素外排泵编码基因的丰度。此外，菌群多样性的降低可以消减抗性基因的宿主以及潜在宿主，从而抑制了抗性基因的垂直转移；同时，菌群代谢速率的显著提高则可以加速对抗性基因水平转移载体的降解[62]。除了风险微生物，市政污泥富集了来自污水处理厂的各种污染物，针对当前层出不穷的新兴污染物，微生物作用可以有效削减某些污染物的数量和毒性[63-64]。同时这些新兴污染物也可能诱导微生物产生新的特异性代谢机制，需要持续予以关注[63-64]。

2.2 餐厨垃圾

餐厨垃圾是餐饮垃圾和厨余垃圾的总称，具有高油、高盐、高含水率、高有机含量等特点[65-66]。在餐厨垃圾厌氧消化过程中大量有机成分往往能高效水解、酸化转化为脂肪酸，但大部分脂肪酸的进一步转化属于 H_2 敏感型代谢而速率不高，转化速率的巨大差异容易造成酸的积累 (尤其是丙酸)[39]，进而导致体系 pH 降低，造成产甲烷菌活性降低，因为大部分产甲烷菌在 $pH<6.7$ 时就已停止代谢[12]。Sun 等发现当 pH 低至 5.1，几乎 90%的乙酸营养和氢营养的产甲烷菌在 6 d 内就会从培养物中消失[67]。尽管脂肪酸同样可以作为系统产出的产品，由于过低 pH 不利于有机酸的解离，持续的酸化会对微生物造成普遍的危害最终同样会影响挥发性脂肪酸 (Volatile fatty acids，VFAs) 的产生，导致整个体系的崩溃[68]。工程上从宏观效应的角度出发，通过投加碱性物质的方式来调节体系 pH，从而保证体系性能和产出效率[69]。然而，为了更有效地调控有机固废的生物转化过程，需要从微观机制如电子传递过程进行精准调控。研究表明，通过建立和强化直接电子传递可以提高 VFAs 利用速率和甲烷产率[70-71]。目前调控电子传递主要有两种途径：电子传递源控制和电子传递过程控制[28,71]。电子传递源控制是以乙醇为产乙酸的前体，这在热力学上更为有利，而且已证实伴随着乙醇的氧化，厌氧消化菌群建立了 DIET 来促进甲烷的生成[29,31]。现有研究中，Li 等[71]和 Gao 等[72]分别通过乙醇型预发酵和原位投加酵母菌实现了以乙醇为电子传递源的 DIET 强化。电子传递过程控制一般是通过投加诸如颗粒活性炭、

生物炭、纳米零价铁或铁氧化物等导电材料强化产甲烷菌和其他细菌间的电子和能量传递[28]。Lim 等发现添加生物炭后，体系富集了梭状芽胞杆菌属 *Clostridia* 等电活性微生物，促进了脂肪酸的降解和乙酸营养型产甲烷过程，最终使得甲烷产率提升了 18%[73]。Dang 等同样证实了碳布和颗粒活性炭促进了体系电子传递从而增加了微生物降解复杂有机质的能力[70]。此外，通过施加电压也可以加速系统中的电子传递，例如微生物电解池 (MEC) 的应用可以显著促进厌氧消化效率[74]。

餐厨垃圾常伴随着高油脂，而高浓度的油脂会限制底物和产物的运输，进而影响到乙酸降解菌和产甲烷菌的活性[75]。传统方法通过油脂分离的方式避免其干扰。事实上，厌氧消化微生物群具备一定程度上抵抗油脂抑制作用的潜力。Amha 等发现虽然油脂的添加在初期造成了餐厨垃圾厌氧消化的代谢滞后和 VFAs 的积累，但随着时间推移微生物群可以通过种间相互作用，转向更有利的代谢途径从而恢复体系功能[76]。此外，研究发现长期的生物驯化能够富集 Firmicutes、Bacteroidetes、互养菌门 Synergistetes 和 Euryarchaeota 等微生物，从而使厌氧消化系统具备快速降解长链脂肪酸的能力[77]。虽然将微生物逐渐暴露于不利环境下可以使群落内部发生适应性变化[78]，但微生物耐受这种极端环境胁迫的分子机制尚不明确。

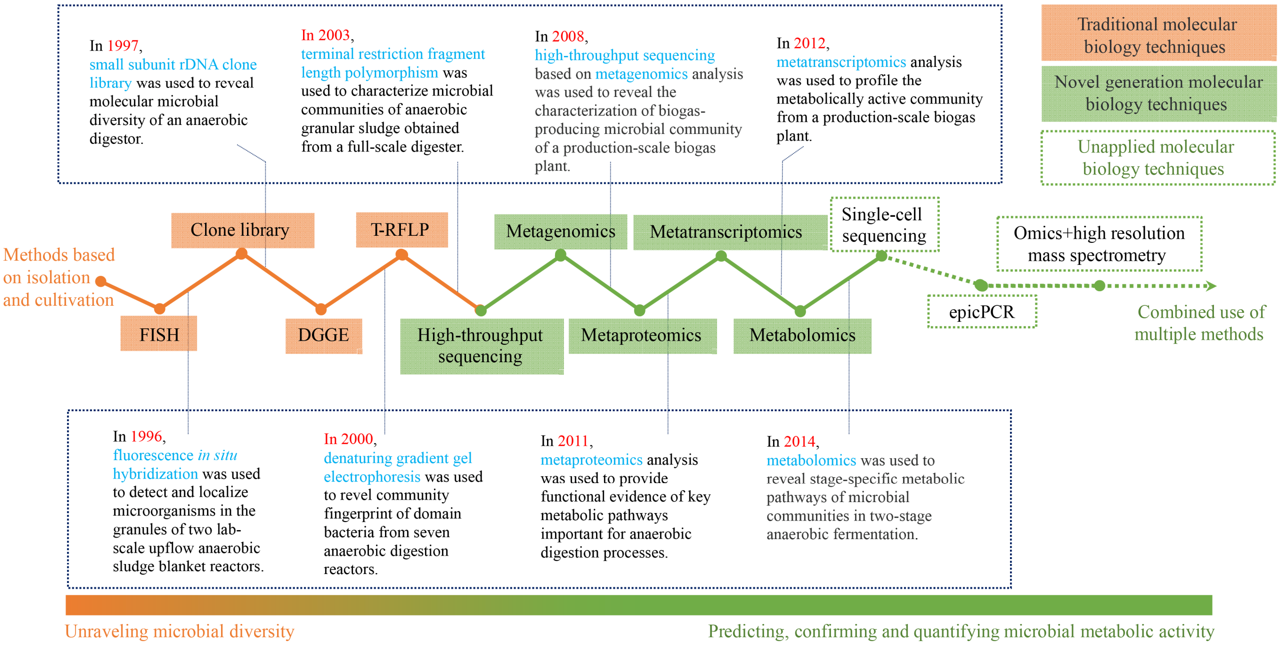
2.3 农业废弃物

农业生产中主要会产生畜禽粪便和以秸秆为代表的植物纤维这两类有机固废。粪便和植物纤维分别是低碳氮比 (C/N) 和高 C/N 的典型案例。C/N 是衡量厌氧消化底物营养平衡的重要参数，厌氧消化过程中微生物消耗碳的速度是氮的 25–30 倍，为实现碳的最大转换效率，体系碳氮比需达到 20∶1–30∶1 的要求[12]。高含氮底物厌氧消化中随着游离氨的积累会显著抑制微生物代谢。Bi 等单独以鸡粪为底物进行厌氧消化时，高浓度的氨态氮抑制乙酸转化和产甲烷过程，与去除氨的处理相比，甲烷产率低约 30%[79]。而 Zhang 等发现秸秆的添加有效缓解了体系的氨抑制[80]。这是因为将富氮 (如粪便) 和富碳 (如植物纤维废物) 的固体废弃物进行共消化恰好平衡了两个体系对营养源组分的需求，同时解除了抑制效应。此外，多个来源的有机固废的共消化可以增加微生物代谢的多样性[14]。Liu 等发现单独以玉米秸秆为底物的厌氧消化过程中物种总丰富度较低，古菌多样性不足[81]。而 Oladejo 等研究表明，粪便的添加为厌氧消化提供了优质的产甲烷菌物种库，混合基质能滋养高丰富度和多样性的微生物组，从而显著提高系统的稳定性和产出效率[82]。共消化以一种极其简便的方式实现了对物质环境和微生物群落结构的调节，使它们彼此契合进行有机固废的高效资源化转化[83]。

物质环境和微生物群落不相适应是厌氧消化系统出现问题的普遍原因，每个厌氧消化系统独特的环境-微生物互作关系导致了不同研究中可能出现迥然不同的结论[84-85]，但在相似的系统中确定存在着“核心功能菌”以及同样的微生物群落构建机制[14]，这样的特征使得基于大数据建模来调控厌氧消化过程成为可能。目前已经能够在宏观上通过改造物质环境 (如预热水解) 和强化微生物功能 (如投加导电材料强化细胞间电子传递) 来提升厌氧消化系统性能，而更精细的定向调控需要依靠解析复杂体系中微生物组代谢机制，这无疑对分析检测方法提出了更高的要求，相关研究依然任重道远。

3 组学技术推动下有机固废微生物组研究发展方向

目前大部分关于厌氧消化的研究已经由单纯的系统性能研究转为同时关注与系统性能密切相关的微生物机制[41,86-88]。自上世纪末以来，组学技术蓬勃发展并逐渐应用到厌氧消化微生物组的研究中 (图 2)，尤其是高通量测序 (指扩增子测序)



In 1997, small subunit rDNA clone library was used to reveal molecular microbial diversity of an anaerobic digestor.

In 2003, terminal restriction fragment length polymorphism was used to characterize microbial communities of anaerobic granular sludge obtained from a full-scale digester.

In 2008, high-throughput sequencing based on metagenomics analysis was used to reveal the characterization of biogas-producing microbial community of a production-scale biogas plant.

In 2012, metatranscriptomics analysis was used to profile the metabolically active community from a production-scale biogas plant.

In 1996, fluorescence *in situ* hybridization was used to detect and localize microorganisms in the granules of two lab-scale upflow anaerobic sludge blanket reactors.

In 2000, denaturing gradient gel electrophoresis was used to revel community fingerprint of domain bacteria from seven anaerobic digestion reactors.

In 2011, metaproteomics analysis was used to provide functional evidence of key metabolic pathways important for anaerobic digestion processes.

In 2014, metabolomics was used to reveal stage-specific metabolic pathways of microbial communities in two-stage anaerobic fermentation.

图 2　现代组学方法应用于厌氧消化系统的时间轴[90,96-102] (FISH：荧光原位杂交；DGGE：变性梯度凝胶电泳；T-RFLP：末端限制性片段长度多态性分析；eqicPCR：细胞内融合基因技术)

Fig. 2　Timeline for the development of omics-based technologies applied to anaerobic digestion[90,96-102]. FISH: fluorescence *in situ* hybridization; DGGE: denaturing gradient gel electrophoresis; T-RFLP: terminal restriction fragment length polymorphism; eqicPCR: emulsion, paired isolation, and concatenation polymerase chain reaction.

的应用极大地丰富了厌氧消化菌群的生物多样性[6,89]。但扩增子测序存在一些不可避免的缺陷，例如引物覆盖度不足等因素造成遗传信息的缺失或偏差，不具备单独用于识别新物种的能力等[6]。宏基因组的应用会克服这些缺陷，尽管 2008 年就有研究者对厌氧消化菌群进行了鸟枪宏基因组测序，但由于分析门槛较高，价格相对昂贵，所以宏基因组的应用远不及扩增子测序[21,90-91]。通过宏蛋白组和宏转录组能够获得体系中具有代谢活性的微生物的转录本表达情况和蛋白分子组成情况，评估当前环境下微生物的基因表达情况和菌群的代谢潜力[92-93]。代谢组的应用通过直接检测小分子代谢产物的方法，可以识别关键代谢途径的信息细节，进一步推断微生物组潜在的代谢机制[92]。一些新兴的技术如单细胞测序技术[94]、基于单细胞分离的细胞内融合基因技术[59] (epicPCR) 在厌氧消化系统中还未见有应用。多组学技术的联用为进一步揭示物种代谢能力和趋向提供了新的方向[95]，如通过培养组学和现有组学技术的结合来解密厌氧消化体系中大量的未培养微生物、通过高分辨质谱技术与组学技术的结合揭示菌群-物质的匹配与互作。

菌群-物质的代谢偶联是解析有机固废生物转化体系中微生物学机制的核心任务，可为污染物的定向转化提供重要的科学依据。由于厌氧消化系统中物质种类繁多、物种多样性高，造成了代谢产物与物种相匹配的困难，生态网络分析[103]等信息学方法的引入为大量有机分子与微生物相匹配带来了新的机会。基于大数据分析构建分子生态网络，一方面可以揭示高度复杂微生物群落中的关键物种以及有机物之间、菌群之间、有机物和菌群的互作关系，另一方面通过引入更为详细的体系运行参数和环境参数，可以建立确定性因素主导下的环境-菌群互作模式。此外，目前有

机固废厌氧消化的微生物组研究还处于发展阶段，还没有一个能够荟萃全球厌氧消化微生物组研究成果的数据库[22]。已有的数据分散在不同的数据存储机构，缺乏统一的存储协议和对厌氧消化微生物组信息专项的管理，亟需建立一个全球性的厌氧消化微生物组数据集和相应的数据库。综上所述，深入研究厌氧消化过程中的微生物组将为提升系统的稳定性、增加能源回收效率提供有效的理论支持和科学依据，是面向未来有机固废减量化、无害化和资源化的必由之路。

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