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生物强化污泥厌氧发酵产酸研究进展*

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摘要　污泥厌氧发酵生产挥发性脂肪酸相较产甲烷,是更具应用价值的污泥稳定途径及资源化利用方式,得到国内外学者的普遍重视。考虑到产酸量低和产酸过程的不稳定性是限制污泥发酵产酸的主要问题,采用生物强化方法实现挥发性脂肪酸的大量积累,与物理和化学方法相比,具有成本低、无二次污染等优点。根据生物强化制剂的类型,归纳了微生物纯培养物、微生物混合培养物及生物酶强化对污泥厌氧发酵产酸的影响,并在此基础上对生物强化技术控制污泥定向产酸、调控奇偶数碳比率等方面的应用进行讨论。此外,分析了影响挥发性脂肪酸产量和组分的因素,如 pH、温度、底物、水力停留时间和污泥龄等。最后对生物强化技术的发展方向进行了展望,以期为深入探究污泥资源化利用提供借鉴。

关键词　生物强化　剩余污泥　厌氧发酵　挥发性脂肪酸

中图分类号　X703

污泥处理是城市发展过程中亟待解决的重大环境问题,我国剩余污泥产量正以每年 6.46% 的比例增长,2025 年前预计将增至 6 500 万 t/a[1]。污泥中包含大量可降解有机物,相较于生物制氢和产甲烷的附加值,污泥厌氧发酵生产挥发性脂肪酸(volatile fatty acids, VFAs)是一种更具前景的资源化技术[2],产出的乙酸、丙酸、正/异丁酸、正/异戊酸等 VFAs,在合成聚羟基脂肪酸酯(polyhydroxyalkanoates, PHAs)[3-4]、提高污水脱氮除磷效率[5]、改善土壤肥力[6]等方面潜力巨大。但由于污泥组分复杂、处理过程影响因素较多,导致厌氧发酵产酸量低且产酸过程不稳定。目前,国内外相关研究大多集中在物理和化学手段促进产酸[7-8],不仅能耗大、成本高,对设备有一定腐蚀性,还会产生二次污染[9]。对于发酵产酸系统而言,其内部微生物群落的变化是促进 VFAs 产生的最关键原因,不同的优势菌群引导着系统向不同的方向发展[10]。因此,关注污泥厌氧发酵产酸相关微生物的进一步研究至关重要。生物强化技术是一类通过向处理系统中引入经过富集、筛选的专属优势菌纯培养物、混合培养物或生物酶等[11],以提高污泥破解程度与水解产酸效率的方法,过程更加绿色、经济。

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本文重点阐述了生物强化手段在污泥厌氧发酵产酸研究中的应用现状。对提高产酸效率的各类生物强化技术进行归纳评述,同时讨论了生物强化技术在定向调控污泥产酸中的典型应用,并分析了 pH、温度、底物、水力停留时间和污泥龄等因素对生物强化污泥产酸效果的影响,以期为深入产业化应用提供参考。

1　生物强化污泥高效产酸

在厌氧发酵体系中,参与污泥有机质水解和产酸过程的微生物种类繁多。根据引入生物制剂的不同类型,生物强化可分为微生物纯培养物、微生物混合培养物及生物酶强化。

1.1　微生物纯培养物

参与水解酸化的细菌涵盖多个菌门,其中拟杆菌门(Bacteroidetes)、变形菌门(Proteobacteria)、绿弯菌门(Cloroflexi)和厚壁菌门(Firmicutes)等相对丰度较高[12]。为建立高效的生物产酸系统,常向污泥中引入有特殊功能的专属优势菌纯培养物。Si 等[13]从污泥中分离出凝血芽孢杆菌(*Bacillus coagulans*),接种 9 天后

VFAs 产量高达 2 594 mg/L，提高了 98.5%。杨春雪[14]采用高温驯化法从剩余污泥中分离出具有胞外水解酶活性的嗜热菌 *Geobacillus* sp. G1，在 35℃ 下发酵 4 天 VFAs 产量达到(2 560 ± 100) mg/L，高出对照组 2 倍。

除细菌外，青霉菌属、曲霉属和酵母菌属等真菌所分泌的胞外酶对污泥中纤维素、脂质及酚类化合物等的降解能力也极强，其在改善污泥脱水性能的同时，还可促进污泥内碳源的释放[15]。Liang 等[16]对污泥水热处理后的上清液进行真菌发酵耦合厌氧发酵产酸两级生物处理，曲霉将有机物转化为菌丝纤维回收，厌氧发酵产生约 2 500 mg COD/L 的 VFAs。Fang 等[17]发现白腐真菌(*P. sajor-caju*)可分泌漆酶和锰过氧化物酶，使 VFAs 产量提高 1.24 倍，达到 240 mg COD/g VSS。目前，优势菌株的开发仍在继续，寻求提高生物强化菌剂稳定性的方式、持续发挥作用的生物强化模式，保证生物的净持有量是生物强化技术走向实用化的关键。

1.2 微生物混合培养物

在实际应用中，相比单一优势菌对特定物质的生物降解效果，人工富集和复配得到的混合优势菌群对污泥产酸的强化效果更为显著。Jie 等[18]从污泥中分离出两株耐碱芽孢杆菌按 1∶1 复配投加，污泥发酵 9 天 VFAs 产量达 3 139 mg/L，远高于单一菌株。胡之弈等[19]通过恒化培养得到了以拟杆菌属为主的藻酸盐降解菌群(alginate-degradingconsortium，ADC)，产酸效率提升了 184%。原因在于 ADC 可高效利用污泥中的酪蛋白、葡聚糖和聚半乳糖醛酸等底物。真菌可在发酵产酸体系上游为细菌的水解酸化功能提供更多的生长基质。王春燕等[20]向污泥中外源投加 10 g/L 酵母菌与 20 g/L 醋酸菌，酵母菌丰富的酶系统刺激胞外聚合物的裂解，其代谢产物被醋酸菌转化为乙酸。发酵第 5 天 SCFAs 累积量达 719 mg COD/g VSS，有机物酸化率由 33% 提高至 74%。

此外，混合菌群的种间关系常常会影响厌氧发酵的整体进程，发酵产酸体系下的互营关系常见于产氢产乙酸菌与同型产乙酸菌之间[21]。江南大学陈坚教授课题组[22-24]通过调节菌群结构与数量，提出了人工重建互营机制的产酸新工艺，同时建立了基于该工艺的动力学模型，使产酸效率最大化。近年来，一些新兴分子生物学技术不断涌现，为产酸功能菌群的研究提供了良好条件。未来如果对产酸阶段生物群落动态演替进行深入研究，尤其在动力学模型优化等方面，势必能建立一定生产强度的产酸体系，具有良好的工程前景。

1.3 生物酶强化

分散在污泥基质中的生物酶通过破碎污泥絮体的交联结构，减弱生物絮凝作用，降解高分子有机物，同时改善污泥发酵[25]。由于碳水化合物和蛋白质是污泥的主要成分，淀粉酶、蛋白酶或两者的组合被广泛测试。刘国华等[26]向初沉污泥的厌氧发酵系统投加碱性蛋白酶，在发酵第 4 天 SCFAs 的产量达 1 508 mg COD/L。Yu 等[27]经过比较认为淀粉酶对污泥溶解和酸化方面的促进效果最优，发酵 7 h 后发酵液中 SCOD 和 VFAs 产量分别提高 78.2% 和 129.6%。

在另一部分研究结果中显示，组合酶的应用对促进污泥水解产酸效果更佳。Luo 等[28]以 3∶1 的比例向污泥中投加蛋白酶和淀粉酶，SCFAs 产量在第 5 天高出对照组 2.04 倍。Xin 等[29]探究了溶菌酶、蛋白酶、淀粉酶和纤维素酶按 1∶1∶1∶1 复配对剩余污泥的溶胞效能及溶出物的释放规律。结果表明，复配酶对有机质的溶出效果显著优于单一酶，4 h 内 SCOD 增至 8 500 mg/L，12 天内 VFAs 有效积累量超过 3 200 mg COD/L，提高了 2.5 倍。单一酶面对络合态的有机物难以寻找到活性位点，多酶体系下更易寻找到目标底物，这些结论差异往往受污泥来源及其有机质组分分布的影响。

由于污泥成分的复杂性，酶活的抑制因子增多，一些商业酶甚至出现自溶现象，因此亟待加强对延长酶活性的研究。且生物酶有较高的经济成本，限制了其大规模应用，建议考虑使用高效产酶微生物或结合其他预处理方法使用。

2 生物强化技术在污泥产酸中的典型应用

污泥厌氧发酵产生的有机酸为混合酸，VFAs 的类型和组分占比直接影响其在化工、制药和食品工业等领域中的应用。生物强化技术可根据后续资源化利用的需求，高效、经济的达成乙酸、丙酸、丁酸等高值酸的定向转化，或实现对 VFAs 中奇数碳与偶数碳比率关系的调控。

2.1 生物强化产偶数酸

污泥厌氧发酵生产的偶数碳挥发性脂肪酸(even carbon volatile fatty acids，ECFA)有乙酸和丁酸等。乙酸化学势能较低，是绝大多数水解产酸菌的代谢产物[30]，在 VFAs 中占比最多。习彦花等[31]将从活性污泥中分离出的双酶梭状芽孢杆菌(*Clostridium bifermentans*)在最佳条件下培养 12 h，乙酸产量达 1 588.7 mg/L。为了在市政污泥厌氧消化过程中获取同

型产乙酸菌,王晋等[32]用40天富集培养得到的菌群对底物利用率达到87%,VFAs中乙酸占比达到100%。除上述水解发酵细菌外,硫酸盐还原菌(sulfate-reducing bacteria,SRB)是一类可利用未完全氧化的小分子短链挥发性脂肪酸还原硫酸盐生成乙酸的菌种。不仅可以促进底物降解,还可提升反应器酸化率。沈志等[33]发现SRB可调控市政污泥厌氧发酵以乙酸型为主导,VFAs中乙酸累积量高达90%以上。在丁酸型发酵体系中,丁酸梭菌属(*Clostridium*)、丁酸弧菌属(*Butyriolbrio*)和梭杆菌属(*Fusobacterium*)等较为常见[34-35]。Liu等[36]筛选出一株酪丁酸梭菌(*Clostridium tyrobutyricum*)RPT-4213,以造纸污泥为原料定向产丁酸,其产量可达8.52 g/L。由于丁酸型发酵常伴有乙酸、丙酸等副产物,丁酸产量低且回收困难成为制约厌氧发酵的主要因素,通过基因工程改造出更强健的丁酸生产菌株[37]或利用混合培养物加速丁酸发酵效率[38]更具应用前景。

污泥厌氧发酵过程中主要生成大量短链脂肪酸,同时也存在少量以己酸为代表的中链羧酸。工业级己酸的价值是乙酸的5倍,其碳氢链更长,具有能量密度高、易于分离回收等特点[39]。近年来链延伸技术兴起,经链延长菌反向β氧化后,乙醇和短链羧酸能被转化为中链羧酸[40]。克氏梭菌(*Clostridium kluyveri*)是少数已被鉴定的混菌体系中主导了己酸生产的关键微生物[41]。Pan等[42]在厌氧膜生物反应器中通过驯化污泥使克氏梭菌丰度由14%增至65%,己酸产量达2.62 g/L,占总VFAs的74%。

2.2　生物强化产奇数酸

丙酸和正/异戊酸是污泥发酵过程中产生的奇数碳挥发性脂肪酸(odd carbon volatile fatty acids,OCFA)通过生物发酵法可实现对OCFA的高性能生产[43]。丙酸杆菌是一种常见的丙酸生产菌[44]。Li等[45]在活性污泥和餐厨垃圾共发酵过程中用酸性丙酸杆菌ATCC4875强化产酸,经两级发酵VFAs产量增至15.3 g COD/L,其中丙酸占比高达69.9%。Wang等[46]通过剩余污泥和废弃油脂共消化富集丙酸杆菌,使OCFA产量增至3 889.24 mg/L,在总VFAs中占88.67%,其中丙酸占比82.80%,戊酸则从初期的1%增至8.96%。研究同时发现,厚壁菌门和拟杆菌门是促进奇数酸产量增加的优势菌门,相对丰度之和达到了88.65%。在发酵产酸体系中,国内外对正/异戊酸的生物合成途径鲜见报道,由于其长链结构易被产氢产乙酸菌利用转化为乙酸,难以在VFAs组分中占据主导。目前,以丙酸和戊酸为原料前体,生物法生产PHAs已成为研究热点[47],大多通过控制反应器参数等提高OCFA的占比[48],但其中合成OCFA的相关微生物群落动态研究并不清晰。寻找高效产丙酸、戊酸的菌株,掌握微生物代谢途径,对于OCFA的生产具有重要意义。

3　生物强化产酸影响因素

在不同条件下,污泥厌氧发酵系统中微生物产短链脂肪酸的效果存在较大差异。其中,pH、温度、底物、水力停留时间(hydraulic retention time,HRT)和污泥龄(sludge retention time,SRT)等因素的影响不容忽视。

3.1　pH的影响

在厌氧发酵产酸过程中pH是最重要的生态因子之一,产酸发酵细菌的生长繁殖速率会受环境pH变化影响,积累不同的酸化产物。厌氧发酵产酸过程可在pH 3.0~12.0进行,而产甲烷菌适宜的生存pH范围在6.6~7.5,因此在酸性或碱性条件下甲烷的产生均会受到抑制,从而促进VFAs的积累[10]。Wu等[49]指出,对剩余污泥分别进行酸和碱预处理后厌氧发酵,可使VFAs产量分别增加15.3倍和12.5倍。酸碱的急剧变化会引起厌氧消化反应器运行效率的显著下降,其内在原因是对酸碱耐受性差的微生物种群数量和活性受到抑制,导致相关的代谢功能出现衰退。因此近年来许多研究发现,逐步调控pH更有利于水解酸化菌群最大限度地进行产酸发酵[50]。Zhao等[51]通过滴加NaOH或HCl逐步控制水解和酸化步骤的最佳pH条件,pH为11时污泥溶胞速率加快且产甲烷菌的活性被抑制,污泥充分水解后在pH为9下产酸速率最高,通过逐步发酵最佳SCFA产量达到2 356 mg COD/L。

不同pH下末端产物的组成也存在较大差异。Atasoy等[52]发现VFAs组成随pH而变化:中性条件下VFAs的主要组分为乙酸;在酸性条件下为乙酸和丁酸,在碱性条件下丁酸含量最多。这与Khan等[53]的结论类似,分析原因可能与酸碱条件下乙酸激酶和丁酸激酶的酶活性有关。综上,pH向碱性偏移,VFAs中低分子酸占比增大;适当减小pH,可增加VFAs中高分子酸占比。

3.2　发酵温度的影响

温度是影响微生物生长、代谢途径、酶活高低和水解速率等的关键因素[54],从而影响产酸发酵效率。厌氧水解酸化细菌可适应的温度范围较广,在低温

(<20℃)、中温(30~40℃)和高温(50~60℃)下都可产酸。但温度过低会限制污泥的水解步骤,当前较多研究更关注在中、高温条件下污泥的产酸效率。不同学者对污泥厌氧发酵研究的最佳产酸温度意见不一。Hao 等[55]认为高温增强了水解酶的活性,使参与水解酸化的菌门比例提高,其 VFAs 产量是中温的 10 倍。与此相反,Zhuo 等[56]在 10~55℃时观察到,污泥水解效率随温度的升高而提高,在 37℃时 VFAs 有最高产量,达 4.6 g COD/L,而 55℃时下降了 40%。李晓玲[57]同样发现中温条件更适合反应器中微生物种群的生长。Vázquez-Fernández 等[58]认为这是由于温度对污泥产酸的作用受污泥组成的影响,碳水化合物更易在中温条件下被微生物分解,高温提高了富含蛋白质污泥的 VFAs 产量。因此应综合考虑污泥的性质及相关条件,选择适宜的发酵温度。

VFAs 组分同样受温度差异影响。Garcia-Aguirre 等[59]在实验中发现,35℃时乙酸和丙酸含量更高,当温度升高到 55℃时,丁酸成为主要产物。同样,Jiang 等[60]在实验中发现,55℃时 VFAs 中丁酸占 81%,而在 35℃和 45℃时,乙酸和丙酸占比达 70%。因此改变温度对控制 VFAs 不同组分的选择性生产是可行的,常采用温度和 pH 双因素共同调控制取不同类型的挥发酸。

3.3 底物的影响

污泥中丰富的有机质为生物产酸提供了条件,由于来源不同,污泥组分存在一定差异。一些研究发现,富含碳水化合物的污泥可使混合酸产物中丙酸和丁酸的占比增加,蛋白质含量高的污泥能促进戊酸生成[61-62]。微生物群落分析表明,营养平衡对维持反应器中产酸菌稳定的生长繁殖环境至关重要。但污泥的碳氮比值(carbon to nitrogen ratio,C/N)偏低,且单一基质可能缺乏一些关键营养元素,因此限制了 VFAs 生产。目前,许多研究者将污泥与有机废弃物(如餐厨垃圾[63-64]、禽畜粪便[65]、作物秸秆[66]等)共发酵调节C/N,平衡各种基质的营养水平,提高产酸潜力。产酸发酵最适 C/N 通常在 20~30[67]。Chen 等[68]将污泥和餐厨垃圾共发酵 6 天,在 pH =8、C/N 为 22 时有最大 VFAs 产量 670 mg COD/g VS_{added}。而在发酵产酸类型及产酸代谢途径方面,刘和等[69]发现当初始 C/N 分别为 12、56 和 156 时,分别会形成以消化链球菌属为主要优势菌群的乙酸型发酵、丙酸杆菌属占优势的丙酸型发酵和梭菌属为主的丁酸型发酵。

有机负荷(organic loading rate,OLR)是常用于代表发酵浓度的指标,OLR 的变化会影响基质降解和产物形成的速度,进而决定酸化程度。在合理范围内可通过增加 OLR 以提高 VFAs 产量并缩短发酵周期[70],若 OLR 超过适当阈值,厌氧发酵的平衡将被打破,会导致产酸量下降甚至反应器失效。另外,Wainaina 等[63]发现 OLR 对 VFAs 种类占比产生影响,高 OLR 使丙酸和丁酸比例增加。核心原因在于控制底物以促进产酸的研究大多基于对微生物群落结构的改变[71],即在发酵系统中迅速富集产酸菌,控制不同产酸途径下菌群的生长速率快于产甲烷菌,从而抑制 VFAs 的消耗,使 VFAs 产量和分布发生变化。

3.4 HRT 和 SRT 的影响

HRT 和 SRT 是厌氧发酵反应器运行控制的重要操作参数之一。在无污泥回流的连续运行反应装置中,HRT 与 SRT 近乎一致,二者共同对系统微生物群落结构和丰度产生影响。Miron 等[72]研究了 HRT 在 3~15 天对污泥厌氧消化的影响。当 HRT<8 天时,产酸菌占主导地位,HRT >8 天则更有利于产甲烷菌的生长。增加 HRT 和 SRT 可在一定程度上促进水解和发酵产酸,但时间过长不利于产酸相和产甲烷相分离,系统内产生的有机酸将转化为甲烷,并且需要更大的反应器。

关于厌氧污泥发酵过程中酸种类分布随停留时间的变化尚未得到一致结论。Luo 等[73]在 SRT 为 4~20 天时观察到乙酸含量从 40.6% 增加到 59.5%,而丙酸则从 49.4% 下降到 23.0%。Mulders 等[74]则认为延长 SRT 增加了丙酸占比,但不利于丁酸产生。以上差异可能与系统中的微生物群落的生长规律有关,这其中的机制尚需进一步探究。

4 结 语

随着对生物强化污泥厌氧发酵产酸研究的深入以及应用的扩展,生物强化技术在污泥资源化领域中取得了一定的研究成果,但也存在不少问题。对生物制剂进行投加时,微生物的选择与组配是一个难点,生物强化菌剂与系统内土著微生物之间的协同代谢关系错综复杂,其中的物质代谢规律及对厌氧发酵代谢途径影响的作用机制有待深入探究。关注水解产酸的关键功能种群,避免冗余种群干扰,依旧是生物强化污泥产酸研究的难点问题。目前对产酸细菌的了解仍然有限,许多具有类似功能的微生物尚未被鉴定。因此,功能微生物的详细鉴定是今后研究的方向之一。此外,

从微生物学到实验应用再到工程实践中反应器的应用,缺乏生物强化作用效果评价体系。许多关于微生物群落结构和演替的研究在实验室规模上进行,实验结果具有局限性,无法做科学的评价及横向比较,应对微生物的特性进行更全面分析,以便更好地发挥其在生物产酸方面的重要作用。

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Biofortification on Acid Production by Anaerobic Fermentation of Sludge: A Review

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Abstract Compared with methane production, producing volatile fatty acids by anaerobic fermentation of sludge is a more valuable way of sludge stabilization and resource utilization, which scholars have widely valued at home and abroad. Considering that the low acid production and the instability of the acid production process are the main problems limiting the acid production by sludge fermentation, the use of bioaugmentation to achieve a large amount of accumulation of volatile fatty acids has the advantages of low cost and no secondary pollution compared with physical and chemical methods. According to the types of bioaugmentation agents, this paper summarized the effects of pure microbial culture, mixed microbial culture, and biological enzyme enhancement on anaerobic fermentation and acid production of sludge, and discussed the application of bioaugmentation technology in controlling directional acid production of sludge and regulating odd-even carbon ratio. In addition, the factors affecting the yield and composition of volatile fatty acids, such as pH, temperature, substrate, hydraulic retention time, and sludge age, were analyzed. Finally, the development prospects for the bioaugmentation technology were discussed in order to provide a reference for further exploration of sludge recycling.

Keywords Bioaugmentation Sewage waste sludge Anaerobic fermentation Volatile fatty acids