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城市污水再生处理中微量有机污染物控制的关键难题与解决思路 王文龙,吴乾元,杜烨,黄南,陆韻,魏东斌,胡洪营







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温度和搅拌对牛粪厌氧消化系统抗生素抗性基因变化 和微生物群落的影响

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摘要:分别设置中温不搅拌、中温搅拌、高温不搅拌和高温搅拌这 4 种牛粪厌氧消化处理,探究温度和搅拌对牛粪厌氧消化抗生素抗性基因(antibiotic resistance genes, ARGs)变化及微生物群落的影响. 以厌氧消化特性为基础,分析 ARGs 和可移动遗传元件(mobile genetic elements, MGEs)的丰度变化和微生物群落结构,并利用网络分析和冗余分析探究影响 ARGs 变化的关键因素. 通过双因素方差分析可知,温度对厌氧消化产气的影响($\eta^2=0.934$)强于搅拌($\eta^2=0.911$),高温总产气量较中温提高了 13.93%,且中温条件下搅拌的总产气量较未搅拌提高了 12.63%. 温度对 ARGs 去除的影响($\eta^2=0.992$)也强于搅拌($\eta^2=0.992$)。高温将 ARGs 和 MGEs 的去除量显著提升至 0.09~1.53 (对数值),但搅拌对 ARGs 和 MGEs 的去除无显著影响. 微生物群落受温度的影响也更为显著,门水平微生物 Firmicutes 成为高温条件下的绝对优势菌,相对丰度高达 86%以上. 属水平微生物 Sedimentibacter、Sphaerochaeta 和 Pseudomonas 等为 ARGs 的潜在宿主菌,直接影响 ARGs 的变化. 理化因子影响了微生物的分布,尤其是总氨氮和总挥发酸,通过影响 ARGs 宿主菌间接影响 ARGs 的变化. 整体来看,高温不搅拌的消化条件有利于气体的产生和 ARGs 的去除.

关键词: 厌氧消化; 搅拌; 温度; 抗生素抗性基因; 微生物群落

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Effects of Temperature and Stirring on the Changes of Antibiotic Resistance Genes and Microbial Communities in Anaerobic Digestion of Dairy Manure

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Abstract: To investigate the effects of temperature and stirring on antibiotic resistance genes (ARGs) and microbial communities during the anaerobic digestion of dairy manure, mesophilic and thermophilic anaerobic digestion experiments were performed with and without stirring. Two-way analysis of variance indicated that temperature affected biogas production more strongly than stirring ($\eta^2 = 0.934 > 0.911$), and thermophilic and stirring increased the total biogas yield by 13.93% and 12.63%, respectively. The effect of temperature on the removal of ARGs was also stronger than that of stirring ($\eta^2 = 0.992 > 0.920$), where thermophilic conditions enhanced the reduction of ARGs and MGEs to 0.09-1.53 (logarithm), while stirring had no significant effects. When temperature was altered from mesophilic to thermophilic, the microbial communities shifted, with Firmicutes becoming the dominant phylum after thermophilic anaerobic digestion, with a relative abundance of > 86%. Network analysis demonstrated that eight genera including Sedimentibacter, Sphaerochaeta, and Pseudomonas were the hosts of ARGs and MGEs, and the redundancy analysis suggested that physicochemical parameters play important roles in shaping microbial communities, especially TAN and TVFAs, which indirectly affected the ARGs by altering their host bacteria.

Key words: anaerobic digestion; stirring; temperature; antibiotic resistance genes (ARGs); microbial communities

随着集约化养殖规模的扩大,畜禽粪便的产量日益增多,随之而来的抗生素抗性问题受到广泛关注^[1~4]. 抗生素抗性基因(antibiotic resistance genes, ARGs)是抗生素抗性传播的重要物质,可在不同种类微生物间水平转移,从而对环境造成污染,甚至对人类健康产生威胁^[5~7]. 畜禽粪便中的 ARGs 是环境中 ARGs 的主要来源之一,养殖场周边空气、土壤、甚至河流中均检出 ARGs^[8~10]. 最大限度地降低畜禽粪便中 ARGs 的丰度,可减缓 ARGs 带来的生态风险.

厌氧消化是处理畜禽粪便的主要方式之一,可将粪便转化为沼气及肥料,也是削减 ARGs 的有效手段之一^[11,12].已有研究表明厌氧消化对 ARGs 的去除效果与微生物群落密切相关^[13-17].温度是厌氧消化系统的重要工艺参数,可通过改变微生物群落影响产沼气性能^[18,19].作为厌氧消化系统中的另一

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重要工艺参数,搅拌可影响传质和传热效率,并影响微生物群落分布^[20,21].因此,温度和搅拌可能作用于微生物群落且产生交互作用,从而影响厌氧消化对 ARGs 的削减作用.

目前,温度和搅拌及二者交互作用对厌氧消化系统中ARGs的影响不甚清楚.本文以大规模奶牛场粪便中的ARGs为研究对象,开展不同温度和不同搅拌速率的厌氧消化试验,主要研究温度和搅拌对ARGs和可移动遗传元件(mobile genetic elements, MGEs)丰度以及微生物群落的影响,分析理化因子、ARGs、MGEs以及微生物群落之间的互作关系,提出有利于削减ARGs的厌氧消化工艺运行参数.

1 材料与方法

1.1 试验材料

新鲜牛粪取自内蒙古一大规模奶牛场,固液分离后,将固体自然风干加入至液体中调节固含率为8%,作为厌氧消化原料. 调节后的原料 pH 值为6.76,可溶性化学需氧量(soluble chemical oxygen demand, SCOD)为8 575.00 mg·L $^{-1}$,总氨氮(total ammonia nitrogen, TAN)浓度为531.55 mg·L $^{-1}$,总挥发酸(total volatile fatty acids, TVFAs)浓度为3 960.49 mg·L $^{-1}$.

1.2 试验设置

厌氧消化反应器为有效容积1 000 mL的厌氧瓶,瓶塞设有采样口和出气口各一个,出气口连接排水集气装置.向每个反应器中加入 400 mL 固含率为8%的消化原料,盖上瓶塞,从采样口通入氮气5 min 营造厌氧环境,随后迅速关闭采样口并连接出气口与排水集气装置,置于恒温水浴锅. 共设置 4 组处理,分别在 35℃ 和 55℃ 下设定搅拌速率 0 和 200 r·min ⁻¹,依次记为 M0、M1、T0 和 T1,详情见表 1,每个处理 3 个平行.

表 1 不同厌氧消化处理参数设置

Table 1 Operational parameters of anaerobic digestion

			·	
处理	处理编号	温度/℃	搅拌速率/r·min-1	
中温不搅拌	MO	35	0	
中温搅拌	M1	35	200	
高温不搅拌	TO	55	0	
高温搅拌	T1	55	200	

1.3 样品采集及理化指标的测定

记录每个反应器每 24 h 的产气量,并用沼气分析仪(MRU Optima7,德国)测定沼气中甲烷含量. 厌氧消化反应运行结束后,混匀消化产物并测定固含率,重复 3 次; 另采取每个反应器中的样品 1 mL 于

离心管内,重复 3 次, 4℃、12 000 $\text{r} \cdot \text{min}^{-1}$ 的条件下 离心 5 min,上清液用于理化性质的测定,沉淀物用于提取 DNA.

总固体含量(total solids, TS)采用 105℃烘干法测定; SCOD 采用重铬酸钾分光光度法测定; TAN浓度采用纳氏试剂分光光度法测定; pH 值采用多功能 pH 计(雷磁 S-3E,上海)测定; TVFAs 采用比色法测定.

1.4 DNA 提取及荧光定量 PCR

1.5 高通量测序

将提取的 DNA 送至上海派森诺生物公司,利用 Illumina NovaSeq 平台进行 16S rRNA 高通量测序,测序区域为 V3 ~ V4 区,引物为 338F(ACTCCTACG GGAGGCAGCAG) 和 806R(GGACTACHVGGGTW TCTAAT).原始序列经质控后得到高质量序列,并在 SILVA 16S rRNA 数据库进行比对.

1.6 数据分析

本试验数据采用 Microsoft Excel 软件进行整理,SPSS 22.0 进行单因素方差分析、双因素方差分析及 Pearson 相关性分析,CANOCO 4.5 软件进行冗余分析,Cytoscape 3.6.0 进行网络分析,Origin Pro 9.0 和 R 3.2.5 作图.

2 结果与讨论

2.1 厌氧消化性能

厌氧消化的产气曲线如图 1 所示,反应后期产气稳定,微生物量达到稳定状态,35℃和55℃厌氧消化分别在第 45 d 和第 30 d 时日产气量小于反应体积的5%(即 20 mL),此时可视为消化结束.以日产气量代表厌氧消化产气速率[图 1(a)],35℃条件下产气集中在第 6~30 d,而55℃条件下产气集中在第 3~16 d,且前 10 d 的产气速率远高于 35℃,说明高温加快了厌氧消化反应进程,缩短了整个周

期[22]. MO、M1、TO 和 T1 的最大产气速率分别为 223、340、703 和 720 mL·d⁻¹. T0 和 T1 的最大产气 速率分别是 MO 和 M1 的 3.15 倍和 2.12 倍,证明高 温可显著(P<0.01)提高产气速率. 分别比较 M0 与 M1 和 T0 与 T1, 发现相较 M0 而言, M1 显著(P < 0.01)提高了最大产气速率, 而 TO 与 T1 之间最大 产气速率无显著差异. 由此说明中温条件下搅拌可 显著提高厌氧消化产气速率,而高温条件下搅拌则 对产气速率无明显影响. 消化后总产气量如图 1(b) 所示, MO、M1、TO 和 T1 的总产气量分别为5 120、 5 767、5 833和5 933 mL. 由双因素方差分析可知温 度和搅拌对总产气量均具有显著影响(P<0.001). 其中, 温度的影响 ($\eta^2 = 0.934$) 大于搅拌 ($\eta^2 = 0.934$) 0.911)及二者交互作用($\eta^2 = 0.845$). TO 和 T1 总产 气量分别较 MO 和 M1 增加了 13.93% 和 2.88%,证 明高温条件可促进产气,与 Lin 等[24]的研究结果一 致.35℃条件下搅拌可促进产沼气,M1 总产气量较 M0 显著(P<0.01)增加 12.63%. 而 T0 与 T1 之间 总产气量无显著差异,表明二者交互作用弱于单独 作用. 这是因为中温条件下搅拌可促进物料的水解, 从而增加了微生物对有机物的利用率[25],而高温本 身可提高有机物利用率,此温度条件下搅拌对总产 气量的影响可忽略不计. 4 组处理甲烷含量均为 50% 左右, 无显著差异, 总产甲烷量则与总产气量规 律一致.

消化结束后理化因子见表 2. 35℃和 55℃条件下的 TS 分别小于 7% 和大于 7%,其中 M0 与 M1 及 T0 与 T1 之间无明显差异. 消化结束后 SCOD 浓度降低了34. 99%~63. 17%,其中高温条件和搅拌处

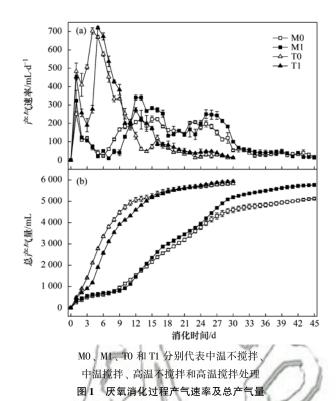


Fig. 1 Biogas production rate and total biogas yield in anaerobic digestion

理的 SCOD 浓度均高于中温条件和未搅拌处理,证明高温和搅拌可促进有机质的水解^[25],这也进一步解释了高温和搅拌可提高总产气量. TVFAs 浓度在消化结束后也降低,但 4 组处理间几乎无显著差异,只有 T1 的浓度显著(P < 0.01) 低于其他 3 组. TAN浓度从初始的 531. 55 mg·L⁻¹ 升高至 866. 26 ~ 953. 92 mg·L⁻¹, 4 组之间无显著差异. 消化结束后4 组处理的 pH 值均接近中性.

表 2 厌氧消化后理化因子

Table 2 Physicochemical parameters after anaerobic digestion

处理	处理编号	TS/%	SCOD/mg·L ⁻¹	TAN/mg·L ⁻¹	TVFAs/mg·L ⁻¹	pH
中温不搅拌	МО	6. 25 ± 0. 42a	3 158. 33 ± 235. 70d	918. 06 ± 73. 69a	3 124. 31 ±62. 23a	6. 52 ± 0. 02a
中温搅拌	M1	6. $86 \pm 0.82a$	$3991.67\pm117.85\mathrm{c}$	953. 92 ± 143. 78a	$3\ 300.\ 33\pm373.\ 39a$	6. $78 \pm 0.29a$
高温不搅拌	TO	$7.08 \pm 0.18a$	$4658.33\pm117.85\mathrm{b}$	$866.26 \pm 36.95a$	$3\ 212.\ 32\ \pm124.\ 46a$	$6.66 \pm 0.02a$
高温搅拌	T1	$7.53 \pm 1.15a$	$5575.00\pm408.25\mathrm{a}$	870. 24 ± 71. 72a	$2508.25\pm285.18\mathrm{b}$	$6.89 \pm 0.13a$

2.2 ARGs 与 MGEs 丰度变化

消化原料中共检测到 7 种 ARGs,包括外排泵基因 tetC 和 tetG,核糖体保护蛋白基因 tetO、tetQ 和 tetT,以及转座酶基因 tetX. 其中 tetQ 的绝对丰度最高,为 7. 45×10^9 copies · mL $^{-1}$,可能是 tetQ 在奶牛病原菌之间可频繁转移 $^{[23]}$. TetC 的绝对丰度最低,为 2. 33×10^6 copies · mL $^{-1}$.

以 16S rRNA 为管家基因, ARGs 与 16S rRNA 绝对丰度的比值作为 ARGs 的相对丰度. 经厌氧消化后, M0~T1 中 ARGs 的总相对丰度分别为

0. 177、0. 309、0. 019 和 0. 020[图 2(a)]. 由双因素方差分析结果可知,温度、搅拌以及二者交互作用对 ARGs 总相对丰度具有显著影响(P < 0.001). 与产气结果相似,温度对 ARGs 总相对丰度的影响($\eta^2 = 0.992$)大于搅拌($\eta^2 = 0.920$)及二者交互作用($\eta^2 = 0.917$), T0 和 T1 产物中总相对丰度显著低于 M0 和 M1,表明高温条件更有利于ARGs 的去除,与 Sun 等[26]的研究结果一致. 将消化产物中每个基因相对丰度的对数值与其消化原料中相对丰度的对数值作差,得到各基因相对丰

度的变化量(对数值),结果如图 2(b)所示. 7 种ARGs 在 TO 和 T1 中的去除量为 0.09 ~ 1.53(对数值),高于 M0 和 M1 中的去除量 0.02 ~ 0.68(对数值),进一步证明高温可促进 ARGs 的去除. 搅拌也可促进部分 ARGs 的去除,例如,tetA 和 tetG 的相对丰度在 M0 中分别增加了 0.07 和 0.43(对数值),而在 M1 中则分别降低了 0.11 和 0.19(对数值). TetC 和 tetO 的相对丰度在 M1 中也显著(P < 0.01)低于 M0.与 T0 相比,T1 中 tetC、tetT 和 tetX 的相对丰度较低.由以上分析可知,搅拌对 ARGs的影响在不同的温度条件下略有差异. 搅拌在中温条件下可显著促进 tetA、tetC 和 tetG 的去除,在高温条件下则促进了 tetC、tetT 和 tetX 的去除,有研究表明中温厌氧消化搅拌速率越高,tetM 和 tetO 的去除效果越好[27],高温条件下搅拌对 ARGs 的

影响还未有报道.

消化原料中 3 种 MGEs 的总相对丰度为 0.006,经厌氧消化后只有 M1 中升高至 0.178,其他 3 组处理均下降[图 2(c)]. 厌氧消化后 3 种 MGEs 相对丰度对数值的变化如图 2(d) 所示. M0 中 intI1 和 intI2 的相对丰度分别降低了 0.72 和 0.67 (对数值),Tn916/1545 的相对丰度几乎无变化.相比之下,T0 使得 intI2 相对丰度降低了 1.81 (对数值),intI1 和 Tn916/1545 的相对丰度变化与 M0 无明显差异. M1 较 M0 显著 (P < 0.01) 增加了整合子 intI1 和 intI2 的相对丰度,而 T1 则是较 T0 显著 (P < 0.01)降低了 Tn916/1545 的相对丰度。由此可见,高温可促进厌氧消化对 intI2 的去除,促进了高温厌氧消化对 Tn916/1545 的去除,

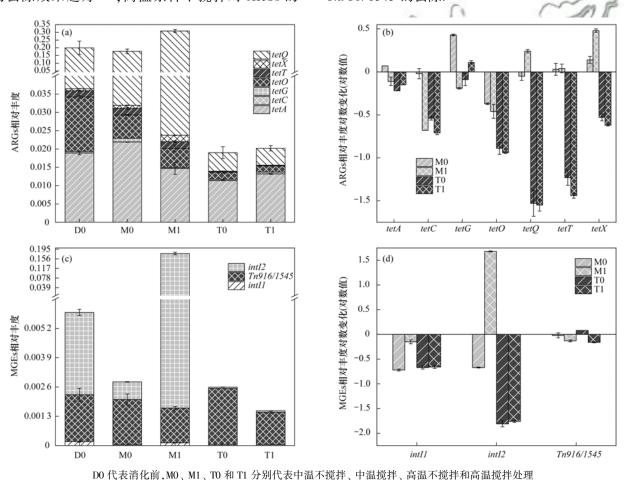
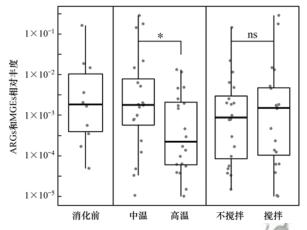


图 2 厌氧消化前后 ARGs 和 MGEs 的相对丰度及其变化 Fig. 2 Relative abundances and changes of ARGs and MGEs before and after anaerobic digestion

为了进一步对比温度和搅拌哪种条件对 ARGs 和 MGEs 去除的影响更大,分别研究中温(M0 和 M1)和高温(T0 和 T1)、未搅拌(M0 和 T0)和搅拌(M1 和 T1)之间各基因相对丰度的差异. 如图 3 所示,图中样点代表各 ARGs 和 MGEs 在消化结束后的相对丰度,箱形内横线代表每种条件下基因相对

丰度的中值. 与初始相比,只有高温和未搅拌条件下的中值减小,说明这两种条件有利于厌氧消化对ARGs的去除. 中温条件下的中值为 0.002,比高温条件下的中值高出 7 倍之多,表明高温更有利于ARGs和 MGEs的去除. 与未搅拌对比,搅拌条件下的中值略高,为 0.001,表明搅拌在一定程度上削弱

了 ARGs 和 MGEs 的去除效果. 整体来看,中温和高温条件之间 ARGs 相对丰度差异显著(P < 0.05),而未搅拌和搅拌之间则不显著,进一步证明温度比搅拌对 ARGs 和 MGEs 去除的影响更大. 从此角度考虑,高温不搅拌的厌氧消化工艺更有利于 ARGs的去除.



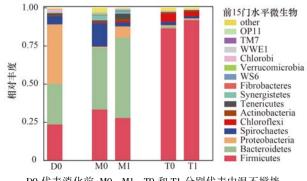
*表示 P < 0.05 的显著差异,ns表示无显著差异

图 3 不同条件厌氧消化后 ARGs 和 MGEs 相对丰度

Fig. 3 Relative abundances of ARGs and MGEs after anaerobic digestion under different conditions

2.3 微生物群落

厌氧消化前后门水平微生物(前15)丰度变化 如图 4 所示. 消化原料中,有 4 种优势菌门,总丰度 占群落结构的 94.56%, 分别为 Proteobacteria (39.03%), Bacteroidetes (26.56%), Firmicutes (23.58%)和 Spirochaetes (5.39%). 厌氧消化结束 后,微生物群落结构发生了明显变化,且不同处理中 变化具有差异. MO 中 Firmicutes、Bacteroidetes 和 Spirochaetes 的丰度分别增加至 33.40%、40.26% 和 15.42%, Proteobacteria 的丰度则降低至1%以下. 而 TO 中这 4 种门水平微生物只有 Firmicutes 丰度升高 至 86.58%, 其他均下降至 3% 以下. Firmicutes 是参 与降解纤维素产挥发酸的主要微生物[28],55℃条 件下 Firmicutes 丰度远高于 35℃,这解释了高温促 进产气的现象. Wu 等[29]的研究发现高温条件下 Firmicutes 的相对丰度降低,这可能是因为与本文厌 氧消化原料不同导致的. 与 MO 相比, M1 中 Bacteroidetes 和 Proteobacteria 丰度分别从 40.26% 和 0.91% 升高至 52.49% 和 7.47%, Spirochaetes 丰 度从 15.42% 下降至 2.66%. Bacteroidetes 也是参与 产 H, 和 CO, 的主要微生物^[28], 35℃条件下搅拌增 加了 Bacteroidetes 的丰度,从而解释了中温条件下 搅拌促进产气的现象. 55℃条件下未搅拌与搅拌处 理微生物相对丰度差异较小,说明搅拌对微生物的 影响小于温度.



D0 代表消化前, M0、M1、T0 和 T1 分别代表中温不搅拌、 中温搅拌、高温不搅拌和高温搅拌处理

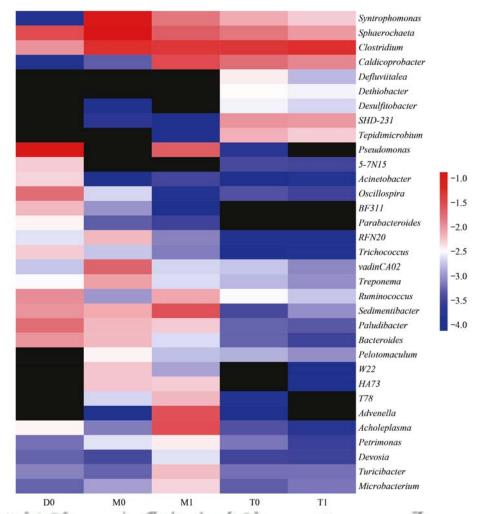
图 4 厌氧消化门水平微生物群落

Fig. 4 Microbial relative abundances in anaerobic digestion at the phylum level

以厌氧消化前后属水平微生物丰度的对数值作 热图,如图 5 所示.消化原料中 Pseudomonas、 Sphaerochaeta、Oscillospira 和 Paludibacter 丰度较高, 为优势属,经消化结束后优势属发生变化.35℃消化 产物中相对丰度较高的属包括 Syntrophomonas、 Sphaerochaeta , Clostridium vadinCA02 Sedimentibacter 等; 55℃消化产物中相对丰度较高 的属则少于 35℃,包括 Clostridium、Caldicoprobacter 和 SHD-231 等. 另外,中温条件下搅拌改变了一些 属水平的相对丰度. M1 中 Pseudomonas 的相对丰度 为 2.30%, M0 中此属完全消失; M1 使得 Sedimentibacter、Advenella 和 Acholeplasma 的相对丰 度从 0.83%、0.01% 和 0.08% 增加至 2.91%、 2.87% 和 3.11%. TO ~ T1 之间的属水平微生物相对 丰度较为接近.

2.4 基因、微生物群落以及理化因子间互作关系

选取每个样本中相对丰度大于1%的属,共11 个属,与ARGs及MGEs作网络分析,结果如图6(a) 所示,找到8个属为ARGs和MGEs的潜在宿主菌, 其在各处理之间丰度影响了 ARGs 的丰度. Ma 等[17] 的研究表明 Trichococcus、Acinetobacter 和 Tissierella 等 12 个属为 tetM、tetW 和 intII 等的潜在 宿主,这些属的相对丰度与 ARGs 的变化规律一致. 本文中 tetG 有 4 个宿主菌 Sphaerochaeta、 Treponema、Syntrophomonas 和 vadinCA02, 其在 MO 中丰度最高,也反映了 tetG 在此条件下相对丰度最 高. TetO 和 intII 有一个共同宿主菌 Pseudomonas, intII 在各处理之间的分布可能间接影响 tetO 的分 布. Sedimentibacter 为 tetQ 和 tetX 的潜在宿主菌,另 外 tetX 和 intI2 共享3 个潜在宿主菌 Sedimentibacter、 Acholeplasma 和 Advenella,这些属的相对丰度在 M1 中最高,这也代表 tetQ 和 tetX 在此条件下丰度增 加. 为了进一步探究影响不同处理中微生物分布的

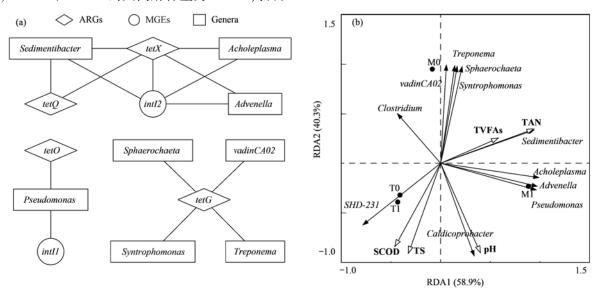


D0 代表消化前, M0、M1、T0 和 T1 分别代表中温不搅拌、中温搅拌、高温不搅拌和高温搅拌处理 图 5 厌氧消化属水平微生物群落

Fig. 5 Microbial relative abundances in anaerobic digestion at the genus level

因子,采用理化因子与微生物间冗余分析,结果见图 6(b). RDA1 和 RDA2 的共同解释量为 99.2%,表明

理化因子较大程度影响了 4 组处理间的微生物分布. 其中, TAN 的解释量最高, 占总解释量的 57%.



(a) 网络分析; (b) 冗余分析; 空心箭头表示理化因子, 实心箭头表示属水平微生物

图 6 理化因子、ARGs、MGEs 以及微生物群落间互作关系

Fig. 6 Interactions among physicochemical parameters, ARGs, MGEs, and microbial communities

而且 TAN 和 TVFAs 与大多数 ARGs 的宿主菌 Sedimentibacter、 Acholeplasma、 Advenella 和 Pseudomonas 等呈正相关关系,由此证明本研究中 TAN 和 TVFAs 通过影响微生物从而影响 ARGs 的变化,与 Zhang 等^[30]的研究结果一致.

3 结论

- (1)温度和搅拌对厌氧消化特性均有影响,高温和搅拌可以促进有机质水解,提高 SCOD 浓度,从而提升总产气量和总甲烷量.与搅拌相比,温度对厌氧消化特性影响更为显著.
- (2)高温可显著提高 7 种 ARGs 和 *intl2* 的去除率,搅拌在不同温度条件下对 ARGs 影响不同. 整体来看,温度比搅拌对 ARGs 和 MGEs 去除的影响更显著,高温不搅拌的厌氧消化工艺有利于 ARGs 的去除.
- (3)微生物群落受温度影响更明显. 高温条件下优势菌门种类减少, Firmicutes 变为绝对优势菌门. 中温条件下搅拌可增加 Bacteroidetes 的丰度,而在高温条件下未搅拌与搅拌的微生物群落无显著差异.
- (4) Sedimentibacter、 Pseudomonas 和 Spharochaeta 等为 ARGs 的潜在宿主菌,其在不同条件下的丰度直接影响了厌氧消化对 ARGs 的处理效果. TAN 和 TVFAs 通过影响宿主菌的丰度,从而间接影响了不同条件下 ARGs 的相对丰度.

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