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红霉素对产甲烷菌的抑制及其驯化

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摘要:红霉素是一类具有一定生物毒性的抗生素类药品,为探明红霉素对产甲烷菌的抑制作用及其可驯化能力,依次在厌氧瓶中进行厌氧毒性试验、在升流式厌氧污泥床反应器 (UASB)中进行连续实验,测定累计甲烷产量、相对产甲烷速率、COD 去除率、甲烷含量. 结果表明,红霉素为 150 $\mathrm{mg}\cdot\mathrm{L}^{-1}$ 时产甲烷速率降为 56. 1%; 250 $\mathrm{mg}\cdot\mathrm{L}^{-1}$ 时反应速率降低 99%以上,活性受到完全抑制. 保持红霉素投加量为 20 $\mathrm{mg}\cdot\mathrm{L}^{-1}$ 连续运行 60 d, COD 去除率、甲烷含量可达到 81. 4%、64. 2%. 红霉素对甲烷菌有抑制作用,半抑制浓度为 150 $\mathrm{mg}\cdot\mathrm{L}^{-1}$. 甲烷菌对红霉素有一定的驯化能力,驯化 60 d 后 COD 去除率、甲烷含量较未驯化时可提高 15. 13%、22. 05%.

关键词:红霉素;产甲烷菌;产甲烷速率;抑制;甲烷含量

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Inhibition of Methanogenium by Erythromycin and Its Domestation

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Abstract: Erythromycin is a kind of antibiotic drugs with certain biological toxicity. In order to investigate the inhibitory effect of erythromycin on methanogens and its acclimation capacity, Anaerobic Toxicity Assay (ATA) and continuous experiment were conducted in anaerobic bottles and the Up-flow Anaerobic Sludge Blanket Reactor (UASB), respectively, to determine the accumulated methane production, ratio of methane production rate, COD removal efficiency, and methane content. The results showed that the methane production ratio was reduced to 56.1% in the presence of 150 mg·L⁻¹ of erythromycin and it was reduced by 99% when the erythromycin reached 250 mg·L⁻¹, indicating that the activity was completely inhibited. Keeping the erythromycin at an concentration of 20 mg·L⁻¹ in the process of continuous operation for 60d, the COD removal efficiency and methane content reached up to 81.4% and 64.2%, respectively. The results suggested that erythromycin had an inhibitory effect on methane bacteria, and the half inhibitory concentration was 150 mg·L⁻¹ (IC₅₀:150 mg·L⁻¹). The COD removal efficiency and methane content were increased by 15.13% and 22.05%, respectively, after domestication for 60 d.

Key words: erythromycin; methanogenium; methane production ratio; inhibition; methane content

制药废水是一类 COD 浓度较高的生产废水,同时由于药品中的内分泌干扰物及某些副作用将最终产生某些特定的生物效应,使得制药废水对水生环境存在一定的潜在危害^[1,2]. 其中抗生素类制药废水由于难降解的、溶解性的有机物质浓度高^[3]、生物毒性物质多等特点已成为国内外水处理的热点问题. 制药废水通常经混凝、过滤、沉淀、离子交换、活性炭吸收、高级氧化、生物处理等方法进行处理^[4,5],其中厌氧生物处理是一种主要的制药废水处理方法,在国内外得到广泛应用.

文献[6~8]研究了抗生素对水体环境的危害、生物降解性及抑制作用. Oktem 等 $[^{9}]$ 研究了实验室规模 UASB(up-flow anaerobic sludge blanket)反应器处理混合制药废水的处理效果,研究结果表明当OLR 为8 kg·(L·d) $^{-1}$ 时可达到最大 COD 去除率

72%. 文献[10~13]研究了升流式污泥床、固定床处理磺胺甲基嘧啶(Sulfamerazine)、安替比林(Antipyrine)等制药废水的处理情况,COD去除率可达到70%~80%左右. 国内也有学者做了相关研究,徐灏龙等[14]和 Zhang等[15]研究了 UASB-MBR、生物膜组合工艺处理制药废水. 这些研究大都从工艺的角度重点考察 COD 的去除情况,未能说明特定种类抗生素对厌氧代谢过程中的重要阶段产甲烷阶段的抑制情况. 本实验通过采用活性实验及连续实验的方法,研究了红霉素对产甲烷菌活性的抑制情况及其驯化能力:实验先通过厌氧毒性试验测得相对产甲烷

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速率,得到红霉素对产甲烷菌活性的抑制程度;随后由于有研究表明氰化物、三氯甲烷等毒性物质可采用间歇投加低浓度的方法对厌氧微生物实现成功驯化,同时由活性实验结果可知红霉素对甲烷菌抑制作用适中,本实验采用连续投加低浓度抑制剂的方法,考察其驯化能力;最后将未接触过抑制剂的污泥、开始接触抑制剂的污泥及驯化后期的污泥进行比较,考察其活性的变化情况.

1 材料与方法

1.1 厌氧毒性试验(anaerobic toxic assay, ATA)

种泥取自实验室规模 UASB 有机酸培养颗粒污泥,培养液组成(表1),红霉素(Erythromycin)是一类大环内酯类抗生素,分子式见图1.向125 mL 厌

氧瓶中投加 1.2 g 颗粒污泥、营养液 80 mL,污泥浓度为 1.5 g·L⁻¹,pH 7.0~7.2. 向瓶中依次投加 50、100、150、200 和 250 mg·L⁻¹的红霉素,充 N_2 2 min 保持厌氧环境,瓶口处使用橡胶塞及螺帽密封,置于恒温振荡箱内培养(35°C、106 r·min⁻¹)[16,17],观察累计甲烷产量与时间的变化关系.

1.2 连续实验

在 UASB(up-flow anaerobic sludge blanket) 反应器中进行连续实验,实验用反应器由有机玻璃制成,总容积 10 L、有效容积 6.28 L、反应区总高度 82 cm(图 2). 采用乙酸、丙酸、丁酸混合有机酸作为营养基质,COD 为5 000 mg·L $^{-1}$,向基质中连续投加 20 mg·L $^{-1}$ 红霉素,观察 COD 去除率及气体中的甲烷含量.

表 1 毒性试验基质组份

			Table 1	Substrate contents in the ATA test				
组份	$C_6 H_{12} O_6$	$\mathrm{KH_{2}PO_{4}}$	$\mathrm{NH_4Cl}$	$CaCl_2$	FeCl_2 . $4\mathrm{H}_2\mathrm{O}$	MgCl ₂ . 6H ₂ O	NaHCO ₃	微量元素
含量/g·L-1	1.4	0.4	1.0	0. 02	0. 4	0. 21	4. 0	10 mL

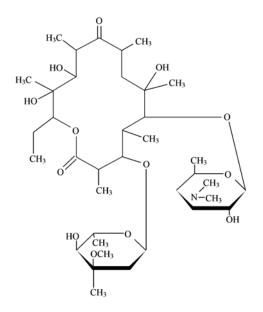


图1 红霉素分子结构式

Fig. 1 Erythromycin molecular structure

1.3 测定方法

气体组成中甲烷含量的测定使用气相色谱仪 (PerkinElmer Clarus500 GC、TCD 检测器)进行测定,载气为氩气,进样口、检测器、色谱柱初始温度分别为 $45 \times 200 \times 45 \times C$.

2 结果与分析

2.1 累计甲烷产量

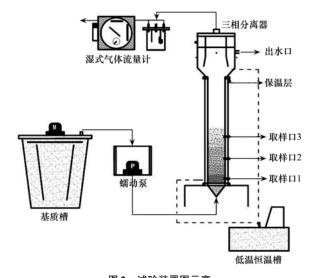


图 2 试验装置图示意

Fig. 2 Equipment setup

甲烷产量随时间的关系曲线[图 3(a)]:同一浓度下甲烷产量随时间的增加而增长,反应初始阶段甲烷产量随时间呈线性增长,在此阶段可得到最大产甲烷速率.相同反应时间下,甲烷产量随红霉素浓度的增加而降低.未投加抗生素的空白与红霉素投加量为50、100、150、200、250 mg·L⁻¹的各组中最大产甲烷量分别为121、101、77、65、48、25 mL.

2.2 产甲烷速率

由累计甲烷产量得出的相对速率与时间关系曲

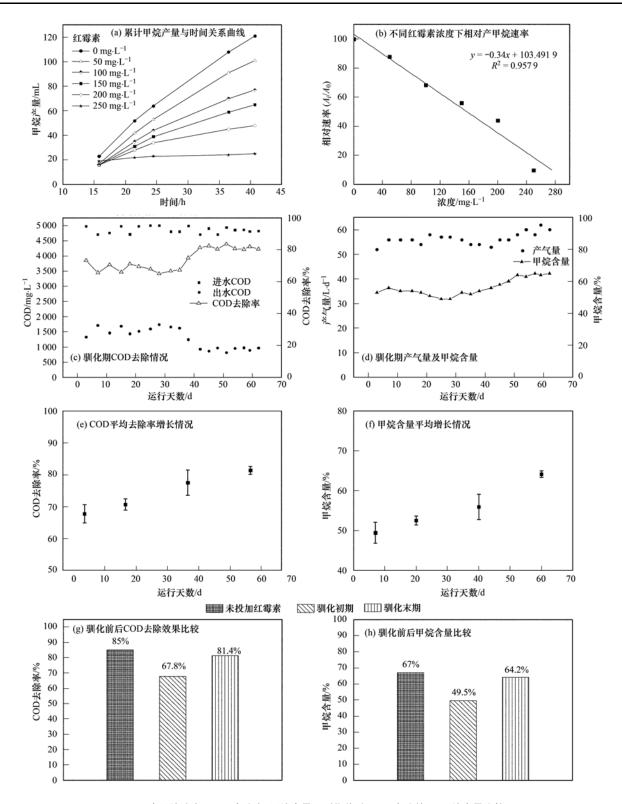


图 3 产甲烷速率、COD 去除率、甲烷含量及驯化前后 COD 去除效果、甲烷含量比较

Fig. 3 Methane production ratio, COD removal efficiency, methane content and comparison the change of COD removal efficiency, methane content before and after domestation

线[图 3(b)]:红霉素投加量为 50、100、150、200 和 250 mg·L⁻¹时与未投加抗生素的空白对照相比,最大产甲烷相对速率分别为 0.878、0.683、0.561、

0.439, 0.097.

2.3 COD 去除率

向混合有机酸营养基质中投加 20 mg·L⁻¹红霉

素后 COD 去除率迅速降至 67.8%. 保持红霉素投加浓度不变连续运行 60 d, COD 去除率有所恢复. 运行到 20、40、60 d 时 COD 去除率依次为 70.7%、77.6%、81.4%,但仍低于未投加红霉素时的 85%的去除效果.

2.4 甲烷含量

向混合有机酸营养基质中投加 20 mg·L⁻¹红霉素后甲烷含量降至 49.5%. 保持红霉素投加浓度不变连续运行 60 d,运行到 20、40、60 d 时甲烷含量增长为 52.6%、56%、64.2%,但仍低于未投加红霉素时的 68%的甲烷含量.

3 讨论

3.1 红霉素对甲烷菌活性抑制作用

累计甲烷产量及相对产甲烷速率随红霉素浓度的增加而降低. 红霉素投加量为 250 mg·L⁻¹时,累计甲烷产量为 25 mL,相对速率仅为 0.097,产甲烷量下降 79.3%,最大产甲烷速率下降 99%,活性受到完全抑制. 红霉素浓度为 150 mg·L⁻¹时,累计甲烷产量降为 53.7%,最大产甲烷速率降为 56.1%,活性降低一半,确定 150 mg·L⁻¹为红霉素对产甲烷菌的半抑制浓度. Sanz 等^[17]研究指出红霉素、氯四环素、氯霉素同属于抑制蛋白质合成的抗生素类药物,氯四环素、氯霉素浓度分别为 152 mg·L⁻¹、41 mg·L⁻¹时甲烷产量菌降低 80%,相比之下红霉素的毒性较弱,更适合用于考察未接触过抗生素的厌氧污泥的驯化能力.

3.2 产甲烷菌驯化能力

Richard 等[18] 研究指出毒物的投加方式对生物 体的驯化程度影响很大,以毒物半抑制浓度的 1/5 ~1/10 为最佳投加量,可达到较好的驯化效果. 根 据活性实验中得到的半抑制浓度 150 mg·L-1,在连 续实验中投加浓度为 20 mg·L-1的红霉素时, COD 去除率由未投加抗生素时85%降为67.8%,甲烷含 量由67%降为49.5%.说明即使抗生素投加量明 显低于半抑制浓度,在连续运行时仍会对产甲烷菌 活性产生影响. 保持红霉素 20 mg·L-1浓度不变,连 续运行60 d 考察甲烷菌对红霉素的可驯化能力. 研 究发现经 60 d 的连续运行, COD 去除率、甲烷含量 均有一定的增长[图 3(c)~3(f)],最终 COD 去除 率达到81.4%、甲烷含量64.2%,相比在基质中投 加红霉素初期时 COD 去除率、甲烷含量分别提高 了 15.13%、22.05% [图 3(g)~3(h)]. 但仍低于 未投加红霉素时的 85% 的 COD 去除率、67% 的甲 烷含量,两者分别下降了 4%、4.2%. Sponza 等^[10]研究了 UASB/CSTR 联合工艺对磺胺类抗生素药物的处理效果,研究指出磺胺类浓度为 10 mg·L⁻¹时,甲烷含量为 76%、COD 去除率为 97%,均高于本实验中驯化后的平均水平:甲烷含量 64.2%、COD 去除率 81.4%,但是实验中抗生素的投加量为 20 mg·L⁻¹高于 Sponza 研究中的 10 mg·L⁻¹投加量,同时本实验采用单一工艺主要考察微生物可驯化能力,并非组合工艺中重点考察处理效果. Sreekanth等^[11]研究了不同条件下抗生素发酵废水的处理效果,指出 OLR 为9 kg·(m³·d) ⁻¹时 COD 去除效果最佳为 65% ~75%,本实验与之相比 COD 去除效果略高.

4 结论

- (1)红霉素会对甲烷菌的活性产生抑制作用, 150 $\text{mg} \cdot \text{L}^{-1}$ 的红霉素可使甲烷产量、产甲烷速率降低 50% 左右,250 $\text{mg} \cdot \text{L}^{-1}$ 时甲烷菌活性基本受到完全抑制.
- (2)甲烷菌对红霉素具有一定的驯化能力,投加 20 mg·L⁻¹红霉素连续运行 60d,可使 COD 去除率、甲烷含量分别提高了 15.13%、22.05%,但未能恢复到未投加红霉素时的水平.

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