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冠状病毒气溶胶传播及环境影响因素

李雪, 蒋靖坤, 王东滨, 邓建国, 贺克斌, 郝吉明



■ 主办 中国科学院生态环境研究中心

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提盐速率对序批式生物反应器性能和微生物群落结构的影响

古柏铭¹, 金春姬^{1,2*}, 温淳¹, 侯金源¹, 赵阳国^{1,2}, 高孟春^{1,2}

(1. 中国海洋大学环境科学与工程学院, 青岛 266100; 2. 中国海洋大学海洋环境与生态教育部重点实验室, 青岛 266100)

摘要: 使用序批式生物反应器驯化耐盐活性污泥, 探究提盐速率对污染物去除效果、活性污泥特性和微生物群落结构的影响。结果表明, 快速提升盐度至 30‰ (30 d 内提升盐度), COD 和 NH_4^+ -N 去除率均出现明显下降, 由最初的 85.5% 和 98.5%, 分别降低至 72.2% 和 81.7%; 缓慢提升盐度至 30‰ (90 d 内提升盐度), COD 和 NH_4^+ -N 去除率受盐度影响较小, 表现出良好的去除性能。在缓慢提升盐度过程中, 盐度为 20‰, 出水 NO_2^- -N 升高至 11.13 $\text{mg}\cdot\text{L}^{-1}$, NO_3^- -N 降低为 0.56 $\text{mg}\cdot\text{L}^{-1}$, 实现短程硝化; 盐度为 30‰, 亚硝酸盐积累率约为 90%, TN 去除率升高至 75% 左右。随着盐度升高, 胞外聚合物中多糖和蛋白质的含量不断增加, 当盐度高于 15‰ 时, 多糖明显增加。高通量测序结果表明, 经快速提盐和缓慢提盐驯化, 微生物多样性明显下降, Shannon 指数由 8.06 分别降为 4.34 和 6.17。随着盐度增加, *Micropruina*、*Denitromonas*、*TM7a* 和 *Marinicella* 表现出良好的耐盐特性。经快速提盐驯化后, *Denitratisoma*、*Defluviimonas*、*Arenimonas* 和 *Denitromonas* 等反硝化菌的相对丰度明显减少。

关键词: 序批式生物反应器 (SBR); 提盐速率; 短程硝化; 胞外聚合物; 微生物群落结构

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Effect of Rate of Salinity Increase on the Performance and Microbial Community Structure of Sequencing Batch Reactors

GU Bai-ming¹, JIN Chun-ji^{1,2*}, WEN Chun¹, HOU Jin-yuan¹, ZHAO Yang-guo^{1,2}, GAO Meng-chun^{1,2}

(1. College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China; 2. Key Laboratory of Marine Environment and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China)

Abstract: In this study, three sequence batch reactors were selected to evaluate the effects of salt-tolerant activated sludge acclimation. The effect of salinity increase rate on pollutant removal, physicochemical characteristics of activated sludge, and microbial community were investigated. The results showed that a rapid salinity increase to 30‰ (within 30 d) reduced removal efficiencies of COD and NH_4^+ -N from 85.5% and 98.5% (18 d) to 72.2% and 81.7% (51 d), respectively. In comparison, a slower salinity increases to 30‰ (within 90 d) had a minor effect on COD and NH_4^+ -N removal. During the rapid salinity increase, a stable shortcut nitrification occurred under 20‰ salinity, in which the effluent NO_2^- -N reached 11.13 $\text{mg}\cdot\text{L}^{-1}$ and NO_3^- -N decreased to 0.56 $\text{mg}\cdot\text{L}^{-1}$. When salinity increased to 30‰, the nitrite accumulation rate was about 90%, and the removal efficiency of total nitrogen increased to approximately 75%. The contents of polysaccharide and protein in extracellular polymer substances increased as salinity increased, and the polysaccharide content increased significantly when the salinity was higher than 15‰. High-throughput sequencing results illustrated that microbial diversity reduced as salinity increased, following the Shannon index decrease from 8.06 (0‰ salinity) to 4.34 (rapid salinity increase) and 6.17 (slower salinity increase). As salinity increased, *Micropruina*, *Denitromonas*, *TM7a*, and *Marinicella* exhibited good salt tolerance. The relative abundance of *Denitratisoma*, *Defluviimonas*, *Arenimonas*, and *Denitromonas* decreased more significantly following the rapid salinity increase compared with that after the slower salinity increase.

Key words: sequencing batch reactor (SBR); salinity promotion rate; shortcut nitrification; extracellular polymeric substances; microbial community structure

在中国沿海城市,因淡水资源短缺,生活用水和工业生产常采用海水直接代替淡水作为原水进行利用^[1]。例如,将海水用于海产品加工、道路清洗、公厕和消防等^[2];海水也常用作工业冷却水,广泛应用于钢铁、石化、机械火电和核电等行业^[3]。同时,海水养殖业的快速发展,也产生大量养殖废水^[4]。这些含盐废水中除含有大量无机盐外,还存在有机物、氮和磷等污染物,若未经处理排放会对沿海生态系统造成巨大破坏^[5]。生物法因具有经济、环保等优点^[6],广泛应用于市政和工业废水的处理。然而,高浓度的无机盐会改变微生物群落结构,影响有机

物和营养物的去除^[7]。有研究表明,无机盐会抑制亚硝酸盐氧化菌(nitrite-oxidizing bacteria, NOB),而氨氧化菌(ammonia oxidizing bacteria, AOB)具有更强的适应能力,在好氧段造成亚硝酸盐的积累^[8],形成短程硝化。

通过耐盐驯化,可提高污泥中微生物对盐度的适应能力^[9],且驯化方式对污泥的耐盐性也会产生

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作者简介: 古柏铭(1997~),男,硕士研究生,主要研究方向为生物法处理海水养殖废水, E-mail: gbm19970311@163.com

* 通信作者, E-mail: jinhou@ouc.edu.cn

影响. 有研究表明, 逐步提升盐度和高盐环境直接驯化污泥对反应器性能和微生物群落会产生不同的影响^[10,11]. 然而, 快速提升盐度和缓慢提升盐度的驯化方式, 对活性污泥特性及微生物群落结构的影响却很少研究. 本实验采用序批式生物反应器 (sequencing batch reactor, SBR) 处理含盐废水, 考察了提盐速率对 COD 降解和脱氮性能的影响, 以及盐度和曝气量对亚硝酸盐积累率 (nitrite accumulation rate, NAR) 的影响. 同时, 本文研究盐度对污泥沉降性能和胞外聚合物 (extracellular polymeric substances, EPS) 的影响, 探究驯化过程中微生物群落结构的演变, 以期快速驯化具有良好特性的耐盐污泥提供理论参考与支撑.

1 材料与方法

1.1 实验装置和进水水质

本实验装置如图 1 所示, SBR 主体是有机玻璃材质的圆柱体, 高 25 cm, 直径 22 cm, 有效容积为 8 L, 温度通过电热丝控制在 30℃ 左右. 接种污泥取自青岛市海泊河污水处理厂, 混合液悬浮固体浓度为 4 000 mg·L⁻¹ 左右. 进水为模拟含盐有机废水, 主要成分为葡萄糖、NH₄Cl 和 KH₂PO₄, 浓度分别约为 200、150 和 9 mg·L⁻¹, pH 为 7.3~8.2 (投加 NaHCO₃), 盐度和微量元素均由海水晶提供.

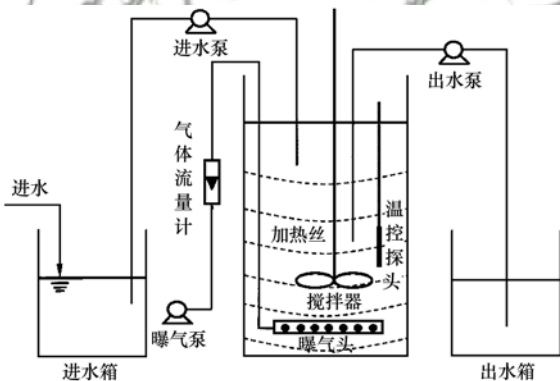


图 1 SBR 装置示意

Fig. 1 Schematic of the SBR

1.2 污泥的耐盐驯化

通过逐步提升盐度来驯化污泥, 设置盐度梯度为 5‰、10‰、15‰、20‰、25‰ 和 30‰, SBR1、SBR2 和 SBR3 每个盐度梯度的驯化时间分别为 5、10 和 15 d, 盐度达到 30‰ 后, 继续运行. 反应器每天运行 4 个周期, 每个周期分 6 个阶段: 进水 (5 min)、缺氧 (60 min)、曝气 (180 min)、沉淀 (30 min)、出水 (5 min) 和闲置 (80 min). 反应器运行 115 d, 其中阶段 1 (1~71 d) 曝气量 1.0 L·min⁻¹, 阶段 2 (71~119

d) 曝气量 0.5 L·min⁻¹.

1.3 EPS 的提取

取 50 mL 污泥混合液, 在 4 000 r·min⁻¹ 下离心 10 min 后, 弃去上清液后加入等体积的 0.9% NaCl 溶液, 摇匀后 80℃ 水浴 30 min, 在 4 000 r·min⁻¹ 下离心 10 min 后, 取上清液经 0.45 μm 滤膜过滤后即为 EPS. EPS 主要成分为蛋白质和多糖, 两者之和即为 EPS 的含量.

1.4 水质指标测定方法

本实验过程中对 COD、NH₄⁺-N、NO₂⁻-N 和 NO₃⁻-N 等常规水质指标进行监测, 每两天测定一次. NH₄⁺-N、NO₂⁻-N 和 NO₃⁻-N 分别采用水杨酸比色法、N-(1-萘基)-乙二胺分光光度法和紫外分光光度法测定; COD 采用重铬酸钾法测定; MLSS 采用重量法测定; 蛋白质采用 Folin-酚分光光度法测定, 多糖采用蒽酮比色法测定.

另外, 当 SBR1 运行第 113 d, SBR3 运行第 18、69 和 113 d 时 (盐度分别为 0‰、15‰ 和 30‰) 取活性污泥样品, 命名为 F-3、T-1、T-2 和 T-3.

1.5 DNA 提取和 PCR 扩增

采用 SDS 方法对样本的基因组 DNA 进行提取, 利用琼脂糖凝胶电泳检测 DNA 的纯度和浓度, 使用无菌水稀释样本 DNA 至 1 ng·μL⁻¹. 以稀释后的基因组 DNA 为模板, 采用引物 515F、806R 和高效高保真酶对 16S rDNA 的 V4 区域进行扩增, 确保扩增效率和准确性.

1.6 高通量测序与 OTUs 聚类

采用 Illumina NovaSeq 测序平台进行双末端测序 (paired end), 经过 Reads 拼接过滤, OTUs (operational taxonomic units) 聚类, 进行物种注释及丰度分析; 通过 α 多样性 (α diversity) 和 β 多样性分析 (β diversity), 可以揭示出样品中物种组成和样本间群落结构的差异.

2 结果与讨论

2.1 不同提盐速率下盐度对 COD 去除效果的影响

如图 2 所示, 各个盐度阶段初期, COD 去除率均出现明显的下降, 表明微生物的活性受到抑制. 经过长时间的驯化, COD 去除率达到 85% 左右. 当 SBR1 盐度低于 20‰, COD 去除率高于 80%; 盐度高于 20‰, COD 去除率降至 70% 左右, 且需较长适应时间达到较好的去除效果. 相较于 SBR1, SBR2 能够很快适应盐度变化. SBR3 仅在 71~83 d (活性污泥恢复期) COD 去除率较低, 其它期间, 均表现出良好的 COD 降解能力. Wang 等^[12] 的研究结果与本研究的类似, 当盐度低于 30‰, COD 去除率高于 90%,

但盐度提升至 60‰, 去除率降至 33%. 也有研究报道, 采用活性污泥法处理皮革废水^[13], 出水 COD 平均为 $225 \text{ mg} \cdot \text{L}^{-1}$, 去除率约为 70%. 这种差异可能是由于实际废水中含有难降解有机物且有机负荷较高.

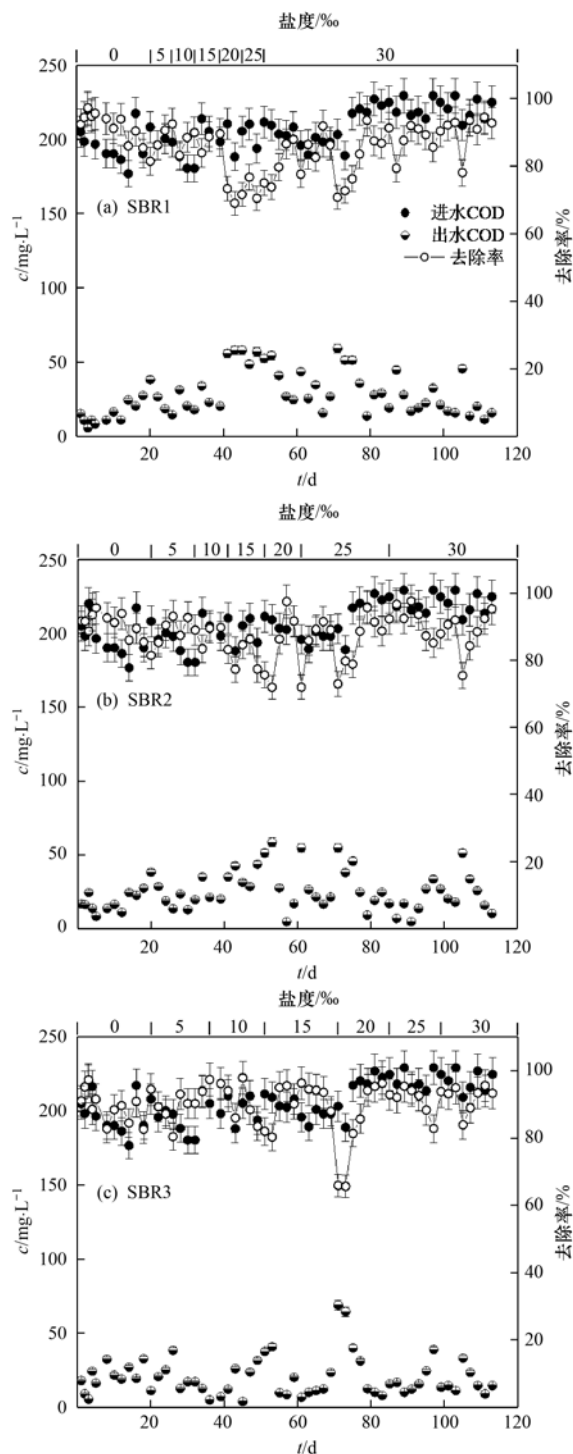


图2 不同提盐速率下水 COD 的变化

Fig. 2 Variation in COD in effluent at different rates of salinity increase

2.2 不同提盐速率下盐度对脱氮性能的影响

图3所示, 提盐速率和盐度对 NH_4^+ -N去除影响较小. 类似地, Navada 等^[14]的研究也发现提盐速率

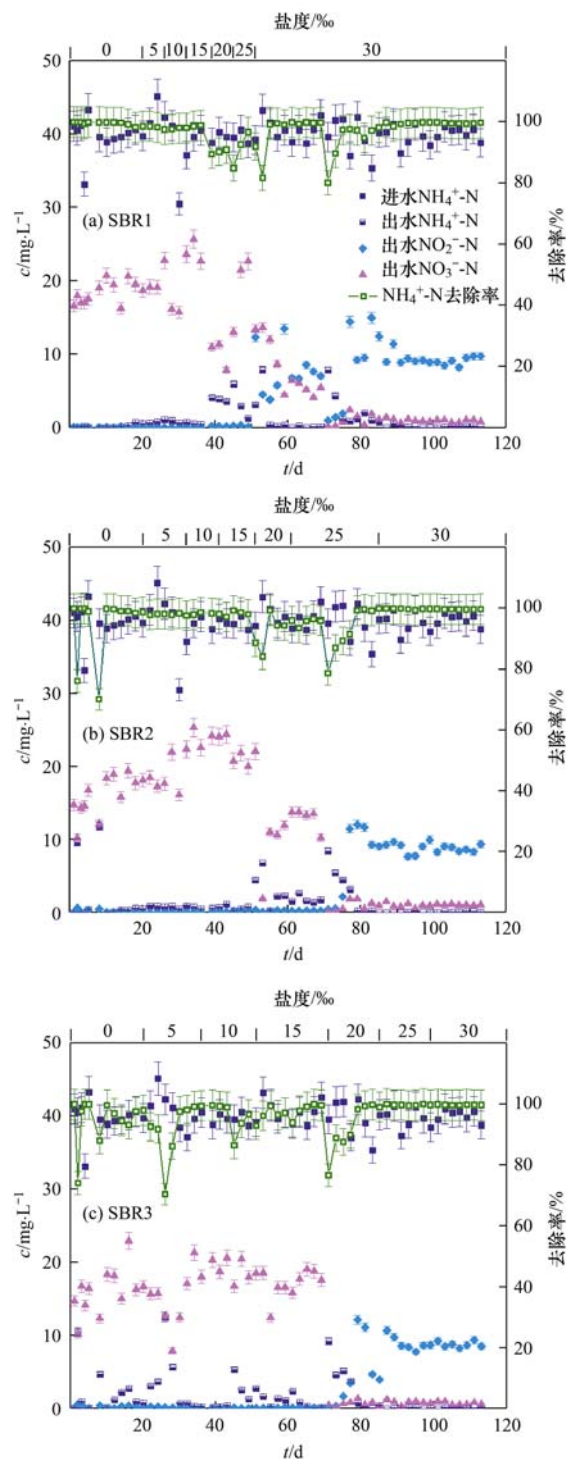


图3 不同提盐速率下水氮素的变化

Fig. 3 Variation in nitrogen in effluent at different rates of salinity increase

对氨氧化能力影响较小. 当 SBR1 盐度提升至 30‰, 出水 NO_2^- -N 浓度高于 NO_3^- -N, 表明高盐抑制 NO_2^- -N 向 NO_3^- -N 的转化^[15], 实现短程硝化. 当 SBR2 盐度为 20‰, NO_2^- -N 出现积累, 但 NAR 较低, 值得注意的是, 出水 NO_3^- -N 大幅减少, 由 $22.67 \text{ mg} \cdot \text{L}^{-1}$ 降至 $13.39 \text{ mg} \cdot \text{L}^{-1}$. SBR3 盐度低于 20‰, 出水以 NO_3^- -N 为主, 脱氮途径仍为传统的硝化-反硝化; 盐度为 30‰, 出水 NO_3^- -N 浓度极低, NO_2^- -N 浓度为 8.69

$\text{mg} \cdot \text{L}^{-1}$.

图4为不同提盐速率下NAR和总氮(total nitrogen, TN)去除率的变化. SBR1盐度为25‰, NAR突然升高; 盐度升至30‰, NAR约为60%; 降低曝气量后NAR明显升高, 稳定在90%左右, TN去除率约为75%. 当SBR2和SBR3盐度高于20‰, 均实现 NO_2^- -N积累, NAR和TN去除率与SBR1相似.

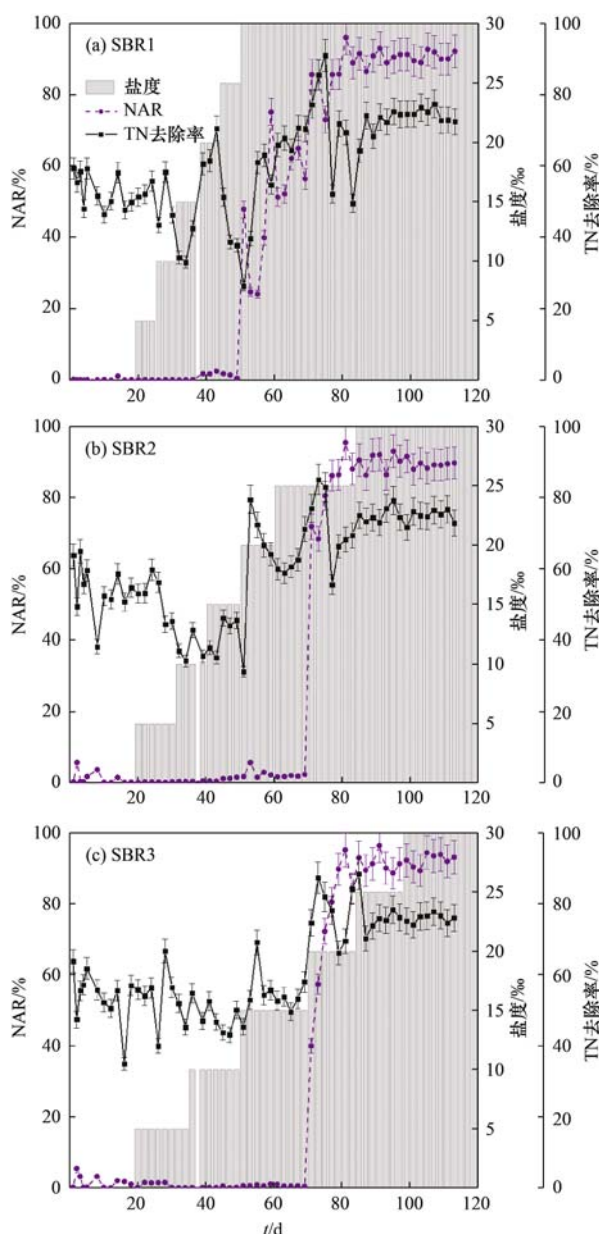


图4 不同提盐速率下NAR和TN去除率的变化

Fig. 4 Variation in NAR and TN removal rates in different rates of salinity increase

图5为特定周期内 NH_4^+ -N、 NO_2^- -N和 NO_3^- -N的变化, 其中图5(a)~5(c)为67 d周期实验, 图5(d)为95 d周期实验. 由图5(a)可知, 盐度为30‰, SBR1实现短程硝化, 且较高曝气量不利于 NO_2^- -N积累^[16]. 图5(b)所示, SBR2好氧段末

期 NO_2^- -N浓度很低, 但整个周期仍有一定的 NO_2^- -N, 推测部分 NH_4^+ -N通过短程硝化-反硝化得以去除, 提高碳源利用率^[17], 降低出水TN. 由图5(c)和5(d)可知, 盐度为实现短程硝化的决定性因素, 盐度为15‰, 缺氧段末期 NO_2^- -N和 NO_3^- -N较高; 盐度为25‰, 周期内 NO_3^- -N浓度较低, 缺氧段反硝化主要为 NO_2^- -N.

上述实验结果表明, 20‰的盐度为实现短程硝化的阈值, 较低的曝气有利于 NO_2^- -N积累, 提高碳源利用率, 提高脱氮性能.

2.3 不同提盐速率下盐度对SVI的影响

通过污泥体积指数(sludge volume index, SVI)表征污泥的沉降性能. 如图6所示, 驯化初期SVI约为 $50 \text{ mL} \cdot \text{g}^{-1}$, 盐度提升后, SBR1和SBR3污泥SVI显著下降, 在50 d降至 $30 \text{ mL} \cdot \text{g}^{-1}$ 左右. 推测高盐环境抑制了丝状菌生长, 污泥絮体紧密结合, 提高污泥的沉降性能^[18]. SBR2污泥SVI随着盐度提升无明显变化, 为 $50 \sim 60 \text{ mL} \cdot \text{g}^{-1}$, 但恢复期出现污泥膨胀现象.

2.4 不同提盐速率下盐度对EPS的影响

图7为不同提盐速率下EPS中蛋白质和多糖含量的变化情况. 结果表明, EPS含量(以VSS计, 下同)随着盐度提升而增加. 最初EPS主要为蛋白质, 多糖很低. SBR1盐度为20‰, 多糖为 $13.9 \text{ mg} \cdot \text{g}^{-1}$ (41 d); SBR2和SBR3盐度为15‰, 多糖升至 $13.5 \text{ mg} \cdot \text{g}^{-1}$ (41 d)和 $14.5 \text{ mg} \cdot \text{g}^{-1}$ (67 d). 有研究发现, 多糖可减轻钠离子对细胞的压力, 在保护细胞中发挥重要作用^[19]. 本实验, 15‰的盐度为微生物大量分泌多糖的阈值. 也有研究报道, 蛋白质随着盐度升高而降低^[20], 本实验中蛋白质含量明显升高, 该差异可能是由于污泥特性和最终的盐度不同所造成.

2.5 微生物群落丰富度和多样性分析

Shannon指数常用来评价微生物多样性, Shannon指数越大, 说明微生物多样性越高^[21]. 如表1所示, 随着盐度提升, SBR3活性污泥Shannon指数由8.06降至6.91(盐度15‰)和6.17(盐度30‰). 而SBR1盐度为30‰, 活性污泥Shannon指数为4.34. 表明快速提盐驯化活性污泥会显著降低微生物多样性.

根据测序结果, 绘制Venn图(图8). 样品T-3与T-2共有物种同T-1与T-2共有物种对比, 明显减少, 表明部分微生物难以适应高盐环境而被淘汰. 当盐度为30‰, 相比SBR1, SBR3的OTUs数目更多, 说明较长的驯化时间能够使微生物更好适应高

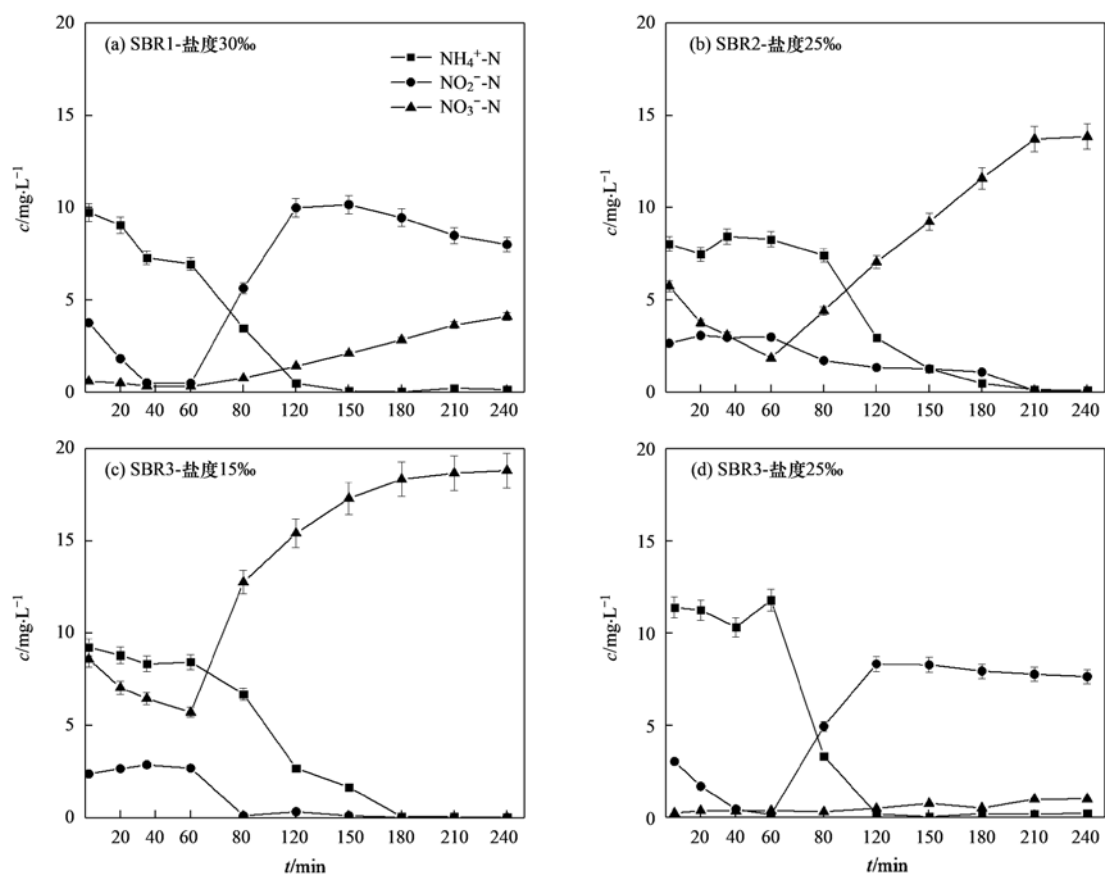


图5 特定周期内SBR1、SBR2和SBR3中氮素的变化
Fig. 5 Variation in nitrogen in specific cycle of SBR1, SBR2, and SBR3

表1 活性污泥多样性指数
Table 1 Diversity of activated sludge samples

样品	OTUs	Ace 指数	Chao 指数	Shannon 指数	Simpson 指数
T-1	2 630	2 978	2 819	8.06	0.979
T-2	2 772	2 978	2 937	6.91	0.894
T-3	2 000	2 247	2 209	6.17	0.947
F-3	1 347	1 619	1 547	4.34	0.842

盐环境^[22].

2.6 微生物群落结构分析

对门水平上相对丰度排名前10的菌门(图9)进行分析,样品中主要包括:Proteobacteria、Actinobacteria、Bacteroidota、Chloroflexi和Planctomycetes菌门.据报道,前4个菌门在很多环境样品中占比较大^[21];Proteobacteria和Bacteroidota存在与脱氮除磷相关的功能菌^[23];Planctomycetes存在脱氮菌属,且在海水微生物群落构成中含量较多^[24].当SBR3盐度由0提升至30‰,Proteobacteria由20.74%(T-1)增加至40.26%(T-3),相应SBR1盐度为30‰时,Proteobacteria占比为34.18%(F-3).快速提盐后,Actinobacteria在SBR1占比为35.42%,高于SBR3的17.43%,表明Actinobacteria能够抵御快速提盐的选择压力,并成为高盐环境下的优势菌门.

本实验还从属水平上对4个样品中前35位的微生物菌属进行分析,绘制相对丰度柱状图(图10).随着盐度增加,Candidatus_Competibacter和Ferruginibacter相对丰度由2.22%和1.69%均降至为0;而Micropruina受盐度影响较小,经快速提盐和缓慢提盐,相对丰度分别为34.1%和11.4%,具有良好的耐盐性.Huang等^[25]的研究发现,当盐度为12‰,在缺氧/好氧交替运行模式下,Candidatus_Competibacter在反硝化过程中起主要作用;据报道,污水处理厂的活性污泥样品中普遍含有Ferruginibacter,能够水解有机物,在高COD浓度的环境中相对丰度较高^[26].Wang等^[27]的研究也发现Micropruina具有很强的耐盐特性,盐度为0~80‰,Micropruina在生物膜中相对丰度较高.

盐度为30‰,优势菌属发生变化,Denitromonas、

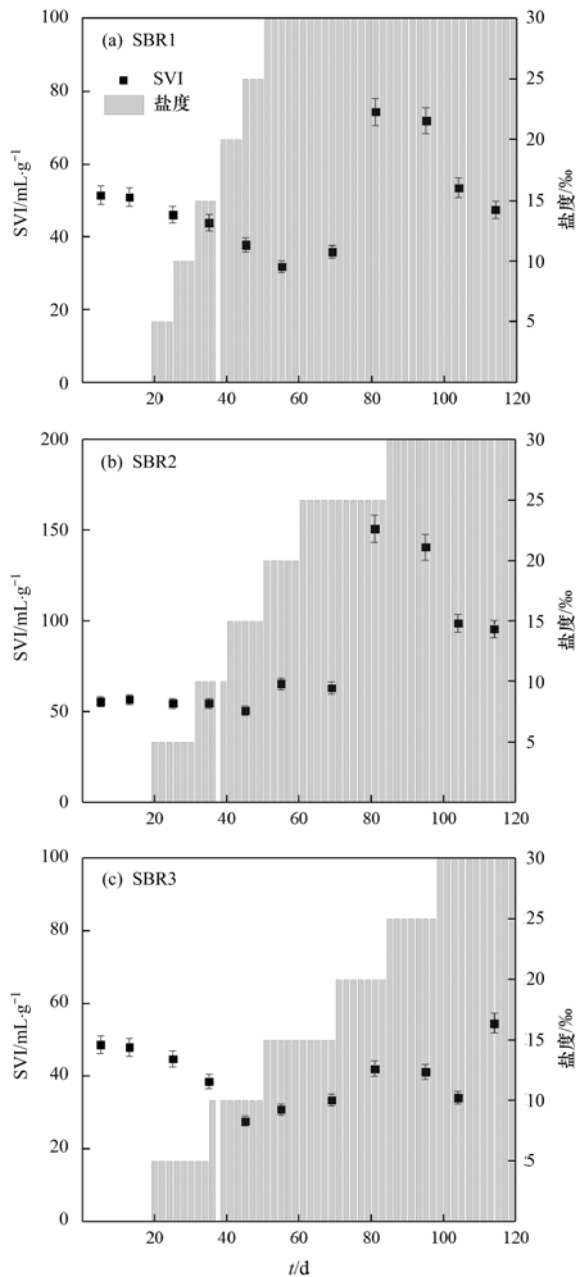


图6 不同提盐速率下 SVI 的变化

Fig. 6 Variation in SVI in different rates of salinity increase

TM7a 和 *Marinicella* 的相对丰度明显升高,快速提盐后占比为 3.91%、13.3% 和 14.5%,缓慢提盐后占比为 7.22%、4.12% 和 11.6%。推测高盐条件造成菌群间的竞争压力减小,有利于 *Denitromonas*、*TM7a* 和 *Marinicella* 的生长。属于红环菌科的 *Denitromonas* 是一种反硝化菌^[28],同时 *Marinicella* 可将 NO_3^- -N 还原为 N_2 ^[29],与该系统较强的反硝化性能相一致。提盐速率对 *Defluviimonas*、*Maribacter*、*Denitratisoma* 和 *Arenimonas* 影响较大。快速提盐至 30‰,占比接近于 0;缓慢提盐至 30‰,占比分别为 0.712%、0.629%、1.09% 和 2.13%。据报道,*Denitratisoma*、*Defluviimonas* 和 *Arenimonas* 均具有反硝化脱氮特性^[30~32]。上述表

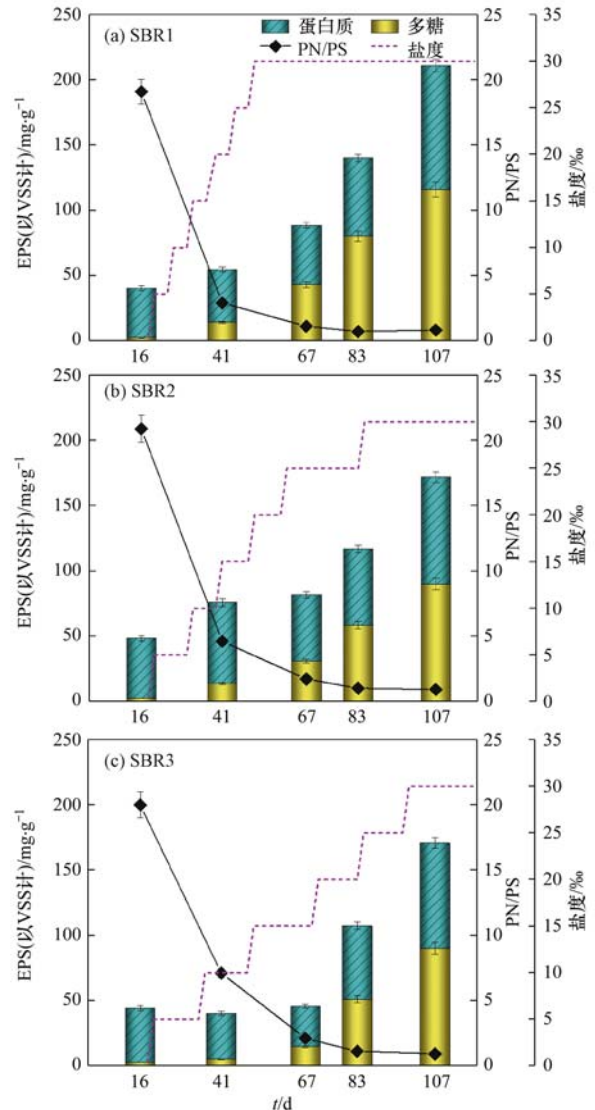


图7 不同提盐速率下 EPS 的变化

Fig. 7 Variation in EPS concentration in different rates of salinity increase

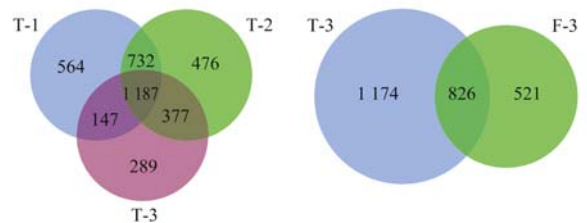


图8 OTUs 分布维恩图

Fig. 8 Venn diagrams showing the distribution of OTUs

明,随着盐度增加,污泥中反硝化菌群结构发生明显变化;对比缓慢提盐,快速提盐后反硝化菌的类别明显减少。

2.7 盐度对氨氧化菌和亚硝酸盐氧化菌的影响

为更深入了解盐度对硝化菌的影响,从属层面对氨氧化菌 (*Nitrosomonas*) 和亚硝酸盐氧化菌 (*Nitrotoga*) 进行分析(图 11)。随着盐度升高,SBR3 污泥样品 *Nitrosomonas* 相对丰度先降低后升高,维

持在0.3%~0.5%；然而，*Nitrotoga*随盐度升高明

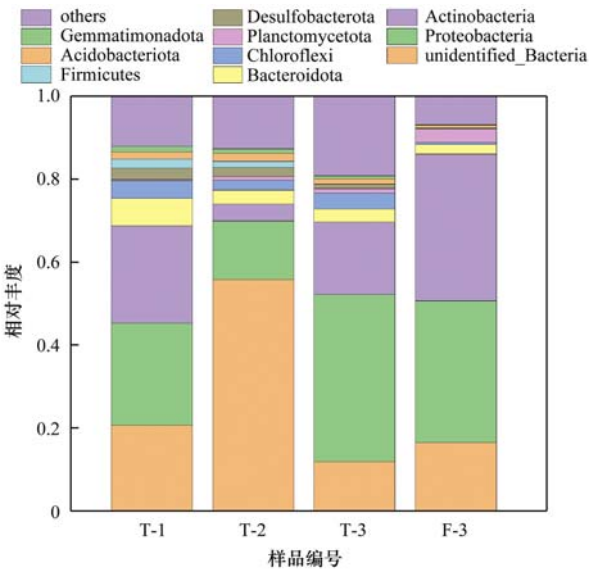
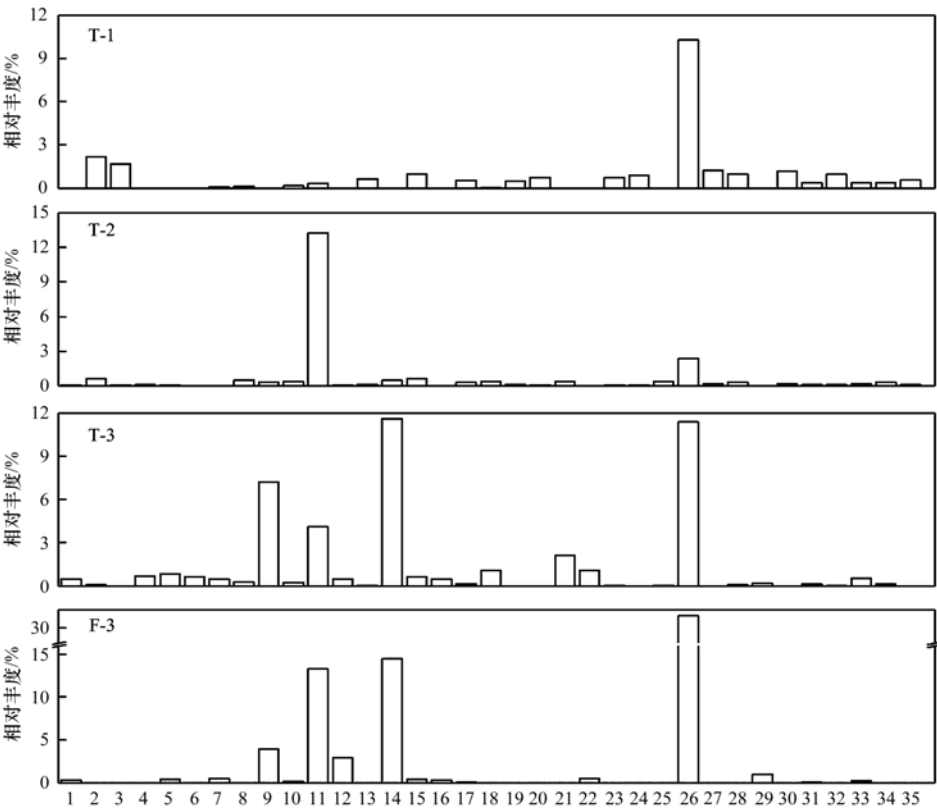


图9 门水平细菌群落结构及分布

Fig. 9 Bacterial community structure and distribution of samples at the phylum level

显减少,由最初0.948‰降至0.327‰(盐度15‰)及0.052‰(盐度30‰). SBR1污泥样品*Nitrotoga*相对丰度为0.086‰,而*Nitrosomonas*相较于SBR3明显减少.上述表明盐度为15‰,NOB还存在一定的活性,出水仍以 NO_3^- -N为主;盐度提升至30‰,NOB无法适应高盐环境,相对丰度急剧减少,造成 NO_2^- -N积累.推测当盐度提升至20‰,由于NOB减少,形成短程硝化.

综上所述,采用逐步提升盐度的方式来驯化活性污泥时,提盐速率对 NH_4^+ -N去除效果影响较小;对比缓慢提盐(90 d),污泥经快速提盐驯化(30 d)后,微生物菌群结构多样性明显降低,多种反硝化细菌的相对丰度明显降低,反硝化菌属比较单一.实际污水处理系统运行条件复杂,且一般需在缺氧段外加大量碳源实现深度脱氮,而快速提盐的驯化方式使得反硝化细菌相对丰度显著降低,进而降低碳源的利用率.在实际工程中,应尽量采用缓慢的提盐速率来驯化活性污泥.



1 表示 *Vitellibacter*, 2 表示 *Candidatus_Competibacter*, 3 表示 *Ferruginibacter*, 4 表示 *Defluviimonas*, 5 表示 *Levinella*, 6 表示 *Maribacter*, 7 表示 *Pseudomonas*, 8 表示 *Sva0081_sediment_group*, 9 表示 *Denitromonas*, 10 表示 *MND1*, 11 表示 *TM7a*, 12 表示 *SM1A02*, 13 表示 *Anaeromyxobacter*, 14 表示 *Marinicella*, 15 表示 *Defluviicoccus*, 16 表示 *Oceanicaulis*, 17 表示 *Pseudolabrys*, 18 表示 *Denitratisoma*, 19 表示 *Mariprofundus*, 20 表示 *Flaviumicrobium*, 21 表示 *Arenimonas*, 22 表示 *Alcanivorax*, 23 表示 *Bradyrhizobium*, 24 表示 *Terrimonas*, 25 表示 *IheB3-7*, 26 表示 *Micropruina*, 27 表示 *9M32*, 28 表示 *Ignavibacterium*, 29 表示 *Arenibacter*, 30 表示 *Oleigrimonas*, 31 表示 *Paracoccus*, 32 表示 *Propionicihla*, 33 表示 *Nitrosomonas*, 34 表示 *unidentified_MBNT15*, 35 表示 *Truepera*

图10 属水平细菌群落相对丰度柱状图

Fig. 10 Bacterial community relative abundance of samples at the genus level

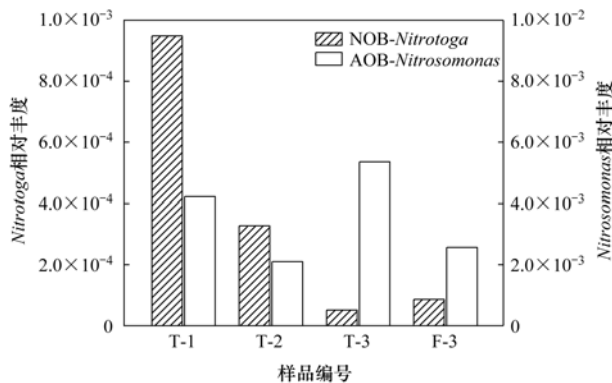


图 11 不同盐度下 *Nitrotoxa* 和 *Nitrosomonas* 相对丰度变化

Fig. 11 Variations in *Nitrotoxa* and *Nitrosomonas* abundance under different salinity levels

3 结论

(1) 快速提盐驯化污泥, COD 和氨氮的去除率明显下降; 缓慢提盐驯化污泥, 各盐度梯度下, COD 和氨氮的去除率均较高. 经过 120 d 驯化, 3 个反应器对污染物的去除效果没有明显差异.

(2) 盐度是实现短程硝化的决定因素, 随着盐度增加, *Nitrosomonas* (AOB) 所受影响较小, 而 *Nitrotoxa* (NOB) 明显减少. 由 SBR3 推测盐度 20‰ 是实现短程硝化的阈值.

(3) 随着驯化时间增加, 污泥沉降性能更好, 且 SVI 与盐度没有明显的相关性; EPS 中蛋白质受盐度影响较低, 当盐度升高至 15‰, 多糖明显增加.

(4) *Micropruina*、*Denitromonas*、*TM7a*、和 *Marinicella* 具有良好的耐盐特性, 盐度为 30‰, 相对丰度较高; 缓慢提盐有利于 *Denitratisona*、*Defluviimonas*、*Arenimonas* 和 *Denitromonas* 等反硝化菌属的生长.

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