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异养硝化-好氧反硝化混合菌对尿素的去除及重金属 和盐度的影响

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摘要:为探究异养硝化-好氧反硝化混合菌对尿素的去除效果,本文考察了混合菌 (DM01 + YH01 + YH02)对尿素的去除特性,以及重金属和盐度对其去除率的影响. 结果表明,当废水中尿素质量浓度为 200.0 $\operatorname{mg·L^{-1}}$,碳源为柠檬酸三钠、C/N 为 10、温度为 30℃、pH 为 7 和转速为 130 $\operatorname{r·min^{-1}}$ 时,尿素在 24 h 可以得到高效降解,去除率为 91.8%;重金属离子 (Ni²+、Cd²+、Cu²+和 Zn²+)会降低混合菌对尿素的去除效果,影响能力的大小顺序为 Cd²+ > Cu²+ > Ni²+ > Zn²+,然而,Fe²+ (< 20.0 $\operatorname{mg·L^{-1}}$)会增强混合菌对尿素的去除效果;盐度 (> 10.0 $\operatorname{mg·L^{-1}}$)会抑制混合菌株对于尿素的去除.

关键词: 异养硝化-好氧反硝化; 混合菌; 尿素; 重金属离子; 盐度

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Removal of Urea by Heterotrophic Nitrification-Aerobic Denitrification Mixed Strains and Effects of Heavy Metals and Salinity

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Abstract: To explore the effect of heterotrophic nitrification-aerobic denitrification mixed strains on urea removal, the removal characteristics of urea and effects of heavy metals and salimity on urea removal by mixed strains (DM01 + YH01 + YH02) were investigated. The results showed that urea could be efficiently degraded by mixed strains at 24 h in 200.0 mg·L⁻¹ urea wastewater when the carbon source was sodium citrate, C/N was 10, temperature was 30°C, pH was 7, and rotation speed was 130 r·min⁻¹. Heavy metal ions (Ni²⁺, Cd²⁺, Cu²⁺, and Zn²⁺) can reduce the removal efficiency of urea by mixed strains, and the degree of influence was Cd²⁺ > Cu²⁺ > Ni²⁺ > Zn²⁺. Fe²⁺ (<20.0 mg·L⁻¹) in urea wastewater can enhance the removal efficiency of urea by mixed strains. Furthermore, a salinity of more than 10.0 mg·L⁻¹ in urea wastewater can reduce the removal efficiency of urea by mixed strains.

Key words: heterotrophic nitrification-aerobic denitrification; mixed bacteria strains; urea; heavy metal ions; salinity

尿素[CO(NH₂)₂]进入水体环境中会快速分解为氨^[1]. 过多尿素进入水体环境后,会增加水体氮素含量,从而对环境造成危害. 环境中尿素主要来源于工业生产废水、农田渗滤液以及养殖场废水. 尿素在生产和使用过程中会不同程度地伴随生产废水和农田渗滤液进入环境^[2]. 养殖场废水也含有大量尿素,废水中的高棕褐色主要来源于动物尿液中的尿素^[3].

目前,对于尿素废水的处理方法主要有:热力学水解法、化学氧化法、脲酶水解法和生物降解法^[2~4].热力学水解法和化学氧化法适用于高质量浓度尿素废水处理,但能耗大^[2].脲酶水解法中如何在常温条件下保持酶的稳定性与活性是一个比较大的挑战^[5].生物降解法是在微生物作用下通过硝化和反硝化作用实现尿素的去除^[6].生物降解法因其经济有效,是现有废水处理技术中的最常用的方法.传统的生物降解包括了好氧硝化与厌氧反硝化两个过程,脱氮途径较长,容易受环境因素的影

响. 异养硝化-好氧反硝化菌的发现, 打破了传统的生物脱氮理论, 因其在好氧条件下同时实现硝化和反硝化过程, 在生物脱氮过程中成为研究热点^[6~8]. 然而, 目前异养硝化-好氧反硝化菌降解尿素的相关研究较少.

因此,本文重点研究异养硝化-好氧反硝化菌株对尿素的去除特性.在实验室前期的研究中发现混合菌株 YH01 + YH02 的脱氮性能优于单菌株YH01 和 YH02^[9]. 所以本文利用实验室筛选出的新型异养硝化-好氧反硝化菌 DM01 和 YH01 + YH02 构成新的混合菌株 DM01 + YH01 + YH02 来去除废水中的尿素,并考察重金属离子(Ni²⁺、

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Cd²⁺、Cu²⁺、Zn²⁺和 Fe²⁺)和盐度对混合菌株脱氮效果的影响,以期为异养硝化-好氧反硝化菌在废水生物脱氮的实际运用提供参考依据.

1 材料与方法

1.1 实验材料

1.1.1 实验菌株与尿素废水

YH01 和 YH02 为实验室前期筛选出的异养硝化-好氧反硝化菌^[9], 异养硝化-好氧反硝化菌 DM01 是由取自广州大坦沙污水处理厂 A₂O 工艺池的活性污泥富集筛选得到. 将异养硝化-好氧反硝化菌 YH01、YH02 和 DM01 分别使用 LB 培养基活化增殖后, 离心收集菌体, 再用无菌水重悬菌体, 按照 1:1:1的比例配制混合菌悬液, 通过平板菌落计数法确定菌液活菌数为 3.2×10⁷ CFU·L⁻¹.

尿素废水: 尿素 200.0 mg·L⁻¹, Na₃C₆H₅O₇·5H₂O 6.2 mg·L⁻¹, 维氏盐 50 mL·L⁻¹, pH 7.

1.1.2 培养基

异养硝化培养基: (NH₄)₂SO₄ 0.6 g·L⁻¹, Na₃C₆H₅O₇·5H₂O 4.3 g·L⁻¹, 维氏盐溶液 50 mL·L⁻¹, pH 7.

维氏盐溶液: K_2HPO_4 5.0 $g \cdot L^{-1}$, NaCl 2.5 $g \cdot L^{-1}$, MgSO₄·7H₂O 2.5 $g \cdot L^{-1}$, FeSO₄·7H₂O 0.05 $g \cdot L^{-1}$, MnSO₄·4H₂O 0.05 $g \cdot L^{-1}$.

1.2 实验方法

1.2.1 菌株 DM01 鉴定

利用细菌通用引物 27F 和 1492R 对菌株 DM01 的 DNA 进行提取与 PCR 扩增,对扩增后的产物进行测序,测序由上海生工生物工程有限公司完成.

1.2.2 混合菌株脱氮实验

将菌株 DM01、混合菌株 YH01 + YH02 和混合菌株 DM01 + YH01 + YH02,以相同的接种量接种于含有相同质量浓度的硝化培养基中,相同条件下培养 48 h,取培养液上清液分析氨氮(NH₄⁺-N)和总氮(TN).

1.2.3 环境因子对尿素去除影响实验

以相同质量浓度的尿素为氮源,控制单一因素,分别在不同碳源(乙酸钠、蔗糖、葡萄糖、丁二酸钠和柠檬酸三钠)、C/N (2、4、6、8、10 和 12)、pH (5、6、7、8、9 和 10)、转速(90、110、130、150 和 180 $\mathbf{r}\cdot\mathbf{min}^{-1}$)、温度(15、20、25、30 和 35°C)和接种量(0%、0.5%、1%、2.5%、5% 和 10%)条件下培养,将混合菌液接入尿素废水中,同时通过平板菌落计数法测定混合菌液活菌数,之后每 12 h 取 1 \mathbf{m} L培养液上清液分析尿素质量浓度.

确定环境因子对尿素去除影响之后,选择合适

的环境因子将混合菌液接入尿素废水中,同时通过平板菌落计数法测定混合菌液活菌数,之后每 12 h取 1 mL 培养液上清液分析尿素、TN、 NH_4^+ -N、硝酸盐氮(NO_3^- -N)、亚硝酸盐氮(NO_2^- -N)、COD 质量浓度和 D_{600} .

1.2.4 重金属离子对尿素去除影响实验

尿素培养基中设置不同质量浓度梯度的金属离子: Ni^{2+} (0、2.0、5.0 和 10.0 mg·L⁻¹)、 Cd^{2+} (0、0.5、1.5、2.0 和 2.5 mg·L⁻¹)、 Cu^{2+} (0、0.5、2.5 和 5.0 mg·L⁻¹)、 Zn^{2+} (0、10.0、25.0 和 50.0 mg·L⁻¹) 和 Fe^{2+} (0、5.0、10.0、20.0 和 50.0 mg·L⁻¹),控制环境因子保持一致,将混合菌液接入尿素废水中,同时通过平板菌落计数法测定混合菌液活菌数,之后每 12 h 取 1 mL 培养液上清液分析尿素、TN 质量浓度和 D_{600} .

1.2.5 盐度对尿素去除影响实验

盐度以尿素废水中 NaCl 质量浓度来表示。尿素培养基中设置不同质量浓度盐度 (0, 10.0, 20.0, 30.0 和 40.0 mg·L⁻¹),控制环境因子保持一致,将混合菌液接入尿素废水中,同时通过平板菌落计数法测定混合菌液活菌数,之后每 12 h 取 1 mL 培养液上清液分析尿素、TN 质量浓度和 D_{600} .

1.3 检测项目及方法

尿素:二乙酰一肟改良法^[10]; TN:碱式过硫酸钾消解紫外分光光度法^[11]; NH₄+-N:纳氏试剂分光光度法^[11]; NO₃-N: 酚二磺酸分光光度法^[11]; NO₂-N: N-(1-萘基)-乙二胺分光光度法^[11]; COD质量浓度:重铬酸钾快速消解法^[11]; D₆₀₀:测定样品在 600 nm 波长时的吸光度^[11].

2 结果与讨论

2.1 菌株 DM01 的鉴定结果

菌株 DM01 测序结果提交到 NCBI, GenBank 的 登录号为 JQ951521, 并通过 MEGA6. 06 构建系统 进化发育树,如图 1 所示. 经 Blast 检索后初步确定 DM01 为假单胞属 (*Pseudomonas*),属于变形菌门 (Proteobacteria).

2.2 混合菌株 DM01 + YH01 + YH02 的脱氮效果

图 2 显示了混合菌株 DM01 + YH01 + YH02 在 48 h 对NH₄⁺-N和 TN 的去除效果. 混合菌株DM01 + YH01 + YH02 对 NH₄⁺-N和 TN 去除效率分别为 98.7%和 90.1%, 脱氮效率明显高于 YH01 + YH02和 DM01. 赵思琪等^[12]的研究发现由不同种属的异养硝化细菌构成的混合菌 YB + YH + YL 具有更优的异养氨氧化和好氧反硝化能力. 黄郑郑等^[13]的

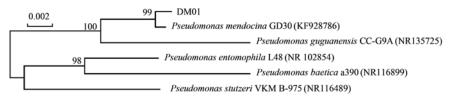


图 1 基于 16S rDNA 基因序列同源性构建 DM01 系统发育树

Fig. 1 Phylogenetic tree of strain DM01 based on the complete sequence of the 16S rDNA gene

研究同样也发现了异养硝化-好氧反硝化混合菌 XH02 + XH03 在异养硝化和好氧反硝化过程中均能 协同脱氮,提高了脱氮效率.这表明一些不同种属 的异养硝化-好氧反硝化细菌存在协助作用,使得混合菌株的脱氮能力以及对环境的耐受性均优于单菌株,在生物脱氮过程中发挥着重要的作用.

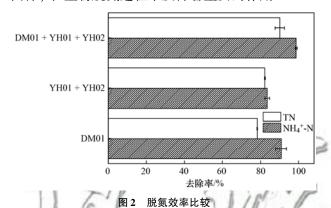


Fig. 2 Comparison of denitrification efficiency

2.3 环境因子对混合菌 DM01 + YH01 + YH02 去 除尿素的影响

2.3.1 碳源

图 3(a)显示了其他单因素(C/N、pH、转速、温 度和接种量)条件保持一致时,不同碳源(乙酸钠、 蔗糖、葡萄糖、丁二酸钠和柠檬酸三钠)条件下混合 菌对尿素的去除情况. 乙酸钠、蔗糖、葡萄糖、丁二 酸钠和柠檬酸三钠在尿素废水中质量浓度分别为: 3.2、2.2、2.6、3.1 和 3.8 g·L⁻¹,添加不同碳源后 尿素废水的 COD 质量浓度分别为1060.0、 1 053. 0、1 054. 0、1 053. 0和1 058. 0 mg·L⁻¹. 混合 菌液经平板菌落计数确定活菌数约为 2.1 × 107 CFU·L-1. 从图 3(a)中可以看出, 蔗糖和葡萄糖作 为碳源时, 尿素几乎没有被去除. 乙酸钠、柠檬酸 三钠和丁二酸钠作为碳源时, 尿素去除率较高. 其 中柠檬酸三钠作为碳源时, 尿素的去除率最高. Silva 等[14]的研究发现, 异养硝化-好氧反硝化菌株 UFV3 以丙酮酸钠、乙酸钠、柠檬酸三钠和琥珀酸钠 作为碳源时对NH,+-N的去除率大于90.0%, 异养硝 化-好氧反硝化菌株 UFV4 仅使用柠檬酸三钠为碳 源时去除率最高. 而葡萄糖和蔗糖作为碳源时,两 株菌都具有较低的生长速度和NH₄-N去除率. Yang 等^[15]的研究发现异养硝化-好氧反硝化菌株 NP5, 在琥珀酸盐作为碳源时NH₄⁺-N去除率最高,对葡萄糖和蔗糖利用率最低.显然不同的异养硝化-好氧 反硝化菌株对碳源的选择是有区别的,但是普遍不能利用蔗糖和葡萄糖等糖类.

2.3.2 C/N

图 3(b)显示了以柠檬酸三钠为碳源,其他单 因素(pH、转速、温度和接种量)条件保持一致时, 在不同 C/N(2、4、6、8、10 和 12)条件下尿素的去 除效果. 混合菌液经平板菌落计数确定活菌数约为 2.2×10⁷ CFU·L⁻¹. 由图 3(b) 可知, C/N 为 8~12 时, 尿素去除率比较接近, 48 h 时的去除率均在 90.0% 左右. 其中当 C/N 为 12 时, 尿素的去除效 率最高. C/N < 4 时, 尿素 48 h 去除率低于 75.7%. Wan 等[16]的研究中也发现异养硝化-好氧反硝化菌 HW-15 在 C/N 为 8 时对NH₄+-N的去除效果最好, C/N < 8 时, 菌株 HW-15 对NH₄+-N的去除率随 C/N 的增大而升高, 然而 C/N 继续增大到 10, NH4+N 的去除率开始下降. 这说明 C/N 过低或过高时, 都 会影响氮的去除. C/N 过低时, 碳源不足, 不能为 菌株生长提供足够的营养, 从而影响氮的去除. C/N过高时, 部分碳源有机物可能会整合入微生物 的酶结构中,从而影响酶活性,进而影响氮的去 除^[17].

2.3.3 pH

图 3(c)显示了以柠檬酸三钠为碳源,其他单因素(C/N、转速、温度和接种量)条件保持一致时,在不同 pH(5、6、7、8、9 和10)的条件下尿素的去除效果.混合菌液经平板菌落计数确定活菌数约为2.8×10⁷CFU·L⁻¹.从图 3(c)中可以看出,pH 为 5 和 10 时,混合菌株对尿素去除效果较差,去除率仅有 40.0%.pH 为 6~9 时,混合菌对尿素的去除效率比较接近,均高于 90.0%,说明混合菌在中性、弱酸性和弱碱性条件下均有较强的尿素去除能力.

2.3.4 转速

摇床的转速直接影响到培养基中溶解氧的含量,提高转速可以加快氧的传递^[18].图 3(d)显示了以柠檬酸三钠为碳源,其他单因素(C/N、pH、温度和接种量)条件保持一致时,在不同转速(90、

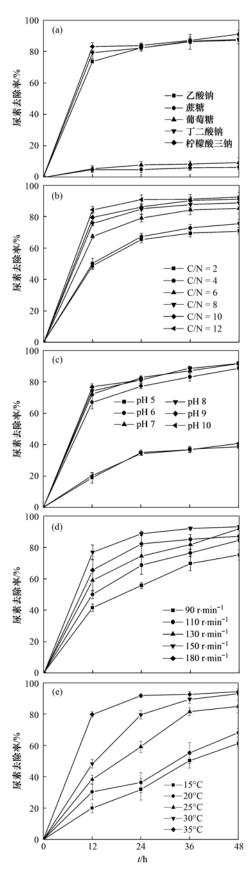


图 3 环境因子(碳源、C/N、pH、转速和温度) 对尿素去除率的影响

Fig. 3 Effects of different environmental factors on urea removal

110、130、150 和 180 r·min⁻¹)的条件下尿素的去

除效果. 混合菌液经平板菌落计数确定活菌数约为 2.6×10^7 CFU·L⁻¹. 从图 3(d) 中可以看出,转速在 $90 \sim 180$ r·min⁻¹时,尿素的去除率先升高后降低. 当转速为 150 r·min⁻¹时,混合菌对于尿素的去除效率最高.

2.3.5 温度

图 3(e)显示了以柠檬酸三钠为碳源,其他单因素(C/N、pH、转速和接种量)条件保持一致时,在不同温度(15、20、25、30 和 35 °C)的条件下混合菌对尿素的去除效果. 混合菌液经平板菌落计数确定活菌数约为 2.2×10^7 CFU·L⁻¹. 温度可以直接影响到生物的酶活性,适宜的温度可以促进混合菌对尿素的去除. 从图 5(e)中可以看出,温度在 $15 \sim 35$ °C 时,尿素的去除效率随着温度的升高而升高. 当温度为 35 °C 时,混合菌对于尿素的去除率最高. 这表明混合菌株在 35 °C 左右对尿素有较好的去除效果.

2.3.6 混合菌 DM01 + YH01 + YH02 接种量对尿素去除的影响

图 4 显示了以柠檬酸三钠为碳源,其他单因素 (C/N、pH、温度和转速)条件保持一致时,不同接种量(0%、0.5%、1%、2.5%、5%和10%)对尿素、TN和 D_{600} 的影响.混合菌液经平板菌落计数确定活菌数约为 2.8 × 10^7 CFU·L⁻¹. 从图 4 可以看出,当接种量为 0.5%~2.5%时,在 12~24 h时,混合菌对尿素和 TN的去除率以及 D_{600} 比较低;在 48 h,混合菌对尿素的去除率可达到 90.0%以上.菌液接种量为 5%~10%,在 12 h时,混合菌对尿素和 TN的去除率以及 D_{600} 即能达到最大值,混合菌对尿素的去除率可达到 90.0%以上.

王田野等[19]的研究发现, 异养硝化-好氧反硝 化菌 SQ2 接种量从 1% 提高到 10% 时, NH₄ -N去除 率显著提高,这一结论是菌株培养 12 h 得到的, 研 究者未在更长时间内监测NH4+N去除率. 黄海洪 等[20]的研究发现在提高蛋白胨培养基中芽孢杆菌 的接种量以后,NH₄+N第1d的去除速率显著提 高. 这与本文研究结果是类似的, 提高异养硝化-好氧反硝化混合菌的接种量, 12~24 h 尿素的去 除率随着接种量的增大而显著提高,但继续培养 到 48 h, 不同接种量的尿素去除率无明显差异. 这表明,混合菌接种量会影响混合菌完全去除尿 素的时间, 但对最终尿素去除率无显著影响. 这 可能是因为混合菌接种量不同时, 短时间内菌株 的菌密度会有较大的差异, 但是经过较长时间的 培养,由于有限的底物质量浓度,混合菌的最终 菌密度差异不大.

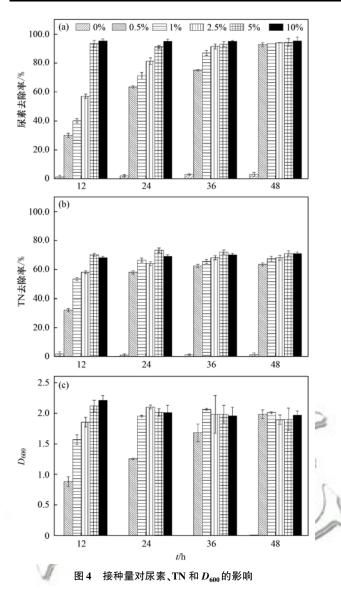


Fig. 4 Effects of inoculated concentration on urea, TN, and D_{600}

2.4 混合菌株 DM01 + YH01 + YH02 对尿素的去除效果

图 5 显示了以柠檬酸三钠为碳源、C/N 为 10、pH 为 7、转速为 130 $\mathbf{r} \cdot \mathbf{min}^{-1}$ 、温度为 30℃和接种量为 1% 时,尿素、TN、NH₄⁺-N、NO₃⁻-N、NO₂⁻-N、COD质量浓度和 D_{600} 的变化. 混合菌液经平板菌落计数确定活菌数约为 3.0 × 10⁷ CFU · L⁻¹. 如图 5 所示,200.0 mg·L⁻¹尿素在 24 h 即可得到高效降解,尿素和 TN 的去除率为 91.8% 和 65.5%. D_{600} 值在 24 h 即达到较大值,说明混合菌在 200.0 mg·L⁻¹ 尿素废水中生长繁殖能力较强。NH₄⁺-N在 24 h 达到峰值质量浓度 22.2 mg·L⁻¹,NO₃⁻-N在 36 h 达到峰值质量浓度 为 24.5 mg·L⁻¹,在 48 h 时 NH₄⁺-N和 NO₃⁻-N的质量浓度分别降低到 4.0 mg·L⁻¹和 3.3 mg·L⁻¹. 而且在尿素去除的整个过程中几乎无NO₇⁻-N积累.

黄海洪等[20]运用1株芽孢杆菌处理尿素废水

时,发现菌株首先通过氨化作用利用尿素,从而避免了NH₄+N的大量积累.这可能是混合菌处理尿素过程NH₄+N峰值较低的原因. Choi 等^[21]在超纯水生产系统中,用紫外线照射过硫酸盐去除尿素,此方法尿素去除率虽然可以达到 90.0%,但是在出水中可以检测到硝酸盐和硫酸盐,这显然是对环境有害的,而且此方法需要额外添加过硫酸盐,较不经济,仅适合小规模处理尿素废水. 本研究中异养硝化-好氧反硝化混合菌 DM01 + YH01 + YH02,不仅可以高效去除废水中尿素,且几乎无污染副产物产生,在实际大规模尿素废水处理中能够有较好地应用.

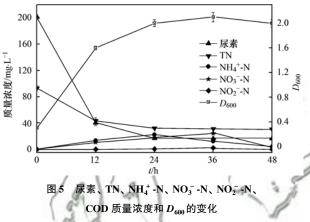


Fig. 5 Changes in urea, TN, NH $_4^+$ -N, NO $_3^-$ -N, NO $_2^-$ -N, COD concentration, and D_{600}

2.5 重金属离子对混合菌 DM01 + YH01 + YH02 去除尿素的影响

图 6 和图 7 显示了以柠檬酸三钠为碳源、C/N 为 10、pH 为 7、转速为 130 r·min $^{-1}$ 、温度为 30 $^{\circ}$ C 和接种量为 1% 时,Ni $^{2+}$ (0、2.0、5.0 和 10.0 mg·L $^{-1}$)、Cd $^{2+}$ (0、0.5、1.5、2.0 和 2.5 mg·L $^{-1}$)、Cu $^{2+}$ (0、0.5、2.5 和 5.0 mg·L $^{-1}$)、Zn $^{2+}$ (0、10.0、25.0 和 50.0 mg·L $^{-1}$) 和 Fe $^{2+}$ (0、5.0、10.0、20.0 和 50.0 mg·L $^{-1}$) 对尿素、TN 和 D_{600} 的影响.混合菌液经平板菌落计数确定活菌数约为 2.8 × 10^7 CFU·L $^{-1}$.

2.5.1 Ni²⁺ 对尿素去除的影响

由图 6 和图 7 可知,当 Ni^{2+} 质量浓度在 0 ~ 10.0 $mg \cdot L^{-1}$ 时,混合菌对于尿素和 TN 的去除效率以及 D_{600} 随离子质量浓度的升高而降低.当 Ni^{2+} 质量浓度为 10.0 $mg \cdot L^{-1}$ 时,与不含 Ni^{2+} 相比,混合菌对尿素和 TN 的去除效率减少了 24.3% 和 19.5%, D_{600} 减少了 1.0 左右.这说明 Ni^{2+} 会通过抑制菌株的生长来影响混合菌对尿素的去除效果. He 等[122]的研究显示 8.0 $mg \cdot L^{-1}$ Ni^{2+} 会造成异养硝

化-好氧反硝化菌 Y-10 对NH₄⁺-N的去除完全停止. Zhang 等^[23]的研究显示 Ni²⁺ 质量浓度在 20.0 ~ 80.0 mg·L⁻¹时,Ni²⁺ 对异养硝化-好氧反硝化菌 ZN1 的抑制作用随 Ni²⁺ 质量浓度增加而增强. 随着 Ni²⁺ 质量浓度从 20.0 mg·L⁻¹增加到 80.0 mg·L⁻¹,NH₄⁺-N去除率从 71.3% 下降到 31.5%. Yang 等^[24]的研究显示,1.0 mg·L⁻¹ 的 Ni²⁺使异养硝化-好氧反硝化菌株 JR1 的NH₄⁺-N去除率轻微降低,而 5.0 mg·L⁻¹ Ni²⁺ 会造成去除率显著降低,10.0 mg·L⁻¹ Ni²⁺则会造成NH₄⁺-N去除过程完全停止. 显然 Ni²⁺会抑制异养硝化-好氧反硝化菌的异养硝化效率,但不同菌株对 Ni²⁺的耐受范围是不同的.

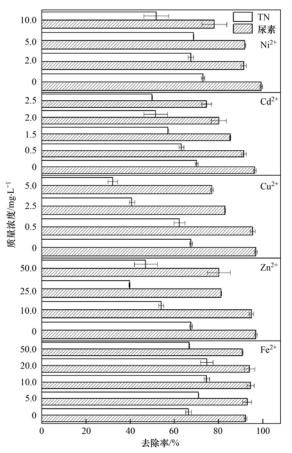


图 6 重金属离子对尿素和 TN 的影响

Fig. 6 Effect of heavy metals on urea and TN

2.5.2 Cd^{2+} 对尿素去除的影响

由图 6 和图 7 可知,当 Cd^{2+} 质量浓度在 0 ~ 2.5 mg·L⁻¹时,混合菌对于尿素和 TN 的去除效率以及 D_{600} 随离子质量浓度的升高而降低。当 Cd^{2+} 质量浓度升高至 2.5 mg·L⁻¹时,与不含 Cd^{2+} 相比,混合菌对于尿素和 TN 的去除率减少了 21.0%和 19.5%, D_{600} 减少了 1.1. 混合菌 D_{600} 的显著下降显示了 Cd^{2+} 会抑制混合菌的生长,从而影响了混合菌对尿素的去除效果。潘民强等[25]的

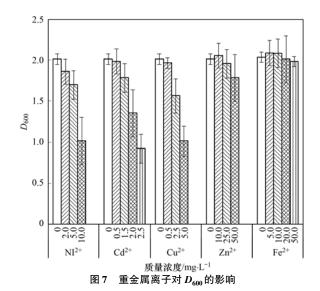


Fig. 7 Effects of heavy metals on D_{600}

研究发现, $0.1 \sim 0.5 \text{ mg} \cdot \text{L}^{-1}$ 的 Cd^{2+} 对生物脱氮除磷影响不明显, $2.0 \text{ mg} \cdot \text{L}^{-1}$ 的 Cd^{2+} 对生物脱氮除磷影响比较明显, 生物反应器脱氮除磷效率显著低于空白组. 马永鹏等 $^{[26]}$ 的研究发现在膜生物反应器中加入 $1.0 \text{ mg} \cdot \text{L}^{-1}$ Cd^{2+} 后, 好氧氨氧化菌的活性受到明显抑制, NH_4^+ -N和 TN 去除率明显下降.

2.5.3 Cu2+对尿素去除的影响

由图 6 和 7 可知,尿素废水中 Cu^{2+} 质量浓度在 $0 \sim 0.5 \text{ mg·L}^{-1}$ 时, Cu^{2+} 对混合菌去除尿素几乎无影响. 随 Cu^{2+} 质量浓度的增加,混合菌对尿素的去除效率降低. Cu^{2+} 质量浓度为 5.0 mg·L^{-1} 时,与不含 Cu^{2+} 相比,混合菌对尿素和 TN 的去除效率分别减少了 19.9% 和 35.3%, D_{600} 减少了 1.0. Lu 等 [27] 的研究发现 0.6 mg·L^{-1} Cu^{2+} 对异养硝化-好氧反硝化菌株 YF1 的好氧反硝化效率几乎无影响,而 Cu^{2+} 质量浓度升高至 3.2 mg·L^{-1} 和 6.4 mg·L^{-1} 时,副球菌 YF1 的好氧反硝化效率,与不含 Cu^{2+} 时相比,降低了 31.1% 和 83.5%. Yang 等 [24] 的研究发现, 1.0 mg·L^{-1} Cu^{2+} 对异养硝化-好氧反硝化菌株 JR1 的 NH_4^+ -N去除率无显著影响,而 5.0 mg·L^{-1} Cu^{2+} 会造成去除率低于 10.0%.

2.5.4 Zn²⁺对尿素去除的影响

由图 6 可知,尿素废水中 Zn^{2+} 质量浓度为 10.0、25.0 和 50.0 mg·L⁻¹时,与 Zn^{2+} 质量浓度为 0 时相比,混合菌对尿素的去除率分别减少了 2.0%、 15.6% 和 16.7%,混合菌对 TN 的去除率分别减少了 13.4%、27.9% 和 20.4%. 值得注意的是,由图 7 可知, Zn^{2+} 质量浓度为 10.0 mg·L⁻¹ 和 25.0 mg·L⁻¹时, D_{600} 无显著变化,说明一定量的 Zn^{2+} 不会影响微生物生长,但可能会影响菌株的脱氮酶活

性,进而影响到尿素的去除. Wang 等^[28]的研究显示 32.5 mg·L⁻¹的 Zn²⁺,好氧硝化细菌 WY-01 对 NH₄⁺-N的去除率会有显著地下降. Sun 等^[29]的研究发现 10.0 mg·L⁻¹的 Zn²⁺ 对异养硝化-好氧反硝化菌株 S1 的硝化效率几乎无影响,即使 Zn²⁺质量浓度达到 20.0 mg·L⁻¹时,菌株 S1 的硝化效率仍有 97.4%.

2.5.5 Fe²⁺对尿素去除的影响

由图 6 可知,当尿素废水中 Fe²⁺质量浓度在 0 ~50.0 mg·L⁻¹范围内时,混合菌对于尿素去除率都可以达到 90.0%以上. 当尿素废水中 Fe²⁺质量浓度在 0 ~20.0 mg·L⁻¹时,混合菌对于尿素去除率随 Fe²⁺质量浓度升高而升高. 当 Fe²⁺质量浓度为20.0 mg·L⁻¹时,与不含 Fe²⁺对比,尿素和 TN 去除率分别提高了 2.3%和 8.6%. 当 Fe²⁺质量浓度继续升高时,尿素和 TN 去除率稍有下降. 由图 7 可知,实验结果显示了 < 20.0 mg·L⁻¹的 Fe²⁺离子可以促进混合菌的生长,从而提高对尿素的去除率. Rajta等^[30]的研究也提出,在 28.0 mg·L⁻¹ Fe²⁺存在下,异养好氧硝化细菌地衣芽孢杆菌增加了NH₄⁺-N和 NO₃⁻-N的利用. Zhao等^[31]的研究发现10.0 mg·L⁻¹ Fe²⁺轻微提高了粪产碱杆菌 NR 在异养条件下对NH₄⁺-N的去除率.

2.6 盐度对混合菌去除尿素的影响

图 8 显示了以柠檬酸三钠为碳源、C/N 为 10 pH 为 7、转速为 130 r·min⁻¹、温度为 30℃ 和接种 量为1%时,盐度(0、10.0、20.0、30.0和40.0 $mg \cdot L^{-1}$) 对尿素、TN 和 D_{600} 的影响. 混合菌液经平 板菌落计数确定活菌数约为 2.7 × 107 CFU·L-1. 从图 8 可知, 尿素废水中盐度小于 10.0 mg·L⁻¹ 时,对混合菌降解尿素、TN和菌株的生长均无显 著影响, 尿素和 TN 的去除率分别为 94.2% 和 68.0%, D₆₀₀为 2.0. 当废水中盐度继续升高, 混 合菌对尿素和 TN 的去除率均会下降,同时菌株 的生长明显受到抑制. 尿素废水中盐度为 40.0 mg·L-1时,混合菌对尿素和TN的去除率仅为 67.0% 和 39.0%, 与盐度为 0 时相比, 下降了 24.5%和30.1%.可见过高的盐度会抑制混合菌 的生长,从而影响对尿素的去除. Chen 等[32]的研 究中显示异养硝化-好氧反硝化菌株 HN-02 在 0~ 10.0 g·L-1盐度下具有较高的NH₄+-N去除率. 这 一结论是在NH₄-N初始质量浓度为 25.0 mg·L⁻¹ 时得到的. 本研究中尿素初始质量浓度为 200.0 mg·L-1, 异养硝化-好氧反硝化混合菌对尿素的去 除在 > 10.0 mg·L⁻¹盐度环境下受到抑制可能与 初始氮质量浓度高有关.

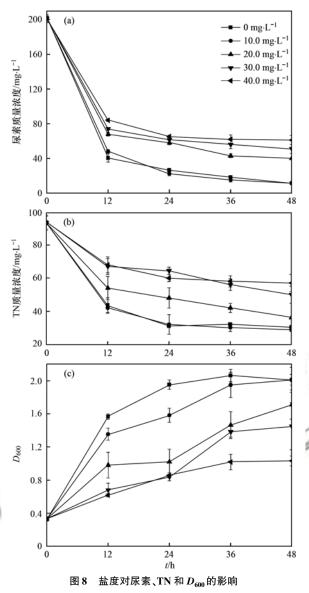


Fig. 8 Effects of salinity on urea, TN, and D_{600}

3 结论

- (1) 异养硝化-好氧反硝化混合菌 DM01 + YH01 + YH02 可以高效去除高质量浓度尿素废水中的尿素. 在 200.0 mg·L⁻¹的尿素废水中, 当碳源为柠檬酸三钠、C/N 为 10、pH 为 7、转速为 130 r·min⁻¹和温度为 30℃的条件下, 尿素在 24 h 可以得到高效降解,混合菌对尿素和 TN 的去除率分别为 91.8% 和 67.4%.
- (2) Ni^{2+} 、 Cd^{2+} 、 Cu^{2+} 和 Zn^{2+} 均会抑制混合菌 DM01 + YH01 + YH02 对废水中尿素和 TN 的去除,影响能力的大小顺序为 $Cd^{2+} > Cu^{2+} > Ni^{2+} > Zn^{2+}$. 尿素废水中 < 20. 0 $mg \cdot L^{-1}$ 的 Fe^{2+} 可促进混合菌对于尿素和 TN 的去除.
- (3) 异养硝化-好氧反硝化混合菌 DM01 + YH01 + YH02 在高质量浓度尿素废水中耐盐性较差. 盐度 $> 10.0 \text{ mg} \cdot \text{L}^{-1}$ 时,混合菌对尿素的去除会

明显受到抑制.

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