

# 环境科学

(HUANJING KEXUE)

ENVIRONMENTAL SCIENCE

第39卷 第2期

Vol.39 No.2

**2018**

中国科学院生态环境研究中心 主办  
科学出版社 出版



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# 内电解人工湿地冬季低温尾水强化脱氮机制

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**摘要:** 针对湿地冬季运行效率低、污染物去除能力差, 本研究通过对比无植物湿地、普通湿地和内电解湿地冬季低水温下(3~12℃)对污水厂尾水的脱氮效能, 深入分析其微生物群落结构组成, 揭示内电解湿地的强化脱氮机制。结果表明, 内电解湿地可以更好地利用尾水中碳源, 脱氮效果优势明显, 出水 TN 浓度维持在 $(9 \pm 0.29) \text{ mg} \cdot \text{L}^{-1}$ , TN 平均去除率达 42.27%, 比无植物湿地和普通湿地分别高出 17.91%、17.33%。冬季低温条件下, 内电解湿地微生物活性仍很高, 荧光显色法测得微生物活性达到  $0.224 \text{ mg} \cdot \text{g}^{-1}$ , 分别是无植物湿地和普通湿地的 2.6、3.4 倍, 反硝化强度分别是无植物湿地和普通湿地的 2.8、3.3 倍。高通量测序表明, 内电解湿地基质微生物群落多样性优于无植物湿地和普通湿地。检测出的脱氮微生物主要有脱氮单胞菌、根瘤菌、生丝微菌、红杆菌, 还有自养反硝化细菌产硫酸杆菌。内电解湿地在脱氮微生物总量上有明显优势, 脱氮微生物占微生物总量的 7.13%, 分别是无植物湿地、普通湿地的 3.8、8.7 倍, 因而脱氮效率更高。

**关键词:** 冬季低温; 污水厂尾水; 人工湿地脱氮; 内电解; 湿地微生物

中图分类号: X703 文献标识码: A 文章编号: 0250-3301(2018)02-0758-07 DOI: 10.13227/j.hjxx.201704155

## Mechanism on Enhanced Nitrogen Removal in Municipal Secondary Effluent via Internal-Electrolysis Constructed Wetlands at Low Temperature in Winter

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**Abstract:** Aiming at the low pollutant removal efficiency of constructed wetlands (CWs) at low temperature in winter, three laboratory-scale vertical-flow CWs, namely unplanted CWs, ordinary CWs, and internal-electrolysis CWs, were used to investigate the nitrogen removal efficiency of municipal secondary effluent when the water temperature was 3–12°C. Moreover, the mechanism of enhanced denitrification of the new wetland was revealed through analysis of the microbial community diversity and community structure. The results showed that the internal-electrolysis CWs could make better use of the carbon sources in the municipal secondary effluent and had a higher removal rate. The effluent TN concentration was maintained at about  $(9 \pm 0.29) \text{ mg} \cdot \text{L}^{-1}$ . The average TN removal rate was 42.27%, which was 17.91% and 17.33% higher than those of the unplanted CWs and ordinary CWs, respectively. The microbial activity was detected using fluorescein diacetate (FDA), and the result revealed that the microbial activity of the internal-electrolysis CWs could reach  $0.224 \text{ mg} \cdot \text{g}^{-1}$ , which was 2.6 times and 3.4 times of that of the unplanted CWs and ordinary CWs, respectively. The microbial denitrification intensity of the internal-electrolysis CWs was 2.8 times and 3.3 times of that of the unplanted and ordinary CWs, respectively. The results of high-throughput sequencing showed that the microbial community diversity of the internal electrolysis CWs was higher than those of the unplanted and ordinary CWs. Denitrification microorganisms were detected, mainly *Dechloromonas*, *Rhizobium*, *Hyphomicrobium*, and *Rhodobacter*, as well as *Thiobacillus*, which is an autotrophic denitrifying bacterium. There were obvious advantages in the total amount of denitrifying microorganisms in the internal-electrolysis CWs, as the denitrification microorganisms accounted for 7.13% of the total microbial biomass, which was 3.8 times and 8.7 times of that of the unplanted CWs and ordinary CWs, respectively.

**Key words:** low temperature in winter; municipal secondary effluent; denitrification of constructed wetland; internal electrolysis; microbial characteristic

我国大部分城镇污水经二级处理后, 直接排入受纳水体。尾水排放量大、污染物含量高, 易加剧受纳水体富营养化<sup>[1,2]</sup>。污水厂尾水作为重要的点源污染, 将尾水进行深度处理, 进一步提高污水处理厂出水水质, 是我国污水处理的重要发展趋势。北京市(DB 11890-2012)、天津市(DB 12599-2015)

收稿日期: 2017-04-14; 修订日期: 2017-08-23

基金项目: 江苏省水利科技项目(2015027); 江苏省自然科学基金项目(BK20161505); 中央高校基本科研业务费专项(2015B16414); 江苏高校优势学科建设工程资助项目(PAPD)

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已先后出台了更为严格的污水厂污染物排放标准。

人工湿地作为一种环境友好型水处理技术，具有污染物去除效果好、运行和维护简单、成本低廉、抗冲击负荷强、环境效益好等优点。将人工湿地作为污水厂尾水深度处理工艺，可以进一步削减尾水中的碳氮磷等污染物<sup>[3, 4]</sup>，减小对受纳水体的不利影响。然而，污水经污水厂生化处理单元处理后，尾水中残留的有机碳源以腐殖酸、富里酸、氨基酸及表面活性剂等为主，这些有机物多含有芳环，可生化性差(B/C 值约为 0.2~0.35)，难以被微生物降解利用<sup>[5]</sup>。而尾水中 TN 含量高，是一种特殊的低碳氮比的污水( $BOD_5/TN$  约为 1)，且  $NO_3^- - N$  是尾水中氮素的主要成分，占 TN 比例可达 80% 以上<sup>[6, 7]</sup>。人工湿地处理尾水时，尾水可利用碳源不足严重影响脱氮效率<sup>[8~10]</sup>，尤其是冬季低温脱氮效率十分不理想，影响了人工湿地的进一步推广应用<sup>[11~13]</sup>。

铁炭内电解是一种高效物理化学水处理技术，广泛用于印染、化工等难降解废水的处理，可有效改善废水可生化性、提高污染物去除效率<sup>[14, 15]</sup>。但铁炭内电解通常单独作为一个处理单元，鲜有与生物处理相结合，特别是与人工湿地相结合进行尾水处理的研究。因此，本文将铁炭内电解与人工湿地技术进行有机结合，将铁炭填料掺杂于湿地基质中，构建新型内电解人工湿地，考察冬季低水温条件下(3~12℃)内电解湿地的脱氮效能，并通过测定基质微生物活性、反硝化强度，结合湿地微生物群落结构分析，揭示内电解人工湿地的强化脱氮机制。

1 材料与方法

1.1 试验装置

试验共构建了 3 组呈圆柱体的下向垂直流人工湿地装置，分别为无植物湿地、普通湿地、内电解湿地。装置材料采用有机玻璃，圆柱体尺寸为  $\Phi \times$

$H = 20\text{ cm} \times 65\text{ cm}$ 。装置底部设有高 5 cm 的集水区，并设置穿孔有机玻璃板进行集水。采用遮光布包裹装置四周，以模拟人工湿地真实的避光状态。人工湿地装置如图 1 所示。人工湿地以粗砂和砾石作为主要基质，湿地植物选用芦苇，湿地构建初期孔隙率为 35%，人工湿地构造如表 1 所示。装置运行时，尾水由蠕动泵分别打入 3 组湿地上部，底部出水，通过流量计控制出水，设计水力停留时间为 2 d。

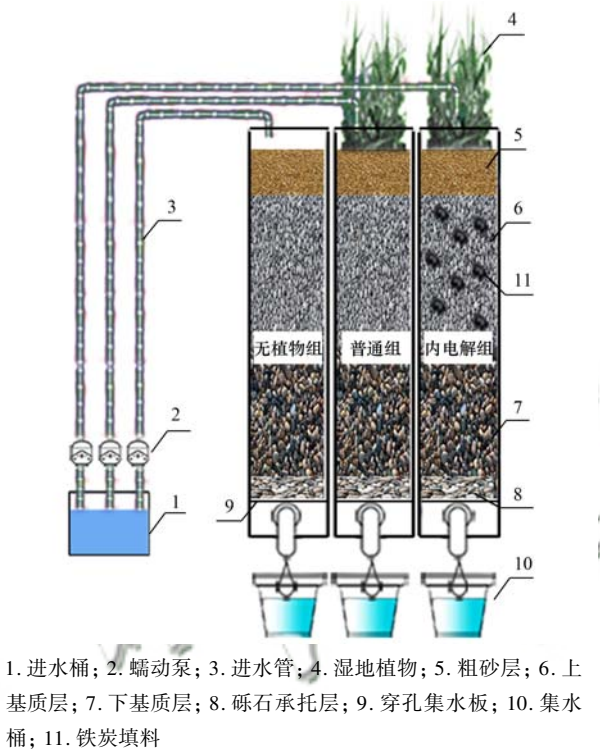


图 1 垂直流人工湿地装置  
Fig. 1 Vertical flow constructed wetland system

1.2 进水水质

试验前采集污水厂尾水进行实测，以明确尾水污染物组成及特征，采用腐殖酸钠、海藻酸钠、牛血清白蛋白等作为尾水中的碳源，以硝酸钠作为主要氮源，在实验室进行污水厂尾水的模拟。试验期间(2016 年 11 月~2017 年 1 月)进水主要污染物浓

表 1 垂直流人工湿地构造<sup>1)</sup>  
Table 1 Structure of vertical flow constructed wetland

| 项目    | 无植物湿地                      | 普通湿地                       | 内电解湿地                               |
|-------|----------------------------|----------------------------|-------------------------------------|
| 植物    | 无                          | 芦苇, 20 株·m <sup>-2</sup>   | 芦苇, 20 株·m <sup>-2</sup>            |
| 粗砂层   | 粒径 2~4 mm, 厚 5 cm          | 粒径 2~4 mm, 厚 5 cm          | 粒径 2~4 mm, 厚 5 cm                   |
| 上基质层  | 粒径 4~8 mm, 厚 20 cm 砾石, 无铁炭 | 粒径 4~8 mm, 厚 20 cm 砾石, 无铁炭 | 粒径 4~8 mm, 厚 20 cm 砾石, 混掺基质质量 1% 铁炭 |
| 下基质层  | 粒径 4~8 mm, 厚 20 cm         | 粒径 4~8 mm, 厚 20 cm         | 粒径 4~8 mm, 厚 20 cm                  |
| 砾石承托层 | 粒径 8~16 mm, 厚 5 cm         | 粒径 8~16 mm, 厚 5 cm         | 粒径 8~16 mm, 厚 5 cm                  |

1) 铁炭填料由铁和炭高温烧结而成，为 10~30 mm 的块状，铁炭质量比为 5:1

度为: COD  $49.72 \sim 53.31 \text{ mg} \cdot \text{L}^{-1}$ 、 $\text{BOD}_5$   $6.51 \sim 12.07 \text{ mg} \cdot \text{L}^{-1}$ 、TN  $14.57 \sim 15.79 \text{ mg} \cdot \text{L}^{-1}$ 、 $\text{NH}_4^+ - \text{N}$   $2.62 \sim 3.81 \text{ mg} \cdot \text{L}^{-1}$ 、 $\text{NO}_3^- - \text{N}$   $11.45 \sim 16.63 \text{ mg} \cdot \text{L}^{-1}$ 、TP  $0.12 \sim 0.23 \text{ mg} \cdot \text{L}^{-1}$ 。

### 1.3 试验方法

常规水质指标: 试验期间, 每2 d采集3组人工湿地进出水水样各200 mL, 采用微孔滤膜过滤水样, 测定水样的COD、TN、 $\text{NH}_4^+ - \text{N}$ 、 $\text{NO}_3^- - \text{N}$ 、TP等, 具体见文献[16], 每组水样取3个平行样。

基质微生物活性: 每7 d检测一次微生物活性, 每组样品取3个平行样。采用荧光显色测试方法<sup>[17]</sup>, 具体方法为: 称取30 g仍保持湿润的基质于100 mL的锥形瓶中, 滴加0.4 mL二乙酸荧光素( $1 \text{ mg} \cdot \text{L}^{-1}$ ), 并加入30 mL的磷酸缓冲液( $\text{KH}_2\text{PO}_4$   $1.3 \text{ g} \cdot \text{L}^{-1}$ ,  $\text{K}_2\text{HPO}_4$   $8.7 \text{ g} \cdot \text{L}^{-1}$ ); 将锥形瓶置于恒温水浴振荡器(水温 $30^\circ\text{C}$ , 振荡频率 $150 \text{ 次} \cdot \text{min}^{-1}$ )上振荡2 h, 静置10 min, 向锥形瓶滴加氯仿/甲醇溶液(2:1)15 mL, 终止微生物的水解; 待溶液静置分层后, 取上层液体离心过滤, 以未添加二乙酸荧光素溶液的样品为空白样, 在494 nm波长下进行比色, 以单位质量基质水解产生的荧光素的量表征微生物活性[1 g基质水解的荧光素的量(mg)]。

基质微生物反硝化强度<sup>[18]</sup>: 每7 d检测一次基质微生物反硝化强度, 每组样品取3个平行样。称取50 g湿地基质, 置于250 mL锥形瓶中, 加入200

mL  $\text{NO}_3^- - \text{N}$ 培养液, 用橡皮塞塞住瓶口, 置于恒温培养中, 于 $20^\circ\text{C}$ 条件下培养3 d, 每间隔24 h取样离心过滤, 测定 $\text{NO}_3^- - \text{N}$ 浓度, 且每次取样后用培养液补足。用单位时间内 $\text{NO}_3^- - \text{N}$ 的浓度变化来表征基质微生物的反硝化强度[1 h内1 kg基质消耗的 $\text{NO}_3^- - \text{N}$ 的量(mg)]。

高通量测序: 从装置基质采样口采集足量湿地基质样品, 于低温条件下保存24 h, 然后用于冰密封保存, 邮寄至上海生工, 样品在7 d内完成测序工作。本试验的高通量测序工作委托上海生工生物有限公司完成, 该公司采用的是MiSeq (Illumina) 平台测序。基质微生物DNA采用Ezup柱式土壤基因组DNA抽提试剂盒进行提取, 对细菌16S rDNA片段的V3区进行扩增。

## 2 结果与讨论

### 2.1 人工湿地脱氮效果

反硝化是人工湿地脱氮的主要途径, 反硝化脱氮需要碳源作为电子供体<sup>[9, 19]</sup>。试验期间, 人工湿地进出水COD浓度及平均去除率如图2所示。3组湿地对COD去除波动幅度较小, 平均去除率分别为 $(34.97 \pm 2.45)\%$ 、 $(33.61 \pm 1.88)\%$ 、 $(47.92 \pm 1.57)\%$ 。冬季低温条件下, 内电解湿地对尾水COD去除效果优于无植物湿地和普通湿地, 无植物湿地和普通湿地差别不大。这主要是湿地基质掺杂

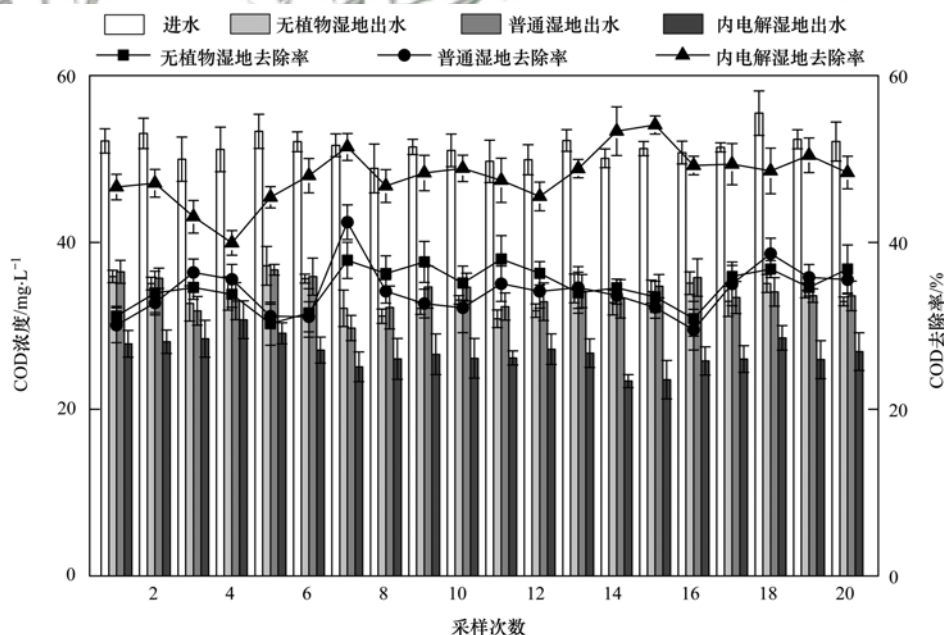


图2 人工湿地进出水COD浓度及平均去除率

Fig. 2 Concentrations of COD in the municipal secondary effluent and effluent of the constructed wetlands and the average removal efficiencies

铁炭, 铁炭内电解过程产生大量活性的  $[H]$  和  $Fe^{2+}$ , 使尾水中的复杂有机物发生开环、断链等作用, 可促进尾水中大分子难降解有机物转变为小分子有机物, 进而被微生物降解<sup>[20, 21]</sup>.

图3为人工湿地进出水 COD 浓度及平均去除率. 从中可知, 冬季低温条件下, 内电解人工湿地脱氮效果优势明显, 出水 TN 浓度维持在  $(9 \pm 0.29) \text{ mg} \cdot \text{L}^{-1}$ , TN 平均去除率达到  $(42.27 \pm 1.70)\%$ , 比无植物湿地和普通湿地分别高出 17.91%、17.33%,

说明冬季植物枯萎后对 TN 的去除作用不大. 无植物湿地、普通湿地、内电解湿地 COD 去除负荷分别为  $0.574$ 、 $0.552$ 、 $0.787 \text{ g} \cdot (\text{m}^3 \cdot \text{d})^{-1}$ , 而 3 组湿地 TN 去除负荷分别为  $0.117$ 、 $0.119$ 、 $0.205 \text{ g} \cdot (\text{m}^3 \cdot \text{d})^{-1}$ , COD、TN 去除负荷之比 (C/N) 分别为 4.91、4.64、3.84. 而通常人工湿地  $C/N > 4$  才表示反硝化碳源较为充足. 由此表明, 湿地微生物进行传统异养反硝化存在碳源不足的现象, 内电解湿地中还可能存在其它形式的脱氮途径<sup>[22]</sup>.

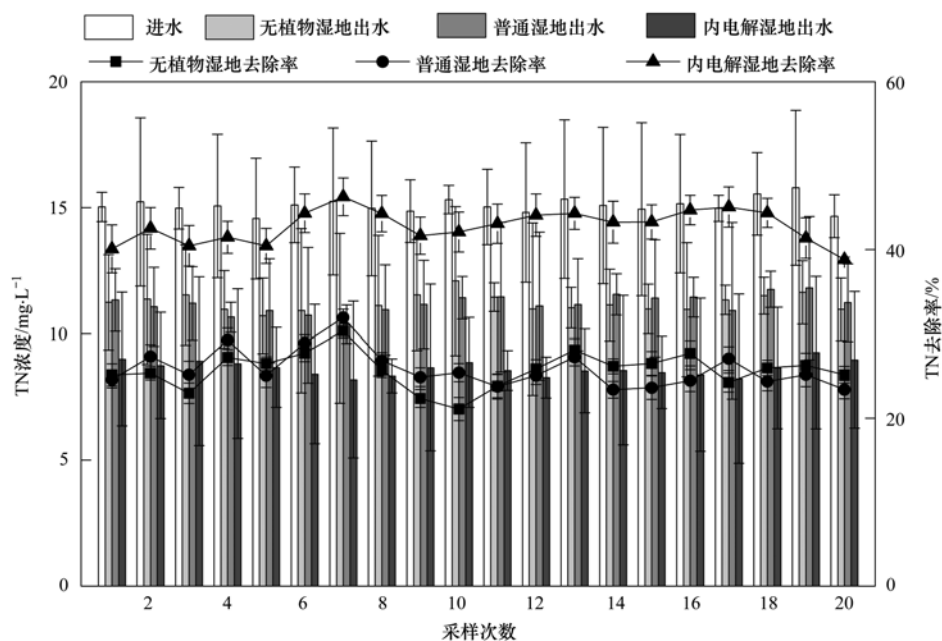


图3 人工湿地进出水 TN 浓度及平均去除率

Fig. 3 Concentrations of TN in the municipal secondary effluent and effluent of the constructed wetlands and the average removal efficiencies

## 2.2 基质微生物活性与反硝化强度

图4为3组湿地系统中基质微生物活性及反硝化强度. 人工湿地微生物是湿地污染物去除的主要承担者, 微生物活性能够直接反映微生物污染物去除能力. 荧光显色法常用于测试基质微生物活性, 微生物活性越高, 水解产生的荧光素量也越大. 由图4知, 无植物湿地、普通湿地、内电解湿地微生物活性分别为  $0.068$ 、 $0.085$ 、 $0.224 \text{ mg} \cdot \text{g}^{-1}$ , 种植植物的普通湿地微生物活性稍高于无植物湿地, 而内电解湿地是普通湿地人工湿地的 2.6 倍. 冬季低温条件下, 内电解人工湿地仍保持很高的微生物活性, 这可能是因为  $Fe^{2+}$  和  $Fe^{3+}$  是微生物生命活动中重要的电子传递体系, 而湿地基质中铁炭填料在内电解过程中产生的  $Fe^{2+}$  和  $Fe^{3+}$  可以参与这种电子传递, 从而加快微生物细胞电子传递速率, 提高微生物活性<sup>[23]</sup>.

$\text{NO}_3^- - \text{N}$  是尾水中氮素的主要成分, 反硝化脱氮

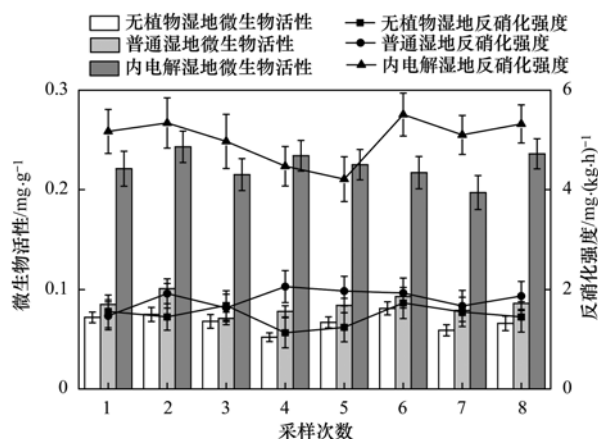


图4 3组湿地系统中基质微生物活性及反硝化强度

Fig. 4 Microbial activity and microbial denitrification intensity in the three laboratory-scale constructed wetlands

作用是决定人工湿地脱氮效率的主要因素. 从图4可以看出, 内电解人工湿地反硝化强度最大  $[5.01 \text{ mg} \cdot (\text{kg} \cdot \text{h})^{-1}]$ , 其次为有植物的普通湿地  $[1.82$

$\text{mg} \cdot (\text{kg} \cdot \text{h})^{-1}$ ], 无植物湿地最低 [ $1.47 \text{ mg} \cdot (\text{kg} \cdot \text{h})^{-1}$ ]. 由于内电解湿地微生物活性很高, 且内电解可为湿地微生物提供更多的可利用碳源, 因而内电解湿地的反硝化强度远高于普通湿地和无植物湿地.

2.3 人工湿地微生物群落结构分析

2.3.1 微生物群落多样性分析

微生物群落多样性是指某种生态系统中菌落所

包含种类多少, 以及个体在种间的分布特征. 在污水处理过程中, 微生物群落多样性越高, 生化处理系统稳定性就越好, 抗冲击负荷能力也越强. 微生物多样性对于维持生态系统稳定是必需的. 目前, 群落生态学中, 主要采用 Shannon、Simpson 等一系列统计学分析指数, 来对环境群落的物种丰度和多样性进行估算分析. 表 2 为本研究中 3 组湿地微生物多样性分析结果.

表 2 3 组湿地微生物多样性分析

Table 2 Biodiversity analysis of the microbial community in the three laboratory-scale constructed wetlands

| 湿地名称  | Out 数目 | Shannon 指数 | Simpson 指数 | 杰卡德距离 |       |       |
|-------|--------|------------|------------|-------|-------|-------|
|       |        |            |            | 无植物湿地 | 普通湿地  | 内电解湿地 |
| 无植物湿地 | 3 931  | 4.42       | 0.10       | 0.000 | 0.372 | 0.832 |
| 普通湿地  | 3 527  | 5.07       | 0.04       | 0.372 | 0.000 | 0.707 |
| 内电解湿地 | 3 634  | 5.41       | 0.03       | 0.832 | 0.707 | 0.000 |

Shannon 指数可表征物种的相对丰度, 其值越大, 说明群落多样性越高<sup>[24]</sup>. 3 组湿地中, 内电解湿地最高(5.41), 其次为普通湿地(5.07), 最低为无植物湿地(4.42), 表明内电解人工湿地微生物群落多样性更高, 种群均匀性好. Simpson 值越大, 表明群落的多样性越差<sup>[25]</sup>, 3 组人工湿地的 Simpson 值分别为 0.10、0.04、0.03, 其结果与 Shannon 指数一致, 内电解湿地的群落多样性高于普通湿地和无植物湿地. 可见, 人工湿地添加铁炭可提高微生物群落多样性. 杰卡德距离(Jaccard distance) 用来研究物种多样性的差异, 其值越接近于 1, 所观察的样品间的差异性越大<sup>[26]</sup>. 无植物湿地与普通砾石层湿地和内电解湿地的杰卡德距离分别为 0.372、0.832, 普通湿地与内电解湿地的杰卡德距离为 0.707, 这表明微生物群落结构的变化受湿地植物和基质添加铁炭的影响, 但主要影响因素是基

质掺杂了铁炭.

2.3.2 人工湿地主导菌群分析

近代生物学将微生物分为域(domain)、门(phylum)、纲(class)、目(order)、科(family)、属(genus)六层, 其中属是分类中最基本的一层. 人工湿地微生物在属级水平的分类较多, 多达 300 余种, 因此按照菌属丰度大小仅选取前 50 种进行分析. 3 组湿地前 50 种菌属的丰度总和分别占总菌属的 85.33%、85.8%、81.55%. 3 组湿地基质微生物在属级上的分布如图 5 所示.

由图 5 可知, 3 组湿地丰度最高的菌属组成相近, 丰度最高的菌属为 *Chlorophyta* (绿藻菌, 分别为 12.15%、30.98%、2.38%)、*Sphingomonas* (鞘氨醇单胞菌, 分别为 23.41%、0.88%、18.74%). 由于试验中人工湿地藻类生长旺盛, 因而检出了较高比例的绿藻菌. 鞘氨醇单胞菌是人工湿地中的优

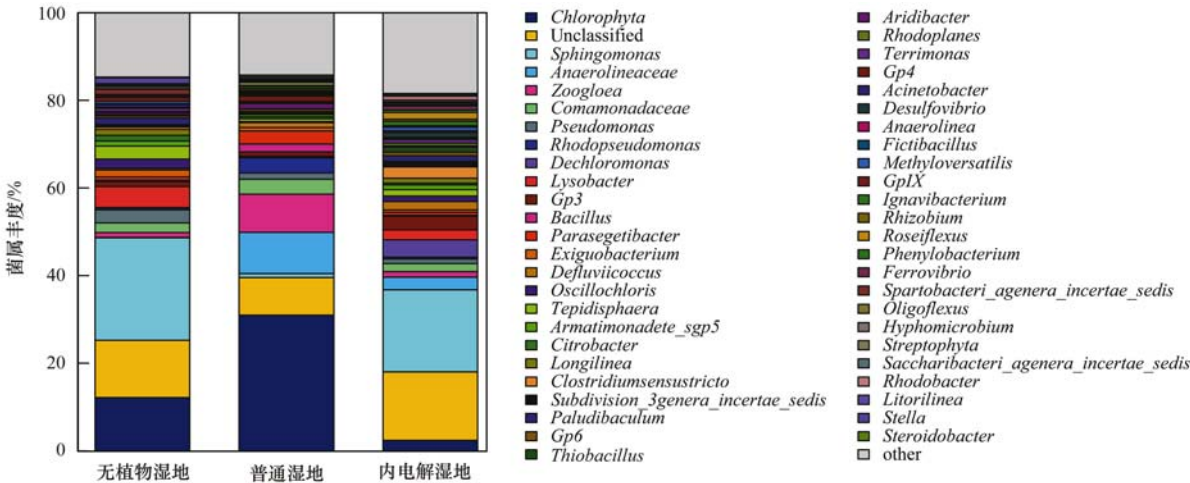


图 5 3 组湿地基质微生物在属级上的分布

Fig. 5 Distribution of microbial strains in the three laboratory-scale constructed wetlands at the genus level

势菌群, 可降解芳香族有机物<sup>[27]</sup>。其他丰度较高的菌属主要包括 *Anaerolineaceae* (厌氧蝇菌)、*Zoogloea* (动胶菌)、*Comamonadaceae* (丛毛单胞菌)、*Dechloromonas* (脱氯单胞菌) 等。厌氧蝇菌是绿弯门的代表菌属, 具有降解碳水化合物和蛋白质的作用<sup>[28]</sup>。动胶菌与菌胶团形成有关, 能促进同步硝化反硝化反应的进行。丛毛单胞菌种类繁多, 不同菌种代谢途径不同, 大多能降解酚类、喹啉类及类固醇类有机物<sup>[29]</sup>。在脱氮微生物方面, 主要检测出了 *Dechloromonas* (脱氯单胞菌)、*Rhizobium* (根瘤菌)、*Hyphomicrobium* (生丝微菌)、*Rhodobacter* (红杆菌)、*Thiobacillus* (产硫酸杆菌) 等菌属。其中, 脱氯单胞菌具有很强的硝酸盐还原能力<sup>[30]</sup>。根瘤菌、生丝微菌、红杆菌是 3 种常见的异养反硝化细菌, 其中, 产硫酸杆菌是一种常见的自养反硝化细菌, 表明湿地中除了传统的异养反硝化作用外, 还存在着自养反硝化。3 组湿地中, 5 种脱氮细菌的总和分别为 1.89%、0.82%、7.13%, 内电解湿地脱氮微生物量分别是无植物湿地、普通湿地的 8.69 倍、3.77 倍, 可见内电解湿地在脱氮微生物总量上有明显的优势, 这也是内电解人工湿地脱氮能力强的原因之一。

### 3 结论

(1) 内电解湿地能更好地利用尾水中碳源, 冬季低温条件下, 脱氮效果优势明显, 出水 TN 浓度维持在  $(9 \pm 0.29) \text{ mg} \cdot \text{L}^{-1}$ , TN 平均去除率达到 42.27%, 比无植物湿地和普通湿地分别高出 17.91%、17.33%, 且冬季植物对 TN 去除几乎没有作用。

(2) 冬季低温条件下, 内电解湿地微生物活性和反硝化强度高于无植物湿地和种植植物的普通湿地。其中, 微生物活性达到  $0.224 \text{ mg} \cdot \text{g}^{-1}$ , 分别是无植湿地和普通湿地的 2.6 倍、3.4 倍; 反硝化强度达到  $5.01 \text{ mg} \cdot (\text{kg} \cdot \text{h})^{-1}$ , 分别是无植物湿地和普通湿地的 2.8 倍、3.3 倍。

(3) 高通量测序表明, 内电解湿地微生物群落多样性优于无植物人工湿地和普通人工湿地, 且湿地基质添加铁炭对生物多样性的影响大于湿地植物的作用。脱氮微生物主要有脱氯单胞菌以及根瘤菌、生丝微菌属、红杆菌属, 以及自养反硝化细菌产硫酸杆菌。内电解人工湿地在脱氮微生物总量上有明显优势, 脱氮微生物占微生物总量的 7.13%, 分别是无植物湿地、普通湿地的 3.8 倍、8.7 倍。

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