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# 生物炭添加对湿地植物菖蒲根系通气组织和根系泌氧的影响

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**摘要:** 在处理污水的潜流人工湿地中, 湿地植物容易受到缺氧胁迫。尽管菖蒲(*Acorus calamus* L.) 是一类对缺氧条件具有显著抵抗能力的湿地植物, 但菖蒲的生理响应并不能完全消除湿地长期缺氧带来的胁迫。生物炭添加能够缓解菖蒲体内超氧化物和过氧化物的积累, 显著降低膜脂过氧化程度, 但生物炭对缓解缺氧胁迫的具体机制尚不清晰。因此, 本研究通过在温室内构建5种不同的生物炭湿地, 采用植物生态学分析方法, 将植物根系通气组织、根孔隙度和根系泌氧相结合, 研究菖蒲根部组织对生物炭添加的响应机制。结果表明, 通过在传统潜流人工湿地中添加生物炭, 有利于菖蒲形成根系通气组织, 增大根孔隙度, 生物炭投加量与根孔隙度具有显著正相关关系。在湿地中添加生物炭将利于 O<sub>2</sub> 通过通气组织传输至地下部分, 并以根系泌氧 (radial oxygen loss, ROL) 的形式扩散至根际, 显著提高根系泌氧量。与其它光强相比, 在 3 000 μmol·(m<sup>2</sup>·s)<sup>-1</sup> 条件下, 菖蒲泌氧能力较强, 生物炭投加比例对植物 ROL 的影响不显著。

**关键词:** 生物炭; 潜流人工湿地; 菖蒲; 根系通气组织; 根系泌氧 (ROL)

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## Influences of Biochar Application on Root Aerenchyma and Radial Oxygen Loss of *Acorus calamus* in Relation to Subsurface Flow in a Constructed Wetland

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**Abstract:** In the subsurface flow of a constructed wetland (CW) used for treating wastewater, low oxygen diffusion results in long-term anoxic or anaerobic surroundings, which cannot meet the needs of plant respiration and poses a threat to the survival of macrophytes. Although sweet sedge (*Acorus calamus* L.) has a significant ability to resist hypoxia, membrane lipid oxidation would still occur in the plant due to the long-term hypoxia in the CW. According to reports in the existing literature, activation of the antioxidative response system could be promoted by adding biochar, thereby significantly decreasing the malonic dialdehyde in the plants. However, the specific reasons why biochar alleviates the stress from anoxia are still not clear. Thus, the responses of macrophyte roots to biochar application were studied in five different CWs built in a greenhouse, using plant ecology analyses combined with root aerenchyma, root porosity, and radial oxygen loss (ROL). The results showed that adding biochar to CW was beneficial for sweet sedge to form root aerenchyma and to increase root porosity. Moreover, there was a significant positive correlation between root porosity and the amount of biochar applied. Photosynthetic metabolism could be indirectly promoted by biochar application by increasing oxygen partial pressure in the blades, helping to transport O<sub>2</sub> to underground parts through aerenchyma, and spreading O<sub>2</sub> to the rhizosphere in the form of ROL. The reduction environment could be improved by applying biochar in CWs, which was also beneficial for ROL. Compared with other light conditions, 3 000 μmol·(m<sup>2</sup>·s)<sup>-1</sup> was more suitable for the growth of *A. calamus* in CWs with biochar, where the ability of the plants to secrete oxygen would be stimulated and enhanced. However, the effect of the biochar application ratio on ROL was not significant.

**Key words:** biochar; subsurface flow constructed wetland; *Acorus calamus* L.; root aerenchyma; radial oxygen loss (ROL)

在处理污水的潜流人工湿地中, 因氧气扩散困难, DO 长期处于较低浓度<sup>[1]</sup>。在这种条件下, 植物受到缺氧胁迫<sup>[2]</sup>。在此状况下, 湿地中的植物会根据所处的缺氧环境呈现出生理的适应性及形态结构的改变<sup>[3, 4]</sup>。抵抗湿地缺氧的一个重要结构特征就是通气组织, 通气组织往往形成于植物根部中央<sup>[5]</sup>, 并通过根系孔隙度表现出来。之前有研究证实, 湿地植物根部的通气组织占全部根系体积的 60% 以上<sup>[6~8]</sup>。湿地植物这种经通气组织释放氧气至根际的行为称为径向氧损失, 又称为根系

泌氧 (radial oxygen loss, ROL)<sup>[9]</sup>。植物所处环境会对 ROL 产生影响, 如污染物<sup>[10]</sup>、重金属<sup>[11, 12]</sup>、盐度<sup>[13]</sup>, ROL 还受到根际环境的氧化还原电位 (oxidation-reduction potential, ORP)、pH、DO 等<sup>[14, 15]</sup>的影响。根据 Lai 等<sup>[16]</sup>采用微电极对 35 种湿地植物 ROL 速率的测定结果发现, 不同植物

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ROL 差异极显著 ( $P=0.000$ )。本文作者前期研究发现, 生物炭添加能够减少菖蒲 (AC) 体内超氧化物和过氧化物的积累, 显著降低 AC 体内丙二醛 (malonaldehyde, MDA) 的含量<sup>[17]</sup>, 但生物炭对缓解缺氧胁迫的具体机制尚不清晰。因此, 本研究拟通过观察植物根系通气组织, 测定根孔隙度和根系 ROL, 探究在生物炭缓解湿地植物缺氧 (或厌氧) 胁迫中, 植物根部组织的响应特征, 以期为生物炭对人工湿地的长期影响研究提供理论参考。

## 1 材料与方法

### 1.1 生物炭潜流人工湿地

本研究的湿地反应器采用圆筒形聚乙烯容器, 每个容器表面积为  $0.1 \text{ m}^2$ , 深为  $35 \text{ cm}$ 。按照生物炭 40%、30%、20%、10% 和 0% 的体积添加比例分别构建微型系统。生物炭采用芦竹作为原材料于  $500^\circ\text{C}$  条件自制, 长度为  $1 \sim 2 \text{ cm}$ <sup>[18]</sup>, 生物炭的比表面积为  $345.92 \text{ m}^2 \cdot \text{g}^{-1}$ , 孔径为  $1.95 \text{ nm}$ , 孔容为  $0.2467 \text{ cm}^3 \cdot \text{g}^{-1}$ , 构建方式按照文献<sup>[17]</sup>进行, 试验装置如图 1 所示。湿地植物取自某生活污水处理厂, 植物经驯化扩培后, 选取长势好且根叶相似的 AC, 分别栽入湿地中 (对应湿地名称分别命名为 AC-40, AC-30, AC-20, AC-10 和 AC-K), 栽种密度为  $30 \text{ 株} \cdot \text{m}^{-2}$ 。湿地微型系统置于温室中运行, 温度 ( $25 \pm 2$ )  $^\circ\text{C}$ , 光照强度 ( $3000 \pm 300$ )  $\mu\text{mol} \cdot (\text{m}^2 \cdot \text{s})^{-1}$ , 光暗比 12 h: 12 h。

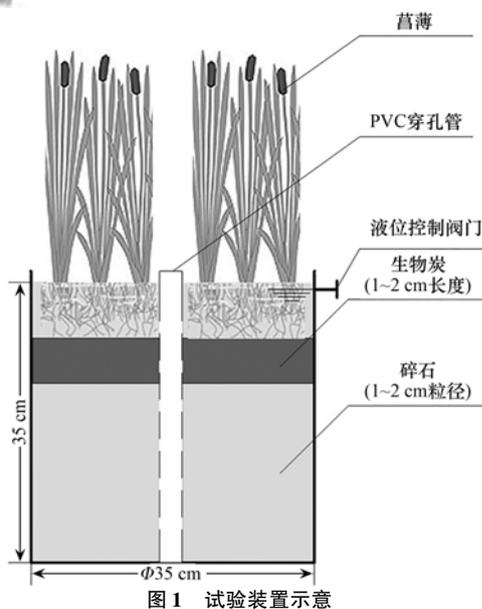


图 1 试验装置示意

Fig. 1 Schematic of the experimental microcosm

人工湿地进水采用自来水配置, 配好的进水化学需氧量 (COD) ( $414.12 \pm 10.67$ )  $\text{mg} \cdot \text{L}^{-1}$ ,  $\text{NH}_4^+ - \text{N}$  ( $19.67 \pm 1.57$ )  $\text{mg} \cdot \text{L}^{-1}$ , 硝态氮 ( $\text{NO}_3^- - \text{N}$ ) ( $31.40 \pm$

$1.02$ )  $\text{mg} \cdot \text{L}^{-1}$  和总磷 ( $5.22 \pm 0.50$ )  $\text{mg} \cdot \text{L}^{-1}$ , 进水 pH 为 ( $7.5 \pm 0.3$ ), DO 为 ( $2.0 \pm 0.5$ )  $\text{mg} \cdot \text{L}^{-1}$ , ORP 为 ( $150 \pm 20$ ) mV。湿地停留时间为 2 d, 处理负荷设定为  $0.05 \text{ m}^3 \cdot (\text{m}^2 \cdot \text{d})^{-1}$ , 有效进水量为 10 L, 进水水位可由顶部水位阀门控制。经过 150 d 左右稳定运行后, 湿地出水 pH 为 ( $7.2 \pm 0.2$ ) ~ ( $7.3 \pm 0.1$ ), 出水 DO 为 ( $0.46 \pm 0.05$ ) ~ ( $0.48 \pm 0.07$ )  $\text{mg} \cdot \text{L}^{-1}$ , 出水 ORP 为 ( $317 \pm 5$ ) ~ ( $348 \pm 5$ ) mV; 湿地主要污染物出水浓度 COD 为 ( $39.73 \pm 8.77$ ) ~ ( $50.51 \pm 11.77$ )  $\text{mg} \cdot \text{L}^{-1}$ ,  $\text{NH}_4^+ - \text{N}$  为 ( $8.04 \pm 2.54$ ) ~ ( $12.71 \pm 3.21$ )  $\text{mg} \cdot \text{L}^{-1}$ , TN 为 ( $9.85 \pm 1.61$ ) ~ ( $14.53 \pm 2.55$ )  $\text{mg} \cdot \text{L}^{-1}$ 。

### 1.2 根系通气组织

取新生的 AC 根系, 用超纯水冲洗干净, 然后用吸水纸将其表面水分彻底吸干。将处理干净的根系用封口袋密封, 保存于  $-20^\circ\text{C}$  条件下备用。用刀片将备用根截断 (于根系中间位置), 用环境扫描电镜 (FEI, Quanta<sup>TM</sup> 650 FEG, 美国) 对根系截断处进行扫描, 加速电压为 10 kV, 放大倍数为 400 倍, 观察成像。

### 1.3 根孔隙度

参照文献<sup>[19]</sup>的改进方法, 取新生的 AC 根系, 用超纯水冲洗干净, 然后用吸水纸将其表面水分彻底吸干。将每条根切成  $2 \sim 2.5 \text{ cm}$  小段之后备用。将 50 mL 比重瓶装满超纯水后称重, 称取 0.3 g 左右的备用根, 置于装满超纯水的比重瓶中后, 称重。将装有备用根的比重瓶抽真空 2 h, 之后取出备用根并置于干燥的研钵中研磨至糊状。将研磨好的根重新置于比重瓶中, 装满超纯水后称重。

根孔隙度计算公式如下:

$$\text{POR} = \frac{P_{\text{gr}} - P_r}{R + P - P_r} \times 100\%$$

式中, POR: 根孔隙度, %;  $P_{\text{gr}}$ : 研磨后的根和装满水的比重瓶的总重量, g;  $P_r$ : 未经研磨的根和装满水的比重瓶的总重量, g;  $R$ : 根重, g;  $P$ : 充满水的比重瓶重量, g。

### 1.4 ROL 测定

本试验利用溶解氧微电极 (Unisense, MM336155, 丹麦) 测定 AC 的 ROL。在烧杯中放入事先灭菌冷却的 1.2 L 琼胶溶液 (1/10 改良 Hoagland 营养液中加入  $1 \text{ g} \cdot \text{L}^{-1}$  琼脂粉,  $0.374 \text{ g} \cdot \text{L}^{-1}$  KCl), 曝入高纯  $\text{N}_2$  15 min, 将刚从湿地拔出的 AC 洗净, 迅速放入溶液中, 将 AC 根部全部没入溶液中; 立即在溶液表面铺上一层 1.0 cm 厚的石蜡, 继续曝高纯  $\text{N}_2$  15 min。然后立即用溶氧微电极连续 1 h 跟踪检测溶液中溶解氧的变化, 以空白为

对照, 单位时间内琼胶溶液中 DO 增加量即为 ROL. 考虑到光照温度对本试验的影响, 光照培养箱中[温度( $25 \pm 2$ ) $^{\circ}\text{C}$ , 光照强度(0、600、1 500、3 000和3 700)  $\mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$ , 植物在测定前预先遮光处理 30 min, 处理过程中培养液采用 1/10 改良 Hoagland 营养液<sup>[20]</sup>.

### 1.5 数据分析

每组试验设置 3 个平行, 试验数据通过 Origin 8.5 整理作图, 并由 PASW Statistics 18.0 进行数据分析. 试验数据表达均采用平均值加或减标准差. 对象之间相互关系采用相关性分析, 并经 Pearson 检验(水平包括显著  $P < 0.05$  和极显著  $P < 0.01$ ). 对象之间的差异性分析采用 One-way ANOVA(水平包括显著  $P < 0.05$  和极显著  $P < 0.01$ ).

## 2 结果与讨论

### 2.1 根系通气组织

不同生物炭湿地中, AC 根系的通气组织成像如图 2 所示. 从中可以直观地看出, 随着生物炭添加量的增加, AC 的根系通气组织逐渐增大, 且 AC-40 和 AC-30 的根系通气组织明显比其它 AC 的通气组织更大.

### 2.2 根孔隙度

经过 150 d 的运行, 不同生物炭湿地中 AC 的根孔隙度如图 3 所示. 相对于 AC-K 而言, 生物炭湿地植物根孔隙度均有所增高. 这与上述各 AC 的根系通气组织成像图是一致的. 由此可知, 生物炭的添加有利于 AC 根系通气组织的形成, 进而可以增强 AC 孔隙度. 经过单因素方差分析( $P > 0.05$ ) 得出, 随着生物炭添加量的增加, AC 的根孔隙度显著增加. 根据相关性检验可知, AC 根孔隙度与生物炭的添加量呈极显著正相关关系( $R^2 = 0.994$ ,  $P < 0.01$ ). 40% 的生物炭添加比例, 使 AC 根孔隙度从( $10.87 \pm 0.71$ )% 增大至( $47.13 \pm 1.41$ )%. 根孔隙度将直接决定 ROL 的速率, 高根孔隙度的湿地植物将会将更多的氧气通过植物体进行运输, 导致更多的 ROL 从根部释放到根际, 进而改变根际氧水平、pH 和根部微区氧化还原环境, 对湿地植物适应淹水和胁迫环境具有重要意义<sup>[21]</sup>.

### 2.3 湿地植物的根系泌氧

不同生物炭湿地 AC 的琼胶溶液中,  $\text{O}_2$  浓度随时间的变化趋势如图 4 所示. 在试验周期内, 随着时间的增加, 溶液中的 DO 浓度逐渐升高, 同时, 还发现不同光照条件下, DO 变化规律不同, 说明光照强度对 ROL 具有影响. 值得注意的是, 在空白对照试验中, DO 随时间也有增大趋势, 从起始的

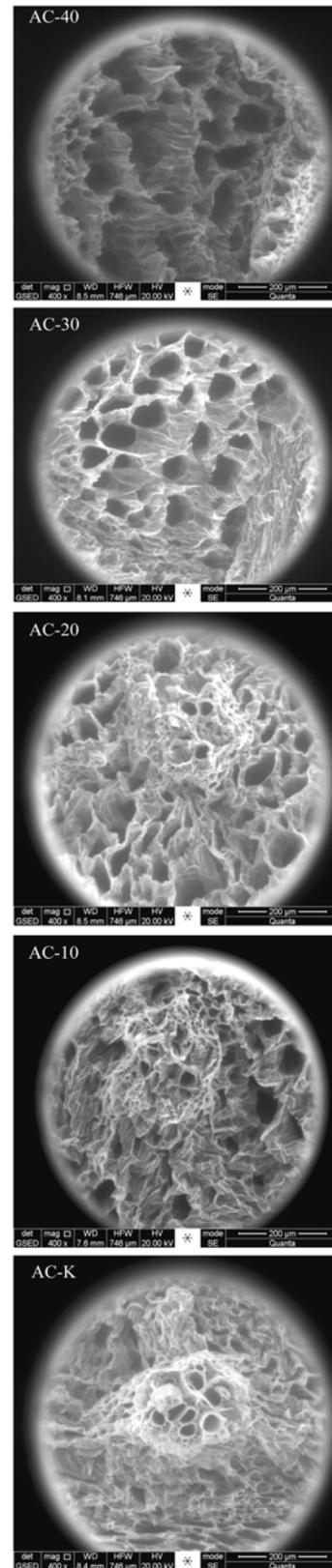


图 2 不同 ACs 的根系通气组织

Fig. 2 Root aerenchyma of different ACs

$0.02 \text{ mg}\cdot\text{L}^{-1}$  增大至  $0.06 \text{ mg}\cdot\text{L}^{-1}$ , 增大幅度较小. 尽管本试验采用石蜡进行液封, 还是会有少量空气穿透进入琼胶溶液. 因此, 在计算 ROL 时将减掉这一部分自然复氧, 以保证结果的可靠性. 通过对琼

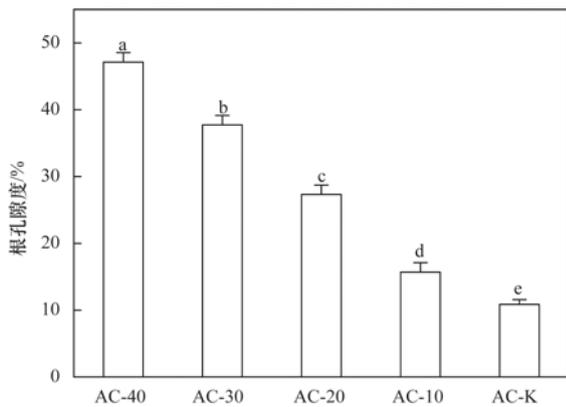


图3 不同ACs的根孔隙度

Fig. 3 Root porosity of different ACs

胶溶液中 DO 随时间变化做线性回归, 得到 DO 变化速率, 即 ROL. 湿地植物在不同光照条件下的拟合方程, 决定系数( $R^2$ ), ROL 如表 1 所示.

除了高生物炭投加比例(40% 和 30%) 在低光照强度下 ROL 不具有显著差异外, 不同湿地植物的 ROL 在 0、600、1 500 和 3 700  $\mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  这 4 种光照强度下均表现出随生物炭投加量的增大呈显著增加的趋势(图 5). 扣除空白对照, 本试验各组湿地中 ROL(以鲜重计) 平均值 AC-40 为  $48.77 \text{ nmol}\cdot(\text{h}\cdot\text{g})^{-1}$ , AC-30 为  $41.27 \text{ nmol}\cdot(\text{h}\cdot\text{g})^{-1}$ , AC-20 为  $32.46 \text{ nmol}\cdot(\text{h}\cdot\text{g})^{-1}$ , AC-10 为  $25.67 \text{ nmol}\cdot(\text{h}\cdot\text{g})^{-1}$ , AC-K 为  $15.69 \text{ nmol}\cdot(\text{h}\cdot\text{g})^{-1}$ . 根

表 1 在不同光照条件下的拟合方程、决定系数和计算 ROL

Table 1 Fitting equations under different light conditions, determination coefficients and calculated ROLs for different ACs

植物	光照强度 $/\mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$	回归方程	$R^2$	ROL(以鲜重计) $/\text{nmol}\cdot(\text{h}\cdot\text{g})^{-1}$
AC-40	0	$y=0.00386x+0.03955$	0.882	31.552
	600	$y=0.00488x+0.09459$	0.933	41.742
	1500	$y=0.00518x+0.11472$	0.971	44.769
	3000	$y=0.00770x+0.07330$	0.988	70.005
	3700	$y=0.00628x+0.00178$	0.974	55.791
AC-30	0	$y=0.00369x-0.00565$	0.974	28.297
	600	$y=0.00500x+0.06231$	0.892	40.806
	1500	$y=0.00532x+0.11360$	0.884	43.785
	3000	$y=0.00640x+0.02510$	0.980	54.126
	3700	$y=0.00527x+0.00721$	0.988	43.335
AC-20	0	$y=0.00231x-0.00399$	0.923	21.470
	600	$y=0.00266x+0.01227$	0.960	26.214
	1500	$y=0.00342x+0.05530$	0.979	36.445
	3000	$y=0.00620x+0.02238$	0.939	74.006
	3700	$y=0.00273x-0.01256$	0.936	27.159
AC-10	0	$y=0.00184x+0.00005$	0.934	16.302
	600	$y=0.00225x+0.05922$	0.978	22.237
	1500	$y=0.00236x+0.04701$	0.930	23.927
	3000	$y=0.00438x+0.04320$	0.980	53.233
	3700	$y=0.00235x-0.00404$	0.975	23.739
AC-K	0	$y=0.00090x+0.02506$	0.977	3.685
	600	$y=0.00090x+0.04252$	0.990	3.809
	1500	$y=0.00243x+0.03594$	0.919	35.287
	3000	$y=0.00258x+0.04963$	0.980	38.479
	3700	$y=0.00160x+0.03081$	0.888	18.092
空白对照	—	$y=0.00072x+0.02392$	0.985	—

据光强对 5 种不同湿地中 AC 的 ROL 影响进行单因素方差分析发现, 有光条件下 AC 的 ROL 能力显著高于黑暗条件. 在 3 000  $\mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  光强条件下, ROL 达到最大且显著大于其余光强. 虽然在光强  $< 3 000 \mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  时, 植物的 ROL 整体呈现随光强增加而增大的趋势, 但当光强  $> 3 000 \mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  时, 植物泌氧能力显著下降. 这是由于光照对植物生长的影响与温度相似, 随着光强(或温度)的升高均能呈现出先增大后降低的趋势,

存在一个最适光强(或温度). 当光强(或温度)超过最适, 则植物体内的叶绿体和线粒体等敏感细胞器会受到损害, 反而不利于植物生长<sup>[22]</sup>.

植物体内气体的流动需要高压和低压形成压力差, 当植物叶片部分的高压与根际附近的氧低压构成压差时, 根系将表现为较强的泌氧能力<sup>[23-25]</sup>. 根据文献[17], 随着生物炭投加量的增加, 植物鲜重和叶片长度增大, 光合色素和可溶性蛋白含量增多, 都有利于植物的光合代谢产生  $\text{O}_2$ . 随着叶片中

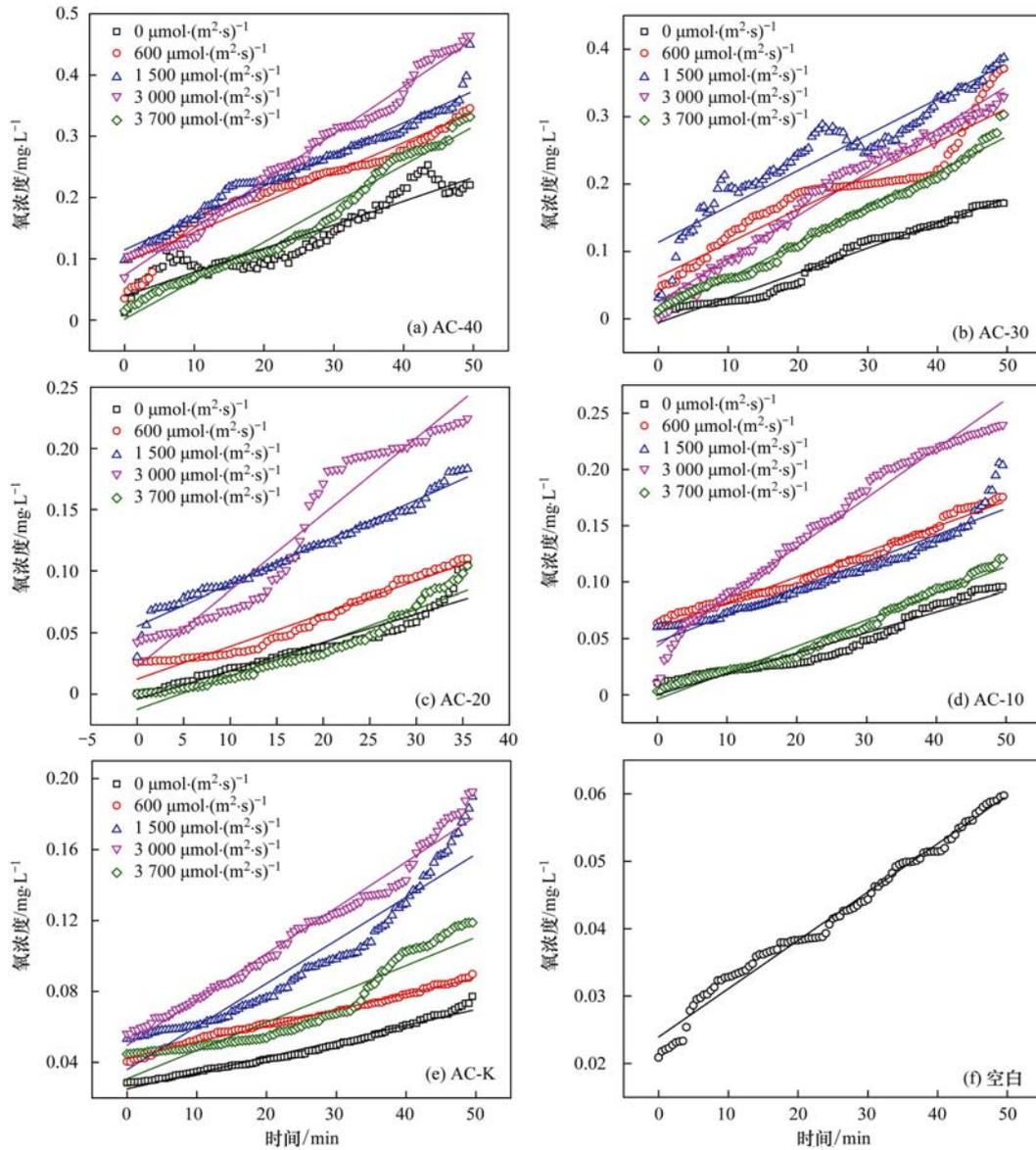
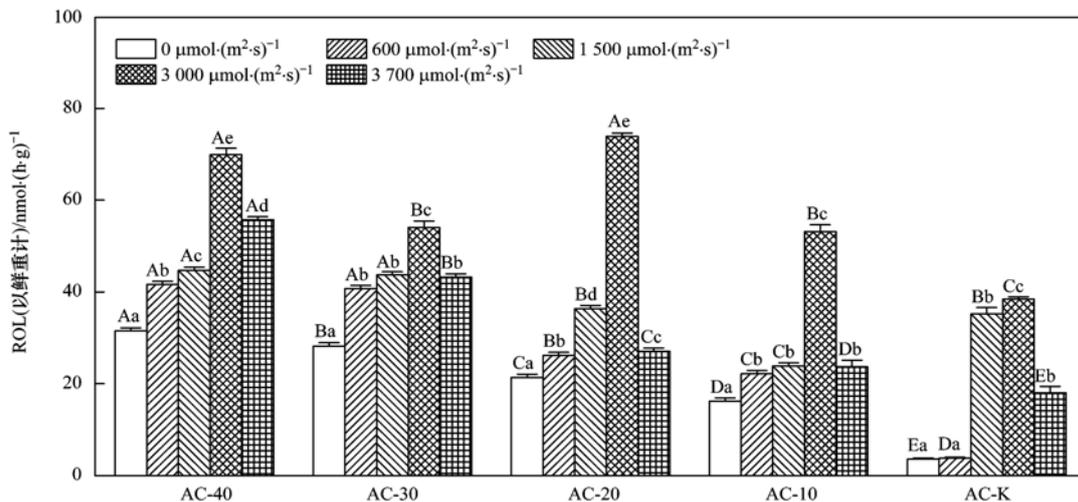


图 4 DO 浓度随时间变化

Fig. 4 DO concentrations changing over time for different ACs



柱状图上不同的字母揭示不同处理的差异性, 其中大写字母揭示组间差异, 小写字母揭示组内差异

图 5 不同光照条件下湿地 AC 的 ROL

Fig. 5 ROLs of ACs under different light intensities in the CWs

氧分压增大, 利于  $O_2$  通过通气组织传输至地下部分, 且以 ROL 的形式扩散至根际<sup>[26]</sup>。

根据光强试验发现,  $3\ 000\ \mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  应该是 AC 的较为适宜生长的光照强度, 在该光强下, 植物表现出代谢旺盛, 泌氧能力增大的特点。在该光照强度下, 虽然生物炭湿地中 ACs 的 ROL 仍显著大于 AC-K, 但由于植物对该光强响应明显, 减弱生物炭的影响, 表现出在生物炭湿地中, 40% 和 20% 的生物炭投加比例差异不显著, 30% 和 10% 的生物炭投加比例差异亦不显著。相关性分析也获得类似的结果, 在  $0$ 、 $600$  和  $3\ 700\ \mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  光强条件下, ROL 与生物炭的投加量呈极显著正相关 ( $R_1^2 = 0.974$ ,  $R_2^2 = 0.959$ ,  $R_3^2 = 0.966$ ,  $P < 0.01$ ), 而在  $3\ 000\ \mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  光强条件下, ROL 与生物炭的投加量呈正相关, 但关系不显著 ( $R^2 = 0.707$ ,  $P > 0.05$ )。

### 3 结论

(1) 通过在传统潜流人工湿地中添加生物炭, 有利于菖蒲形成根系通气组织, 增大菖蒲的根孔隙度。

(2) 在湿地中添加生物炭将间接促进植物的光合代谢产生  $O_2$ , 利于  $O_2$  通过通气组织传输至地下部分, 并以根系泌氧的形式扩散至根际。

(3) 在光照强度小于  $3\ 000\ \mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  时, 菖蒲的根系泌氧量整体呈现随生物炭投加量增加而增大的趋势。  $3\ 000\ \mu\text{mol}\cdot(\text{m}^2\cdot\text{s})^{-1}$  为菖蒲的较为适宜生长的光照强度, 在该光强下, 植物泌氧能力较强, 减弱了生物炭投加比例对植物 ROL 的影响。

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## 《环境科学》多项引证指标名列前茅

2018年11月1日,中国科学技术信息研究所在中国科技论文统计结果发布会上公布了2017年度中国科技论文统计结果.统计结果显示《环境科学》2017年度总被引频次11 228,影响因子1.958,多项引证指标位居环境科学技术及资源科学技术类科技期刊前列.



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