

环境科学

(HUANJING KEXUE)

ENVIRONMENTAL SCIENCE



第35卷 第4期

Vol.35 No.4

2014

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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再生水中5种抗生素抗性菌的紫外线灭活及复活特性研究

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摘要: 抗生素抗性菌作为再生水中的新兴污染物而受到广泛关注。为探明紫外线对抗生素抗性菌的灭活和消毒后抗性菌的复活潜能, 研究了以城市污水为水源的再生水(简称“再生水”)中青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌、氯霉素抗性菌和利福平抗性菌的紫外线灭活特性, 并考察了再生水中的抗生素抗性菌在黑暗条件下的复活潜能。结果表明, 20 $\text{mJ} \cdot \text{cm}^{-2}$ 紫外线消毒剂量下, 实际再生水中青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌和氯霉素抗性菌的灭活率均高于4-log, 与总异养菌群灭活率相当, 而利福平抗性菌的灭活率(3.7-log)略低于总异养菌群。紫外线消毒后, 再生水静置22 h后, 抗生素抗性菌普遍出现复活现象, 当紫外线消毒剂量为常规剂量20 $\text{mJ} \cdot \text{cm}^{-2}$ 时, 消毒后再生水中的抗生素抗性菌菌落形成能力高达3-log。因此, 常规的紫外线消毒剂量不能有效控制再生水储存或运输过程中抗生素抗性菌的复活。

关键词: 抗生素抗性菌; 紫外线消毒; 紫外线灭活; 复活特性; 再生水处理

中图分类号: X172 文献标识码: A 文章编号: 0250-3301(2014)04-1326-06 DOI: 10.13227/j.hjxk.2014.04.017

Inactivation and Reactivation of Antibiotic-Resistant Bacteria During and After UV Disinfection in Reclaimed Water

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Abstract: Prevalence of antibiotic-resistant bacteria in wastewater effluents is concerned as an emerging contaminant. To estimate inactivation and reactivation potentials of antibiotic-resistant bacteria by UV disinfection, inactivation and reactivation of penicillin-, ampicillin-, cephalaxin-, chloramphenicol-and rifampicin-resistant bacteria in the secondary effluent were studied under different UV doses. The results showed that the inactivation ratios of penicillin-, ampicillin-, cephalaxin-and chloramphenicol-resistant bacteria were higher than 4-log, which was closed to that of total heterotrophic bacteria; however, the inactivation ratio of rifampicin-resistant bacteria was lower (3.7-log) under 20 $\text{mJ} \cdot \text{cm}^{-2}$ UV exposure. After 22 h standing incubation, antibiotic-resistant bacteria widely reactivated. The colony forming ability of antibiotic-resistant bacteria was as high as 3-log when exposed to 20 $\text{mJ} \cdot \text{cm}^{-2}$ UV light. Hence, conventional UV dose can not effectively control reactivation of antibiotic-resistant bacteria in reclaimed water by UV disinfection.

Key words: antibiotic-resistant bacteria; UV disinfection; inactivation; reactivation; reclaimed water treatment

再生水可应用于市政杂用、景观回用、娱乐用水等方面, 在城市水循环体系中扮演着越来越重要的角色^[1]。以城市污水为水源的再生水中存在着多种水生病原微生物, 在再生水利用过程中存在微生物安全风险^[2~5]。消毒是控制再生水中病原微生物的必要措施之一。

前期的研究结果表明, 城市污水处理厂二级出水中抗生素抗性菌浓度较高, 且这些抗性菌能在再生水中较长时间内稳定存在^[6]。再生水中抗生素抗性菌的传播可能引起健康风险, 因此有必要利用消毒对再生水中抗生素抗性菌进行控制。抗生素抗性菌相关的消毒研究可追溯至20世纪70年代。一些

研究表明, 氯消毒、紫外线消毒均会改变二级出水或饮用水中的抗生素抗性菌比例, 而过氧乙酸消毒会改变二级出水中抗性菌所携带质粒的分布^[7~12]。但国内外对于各种消毒方法对抗生素抗性菌的灭活特性研究较少。紫外线消毒是最常用的污水消毒工艺之一, 由于紫外线同时具有灭菌效果好、消毒副产物生成少而在污水消毒领域里应用广泛。

本研究主要探讨不同紫外线消毒剂量下实际再

收稿日期: 2013-08-08; 修订日期: 2013-09-23

基金项目: 国家自然科学基金项目(51078209)

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生水中抗生素抗性菌灭活特性,以及基于抗生素抗性菌灭活率达标的紫外线消毒剂量,在此基础上,针对实际再生水中的抗生素抗性菌在黑暗条件下的复活特征,考察抗生素抗性菌的复活潜能,基于复活率进一步提出紫外线消毒的实际灭活率,以期为再生水储存和输配提供基础数据支撑。

1 材料与方法

1.1 水样

取自北京市一座实际运行的城市污水处理厂(WWTP-X)的二级出水作为再生水水样。取样时段为2010年4月~2011年6月。该城市污水处理厂的主要处理工艺包括一级沉淀池、A/A/O生化处理系统和二级沉淀池。取样设备均经过消毒处理,样品

保存于冰盒运回实验室,保存于4℃冰箱,在4 h内进行抗生素抗性菌及相关水质指标测定工作。水样相关的水质参数见表1。

1.2 紫外线消毒试验与灭活评价方法

1.2.1 紫外线消毒试验

紫外线消毒试验在实验室自主设计的紫外线平行光束仪装置内进行。平行光束仪内装有1根40 W低压紫外灯管,灯管水平中心线距离试验水样表面40 cm^[13]。用分光光度计(型号UV-2401PC)测定试验水样在254 nm下的吸光度(cm⁻¹),根据紫外线消毒试验中平均光强计算确定水样实际接受的平均光强^[14]。本试验中,所用紫外装置的平均光强为0.034 mW·cm⁻²。紫外线剂量为水样接受的平均光强与照射时间的乘积。

表1 消毒用水样的水质参数

Table 1 Water quality of reclaimed water sample used in UV disinfection

pH	COD/mg·L ⁻¹	TOC/mg·L ⁻¹	UV ₂₅₄ /cm ⁻¹	NH ₄ ⁺ -N/mg·L ⁻¹
8.0~8.1	82.0~110.7	9.0~15.9	0.14~0.21	8.8

取15 mL水样于携带磁力转子的直径60 mm的灭菌培养皿中,取下培养皿盖,并将培养皿置于平行光束仪正下方,启动搅拌器,使水样混合15 s后,打开遮光板,使得水样接受一定时间的紫外线照射^[14]。消毒试验结束后迅速合上遮光板,并取出培养皿。紫外线消毒试验在常温下(25℃±2℃)进行。

1.2.2 灭活评价方法

采用对数灭活率表征紫外线消毒对水样中总异养菌群和抗生素抗性菌的灭活效率,计算方法如下式:

$$\text{灭活率} = \lg\left(\frac{N_0}{N_i}\right)$$

式中,N₀:紫外线照射前的细菌数(CFU·mL⁻¹),N_i:紫外线照射后水样中的细菌数(CFU·mL⁻¹)。

1.3 复活试验与评价方法

1.3.1 复活试验

将紫外线消毒后的水样置于黑暗常温(25℃±2℃)下,经过22 h后测定水样中的总异养菌群和各抗生素抗性菌。

1.3.2 复活潜力评价方法

为了评价消毒后水样中总异养菌群和抗生素抗性菌的复活程度,文献中光复活率的评价方法,将之拓宽为复活百分比。复活百分比(PR)反映了消毒后灭活细菌中被修复细菌的比例^[15,16],其计算公式如

式(1)所示。

$$PR = (N_r - N_i)/(N_0 - N_i) \times 100\% \quad (1)$$

同时,利用水样中细菌的菌落形成能力(colony forming ability,CFA)来评价再生水中总异养菌群和抗生素抗性菌的复活潜能,以比较不同初始浓度下各类细菌的复活潜能的差别,其计算公式如式(2)所示。

$$\lg\left(\frac{N_r}{N_i}\right) = \lg\left(\frac{N_r}{N_0}\right) - \lg\left(\frac{N_i}{N_0}\right) \quad (2)$$

式中,N₀:紫外线照射前的细菌数(CFU·mL⁻¹),N_i:紫外线照射后水样中的细菌数(CFU·mL⁻¹),N_r:复活试验后水样中的细菌数(CFU·mL⁻¹)。

1.4 微生物检测方法

1.4.1 总异养菌群测定

采用营养琼脂培养基平板法测定污水中的总异养菌群数^[17]。在培养皿(Φ90 mm)中加入1 mL磷酸缓冲盐溶液(phosphate buffered saline,PBS)10倍梯度稀释水样,加入10 mL的营养琼脂培养基,摇匀凝固后,倒置放于37℃培养箱内培养24 h,计所有菌落数,用单位体积水样的菌落形成单位(CFU·mL⁻¹)表示。检出限为1 CFU·mL⁻¹,每次测定设3个平行样。

1.4.2 抗生素抗性菌测定

采用含一定浓度抗生素的营养琼脂培养基平板法测定污水中的各类抗生素抗性菌^[18]。在培养皿

(Φ90 mm)中加入1 mL PBS 10倍梯度稀释水样,加入一定量的抗生素溶液,加入10 mL的营养琼脂培养基,使培养皿体系中抗生素达到一定浓度,摇匀凝固后,倒置放于37℃培养箱内培养24 h,计菌落数,用单位体积水样的菌落形成单位(CFU·mL⁻¹)表示。

表2 抗生素抗性菌检测用抗生素浓度

Table 2 Concentrations of antibiotics used in antibiotic-resistant bacteria detection

抗生素	青霉素 (PEN)	氨苄青霉素 (AMP)	头孢氨苄 (CEL)	氯霉素 (CHL)	利福平 (rif)
抗生素浓度/mg·L ⁻¹	16	32	32	32	4

2 结果与分析

2.1 紫外线消毒对抗生素抗性菌的灭活

2.1.1 抗生素抗性菌的灭活特性

紫外线对再生水中各类抗生素抗性菌灭活效率的差异可能导致增大或降低抗生素抗性菌在再生水中存活的可能性。不同紫外线消毒剂量下,水样中青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌、氯霉素抗性菌和利福平抗性菌的剂量响应曲线如图1所示,灭活曲线如图2所示。

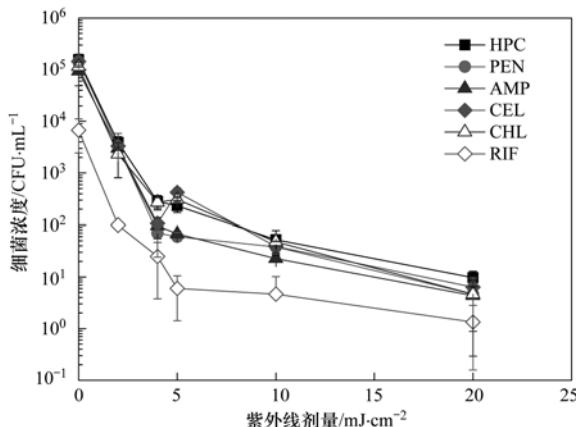


图1 不同紫外线消毒剂量下抗生素抗性菌的剂量响应曲线
Fig. 1 Response curves of antibiotic-resistant bacteria under series of UV exposures

水样中总异养菌群、青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌、氯霉素抗性菌和利福平抗性菌的浓度范围分别为 $1.1 \times 10^5 \sim 1.9 \times 10^5$ 、 $1.3 \times 10^5 \sim 1.6 \times 10^5$ 、 $4.3 \times 10^4 \sim 1.3 \times 10^5$ 、 $1.2 \times 10^5 \sim 1.6 \times 10^5$ 、 $7.8 \times 10^4 \sim 1.4 \times 10^5$ 和 $2.8 \times 10^3 \sim 1.1 \times 10^4$ CFU·mL⁻¹,其中青霉素抗性菌、氨苄青霉素抗性菌、先锋霉素抗性菌和氯霉素抗性菌的浓度

示。检出限为 $1 \text{ CFU} \cdot \text{mL}^{-1}$,每次测定设3个平行样。测定体系的抗生素浓度采用CLSI (clinical and laboratory standards institute, 美国临床与实验室标准协会)药敏试验标准中病原菌抗性界定浓度的最大值,如表2所示^[19]。

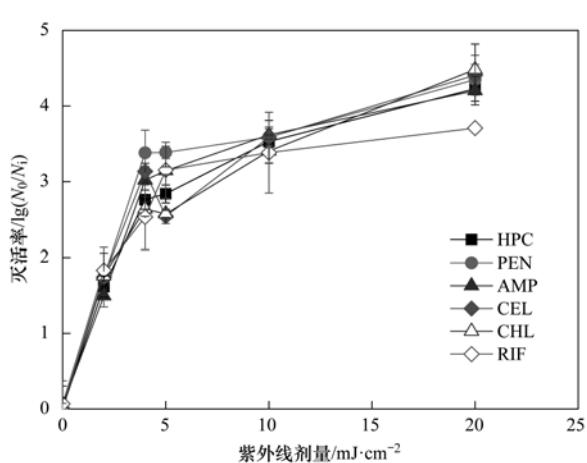


图2 不同紫外线消毒剂量下抗生素抗性菌的灭活曲线

Fig. 2 Inactivation curves of antibiotic-resistant bacteria under series of UV exposures

显著高于利福平抗性菌浓度(One-way ANOVA, $P < 0.05$)。

从灭活曲线来看,青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌和氯霉素抗性菌的灭活曲线接近于总异养菌群的灭活曲线。当再生水暴露于 $4 \text{ mJ} \cdot \text{cm}^{-2}$ 紫外线剂量时,青霉素抗性菌和头孢氨苄抗性菌的灭活率分别达到 3.38-log 和 3.13-log ,显著高于总异养菌群的灭活率(2.76-log) (One-way ANOVA, $P < 0.05$);然而,在其余紫外线暴露剂量下,青霉素抗性菌和头孢氨苄抗性菌灭活率与总异养菌群无显著性差别。利福平抗性菌的灭活率与总异养菌群无显著性差别。当紫外线剂量达到 $20 \text{ mJ} \cdot \text{cm}^{-2}$ 时,再生水中的青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌和氯霉素抗性菌的灭活率均高于 4-log ,与总异养菌群的灭活率相当,而利福平抗性菌的灭活率为 3.7-log ,稍低于总异养菌群。

以上研究结果表明,再生水中此5类抗生素抗性菌对紫外线的响应与总异养菌群类似,并没有显著表现出对紫外线的耐受性或敏感性。这一结果与

文献[20]的结论——紫外线消毒对抗生素敏感大肠杆菌与氨苄青霉素抗性大肠杆菌的灭活无显著差别类似。

2.1.2 紫外线消毒后抗生素抗性菌比例的变化

紫外线消毒后水样中抗生素抗性菌的比例变化如图3所示。从中可知, 经过紫外线消毒后, 青霉素抗性菌、氨苄青霉素抗性菌和头孢氨苄抗性菌的比例较其各自的初始比例低; 而氯霉素抗性菌和利福平抗性菌的比例变化不大。经紫外线消毒后, 利福平抗性菌比例升高; 暴露于 $20\text{ mJ}\cdot\text{cm}^{-2}$ 紫外线照射后, 利福平抗性菌比例由初始的4.33%升高至13.79%。这一结果表明, 紫外线消毒对利福平抗性菌可能存在选择作用。

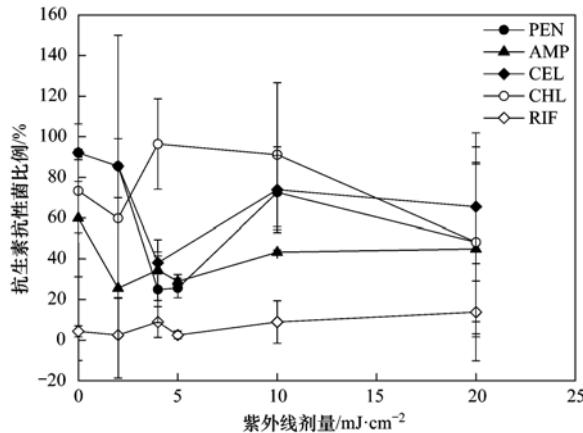


图3 紫外线消毒后水样中抗生素抗性菌在总异养菌群中的比例变化

Fig. 3 Proportions of antibiotic-resistant bacteria after UV disinfection

2.2 紫外线消毒后抗生素抗性菌的复活

研究表明, 紫外线消毒灭活的细菌能够通过自身体内的酶激活与反应在光照或黑暗条件下进行自我修复^[15,21]。由于紫外线没有持续的消毒能力, 当经过紫外线消毒后的再生水储存于水箱或输送水管中时, 细菌会出现复活或再生长的现象。一般来讲, 再生水储存于水箱或管道中的时间从几小时到几天不等。因此, 本研究选取了22 h作为抗性菌在黑暗条件下的修复时间。

在再生水储存过程中, 水中增加的细菌数可能包括活细菌的繁殖再生长和灭活的细菌的复活及其繁殖再生长。前期研究结果表明, 二级出水静置22 h后, 总异养菌群和抗生素抗性菌均无显著性生长, 说明其中的碳、氮源等营养物质在此时间段内难以支撑细菌的自我繁殖^[22]。Power等^[23]也观察到在48 h内细菌在消毒前的二级出水中无明显变化。因此, 消毒后二级出水中抗性菌菌落个数的增长可粗略认为

是由于被灭活细菌复活所引起的。

在本研究中, 同时采用复活百分比和菌落形成能力2个指标评价总异养菌群和各类抗生素抗性菌的暗修复潜能。紫外线消毒后总异养菌群和抗生素抗性菌的复活百分比如图4所示。

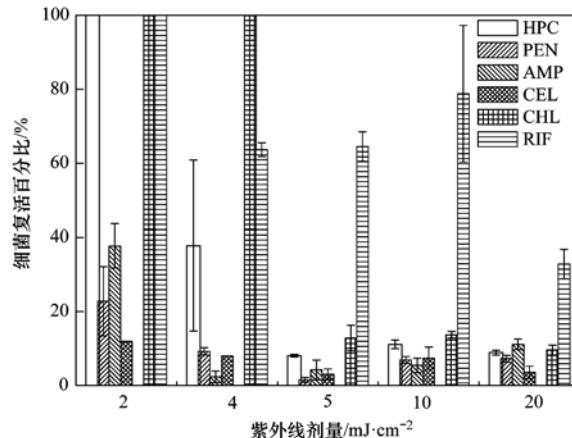


图4 紫外线消毒后总异养菌群和抗生素抗性菌的复活百分比

Fig. 4 Reactivation of antibiotic-resistant bacteria and heterotrophic bacteria after UV disinfection

总体而言, 总异养菌群的复活百分比随着紫外线消毒剂量的增大而减小。当紫外线消毒剂量为5、10和20 $\text{mJ}\cdot\text{cm}^{-2}$ 时, 总异养菌群的复活百分比稳定在10%左右。相比之下, 在相同的紫外线剂量照射后, 青霉素抗性菌、氨苄青霉素抗性菌和头孢氨苄抗性菌的复活百分比低于总异养菌群; 随着紫外线剂量的升高, 复活百分比的差距减小。当暴露于 $20\text{ mJ}\cdot\text{cm}^{-2}$ 紫外线照射后, 青霉素抗性菌、氨苄青霉素抗性菌和头孢氨苄抗性菌的百分比仍分别高达7.2%、11.1%和3.6%。

在相同的紫外线剂量照射后, 氯霉素抗性菌和利福平抗性菌的复活百分比高于总异养菌群。尽管随着紫外线消毒剂量的增大, 这2类抗生素抗性菌的复活百分比随之降低, 但当暴露于 $20\text{ mJ}\cdot\text{cm}^{-2}$ 紫外线照射后, 氯霉素抗性菌和利福平抗性菌的复活百分比仍分别高达9.6%和32.9%。此外, 利福平抗性菌的复活百分比显著高于总异养菌群的复活百分比(One-way ANOVA, $P < 0.05$)。

菌落形成能力(colony forming ability, CFA)同样能够反映消毒后总异养菌群和抗生素抗性菌的复活潜能。紫外线消毒后, 总异养菌群和抗生素抗性菌的菌落形成能力如图5所示。再生水中青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌和氯霉素抗性菌与总异养菌群的菌落形成能力差别与两者的复活百分比差别类似。然而, 利福平抗性菌的菌落形成能

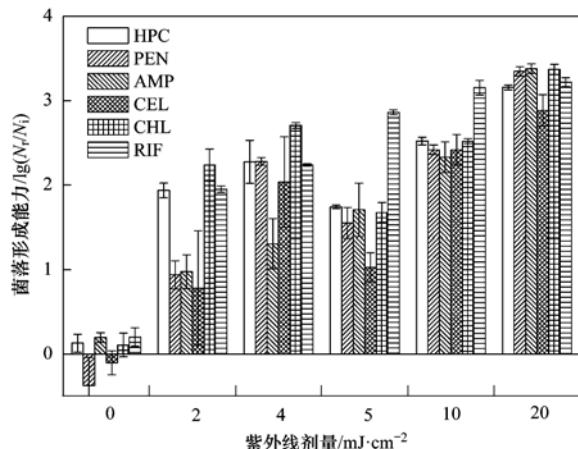


图5 紫外线消毒后总异养菌群和抗生素抗性菌的菌落形成能力

Fig. 5 Colony forming ability of antibiotic-resistant bacteria after UV disinfection

力与总异养菌群无显著性差异(One-way ANOVA, $P > 0.05$).

复活百分比与菌落形成能力结果之间差异的主要原因在于两种分析指标之间的差别,复活百分比的计算基于再生水中抗性菌的初始浓度,而菌落形成能力恰好避免了再生水中不同抗性菌的初始浓度不同这一缺陷.本研究中,再生水中利福平抗性菌的初始浓度比总异养菌群低1-log,而再生水中青霉素抗性菌、氨苄青霉素抗性菌、先锋霉素抗性菌和氯霉素抗性菌的初始浓度与总异养菌群在同一水平.由于一般情况下,实际再生水中各类抗生素抗性菌的初始浓度均有所差异,因此,通过测定复活后再生水中的细菌浓度计算得到其菌落形成能力来描述实际再生水中抗生素抗性菌的复活潜能更为合理.

同时,研究结果表明,当再生水接受不大于20 $\text{mJ}\cdot\text{cm}^{-2}$ 紫外线照射后,各类抗生素抗性菌的复活现象较为普遍.当紫外线剂量较低时,再生水中青霉素抗性菌、氨苄青霉素抗性菌和头孢氨苄抗性菌的复活潜能小于总异养菌群;然而,总体而言,再生水中抗生素抗性菌的复活潜能与总异养菌群差别不大.当紫外线消毒剂量达到20 $\text{mJ}\cdot\text{cm}^{-2}$ 时,经过22 h静置,再生水中的抗生素抗性菌的菌落形成能力比消毒结束时的高近3-log,这说明当再生水处理后经过一定时间的储存与运输,20 $\text{mJ}\cdot\text{cm}^{-2}$ 紫外线消毒剂量用于再生水中的抗生素抗性菌控制是不够的.

3 结论

紫外线消毒对再生水中利福平抗性菌的灭活率

略低于总异养菌群,但差别并不显著;青霉素抗性菌、氨苄青霉素抗性菌、头孢氨苄抗性菌和氯霉素抗性菌的灭活率与总异养菌群灭活率相当.当再生水接受不大于常规紫外线消毒剂量20 $\text{mJ}\cdot\text{cm}^{-2}$ 消毒后,抗生素抗性菌的复活现象较为普遍,因此,常规的紫外线消毒剂量不能有效控制再生水储存或运输过程中抗生素抗性菌的复活.当紫外线剂量较低时,青霉素抗性菌、氨苄青霉素抗性菌和头孢氨苄抗性菌的复活潜能小于总异养菌群,氯霉素抗性菌与利福平抗性菌的复活潜能与总异养菌群差别不大.

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(HUANJING KEXUE)

(月刊 1976年8月创刊)

2014年4月15日 35卷 第4期

ENVIRONMENTAL SCIENCE

(Monthly Started in 1976)

Vol. 35 No. 4 Apr. 15, 2014

主	管	中国科学院	Superintended	by Chinese Academy of Sciences
主	办	中国科学院生态环境研究中心	Sponsored	by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences
协	办	(以参加先后为序)	Co-Sponsored	by Beijing Municipal Research Institute of Environmental Protection
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		E-mail:hjkx@rcees.ac.cn		http://www.hjkx.ac.cn
		http://www.hjkx.ac.cn		
出	版	科学出版社	Published	by Science Press
		北京东黄城根北街16号		16 Donghuangchenggen North Street,
		邮政编码:100717		Beijing 100717, China
印	刷	北京北林印刷厂	Printed	by Beijing Bei Lin Printing House
装	订		Distributed	by Science Press
发	行	科学出版社		Tel:010-64017032
		电话:010-64017032		E-mail:journal@mail.sciencep.com
		E-mail:journal@mail.sciencep.com		
订	购	全国各地邮局	Domestic	All Local Post Offices in China
购	处	中国国际图书贸易总公司	Foreign	China International Book Trading Corporation (Guoji Shudian), P. O. Box 399, Beijing 100044, China
国	外	(北京399信箱)		

中国标准刊号: ISSN 0250-3301
CN 11-1895/X

国内邮发代号: 2-821

国内定价: 90.00 元

国外发行代号: M 205

国内外公开发行