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### ENVIRONMENTAL SCIENCE

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### 链霉菌 FX645 对偶氮染料红 AR30 的降解机制研究

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摘要:从印染厂污泥中筛选到1株对偶氮染料红30(AR30)具有较强脱色降解作用的菌株 Streptomyces sp. FX645.通过降解产物的紫外-可见吸收光谱、LC-MS分析及各降解产物在发酵体系中浓度随时间变化的规律,探讨了菌株 FX645 对 AR30 的可能降解途径,首先在偶氮还原酶的作用下 AR30 发生偶氮键的裂解,生成4-硝基-2,6-二氯苯胺与2-[(4-胺苯基)-(2-氰乙基)-氨基]乙酸乙酯,然后分别发生硝基还原、氨基酰化、氰基水解作用,生成一系列的苯胺类化合物.

关键词:偶氮染料; 脱色降解; 链霉菌; 降解机制; 偶氮还原酶

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# Microbial Degradation Mechanism of Disperse Azo Dye Red 30 by *Streptomyces* sp. FX645

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**Abstract:** One strain, identified as *Streptomyces* sp. FX645 which was isolated from the sludge collected in a printing and dyeing mill, had high potency of degradation and decolourisation of azo dye Red 30 (AR30). The microbial degradation mechanism on AR30 by strain FX645 was proposed through analyzing the UV-vis spectra and LC-MS spectra of the degradation products and investigating the variations in the concentrations of the degradation products in the culture. It is suggested that the azo bond of AR30 was initially cracked by azo reductase to produce 2,6-dichloro-4-nitrobenzenamine and 2-[ (4-aminophenyl)-(2-cyanoethyl) amino] ethylacetate, which then generated several aromatic amine compounds under the actions of nitroreduction, aminoacylation and cyano hydrolysis, respectively.

Key words: azo dye; decolourisation and degradation; Streptomyces sp.; degradation mechanism; azo reductase

偶氮染料广泛应用于纺织、食品、造纸、化妆品、制药等行业,是指含有芳环或杂环母核与偶氮双键的一类化合物,很难在环境中降解<sup>[1]</sup>,属于"三致"化合物<sup>[2~4]</sup>.研究偶氮染料化合物的生物降解作用,对构建经济高效与环境友好的偶氮染料废水处理工艺具有重要指导意义<sup>[5~7]</sup>.目前,报道的偶氮染料降解微生物种类主要有真菌<sup>[8,9]</sup>、细菌<sup>[10,11]</sup>和藻类<sup>[12~14]</sup>,作用机制主要是吸附和降解等<sup>[15]</sup>,而且效果非常明显.本研究从印染厂污泥中筛选到1株对偶氮染料红30(AR30)具有较强脱色降解作用的菌株 Streptomyces sp. FX645,并对降解机制进行了初步探讨.

#### 1 材料与方法

#### 1.1 污泥样品

本研究中污泥样采自湖南省株洲市棉纺厂印染车间废水滤池,运输时使用冰袋保护包装,使用前放入实验室-20℃冰箱中冷冻保存.

#### 1.2 试剂与仪器

#### 1.2.1 试剂与培养基

偶氮染料  $AR30(C_{19}H_{17}O_4N_5Cl_2)$ ,分析纯,由浙 江润土染料有限公司赠送,相对分子质量为 449,结 构式如下:

$$O_2N - \bigvee Cl \\ N = N - \bigvee CH_2CH_2CN \\ CH_2CH_2OCCH_3 \\ Cl$$

染料母液:用二甲亚砜 (DMSO) 配制成 50 g·L<sup>-1</sup>的 AR30 母液,经 0.22 μm 滤膜过滤除菌备用.

筛选培养基:琼脂 20 g,染料母液 2 mL,无菌水 定容至 1 L.

富集培养液:葡萄糖 5 g,蛋白胨 2 g,牛肉膏 3 g, Na<sub>2</sub>HPO<sub>4</sub> 3.5 g, KH<sub>2</sub>PO<sub>4</sub> 1.5 g, MgSO<sub>4</sub> 0.2 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01 g,无菌水 1 L,调 pH 为 7.0.

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脱色降解培养液: NH<sub>4</sub>Cl 5 g, Na<sub>2</sub>HPO<sub>4</sub> 3.5 g, KH<sub>2</sub>PO<sub>4</sub> 1.5 g, MgSO<sub>4</sub> 0.2 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01 g,染料母液 2 mL,无菌水 1 L,调 pH 为 7.0.

#### 1.2.2 主要仪器

YXQ-LS-75S 自动立式高压灭菌锅(苏州江东精密仪器有限公司),YJ-1000 型超净工作台(吴江天润净化设备有限公司),D2F-6050 型真空干燥箱(上海精宏实验设备有限公司),HYG-A 恒温培养箱(江苏太仓市实验设备厂),UV-2100 型紫外可见光光谱仪(北京北分瑞利分析仪器公司).降解产物LC-MS 数据经送样外测获得.

#### 1.3 实验方法

#### 1.3.1 菌株的筛选及鉴定

称取污泥样 5.0 g,放入盛有 50 mL 无菌水并带有玻璃珠的三角烧瓶中,250 r·min  $^{-1}$  振荡约 20 min 后进行梯度稀释,得到稀释  $10 \times 10^{2} \times 10^{3} \times 10^{4} \times 10^{5} \times 10^{6} \times 10^{7}$  倍的稀释液.分别取稀释液 200  $\mu$ L 涂布于筛选培养基中,32℃培养 3 ~5 d,挑选脱色圈较大的菌落进一步分离纯化,并对照文献[16]进行初步鉴定.

#### 1.3.2 脱色降解菌的富集与染料共培养发酵

用接种环挑取纯化的菌株于装有 100 mL 富集培养液的 250 mL 三角瓶中,于 32% 培养 2 d. 取 10 μL 菌液接种于 100 mL 染料浓度为  $100 \text{ mg} \cdot \text{L}^{-1}$ 的脱色降解培养液中,厌氧培养 5 d. 发酵液备用.

#### 1.3.3 紫外-可见光谱分析

取上述 5 mL 染料共培养发酵液,8 000 r·min<sup>-1</sup> 离心,上清液用乙酸乙酯萃取 3 次,合并萃取液,以乙酸乙酯为参比,在 200~900 nm 范围内进行紫外-可见扫描,观察 AR30 的紫外-可见光谱变化.

#### 1.3.4 LC-MS 分析

将上述染料共培养发酵液 500 mL 于8 000 r·min<sup>-1</sup>下离心,用乙酸乙酯萃取上清液 3 次,合并萃取液,浓缩干燥得降解混合产物,进行液相色谱-质谱分析,并与初始染料进行对照.

液相色谱条件:进样 10 μL,波长 289 nm,流动相流速 1 mL·min<sup>-1</sup>;流动相为  $H_2O$  与  $CH_3CN$  的比例在 30min 内由 80: 20 变为 20: 80;色谱柱为 Agilent XDB- $C_{18}$ ( $\Phi$  4.6 mm × 250 mm; 5 μm).

质谱条件:离子源为电喷雾离子源(ESI);扫描方式为正离子扫描(Positive);毛细管电压 4.0 kV;去溶剂温度 350%;去溶剂流速  $10 \text{ L·min}^{-1}$ .

#### 2 结果与讨论

#### 2.1 菌株鉴定结果

从株洲棉纺厂污泥样品中分离筛选到了1株对偶氮染料 AR30 具有较强脱色效果的菌株. 该菌株在高氏 I 号培养基上菌丝较细,生长缓慢,菌落质地致密,表面呈较紧密的绒状. 营养菌丝匍匐生长于培养基内,菌落与培养基结合紧密,不易挑起. 气生菌丝叠生在营养菌丝上,覆盖整个菌落表面. 孢子丝呈弯曲状,孢子为球形,表面光滑,灰黄色,不产生色素,对照文献[16],这些生理生化特点基本符合链霉菌生长形态特征. 另外通过 16S rDNA 分析,测得的 800 bp 碱基对中的 30~650 bp 与 Streptomyces variabilis (AB184884)同源性 100%. 最后选取 11 株模式菌株进行系统发育分析,用 Clustal X 软件和MEGA4. 0 软件构建系统发育树(见图 1),并将其定名为 Streptomyces sp. FX645.

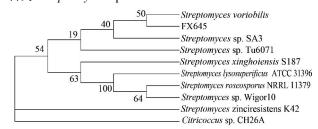


图 1 FX645 和相关菌株的系统发育树

Fig. 1 Phylogenetic tree of strain FX645 and its relatives

#### 2.2 紫外-可见光谱分析结果

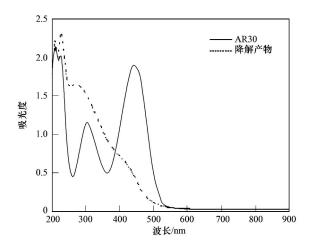
图 2 描述了 AR30 降解前后的紫外-可见吸收光谱变化情况. 降解前, AR30 的特征吸收峰在 289 nm 与 445 nm 处,其中 445 nm 吸收峰是由偶氮染料的发色基(偶氮键)、助色基(硝基、氰基等)产生的. 对 AR30 降解产物而言,紫外光区 289 nm 和可见光区 445 nm 处吸收峰均基本消失,而在 220~250 nm 范围吸光强度较大. 这说明 AR30 已经被链霉菌 FX645 降解,在降解过程中产生了其他化合物,这些化合物在 250 nm 处的吸光度很强,说明可能仍具有苯环结构.

#### 2.3 LC-MS 分析结果

偶氮染料 AR30 质谱图与降解产物液质联用色谱、质谱图如图 3~5 所示.

由质谱图(图3)可以看出,染料 AR30 的 [M+H] <sup>+</sup>与[M+Na] <sup>+</sup>峰的丰度都较大,相对分子质量为449. 从降解产物的液相色谱图(图4)可以看出,5个色谱峰的保留时间分别为2.506、5.908、6.892、9.716、11.013和13.841min,而在AR30的保留值25min处的峰已经消失,说明AR30已经基本降解完全,并生成5种降解产物.

根据降解产物的质谱图(图5),与AR30结构



#### 图 2 降解前后 AR30 的紫外-可见吸收光谱

Fig. 2 UV-Vis absorption spectra of AR30 before and after degradation

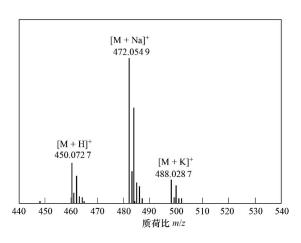


图 3 染料 AR30 质谱图

Fig. 3 Mass Spectrum of AR30

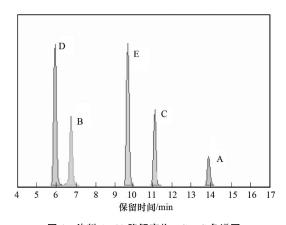


图 4 染料 AR30 降解产物 LC-MS 色谱图

Fig. 4 Chromatography in LC-MS of AR30 degradation products 式加以比较,分析得到 5 个降解产物的结构式, 如下.

产物 A: 4-硝基-2,6-二氯苯胺(2,6-dichloro-4-nitrobenzenamine, $M_r = 206$ );产物 B: 2,6-二氯-1,4-

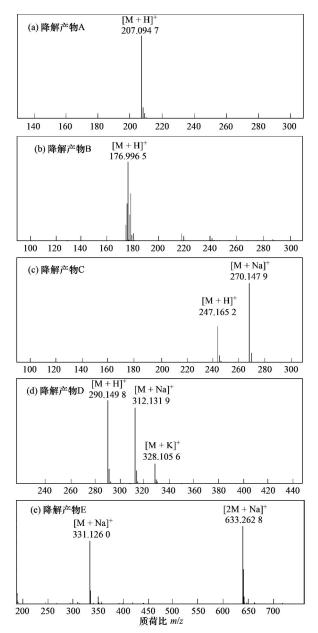


图 5 链霉菌 FX645 降解 AR30 的 5 种产物质谱图

Fig. 5 Mass spectra of 5 degradation products of AR30 by FX645

苯二胺(2,6-dichlorobenzene- 1,4-diamine, $M_r=176$ );产物 C: 2-[(4-胺苯基)-(2-氰乙基)-氨基] 乙酸 乙酯 [2-[(4-aminophenyl) (2-cyanoethyl) amino]ethyl acetate, $M_r=247$ ];产物 D: 2-[(4-乙酰胺苯基)-(2-氰乙基)-氨基] 乙酸乙酯 [2-[(4-acetamidophenyl) (2-cyanoethyl)amino]ethyl acetate, $M_r=289$ ];产物 E: 3-[(4-乙酰胺苯基)-(2-乙酰氧乙基)-氨基] 丙酸 [3-[(4-acetamidophenyl) (2-acetoxyethyl)amino]propanoic acid, $M_r=308$ ].

在降解发酵体系中,12 h 时 5 种产物中的 A、C 浓度较高,而 E 浓度很低; 随降解时间的延长,B、E 浓度逐渐升高,而 A、C 浓度则急剧下降; 36 h 时体

系中 AR30 浓度几近于 0,而只含有 B、D 与 E 化合物. 据此,推测出 *Streptomyces* sp. FX645 降解分散染料 AR30 的可能途径,如图 6 所示.

图 6 菌株 FX645 对 AR30 可能降解途径

Fig. 6 Proposed pathway of AR30 degradation by *Streptomyces* sp. FX645

有研究者认为,偶氮化合物在偶氮还原酶的作用下,可能经过了一个中间过渡态——加氢偶氮苯,也就是说偶氮键的断裂是经由两步还原完成<sup>[17~20]</sup>.由于加氢偶氮苯类物质在整个反应过程中的相对丰度一直较低,不能累积到一定的量,说明它在反应体系中不太稳定,可以推断它们一旦生成就会很快分解.同样,本研究中 AR30 可能也是在偶氮还原酶作用下生成了化合物 A 与 C. 然后发生硝基还原反应 A 生成了 B,C 发生氨基酰化反应生成 D,D 继而发生氰基水解反应生成了 E.

通过以上谱图分析, AR30 脱色降解机制为:在 厌氧条件下, 偶氮染料的偶氮双键被链霉菌 FX645 的偶氮还原酶还原裂解, 并产生一系列中间产物芳 香胺类化合物, 其中偶氮双键的还原是偶氮染料脱 色降解的关键一步.

#### 3 结论

从株洲棉纺厂印染车间污泥样品中分离筛选到了1株对偶氮染料 AR30 具有较强降解脱色能力的菌株 Streptomyces sp. FX645. 根据降解产物的光谱数据,可知 Streptomyces sp. FX645 可将 AR30 降解

为 4-硝基-2,6-二氯苯胺、2,6-二氯-1,4-苯二胺、2-[(4-胺苯基)-(2-氰乙基)-氨基]乙酸乙酯、2-[(4-乙酰胺苯基)-(2-氰乙基)-氨基]乙酸乙酯和 3-[(4-乙酰胺苯基)-(2-乙酰氧乙基)-氨基]丙酸等 5 种苯胺类物质,并根据各降解产物浓度变化规律,提出在偶氮还原酶作用下的降解途径. 生成的苯胺类物质可以转入具有芳香胺降解矿化作用的微生物体系中,进一步降解矿化. 如果将两类微生物加以结合建立组合生物反应器,用于偶氮染料的微生物降解与矿化,有望成为处理偶氮染料废水的重要方法之一. 参考文献:

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