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### 群体感应淬灭菌的分离及其膜污染控制性能

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摘要:通过淬灭细菌的群体感应系统来抑制生物膜形成、防止膜生物污染的方法近年来受到广泛关注.本实验从实际运行污水处理厂活性污泥中分离出 5 株具有群体感应淬灭功能的菌株,其中菌株 HG10 对信号分子 N-乙酰高丝氨酸环内酯(C6-HSL)分解能力最强.经 16S rRNA 基因序列比对,初步鉴定为蜡样芽孢杆菌(Bacillus cereus).用海藻酸钠将菌株 HG10 进行包埋固定,以探究其在膜过滤系统中对膜污染防治的效果.结果表明,经过 8 d 培养,添加细菌包埋珠(SA-HG10)的实验组 B中膜通量为 181.29 L·(m²·h)<sup>-1</sup>,未投加包埋珠的对照组 A 膜通量为 110.64 L·(m²·h)<sup>-1</sup>,B 组膜通量比 A 组高出 63.86%;对微滤膜片上生物膜中 EPS 含量测定表明,实验组 B 中 EPS 多糖和蛋白质含量较对照组 A 分别减少了 29% 和 48%,疏水性蛋白质含量的大量减少是造成膜污染减弱的主要原因;膜表面胞外聚合物(EPS)总含量减少了 43%,表明投放 SA-HG10 细菌包埋珠对过滤膜片上生物膜形成具有明显抑制作用,改善了膜过滤性能.

关键词: 群体感应淬灭; 蜡样芽孢杆菌; 固定化; 膜污染; 胞外聚合物

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# Isolation of Quorum Quenching Bacteria and Their Function for Controlling Membrane Biofouling

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**Abstract:** Interspecies quorum quenching by bacterial cells has been reported as a novel approach for mitigating the biofouling via restraining quorum sensing (QS). Five indigenous quorum quenching bacterial strains were isolated from activated sludge taken from wastewater treatment plant. Strain HG10 showed high degrading activity against C6-HSL (N-hexanoyl-L-Homoserine lactone). The result of 16S rDNA sequencing showed that the isolated strain HG10 belonged to the genus *Bacillus cereus*. Strain HG10 was immobilized in sodium alginate (SA), and the biofouling inhibition of SA-HG10 in membrane filtration treatment system was investigated. The results showed that the membrane flux in experimental group B (provided with SA-HG10) was 181. 29 L·( $m^2 \cdot h$ )<sup>-1</sup>, and the membrane flux in control group A (without the beads) was 110. 64 L·( $m^2 \cdot h$ )<sup>-1</sup>, The membrane flux in group B was 63. 86% higher than group A. The content of extracellular polymeric substances (EPS) in the biofilm on the membrane filter was also measured. The results showed that the contents of polysaccharides and proteins in the experimental group B decreased by 29% and 48% respectively than those of group A. A large decrease in the content of hydrophobic proteins was the main reason for the decrease of membrane fouling. The content of EPS in the membrane surface decreased by 43%, indicating that SA-HG10 could inhibit biofilm formation on the membrane filter and effectively improve the filtering performance.

**Key words:** quorum quenching; *Bacillus cereus* HG10; immobilization; membrane biofouling; extracellular polymeric substances (EPS)

膜过滤处理工艺因其出水浊度低、处理效率高等优势在污水处理中有着广泛应用[1]. 但膜污染成为限制其长久运行的主要问题,其中膜表面生物膜形成导致的生物污染占据重要原因[2]. 目前膜污染防治的方法主要有:①膜表面改性等物理法<sup>[3]</sup>;②利用强酸、强碱、氧化剂进行清洗等化学法<sup>[4,5]</sup>;③酶干扰及群体感应抑制法等生物法<sup>[6]</sup>. 物理法和化学法换膜成本高、风险大,且易造成抗性菌株的产生及新的环境与健康危害<sup>[7]</sup>. 生物法的出现为从根本上防止及控制生物淤积提供了一条可能的途径,由于其环境友好,安全性高及可持续性等优势在

近年来迅速崛起,成为近期膜污染研究的热点[8].

群体感应淬灭法是指通过抑制细菌之间的信号交流(群体感应, quorum sensing, QS)来控制细菌生物膜形成的方法<sup>[9]</sup>. 在群体感应调控的生物膜形成过程中, AHL 类自诱导物(*N*-酰基高丝氨酸环内酯, *N*-acyl homoserine lactone, AHLs)是一种非常重要的信号分子. 近年来,基于群体感应淬灭理论(quorum

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quenching,QQ) 控制膜污染的生物法受到广泛关注<sup>[9,10]</sup>. Oh 等<sup>[11]</sup>尝试将能分解 AHLs 的重组大肠杆菌封装入中空纤维膜的微孔中,有效控制了生物淤积. Kim 等<sup>[12]</sup>从 MBR 活性污泥及膜表面泥饼层中分离出红球属菌(*Rhodococcus* sp. BH4),通过制成细胞包埋珠有效验证了其群体感应淬灭效应.

然而,目前应用于膜污染防治的群体感应淬灭菌只有为数几株且分解信号分子 AHLs 的种类有限,在不同污水环境中,对膜污染的防治效果仍处于探索阶段<sup>[13~15]</sup>. 更多用于膜污染防治的群体感应淬灭菌仍待进一步分离<sup>[16]</sup>. 本研究从实际污水处理厂活性污泥中分离纯化出 1 株群体淬灭功能菌株蜡样芽孢杆菌 HG10,该种菌在土壤、生活污水和垃圾渗滤液等环境中均有发现且已被证实具有群体感应淬灭功能<sup>[17~19]</sup>. 但目前关于该菌在防止膜污染方面的研究尚未见报道;基于此,本实验通过对菌株 HG10 进行包埋固定,验证其群体感应淬灭功能在控制生物膜形成方面的可行性及效果,以期为该种菌在膜污染防治中应用提供数据参考和理论依据.

#### 1 材料与方法

#### 1.1 材料

#### 1.1.1 样品来源

用于菌株分离的活性污泥样取自深圳某污水处理厂.

#### 1.1.2 LB 培养基

蛋白胨 10 g、酵母膏粉 5 g、氯化钠 5 g、葡萄糖 1 g; 蒸馏水1 000 mL,  $121 \degree$  灭菌 15 min, 保存备用. 固体培养基加琼脂 1.5%. 1/2 LB 培养基的各成分含量为上述 0.5 倍.

#### 1.1.3 试剂及菌株

N-乙酰高丝氨酸环内酯(C6-HSL)和 N-癸酰高 丝氨酸环内酯(C10-HSL)均购于 Cayman Chemical (USA); PCR 反应所用 Premix Taq 混合酶购于 TaKaRa(Japan); 包埋菌株所用载体为海藻酸钠 (aladdin,AR); 实验所用微滤膜为 Millipore 微孔过 滤膜(GVWP04700, 0. 22 μm, 47 mm); 报告菌 Chromobacterium violaceum CV026 和 Chromobacterium violaceum VIR07 为实验室保存; 人 工配置污水配方如表 1<sup>[20]</sup>.

#### 1.2 菌株分离纯化

采用基本培养基法定向分离具有 QQ 功能的细菌[13],配置 C6- $HSL(2.5 mmol·L^{-1})$ 为唯一碳源的

表1 合成污水配方

Table	1	Components	of s	vnthetic	wastewater

组分	浓度/mg·L-1
葡萄糖	1 600
酵母提取物	56
蛋白胨	460
$\mathrm{NH_4Cl}$	340
$\mathrm{KH_{2}PO_{4}}$	87
$CaCl_2$	9.8
$MgSO_4 \cdot 7H_2O$	128
$MnCl_2 \cdot 4H_2O$	9. 44
$FeCl_3 \cdot 6H_2O$	8
NaHCO <sub>3</sub>	1 022
pH	7.0 ~ 7.5

基本培养基,将污泥 2 μL 接种于 100 μL 基本培养基中,恒温振荡(700 r·min<sup>-1</sup>,30℃)培养 3 d 后,取 1 μL 接种到新的 C6-HSL 为唯一碳源的基本培养基中,将连续 3 次富集培养后的菌液涂布于 LB 琼脂培养基 30℃恒温培养,于培养 24 h、48 h 时挑取不同形态单菌落进行分离纯化. 对所有分离菌落进行QQ 功能检测.

#### 1.3 分离菌株 QQ 和 QS 功能检测

使用报告菌 CV026 对分离菌株进行 QQ 功能检测:滴加的样品中含有 C6-HSL 时,含有报告株 CV026 的 LB 培养基上呈现紫色反应 [21]. 将分离菌株于 1/2 LB 培养基前培养 12 h后,取菌液以 1:100 体积比转接到新的 1/2 LB 培养基中(其中含有浓度为 10  $\mu$ mol·L<sup>-1</sup> 的 C6-HSL 溶液);在 30%,170 r·min <sup>-1</sup>条件下培养 8、10、12、24 h时,取菌液 500  $\mu$ L 并离心(13 500 r·min <sup>-1</sup>,5 min),上清液经 0.22  $\mu$ m 滤膜过滤后 -20% 冻存;将无菌滤纸片放置于含有 CV026 的 LB 平板上,在滤纸片上滴加冻存滤液 20  $\mu$ L 后,将 LB 平板恒温 30% 培养 24 h,观察滤纸周围显色反应.

分离细菌的 QS 功能检测,使用 CV026 和 VIR07 为报告菌,分别对短链类 AHLs(C4-C8)和长链类 AHLs(C10-C16)进行验证<sup>[21]</sup>. 将分离纯化的 QQ 菌株菌落与报告菌 CV026 和 VIR07 以"T"型划线的方式接种于同一 LB 平板,恒温(30°C)培养 24 h 后观察报告菌显色反应. 10  $\mu$ L 的 C6-HSL 和 C10-HSL(10  $\mu$ mol·L<sup>-1</sup>)分别被划线在指示菌平板上,作为对照.

#### 1.4 QQ 菌株 16S rRNA 基因序列测定

使用通用引物 27F (5'-AGAGTTTGATCCTGG CTCAG-3') 和 1492R (5'-GGCTACCTTGTTACGAC TT-3')进行目的基因片段扩增. 扩增条件: 94℃预

变性 3 min,94℃变性 1 min,54℃退火 30 s,72℃延伸 1.5 min,35 次循环,最后 72℃延伸 10 min.将 PCR 产物进行琼脂糖凝胶电泳后由华大公司进行基因测序,测定序列通过 GenBank 数据库比对 鉴定.

#### 1.5 菌株固定化及 QQ 功能验证

菌株 HG10 与质量-体积浓度为 4% 的海藻酸钠溶液 (SA)按 100 mg·mL⁻¹比例混匀后,滴加到质量-体积浓度为 3% 的 CaCl₂溶液中,4℃交联后低温保存. 制备无细菌包埋的空海藻酸钠珠 (Vacantbeads)为对照组,以探究包埋珠对群体感应信号分子的物理吸附作用.

配置 C6-HSL 浓度为 10 μmol·L<sup>-1</sup>的 LB 培养基 20 mL,设置 3 组实验: 加入 8 颗海藻酸钠细菌包埋珠(SA-HG10)的培养基,加入 8 颗无细菌包埋的空海藻酸钠珠的培养基(Vacant-beads),不加任何包埋珠的培养基作为空白对照(Control). 3 组实验在 70 r·min<sup>-1</sup>,30℃条件下振荡培养,分别于 15、17、19、21、24、26、34、54 h 进行取样,并依据 1. 2 节群体淬灭检测所述方法,测量紫色圆直径,根据公式(1)计算投加不同包埋珠的 C6-HSL 浓度随时间的变化情况<sup>[20,22]</sup>.

 $c = 0.0825 \times e^{0.1533x}$   $(r^2 = 0.98)$  (1) 式中,c 为 C6-HSL 浓度( $\mu mol \cdot L^{-1}$ ); x 为显紫色圆直径(mm).

#### 1.6 生物膜生长鉴定

将 PVDF 材质微滤膜片放入锥形瓶中,依次加入 49 mL 合成污水,1 mL 实验室驯化良好的活性污泥(MLSS = 10 g·L<sup>-1</sup>),1.4 节实验结果证明无细菌包埋的海藻酸钠珠对 C6-HSL 的降解能力与空白对照组无显著差异可忽略不计(2.2 节),故本节设置两组实验:不加包埋珠的锥形瓶作为对照组(A),投加 15 颗 SA-HG10 的锥形瓶作为实验组(B). 在70 r·min<sup>-1</sup>恒温(30℃)条件下培养,每 24 h 进行人工配置污水更换,以提供反应系中微生物生长所需营养成分. 经过一个生物膜生长周期 8 d 后,取出微滤膜片,进行膜通量和生物膜 EPS 测量.

膜通量测定采用超滤杯(UFSC05001, Amicon, USA)自行搭建(图1),氮气提供稳定正压15 kPa±1 kPa,膜片置于超滤杯膜固定装置内,采用超纯水(MilliQ water)作为过滤用水,过滤体积为50 mL,使用量筒和秒表分别记录过滤水量和所需时间.

膜表面生物膜中 EPS 的提取,采用热提取法<sup>[23]</sup>:将反应后膜片剪碎于 15 mL 0.9% NaCl 溶

液中,超声 10 min,150 r·min <sup>-1</sup>摇匀 10 min,超声 5 min,80℃ 水浴 30 min,取出碎膜片后,12 000 r·min <sup>-1</sup>离心 20 min,取上清液测定 EPS 含量,EPS 总量用多糖与蛋白质之和表征.多糖和蛋白质分别采用苯酚-硫酸法和考马斯亮蓝法进行测定<sup>[24]</sup>.

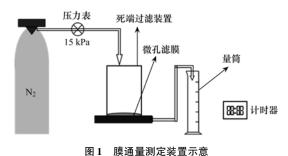


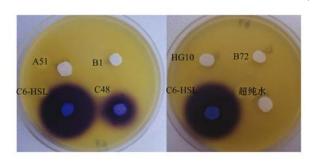
Fig. 1 Schematic of membrane flux test apparatus

#### 2 结果与讨论

#### 2.1 QQ 功能菌株分离及鉴定结果

本研究共分离 18 株优势菌,对分离菌株全部进行群体感应淬灭功能验证,得到 5 株具有群体淬灭功能的细菌. 图 2 为培养 24 h 时 5 株细菌的 QQ 功能检验,菌株 HG10、A51、B1 和 B72 培养 24 h 时,其上清液在滤纸周围无显紫色反应,表明菌株在 24 h 内可将 C6-HSL 完全分解;而菌株 C48 在培养 24 h 后,其上清液在滤纸片周围出现部分紫色,表明C48 在培养 24 h 时能分解部分 C6-HSL,群体淬灭功能较弱. 5 种分离菌的 16S rRNA 基因鉴定结果如表 2 所示.

在天然环境中存在同时具有 QS 和 QQ 功能的细菌<sup>[25]</sup>,对分离菌株的 QS 功能进行检测. 结果如图 3,同属于 *Burkholderia* sp. 属的 B72 和 C48 自身可产生信号分子 AHLs,其中菌株 B72 自身可生产短链类信号分子,C48 可同时生产短链类和长链类信号分子. 排除这两株可自身生成 AHLs 的细菌,



培养时间: 24 h, C6-HSL 溶液(10  $\mu$ mol·L $^{-1}$ ) 和超纯水分别为阳性对照和阴性对照

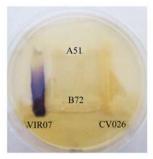
#### 图 2 分离 5 种细菌的群体淬灭功能验证

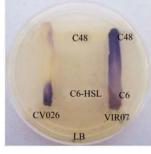
Fig. 2 Bio agar assay for quorum quenching of each strain

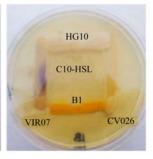
#### 表 2 已分离群体感应淬灭菌株

Table 2	Lists	of	isolated	quorum	quenching	bacterial	strains

菌株	序列号	相似菌株	同源相似性/%
HG10	KX430857	Bacillus cereus strain ATCC 21281	99
A51	KX430853	Delftia tsuruhatensis strain NBRC 16741 [26]	99
B1	KX430854	Chryseobacterium indologenes strain NBRC 14944	99
B72	KX430855	Burkholderia cepacia strain ATCC 55792	100
C48	KX430856	Burkholderia cepacia strain ATCC 51671	99







C6-HSL 和 C10-HSL 作为标准样品

#### 图 3 分离细菌的群体感应功能检测

Fig. 3 Bio agar assay for quorum sensing of each strain

并结合之前 QQ 功能的检验,从余下 3 种菌株 HG10、B1 和 B51 中选取分解 C6-HSL 能力最强的 HG10 作为微生物固定包埋的实验菌种.

#### 2.2 固定化包埋菌的 QQ 功能检验

微生物在固定化包埋过程中,海藻酸钠溶液浓度、交联时间及交联剂浓度等包埋条件均会影响固定化微生物性能,导致包埋菌活性降低<sup>[27]</sup>.图 4为投放 SA-HG10 和空海藻酸钠珠(Vacant-beads)的 LB 培养基中,C6-HSL 浓度随时间的变化曲线.含有菌株 HG10 的海藻酸钠包埋珠在培养 17、19和 21 h 时,对溶液中 C6-HSL 的降解率分别为

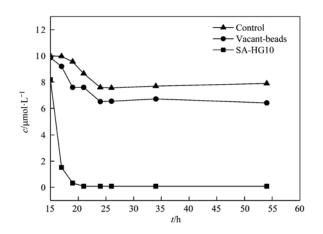


图 4 细菌包埋珠(SA-HG10)、无细菌空海藻酸钠珠 (Vacant-beads)对 C6-HSL 降解能力检测

Fig. 4 Comparison of QQ activity of beads with strain HG10, vacant beads and control

85%、96%和99%,表现出极强的群体感应淬灭功能;投放空海藻酸钠珠(Vacant-beads)的一组C6-HSL减少量仅为20%~26%,这可能是由于空海藻酸钠珠的物理吸附作用造成<sup>[12]</sup>. Khan等<sup>[14]</sup>在对甲基状芽孢杆菌研究中得出类似结果:在培养时间为16~23h内,甲基状芽孢杆菌对信号分子C12-HSL的降解率可达到90%~96%.本研究中,用海藻酸钠固定的菌株 HG10在24h对信号分子C6-HSL降解率达到99%以上,具有极强的群体淬灭功能.

#### 2.3 固定化菌株对膜污染的控制研究

#### 2.3.1 微滤膜片外观变化

于锥形瓶中取出培养 8 d 后的过滤膜片,微滤膜片表观外貌如图 5 所示. 未投加包埋珠的对照组 A 膜表面观察到明显的鞭毛状絮体<sup>[15]</sup>,已形成成熟生物膜. 实验组 B 滤膜表面微生物絮体附着量较少,表明投加 SA-HG10 细菌包埋珠对滤膜表面微生物的附着生长产生了抑制作用,进而控制膜污染的加剧.

#### 2.3.2 膜通量变化

图 6 为不同反应体系中微滤膜过滤液体积随时间的变化曲线,相比于新膜片过滤 50 mL 超纯水需要 90 s 时间,实验组 B 需要 653 s,未投加任何包埋珠的对照组 A 则需更长时间(1070 s),膜过滤渗透性能开始下降,已出现明显膜污染. 通过公式(2)计算不同反应体系内过滤膜通量.



A 组和 B 组分别为未投加包埋珠和投加细菌包埋珠 SA-HG10 的测试滤膜,下同 **图 5** 不同实验条件下过滤膜片对比

Fig. 5 Comparison of membrane filters under different test conditions

$$LMH = \frac{V_{50}}{A \times t} \tag{2}$$

式中,LMH 为膜通量[ $L \cdot (m^2 \cdot h)^{-1}$ ], $V_{50}$ 为过滤液体积(50 mL),A 为膜有效过滤面积(13.4 cm<sup>2</sup>),t 为过滤时间(s).

得出 B 组膜通量为 181. 29 L·(m²·h) -1,比对 照组 A 的 110. 64 L·(m²·h) -1高出 63. 9%,表明通 过群体感应淬灭机制有效提高了膜通量. Monzon 等[28]提出,环境中微生物细菌高密度聚集并通过群体感应机制形成生物膜. 本研究中,群体感应淬灭菌株 HG10 通过抑制上述过程的发生,使膜表面微生物的附着减少,膜污染程度显著减缓.

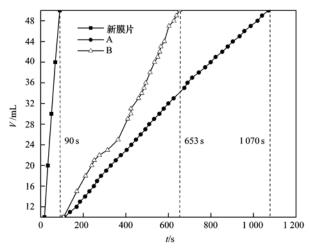


图 6 不同反应体系膜过滤性能比较

Fig. 6 Comparison of membrane flux of different fouled membranes

#### 2.3.3 EPS 含量变化

大量研究表明,生物膜中胞外聚合物(EPS)是引起膜污染的主要污染物<sup>[23,29]</sup>. 微生物分泌而形成的胞外聚合物中,多糖和蛋白质的含量可以占到其主要成分的60%~70%<sup>[30]</sup>,对过滤膜表面生物膜中EPS进行提取,并测定其多糖和蛋白质含量,结果如图 7 所示. 对照组(A)膜表面多糖和蛋白质含量分

别为 11. 43 μg·cm<sup>-2</sup>和 37. 07 μg·cm<sup>-2</sup>, 而含有蜡样 芽孢杆菌包埋珠的实验组(B)滤膜片上生物膜中多 糖和蛋白质含量分别为 8.09 μg·cm<sup>-2</sup> 和 19.32 μg·cm<sup>-2</sup>, 较对照组 A 分别减少了 29% 和 48%. 蛋 白质含量的大量减少很可能是造成生物膜形成减少 的主要原因:蛋白质属疏水性物质,Le-Clech等[31] 研究发现较多的疏水性物质将会加强微生物絮体在 膜表面的附着,同时提出相对于多糖,蛋白质更易导 致微生物絮体的疏水性. B 组疏水性蛋白质含量较 多糖减少更多,导致膜表面疏水性物质的大量减少, 进而导致生物膜附着量的减少: B 组胞外聚合物 EPS 较对照组 A 组减少了 43%; Jiang 等[32] 将海藻 酸钠包埋群体淬灭酶运用在膜生物反应器中,也发 现膜组件表面 EPS 较对照组相比减少了大约 50%, 表明在本实验中,群体感应淬灭技术对膜污染的减 缓很可能是由于减少了生物膜中 EPS 含量而造成.

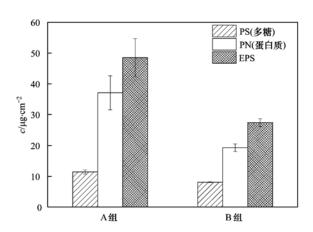


图 7 无细菌包埋珠的对照组(A组)和含有细菌包埋珠的实验组(B组)过滤膜表面多糖和蛋白质含量

Fig. 7 PS and PN concentrations on the surface of membrane filter in control group (A) and experiment group (B)

#### 3 结论

(1)从实际运行的污水处理厂活性污泥中分离

出1株新型群体感应淬灭菌 HG10,初步鉴定为蜡样 芽孢杆菌 Bacillus cereus.

(2) HG10 制成的包埋珠(SA-HG10) 对群体感应信号分子 C6-HSL 具有 99% 的降解能力; 其投加到模拟废水过滤系统中,提高了 63.9% 的膜通量,改善了膜过滤性能,为该菌在实际工程应用中节省能耗和成本提供了理论基础.

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### **HUANJING KEXUE**

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