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外源 Ca^{2+} 对 SBR 启动期活性污泥胞外多聚物的动态影响

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摘要: 利用无机物如 Ca^{2+} , 加快活性污泥反应器启动, 强化活性污泥絮体性能和结构稳定性, 受到越来越多的重视. 采用序批式反应器, 研究进水中添加 Ca^{2+} 对反应器启动期活性污泥沉降性能和胞外多聚物的影响. 结果表明运行至 28 d, 与进水中不添加 Ca^{2+} 的反应器(对照反应器)相比, 进水中添加 $150 \text{ mg} \cdot \text{L}^{-1}$ 外源 Ca^{2+} 的反应器中活性污泥 MLSS 和 MLVSS 值分别高出了 89.6% 和 75.6%, SVI 值则降低了 47.9%; 活性污泥胞外多聚物总量增加了 76.4%, 多糖增加了 28.8%, 蛋白质减少了 31.6%, 添加 $150 \text{ mg} \cdot \text{L}^{-1}$ 外源 Ca^{2+} 的反应器中污泥胞外多聚物中多糖/蛋白质值为 68.8, 对照反应器的活性污泥胞外多聚物多糖/蛋白质值仅为 36.6. 三维荧光光谱和红外光谱分析表明外源 Ca^{2+} 导致活性污泥胞外多聚物组分发生了变化. 实验结果为进水中添加外源 Ca^{2+} 改善活性污泥沉降性能提供了基础数据.

关键词: 活性污泥; 启动期; 胞外多聚物; 沉降性能; 动态变化

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Evolution of Extracellular Polymeric Substances of the Activated Sludge with Calcium Ion Addition During Set-up Period of Sequencing Batch Reactors

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Abstract: Great attention has been paid to accelerate the start-up period and enhance floc properties and structural stability in activated sludge reactors with the aid of inorganic chemical agents such as calcium ion. The laboratory-scale sequencing batch reactors (SBRs) were operated continuously for 35 days to investigate the effect of calcium ion (Ca^{2+}) on the physicochemical properties and evolution of extracellular polymeric substances (EPS) of activated sludge during set-up period. When compared to the control (non-calcium ion addition), the addition of $150 \text{ mg} \cdot \text{L}^{-1} \text{Ca}^{2+}$ to the influent significantly increased the mixed liquid suspended solids (MLSS) and the mixed liquid volatile suspended solids (MLVSS) by 89.6% and 75.6% on 28 d, respectively, and decreased the sludge volume index (SVI) by 47.9% following SBRs set-up. Compared with the control system, the contents of EPS, polysaccharides (PS) and proteins (PN) were increased by 76.4%, 28.8% and 31.6% under the condition of Ca^{2+} dosage of $150 \text{ mg} \cdot \text{L}^{-1}$. The PS/PN ratio was 68.8 for Ca^{2+} addition, compared to only 36.6 for the control reactor. Analysis using three-dimensional excitation emission matrix fluorescence spectroscopy and Fourier transform infrared spectrum revealed that Ca^{2+} addition changed the compositional characteristics of EPS. Results from this study provided a fundamental knowledge basis for the improvement of the settling properties of activated sludge with calcium ion addition.

Key words: activated sludge; set-up period; extracellular polymeric substances; settling properties; dynamic change

活性污泥工艺是目前应用最广泛的废水生物处理技术,它利用活性污泥(微生物聚集体)去除水中的各种污染物^[1,2]. 活性污泥法废水处理工艺中,二沉池内的活性污泥通过絮凝和沉淀实现泥水分离,是决定出水水质、保证废水处理构筑物内持有稳定数量微生物的关键步骤之一. 然而,活性污泥不能有效絮凝形成大、密实、强度高的污泥絮体几乎是每个污水处理厂都可能遇到且难以快速解决的问题. 研究表明在水中投加适量阳离子如 Ca^{2+} 、 Mg^{2+} 、 Fe^{3+} 等能够强化活性污泥的絮凝性能、改善污泥沉降性能,促进活性污泥微生物多样性等^[3~6].

活性污泥絮体主要由细菌、水、胞外聚合物

(extracellular polymeric substances, EPS)等组成^[7]. 研究表明 EPS 在活性污泥的生物絮凝中起着关键作用^[8,9]. EPS 与细菌菌体表面均带负电荷^[10],金属阳离子如 Ca^{2+} 可基于 DCB (divalent cation bridging)理论与 EPS 相互作用,形成 $\text{EPS}-\text{Ca}^{2+}-\text{EPS}$ 联接物,构成絮体骨架^[11],促进活性污泥絮体形成与稳定^[5,12].

尽管有许多外源阳离子促进活性污泥微生物自

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固定化(污泥颗粒化)的研究,这些研究着眼于阳离子投加量^[13]、反应器工艺参数^[14,15]、污染物去除性能^[16]等方面,为人们深入理解外源阳离子强化/改进活性污泥絮凝性能的机制奠定了重要的基础。然而,对于金属离子如何影响反应器启动期活性污泥沉降性能,EPS 产生量、组分变化等鲜有报道。本文在活性污泥反应器启动期,外源添加 Ca^{2+} ,分析 Ca^{2+} 对活性污泥生物量、沉降性能,EPS 含量与组成随运行时间的动态变化,以期外源 Ca^{2+} 改进和强化活性污泥絮凝性、提升污水生物处理系统稳定性的技术应用提供理论基础。

1 材料与方法

1.1 实验装置

接种污泥取自无锡某城市污水处理厂,污泥混合液悬浮固体浓度 MLSS 为 $3\,680\text{ mg}\cdot\text{L}^{-1} \pm 2\text{ mg}\cdot\text{L}^{-1}$,SVI 为 $76\text{ mL}\cdot\text{g}^{-1} \pm 3\text{ mL}\cdot\text{g}^{-1}$ 。实验装置采用模拟 SBR,有效容积为 1 L。SBR 运行周期为 6 h:进水 5 min、曝气 320 min、沉降 30 min、排水 5 min。

实验废水为人工配置,模拟中等强度城市生活污水水质。人工废水组成: $\text{C}_6\text{H}_{12}\text{O}_6$, $375\text{ mg}\cdot\text{L}^{-1}$; CH_3COONa , $586\text{ mg}\cdot\text{L}^{-1}$; NH_4Cl , $200\text{ mg}\cdot\text{L}^{-1}$; $\text{KH}_2\text{PO}_4 \cdot 3\text{H}_2\text{O}$, $700\text{ mg}\cdot\text{L}^{-1}$; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $500\text{ mg}\cdot\text{L}^{-1}$,微量元素 $1\text{ mL}\cdot\text{L}^{-1}$ 。微量元素组成见文献[6]。使用 NaHCO_3 调节废水 pH 使其保持在 6.5 ~ 7.5 范围。

前期实验,在进水中外源添加不同质量浓度 Ca^{2+} (0、30、50、100、150、200 $\text{mg}\cdot\text{L}^{-1}$),当 Ca^{2+} 添加量为 100 ~ 200 $\text{mg}\cdot\text{L}^{-1}$ 时,明显改善了活性污泥沉降性能和污染物去除性能^[6],因此本研究中外源 Ca^{2+} 添加量设置为 150 $\text{mg}\cdot\text{L}^{-1}$ (在图中以 Ca 表示),同时以进水中不添加 Ca^{2+} (0 $\text{mg}\cdot\text{L}^{-1}$) 的 SBR 作为对照(以 CK 表示),研究外源添加 Ca^{2+} 对活性污泥启动期污泥性能及 EPS 产生的动态影响。

1.2 EPS 提取

EPS 采用超声-阳离子交换树脂法从活性污泥中提取^[17,18]。将 10 mL 活性污泥用超声波清洗仪(KQ-700DE,昆山市超声仪器公司)在 40 kHz、100 W 条件下超声 2 min。每 10 mL 活性污泥加入 1 g 阳离子交换树脂(Dowex 50WX8, hydrogen form, 200 ~ 400 mesh, Sigma-Aldrich, USA);置于摇床(HYL-A,太仓强乐),200 $\text{r}\cdot\text{min}^{-1}$ 转速,振荡 4 h;污泥和树脂混合液在 6 000 $\text{r}\cdot\text{min}^{-1}$ (Centrifuge 5810R, Eppendorf)、4℃ 下离心 30 min,所得上清液即为

EPS 溶液。

EPS 数量以总有机碳(total organic carbon, TOC)来表征,采用 TOC 分析仪(TOC-VCPN analyzer, Shimadzu, Japan)测定。EPS 中多糖(polysaccharides, PS)采用苯酚-硫酸法测量,标准物质为葡萄糖;蛋白质(proteins, PN)采用考马斯亮蓝法测量,牛血清白蛋白作为标准物质^[19]。

1.3 三维荧光光谱分析

EPS 的三维荧光光谱(three-dimensional excitation emission matrix fluorescence spectroscopy, 3D-EEM)采用荧光分光光度计(LS-55, Perkin-Elmer Co., USA)测定。使用氙弧灯为激发光源,激发波长 E_x 为 200 ~ 400 nm,发射扫描波长 E_m 为 280 ~ 500 nm;激发与发射狭缝宽度均为 5 nm,激发波长扫描间隔为 10 nm,扫描速度为 1 200 $\text{nm}\cdot\text{min}^{-1}$; Origin 8.0 软件进行数据分析。

1.4 红外光谱分析

反应器中活性污泥浓度、污泥沉降性能及污染物(C、N、P 等)去除性能保持稳定时,反应器进入启动末期或者稳定期^[4]。在反应器启动末期,采集活性污泥样品提取 EPS,进行红外光谱(fourier transform infrared spectrum, FT-IR)分析。EPS 提取物在 -80℃ 冰箱(SANYO, 日本)冷冻 24 h,然后置于冷冻干燥仪(Christ ALPHA 2-4 LD, 德国)干燥处理。完全干燥后,EPS 与 KBr 以 1:100(质量比)的比例混合,放在玛瑙坩埚里研磨均匀。取约 150 mg 样品,压缩成薄片,采用 NEXUS 870FT 红外光谱仪(Nicolet, USA)分析。红外光谱分析仪扫描波长为 4 000 ~ 400 cm^{-1} ,扫描 32 次,指定分辨率为 4 cm^{-1} 。

1.5 污泥生物量与沉降性能分析方法

混合液悬浮固体浓度(mixed liquor suspended solids, MLSS)、混合液挥发性悬浮固体浓度(mixed liquor volatile suspended solids, MLVSS)是计量活性污泥反应器中生物量的指标,污泥容积指数(sludge volume index, SVI)是表征活性污泥沉降、浓缩性能的指标^[20],其测定方法采用标准方法测定^[21]。SBR 中泥水混合液溶解氧(dissolved oxygen, DO)采用 Thermo 公司 Orion 3star DO 仪测定,混合液 pH 用雷磁 PHS-3C 精密 pH 计测定,确保反应器运行参数稳定。

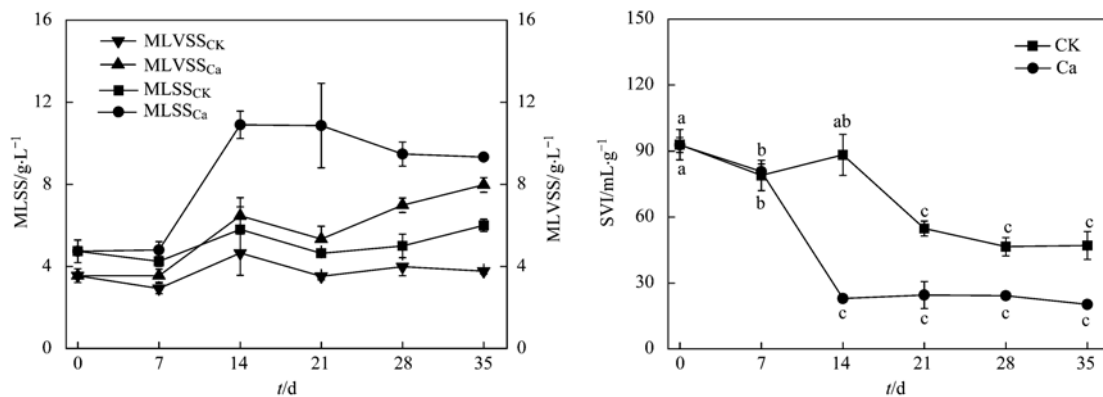
2 结果与讨论

2.1 启动期 SBR 活性污泥生物量及沉降性能的动态变化

在进水中分别添加 0 和 150 $\text{mg}\cdot\text{L}^{-1}$ Ca^{2+} ,对

SBR 启动期活性污泥生物量、沉降性能的影响如图 1 所示. SBR 接种污泥的 SVI、MLSS 和 MLVSS 分别是 $92.9 \text{ mL} \cdot \text{g}^{-1}$ 、 $4.7 \text{ g} \cdot \text{L}^{-1}$ 和 $3.6 \text{ g} \cdot \text{L}^{-1}$. SBR 运行前 7 d 中, 2 个反应器中活性污泥生物量没有明显变化, 活性污泥微生物处在接种后的适应期; 但 2 个反应器中活性污泥 SVI 值与接种污泥相比均有明显降低. 运行 7 d 后, 与接种污泥相比, 2 个反应器中活性污泥生物量都有显著增加. 运行至 28 d, 与接种污泥相比, 进水中添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{ Ca}^{2+}$ 的 SBR 中活性污泥生物量大幅度提高, MLVSS 增长率为 96.9%, SVI 显著降低, 为 $24.3 \text{ mL} \cdot \text{g}^{-1}$; 进水中没有添加外源 Ca^{2+} 的 SBR, 活性污泥生物量也有增长, MLVSS 增长率为 12.1%, SVI 值为 46.5

$\text{mL} \cdot \text{g}^{-1}$. 运行至 28 d, 添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{ Ca}^{2+}$ 的 SBR 中活性污泥生物量 MLVSS 与 SVI 分别是对照反应器中污泥的 1.8 倍和 0.5 倍. 可见, 进水中添加外源 Ca^{2+} 在活性污泥启动期明显促进了活性污泥微生物的生长, 改善了污泥的沉降性能. Yu 等发现 $300 \text{ mg} \cdot \text{L}^{-1}$ 以下外源 Ca^{2+} 促进了活性污泥系统中微生物的生长和沉降性能, 但超过 $300 \text{ mg} \cdot \text{L}^{-1}$ 时, 活性污泥的浓度反而降低, 沉降性能下降, 系统对污染物的去除率也会降低^[12]. Liu 等^[22] 在反应器中加入外源 Ca^{2+} 和 Mg^{2+} , 发现 Ca^{2+} 能够有效促进活性污泥颗粒的形成, 使活性污泥表现出更好的沉降性能, 而 Mg^{2+} 主要影响活性污泥微生物的多样性.



CK: 进水中不添加 Ca^{2+} 的反应器; Ca: 进水中添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{ Ca}^{2+}$ 的反应器

图 1 启动期活性污泥生物量与沉降性能的动态变化

Fig. 1 Dynamic changes of biomass and settling property of activated sludge during the set-up period of SBRs

2.2 启动期活性污泥 EPS 含量及组分的变化

EPS 在活性污泥絮体的形成及絮体结构稳定性的维持有着重要作用^[23]. 在 SBR 启动期间, 活性污泥絮体产生的 EPS 动态变化如图 2 所示. SBR 运行至 21 d, 与接种污泥相比, 进水中添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{ Ca}^{2+}$ 的活性污泥, EPS 产生量显著增加, 达 $71.3 \text{ mg} \cdot \text{L}^{-1}$, 与接种污泥相比, 增加了 25.2%; 进水中不添加 Ca^{2+} 的活性污泥, EPS 产生量反而降低, 与接种污泥相比, 降低了 13.0%. 随着运行时间增加, 2 个 SBR 活性污泥产生的 EPS 没有显著性增加或降低. 运行第 28 d, 进水中添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{ Ca}^{2+}$, 其活性污泥 EPS 数量为 $80.2 \text{ mg} \cdot \text{L}^{-1}$, 对照反应器中活性污泥 EPS 仅有 $45.5 \text{ mg} \cdot \text{L}^{-1}$.

在 SBR 启动期, Ca^{2+} 不仅对 EPS 数量有影响, 对 EPS 组成也有明显影响 (图 3). 总体上, 进水中无论是添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{ Ca}^{2+}$ 还是不添加 Ca^{2+} , 与接种污泥相比, EPS 中多糖含量呈明显增加趋势, 蛋白质含量呈明显下降趋势. 运行至 28 d, 进水中添

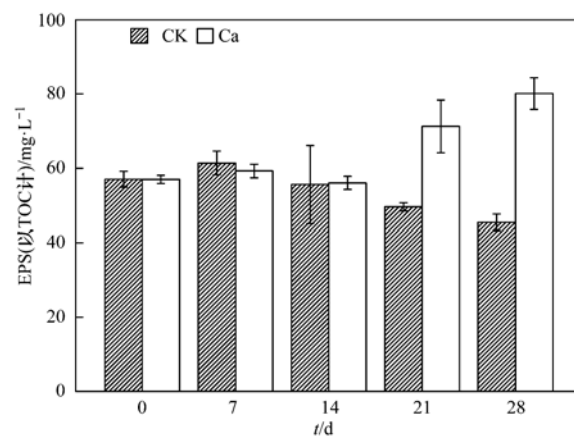


图 2 启动期活性污泥 EPS 数量的动态变化

Fig. 2 Dynamic changes of EPS of the activated sludge during the set-up period of SBRs

加 $150 \text{ mg} \cdot \text{L}^{-1} \text{ Ca}^{2+}$ 的活性污泥, 与接种污泥相比, 多糖增加了 90.3%, PS/PN 值由 6.4 增加到 68.8, 而蛋白质下降了 82.4%; 不添加 Ca^{2+} 的活性污泥, 多糖增加了 53.5%, PS/PN 值由 5.2 增加到 36.6,

而蛋白质下降了 78.1%。进水中添加 Ca^{2+} , 明显促进了胞外多聚物中多糖含量的增加。活性污泥絮体中的多糖在细胞间黏附以及微生物絮凝中起重要作用。多糖官能团例如 $-\text{OH}$, 可以和 Ca^{2+} 作用, 形成紧密、不易变形的胶状介质, 提高污泥絮体的稳定性^[12], 从而使反应器有更高的抗冲击负荷性能。Adav 等^[24] 认为多糖类物质是好氧颗粒污泥形成的重要物质, 他们在研究中发现, 与接种污泥相比, 好氧颗粒污泥 EPS 的 PS/PN 比例明显升高, 由 3.4 升到 6.2。Jiang 等^[25] 在进水中添加 $100 \text{ mg} \cdot \text{L}^{-1} \text{Ca}^{2+}$, 颗粒污泥形成时间由 32 d 缩短为 16 d, EPS 中 PS

含量由 $41 \text{ mg} \cdot \text{g}^{-1} \text{VSS}$ 增加到 $92 \text{ mg} \cdot \text{g}^{-1} \text{VSS}$ 。但也有研究表明蛋白质是影响活性污泥絮凝性能的主要因素。张燕等^[26] 研究 Ca^{2+} 对生物膜形态结构及其组分的影响, 发现生物膜 EPS 中胞外蛋白质含量为 $1.7 \text{ mg} \cdot \text{mL}^{-1}$, 而多糖含量仅为 $0.37 \text{ mg} \cdot \text{mL}^{-1}$ 。Mcswain 等^[27] 在培养好氧颗粒污泥时发现 EPS 中蛋白质/多糖的比例始终维持在 6.6 ~ 10.9 之间, 甚至有研究者认为多糖类物质的大量生成是代谢阻滞现象, 可能会阻止微生物间的聚集^[28]。多糖和蛋白质是 EPS 中主要的组成部分, 其种类和含量直接决定了活性污泥的表面性质, 絮凝和沉降性能^[29]。

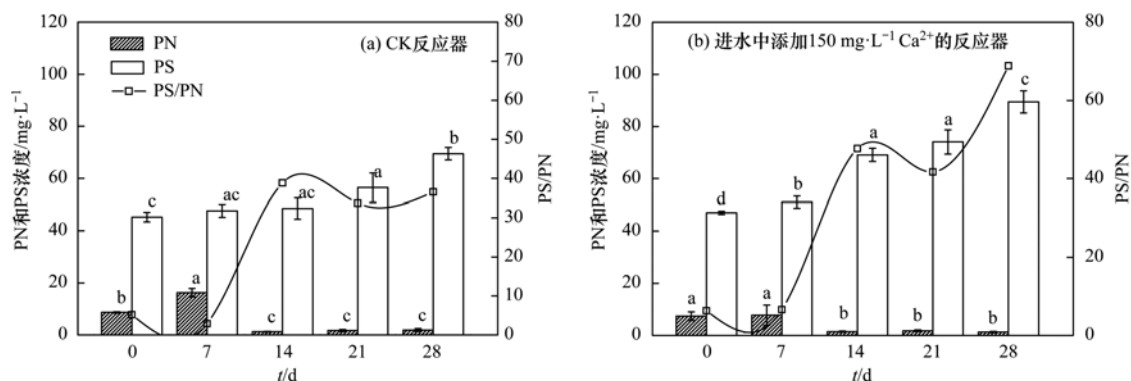


图3 启动期活性污泥 EPS 组分的动态变化

Fig. 3 Dynamic changes of EPS constituents in the activated sludge during the set-up period of SBRs

2.3 启动期活性污泥 EPS 三维荧光光谱的动态变化

3D-EEM 被认为是一种选择性高、灵敏度好的光谱技术, 而且其所需样品量少, 不破坏被测物质结构, 广泛应用于 EPS 组分、官能团鉴定^[30]。进水中添加外源 Ca^{2+} 与不添加 Ca^{2+} 活性污泥 EPS 的三维荧光光谱见图 4。进水主要成分为葡萄糖、乙酸钠和无机盐类, 这些物质均不产生荧光^[31], 因此三维荧光光谱检测到的物质来源于 EPS 产物。在 SBR 启动期, 活性污泥三维荧光光谱中具有 4 个荧光峰, A 峰 ($E_x/E_m = 225 \sim 230/250 \sim 343 \text{ nm}$), B 峰 ($E_x/E_m = 250 \sim 300/340 \sim 350 \text{ nm}$), C 峰 ($E_x/E_m = 250 \sim 280/280 \sim 300 \text{ nm}$), D 峰 ($E_x/E_m = 200 \sim 250/250 \sim 300 \text{ nm}$), 这些峰均属于芳香族类蛋白质, 其中, D 峰为酪氨酸^[32]。本实验采用阳离子交换树脂作为 EPS 的提取剂, 腐殖酸及富里酸类化合物没有在三维荧光光谱中检测到, 这可能是阳离子交换树脂对 EPS 中腐殖酸类物质提取效率较差的缘故^[33]。EPS 组分测定结果显示进水中含 Ca^{2+} 的活性污泥 EPS 中总蛋白质含量较 CK 反应器中活性污泥 EPS 总蛋白质含量低, 但从 3D-EEM 光谱图中, 与对照反应器相比, 外源加入 Ca^{2+} 活性污泥 EPS 中芳香族类蛋白质

更多, 酪氨酸具有稳定长链环状结构, 可以稳定污泥絮体^[30]。Sheng 等^[34] 通过外源加入不同浓度 Ca^{2+} , 发现 Ca^{2+} 对好氧污泥及厌氧污泥 EPS 的三维荧光图谱、峰强度和峰位置影响较小, Hg^{2+} 对污泥 3D-EEM 图谱影响较大, 虽然图谱中峰位置没有发生明显变化, 但峰强度随着 Hg^{2+} 浓度的增加而显著降低, Cu^{2+} 也会有相同的影响^[35]。

2.4 启动末期活性污泥 EPS 的红外光谱分析

红外光谱分析可以表征 EPS 中官能团变化^[30], 当 SBR 运行至启动末期即稳定期时 (28 d), 将 2 个 SBR 中活性污泥 EPS 进行了红外光谱分析 (图 5)。红外谱图中 $1800 \sim 900 \text{ cm}^{-1}$ 与氨基、羰基等官能团相关^[36], 1517 cm^{-1} 表征酪氨酸苯酚结构中芳香环振动引起的肩峰^[30], 2 组污泥 EPS 中均存在此峰, 但在 CK 污泥中此峰强度低于进水中添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{Ca}^{2+}$ 的污泥, 吸收峰越高, 表明该峰代表的基团浓度越高^[30]。进水中添加 $150 \text{ mg} \cdot \text{L}^{-1} \text{Ca}^{2+}$ 的活性污泥 EPS 红外光谱与 CK 相比, 存在 3 处有差异的吸收峰, 酰胺蛋白质肽键分别出现在 1710 cm^{-1} (CK 反应器)、 1730 cm^{-1} ($150 \text{ mg} \cdot \text{L}^{-1} \text{Ca}^{2+}$ 反应器) 处, 脂碳链 C—H 分别出现在 1950 cm^{-1}

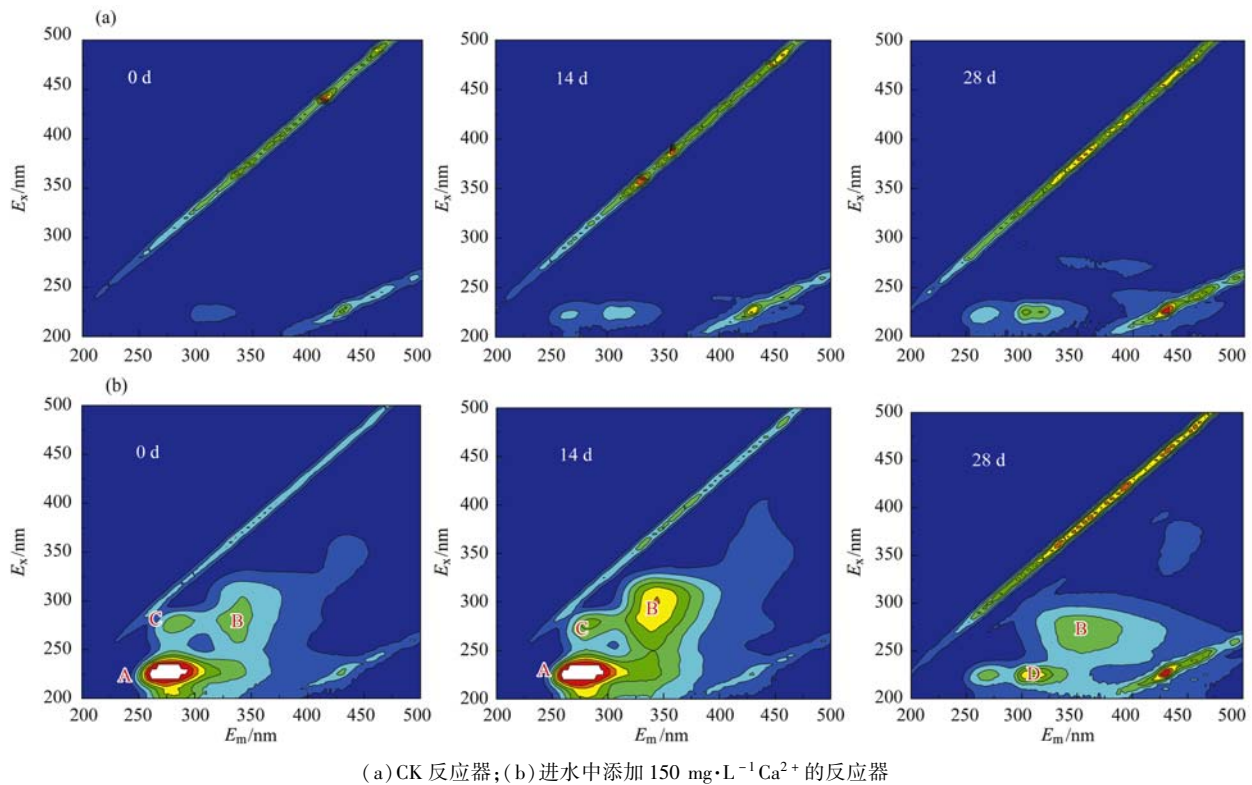


图 4 启动期活性污泥三维荧光光谱的动态变化

Fig. 4 Dynamic changes of 3D-EEM spectroscopy of activated sludge during the set-up period of SBRs

(CK 反应器)、 $1\,940\text{ cm}^{-1}$ ($150\text{ mg}\cdot\text{L}^{-1}\text{ Ca}^{2+}$ 反应器), 缔合—OH 及氨基的峰则分别出现在 $3\,390\text{ cm}^{-1}$ (CK 反应器)、 $3\,410\text{ cm}^{-1}$ ($150\text{ mg}\cdot\text{L}^{-1}\text{ Ca}^{2+}$ 反应器) 处, 这表征 C=O 及 C—H 在相应位置已发生变化. 这些变化显示 Ca^{2+} 可能导致 EPS 中官能团发生变化, EPS 中官能团的改变对活性污泥的形成与结构稳定性影响较大^[37]. Zhu 等^[30] 也发现污泥沉降性能良好时, 与接种污泥相比, 活性污泥 EPS 的红外光谱发生变化, 多糖成分、含量的改变及在颗粒污泥形成过程起重要作用.

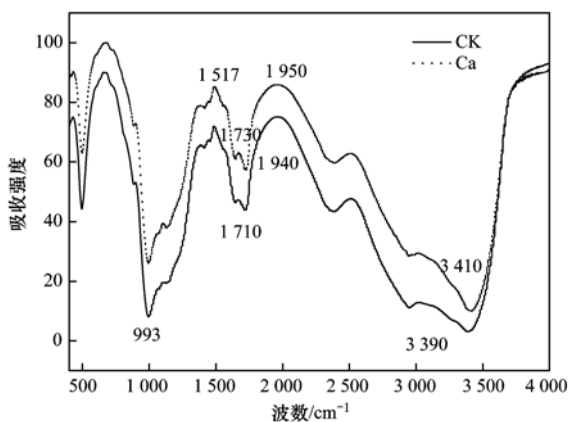


图 5 启动末期活性污泥 EPS 的红外图谱

Fig. 5 FT-IR spectroscopy of the EPS of the activated sludge at the end of set-up period

3 结论

(1) 与对照相比, 进水中添加 $150\text{ mg}\cdot\text{L}^{-1}$ 外源 Ca^{2+} 可以明显促进活性污泥生物量增加、改善污泥沉降性能. 运行约 28 d, 反应器进入稳定运行期, 与对照相比, 添加 Ca^{2+} 的反应器活性污泥 MLSS、MLVSS 分别增加了 89.6%、75.6%, SVI 降低了 47.9%.

(2) Ca^{2+} 促进活性污泥微生物分泌更多的胞外多聚物, 相同工况下运行 28 d, 进水中加入 $150\text{ mg}\cdot\text{L}^{-1}\text{ Ca}^{2+}$ 的活性污泥 EPS 比对照活性污泥 EPS 增加 $34.7\text{ mg}\cdot\text{L}^{-1}$, EPS 中多糖量增加 28.8%, 有利于增加细胞间粘附以及微生物絮凝.

(3) 活性污泥 EPS 三维荧光光谱和红外光谱分析表明, 添加 $150\text{ mg}\cdot\text{L}^{-1}$ 外源 Ca^{2+} 改变了 EPS 组分, 促进多糖及芳香族类蛋白质的产生, 有利于活性污泥絮体结构稳定性及改进活性污泥沉降性能.

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