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微塑料诱导下污泥造粒潜能变化及微生物富集特征

谢晴帆¹, 俞楠¹, 张妮^{1,2}, 谢周云¹, 单珂欣¹, 吴亦馨¹, 唐力¹, 夏静芬¹, 杨国靖^{1,2*}

(1. 浙江万里学院生物与环境学院, 宁波 315100; 2. 湖南大学环境科学与工程学院, 长沙 410082)

摘要: 微塑料(MPs)是污水处理厂中普遍检出的新兴污染物之一, 目前研究主要集中于传统污水处理系统的污染水平及分布特征, 但有关微塑料暴露对污泥颗粒化过程的研究鲜见报道. 为探究微塑料对污泥颗粒化的诱导影响, 选用环境中广泛检出的聚对苯二甲酸乙二醇酯微塑料(PET-MPs)作为研究对象, 通过微塑料暴露试验研究 PET-MPs 对污泥造粒过程中系统潜能、胞外聚合物(EPS)组成和菌群富集特征的影响. 结果表明, PET-MPs 暴露显著加快污泥颗粒化进程, 同时以蛋白质(PN)为主导的 EPS 含量上升会增强污泥表面疏水性, 造粒速度和 EPS 分泌量与暴露粒径成正比, 微塑料和 EPS 协同促进颗粒污泥的形成. 然而微塑料持续暴露会导致系统除污性能恶化, 250 μm PET-MPs 暴露下亚硝酸盐氮积累的负面影响最严重, 浓度高达 $(5.08 \pm 0.24) \text{ mg} \cdot \text{L}^{-1}$. 高通量测序结果进一步表明, 变形菌门(Proteobacteria)和拟杆菌门(Bacteroidota)是促进颗粒污泥形成的主要优势门; 红环菌科(Rhodocyclaceae)、鞘氨醇杆菌科(Sphingomonadaceae)、黄杆菌科(Flavobacteriaceae)和红细菌科(Rhodanobacteraceae)通过增加分泌 EPS 促进污泥絮体凝聚; 但丛毛单胞菌科(Comamonadaceae)和几丁质菌科(Chitinophagaceae)的相对丰度降低会削弱氨氧化和亚硝酸盐氧化能力; 红杆菌科(Rhodobacteraceae)、丝生单胞菌科(Hyphomonadaceae)和黄单胞菌科(Xanthomonadaceae)丰度降低则抑制硝态氮的去除.

关键词: 微塑料(MPs); 污泥; 造粒; 系统潜能; 微生物富集

中图分类号: X703 文献标识码: A 文章编号: 0250-3301(2023)07-3997-09 DOI: 10.13227/j.hjxx.202208196

Change in Granulation Potential and Microbial Enrichment Characteristics of Sludge Induced by Microplastics

XIE Qing-fan¹, YU Nan¹, ZHANG Ni^{1,2}, XIE Zhou-yun¹, SHAN Ke-xin¹, WU Yi-xin¹, TANG Li¹, XIA Jing-fen¹, YANG Guo-jing^{1,2*}

(1. College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, China; 2. College of Environmental Science and Engineering, Hunan University, Changsha 410082, China)

Abstract: Microplastics (MPs), as a new type of pollutant, are widely detected in sewage treatment plants. Currently, research on MPs in traditional sewage treatment systems has mainly been focused on the pollution level and distribution characteristics, with a lack of studying the impact of MPs on the sludge granulation. In order to explore the effect of MPs on the granulation process, a microplastic exposure test was conducted by adding polyethylene terephthalate microplastics (PET-MPs), which are widespread in the environment. The operating performance of the system, extracellular polymeric substance (EPS) composition, and flora enrichment were analyzed on the sludge granulation. The results showed that the exposure of PET-MPs significantly accelerated the sludge granulation process, whereas the increase in EPS content dominated by PN enhanced the sludge surface hydrophobicity; the granulation rate and EPS secretion were proportional to the exposed particle size. Microplastics and EPS secretions synergistically promoted the formation of granular sludge. However, continuous microplastic exposure led to deterioration of the system decontamination performance and inhibited the degradation process of pollutants, with the most negative effect of nitrite nitrogen accumulation under 250 μm PET-MPs exposure, as high as $(5.08 \pm 0.24) \text{ mg} \cdot \text{L}^{-1}$. The high-throughput sequencing revealed that the microbial community diversity fell in the experimental group. The dominant bacteria at the phylum level were Proteobacteria and Bacteroidota on the sludge granulation. Rhodocyclaceae, Sphingomonadaceae, Flavobacteriaceae, and Rhodanobacteraceae promoted flocculation by increasing EPS secretion. The decrease in Comamonadaceae and Chitinophagaceae weakened the ammonia and nitrite oxidation capacity of the system, whereas the decrease in Rhodobacteraceae, Hyphomonadaceae, and Xanthomonadaceae inhibited the removal of nitrate nitrogen.

Key words: microplastics (MPs); sludge; granulation; system performance; microbial enrichment

微塑料(microplastics, MPs)是指粒径小于 5 mm 的塑料颗粒, 由环境中大塑料经过紫外线光解、物理分裂和生物降解等长期作用下形成, 已成为环境中新兴污染物之一^[1,2]. 现阶段关于污水处理厂中 MPs 的研究主要集中于其污染水平, 且进水丰度大多分布在 $1.80 \times 10^2 \sim 1.20 \times 10^4$ 个 $\cdot \text{L}^{-1}$, 其中聚对苯二甲酸乙二醇酯微塑料(polyethylene terephthalate microplastics, PET-MPs)的检出最为普遍^[3~5]. 同时 MPs 也会被截留在污泥中, 随着微塑料截留量的逐步积累, 会对污泥微生物的生长发育过程造成负面作用^[6~8], 有学者研究发现 MPs 蓄积会导致轮虫生

长滞缓^[9]及沉积物丰度下降^[10]. 因此, MPs 的长期暴露可能会对污水生物处理系统的稳定运行产生潜在影响.

好氧颗粒污泥(aerobic granular sludge, AGS)作为传统活性污泥改良后的一种特殊生物膜, 因具备优良沉降性能、结构密实和微生物含量高等优势而

收稿日期: 2022-08-21; 修订日期: 2022-10-13

基金项目: 国家自然科学基金项目(51408551); 浙江省自然科学基金项目(LY21E080028); 浙江省一流学科课题项目(CX2021045); 浙江省大学生科技创新(新苗计划)项目(2021R420015); 浙江万里学院科研创新团队项目

作者简介: 谢晴帆(1997~), 女, 硕士研究生, 主要研究方向为污水生物处理与资源化, E-mail: 771053440@qq.com

* 通信作者, E-mail: guojing_yang@163.com

拥有广阔的工程应用前景^[11].但其颗粒化过程较为复杂,会受诱导晶核、胞外聚合物(extracellular polymeric substances, EPS)和水力条件等因素的影响^[11,12]. MPs 因具有比表面积大^[13]、疏水性^[14]和难降解^[15]等特性,不仅在水环境中易于团聚^[14],而且能为微生物提供生态位^[16,17].然而有关 MPs 是否会作为外源晶核对污泥颗粒化过程的造粒进程、物质循环和微生物菌群产生作用至今尚未见报道,因此开展微塑料暴露对污泥颗粒化过程的影响研究具有重要意义.

基于此,本文拟选用 PET-MPs 为研究对象,通过外源投加不同粒径(13、48 和 250 μm) PET-MPs 模拟污泥颗粒化过程中的微塑料暴露污染,考察颗粒化过程中氮素转化规律、污泥结构差异和 EPS 分泌变化,揭示颗粒化进程中微塑料暴露下系统脱氮效能、污泥理化特性和微生物富集响应机制,旨在为微塑料暴露对 AGS 形成过程的影响和稳定运行提供理论依据与技术支持.

1 材料与方法

1.1 接种污泥与试验用水

本试验所需接种污泥取自宁波市福明净化水厂的二沉池,呈黑褐色絮体状,接种后混合液污泥浓度(mixed liquor suspended solids, MLSS)约为 6 000 $\text{mg}\cdot\text{L}^{-1}$,污泥沉降指数(sludge index, SVI)在 55 $\text{mL}\cdot\text{g}^{-1}$ 左右.取回种泥并空曝 24 h,去除其中的残余污染物质后进行好氧颗粒污泥培养试验.废水中检测到微塑料的平均粒径为 200 μm ,但经环境长期作用会进一步裂解成更小尺寸的微粒^[18],因此本研究设置 4 种处理方式: R_0 (Control)、 R_s (投加 13 μm PET-MPs)、 R_m (投加 48 μm PET-MPs)和 R_L (投加 250 μm PET-MPs),其中 PET-MPs 购自中新塑料有限公司,投加浓度均为 100 $\text{mg}\cdot\text{L}^{-1}$,以探明微塑料在环境水平浓度暴露下对 AGS 形成过程的影响.试验用水的主要成分如下:COD($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$)为 200 ~ 250 $\text{mg}\cdot\text{L}^{-1}$; NH_4^+-N (NH_4Cl)为 25 ~ 35 $\text{mg}\cdot\text{L}^{-1}$; SOP(KH_2PO_4)为 7 ~ 8 $\text{mg}\cdot\text{L}^{-1}$; NaHCO_3 为 110 $\text{mg}\cdot\text{L}^{-1}$; $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ 为 40 $\text{mg}\cdot\text{L}^{-1}$; CaCl_2 为 30 $\text{mg}\cdot\text{L}^{-1}$.

1.2 试验装置与运行条件

本试验在 4 个构造相同的 SBR 反应器中进行,如图 1 所示.反应器整体由有机玻璃制成,上部呈圆柱体,底部呈圆锥体,反应区高度为 90 cm,内径为 8 cm,有效容积为 4.5 L;采用上部进水、中部出水和底部曝气的形式,排水比为 50% ~ 60%;采用好氧/缺氧模式,每天运行 2 ~ 3 个周期,具体过程如下:进

水 15 min、好氧 240 min、缺氧 180 min、沉降 5 ~ 30 min 和排水 10 ~ 15 min.

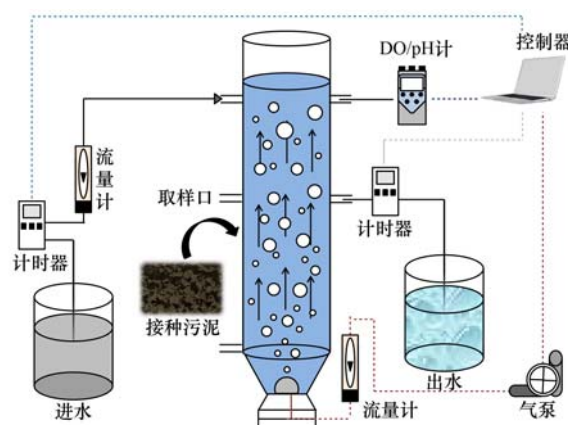


图 1 SBR 试验装置示意

Fig. 1 SBR experiment device

1.3 试验项目与分析方法

NH_4^+-N 、 NO_2^--N 和 NO_3^--N 分别采用纳氏试剂分光光度法、 N -(1-萘基)-乙二胺分光光度法和酚二磺酸分光光度法测定^[12], TN 通过加和法计算; COD 采用微波消解-重铬酸钾法测定^[12]; 典型周期内污染物的测定方法:待好氧颗粒污泥基本稳定后,从各母反应器中移取相同污泥浓度的泥样至有效容积为 4.5 L 的 4 个子反应器,多次淘洗后加试验用水至刻度线处,试验用水的主要成分见 1.1 节,分别每隔 30 min 进行取样检测; SV_5 和 SV_{30} 采用重力沉降法测定^[12]; MLSS、MLVSS 和 ESS 采用重量法测定^[12]; 利用光学显微镜、数码相机和扫描电镜(scanning electron microscope, SEM)对污泥外观及微观形态进行观察分析;通过热处理法提取 EPS,并分别采用改良型 BCA 蛋白质测定试剂盒和苯酚-硫酸法测定其中的蛋白质(protein, PN)和多糖(polysaccharide, PS)^[11,19];通过上海美吉生物医药科技有限公司的 Illumina MiSeq 平台对污泥样品进行高通量测序及分析.

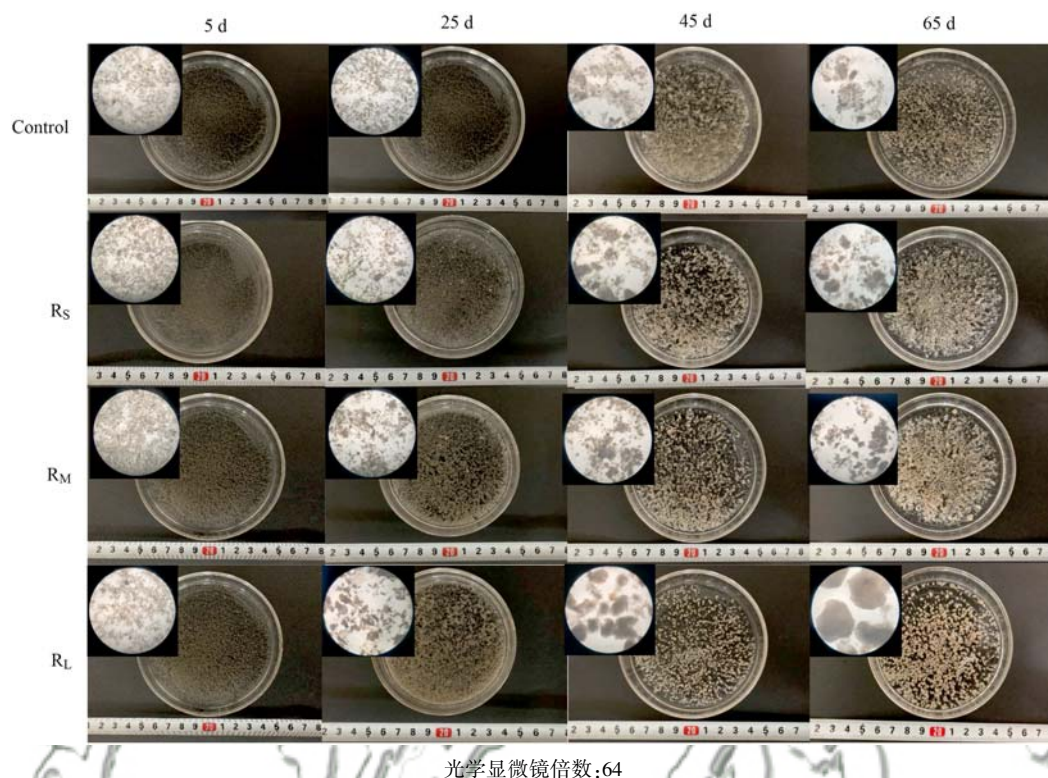
2 结果与讨论

2.1 污泥造粒进程的形态变化

为了直观比较不同粒径 PET-MPs 暴露下 AGS 形成过程的差异,本试验分别在 5、25、45 和 65 d 时对污泥形貌进行考察(图 2).由图 2 可见,驯化初期污泥为色泽暗黄、结构疏松的絮状污泥.经过 25 d 的培养,试验组各反应器内粒状晶核数量增多,但 R_0 反应器中仍以絮状污泥为主体.当反应器运行至 45 d 时, R_0 反应器出现污泥晶核,而此时 R_s 和 R_m 反应器内形成污泥小颗粒, R_L 反应器则基本实现污泥颗粒化.随着污泥造粒程度持续推进至 65 d 左

右, 颗粒污泥粒径进一步增大, R_L 反应器的颗粒污泥结构密实规则, R_S 和 R_M 反应器内颗粒污泥细小、结构较松散, 而此时 R_0 反应器中仍有少量絮状污泥环绕在颗粒周围. 由此可见, 微塑料暴露对污泥颗粒

化进程具有促进作用, 且造粒速度与微塑料粒径呈正相关. 分析其原因, 这可能是因为疏水性强的 MPs 能够作为载体供微生物黏附生长^[16], 且粒径越大能够提供越多的附着位点, 从而加快污泥造粒进程.



光学显微镜倍数: 64
图 2 颗粒化过程中污泥外观变化

Fig. 2 Changes in the appearance of sludge during granulation

2.2 颗粒化进程中污泥特性的差异分析

根据污泥形貌差异将颗粒化过程分为 3 个阶段, 阶段 I (0 ~ 15 d) 为接种驯化期, 阶段 II (16 ~ 60 d) 为颗粒形成期, 阶段 III (61 ~ 80 d) 为颗粒稳定期. 为探究 AGS 颗粒化进程中污泥特性对微塑料暴露的响应机制, 考察了不同粒径 PET-MPs 暴露下不同生长时期的污泥浓度及絮凝沉降性能的变化, 结果如图 3 所示.

由图 3 可见, 驯化期由于沉淀时间调整, 沉降性能较差的污泥会被选择性排出, 各反应器的 MLSS 迅速下降, MLVSS/MLSS 随之升高, 同时 SV_{30}/SV_5 也呈上升趋势; 对照组 R_0 反应器 ESS 浓度无明显变化, 试验组则先增加后减少. 这可能是因为启动初期各反应器内为絮状污泥, 经微塑料暴露后污泥絮凝性下降, 但随着微塑料持续暴露和颗粒的逐步形成, 颗粒污泥对微塑料具有更好的耐受性, 絮凝能力有所恢复. 形成期 R_0 污泥浓度趋于稳定至 $3602.3 \text{ mg} \cdot \text{L}^{-1}$, 但试验组各反应器的 MLSS 均低于对照组, 最低值 ($\text{mg} \cdot \text{L}^{-1}$) 分别下降至 1 650.5 (R_S)、1 886.4 (R_M) 和 546.6 (R_L), 这表明微塑料持续暴露会抑制微生物生长, 且大粒径 PET 暴露下抑制作用最显

著. 然而 R_L 反应器的 SV_{30}/SV_5 在 40 d 时最先达到 1.0, 第 60 d 时 R_L 的 MLVSS/MLSS 增至 97.2%, 同时 ESS 降至 $137.3 \text{ mg} \cdot \text{L}^{-1}$; R_S 和 R_M 反应器的 SV_{30}/SV_5 均在 50 d 后趋于 1.0, R_0 反应器则在 70 d 后趋于 1.0. 已有相关研究表明微塑料作为载体可为微生物提供附着位点^[17,20], 由此推测在整个造粒过程中, 投加微塑料可以诱导污泥微生物定殖和污泥晶核形成, 同时发现较大粒径微塑料能够促进游离细菌向污泥晶核靠拢, 进而更有利于实现污泥颗粒化.

2.3 颗粒化进程中胞外聚合物的分泌规律

胞外聚合物 (EPS) 是微生物分泌并黏附于细胞壁外的一类高分子聚合物^[21], 包含蛋白质 (PN)、多糖 (PS)、腐殖酸和核酸等, 其中 PN 和 PS 为主要组成部分^[22], PN/PS 值则与细胞表面疏水性密切相关^[23,24].

已有相关研究表明 EPS 可以通过架桥等作用连接和粘附微生物细胞, 有利于颗粒污泥的形成^[25]. 图 4 为污泥颗粒化进程中 PN、PS 含量和 PN/PS 值在不同粒径 PET-MPs 暴露影响下的变化特征. 从中可见, 驯化期各反应器 PN、PS 含量和

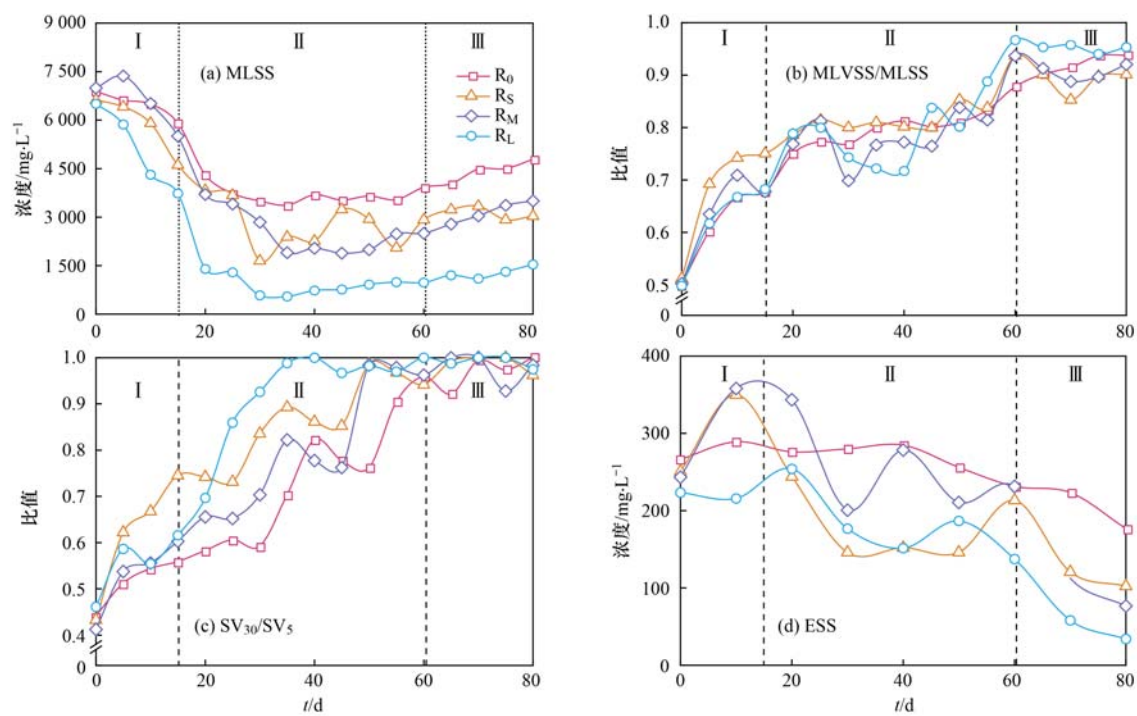


图3 颗粒化过程中污泥浓度、沉降性和絮凝性变化

Fig. 3 Changes in sludge concentration, sedimentation, and flocculation during granulation

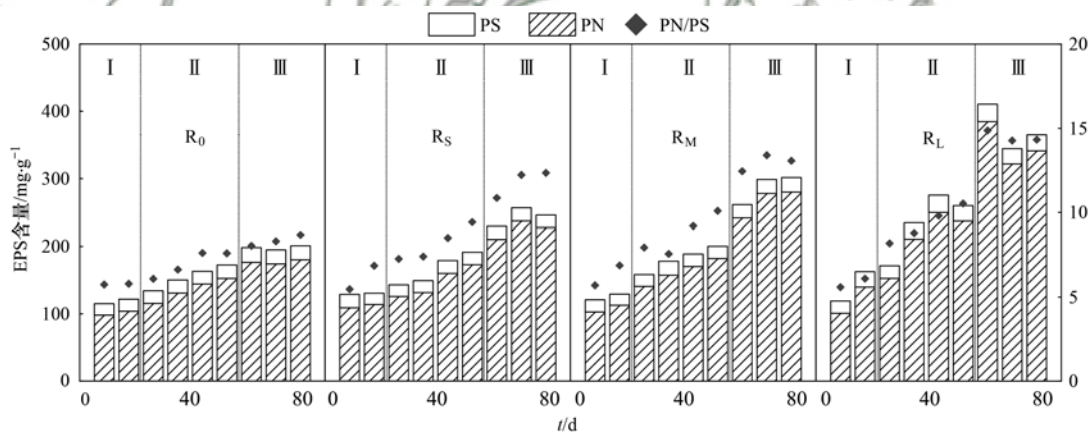


图4 颗粒化过程中 EPS 分泌变化

Fig. 4 Changes in EPS secretion during granulation

PN/PS 值无明显变化;形成期试验组各反应器的 EPS 总量均呈上升趋势,而对照组 R_0 反应器增幅不显著,可见微塑料暴露下有助于刺激污泥微生物分泌 EPS,这意味着污泥粒径的增加不仅受益于微塑料提供附着生长的环境,也受益于微塑料刺激下 EPS 分泌量的上升.随着污泥颗粒化程度的提高,稳定期试验组各反应器 EPS 总量均高于对照组,其中 R_L 反应器在 65 d 时陡增至 $410.59 \text{ mg} \cdot \text{g}^{-1}$,这可能是微生物处于微塑料蓄积压力下的一种自我保护行为^[26~28].同时可以发现,在整个颗粒化过程中 PN 占据主导地位,因其带正电荷而更易通过静电作用与污泥键合,是影响微生物聚集的关键因素^[29,30].此外,试验组各反应器中的 PN/PS 值在颗粒化过程

中增加趋势明显快于对照组,分别由最初的 5.57 逐渐增至 12.33、13.06 和 14.32,这表明微塑料的引入造成 PN/PS 值的提升,从而增强细胞表面疏水性.

2.4 颗粒化进程中除污性能影响及典型周期分析
为明晰不同粒径微塑料对颗粒化过程中除污性能的影响,各反应器 COD、 $\text{NH}_4^+ \text{-N}$ 和 TN 出水情况变化如图 5 所示.从中可见,在整个颗粒化进程中对 照 组 R_0 反应器的除污性能逐步提升.与对照组相比,驯化期试验组各反应器的 $\text{NH}_4^+ \text{-N}$ 和 TN 去除均未受到明显影响,COD 去除则在反应器运行至 12 d 时开始恶化,表明微塑料暴露会抑制有机污染物的降解,这可能是由于微塑料自身具备特殊的表面性

质能够吸附废水中的部分污染物^[31~33],从而干扰微生物对污染物的去除.随着微塑料的持续暴露,试验组各反应器 COD、 $\text{NH}_4^+\text{-N}$ 和 TN 均呈现不断恶化趋势,其中 $\rho(\text{TN})$ 出水峰值最高可达 $(13.94 \pm 1.78) \text{ mg} \cdot \text{L}^{-1}$,较大粒径暴露下的抑制作用更显著,这显示出污泥造粒速度的加快并没有提升系统除污性能,与 Guo 等^[34]以碳酸钙和羟基磷灰石为外源晶核促进污泥颗粒化的研究结果相一致.

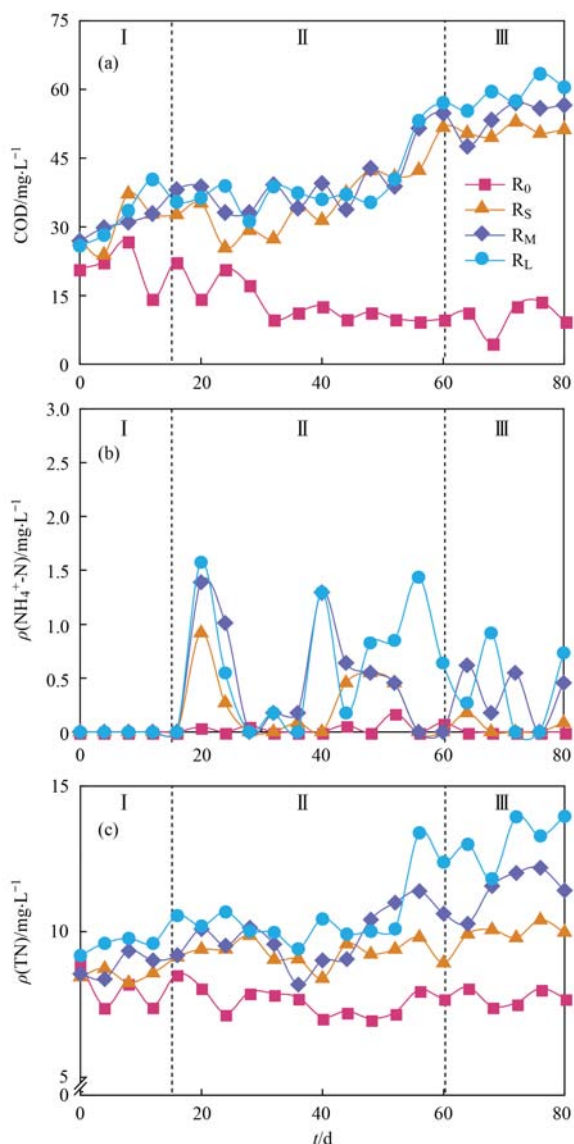


图5 颗粒化过程中 COD、 $\text{NH}_4^+\text{-N}$ 和 TN 出水情况变化

Fig. 5 Variations in COD, $\text{NH}_4^+\text{-N}$, and TN effluent during granulation

为进一步探析各反应器除污性能恶化的原因,对稳定期典型周期内各系统的 COD、 $\text{NH}_4^+\text{-N}$ 、 $\text{NO}_2^-\text{-N}$ 和 $\text{NO}_3^-\text{-N}$ 的转化过程进行考察,结果如图 6 所示,其转化过程主要集中在好氧阶段.从图 6 可以看出各反应器对 COD 的转化利用无明显差异[图 6 (a)].对照组 R_0 反应器的 $\text{NH}_4^+\text{-N}$ 浓度在 120 min 时最先基本去除殆尽, R_S 和 R_M 反应器分别在反应进

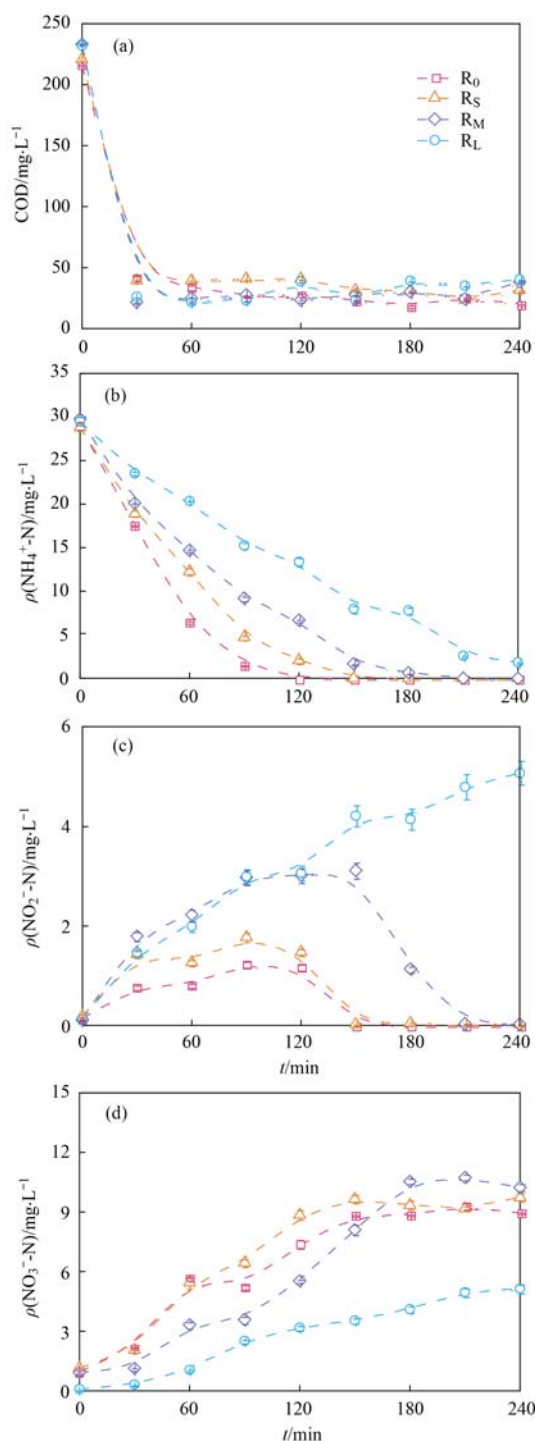


图6 稳定期 COD、 $\text{NH}_4^+\text{-N}$ 、 $\text{NO}_2^-\text{-N}$ 和 $\text{NO}_3^-\text{-N}$ 典型周期内的变化

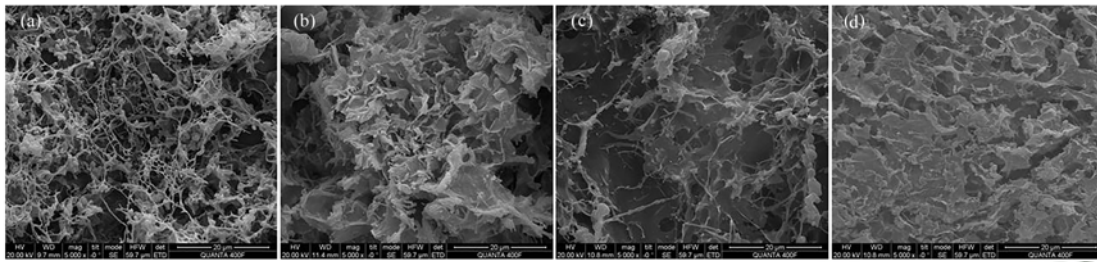
Fig. 6 Variations in the typical cycle of COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ in the stable stage

行至 150 min 和 210 min 时亦几乎检测不出,而 R_L 反应器在好氧结束时(240 min)的 $\rho(\text{NH}_4^+\text{-N})$ 为 $(1.85 \pm 0.09) \text{ mg} \cdot \text{L}^{-1}$,表明较大粒径微塑料暴露明显抑制了氨氮的去除过程.此外通过对比各反应器 $\text{NH}_4^+\text{-N}$ 浓度随时间变化的趋势发现, $\text{NH}_4^+\text{-N}$ 去除速率随微塑料粒径的增大而减缓,该现象直观显示微塑料暴露降低了氨氧化速率,其中较大粒径抑制作

用更明显[图 6(b)]. 对照组 R_0 和试验组 R_S 、 R_M 的 NO_2^- -N 浓度均呈现先升高后下降的趋势, 同时 NO_3^- -N 浓度始终维持在较低的浓度, 呈现显著的同步硝化反硝化作用; 各反应器 NO_2^- -N 的最大积累量随着微塑料粒径的增大而上升, 其中 R_L 反应器的 $\rho(\text{NO}_2^-$ -N) 持续升至 $(5.08 \pm 0.24) \text{ mg} \cdot \text{L}^{-1}$, 且 NO_3^- -N 浓度均低于其余反应器, 这表明 NO_2^- -N 氧化为 NO_3^- -N 的过程明显受阻, 系统脱氮过程主要以短

程硝化反硝化的方式进行[图 6(c) ~ 6(d)].

各反应器污泥微观结构的 SEM 结果显示, 对照组 R_0 污泥结构规则且孔隙率高, 而微塑料暴露下的颗粒污泥内部结构受到严重破坏, 污泥骨架趋于松散, 孔隙率明显下降(图 7). 这表明 PET-MPs 的持续暴露不仅改变了污泥内部的结构, 也阻碍了传质通道中营养物质和 O_2 的运输传递, 从微观角度解释了微塑料暴露下污染物转化过程受阻的原因.



(a) R_0 ; (b) R_S ; (c) R_M ; (d) R_L

图 7 稳定期的污泥微观形态

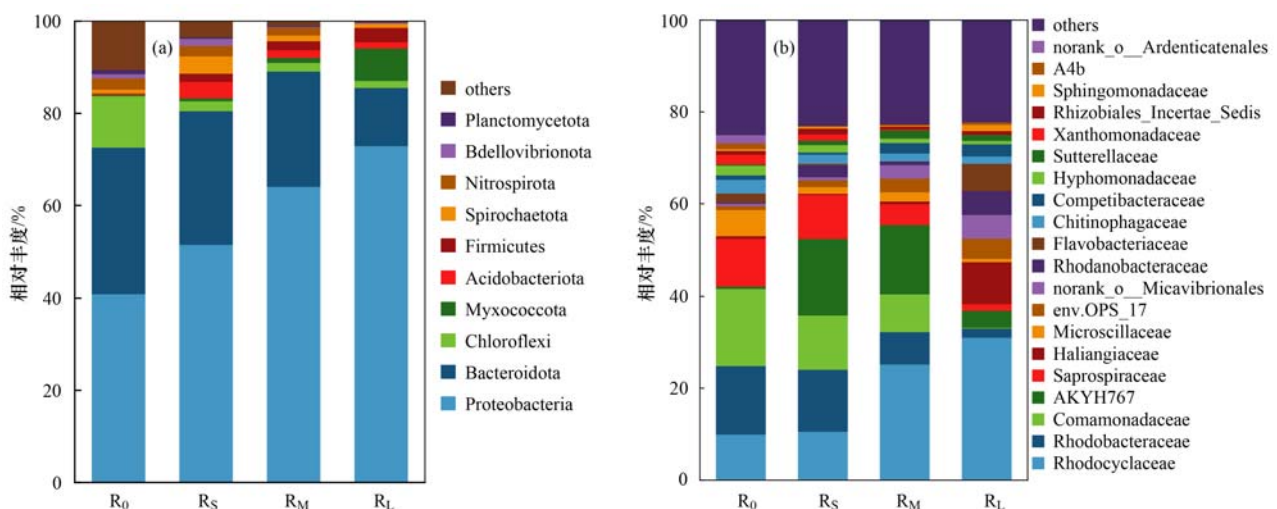
Fig. 7 Morphology of sludge in the stable stage

2.5 污泥造粒进程的微生物富集响应分析

为探明微塑料对污泥颗粒化过程的影响机制, 考察了稳定期门、科水平上前 10 和前 20 的菌群富集特征(图 8). 从图 8(a)中可以看出, 变形菌门(Proteobacteria)和拟杆菌门(Bacteroidota)是颗粒化过程中相对丰度最高的 2 个优势门, 但微塑料暴露使其产生明显差异. 由于微塑料具备疏水特性, 在运行期间增加了其与污泥微生物的接触, 这进一步表明微塑料有利于微生物富集定殖. 有研究表明, Proteobacteria 是分泌 EPS 的主要菌门之一^[35,36], 其在试验组各反应器中的相对丰度较对照组分别上升了 10.58% (R_S)、23.19% (R_M) 和 32.02% (R_L), 可

见 Proteobacteria 在微塑料暴露下通过分泌 EPS 提升了污泥疏水性, 从而加快污泥造粒进程. 微塑料持续暴露后使厚壁菌门(Firmicutes)相对丰度增加, 产生更多具有较强抗逆性的内生孢子形成保护^[37,38], 以防微塑料造成进一步破坏. Bacteroidota 是降解有机化合物的功能性微生物^[39], 硝化螺旋菌门(Nitrospirata)则是一类具备硝化能力的菌门^[40], 试验组各反应器的相对丰度较对照组均出现不同程度的下降, 与试验组中出现 COD 上升[图 5(a)]和亚硝酸氮积累现象[图 6(c)]相符.

在科水平上进一步探析了颗粒形成过程中各反应器的微生物富集差异, 分布情况见图 8(b). 有研



(a) 门水平; (b) 科水平

图 8 门和科水平的微生物群落相对丰富度变化

Fig. 8 Changes in relative abundance of microbial community at phylum and family level

究表明,红环菌科(Rhodocyclaceae)、鞘氨醇杆菌科(Sphingomonadaceae)、黄杆菌科(Flavobacteriaceae)和红细菌科(Rhodanobacteraceae)都能够分泌EPS以促进污泥絮体附着和好氧颗粒的形成^[41,42]。这些菌科在微塑料暴露下均呈现上升趋势,共聚集后能够良好共存,对于颗粒污泥的形成起到关键作用。根据已有研究发现丛毛单胞菌科(Comamonadaceae)是硝化菌的一种^[43~45],能够转化去除污水中的 NH_4^+-N 和 NO_2^--N ,然而其在试验组各反应器的相对丰度由对照组的16.77%分别下降为11.83%(R_s)、8.22%(R_m)和0.13%(R_L);几丁质菌科(Chitinophagaceae)则是一种氨氧化细菌^[46],在试验组各反应器中的相对丰度均低于对照组,这进一步从微生物学角度证明微塑料可抑制氨氮和亚硝酸盐氮氧化过程及效率。此外,微塑料的持续暴露还阻碍了一些反硝化细菌的生长,如红杆菌科(Rhodobacteraceae)、生丝单胞菌科(Hyphomonadaceae)和黄单胞菌科(Xanthomonadaceae)^[47,48],其相对丰度在微塑料暴露后均出现不同程度的下降,进而抑制了各试验组反应器TN的去除。

3 结论

(1)不同粒径PET-MPs暴露均可促进污泥颗粒化,PET暴露粒径越大,可以提供更多微生物生态位,同时有助于刺激分泌EPS,显著增强细胞表面疏水性,更易于诱导污泥造粒。

(2)在污泥颗粒化过程中,当颗粒晶核形成后,PET-MPs的持续暴露会堵塞内部传质通道和吸附污染物质,抑制系统硝化过程,从而导致氮素蓄积,其中250 μm PET组的影响最为明显。

(3)在AGS形成过程中,Rhodocyclaceae、Sphingomonadaceae、Flavobacteriaceae和Rhodanobacteraceae会富集分泌EPS,从而促进污泥造粒;但微塑料持续暴露会导致Comamonadaceae、Chitinophagaceae、Rhodobacteraceae、Hyphomonadaceae和Xanthomonadaceae丰度降低,进而削弱系统的脱氮性能。

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