



汪流伟,肖小双,安雪姣,等.硫氰酸盐高效降解菌群的动力学及微生物多样性研究[J].江西农业大学学报,2023,45(2):444-452.

WANG L W,XIAO X S,AN X J,et al.Studies on the kinetics and microbial diversity of a microbial consortium with highly efficient thiocyanate degradation ability[J].Acta agriculturae universitatis Jiangxiensis,2023,45(2):444-452.

# 硫氰酸盐高效降解菌群的动力学及微生物多样性研究

汪流伟,肖小双,安雪姣,李宁健,谢东,张庆华\*

(江西农业大学 生物科学与工程学院,江西 南昌 330045)

**摘要:**【目的】硫氰酸盐( $SCN^-$ )是一种常见的金矿、纺织、印染和焦化工业污染物,具有较强毒性和化学结构稳定性,其所造成的环境污染已成为近年来亟待解决的难题。【方法】在前期构建的一组高效硫氰酸盐降解复合菌群的基础上,探究在不同初始硫氰酸盐浓度下复合菌群对硫氰酸盐降解速率及复合菌群生长速率的影响,并对其进行动力学分析;根据细菌16S rRNA的序列结果进行群落结构组成分析;通过OTUs、Chao1指数、Shannon指数和Simpson指数对原始污泥和复合菌群进行多样性分析。【结果】(1)复合菌群降解硫氰酸盐及复合菌群生长符合底物抑制模型-Haldane方程,硫氰酸盐最佳降解速率( $V_{opt}$ )与硫氰酸盐浓度( $S_{opt}$ )分别为 $0.137\ 85\ g/(L\cdot h)$ 和 $0.121\ 26\ g/L$ 、复合菌群大生长速率( $V_{opt}$ )与硫氰酸盐浓度( $S_{opt}$ )分别为 $1.278\ 28\ g/(L\cdot h)$ 和 $2.784\ 68\ g/L$ 。此外,复合菌群在30 h内可完全降解 $4\ g/L$ 硫氰酸盐,最高可耐受 $15\ g/L$ 的硫氰酸盐,培养6 d,降解率可达100%,生物量增加 $8.65\ g/L$ 。(2)该复合菌群细菌多样性及结构与原始污泥发生了变化,细菌多样性及结构在添加硫氰酸盐定向富集驯化后发生了改变,生物多样性在减少;活性污泥中的主要菌属有Uncultured\_bacterium\_f\_Blastocatellaceae、Alicyciphilus、uncultured\_bacterium\_f\_NS9\_marine\_group、Diaphorobacter、uncultured\_bacterium\_f\_Mitochondria、Thiobacillus,而复合菌群样品中主要的菌属有uncultured\_bacterium\_f\_Saccharimonadaceae、uncultured\_bacterium\_f\_Chitinophagaceae、uncultured\_bacterium\_f\_NS9\_marine\_group、Dokdonella、Thiobacillus。其中Thiobacillus从活性污泥中的劣势菌属(丰度大约为2.69%)变为复合菌群中的优势菌属(丰度大约为77.73%),被认为是复合菌群降解硫氰酸盐的关键菌属。【结论】研究结果初步揭示了复合菌群降解硫氰酸盐规律,并解析了其微生物演变过程,挖掘了功能微生物,为后续硫氰酸盐废水的高效处置提供一定的研究基础和理论依据。

**关键词:**复合菌群;硫氰酸盐;废水的高效处置;降解;动力学;微生物群落结构

**中图分类号:**Q939.9    **文献标志码:**A

**开放科学(资源服务)标识码(OSID):**



**文章编号:**1000-2286(2023)02-0444-09

**收稿日期:**2022-08-18    **修回日期:**2022-10-18

**基金项目:**国家自然科学基金项目(32160027)、江西省自然科学基金重点项目(20212ACB205003)、江西省高层次高技能领军人才项目和江西省研究生创新专项资金(YC2022-S401)

Project supported by the National Natural Science Foundation of China(32160027),the Key Project of Natural Science Foundation of Jiangxi Province,China(20212ACB205003),the High-level and High-skilled Leading Talents Training Project of Jiangxi Province,China and Innovation Fund for Postgraduate Students in Jiangxi Province(YC2022-S401)

**作者简介:**汪流伟,硕士生,orcid.org/0000-0002-7994-6485,1909669580@qq.com;\*通信作者:张庆华,副教授,博士,主要从事有害物的生物降解与转化研究,orcid.org/0000-0003-1067-4343,zqh\_net@163.com。

## Studies on the Kinetics and Microbial Diversity of Microbial Consortium with Highly Efficient Thiocyanate Degradation Ability

WANG Liuwei, XIAO Xiaoshuang, AN Xuejiao,  
LI Ningjian, XIE Dong, ZHANG Qinghua<sup>\*</sup>

(College of Bioscience and Biotechnology, Jiangxi Agricultural University, Nanchang 330045, China)

**Abstract:** [Objective] As a common pollutant in gold mining, textile, printing and dyeing and coking industries, Thiocyanate ( $\text{SCN}^-$ ) has strong toxicity and chemical structure stability, and its environmental pollution has become an urgent problem to be solved in recent years. [Method] Based on a group of high-efficiency thiocyanate degradation microbial community constructed in the previous stage, the effects of the microbial community on thiocyanate degradation rate and growth rate of the microbial community under different initial thiocyanate concentrations were explored. Kinetic analysis was implemented. According to the sequence results of 16S rRNA of bacteria, the community structure was analyzed. OTUs, Chao1 index, Shannon index, and Simpson index were used to analyze the diversity of raw sludge and microbial community. [Result] (1) The degradation of thiocyanate and growth of the microbial community conformed to the substrate inhibition model-Haldane equation. The optimum degradation rate of thiocyanate and thiocyanate concentrations were 0.137 85 g/(L·h) and 0.121 26 g/L, respectively. The maximum growth rate of microbial community and thiocyanate concentrations were 1.278 28 g/(L·h) and 2.784 68 g/L, respectively. In addition, the microbial community could completely degrade 4 g/L thiocyanate within 30 hours, the maximum tolerance of the microbial community was 15 g/L thiocyanate, and the degradation rate reached 100% after 6 days of culture. Biomass was increased by 8.65 g/L. (2) The bacterial diversity and structure of the microbial community changed from those of the original sludge, the diversity and structure of bacteria were changed after thiocyanate enrichment and acclimation, and the biodiversity decreased again. *Uncultured\_bacterium\_f\_Blastocatellaceae*, *Alicycliphilus*, *uncultured\_bacterium\_f\_NS9\_marine\_group*, *Diaphorobacter*, *uncultured\_bacterium\_f\_Mitochondria*, *Thiobacillus* are the main genera in the original sludge samples. *Dokdonella*, *Thiobacillus*, *uncultured\_bacterium\_f\_Saccharimonadaceae*, *uncultured\_bacterium\_f\_NS9\_marine\_group*, and *uncultured\_bacterium\_f\_Chitinophagaceae* are the main genera in the microbial community samples. Among them, *Thiobacillus* changed from the inferior genus in activated sludge (the abundance was about 2.69%) to the dominant genus in microbial community (the abundance was about 77.73%), which was considered as the key genus for thiocyanate degradation by microbial community. [Conclusion] The research results preliminarily revealed the rule of thiocyanate degradation by microbial community, analyzed its microbial evolution process, and revealed functional microorganisms, which provided research basis and theoretical foundation for the efficient disposal of thiocyanate wastewater.

**Keywords:** microbial consortium; thiocyanate; efficient disposal of wastewater; degradation; dynamics; microbial community structure

**【研究意义】**硫氰酸盐是一种在金矿开采和焦化工业过程产生的含碳、氮、硫等元素的常见化合物，排放浓度高达5 000 mg/L，具有较强的毒性和化学稳定性<sup>[1-2]</sup>，其主要是通过抑制多种酶的活性，使得多种生物受到迫害<sup>[3]</sup>，对生活环境造成了极大的危害。鉴于其毒性和来源广泛，工业排放指令规定，硫氰酸盐在焦化废水中的排放浓度必须降低至4 mg/L，为此，硫氰酸盐降解研究就显得尤为重要<sup>[4]</sup>。**【前人研究进展】**经研究发现，通过物理、化学和生物方法可有效去除硫氰酸盐<sup>[5]</sup>，其中物化法效果较差，实施成本较高，有时还会产生二次污染，不能达到排放的标准<sup>[6-9]</sup>；相比之下，由微生物介导的生物法去除硫氰酸盐具有低成本、高效率、降解完全、不产生二次污染等优点，也是当前国内外研究的主要方法之一。具备降解硫氰酸盐的微生物已从多种来源中分离和鉴定，如 *Arthrobacter*、*Klebsiella*、*Pseudomonas*、*Ralstonia*、*Thioba-*

*cillus thioparus*、*Thioalkalivibrio thiocyanoxidans*、*Paracoccus thiocyanatus* 等<sup>[10-13]</sup>, 这些微生物利用硫氰酸盐作为能量和碳、氮、硫源<sup>[14-15]</sup>, 可在不同程度上实现硫氰酸盐的降解。【本研究切入点】与仅借助单一微生物降解硫氰酸盐相比, 含有多种微生物的活性污泥的去除具有更稳定、更高的去除率<sup>[16]</sup>。有研究表明, 以硫杆菌为主的微生物群落在连续流动反应器中的最大硫氰酸盐去除率为 1.07 mmol/(L·h)<sup>[17]</sup>; 用于自养硫氰酸盐去除的微生物群落也以硫杆菌属物种为主, 能够在 5 d 内将 2 109 mg/L 降解到 890 mg/L<sup>[18]</sup>。这些研究始终检测到硫杆菌属的细菌是降解菌群中的关键菌群, 主要参与硫氰酸盐的降解, 但是关于复合菌群的降解动力学和生长动力学及降解硫氰酸盐的细菌群落结构了解甚少, 对其进行分子生态学研究, 为处理实际含硫氰酸盐废水提供新见解。【拟解决的关键问题】本课题组前期从焦化工业废水中富集驯化获得了一组降解速率快、酶活高且结构稳定的硫氰酸盐高效降解复合菌群。该菌群可在 27 h 内将 3 g/L 硫氰酸盐完全降解, 且可在 11 d 内将 12 g/L 硫氰酸盐完全降解, 远超现有文献报道的最高水平 (7 g/L, 12 d)<sup>[19]</sup>。在此基础上, 对复合菌群降解硫氰酸盐速率和菌群生长速率进行动力学分析; 采用高通量测序技术, 对复合菌群微生物多样性分析及其驯化富集前后的群落结构的演替变化, 确定其优势菌群, 以期为含高浓度硫氰酸盐废水处理提供理论支持。

## 1 材料和方法

### 1.1 材料

1.1.1 复合菌群来源 本课题组前期从江西省丰城市新高焦化厂的初曝池取样后富集驯化得到的高效降解复合菌群。

1.1.2 培养基 无机盐培养基<sup>[20]</sup>: NaCl 0.15 g/L, MgSO<sub>4</sub> 0.15 g/L, FeSO<sub>4</sub> 0.075 g/L, CaCl<sub>2</sub> 0.01 g/L, K<sub>2</sub>HPO<sub>4</sub> 1.1 g/L, KH<sub>2</sub>PO<sub>4</sub> 1.1 g/L, pH 7.2。

### 1.2 方法

1.2.1 硫氰酸盐检测方法 硫氰酸盐检测采用铁比色法<sup>[21]</sup>。

1.2.2 生物量检测 将发酵液在 4 °C 下 8 000 r/min 离心 5 min 后收集复合菌群, 菌群经蒸馏水 3 次润洗, 离心后再次收集菌群。菌群在室温下干燥后称重。复合菌群生物量计算公式:

$$\text{生物量(DCW)} = \text{检测量} - \text{接种量} \quad (1)$$

### 1.3 不同初始浓度对复合菌群的影响

将活化的复合菌群按 10% (V/V) 的接种量接种至分别以硫氰酸盐初始浓度为 0.1~15 g/L 的无机盐培养基中, 在其他条件保持一致的情况下探究复合菌群降解硫氰酸盐及菌群生长的影响。

### 1.4 硫氰酸盐降解复合菌群的微生物多样性分析

将原始活性污泥与复合菌群菌液离心, 将洗涤后的污泥与复合菌群由北京百迈克公司用于细菌总 DNA 的提取、PCR 扩增和 16S rRNA 测序分析。采用引物 27F5'-AGR GTT GAT YNT GGCT CAG-3' 和 1492R5'-TASGGHTACCTTGTTASGACTT-3' 进行 PCR 扩增。总 DNA 提取按照土壤基因组 DNA 抽提试剂盒进行。通过数据处理对物种进行注释及分类学分析、Alpha 多样性分析、Beta 多样性分析、组间差异显著性分析、相关性与关联性分析<sup>[22]</sup>。

### 1.5 试验设计与数据统计分析

采用 origin 绘图软件进行绘图, 利用 SPSS Statistics 25.0 进行数据统计分析, 每组试验 3 次重复。

## 2 结果和讨论

### 2.1 不同初始硫氰酸盐浓度对复合菌群的影响

硫氰酸盐在一定浓度下对多种酶有抑制作用, 因此, 硫氰酸盐初始浓度过高时, 研究其耐受性是必要的<sup>[23]</sup>。为了深入探究硫氰酸盐初始浓度对降解率及生长速率的影响, 选取浓度范围 0.1~15 g/L 的硫氰酸盐进行分析, 其结果如图 1 所示, 当硫氰酸盐初始浓度在 0.1~1 g/L 时, 复合菌群降解速率越来越快; 但随着硫氰酸盐初始浓度的提高 (2~15 g/L), 复合菌群降解速率越来越慢, 这可能是复合菌群经历了一段适应期, 且硫氰酸盐浓度越高, 适应期越长。同样, 当硫氰酸盐初始浓度在 1~4 g/L 时, 复合菌群生长速率越来越快; 当浓度进一步提高时 (5~15 g/L), 复合菌群生长速率越来越慢。在低浓度时 (1 g/L), 复合菌

群降解硫氰酸盐及其生长不受底物限制,当提高浓度(4 g/L),复合菌群的生长速率依旧处于上升的趋势,但降解速率却在降低,结果表明复合菌群生长速率并不完全吻合于复合菌群降解硫氰酸盐速率。

由图1可知,随着硫氰酸盐浓度的逐渐增加,降解所需时间显著延长,硫氰酸盐降解速率和复合菌群生长速率均呈先增后减的趋势。由此说明高浓度硫氰酸盐对该复合菌群的降解及生长具有一定的抑制作用。因此,可以采用底物抑制模型-Haldane方程来描述该复合菌群的降解及生长的动力学方程(方程式如下)。随后利用硫氰酸盐降解时间和降解速率(复合菌群生长速率)进行拟合,结果如表1所示,硫氰酸盐降解速率(菌群生长速率)和降解时间符合线性关系,故该复合菌群对硫氰酸盐的降解动力学和菌群生长动力学符合一级反应动力学。

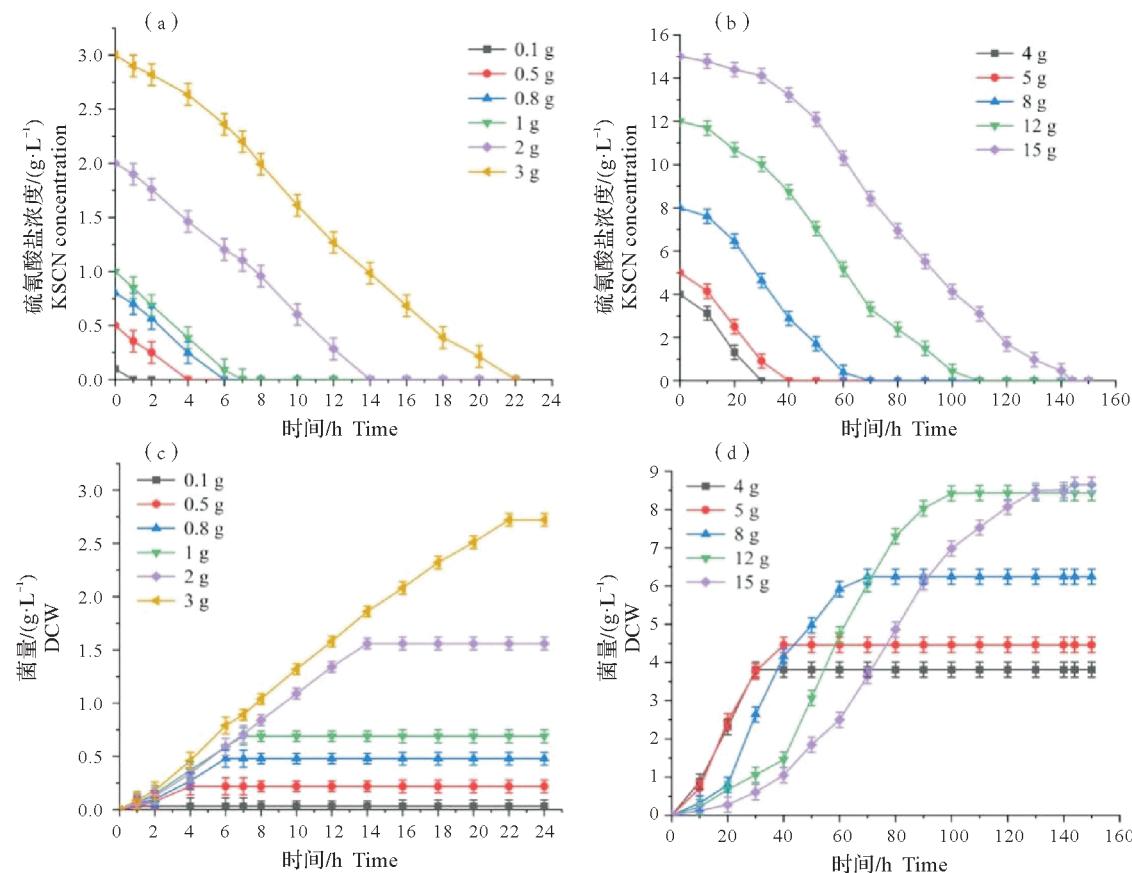
$$V = \frac{V_{\max} \cdot S}{S + S^2/K_i + K_s} \quad (2)$$

$$V_{\text{opt}} = \frac{V_{\max}}{1 + 2 \sqrt{\frac{K_s}{K_i}}} \quad (3)$$

$$S_{\text{opt}} = \sqrt{K_i K_s} \quad (4)$$

式中, $V$ 为降解速率[g/(L·h)], $S$ 为硫氰酸盐初始浓度(g/L), $V_{\max}$ 为硫氰酸盐的最大降解率[(g/(L·h)], $K_i$ 为底物抑制常数(g/L), $K_s$ 为底物饱和常数(g/L)。 $V_{\text{opt}}$ 为硫氰酸盐的最佳降解速率/最佳生长速率[g/(L·h)], $S_{\text{opt}}$ 为硫氰酸盐的最佳降解/生长浓度(g/L)。

分别对不同硫氰酸盐浓度下初始阶段降解速率和菌体生长速率的实验值与理论值进行拟合,结果



(a)硫氰酸盐初始浓度为0.1~3 g/L的降解速率,(b)硫氰酸盐初始浓度为4~15 g/L的降解速率,(c)硫氰酸盐初始浓度为0.1~3 g/L的菌体生长速率,(d)硫氰酸盐初始浓度为4~15 g/L的菌体生长速率。

(a) Degradation curve with initial thiocyanate concentration of 0.1~3 g/L, (b) degradation curve with initial thiocyanate concentration of 4~15 g/L, (c) Growth rate of bacteria with initial thiocyanate concentration of 0.1~3 g/L, (d) Growth rate of bacteria with initial concentration of thiocyanate at 4~15 g/L.

图1 复合菌群对不同浓度硫氰酸盐的降解性能

Fig.1 Degradation of thiocyanate at different concentrations by microbial community

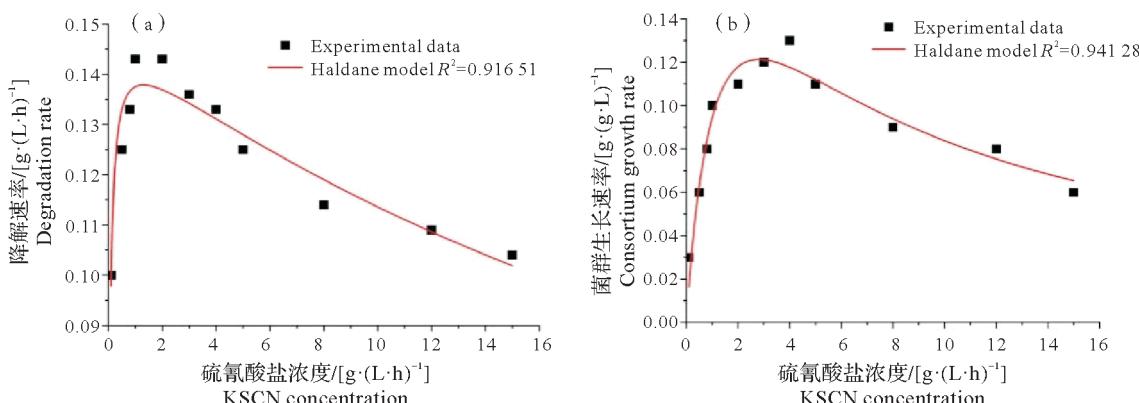
**表1 复合菌群对硫氰酸盐降解及细菌生长的动力学方程及参数**  
**Tab.1 Kinetic equation and parameters of thiocyanate degradation and microbial growth of the microbial community**

KSCN concentration (mmol·L <sup>-1</sup> )	硫氰酸盐降解			微生物生长	
	Thiocyanate degradation		$R^2$	Microbial growth	
	动力学方程	Kinetic equation		动力学方程	Kinetic equation
0.1	$c=-0.05t+0.1$		1	$c=0.0165t$	1
0.5	$c=-0.125t+0.5004$		1	$c=0.055t-0.01$	0.9758
0.8	$c=-0.1358t+0.8111$		0.9969	$c=0.0805t-0.029$	0.9769
1	$c=-0.145t+0.9838$		0.9970	$c=0.091t-0.004$	0.9870
2	$c=-0.144t+2.042$		0.9976	$c=0.1159t-0.075$	0.9953
3	$c=-0.1418t+3.3234$		0.9775	$c=0.1278t-0.0004$	0.9968
4	$c=-0.1381t+4.1823$		0.9845	$c=0.1155t-0.0917$	0.9815
5	$c=-0.1324t+5.1596$		0.9877	$c=0.1197t-0.116$	0.9788
8	$c=-0.1287t+8.4673$		0.9798	$c=0.102t-0.116$	0.9660
12	$c=-0.1237t+12.888$		0.9811	$c=0.0925t-0.9678$	0.9554
15	$c=-0.1188t+16.69$		0.9784	$c=0.0715t-1.0108$	0.9661

如图3所示,相关系数 $R^2$ 分别为0.91651和0.94128,说明拟合效果较好。随着硫氰酸盐浓度的不断升高,降解速率和生长速率也依次增大,直到达到最高点即存在最大降解速率 $V_{max}$ 和复合菌群最大生长速率 $V_{max}$ 分别为0.14879 g/(L·h)和0.23646 g/(L·h),底物抑制常数 $K_i$ 分别为32.8455 g/L和5.9280 g/L,底物饱和常数 $K_s$ 分别为0.05174 g/L和1.33759 g/L,如表2所示。代入公式计算,得到最佳降解速率 $V_{opt}$ 与最佳硫氰酸盐浓度 $S_{opt}$ 分别为0.13785 g/(L·h)、0.12126 g/L,最佳菌群生长速率 $V_{opt}$ 和最佳硫氰酸盐浓度 $S_{opt}$ 分别为1.27828 g/(L·h)、2.78468 g/L,这些结果与图2的峰值点基本一致。

**表2 复合菌群降解硫氰酸盐动力学及细菌群落生长动力学参数**  
**Tab.2 Parameters of thiocyanate degradation and microbial community growth kinetics**

Kinetic models	参数 Parameters					
	$V_{max}$	$K_i$	$K_s$	$R^2$	$V_{opt}$	$S_{opt}$
降解硫氰酸盐动力学 Kinetics of thiocyanate degradation	0.14879	32.8455	0.05174	0.91651	0.13785	1.27828
细菌群落生长动力学 Kinetics of microbial community growth	0.23646	5.9280	1.33759	0.94128	0.12126	2.78468



(a) 硫氰酸盐降解动力学,(b)复合菌群生长动力学。  
(a)Degradation kinetics,(b)microbial community growth kinetics.

**图2 复合菌群的霍尔丹模型拟合曲线**  
**Fig.2 Fitted curves of Haldane model of microbial community**

高浓度硫氰酸盐降解速率的降低可能是因为底物浓度高、毒性大,难以被微生物快速利用,其次还可能是高浓度硫氰酸盐阻止了酶的活性区域,降低酶活,从而阻碍了生物降解和复合菌群生长。表明复合菌群在硫氰酸盐降解过程中符合底物抑制模型,进一步明确了硫氰酸盐降解模型的适用性。这与黄会静<sup>[24]</sup>等研究结果相似,但是其研究的硫氰酸盐底物浓度最大为3 227.21 mg/L,本研究的最高浓度为15 g/L。众所周知硫氰酸盐在实际废水中的含量仅有0.5~3 g/L,但该复合菌群可降解15 g/L的硫氰酸盐,远超报道的最高水平70 mmol<sup>[19]</sup>,并且能保持较高的降解效率。这为后期利用复合菌群处理高浓度复杂的实际硫氰酸盐废水提供了重要的理论支持。

## 2.2 硫氰酸盐降解复合菌群微生物多样性分析

**2.2.1 硫氰酸盐降解复合菌群群落结构多样变化** 为了分析硫氰酸盐降解复合菌群与原始活性污泥的细菌群落结构以及丰富度,在97%相似水平上计算了常见的多样性指数。原始活性污泥和复合菌群细菌多样性指数(表3),覆盖率指数是样品的测序深度也表明样品的覆盖率,6个样品的覆盖率分别是0.997 4、0.998 5、0.998 2、0.999 2、0.999 2和0.998 7,表明绝大部分的细菌种群都被检测出来。随着硫氰酸盐降解菌群的富集驯化,降解菌群中的细菌多样指数随之减少,如原始的活性污泥经过培养驯化后,生物分类单元(OTUs)从87、89、84降为44、44、40,香农指数也从2.667 8、2.857 4、2.714 3减为1.512 6、1.813 8、1.393 1,这表明细菌多样性及结构在复合菌群中发生了改变,不断提高硫氰酸盐浓度对群落结构产生胁迫压力,使其生物多样性减少。而保存下来的这部分微生物有些可能是硫氰酸盐降解菌,还有些可能与保持污泥稳定性相关,也有可能是它们可以利用硫氰酸盐降解的产物或者次级产物进行生存。

**表3 细菌群落多样性指数**  
**Tab.3 Diversity indices of bacterial community**

组别 Group	样品编号 Sample ID	谱系多样性 PD_whole_tree	ASV数量 ASVs	香农指数 Shannon	覆盖率 Coverage	辛普森指数 Simpson	Chao1指数 Chao1
CK	CK1	9.706 2	87	2.667 8	0.997 4	0.618 3	96.230 8
	CK2	9.761 7	89	2.857 4	0.998 5	0.653 4	91.769 2
	CK3	9.083 4	84	2.714 3	0.998 2	0.651 9	88.583 3
DK	DK1	5.606 7	44	1.512 6	0.999 2	0.356 0	45.428 6
	DK2	5.385 7	44	1.813 8	0.998 2	0.465 8	99.0
	DK3	5.296 1	40	1.393 1	0.998 7	0.341 3	49.333 3

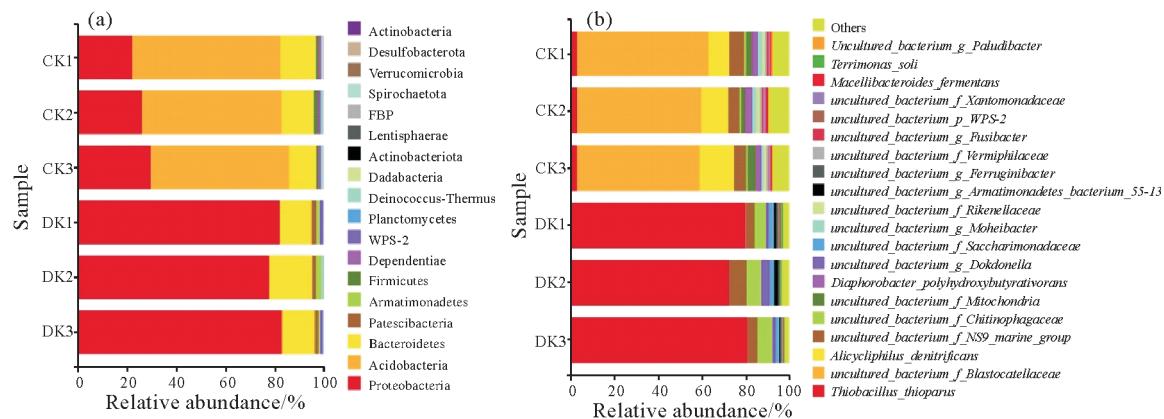
CK为活性污泥,DK为复合菌群,CK1、CK2、CK3和DK1、DK2、DK3分别代表每组的3个平行样本。

CK represents activated sludge, DK represents microbial community, CK1, CK2, CK3 and DK1, DK2, and DK3 respectively represent three parallel samples of each group.

**2.2.2 硫氰酸盐降解复合菌群群落组成变化** 如图3(a)所示,原始污泥的优势菌门主要有 Acidobacteria、Proteobacteria、Bacteroidetes、Firmicutes。其丰度大约为:57.95%、22.05%、12.49%、1.32%;复合菌群的优势菌门主要有 Proteobacteria、Bacteroidetes、Patescibacteria、Armatimonadetes,其丰度大约为:81.04%、14.46%、1.80%、1.34%,这与Wang等<sup>[19]</sup>的研究结果相似。其中复合菌群独有的 Patescibacteria 已被报道其常与反硝化菌共存<sup>[25]</sup>,Armatimonadetes 对废水中酚类物质具有较好的降解效果<sup>[26-27]</sup>。Acidobacteria 在复合菌群中消失的原因可能是在硫氰酸盐胁迫下由于不能有效的利用硫氰酸盐而逐渐被淘汰,有研究表明 Acidobacteria 与环境中的 C 和 N 含量成反比<sup>[28]</sup>,这也解释了在硫氰酸盐降解过程中氨氮浓度越来越高的原因。Proteobacteria 在原始污泥和复合菌群中都是优势菌门,且在富集驯化过程中丰度明显提高,这说明其在硫氰酸盐的胁迫下不受影响,而且能很好地代谢繁殖<sup>[29]</sup>。同样 Bacteroidetes 也是原始污泥和复合菌群中共有的优势菌门,在后期富集驯化过程中丰度无明显差异,说明 Bacteroidetes 能适应硫氰酸盐的胁迫,唐涛涛<sup>[30]</sup>发现其可降解多环芳烃。

如图3(b)所示,原始污泥中的优势菌属及其丰度为:*Uncultured\_bacterium\_f\_Blastocellaceae*、*Alicy-*

*cliphilus*、*uncultured\_bacterium\_f\_NS9\_marine\_group*、*Diaphorobacter*、*uncultured\_bacterium\_f\_Mitochondria*、*Thiobacillus*。其丰度大约为:57.95%、12.40%、5.98%、2.80%、2.93%、2.69%;复合菌群中优势菌属和丰度分别为:*Thiobacillus*、*uncultured\_bacterium\_f\_Chitinophagaceae*、*uncultured\_bacterium\_f\_NS9\_marine\_group*、*Dokdonella*、*uncultured\_bacterium\_f\_Saccharimonadaceae*。其丰度大约为:77.73%、6.16%、5.63%、2.09%、1.80%。在复合菌群中,原始污泥中的优势菌群*Alicycliphilus*、*uncultured\_bacterium\_f\_Blastocatellaceae*和*Diaphorobacter*均未出现,表明这些菌属不能生存在硫氰酸盐的胁迫下。*Thiobacillus*已被广泛报道能有效降解硫氰酸盐,且是含硫氰酸盐废水处理系统中的关键菌属,亦可利用H<sub>2</sub>S作为能源生长<sup>[31-32]</sup>,在复合菌群中其丰度大约为77.73%,而在原始活性污泥中其丰度大约为2.69%,结果表明*Thiobacillus*可能是硫氰酸盐主要的降解菌属。*Dokdonella*、*uncultured\_bacterium\_f\_Saccharimonadaceae*和WPS-2在菌群富集驯化过程中出现,说明这些菌属可以协同利用硫氰酸盐或其降解物生长代谢。*uncultured\_bacterium\_f\_NS9\_marine\_group*丰度在富集驯化过程中没有明显改变,这可能是该菌属可以在高浓度硫氰酸盐胁迫下生长代谢。不同的细菌具有不同污染物降解的功能,同一种细菌也可具有降解多种不同污染物的功能,如*Thiobacillus*不仅能降解硫氰酸盐,还具有很强的反硝化能力<sup>[33-34]</sup>。总的结果表明两组样品中不同属在不同样品中的丰度不同,有些菌属的丰度会有变化,说明细菌种群在高浓度硫氰酸盐胁迫下有一定的适应性,群落结构可以通过自身的组成变化适应高浓度硫氰酸盐环境。这也进一步说明该复合菌群的适应性强,这对处理成分复杂的真实硫氰酸盐废水具有极大的优势。



CK 为活性污泥组,DK 为复合菌群组,(a)门水平上的细菌组成,(b)属水平上的细菌群落组成。  
CK is the activated sludge group, DK is the compound bacteria group, (a)Bacterial composition at the phylum level,  
(b)bacterial community composition at the genus level.

图3 门/属水平上的细菌群落组成

Fig.3 Composition of bacterial community at phylum/genus level

### 3 结 论

(1)研究发现硫氰酸盐降解菌群的降解动力学和生长动力学均符合 Haldane 模型,最佳降解速率V<sub>opt</sub>值和最佳硫氰酸盐浓度S<sub>opt</sub>为0.137 85 g/(L·h)、0.121 26 g/L;最佳复合菌群生长速率V<sub>opt</sub>值和最佳硫氰酸盐浓度S<sub>opt</sub>分别为1.278 28 g/(L·h)、2.784 68 g/L。

(2)复合菌群中发现*Thiobacillus*、*uncultured\_bacterium\_f\_Chitinophagaceae*、*uncultured\_bacterium\_f\_NS9\_marine\_group*、*uncultured\_bacterium\_f\_Saccharimonadaceae*、*Dokdonella*等菌属的丰度较高,其中,*Thiobacillus*由活性污泥的劣势菌属(丰度大约为2.69%)变为复合菌群的优势菌属(丰度大约为77.73%),被认为是复合菌群降解硫氰酸盐的关键菌属。

本研究结果可为复合菌群在硫氰酸盐废水的实际处置提供研究基础和技术支撑。

### 参考文献 References:

- [1] WANG X G, LI B C, CHEN J S, et al. Cyclic degradation of thiocyanate in cyanide barren solution by manganese oxides [J].

- Minerals engineering, 2022, 176: 107314.
- [2] NI G, CANIZALES S, BRONMAN E, et al. Microbial community and metabolic activity in thiocyanate degrading low temperature microbial fuel cells [J]. Frontiers in microbiology, 2018, 9: 2308.
- [3] KATZ A I, EPSTEIN F H. Effect of anions on adenosine triphosphatase of kidney tissue [J]. Enzyme, 1971, 12(4): 499-507.
- [4] ROUDIER S, SANCHO L D, REMUS R, et al. Best available techniques (BAT) reference document for iron and steel production: industrial emissions directive 2010/75/EU: integrated pollution prevention and control [R]. Seville site: Joint research centre, 2013.
- [5] GOULD W D, KING M, MOHAPATRA B R, et al. A critical review on destruction of thiocyanate in mining effluents [J]. Minerals engineering, 2012, 34: 38-47.
- [6] 刘二博, 赵兵强, 张明亮, 等. 化学沉淀法脱除HPF脱硫废液中的硫氰酸盐 [J]. 环境工程学报, 2016, 10(3): 1328-1332.
- LIU E B, ZHAO B Q, ZHANG M L, et al. Removal of thiocyanate from HPF desulfurization waste solution with chemical precipitation [J]. Chinese journal of environmental engineering, 2016, 10(3): 1328-1332.
- [7] OULEGO P, COLLADO S, GARRIDO L, et al. Wet oxidation of real coke wastewater containing high thiocyanate concentration [J]. Journal of environmental management, 2014, 132: 16-23.
- [8] WANG J J, HAN Y J, LI J, et al. Selective adsorption of thiocyanate anions using straw supported ion imprinted polymer prepared by surface imprinting technique combined with RAFT polymerization [J]. Separation and purification technology, 2017, 177: 62-70.
- [9] ZAIA D A M, DE CARVALHO P C G, SAMULEWSKI R B, et al. Unexpected thiocyanate adsorption onto ferrihydrite under prebiotic chemistry conditions [J]. Origins of life and evolution of the biosphere, 2020, 50: 57-76.
- [10] RAHMAN S F, KANTOR R S, HUDDY R, et al. Genome-resolved metagenomics of a bioremediation system for degradation of thiocyanate in mine water containing suspended solid tailings [J]. Microbiology open, 2017, 6(3): e00446.
- [11] PAN J X, WEI C H, FU B B, et al. Simultaneous nitrite and ammonium production in an autotrophic partial denitrification and ammonification of wastewaters containing thiocyanate [J]. Bioresource technology, 2018, 252: 20-27.
- [12] SPURR L P, WATTS M P, GAN H M, et al. Biodegradation of thiocyanate by a native groundwater microbial consortium [J]. PeerJ, 2019, 7: e6498.
- [13] OSHIKI M, FUKUSHIMA T, KAWANO S, et al. Thiocyanate degradation by a highly enriched culture of the neutrophilic halophile *Thiohalobacter* sp. strain FOKN1 from activated sludge and genomic insights into thiocyanate metabolism [J]. Microbes and environments, 2019, 34(4): 402-413.
- [14] HUDDY R J, VAN ZYL A W, VAN HILLE R P, et al. Characterisation of the complex microbial community associated with the ASTERTM thiocyanate biodegradation system [J]. Minerals engineering, 2015, 76: 65-71.
- [15] 陆洪宇, 孙亚全, 董春娟, 等. 焦化废水中 COD、挥发酚和硫氰化物同步高效去除 [J]. 环境工程学报, 2014, 8(7): 2848-2852.
- LU H Y, SUN Y Q, DONG C J, et al. Removal of COD, volatile phenol and thiocyanate from coking wastewater [J]. Chinese journal of environmental engineering, 2014, 8(7): 2848-2852.
- [16] RYU B G, KIM W, NAM K, et al. Comprehensive study on algal - bacterial communities shift during thiocyanate degradation in a microalga-mediated process [J]. Bioresource technology, 2015, 191: 496-504.
- [17] KANTOR R S, HUDDY R J, IYER R, et al. Genome-resolved meta-omics ties microbial dynamics to process performance in biotechnology for thiocyanate degradation [J]. Environmental science & technology, 2017, 51(5): 2944-2953.
- [18] RAPER E, STEPHENSON T, FISHER R, et al. Characterisation of thiocyanate degradation in a mixed culture activated sludge process treating coke wastewater [J]. Bioresource technology, 2019, 288: 121524.
- [19] WANG X Y, LIU L T, LIN W T, et al. Development and characterization of an aerobic bacterial consortium for autotrophic biodegradation of thiocyanate [J]. Chemical engineering journal, 2020, 398(15): 125461.
- [20] 王书萍. 焦化废水中硫氰化物、苯酚高效降解菌的分离鉴定及降解特性研究 [D]. 武汉: 武汉科技大学, 2014.
- WANG S P. Isolation, identification and biodegradation characteristics research of strains for phenol and thiocyanate degradation [D]. Wuhan: Wuhan University of Science and Technology, 2014.
- [21] MARY T M U, SWAMINATHAN M. Enhanced biodegradation of thiocyanate by immobilized *Bacillus brevis* [J]. Journal of Indian chemical society, 2022, 99(8): 100588.

- [22] 雷菲, 张冬明, 吴宇佳, 等. 化肥减量配施有机肥对樱桃番茄产量、品质和微生物群落结构的影响[J]. 江西农业大学学报, 2021, 43(6): 1269-1277.  
LEI F, ZHANG D M, WU J Y, et al. Influences of chemical fertilizer reduction combined with organic fertilizer application on yield and quality of cherry tomato and structure of soil microbial community[J]. Acta agriculturae universitatis Jiangxiensis, 2021, 43(6): 1269-1277.
- [23] 潘霞霞, 李媛媛, 黄会静, 等. 焦化废水中硫氰化物的生物降解及其与苯酚, 氨氮的交互影响[J]. 化工学报, 2009, 60(12): 3089-3096.  
PAN X X, LI Y Y, HUANG H J, et al. Biodegradation of thiocyanide in coking wastewater and its interaction with phenol and ammonia nitrogen[J]. Journal of chemical industry, 2009, 60(12): 3089-3096.
- [24] 黄会静. 焦化废水处理的硫氰化物降解功能菌特性研究[D]. 广州: 华南理工大学, 2011.  
HUANG H J. Study on characteristics of thiocyanate degrading functional bacteria in coking wastewater treatment [D]. Guangzhou: South China University of Technology, 2011.
- [25] 李佳萍. 硫酸盐对反硝化型甲烷厌氧氧化过程影响研究[D]. 杭州: 浙江工商大学, 2020.  
LI J P. Effect of sulfate on anaerobic oxidation of denitrifying methane[D]. Hangzhou: Zhejiang Gongshang University, 2020.
- [26] 王迪. 焦化污染土壤中降解苯酚的厌氧反硝化菌群结构及bamA基因多样性研究[D]. 太原: 太原理工大学, 2018.  
WANG D. Study on the structure and bamA gene diversity of anaerobic denitrifying bacteria for phenol degradation in coking contaminated soil[D]. Taiyuan: Taiyuan University of Technology, 2018.
- [27] LIAO J Y, HU A, ZHAO Z W, et al. Biochar with large specific surface area recruits N<sub>2</sub>O-reducing microbes and mitigate N<sub>2</sub>O emission[J]. Soil biology and biochemistry, 2021, 156: 108212.
- [28] ZHU X B, TIAN J P, LIU C, et al. Composition and dynamics of microbial community in a zeolite biofilter-membrane bioreactor treating coking wastewater[J]. Applied microbiology and biotechnology, 2013, 97(19): 8767-8775.
- [29] MA Q, QU Y Y, SHEN W L, et al. Bacterial community compositions of coking wastewater treatment plants in steel industry revealed by Illumina high-throughput sequencing[J]. Bioresource technology, 2015, 179: 436-443.
- [30] 唐涛涛. 不同类型秸秆厌氧共代谢降解污泥中多环芳烃的效能及机制研究[D]. 贵州: 贵州大学, 2019.  
TANG T T. The effect of different straws on anaerobic removal of ficiency and mechanism of polycyclic aromatic hydrocarbons in sludge by co-metabolism Pathway[D]. Guizhou: Guizhou University, 2019.
- [31] MEKUTO L, NTWAMPE S K O, MUDUMBI J B N. Microbial communities associated with the co-metabolism of free cyanide and thiocyanate under alkaline conditions[J]. 3 Biotech, 2018, 8(2): 93.
- [32] KELLY D P, WOOD A P. Confirmation of *Thiobacillus denitrificans* as a species of the genus *Thiobacillus*, in the β-subclass of the *Proteobacteria*, with strain NCIMB 9548 as the type strain[J]. International journal of systematic and evolutionary microbiology, 2000, 50(2): 547-550.
- [33] DI BIASE A, WEI V, KOWALSKI M S, et al. Ammonia, thiocyanate, and cyanate removal in an aerobic up-flow submerged attached growth reactor treating gold mine wastewater[J]. Chemosphere, 2020, 243: 125395.
- [34] RAPER E, FISHER R, ANDERSON D R, et al. Nitrogen removal from coke making wastewater through a pre-denitrification activated sludge process[J]. Science of the total environment, 2019, 666(20): 31-38.