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模拟电镀污泥阴离子浸出液对氧化亚铁硫杆菌活性的 影响

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摘要:利用课题组分离得到的氧化亚铁硫杆菌 WZ-1 (GenBank 序列登录号: JQ968461) 研究电镀污泥浸出液中无机阴离子对 WZ-1 活性的影响,考察了 Cl ¯、NO $_3$ ¯、F ¯ 这 3 种单一阴离子以及 4 种不同模拟电镀污泥阴离子浸出液对 WZ-1 Fe 2 *氧化活性 和表观呼吸速率的影响,结果表明菌株在接种量为 6.7%、初始 pH 2.0、温度 30 °C、转速 150 r·min $^-$ 1的条件下,浓度分别为 5.0 g·L $^-$ 1、1.0 g·L $^-$ 1 的 Cl ¯、NO $_3$ ¯ 对 WZ-1 的活性没有影响;WZ-1 对 Cl ¯、NO $_3$ ¯、F $^-$ 的最大耐受浓度分别为 10.0 g·L $^-$ 1、5 g·L $^-$ 1、25.0 mg·L $^-$ 1;4 种模拟电镀污泥阴离子浸出液对 WZ-1 活性的影响有明显差异,强弱顺序为:Cl $^-$ /NO $_3$ ¯/F $^-$ > NO $_3$ ¯/F $^-$ > Cl $^-$ /F $^-$ > Cl $^-$ /NO $_3$ ¯.

关键词:氧化亚铁硫杆菌;亚铁;活性;表观呼吸速率;电镀污泥阴离子浸出液

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Effect of Simulated Inorganic Anion Leaching Solution of Electroplating Sludge on the Bioactivity of *Acidithiobacillus ferrooxidans*

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Abstract: An Acidithiobacillus ferrooxidans strain WZ-1 (GenBank sequence number; JQ968461) was used as the research object. The effects of Cl⁻, NO₃⁻, F⁻ and 4 kinds of simulated inorganic anions leaching solutions of electroplating sludge on the bioactivity of Fe²⁺ oxidation and apparent respiratory rate of WZ-1 were investigated. The results showed that Cl⁻, NO₃⁻ didn't have any influence on the bioactivity of WZ-1 at concentrations of 5.0 g·L⁻¹, 1.0 g·L⁻¹, respectively. WZ-1 showed tolerance to high levels of Cl⁻ and NO₃⁻ (about 10.0 g·L⁻¹, 5.0 g·L⁻¹, respectively), but it had lower tolerance to F⁻ (25 mg·L⁻¹). Different kinds of simulated inorganic anions leaching solutions of electroplating sludge had significant differences in terms of their effects on bioactivity of WZ-1 with a sequence of Cl⁻/NO₃⁻/F⁻ \geq NO₃⁻/F⁻ \geq Cl⁻/F⁻ \geq Cl⁻/F⁻ \geq Cl⁻/F⁻ \geq Cl⁻/NO₃⁻.

Key words: Acidithiobacillus ferrooxidans; ferrous; bioactivity; apparent respiratory rate; simulated inorganic anions leaching solution of electroplating sludge

嗜酸性氧化亚铁硫杆菌 (Acidithiobacillus ferrooxidans, A. f)是化能自养菌,能生长在亚铁、单质硫及硫化物矿物上^[1],且能通过直接作用或其代谢产物的间接作用,产生氧化、还原、络合、吸附或溶解作用^[2],达到浸出固相中重金属的效果.目前已在市政污泥^[3,4]、制革污泥^[5]重金属脱除、矿山冶金^[6,7]等领域成熟应用.同时,在 A. f 浸出废弃电子材料^[8,9]、废旧电池^[10,11]、熔炼粉尘^[12,13]中重金属方面取得较好试验效果.

为较好地利用 A.f 浸出固相中重金属,需要对处理对象或浸出液中主要成分对功能菌株活性的影响进行研究,为之后的浸出试验研究提供理论支持,以较好地指导后续的工程推广应用. 目前,研究人员进行了 Ni^{2+} 、 $Co^{2+[14]}$, $Cu^{2+[15]}$, $Mg^{2+[16]}$, Cr^{3+} 、 Cr^{6+} 、 $Mn^{2+[17]}$ 单一重金属离子; Cu^{2+} 、 Zn^{2+} 、 Fe^{3+}

复合重金属离子^[18];甲酸、乙酸、丙酸、苹果酸等低分子有机物^[2,19]; NO_3 、 SO_4 、Cl 和 PO_4 等阴离子^[20];闪锌矿、黄铜矿、黄铁矿、方铅矿等硫化矿^[1,21]对 A. f活性影响的研究.

为了研究、开发电镀污泥生物浸出技术,需考察电镀污泥浸出液对 A.f 活性的影响,本课题组之前研究了以 Ni^{2+} 、 Cr^{3+} 、 Cu^{2+} 、 Zn^{2+} 为主要组分的模拟电镀污泥重金属浸出液对自主分离纯化的氧化亚铁硫杆菌菌株 WZ-1 (GenBank 序列登录号: JQ968461)活性的影响^[24],获得了不同浓度、不同种类重金属离子分别在单一和复合条件下对氧化亚

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铁硫杆菌 WZ-1 活性的不同影响结果. 本研究在上述试验基础上,以电镀污泥浸出液中常见的无机阴离子作为影响因素,考察分析单一无机阴离子和模拟电镀污泥阴离子浸出液对 WZ-1 活性的影响,以获得电镀污泥浸出液中无机阴离子对 A. f 活性的影响规律,以期为后续理论试验研究及技术开发应用奠定基础.

1 材料与方法

1.1 菌株的培养及试验装置

(1)菌株的培养

氧化亚铁硫杆菌 WZ-1 分离自浙江省温州市龙湾区某制革污水处理站二沉池污泥,经细菌形态观察、革兰氏染色观察以及 16S rDNA 的 PCR 扩增、序列测定和系统发育树分析,确定该菌为 1 株氧化亚铁硫杆菌,取名 WZ-1, GenBank 中的序列登录号为 JQ968461. 氧化亚铁硫杆菌 WZ-1 的培养以及相关试验均于 9K 液体培养基中进行,培养基成分组成如下 $(g \cdot L^{-1}): (NH_4)_2SO_4$ 3. 00, $MgSO_4 \cdot 7H_2O$ 0. 50, K_2HPO_4 0. 50, KCl 0. 10, Ca $(NO_3)_2$ 0. 01, FeSO₄ $\cdot 7H_2O$ 44. 20, pH 2. 0. 于 30℃摇床中培养或试验(150 $\mathbf{r} \cdot \mathbf{min}^{-1})^{[22]}$.

(2)试验装置

试验采用 200 mL 已灭菌的锥形瓶作为培养容

器,并选用恒温摇床为试验培养提供恒温及规律摇晃环境.图1为试验期间,某一时刻各试验组溶液的不同颜色区别.



图1 试验期间某一时刻各试验组溶液的颜色区别

Fig. 1 Color difference between different liquids of experimental group at one point

1.2 单一无机阴离子对 WZ-1 活性影响试验

试验选用 Cl^- 、 NO_3^- 、 F^- 作为试验用无机阴离子,向 140 mL 9K 液体培养基中投加不同量的金属盐配制不同浓度试验组. 接种对数期 WZ-1 到试验组,用 $l.0 \text{ mol} \cdot L^{-1}$ 稀硫酸调节体系初始 pH 至 $l.0 \text{ col} \cdot L^{-1}$ 稀硫酸调节体系初始 pH 至 $l.0 \text{ col} \cdot L^{-1}$ 稀硫酸调节体系初始 pH 至 $l.0 \text{ col} \cdot L^{-1}$ 条件下培养. 培养过程中,每 $l.0 \text{ l.d} \cdot L^{-1}$ 条件下培养. 培养过程中,每 $l.0 \text{ col} \cdot L^{-1}$ 条件下培养 $l.0 \text{ col} \cdot L^{-1}$ 移成酸调节体系初始 pH 值、ORP 以及 $l.0 \text{ col} \cdot L^{-1}$ 的累计耗氧量. 试验用金属盐分别为: $l.0 \text{ col} \cdot L^{-1}$ 的累计耗氧量. 试验组设置见表 $l.0 \text{ col} \cdot L^{-1}$ 的累计耗氧量. 试验组设置见表 $l.0 \text{ col} \cdot L^{-1}$ 的。

表 1 单一无机阴离子对 WZ-1 活性影响试验试验组设置1)

试验组编号	蒸馏水/mL	菌液/mL	Cl - 浓度/g·L - 1	NO ₃ - 浓度/g·L ⁻¹	F-浓度/mg·L-1
CK-1	10	_	_	_	_
CK-2	_	10	_	_	_
Cl ⁻ -1	_	10	5. 0	_	_
Cl2	_	10	10.0	_	_
Cl3	_	10	20. 0	_	_
Cl4	_	10	30.0	_	_
NO ₃ - 1	_	10	_	1.0	_
NO ₃ -2	_	10	_	3.0	_
NO ₃ -3	_	10	_	5. 0	_
NO ₃ -4	_	10	_	10.0	_
F1	_	10	_	_	25. 0
F2	_	10	_	_	50. 0
F3	_	10	_	_	75. 0
F4	_	10	_	_	100.0

^{1)&}quot;一"表示未添加该组分,下同

1.3 模拟电镀污泥阴离子浸出液对 WZ-1 活性影响试验

该试验以1.2 节试验结果为设计依据,分别选取对 WZ-1 活性影响最小的无机阴离子浓度作为模

拟电镀污泥阴离子浸出液中各无机阴离子浓度,其中,试验组初始条件、试验过程和方法同 1.3 节,试验组设置见表 2.

1.4 指标监测分析方法

表 2 模拟电镀污泥无机阴离子浸出液对 WZ-1 活性影响试验试验组设置¹⁾

Table 2 Group settings of the experiment for investigating the influence of simulated anion leaching solution

of electroplating sludge on WZ-1's bioactivity

试验组编号	蒸馏水/mL	菌液/mL	Cl⁻浓度/g·L⁻¹	NO ₃ 浓度/g·L ⁻¹	F-浓度/mg·L-1
CK-1	10	_	_	_	_
CK-2	_	10	_	_	_
Cl -/NO ₃	_	10	2.5	0. 5	_
Cl -/F -	_	10	2.5	_	12. 5
NO ₃ /F -	_	10	_	0. 5	12. 5
Cl - /NO ₃ - /F -	_	10	2.5	0.5	12. 5

(1)pH、ORP 和 Fe²⁺监测方法^[22]

pH、ORP 采用 FE20K 精密 pH 计测定, pH 电极型号为 LE438, ORP 电极型号为 LE510(梅特勒-托利多仪器(上海)有限公司); Fe²⁺浓度测定采用邻菲啰啉分光光度法^[23].

(2) WZ-1 累计耗氧量和表观呼吸速率测定方 法^[22]

WZ-1 累计耗氧量通过 BI-2000 电解呼吸仪测 定,表观呼吸速率为累计耗氧量曲线斜率. WZ-1 在 液体培养基中进行好氧呼吸,同时进行 Fe²⁺氧化作 用,两者的共同作用会导致水封瓶内 0。被消耗,导 致瓶内压力降低,外腔与大气相通,由于内外气压 差,电解单元内腔液面上升、外腔液面下降,当外腔 探针离开液面时,系统自动接通电解单元电源,开始 电解稀硫酸生成 O,,补充瓶内被消耗的 O,,外腔产 生的 H, 逸出至大气中, 压力恢复后, 外腔液面上 升, 当探针重新接触液面后, 电解单元停止工作. 测 试数据直接通过计算机记录,每3 min 记录1次. BI-2000 电解呼吸仪具有自动磁力搅拌功能,转速为 100 r·min⁻¹, 水浴温度为 30℃. 试验时, 准确量取 100 mL 混合培养液于 150 mL 水封瓶内,组装完毕 后进行系统检漏,检漏成功后运行1h,待系统温度 完全稳定后,正式启动呼吸仪开始监测.

2 结果与讨论

2.1 单一无机阴离子对 WZ-1 活性的影响

2.1.1 单一无机阴离子对 WZ-1 Fe^{2+} 氧化活性的 影响

Acidithiobacillus ferrooxidans 在生物浸出过程中,依靠将 Fe^{2+} 氧化成 Fe^{3+} 这一过程来获得自身生长繁殖所需能量, Fe^{2+} 的氧化程度能表征其代谢活性[^{23]}, Fe^{2+} 氧化率依照式(1)计算.

$${\rm Fe}^{2+}$$
氧化率 = $(\,c_{_{\rm Fe}^{2+}\bar\eta y \dot h}\,-\,c_{_{\rm Fe}^{2+}\bar\eta h \dot h}\,)/c_{_{\rm Fe}^{2+}\bar\eta y \dot h}}\times 100\%$

图 2 为 Cl $^-$ 、NO $_3^-$ 、F $^-$ 对 WZ-1 氧化 Fe $^{2+}$ 的影响作用.

从图 2 可知,空白组 CK-2 中 WZ-1 经 12 h 后即进入对数增长期,培养液中的 Fe^{2+} 在 60 h 内被完全氧化, Fe^{2+} 氧化平均速率为 114. 44 $mg \cdot h^{-1}$,表明 WZ-1 具有较好的 Fe^{2+} 氧化活性.

由图 2(a)、2(b) 分别可知,低浓度 Cl-(5.0 g·L⁻¹)、NO₃⁻(1.0 g·L⁻¹)对 WZ-1 的 Fe²⁺氧化活 性几乎没有影响. 培养 60 h 时, Cl-1、NO, -1 已氧化 99%以上的 Fe²⁺, Fe²⁺平均氧化速率分别为 115.41 mg·h⁻¹、120.69 mg·h⁻¹,较空白组 CK-2 几乎没有 差别. 这一结果与张成桂等[20]的研究结果有所不 同,其研究表明:0.05 mol·L⁻¹(1.775 g·L⁻¹)的Cl⁻ 完全抑制氧化亚铁硫杆菌活性,整个试验过程中,氧 化亚铁硫杆菌数量没有增长趋势,pH 亦无下降变 化; 3.1 g·L⁻¹的 NO₃ 亦对氧化亚铁硫杆菌产生完 全抑制作用,而 WZ-1 在 3.0 g·L⁻¹甚至 5.0 g·L⁻¹ 的 NO, 浓度条件下仍具有较好的 Fe²⁺氧化活性. 当 Cl⁻、NO₃ 浓度上升, WZ-1 的 Fe²⁺ 氧化活性则 开始受到抑制,至浓度分别达到 20.0 g·L⁻¹、10.0 g·L-1时,WZ-1 完全丧失 Fe2+氧化活性. 这或许由 于 WZ-1 分离于制革污泥,制革污水中各种阴离子 较为复杂,包括 Cl-和 NO3, WZ-1 长期生活于该环 境中,对其已产生一定的耐性,但当浓度上升至一定 阶段后, WZ-1 还是无法适应高浓度 Cl - 和 NO, 1.

由图 2(c) 可知, WZ-1 在 25.0 mg·L⁻¹ F⁻ 存在的条件下,活性受到一定的抑制,但经过近 50 h 的停滞期后, WZ-1 逐渐适应该环境,并开始快速氧化培养液中的 Fe^{2+} ,至第 96 h 时, Fe^{2+} 氧化率达到 85.87%, 亚铁平均氧化率为 66.72 mg·L⁻¹. 随着 F⁻ 浓度的不断上升, WZ-1 的 Fe^{2+} 氧化活性受到完全抑制. 研究表明: WZ-1 较一般的氧化亚铁硫杆菌对 $C1^-$ 和 NO_3^- 有更好的耐受能力, 5.0 g·L⁻¹的 $C1^-$ 和 1.0 g·L⁻¹的 NO_3^- 对 WZ-1 的活性几乎没有影响,

25. 0 $mg \cdot L^{-1} F^-$ 对 WZ-1 的活性有一定的抑制作用,随着浓度的上升, WZ-1 活性开始受到明显抑制,当 Cl⁻、NO₃⁻ 和 F⁻ 浓度分别达到 20. 0 $g \cdot L^{-1}$ 、10. 0 $g \cdot L^{-1}$ 和 50. 0 $mg \cdot L^{-1}$ 时, WZ-1 完全失活.

根据上述试验结果可知,试验所用 3 种无机阴离子对 WZ-1 Fe^{2+} 氧化活性的影响顺序为: F^{-} > NO_{3}^{-} > Cl^{-} .

2.1.2 单一无机阴离子对 WZ-1 累计耗氧量和表 观呼吸速率的影响

针对 2.1.1 节的试验结果,本研究利用 BI-2000

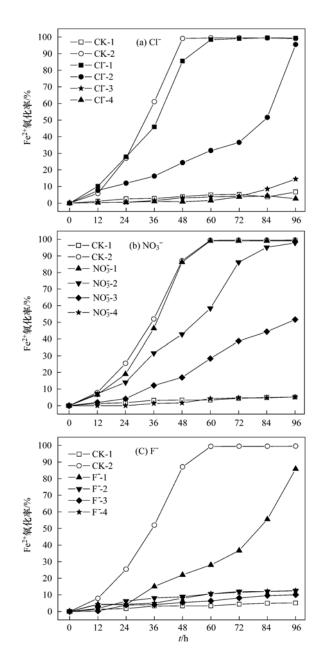


图 2 不同浓度 Cl - 、NO₃ 、F - 对 WZ-1 氧化 Fe²⁺ 的影响

Fig. 2 Effects of different concentrations of Cl $^{-}$, NO $_3^-$, $F^- \ \, on \ \, Fe^{2\,+} \ \, oxidation \, of \ \, WZ-1$

电解呼吸仪测定 WZ-1 在不同试验阶段的累计耗氧量并计算其表观呼吸速率,以验证无机阴离子对WZ-1 活性的影响规律.

图 3 分别为 Cl^- 、 NO_3^- 、 F^- 各浓度梯度试验组在第 0 h、第 48 h、第 96 h 时的表观呼吸速率. 从中可以看出,空白组 CK-2 中 WZ-1 的表观呼吸速率由第 0 h 的 12. 5 $mg \cdot (L \cdot h)^{-1}$ 上升至第 48 h 时的 27. 5 $mg \cdot (L \cdot h)^{-1}$,第 96 h 时,其表观呼吸速率又下降到 9. 5 $mg \cdot (L \cdot h)^{-1}$,变化趋势呈抛物线. 比对各试验组的表观呼吸速率变化和图 2 的 Fe^{2+} 氧化曲线,发

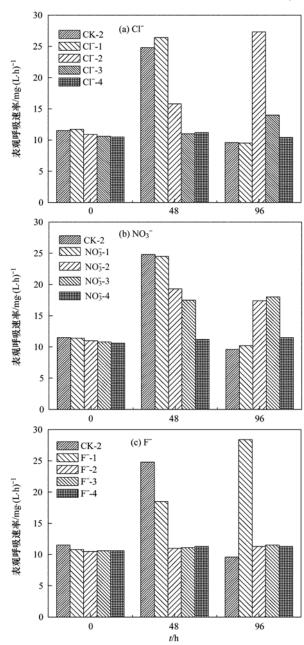


图 3 不同浓度 Cl^- 、 NO_3^- 、 F^- 对 WZ-1 表观呼吸速率的影响

Fig. 3 Effects of different concentrations of Cl $^{\rm -}$, NO $_3^{\rm -}$, F $^{\rm -}$ on the apparent respiratory rate of WZ-1

现 WZ-1 的表观呼吸速率变化趋势及变化幅度与对 应 Fe2+氧化曲线切线斜率的变化趋势和变化幅度 一致,且各变化时刻点与 Fe²⁺ 氧化速率变化时刻点 相吻合.

通过测定分析 WZ-1 的累计耗氧量和表观呼吸 速率,从细菌利用 0。进行生命活动的能力方面验证 了单一无机阴离子对 WZ-1 活性的影响. WZ-1 呼吸 试验的结果与 2.1.1 节的试验结果一致.

2.2 模拟电镀污泥阴离子浸出液对 WZ-1 活性的 影响

为了考察电镀污泥阴离子浸出液对 WZ-1 活性 的影响,根据 2.1 节试验结果,选取对 WZ-1 活性抑 制作用较小的各无机阴离子浓度配制模拟电镀污泥 阴离子浸出液,而预试验结果显示:Cl-、NO;、F-浓度分别为 5.0 g·L⁻¹、1.0 g·L⁻¹、25 mg·L⁻¹时, WZ-1 完全失活, 所有试验组无试验差异, 因此, Cl⁻、NO₃⁻、F⁻的配制浓度改为: 2.5 g·L⁻¹、0.5 $g \cdot L^{-1} \setminus 25 \text{ mg} \cdot L^{-1}$.

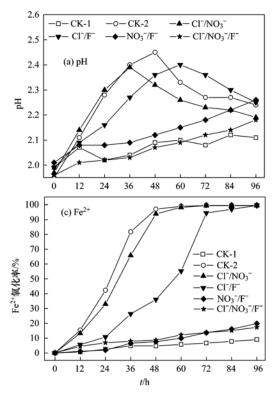
氧化亚铁硫杆菌被接种到新鲜的 9K 液体培养 基后,能快速氧化其中的 Fe2+,这一过程需要消耗 培养液中的 H+ [式(2)], 当培养液中大部分 Fe²⁺ 被氧化为 Fe3+ 后, Fe3+ 开始水解, 并产生 H+[式 因[24],因此,pH 值是氧化亚铁硫杆菌培养过程中的 一个重要指标.

(3)],这就是培养过程中 pH 先升后降的主要原

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{A.f} 4Fe^{3+} + 2H_2O$$
 (2)

$$Fe^{3+} + 3H_2O \longrightarrow Fe(OH)_3 + 3H^+$$
 (3)

图 4 反映了不同模拟电镀污泥阴离子浸出液对 培养液 pH、ORP、Fe2+氧化率以及 WZ-1 表观呼吸 速率变化的影响. 所有试验组的 pH 值在 12 h 内均 有所上升,对比 CK-1 的 pH 变化趋势,可知这不是 由 WZ-1 氧化 Fe²⁺ 所引起的 pH 值上升, 而是由于 培养基具有一定的 pH 滞后性所造成的. 接种 12 h 后,CK-2的pH值快速上升,至第48h时,pH达到 最大值 2.45,之后由于 Fe3+水解作用不断释放 H+, 培养液的 pH 值持续下降. 相应的, CK-2 的 ORP 在 前 36 h 缓慢上升,由最初的 356 mV 上升至 448 mV,之后快速上升,至第96 h 达到614 mV. 比较不 同试验组的 pH 值、ORP 变化曲线,可以明显看出 不同模拟电镀污泥阴离子浸出液对培养液 pH 值和 ORP 变化的影响差异. 结合图 4(c) 中各试验组 Fe2+氧化曲线,可见试验组进入Fe2+快速氧化期的 拐点与 pH 快速上升的拐点处于相同时刻,而当培



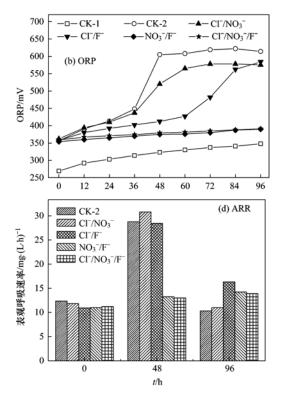


图 4 模拟电镀污泥阴离子浸出液对培养液 pH 值、ORP、Fe2+氧化及 WZ-1 表观呼吸速率变化的影响

Fig. 4 Effects of simulated inorganic anion leaching solution of electroplating sludge on changes of pH, ORP, Fe2+ oxidation and apparent respiratory of WZ-1

养液中 Fe^{2+} 氧化率达到 50% 以上时,溶液的 ORP 快速上升.

图 4(c) 中不同模拟电镀污泥阴离子浸出液对 WZ-1 Fe²⁺氧化活性影响的差异明显. Cl⁻/NO₃-/ F 和 NO, 7F 这 2 种模拟电镀污泥阴离子浸出液 对 WZ-1 的 Fe²⁺氧化活性抑制最显著,Fe²⁺氧化率 在试验结束时分别仅为 17. 32%、19. 82%, 比较 CK-1 可知 WZ-1 几乎完全丧失 Fe^{2+} 氧化活性. 而 分别由 Cl-、NO; 和 Cl-、F-组成的模拟电镀污 泥阴离子浸出液对 WZ-1 Fe^2+ 氧化活性影响较小, 试验中, WZ-1 虽然分别有近 12 h 和 24 h 的停滞 期,但随后即进入对数生长期,其 Fe2+氧化速率较 快,分别于第72 h 和第96 h 达到99% 以上的 Fe2+ 氧化率,与此同时,CK-2 达到 99% Fe2+氧化率的 耗时为60 h. 图4(d)中各试验组 WZ-1 的表观呼 吸速率大小顺序及变化趋势与图 4(c) 的 Fe^{2+} 氧 化曲线在对应时刻的斜率大小及其变化趋势 一致.

根据上述试验结果可知,不同模拟电镀污泥阴离子浸出液对 WZ-1 活性影响的差异明显,Cl⁻、 NO_3^- 、 F^- 配制浓度分别为 2.5 g·L⁻¹、0.5 g·L⁻¹、25 mg·L⁻¹时,Cl⁻/ NO_3^- / F^- 和 NO_3^- / F^- 这 2 种模拟电镀污泥阴离子浸出液对 WZ-1 活性产生完全抑制,Cl⁻/ NO_3^- 和 Cl⁻/ F^- 这 2 种模拟电镀污泥阴离子浸出液对 WZ-1 活性的影响较小,5 种模拟电镀污泥阴离子浸出液对 WZ-1 活性影响的大小为:Cl⁻/ NO_3^- / F^- > NO_3^- / P^- > $NO_3^ P^-$ > $NO_3^ P^ P^ NO_3^ NO_3^-$

3 结论

- (1) WZ-1 较一般的氧化亚铁硫杆菌对 Cl⁻和 NO₃⁻有更好的耐受能力,5.0 g·L⁻¹的 Cl⁻和 1.0 g·L⁻¹的 NO₃⁻对 WZ-1 的活性几乎没有影响. WZ-1 对单一 Cl⁻、NO₃⁻、F⁻的最大耐受浓度分别为 10.0 g·L⁻¹、5.0 g·L⁻¹、25.0 mg·L⁻¹.
- (2) 根据模拟电镀污泥阴离子浸出液对 WZ-1 活性影响大小的顺序,从阴离子这一影响因素角度 考虑,WZ-1 较适合处理含一定量 Cl^-/F^- 或 Cl^-/NO_3^- 为主要阴离子组分的电镀污泥,对同时含有 $Cl^-/NO_3^-/F^-$ 或者 NO_3^-/F^- 的电镀污泥则完全失效.

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