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### 目 次

深圳大气颗粒物中卤代多环芳烃污染研究 ····································	
	3)
北京市曲刑祭协众 JU VOC 批讲性红斑穴 出版 和蛙目 何下達 化块芒 耳石 公太鄉 迷溪(152	2)
北京中央望食队企业 VOUS 排放付证明九	(3)
2006~2010 年珠三角地区 SO <sub>2</sub> 特征分析	80)
环境空气 PM、连续监测系统手工采样比对测试 ························· 王强、钟琪、迟颖、张杨、杨凯(153	88)
燃煤由厂可凝结颗粒物的测试与排放   ***	۱ <u>۵</u> )
然外电产的观点软件的的现在分词从 主治无比学生的家庭化步入强压破损用地继续基础是自由了供使奶帕克	7
南海水坝不同深及非尤合似生物的直恢浴能及其对不同电士供体的响应····································	
	(0)
基于 GOCI 影像和水体光学分类的内陆湖泊叶绿素 a 浓度遥感估算 冯驰,金琦,王艳楠,赵丽娜,吕恒,李云梅(155贵州清水江流域丰水期水化学特征及离子来源分析 吕婕梅,安艳玲,吴起鑫,罗进,蒋浩(156东莞石马河流域水化学特征时空差异及来源辨析 高磊,陈建耀,王江,柯志庭,朱爱萍,许凯(157河东源)[河京水水北岩]	7)
告州清水汀流域主水期水化学转征及离子平源分析	55)
- 央川田小江加坡十小河小化寸竹皿及南 J 不極力切 - ロ灰梅, ×花々, 大尺鐘, 少址, 竹垣 (150 キャプコンドレ J ル サイル・シャリー 1 - マー・ サー 1 - マー・ 1	12)
乐完石马河流域水化字特征时仝差异及米源辨析 尚磊,陈廷雄,土江, 何态庭, 朱发泙, 计凯(15/	(3)
河南鸡冠洞洞穴水对极端气候的响应及其控制因素研究 ·····	
	(2.)
石漠化治理对岩溶地下水水化学和溶解无机碳稳定同位素的影响 肖时珍,熊康宁,蓝家程,张晖,杨龙(159	(0)
旱季不同土地利用类型下岩溶碳汇效应差异 赵瑞一,梁作兵,王尊波,于正良,江泽利 (159	98)
有机氯农药在岩溶区上覆土壤中的垂直迁移特征及对地下水的影响 孙玉川,王永啟,梁作兵,袁道先(160	)5)
山东南四湖沉积物中汞的污染现状及迁移研究 曹霏霏,杨丽原,庞绪贵,王炳华,王云倩(161	5)
摇蚊幼虫扰动下沉积物微环境和微界面对物理扰动强度的响应 史晓丹,李勇,李大鹏,王忍,邓猛,黄勇(162	2)
蓝玻矾式机矾于0.10个的成分另种成介田内的连轨到加度的响应	22)
南万红壤区氮湿沉降特征及具对流域氮输出的影响	80)
不同紫色母岩对景观水体氮磷及有机物去除的影响 黄雪娇,刘晓晨,李振轮,石纹豪,杨珊(163	39)
荔枝落叶对铜绿微囊藻牛长和光合作用的影响	18)
带连相节温相物对是菜的丰田作用	5 )
與比似全位從例列球條的母连[F/用] 除亚洲,表对(103	)
南方红壤区氮湿沉降特征及其对流域氮输出的影响	2)
水中利谷隆氯化降解动力学和消毒副产物生成特性 凌晓, 胡晨燕, 程明, 谷建(166	(8)
化学消毒的中和剂对水中内毒素活性检测的影响 ····································	/4 )
11 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	10 \
十美生物灰外小中氨氮的吸附行性	0)
丁二酸改性茶油树木屑吸附铀的研究 张晓峰,陈迪云,彭燕,刘永胜,熊雪莹(168	36)
SPG 膜表面润湿性对膜污染和化学耐受性的影响 ·················· 张静,肖太民,张晶,曹丽亚,杜亚威,刘春,张磊 (169	94)
TiO. 诱导下左旋氧氟沙星的可见光降解及其机制	00.
$TiO_2$ 诱导下左旋氧氟沙星的可见光降解及其机制 郭宏生,刘亚楠,乔琪,魏红,董呈幸,薛洁,李克斌(170新型高分子絮凝剂对废水中 $Cr(VI)$ 的捕集性能 王刚,杜凤龄,常青,徐敏(170	77 \
利望向分丁系娱利利及小中Cr( VI)的佣果性能	"
基于 OUR-HPR 测量在线估计活性污泥合成 PHA 量 曾善文,王泽宇,高敬,刘东,张代钧,卢培利(171	.3)
分离高浓度污泥产酸发酵液的自生动态膜形成机制 ····································	20)
通风强度对市政污泥生物干化中试效果的影响	
$\mathbb{Z}_{\mathcal{N}}$	7)
上版可收阅教人刘公复歌 N N 二乙酸四种对泛混由金人昆荽取盐或的研究	27)
生物可降解螯合剂谷氨酸 N,N-二乙酸四钠对污泥中重金属萃取效率的研究	
	(3)
	(3)
	(3)
	(3)
三年,崔延瑞,汤晓晓,杨慧娟,孙剑辉(173百乐克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析············· 田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173异养硝化-好氧反硝化菌 YL 的脱氮特性····································	33) 39) 49)
三年,崔延瑞,汤晓晓,杨慧娟,孙剑辉(173百乐克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析············· 田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173异养硝化-好氧反硝化菌 YL 的脱氮特性····································	33) 39) 49)
三年,崔延瑞,汤晓晓,杨慧娟,孙剑辉(173百乐克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析············· 田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173异养硝化-好氧反硝化菌 YL 的脱氮特性····································	33) 39) 49)
三年,《中国 一年	33) 39) 49) 57) 53)
三年,《中国 一年	33) 39) 49) 57) 53)
三年克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析 田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173) 异养硝化-好氧反硝化菌 YL 的脱氮特性 梁贤,任勇翔,杨全,赵思琪,夏志红(174) 菌株 Arthrobacter sp. CN2 降解对硝基苯酚的特性与动力学 任磊,史延华,贾阳,姚雪松,Ruth Nahurira,弥春霞,闫艳春(175) 短短芽胞杆菌及其芽胞对芘的降解 刘芷辰,叶锦韶,彭辉,刘则华,邓庭进,尹华,廖丽萍(176) 垃圾填埋场抗生素抗性基因初探 李蕾,徐晶,赵由才,宋立岩(176) 不同构型人工湿地基质中土著菌的耐药性及整合子丰度调查 麦晓蓓,陶然,杨扬,张敏,林剑华,满滢(177)	33) 39) 49) 57) 53) 59)
三年克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析 田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173) 异养硝化-好氧反硝化菌 YL 的脱氮特性 梁贤,任勇翔,杨全,赵思琪,夏志红(174) 菌株 Arthrobacter sp. CN2 降解对硝基苯酚的特性与动力学 任磊,史延华,贾阳,姚雪松,Ruth Nahurira,弥春霞,闫艳春(175) 短短芽胞杆菌及其芽胞对芘的降解 刘芷辰,叶锦韶,彭辉,刘则华,邓庭进,尹华,廖丽萍(176) 垃圾填埋场抗生素抗性基因初探 李蕾,徐晶,赵由才,宋立岩(176) 不同构型人工湿地基质中土著菌的耐药性及整合子丰度调查 麦晓蓓,陶然,杨扬,张敏,林剑华,满滢(177)	33) 39) 49) 57) 53) 59)
三年克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析 田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173) 异养硝化-好氧反硝化菌 YL 的脱氮特性 梁贤,任勇翔,杨全,赵思琪,夏志红(174) 菌株 Arthrobacter sp. CN2 降解对硝基苯酚的特性与动力学 任磊,史延华,贾阳,姚雪松,Ruth Nahurira,弥春霞,闫艳春(175) 短短芽胞杆菌及其芽胞对芘的降解 刘芷辰,叶锦韶,彭辉,刘则华,邓庭进,尹华,廖丽萍(176) 垃圾填埋场抗生素抗性基因初探 李蕾,徐晶,赵由才,宋立岩(176) 不同构型人工湿地基质中土著菌的耐药性及整合子丰度调查 麦晓蓓,陶然,杨扬,张敏,林剑华,满滢(177)	33) 39) 49) 57) 53) 59)
三年克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析 田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173) 异养硝化-好氧反硝化菌 YL 的脱氮特性 梁贤,任勇翔,杨全,赵思琪,夏志红(174) 菌株 Arthrobacter sp. CN2 降解对硝基苯酚的特性与动力学 任磊,史延华,贾阳,姚雪松,Ruth Nahurira,弥春霞,闫艳春(175) 短短芽胞杆菌及其芽胞对芘的降解 刘芷辰,叶锦韶,彭辉,刘则华,邓庭进,尹华,廖丽萍(176) 垃圾填埋场抗生素抗性基因初探 李蕾,徐晶,赵由才,宋立岩(176) 不同构型人工湿地基质中土著菌的耐药性及整合子丰度调查 麦晓蓓,陶然,杨扬,张敏,林剑华,满滢(177)	33) 39) 49) 57) 53) 59)
是青,崔延瑞,汤晓晓,杨慧娟,孙剑辉(173 百乐克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析	33) 39) 39) 37) 33) 36) 35)
早青,崔延瑞,汤晓晓,杨慧娟,孙剑辉(173   百乐克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析   田美,刘汉湖,申欣,赵方庆,陈帅,姚永佳(173   异养硝化-好氧反硝化菌 YL 的脱氮特性   梁贤,任勇翔,杨全,赵思琪,夏志红(174   菌株 Arthrobacter sp. CN2 降解对硝基苯酚的特性与动力学   任磊,史延华,贾阳,姚雪松,Ruth Nahurira,弥春霞,闫艳春(175 短短芽胞杆菌及其芽胞对芘的降解   刘芷辰,叶锦韶,彭辉,刘则华,邓庭进,尹华,廖丽萍(176   垃圾填埋场抗生素抗性基因初探   李蕾,徐晶,赵由才,宋立岩(176   不同构型人工湿地基质中土著菌的耐药性及整合子丰度调查   麦晓蓓,陶然,杨扬,张敏,林剑华,满滢(177   硝酸盐和甲烷对覆土中苯系物厌氧氧化的影响   柳蓉,龙焰,王立立,何婷,叶锦韶(178   山西高原落叶松人工林土壤呼吸的空间异质性   严俊霞,李洪建,李君剑,武江星(179   施氮对黄土旱塬区春玉米土壤呼吸和温度敏感性的影响   美继韶,郭胜利,王蕊,刘庆芳,王志齐,张彦军,李娜娜,李如剑,吴得峰,孙棋棋(180	33) 39) 39) 37) 33) 36) 35)
是青,崔延瑞,汤晓晓,杨慧娟,孙剑辉(173 百乐克(BIOLAK)活性污泥宏基因组的生物多样性及功能分析	33) 39) 49) 57) 53) 59) 76) 35)
「日のLAK   活性汚泥宏基因组的生物多样性及功能分析   日美、刘汉湖、申欣、赵方庆、陈帅、姚永佳 (173   早养硝化-好氧反硝化菌 YL 的脱氮特性   深景、任勇翔、杨全、赵思琪、夏志红 (174   菌株 Arthrobacter sp. CN2 降解对硝基苯酚的特性与动力学   任磊、史延华、贾阳、姚雪松、Ruth Nahurira、弥春霞、闫艳春 (175   短短芽胞杆菌及其芽胞对芘的降解   刘芷辰、叶锦韶、彭辉、刘则华、邓庭进、尹华、廖丽萍 (176   垃圾填埋场抗生素抗性基因初探   李曹、徐晶、赵由才、宋立岩 (176   不同构型人工湿地基质中土著菌的耐药性及整合子丰度调查   表晓蓓、陶然、杨杨、张敏、林剑华、满滢 (177   硝酸盐和甲烷对覆土中苯系物厌氧氧化的影响   柳蓉、龙焰、王立立、何婷、叶锦韶 (178   山西高原落叶松人工林土壤呼吸的空间异质性   严俊霞、李洪建、李君剑、武江星 (179   施氮对黄土旱塬区春玉米土壤呼吸和温度敏感性的影响   一一一一一一一一一一一一一一一一一一一一一一一一一一一一一一一一一一一	33) 39) 39) 37) 33) 36) 35) 33) 36) 37) 36)
三成街区AK)活性污泥宏基因组的生物多样性及功能分析	33) 39) 39) 37) 33) 35) 35) 33) 32) 36) 88) 87) 86)
三成街区AK)活性污泥宏基因组的生物多样性及功能分析	33) 39) 39) 37) 33) 35) 35) 33) 32) 36) 88) 87) 86)
「日の日の日の日の日の日の日の日の日の日の日の日の日の日の日の日の日の日の日の	33) 39) 39) 37) 33) 35) 35) 35) 32) 36) 36) 37) 36)
日	33) 39) 39) 37) 33) 35) 35) 33) 32) 36) 36) 36) 36) 36)
	33) 39) 39) 39) 37) 33) 35) 33) 32) 36) 36) 36) 36) 36) 36) 36) 36) 36) 36
	33) 39) 39) 39) 37) 33) 35) 33) 32) 36) 36) 36) 36) 36) 36) 36) 36) 36) 36
	33) 39) 39) 39) 37) 33) 35) 33) 32) 36) 36) 36) 36) 36) 36) 36) 36) 36) 36
	33) 39) 39) 39) 37) 33) 35) 33) 32) 36) 36) 36) 36) 36) 36) 36) 36) 36) 36
展青、崔延瑞、汤晓晓、杨慧娟、孙剑辉(173	33) 39) 39) 39) 37) 33) 35) 33) 32) 36) 36) 36) 36) 36) 36) 36) 36) 36) 36
	33) 39) 39) 39) 37) 33) 35) 33) 32) 36) 36) 36) 36) 36) 36) 36) 36) 36) 36

## 异养硝化-好氧反硝化菌 YL 的脱氮特性

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(1. 西安建筑科技大学环境与市政工程学院,西安 710055; 2. 陕西省建筑设计研究院有限责任公司,西安 710016) 摘要:针对传统自养硝化-厌氧反硝化工艺流程长、脱氮效率低的问题,从驯化成熟且具有高效同步硝化反硝化作用的 SBR 反应器中筛得 1 株异养硝化菌 YL,经鉴定为铜绿假单胞菌(*Pseudomonas aeruginosa*),并通过单因子试验和正交试验对其异养硝化和好氧反硝化特性进行了研究. 结果表明,菌株 YL 进行氨氧化作用的最适条件为:碳源为琥珀酸钠、C/N 为 10、pH 为 7.0、温度为 30℃、转速为 160~200 r·min⁻¹,此时氨氧化速率为 5.05 mg·(g·h)⁻¹,TOC 转化速率为 45.95 mg·(g·h)⁻¹,氦氮和 TOC 去除率分别为 100% 和 90.8%;菌株 YL 还能够利用亚硝酸盐、硝酸盐和羟胺进行生长代谢,去除率分别为 92.7%、93.6% 和 94.8%;影响菌株 YL 好氧反硝化性能最主要的因素为 C/N,在最优条件(C/N = 10, T = 30℃, r = 200 r·min⁻¹, pH = 7)下,硝氮去除率为 94.6%,总氮去除率 76.3%.表明菌株 YL 能够独立快速高效地完成异养硝化和好氧反硝化脱氮过程. 关键词:异养硝化;好氧反硝化;铜绿假单胞菌;脱氮;正交试验

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# Characteristics of Nitrogen Removal by a Heterotrophic Nitrification-Aerobic Denitrification Bacterium YL

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**Abstract**: Traditional process of autotrophic nitrification-anaerobic denitrification usually has problems of long procedure and low efficiency. To overcome these problems, a heterotrophic nitrification-aerobic denitrification bacterium YL was isolated from a domesticated mature SBR reactor with efficient simultaneous nitrification and denitrification ability, and was identified as *Pseudomonas aeruginosa* YL. Meanwhile, the characteristics of the nitrogen removal of strain YL was investigated through single – factor experiments and an orthogonal experiment. The results showed that the preferred conditions were: succinate as the carbon source, C/N ratio of 10, pH of 7.0, temperature of 30°C, and the shaking speed of 160-200  $\mathbf{r} \cdot \mathbf{min}^{-1}$ , while the removal rate of ammonia oxidation was 5.05  $\mathbf{mg} \cdot (\mathbf{g} \cdot \mathbf{h})^{-1}$ , the transformation rate of TOC was 45.95  $\mathbf{mg} \cdot (\mathbf{g} \cdot \mathbf{h})^{-1}$ , and the removal rates of nitrogen and TOC were 100% and 90.8%, respectively. Nitrite, nitrate and hydroxylamine could also be metabolized by strain YL, and the removal rates were 92.7%, 93.6% and 94.8%, respectively. The most important influencing factor on aerobic denitrification of strain YL was C/N ratio. Under the optimal conditions (C/N = 10, T = 30°C, r = 200 r·min<sup>-1</sup>, pH = 7), the removal rates of nitrate and total nitrogen were 94.6% and 76.3%, respectively. Hence, strain YL could remove nitrogen by heterotrophic nitrification-aerobic denitrification independently, quickly, and effectively.

Key words: heterotrophic nitrification; aerobic denitrification; Pseudomonas aeruginosa; nitrogen removal; orthogonal experiment

传统的生物脱氮工艺即自养硝化-厌氧反硝化工艺,存在基建费用高、水力停留时间长、能耗大、抗冲击能力弱等不足. 近年越来越多的异养硝化好氧反硝化 (heterotrophic nitrification-aerobic denitrification, HN-AD) 菌被发现,主要包括泛养硫球菌 (Thiosphaera pantotropha) [1]、产碱菌属 (Alcaligenes) [2]、假单胞菌属(Pseudomonas) [3]、芽孢杆菌(Bacillus) [4]、不动杆菌(Acinetobacter) [5]等.这些细菌因具有生长快、活性高、增殖底物广泛、能同时进行异养硝化和好氧反硝化等优势而具有显著的工程应用价值. Joo 等[6]将 Alcaligenes faecalis strain No. 4应用于养猪场废水处理,在高有机和高氨负荷下取得了良好的碳氮去除效果; Yao 等[7]通

过向实验室人工配水 SBR 反应器中投加耐寒 HN-AD 菌,显著改善了低温时氮的去除. 但是国内外对 HN-AD 菌的研究尚处于起步阶段,因此筛选出更多可同步脱氮除碳的 HN-AD 菌并对其生物学特性进行深入研究,对丰富生物脱氮理论和指导工程实践具有重要意义.

本研究从实验室长期稳定运行的 SBR 反应器中筛得1 株异养硝化菌株 YL 并对其进行了鉴定.

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同时对其脱氮特性进行了分析.

#### 1 材料与方法

#### 1.1 菌株的分离及鉴定

从稳定运行的 SBR 反应器(进水NH<sub>4</sub><sup>+</sup>-N 100 mg·L<sup>-1</sup>, COD 1200 mg·L<sup>-1</sup>)中取活性污泥样品 10 mL 充分悬浮于 90 mL 0.9% 的无菌生理盐水中,在异养硝化培养基上进行稀释涂布,于生化培养箱30℃下培养.进行多次纯化分离,挑选出形成的单菌落进行异养硝化能力测试<sup>[8]</sup>.将筛选出的具有较高异养硝化特性的菌株悬浮在 25% 的甘油中,于-80℃保存.

观察菌株的菌落特征,个体形态和革兰氏染色结果. 菌株的 DNA 提取采用细菌基因组提取试剂盒(MO BIO, USA),将提取菌株的 DNA 作为 16S rDNA 的扩增模板,采用德国 Eppendorf 银制梯度PCR 仪(型号:5345 000.579)进行扩增,反应引物为 27F: 5'-AGAGTITGATCCTGGCTCTAG-3'和1492R:5'-GGTTACCTTGTTACGACTT-3'.采用北京六一仪器厂生产的 DYY-6D DYCP-31DN 水平电泳系统琼脂糖凝胶电泳分离检测 PCR 产物,产物的测序由上海生工生物技术有限公司完成.测序结果用BLAST 程序和 GenBank 中已登录的 16S rDNA 序列进行核苷酸同源性比较,然后应用 MEGA 5.0 软件构建该菌株系统发育树.

#### 1.2 培养基

异养硝化培养基(g·L<sup>-1</sup>):  $(NH_4)_2SO_40.47$ , 琥珀酸钠 5.62, 维氏盐溶液 50 mL, C/N = 10, pH = 7.0.

羟胺培养基( $g \cdot L^{-1}$ ): HONH<sub>3</sub>Cl 0.25, 琥珀酸钠 2.81, 维氏盐溶液 50 mL, pH = 7.0.

亚硝氮培养基 $(g \cdot L^{-1})$ : NaNO<sub>2</sub> 0. 49, 琥珀酸钠 5. 62, 维氏盐溶液 50 mL, pH = 7. 0.

硝氮培养基 $(g \cdot L^{-1})$ : KNO<sub>3</sub> 0.72, 琥珀酸钠 5.62, 维氏盐溶液 50 mL, pH = 7.0.

维氏盐溶液 (g·L<sup>-1</sup>): K<sub>2</sub>HPO<sub>4</sub> 5.0, MgSO<sub>4</sub>·7H<sub>2</sub>O 2.5, NaCl 2.5, MnSO<sub>4</sub>·4H<sub>2</sub>O 0.05, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.05.

#### 1.3 菌株 YL 的脱氮特性研究

#### 1.3.1 不同反应条件对菌株 YL 异养硝化的影响

分别研究碳源、C/N、pH、温度和转速对氨氮 去除效果的影响. 碳源包括葡萄糖、乙酸钠、蔗糖、柠檬酸钠和琥珀酸钠; C/N 分别为 2、5、10 和 15; pH 分别为 4、5、6、7、8、9、10 和 11; 温度分别为

10、20、30 和 37℃;摇床转速分别为 80、120、160 和 200 r·min<sup>-1</sup>;其中氨氮浓度均为 100 mg·L<sup>-1</sup>. 固定单一影响因子,其余条件分别为温度 30℃、pH = 7.0、C/N = 10 以及转速 160 r·min<sup>-1</sup>. 将处于对数生长期的菌液 ( $D_{600}$  = 1.000)以 1% 接种量接种于 100 mL 的不同培养基中,各培养基分装于 250 mL 锥形瓶中经高压灭菌. 摇床培养约 24 h,测定  $D_{600}$ 、氨氮和 TOC 浓度变化.

#### 1.3.2 菌株 YL 对不同氮源的利用

碳源为琥珀酸钠,氮源分别为氨氮、羟胺、亚硝酸盐和硝酸盐,培养基对应的浓度为: 氨氮 100  $\text{mg}\cdot\text{L}^{-1}$ 、羟胺 50  $\text{mg}\cdot\text{L}^{-1}$ 、亚硝氮和硝氮均为 200  $\text{mg}\cdot\text{L}^{-1}$ . 接种量为 1%、C/N=10、30°C、160  $\text{mg}\cdot\text{L}^{-1}$ 条件下培养 48 h,定时测定其  $D_{600}$ 、pH、TOC 以及氮浓度,判断菌株 YL 对 4 种氮源的利用情况.

#### 1.3.3 正交试验设计

为优化菌株 YL 好氧反硝化性能的条件,以硝酸盐为利用氮源,设计 L<sup>16</sup>(4<sup>4</sup>)正交试验,因素水平表见表 1. 将菌液以 1%的接种量接种于装有 100 mL 培养基的 250 mL 锥形瓶中,采用恒温摇床培养,取上清液测定硝氮浓度及总氮浓度.

表 1 正交试验因素水平表

Table 1 Factors and levels of the orthogonal experiment

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因素	转速/r·min <sup>-1</sup>	C/N	温度/℃	рН
水平1	80	5	15	6
水平2	120	10	20	7
水平3	160	15	30	8
水平4	200	20	37	9

#### 1.4 分析方法

 $NH_4^+$ -N、 $NO_2^-$ -N、 $NO_3^-$ -N和 TN 浓度均采用标准方法测定<sup>[9]</sup>;  $NH_2OH$ -N 浓度采用间接分光光度法<sup>[10]</sup>; 菌体生长吸光度( $D_{600}$ )采用吸光度法测定; TOC 采用日本岛津 TOC-LCPN 分析仪测定; pH 值采用雷磁 DHS-3D pH 计测定.

#### 2 结果与讨论

#### 2.1 菌株的鉴定

YL的菌落表面扁平湿润、不透明,光泽度好. 菌落颜色为蓝绿色,表明在培养过程中菌株分泌了某种色素. 革兰氏染色为阴性,大小为 0.5 μm×(1.0~1.5)μm. 电镜扫描照片见图 1.

经 16S rDNA 测序(序列号: KJ765709) BLAST 同源性检索, 发现 YL 与 Pseudomonas aeruginosa

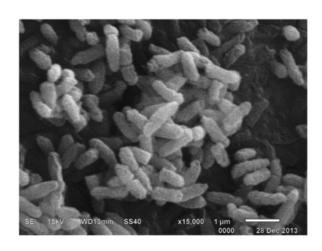


图 1 菌株 YL 的电镜扫描照片

Fig. 1 Scanning electron micrograph of strain YL

(M34133)的相似性在 99% 以上,结合菌株的形态 学特征,可以初步确定菌株为铜绿假单胞菌,命名为 Pseudomonas aeruginosa YL. 使用 MEGA 5.0 核酸分析软件将菌株 YL 与某些异养硝化细菌和其他假单胞菌属进行系统发育分析,得到 YL 的系统进化树(图 2),进一步表明菌株 YL 为 Pseudomonas aeruginosa.

#### 2.2 不同反应条件对菌株 YL 异养硝化的影响

图 3(a)表明,当碳源是乙酸钠、琥珀酸钠和柠檬酸钠时,24 h 后氨氮去除率均达到 90.0% 以上, TOC 去除率均达到 80% 以上, $D_{600}$ 均在 0.735 以上. 其中利用琥珀酸钠时氨氮去除率为 98.9%、TOC 去除率为 90.8%,且  $D_{600}$ 达到最大 1.382. 而碳源为葡萄糖和蔗糖时,氨氮去除率仅为 26.3% 和 16.8%,

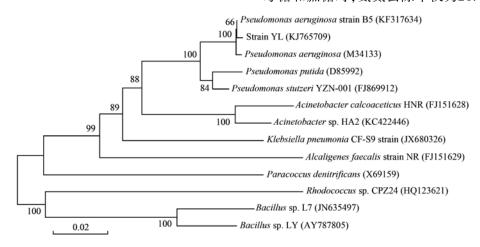


图 2 YL 的系统进化树

Fig. 2 Phylogenetic tree of strain YL

TOC 去除率仅为 8.1% 和 8.9%. 可见菌株 YL 利用有机酸优于像葡萄糖和蔗糖这种常用糖类,这与姜磊等<sup>[11]</sup>研究的菌株 *Pseudomonas* sp. N6 对乙酸钠和琥珀酸钠利用率高于对大分子有机物的利用一致. 所以琥珀酸钠可作为菌株 YL 的最佳碳源.

从图 3 (b) 可以看出, C/N = 2 和 C/N = 5 时,  $D_{600}$ 分别为 0. 303 和 0. 707, 氨氮去除率仅为 39. 4% 和 67. 6%, TOC 几乎完全去除, 说明碳源不足会使菌体的生长受到抑制, 氨氮去除效果差. C/N 为 10 和 15 时, 氨氮去除率分别为 98. 9% 和 99. 2%, TOC 去除率分别为 90. 8% 和 90. 1%, 对应的  $D_{600}$ 分别为 1. 382 和 1. 229. 菌体在较高 C/N = 15 下生长快速, 更快进入内源呼吸期,  $D_{600}$ 较 C/N = 10 时有所下降. 所以适宜菌株 YL 生长及反应的 C/N 为 10.

由图3(c)可知,在弱碱环境(pH7~9)下,菌株YL有着很好的氨氧化效果,对应 $D_{600}$ 值分别为

1.382、1.387 和 1.476, 氨氮去除率分别高达98.9%、98.3% 和 97.8%, TOC 去除率分别为90.8%、85.6%和80.3%. 在偏酸条件(pH = 5)及碱性条件(pH = 10)下, 氨氮去除率降至 85.5%和65.5%. 而在 pH < 5 以及 pH > 10 这种过酸过碱的环境下, 菌株 YL 几乎不生长. 可见菌株 YL 耐受pH 范围为5~10, 最佳 pH 为7.0. 菌株 YL 适应的pH 范围与王兆阳等[12]研究的耐冷兼性嗜碱性菌株 Pseudomonas sp. GL19 (pH 6~10)大致相同.

由图 3(d) 可见,温度为 10 ℃ 时,菌株 YL 几乎不生长.随着温度升高,菌体生长量和氨氮去除率增加,30 ℃下氨氮去除率为 98.9%, TOC 去除率为 90.8%, $D_{600}$ 值为 1.382. 但在 37 ℃, $D_{600}$ 明显降低,氨氮和 TOC 去除率也下降,这是因为随着温度升高,生物活性物质变性,细胞功能下降甚至死亡,从而影响反应效果 $^{[13]}$ .可见菌株 YL 最适温度为

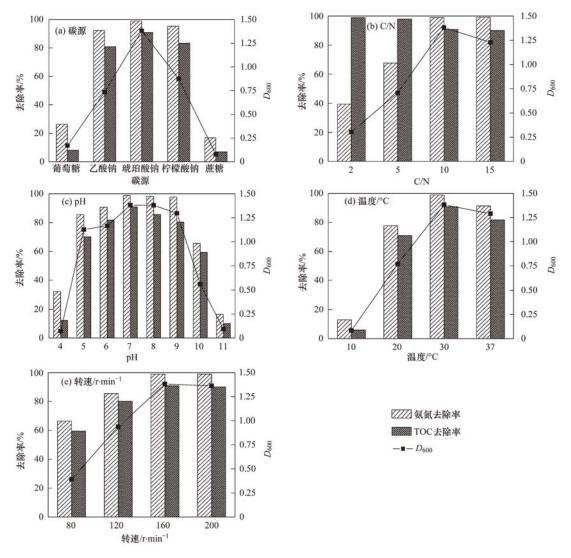


图 3 不同反应条件对菌株 YL 异养硝化的影响

Fig. 3 Effects of various reaction conditions on heterotrophic nitrification of strain YL

30℃.

如图 3(e) 所示,随着转速的增加,菌体的  $D_{600}$  和氨氮去除率增加. 在转速为  $160 \text{ r·min}^{-1}$  和  $200 \text{ r·min}^{-1}$ 时,氨氮都接近完全去除,去除率分别为 98.9% 和 99.0%, TOC 去除率分别为 90.8% 和 90.2%. 但转速  $200 \text{ r·min}^{-1}$ 下的  $D_{600}$  相比略低,分别为 1.382 和 1.362. 转速太低,溶解氧不足,反应效果差;提高转速可以加快氧的传递,反应完全. 所以,转速在  $160 \sim 200 \text{ r·min}^{-1}$ 之间,菌株生长、氨氧化效果及 TOC 去除效果好.

#### 2.3 菌株 YL 对不同氮源的利用

分别以硫酸铵、亚硝酸钠、硝酸钾和盐酸羟胺作为单一氮源培养 48 h,考察菌株 YL 对不同氮源的利用情况,结果见图 4.

图 4(a) 表明, 氨氮和总氮在 18 h 去除率均达

到 100%. 反应 24 h 后  $D_{600}$  值达到最大 1. 382, pH 从 7. 0 增至 9. 0. TOC 随着氨氮的降解而显著下降,TOC 去除率和 TOC 转化速率分别为 90. 8% 和 50. 55 mg·(L·h)  $^{-1}$  [45. 95 mg·(g·h)  $^{-1}$ ]. 反应过程中出现明显的羟胺转化, 9 h 时 NH<sub>2</sub>OH-N 浓度达到最大, 积累量为 2. 06 mg·L $^{-1}$ , 此后逐渐降低. 反应 20 h 后氨氮浓度逐渐增加, 说明此时菌群进入内源呼吸期, 之前用于细胞合成的氮源分解为氨氮再次回到水中. 通过菌生长合成所去除的氨氮占总去除氨氮的 50. 2%. 菌株 YL 最大比生长速率为 0. 19 h $^{-1}$ , 而自养硝化细菌的比生长速率通常为 0. 008 ~ 0. 045 h $^{-1}$ [14], 可见菌株 YL 增殖速率较自养硝化细菌的比生大速率,2 mg·(L·h)  $^{-1}$  [7. 61 mg·(g·h)  $^{-1}$ ], 平均氨氧化速率为 5. 55 mg·(L·h)  $^{-1}$  [5. 05

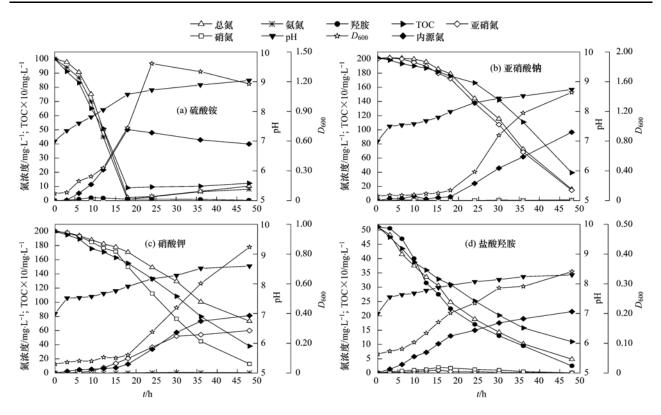


图 4 菌株 YL 对不同氮源的降解

Fig. 4 Biodegradation of nitrogen sources by strain YL

mg·(g·h)<sup>-1</sup>],与自养硝化细菌的平均氨氧化速率7.0 mg·(g·h)<sup>-1[14]</sup>相比在同一数量级. 另外,反应过程无亚硝氮积累且总氮去除完全,这不同于同等条件时菌株筛选所用 SBR 中的硝化污泥,即脱氮时出现明显亚硝氮积累(最高浓度为 28.5 mg·L<sup>-1</sup>),总氮去除率仅为 56.4%. 故菌株 YL 可以用于处理高氨废水或作为生物添加的候选菌株.

由图 4(b)和 4(c)可见,在初始亚硝氮浓度和 硝氮浓度均为200 mg·L-1时,菌株YL均生长良好, 内源氮和 $D_{600}$ 值同时在48 h 时达到最大,pH 由7.0 分别上升至 8.7 和 8.6, TOC 去除率分别为 80.3% 和 81.0%,说明 YL 能够利用亚硝酸盐和硝酸盐生 长及代谢. 这与已报道的一些异养硝化菌一致,如 Pseudomonas stutzeri F1<sup>[15]</sup>, P. mendocina TN-05<sup>[16]</sup>, P. stutzeri YZN-001<sup>[17]</sup>等. 以亚硝酸盐为唯一氮源 时,反应9h内亚硝氮浓度没有明显变化,9h后才 显著下降,说明高浓度亚硝酸盐虽对微生物活性有 抑制作用,但菌株 YL 对其有适应性. 最大比生长速 率在 24~30 h 时, 为 0.09 h-1. 最大亚硝氮降解速 率在 30 ~ 36 h, 为 6.46 mg·(L·h)<sup>-1</sup> [5.87 mg·(g·h)<sup>-1</sup>],平均降解速率为 3.86 mg·(L·h)<sup>-1</sup> [3.51 mg·(g·h)<sup>-1</sup>],与 Klebsiella pneumoniae CF-S9<sup>[18]</sup>、Acinetobacter sp. TN-14<sup>[19]</sup>等相当. 反应中有

痕量氨氮和硝氮的产生,最终亚硝氮去除率及总氮 去除率分别为 92.7% 和 91.9%. 相比于 Bacillus sp. YX-6 在亚硝氮浓度超过 20 mg·L-1 时反硝化活 性便受到抑制<sup>[20]</sup>,以及 Acinetobacter sp. HA2 在起 始亚硝氮浓度为 80 mg·L-1 时的去除率仅为 83.6% [21], 菌株 YL 更能高效利用较高浓度的亚硝 氮. 以硝酸盐为唯一氮源时,菌株 YL 最大比生长速 率在 18~24 h 时, 为 0.10 h-1. 最大硝氮降解速率 在 15 ~ 18 h 时, 为 7.20 mg·(L·h)<sup>-1</sup> 「6.55 mg·(g·h)<sup>-1</sup>]. 平均降解速率为 3.90 mg·(L·h)<sup>-1</sup> [3.55 mg·(g·h)<sup>-1</sup>],最终硝氮去除率为93.6%.对 硝氮的去除率高于利用亚硝氮. 去除硝氮过程中亚 硝氮逐渐累积,在48 h 时积累量为59.9  $mg \cdot L^{-1}$ ,总 氮去除率为63.4%.说明硝氮只是快速地转化为亚 硝氮,没有达到完全的氮去除,这是因为硝氮初始浓 度高达 200 mg·L<sup>-1</sup>, 高浓度硝氮大量转化为亚硝 氮、而亚硝氮反硝化不及时所致.

在以羟胺为唯一氮源时,如图 4(d), $D_{600}$  值在 48 h 可达到 0.341,最大比生长速率在  $9 \sim 12 h$  时,为  $0.07 h^{-1}$ . 羟胺去除率达到 94.8%,平均降解速率为  $1.0 \text{ mg} \cdot (\text{L} \cdot \text{h})^{-1}$  [  $0.91 \text{ mg} \cdot (\text{g} \cdot \text{h})^{-1}$  ],TOC 去除率为 78.1%. pH 从 7.0 增至 8.3. 通过氮平衡分析,内源氮占消耗羟胺的 45.2%,其余

54.8% 用于反硝化.反应 15 h 内亚硝氮和硝氮浓度逐步增加,15 h 后逐渐降低.可见菌株 YL 能够利用羟胺生长,这与一些已报道的菌株不同,如 Acinetobacter calcoaceticus HNR<sup>[22]</sup>既不能利用亚硝酸盐、硝酸盐,也不能利用羟胺生长; Thiosphaera pantotropha<sup>[23]</sup>能利用亚硝酸盐和硝酸盐生长,却不能利用羟胺生长.

综上,菌株 YL 能够利用氨氮、亚硝氮、硝氮和 羟胺这4种氮源,但以氨氮为增殖底物时菌株 YL 的最大比生长速率最快,其次分别为硝氮、亚硝氮 和羟胺.

#### 2.4 菌株 YL 的好氧反硝化性能优化

对正交试验结果进行分析,见表 2. 在初始硝氮浓度为 100 mg·L<sup>-1</sup>,培养 1 d 后,有 9 组条件下硝氮去除率达到 50% 以上,说明菌株 YL 环境适应性良好. 分别以硝氮去除率和总氮去除率为指标,通过极差分析法分析各因素对菌株 YL 好氧反硝化性能的影响顺序.

表 2 正交试验结果分析

Table 2 Analysis of orthogonal experiment results										
试验编号	转速/r·n	nin <sup>-1</sup>	C/	N	温度	€/°C	p	Н	硝氮去除率/%	总氮去除率/%
1	80		5		1	5	6	. 0	12. 6	9. 1
2	80		20		3	0	9	. 0	66. 4	50. 5
3	80		15		2	0	8	. 0	57. 6	40. 3
4	80		10		3	7	7	. 0	59. 2	42. 8
5	120		10		2	0	6	. 0	47. 4	43. 9
6	120		20		1	5	8	. 0	33. 9	29. 6
7	120		15		3	7	9	. 0	51. 5	44. 6
8	120		5		3	0	7	. 0	56. 1	49. 3
9	160		15		3	0	6	. 0	67. 7	58. 8
10	160		10		1	5	9	. 0	64. 9	59. 7
11	160		5		3	7	8	. 0	32. 3	27. 9
12	160		20		2	0	7	. 0	65. 7	54. 7
13	200		20		3	7	6	. 0	54. 7	48. 3
14	200		15		1	5	7	. 0	64. 6	58. 3
15	200		5		2	0	9	. 0	52. 3	47. 8
16	200		10		3	0	8	. 0	87. 3	67. 8
K1	49.0	35. 7	38. 3	33. 5	44. 0	39. 2	45.6	40.0		
K2	47.2	41.9	64. 7	53.6	55. 8	46. 7	61.4	51.3		
К3	57.7	50.3	60.4	50. 5	69. 4	56. 6	52.8	41.4		
K4	64. 7	55.6	55. 2	45.8	49. 4	40. 9	58.8	50. 7		
极差R	17.5	19. 9	26. 4	20.0	25. 4	17. 4	15.8	11.3		

从表 2 看出,以硝氮去除率为指标,各因素影响 好氧反硝化性能的主次顺序为 C/N >温度 >转速 > pH,以总氮去除率为指标,各因素影响的主次顺序为 C/N >转速 >温度 > pH,说明好氧反硝化时 YL 对 C/N 的敏感度大. 另外,充足的碳源虽能保证好氧反硝化的有效进行,但 C/N 过高会使有机物嵌入酶结构而影响酶活性,从而抑制反应 [24]. 转速和温度影响次之,pH 影响最小. 而且通过这两个指标分析推出的最优条件均为: C/N = 10, T = 30 °C, r = 200 r·min  $^{-1}$ , pH = 7. 0.

为验证正交试验结果的有效性,在最优水平组合条件下对菌株 YL 进行硝氮降解试验,结果如图 5 所示. 反应 24 h 内,硝氮浓度持续下降, $D_{600}$ 值和 pH 持续增大,说明菌体生长处于增殖期,菌株生长良好. 24 h 后进入内源呼吸期, $D_{600}$ 值开始下降,出

现少量氨氮累积. 最大硝氮降解速率在  $12 \sim 15 \text{ h}$ 时,为  $8.47 \text{ mg} \cdot (\text{L} \cdot \text{h})^{-1} [7.70 \text{ mg} \cdot (\text{g} \cdot \text{h})^{-1}]$ ,降解速率较之前有所提高. 反应 48 h 后,硝氮去除率达

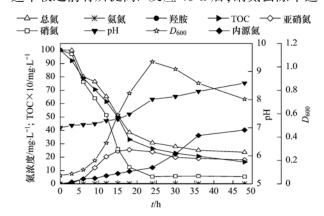


图 5 优选条件下菌株 YL 的反硝化特性

Fig. 5 Denitrification characteristics of strain YL under the optimal condition

到 94.6%, TOC 去除率为 83.6%, 虽整个反应过程中亚硝氮逐渐积累, 但总氮的去除率达到 76.3%. 通过氮平衡计算得出, 内源氮占去除硝氮的 42.6%. 内源呼吸期时硝氮值稳定在 5.4  $\mathrm{mg} \cdot \mathrm{L}^{-1}$ 左右, 无明显降解现象, 相比于 HN-AD 菌 *Rhodococuus pyridinivorans* CPZ24 只能将硝氮浓度由初始的 50  $\mathrm{mg} \cdot \mathrm{L}^{-1}$ 降解到 16.6  $\mathrm{mg} \cdot \mathrm{L}^{-1}$ [25], 菌株 YL 对硝氮的利用更具优势.

#### 3 结论

- (1)从高氨环境的 SBR 反应器中筛得 1 株异养 硝化-好氧反硝化菌 YL,鉴定为铜绿假单胞菌 (Pseudomonas aeruginosa).
- (2)菌株 YL 氨氧化最适宜的碳源是琥珀酸钠、C/N 为 10、pH 为 7.0、温度为 30℃,以及转速 160~ 200 r·min<sup>-1</sup>. 此 时 氨 氧 化 速 率 为 5.05 mg·(g·h)<sup>-1</sup>,TOC 转化速率为 45.95 mg·(g·h)<sup>-1</sup>. 氨氮和 TOC 去除率分别为 100% 和 90.8%.
- (3)菌株 YL 不仅能去除氨氮,还能利用亚硝酸盐、硝酸盐和羟胺生长代谢. 相比于其它氮源,以氨氮为增殖底物时菌株 YL 的增殖速率最大,且无亚硝氮和硝氮的积累.
- (4)影响菌株 YL 好氧反硝化特性最主要的因素是 C/N,优化条件为: C/N = 10, T = 30  $^{\circ}$ C, r = 200 r·min  $^{-1}$ , pH = 7.

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### **CONTENTS**

Pollution of Halogenated Polycyclic Aromatic Hydrocarbons in Atmospheric Particulate Matters of Shenzhen	
Emission Characteristics of VOCs from Typical Restaurants in Beijing	
Characteristics Analysis of Sulfur Dioxide in Pearl River Delta from 2006 to 2010	
Comparison Test Between PM <sub>2.5</sub> Continuous Monitoring System and Manual Sampling Analysis for PM <sub>2.5</sub> in Ambient Air  Determination and Emission of Condensable Particulate Matter from Coal-fired Power Plants	WANG Qiang, ZHONG Qi, CHI Ying, et al. (1538)
Potential Carbon Fixation Capability of Non-photosynthetic Microbial Community at Different Depth of the South China Sea and Its	
rotential Carbon Fixation Capability of Non-photosynthetic successful Community at Different Depth of the South China Sea and its	Response to Different Electron Donois
Remote Sensing Estimation of Chlorophyll-a Concentration in Inland Lakes Based on GOCI Image and Optical Classification of Wat	
Hydrochemical Characteristics and Sources of Qingshuijiang River Basin at Wet Season in Guizhou Province	
Temporal-spatial Variation and Source Identification of Hydro-chemical Characteristics in Shima River Catchment, Dongguan City	
Response and Control Factors of Groundwater to Extreme Weather, Jiguan Cave, Henan Province, China	
Impact of Rocky Desertification Treatment on Underground Water Chemistry and Dissolved Inorganic Carbon Isotope in Karst Areas	······
Difference of Karst Carbon Sink Under Different Land Use and Land Cover Areas in Dry Season Vertical Migration Characteristics of Organochlorine Pesticides in Overlying Soil in Karst Terranes and Its Impact on Groundwater	
	·· SUN Yu-chuan, WANG Yong-qi, LIANG Zuo-bing, et al. (1605)
Pollution Status and Migration of Mercury in the Sediments of Nansi Lake in Shandong Province	
Response of Sediment Micro Environment and Micro Interface to Physical Disturbance Intensity Under the Disturbance of Chironom	nus plumosus ·····
Characteristics of Atmospheric Nitrogram Was Describing and Associated Laurest on N. Taracterist in the Watershed of Ded Scil Associated	
Characteristics of Atmospheric Nitrogen Wet Deposition and Associated Impact on N Transport in the Watershed of Red Soil Area i	
Effect of Different Purple Parent Rock on Removal Rates of Nitrogen, Phosphorus and Organics in Landscape Water	
Effects of Litchi chinensis Defoliation on Growth and Photosynthesis of Microcystis aeruginosa	
Effects of Literal crunensis Detoliation on Growth and Photosynthesis of Microcystis aeruginosa  Toxicity of Coptis chinensis Rhizome Extracts to Green Algae	··· WAING Alao-xiong, JIAING Chen-chun, Li Jin-wei, et al. (1048)
Formation Mechanism of the Disinfection By-product 1,1-Dichloroacetone in Drinking Water	
Degradation Kinetics and Formation of Disinfection By-products During Linuron Chlorination in Drinking Water	
Interference for Various Quench Agents of Chemical Disinfectants on Detection of Endotoxin Activities in Water	
Ammonium Adsorption Characteristics in Aqueous Solution by Dairy Manure Biochar	
Absorption of Uranium with Tea Oil Tree Sawdust Modified by Succinic Acid	
Effect of Membrane Wettability on Membrane Fouling and Chemical Durability of SPG Membranes	
TiO <sub>2</sub> -Induced Photodegradation of Levofloxacin by Visible Light and Its Mechanism	
Performance of Novel Macromolecule Flocculant in the Treatment of Wastewater Containing Cr( VI) Ions	
On-line Estimation for the Amount of Stored PHA in Activated Sludge Based on OUR-HPR Measurements	
Formation Mechanism of Self-forming Dynamic Membrane During Separation of High-concentration Sewage Sludge Fermented for Ac	CIG Production
	HITANC Shari THI Hong by VIN Do at al. (1720)
Influence of Air Flux on Municipal Studeo Biodesine in a Pilat Scale Test	HUANG Shuai, LIU Hong-bo, YIN Bo, et al. (1720)
Influence of Air Flux on Municipal Sludge Biodrying in a Pilot Scale Test	
Influence of Air Flux on Municipal Sludge Biodrying in a Pilot Scale Test  Extraction of Heavy Metals from Sludge Using Biodegradable Chelating Agent N, N-bis(carboxymethyl) Glutamic Acid Tetrasodium	
Influence of Air Flux on Municipal Sludge Biodrying in a Pilot Scale Test  Extraction of Heavy Metals from Sludge Using Biodegradable Chelating Agent N, N-bis(carboxymethyl) Glutamic Acid Tetrasodium  Biodiversity and Function Analyses of BIOLAK Activated Sludge Metagenome	
Influence of Air Flux on Municipal Sludge Biodrying in a Pilot Scale Test  Extraction of Heavy Metals from Sludge Using Biodegradable Chelating Agent N, N-bis(carboxymethyl) Glutamic Acid Tetrasodium Biodiversity and Function Analyses of BIOLAK Activated Sludge Metagenome  Characteristics of Nitrogen Removal by a Heterotrophic Nitrification-Aerobic Denitrification Bacterium YL	
Influence of Air Flux on Municipal Sludge Biodrying in a Pilot Scale Test  Extraction of Heavy Metals from Sludge Using Biodegradable Chelating Agent N, N-bis(carboxymethyl) Glutamic Acid Tetrasodium Biodiversity and Function Analyses of BIOLAK Activated Sludge Metagenome  Characteristics of Nitrogen Removal by a Heterotrophic Nitrification-Aerobic Denitrification Bacterium YL  Biodegradation Characteristics and Kinetics of p-nitrophenol by Strain Arthrobacter sp. CN2	
Influence of Air Flux on Municipal Sludge Biodrying in a Pilot Scale Test  Extraction of Heavy Metals from Sludge Using Biodegradable Chelating Agent N, N-bis(carboxymethyl) Glutamic Acid Tetrasodium Biodiversity and Function Analyses of BIOLAK Activated Sludge Metagenome  Characteristics of Nitrogen Removal by a Heterotrophic Nitrification-Aerobic Denitrification Bacterium YL  Biodegradation Characteristics and Kinetics of p-nitrophenol by Strain Arthrobacter sp. CN2  Biodegradation of Pyrene by Intact Cells and Spores of Brevibacillus brevis	
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