

假单胞菌磷代谢特性的研究

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摘要 本文考察了处理效果良好的循序间歇式废水生物脱氮除磷试验装置内活性污泥优势菌——假单胞菌 (*Pseudomonas* sp.) 的磷代谢特性。试验结果表明, 在好氧条件下, 以乙酸盐为基质培养时, 向培养基内滴加 H_2SO_4 可导致假单胞菌产生磷释放现象, 滴加 $NaOH$ 或 $NaHCO_3$ 时, 则发生过量的摄磷现象, 且加 $NaOH$ 较加 $NaHCO_3$ 所引起的磷摄取量大。另外, 当以乙酸盐为基质时, 假单胞菌于好氧培养过程中能明显除磷, 其效果与厌氧-好氧培养过程相近。

关键词 废水处理, 假单胞菌, 生物脱氮, 生物除磷。

废水生物除磷是一项新型废水生物处理技术。其基本原理为通过除磷菌过量摄取废水中的磷酸盐于细胞内形成聚磷酸盐内含物, 以剩余污泥排出处理系统。笔者在研究循序间歇式生物脱氮除磷试验装置内活性污泥的微生物组成时, 发现其优势菌为假单胞菌属和气单胞菌属^[1]。目前, 人们对气单胞菌属进行了较全面的研究^[1,2], 弄清了其生理特性及除磷作用。对假单胞菌属的磷代谢生理特性虽也进行了一些探讨^[3], 认为该菌是一种除磷菌, 能过量摄磷, 但对其摄磷特点及与环境因素之间的关系等尚未见报道。

本文对从试验装置活性污泥中分离的优势菌(假单胞菌), 进行纯种培养, 考察酸、碱及氧量等对假单胞菌磷代谢的影响。为阐明废水生物除磷机理提供依据。

一、试验材料及方法

(一) 试验材料

1. 试验装置

循序间歇式废水生物脱氮除磷试验装置见图1。原水为自来水中投加葡萄糖、少量氯化铵、硫酸镁、磷酸二氢钾、氯化钙、氯化钠及碳酸氢钠。其水质大致为: $TOC=160mg/L$; $TN=20-25mg/l$; $TP=8-18mg/L$ 。

试验装置按标准时序^[4]稳定运行51天, TOC 、 TN 及 TP 的去除率分别约为90%、

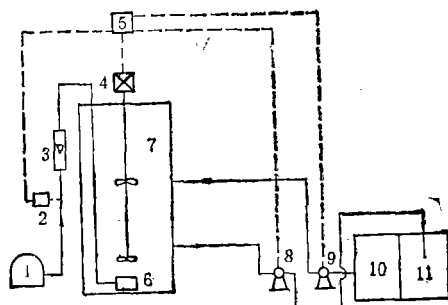


图1 试验装置示意图

1. 空压机 2. 电磁阀 3. 转子流量计 4. 搅拌电机
5. 控制装置 6. 微孔曝气头 7. 反应器 8. 排水泵
9. 进水泵 10. 配水箱 11. 出水箱

80—85%和95%以上。

2. 菌种来源

假单胞菌是由试验装置稳定运行期活性污泥中分离获得。

3. 培养基

(1) 肉汁胨培养基^[5]

(2) 富磷培养基^[6]

无水乙酸钠 5g; 硫酸镁 0.5g; 硫酸铵 2g; 磷酸二氢钾 0.25g; 氯化钙 0.2g; 酪酸铵 0.6g; 蒸馏水 1000 ml; pH 7.0—7.2。1 kg/cm² 灭菌 20min。

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1) 周岳溪, 废水生物除磷机理及间歇式生物处理工艺的研究(博士论文), 清华大学, 1990年

(二) 试验方法

1. 预培养

在进行各项设定的实验之前,细菌须经过一定时间的预培养。将假单胞菌新鲜斜面培养物转接到肉汁胨液体培养基内,30℃,好氧培养24h,然后在10℃,4500r/min条件下,离心(K70D Beckman USA)10min。再将沉积培养物悬液按规定量转接到下列试验所采用的培养基内培养。

2. 酸、碱对摄磷量的影响

将预培养后的假单胞菌分别转接到3个富磷培养基内并于30℃、好氧培养。自开始至第2小时,向其中的一个培养液内滴加0.025mol/L H_2SO_4 (隔半小时滴一次,每次2.5ml,共滴4次);另一个内滴加0.1mol/L NaOH (隔半小时滴一次,每次1.0ml,共滴4次);第三个培养基内滴加0.1mol/L $NaHCO_3$ (隔半小时滴一次,每次1.5ml,共滴4次)。分别考察相应的培养液中细菌摄磷量与培养时间的关系。

3. 氧量对摄磷量的影响

将预培养后的假单胞菌分别转接到2个富磷培养基内。其中一个在30℃下好氧培养,另

一个则在相同温度下自开始至第2小时厌氧培养,然后再转为好氧培养。分别考察相应培养液中细菌摄磷量与培养时间的关系。

4. 采用细菌培养液浊度(A 650 nm)和细菌细胞干重校正曲线法计算培养液中细菌浓度(g/L)。

二、结果和讨论

1. 酸、碱的影响

向培养基中滴加 H_2SO_4 、NaOH 及 $NaHCO_3$ 对该菌好氧生长(30℃)过程中摄磷量影响的研究结果分别示于图2—4。

结果表明,向培养液中滴加 H_2SO_4 诱导细菌产生磷释放现象(摄磷量呈负值);而滴加NaOH时,细菌的摄磷量则明显提高, $NaHCO_3$ 的作用次之。这可能是因为,细菌降解废水中有机物通常是首先通过细胞膜的基质传输系统,将基底摄入细胞,再沿着相应的代谢途径降解。根据基质的性质,细胞对基质的摄取方式有4种形式,即自由扩散、主动传输、促进扩散和伴随传输^[7]。本试验是以乙酸盐作为基质,而乙酸盐是以中性乙酸分子形式^[8-9]通过自由扩

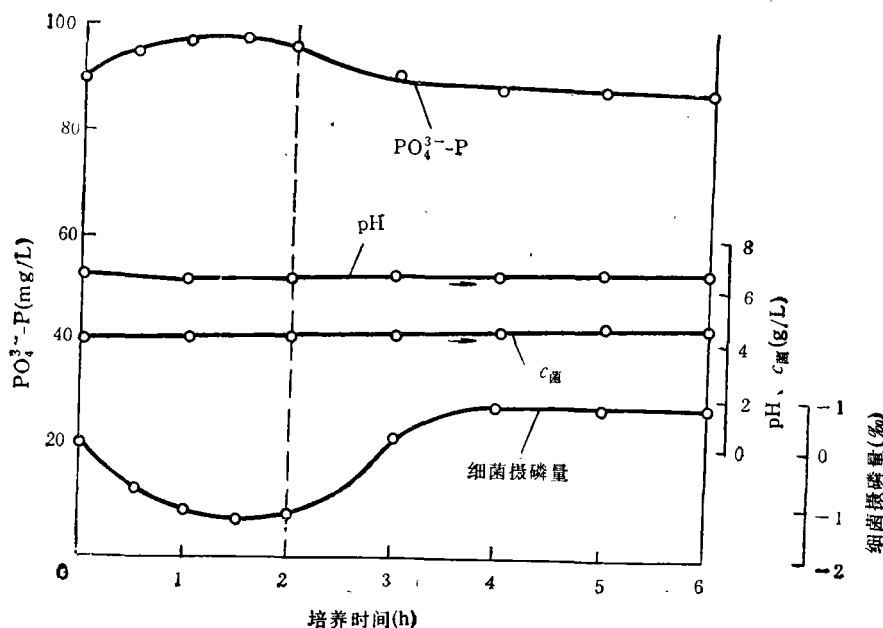


图2 H_2SO_4 对假单胞菌摄磷的影响

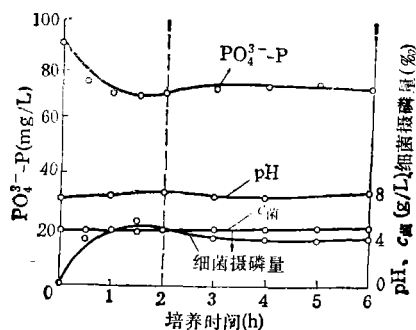
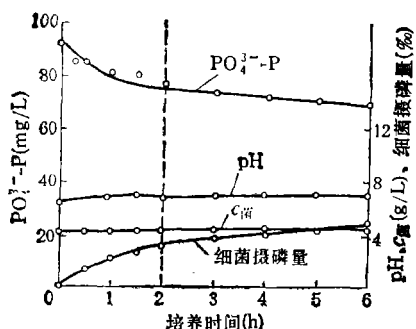
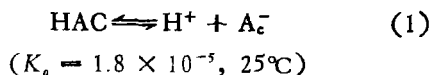


图3 NaOH 对假单胞菌摄磷的影响

图4 NaHCO₃ 对假单胞菌摄磷的影响

散传输方式传入细胞^[10]。在水中,乙酸呈下列电离平衡^[11]:

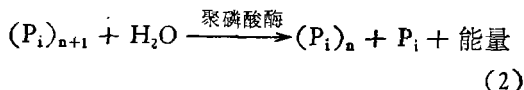


通常,培养液的 $\text{pH} \geq 6.5$ 。

由式 1 可以得出,这时乙酸的电离度大于 98.4%,也就是说 98.4% 以上的乙酸呈离子状态存在于水中,中性状态的乙酸分子极少,且受式(1)的电离平衡约束,故能进入细胞的乙酸很少且传入速率缓慢。另外,细胞膜内呈碱性^[9],所以,乙酸一旦传进细胞便重新离解为 H^+ 和 Ac^- 。

向培养液中滴加 H_2SO_4 破坏了式(1)的平衡,水中的中性乙酸分子急剧增多,从而使细胞内的乙酸增加,由于细胞膜内呈碱性状态,乙酸进入细胞后将重新离解。因此,加入 H_2SO_4 后会减弱细胞膜内的碱性,使细胞膜内外两侧的 pH 梯度减小^[8],破坏了维持细胞正常生长的能量平衡。为了抑制细胞内 pH 的变化以维持正常的生长、繁殖,细菌可能通过胞内聚磷酸盐的磷源作用,经聚磷酸酶催化聚磷酸盐水解产

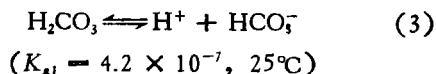
生磷酸并释放能量^[12]:



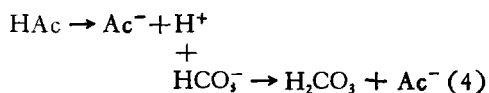
在此能量作用下,细胞通过细胞膜上的 H^+ 泵将胞内过量的 H^+ 排到胞外,同时释放磷。

与 H_2SO_4 的作用相反,向培养液中滴加 NaOH 会使其中的 Ac^- 量增加(式(1)),因此扩散进入细胞的 HAC 减少,细菌可以摄取更多的磷酸,其结果是,在 NaOH 的滴加过程中,培养基内的磷酸明显减少。

当向培养基内滴加 NaHCO_3 时,细菌的摄磷量也缓慢增加,这可能是因为培养液内存在着两个电离平衡,一个是乙酸电离平衡(式(1)),另一个电离平衡为^[11]:



与乙酸相比,碳酸是一种更弱的酸,因此向培养基内滴加 NaHCO_3 会使原来的电离平衡(式(1))破坏,反应如下:



结果,使乙酸分子减少。但反应式(4)的进行过程受 H_2CO_3 和 HAc 电离平衡的控制,所以滴加 NaHCO_3 不象 NaOH 那样造成 HAc 数量急剧减少,而呈现出 HAc 迟缓减少过程。故细菌摄磷量增加较迟缓。

试验还表明,在实际的工艺运行过程中,应尽可能避免废水处于酸性状态,以便维持除磷菌正常的能量平衡,提高工艺的除磷效率。

2. 氧的影响

在培养过程中,氧量对假单胞菌摄磷量影响的研究结果(见图 5)表明,在厌氧-好氧培养过程的厌氧阶段,假单胞菌产生磷释放现象,进入好氧培养阶段时,该菌又重新从培养液中摄取较多的磷,这说明此菌具有一般除磷菌的共性。当开始就进行好氧培养时,虽然该细菌没有产生磷释放现象,但它同样具有明显的摄磷能力。然而,当培养到第 4 小时后两者的除磷效果相近,这可能是因为是在废水生物除磷过程

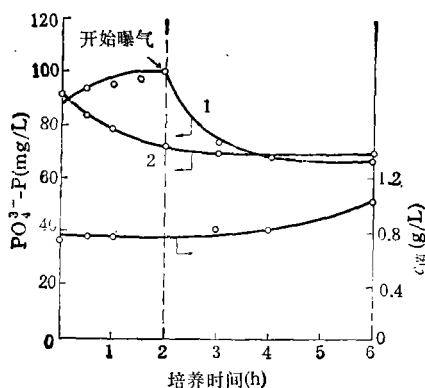


图 5 氧对假单胞菌摄磷的影响 (30°C)

1 开始至第 2 小时厌氧培养, 然后好氧培养 2 全部好氧培养

中, 厌氧的作用是通过酵解菌的作用使水中的有机物降解成短链脂肪酸, 以充当除磷菌摄磷所需的基质^[13]。本文研究结果也证实了近年来国外研究者提出的理论假设^[13], 只要废水中存在着足够的乙酸盐, 即使是全部好氧过程, 除磷菌也能有效地摄取磷酸而达到除磷效果。

三、结 论

本文通过纯种培养试验, 研究了假单胞菌的磷代谢特点, 得出了下述结论:

1. 在好氧条件下, 以乙酸盐为基质培养时, 向培养基内滴加 H_2SO_4 可使假单胞菌产生磷释放现象。滴加 $NaOH$ 或 $NaHCO_3$ 时则产生过量摄磷现象, 且滴加 $NaOH$ 较 $NaHCO_3$ 所

引起的磷摄取量要大。因此, 在实际的工艺运行过程中, 应尽可能避免废水处于酸性状态, 以便维持除磷菌正常的能量平衡, 提高除磷效率。

2. 当以乙酸盐为基质时, 假单胞菌在好氧培养过程中能明显除磷, 其效果与厌氧-好氧培养过程相近。证实了最近人们提出的假设, 即若废水中存在足够的乙酸盐, 除磷菌在直接好氧培养过程中也能有效地除磷。

致谢 本文得到了张红同志的热情帮助, 致以衷心感谢。

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· 环境信息 ·

以 150 万伏脉冲电子束“攻击”废物流

这是一种用以消除危险有机化合物的最新方法。来自洛斯阿拉莫斯国家实验室、佛罗里达国际大学和迈阿密大学的研究人员已发现, 他们能消除 99% 以上的很多危险化学品和多达 85% 的难处理物质, 例如酚。在这一方法过程中, 一台加速器释放 100 毫微秒电子脉冲, 将水分子分解成自由基, 后者再将溶解有机

物氧化成 CO_2 、 H_2O 和盐类。据研究人员称, 在一家城市废物处理厂进行的实验研究表明, 这一技术可以用来净化饮用水。该技术的研制者们还设想: 将这一技术用来处理来自能源部净化场的放射性混合废物。

淮海译自 *ES&T*, 1992, 25(5): 849

Abstracts

Chinese Journal of Environmental Science

dyeing wastewater, microbial distribution.

Preliminary Studies on the Buffering Effects of Forest Litter on Soil Acidification. Wang Silong, Chen Chuying (Institute of Applied Ecology, Academia Sinica, Shenyang 110015): *Chin. J. Environ. Sci.*, 1992, 13(5), pp. 25—30

The ash element content and decomposition rate of the litter of *S. Superba* were found to be much higher than those of the litter of *C. lanceolata*. Subsequently, it is inferred that the former possesses a higher potential to buffer acidification of the soil compared with the latter. When the soil was covered with litter of *S. superba*, the base cation content and pH-value of leachate down from the litter and soil were evidently higher than the control; the basesaturation percentage and pH-value of the soil itself were also markedly higher, and the acidity and Al/Ca of the soil were far lower than the control. That is, the buffering ability of litter of *S. superba* to soil acidification is very strong, while the litter of *C. lanceolata*, is not.

Key words: *Cunninghamia lanceolata* (Lamb.) hook, *Schima superba* gardn. et champ, litter, buffering ability, soil acidification.

Studies on the Effects of Heavy Metals on the Contents of Nucleic Acids and Activities of Nucleases in the Root Tips of *Vicia faba*. Duan Changqun, Wang Huanxiao, [Qu Zhongxiang] (Biology Department, Yunnan University, Kunming 650091): *Chin. J. Environ. Sci.*, 1992, 13(5), pp. 31—35

Under the treatment of Pb^{2+} , Cd^{2+} , Zn^{2+} ions at low concentrations the contents of DNA (treated with the concentration less than 10.0, 0.50 and 50.0 ppm), and RNA (treated with the concentration less than 10.0, 50.0 and 50.0 ppm) and the activities of DNase (all less than 10.0 ppm) and RNase (less than 20.0, 10.0 and 50.0 ppm) in the root tips of *Vicia faba* L. increased with the increase of the concentrations of metal ions; however, the contents of DNA and RNA and the activities of DNase and RNase decreased when the concentrations of the metal ions were higher than the concentrations stated above. Hg^{2+} did not exert regular effects except that the activities of RNase increased with the treatment concentration of Hg^{2+} below 0.05 ppm, and decreased when the concentration of Hg^{2+} was above 0.05 ppm. The results demonstrate that the effects of heavy metals on nucleic acids metabolism depend on the interaction between the extents of the adaptive response of plant tissues and the toxic action of heavy metal ions. Metal ion characteristics and the tolerant inducible system of different metabolism that might exist in plants, and the effects of protein metabolism on the adaptive actions of plants were also discussed.

Key words: *vicia faba* L., heavy metals, nucleic acids, nucleases, adaptive responses.

Research on Integer Programming Model of Urban Waste Water Reuse System. Wu Limei et al. (Beijing Municipal Research Institute of Environmental Protection, Beijing 100037): *Chin. J. Environ.*

Sci., 1992, 13(5), pp. 36—41

A 0—1 integer programming model for the planning of urban wastewater reuse system were established. The planning of the wastewater collection system, location of the treatment plant and the efficiency of the treatment were formulated. Emphases were put on discussions of how to determine the user of the renovated water, the quantity of the reused water and the scale of additional advanced treatment so as to establish renovated water allocation system. The model was validated to be rational and applicable by examining a wastewater land treatment system.

Key words: urban wastewater reuse, integer programming, water allocation system, wastewater treatment. **A Study on the Characteristics of Phosphate Metabolism of *Pseudomonas* sp.** Zhou Yuexi, Qian Yi, Gu Xiasheng (Department of Environmental Engineering, Tsinghua University, Beijing 100084): *Chin. J. Environ. Sci.*, 13(5), 1992, pp. 42—45

The characteristics of the phosphate metabolism of *Pseudomonas* sp., the predominate genus in the activated sludge mixed liquor from the sequencing biological batch system which effectively removes nitrogen, phosphorus and organic matter, were investigated. Experimental results demonstrate: (1) Under aerobic conditions and with acetates as carbon source, the addition of sulphuric acid causes *Pseudomonas* sp. to release phosphates, and the addition of NaOH and $NaHCO_3$ causes *pseudomonas* sp. to excessively take phosphate. (2) The phosphate uptake capacity of *Pseudomonas* sp. is nearly the same in the aerobic system as in the anaerobic-aerobic system with acetate as carbon source.

Key words: waste water treatment, *pseudomonas* sp., phosphorus.

Study on Comprehensive Treatment of Waste Water from Furfural Production. Chen Yulian et al. (Department of Chemical Engineering, Taiyuan University of Technology, Taiyuan 030024): *Chin. J. Environ. Sci.*, 1992, 13(5), pp. 46—49

During the process of furfural production, waste liquor containing 1—2.5 % acetic acid is discharged. Not only is acetic acid wasted but also the surrounding environment is polluted. In the operation described in this paper, the waste water passes through a 400×1600 mm electrodialyzer equipped with 90—120 pairs of membrane modules, recovering acetic acid with concentrations of 18—20%. The treated waste water contains only 0.02% of acetic acid, and could be safely discharged or reused. Furthermore, an industrial graded acetic acid with purity of 99% could be produced by means of extraction-distillation of the recovered acetic acid solution. Industrialization of the comprehensive technology of electrodialysis-extraction-distillation for the treatment of the waste water from furfural production mentioned here has been achieved.

Key words: waste water treatment, membrane separation, electrodialysis, acetic acid, recovery.

Effect of Sulfate Ion on Anaerobic Biological Treatment of Waste water. Liu Yan (Department of