

钴(Ⅱ)和镍(Ⅱ)对长春花 C. roseus 细胞悬浮生长的影响

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摘要 研究了 pH5.5条件下,钻(1)和镍(1)对长春花 C. roseus 细胞悬浮培养生长的影响。当介质中钻的起始浓度为 0.50 2.0 μ g/m1时,能轻微刺激 C. roseus 细胞的生长,[Co(1)]>4.0 μ g/m1,[Ni(1)]>2.0 μ g/m1时,阻碍细胞生 长,Ц即制程度与介质中钻(1)。镍(1)的浓度相关;它们的最小致死浓度分别为 50.0 μ g/m1 Co(1)和 10.0 μ g/m1 Ni (1)。钻(1)较容易被 C. roseus 所吸收,面镍(1)的毒性相对较大。

关键词 钻,镍,长春花C.rseus,毒性效应。

钴和镍在生物有机体中具有重要作用,是许 多植物生长的必需元素。不少文献报道微量镍可 以轻度刺激植物生长。1946年,Roach 和 Barchay 发现在植株上喷洒很稀的镍盐溶液可以增 加土豆的产量^[1];Dobrolyubskii 和 Slavvo 1957 年报道,喷洒微量镍可以增加葡萄的产量^[2]。最 近,Dixon 和 Polacco 等研究表明镍是许多酶体 系所必需的^[3,4]。人们早就知道钴是反刍动物所 必需的元素。1948年,发现钴是所有动物所必需 的维生素 B 的组成部分;1954年,Holm-Hansen 等发现钴是淡水兰绿藻生长所必需的元素^[5];此 后,Ahmed 和 Evans 等证实钴是共生固氮所不 可缺少的^[6,7]。但迄今,未曾见过钴,镍等重金属 对细胞悬浮培养生长影响的报道。

本文主要从环境科学的角度研究 Co(1), Ni(1)对长春花 C. roseus 细胞悬浮培养生长的 影响;同时为选择代谢诱导物提供参考。

1 实验材料和方法

1.1 主要试剂

Co(I).Ni(I)标准溶液(1mg/ml):将 4.0375g CoCl₂.6H₂O 或 4.0494g NiCl₂.6H₂O 分别溶解 在1000ml 蒸馏水中,即可配得。

1.2 C. roseus 细胞悬浮培养

往每只 500ml 三角锥瓶中加入 100ml APM 介质(表 1),再加入不同量的钴(Ⅰ)或镍(Ⅰ)标 准溶液及蒸馏水,使其溶液总体积均为185ml, 用 NaOH 或 HCl 溶液调介质 pH 为 5.5。然后用 棉纱塞子塞住瓶口,铝箔纸封好口。 将盛有培养 介质的三角烧瓶放入高温高压消毒器中,在 121 C, 20Psi 下消毒 15min, 取出后冷却至室温, 在植物组织培养箱中分别移入 15ml 已在 1-B5 介质[13] 中培养 10d 的 C. roseus 细胞悬浮液;此 时,培养介质中 Co(I)和 Ni(I)的浓度 均为 0-50.0µg/ml 将所有锥形瓶固定在旋转摇瓶机 中(135r/min),在25°C下避光培养;每隔几日取 1ml 培养液,测定其上层清液的折光率,以监示 C. roseus 细胞悬浮生长的情况;待细胞孵化完全 (15d)后,用 Miracloth 滤纸真空过滤悬浮液,用 蒸馏水洗涤 2 次,滤干后即可得湿重,将培养产 物放入冰箱中冷冻,经低温真空干燥后可得干 重,计算可得不同钴(工)或镍(工)浓度下

C. roseus 的生物产率(相对于对照组)。C. roseus (I)浓度低于 0.10µg/ml 时,C. roseus 细胞生长 细胞悬浮培养实验均重复一次,生物产率取其平 正常;当介质中钴的起始浓度在 0.50-2.0µg/ 均值。 ml 时,对C. roseus 细胞生长有一定的促进作用,

表1 C. roseus 细胞悬浮培养液(APM)的组成

成分	含量(mg/L)
KH-PO;	68
KNO_3	950
$\mathbf{NH}_{3}\mathbf{NO}_{3}$	720
CaCl ₂	220
MgSO:	185
Fe ¹	18. 6ml
KI(75mg/100ml)	0. 5ml
微量营养成分	1. 0ml
维生素	5. Oml
]蔗糖	50 g
富里酸(25mg/100ml)	1. 0ml
维生素 H(5mg/100ml)	1. 0ml
6- 苄胺基嘌呤(112-5mg/ml)	1. 0ml
吲哚-3-乙酸(17.52mg/ml)	1. Oml

1) $Fe_1Na_2EDTA(4, 0g/L) - FeSO_4, 7H_2O(3, 0g/l)$

- 微量营养成分(mg/100ml): MnSO₄. H₂O, 700; Zn-SO₄. 7H₂O, 405; H₃BO₃, 240; 甘氨酸, 200; (NH₄), Mo;O₂₁. 4H₂O, 9. 25
- 3) 维生素(mg/100ml):肌醇,2000;烟酸,100;维生素
 B6,10;盐酸硫胺素,10

1.3 样品分析

应用 AA1275 型原子吸收分光光度计测定 滤液中 Co(I).Ni(I)的残留量(石墨炉原子吸 收法的检出限分别为 Co:2ng/ml,Ni:5ng/ml)。 干燥细胞样品的分析步骤如下:取 0.2g 干燥样 品于 100ml 烧杯中,加入 5ml 浓 HNO3 和 1ml 浓 H₂SO₄,放置 1h,然后在电热板上加热,直到溶液 变清,消解完全,冷却后转移到 50ml 容量瓶中, 用蒸馏水稀释至刻度,摇匀。有部分样品溶液还 需进一步稀释。用原子吸收法测定 Co 和 Ni 的含 量。结果表明,干燥细胞样品中镍(或钴)的含量 与相应滤液中镍(或钴)的残留量之和基本等于 培养介质中起始镍(或钴)的总量;说明细胞悬浮 培养过程中锥形瓶对镍或钴的吸附可忽略不计。

2 结果与讨论

2.1 钻、镍对C. roseus 细胞悬浮生长的影响

钴(1)镍(1)对 C. roseus 细胞悬浮培养生 长的影响见图 1。实验表明,在 PH5.5条件下,钻

正常;当介质中钴的起始浓度在 0.50-2.0µg/ ml 时,对 C. roseus 细胞生长有一定的促进作用, 其生物产率为对照组的 105%-110%; 当[Co (Ⅱ)]>4.0µg/ml 时,C. roseus 细胞生长受到抑 制,其生物产率随着介质中Co(I)起始浓度的 增大而下降;当培养介质中Co(I)浓度为 50µg/ml 时,C. roseus 细胞不生长,随即死亡。Co (**Ⅰ**)对 C. roseus 细胞生长的最小抑制浓度 (MIC)和最小致死浓度(MLC)分别为 4. 0µg/ml 和 50.0µg/ml, IC50(生物产率降低 50%时金属 离子的起始浓度)为 8.17µg/ml。 镍对 C. roseus 细胞悬浮培养生长有较大的影响;当[Ni(I)]< 1.0µg/ml时,C.roseus 细胞生长正常;较高浓度 的 Ni(I)将抑制 C. roseus 细胞生长: Ni(I)浓 度为 10.0µg/ml 时,C. roseus 细胞不生长,随即 死亡。Ni(Ⅱ)的 MIC 和 MLC 值分别为 1.0µg/ ml 和 10.0µg/ml, IC50 为 6.17µg/ml, 由此可见, 就C.roseus 细胞悬浮培养体系而言,Ni(I)比 Co(I)有较大的毒性。

2.2 C. roseus 对钴、镍的吸收和富集作用

Co(I)和 Ni(I)可以被各种植物所吸收。 图 2 是 C. roseus 细胞在不同金属浓度培养介质 中吸收 Co(I)和 Ni(I)的情况。通过计算培养 介质中Co(I)或Ni(I)的起始浓度与收获时介 质中的残留浓度之差,可得 C. roseus 细胞对 Co (Ⅰ)或 Ni(Ⅰ)的百分吸收率。实验表明, C. roseus 细胞对 Co(I), Ni(I)的百分吸收率 随着介质中相应金属浓度的增加而逐渐减小。在 金属离子浓度较低时,C.roseus 细胞呈现较高的 吸收率;相对而言,Co(I)较容易被C.roseus 细 胞所吸收。C. roseus 细胞中金属的含量与其在培 养介质中的浓度成正相关(表 2)。将干燥细胞样 品中金属的含量与它们在介质中的起始浓度之 比称为C. roseus 对Co(I)或Ni(I)的生物富集 系数;生物富集系数与培养中Co(1),Ni(1)浓 度有关,其最大生物富集系数见表 2。实验结果 表明,当介质中Co(I),Ni(I)的起始浓度为 50.0µg/ml 时,它们在干燥 C. roseus 细胞样品中 的含量分别为 22. 0mg/g 和 1. 87mg/g。说明,Co





图 1 Co(1). Ni(1)对 C. roseus 细胞悬浮生长的影响



图 2 C. roseus 细胞对 Co(1)、Ni(1)的吸收 表 2 干燥 C. roseus 样品中 Co(1)、Ni(1) 的含量及最大生物富集系数

金属离子	金屬浓度	干燥 C. roseus 样品中	最大生物
	(µg/ml)	金属含量(mg/g)	富集系数
Co(1)	0.10-50.0	0.0008-22.0	440
Ni(I)	0.10-50.0	0.0007-1.87	53

综上所述,在所试的金属浓度范围内,Ni(I)不 能促进C.roseus 细胞悬浮培养生长;当培养介质 中Co(I)为0.50-2.0µg/ml时,对C.roseus 细胞生长有一定的促进作用;因此,Co(I)有可 能被用作诱导物,以促进C.roseus 细胞悬浮培养 过程中代谢产物的积聚,提高生物碱的产量。这 个问题正在进一步研究之中。无论是Co(I)还 是 Ni(I),当浓度过高时,都会抑制C.roseus 细 胞生长,乃至死亡;故在利用C.roseus 细胞悬浮 培养体系来制备生物碱时,应严格控制培养介质 中Co(I),Ni(I)等的含量。

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The environmental deterioration caused by human activities and overlapped by the natural changes has become one of the major problems for current environmental protection cause. The desertification of large lands and the sand-dust storms occurred in the deserts and the desertificated regions are such typical problems. This paper describes the major sources of the sand-dust storms in China and the transportation path of the Kosa aerosol. A brief description of the transportation path of the sand-dust storm generated around the region of Jinchang City, Gansu Province on May 5, 1993 is also given.

Key words: Sand-dust storms, Northwest Region, China, high-altitude transportation.

New Type Nonpollution Antifouling Coating for Marine Application. Tian Jun et al. (Laboratory of Solid Lubrication, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000): Chin. J. Environ. Sci., 14(5), 1993, pp. 65-68

This paper deals with the major affecting factors of the fouling living things, the present development situation of antifouling coating for marine application and the important role of initial mucosa in the adherent mechanism. The antifouling mechanism and application of the new type nonpollution antifouling coating have been emphatically discussed, based on the material of low surface free energy, hydrophilic material, biochemical material, and low toxic material. It is suggested that the low toxic material is the recent goal to solve the marine organotin pollution, but nontoxic antifouling coating of the new generation, particularly the material of low surface free energy, is at the initial stage of development. The application of this material is almost impossible in the near future.

Key words: marine nonpollution antifouling coating, antifouling mechanism, marine environment.

Effects of Cobalt (II) and Nickel (II) on Catharanthus roseus Cell Suspension Cultures. Zhu Lizhong (Department of Chemistry, Hangzhou University, Hangzhou 310028); Chin. J. Environ. Sci., 14 (5), 1993, pp. 69 71

The effects of cobalt(II) and nickel(II) on the growth of *catharanthus roseus* (*C. roseus*) cell suspension cultures at pH 5.5 were investigated. Cell growth and cobalt or nickel uptake were monitored. When the concentrations

of Co(II) and Ni(II) were more than 4.0 μ g/ml and 2.0 μ g/ml respectively, the growth of *C. roseus* was inhibited. Minimum lethal concentrations of Co(II) and Ni(II) were 50.0 μ g/ml and 10.0 μ g/ml respectively. Cobalt (II) was more easily to be uptaken and enriched by *C. roseus* cells than nickel(II).

Key words: Catharanthus roseus, cell culture, cobalt, nickel, toxicity test.

Study on the Some Environmentally Chemical Natures of Ether Amines $R-O-(CH_2)_3NH_2$ in Mineral Separation Wastewater. Tao Yinhe et al. (General Prospecting and Research Institute, Ministry of Metallurgical Industry, Baodin 071069); Chin. J. Environ. Sci., 14(5), 1993, pp. 72-75

Some tests have been made on soil-column leach, degradation and adsorption to study some environmentally chemical natures of the ether amines R-O-(CH₂)₃NH₂ in mineral separation wastewater. The results indicated that: ① in water, the R-O-(CH₂)₃NH₂ was degraded by following an equation of first-order biochemical reaction, $c-c_0exp(-kt)$, where the temperature and pH value are two major, factors affecting the degradation coefficient; ② the soil adsorbed the ether amines R-O-(CH₂)₃NH₂ at a rate of over 92% and the adsorption equilibrium equation was q = 5.335 + 63.29C; and ③ in soil, the ether amines R-O-(CH₂)₃NH₂ moved in the vertical direction by following the c=aexp(-b/t). These results provided a scientific basis for water quality calculation and environmental protection designing.

Key words: ether amines, R-O-(CH_2)₃ NH_2 , degradation law, degradation coefficient, soil adsorption ratio, distribution coefficient, vertical direction movement.

Collection with a Graphite Probe Filter/Determination of Trace Copper in Atmospheric Particulate Matter by Graphite Probe furnace Atomic Absorption Spectrometry. Zhang Bicheng (Department of Chemistry, Hubei University, Wuhan 430062); Chin. J. Environ. Sci., 14 (5), 1993, pp. 76 - 78

Direct determination of trace copper in atmospheric particulate matter was done by graphite probe furnace atomic absorption spectrometry after collection with a graphite probe filter. The new method was simple and rapid. The concentration of copper and absorbance (peak-area mode) showed excellent linear relation in the range of 0 - 550