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硕士学位论文

生态浮床中植物根际的酶学研究

Study on Enzymology in the Rhizosphere of the

Plant on the Ecological Floating Bed

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## 摘要

自然水体中的有机物通常以大分子高聚物的形式存在,只有通过胞外酶的水解作用,降解为小分子后才能被异养微生物吸收利用。胞外酶在海洋环境中起着非常重要的作用,是微生物降解和利用有机化合物的关键。胞外酶活性的大小是有机物降解的关键影响因子。酶活性的大小能从一定程度上反应出有机物的降解速率。同时胞外酶活性还可以作为反映富营养化的一个非常合适的指标。胞外酶的研究,对于全面理解生态系统的物质循环、评估水域环境质量、探讨生态修复机制等都具有重要意义。

根际是指受到植物根系影响的、在植物生长、吸收和分泌过程中形成的物理、化学和生物学性质不同于周围环境的、复杂动态的微型生态系统,是一个重要的环境界面。其独特的物理、化学、生物学性质直接影响着污染物的循环、转化及其归宿。根际环境中具有较高的生物量,微生物数量也显著高于非根际环境,从而形成了“根际效应(Rhizosphere Effect)”,微生物的根际效应可以有效地促进根际中有机物的降解,植物根际降解已经成为植物修复领域中的研究热点。

本研究在筲箕湖干渠、内湖和外湖范围内选择了 14 个站位,选取了碳、氮和磷循环中的四种限速酶:-D-葡萄糖苷酶、磷酸酶、脂肪酶和亮氨酸氨基肽酶为研究对象,使用高灵敏度的荧光模拟底物法,对其在筲箕湖中的时空变化、浮床中植物根际和非根际水体中的活性进行了相关研究;并在实验室内着重研究了添加有机氮后亮氨酸氨基肽酶活性和根际细菌数量的变化以及蛋白质的降解效率;旨在阐述植物根系在有机污染物降解中所发挥的重要作用。主要的研究结果如下:

(1)筲箕湖水体中胞外酶活性总体上呈现出干渠>内湖、外湖>进、出水口的趋势,干渠处的 8#和 14#两个站位的酶活性远远高于其他站位,这可能是由于干渠区域污染严重,湖水中的有机污染物较多,从而造成微生物活性较高、酶活性亦高。

(2)筲箕湖水体中的胞外酶活性呈现出明显的季节变化特征,春、夏、秋、冬四季中,整个湖区 -D-葡萄糖苷酶活性分别是 0.032  $\mu\text{mol/L/h}$ 、0.065  $\mu\text{mol/L/h}$ 、

0.013  $\mu\text{mol/L/h}$  和 0.007  $\mu\text{mol/L/h}$  ; 磷酸酶活性分别是 0.033  $\mu\text{mol/L/h}$ 、0.674  $\mu\text{mol/L/h}$ 、0.037  $\mu\text{mol/L/h}$  和 0.007  $\mu\text{mol/L/h}$  ; 脂肪酶活性分别是 0.052  $\mu\text{mol/L/h}$ 、0.095  $\mu\text{mol/L/h}$ 、0.011  $\mu\text{mol/L/h}$  和 0.059  $\mu\text{mol/L/h}$  ; 亮氨酸氨基肽酶活性分别是 0.088  $\mu\text{mol/L/h}$ 、0.252  $\mu\text{mol/L/h}$ 、0.061  $\mu\text{mol/L/h}$  和 0.033  $\mu\text{mol/L/h}$ 。胞外酶活性总体上呈现出夏季>春季、秋季>冬季的趋势。由此可以看出温度是影响胞外酶活性的主要因素。

(3)在浮床系统中,海马齿根际水体中的胞外酶活性显著高于非根际水体,根际水体中的细菌丰度也远远高于非根际水体,是非根际水体中的 43 倍。根际效应非常明显。

(4)四种胞外酶活性的粒级分布也有明显差异,根际水体中以溶解态( $<0.22 \mu\text{m}$ )和颗粒态( $>3 \mu\text{m}$ )形式存在的  $\alpha$ -D-葡萄糖苷酶活性分别占总活性的 50.73%和 45.67%,非根际水中约有 65.87%的酶活性以颗粒态的形式存在;根际水中颗粒态的磷酸酶活性大约占 65.02%,非根际水中 68.75%的磷酸酶活性以细菌态(0.22-3  $\mu\text{m}$ )的形式存在;根际水中细菌态的脂肪酶活性比例为 93.02%,非根际水中脂肪酶活性全部为颗粒态的形式存在;根际水体中颗粒态的亮氨酸氨基肽酶活性为 59.58%,非根际水中颗粒态和细菌态的亮氨酸氨基肽酶活性相当,分别为 44.06%和 40.23%。

(5)对有机氮化合物的降解,植物根系和根际细菌的联合作用具有更高的降解效率。在实验过程中,连续三次添加了蛋白质,植物组在添加蛋白质后的 36 h 内能将蛋白质彻底降解,而细菌组的降解效率则远远低于植物组,添加抗生素后,单独的植物根系无法降解蛋白质。因此植物根系及其根际细菌组成了互惠共生关系。蛋白质的降解过程伴随着酶活性的升高以及细菌丰度的增加,整个实验过程中植物组的亮氨酸氨基肽酶活性始终高于细菌组。实验后期,随着蛋白质含量的降低,酶活性也逐渐下降。此外,蛋白质降解过程中,微生物的群落结构发生了变化,由以球菌为主的细菌群落变为以杆菌为主的细菌群落。

关键词:胞外酶活性;海马齿;根际;微生物

## Abstract

The organic compounds in the natural water existed in the form of high molecule, and could be assimilated by the heterotrophic microorganisms only through the decomposition of the extracellular enzymes. Therefore, extracellular enzymes played vital role in the marine environment, and were the key factor of the microbial degradation and the utilization of the organic compounds. To some extent, extracellular enzyme activity (EEA) could reflect the degradation rate of the organic compounds. At the same time, EEA was the appropriate index for eutrophication. The exploration of EEA had significant meaning in the comprehensive understanding of material cycle of the ecosystem, assessment of the quality of the aquatic environment and mechanism of the bioremediation.

Rhizosphere was referred to the dynamic and complex microenvironment that was different from the bulk in the physical, chemical and biological property under the effect of growth, absorption and secretion of the plants. It was a quite important environmental interface. The unique character of rhizosphere could affect the circulation, transformation and fate of the pollution directly. The significant higher biomass and microbial abundance in the rhizosphere form the rhizosphere effect, thus could facilitate the degradation of the toxicants in the rhizosphere. Rhizoremediation had become the hot pot in the phytoremediation.

In the 14 sites of the Yundang Lagoon, model fluorescent substrate was used to assess the spatial and temporal variation of the activities of  $\alpha$ -D-glucosidase, phosphatase, lipase and leucin-aminopeptidase that were the key enzyme in the carbon, nitrogen and phosphorus cycle. Comparison of the EEA in the rhizosphere and non-rhizosphere of the *Sesuvium portulacastrum* on the floating-bed was also studied. In the experiment, the variation of leucine-aminopeptidase activity, bacterial abundance after organic nitrogen added and degradation rate were carried out to explore the function of the root and rhizospheric bacteria in the rhizoremediation. The main results were as follows:

(1) The trend of the EEA in the water of Yundang Lagoon was:  $EEA_{\text{canal}} > EEA_{\text{inner lake}}, EEA_{\text{outer lake}} > EEA_{\text{intake}}, EEA_{\text{outlet}}$ . The EEA of 8# and 14# station in the canal were higher than other stations in most time of year, probably because of the series organic pollution, higher microorganism activities and enzyme activities.

(2) The EEA in the Yundang Lagoon showed significant seasonal variations: in the spring, summer, autumn and winter, the  $\alpha$ -D-glucosidase activities of the lagoon were 0.032  $\mu\text{mol/L/h}$ , 0.065  $\mu\text{mol/L/h}$ , 0.013  $\mu\text{mol/L/h}$  and 0.007  $\mu\text{mol/L/h}$ ; the phosphatase activities were 0.033  $\mu\text{mol/L/h}$ , 0.674  $\mu\text{mol/L/h}$ , 0.037  $\mu\text{mol/L/h}$  and 0.007  $\mu\text{mol/L/h}$ ; lipase activities were 0.052  $\mu\text{mol/L/h}$ , 0.095  $\mu\text{mol/L/h}$ , 0.011  $\mu\text{mol/L/h}$  and 0.059  $\mu\text{mol/L/h}$ ; the leucine-aminopeptidase activities were 0.088  $\mu\text{mol/L/h}$ , 0.252  $\mu\text{mol/L/h}$ , 0.061  $\mu\text{mol/L/h}$  and 0.033  $\mu\text{mol/L/h}$ . The trend was summer > spring, autumn > winter. Temperature was the major factor that affected EEA.

(3) In the floating-bed system, EEA in the rhizospheric water were significant higher than that in the bulk water. The rhizospheric bacteria abundance was 43 times more than that in the non-rhizosphere water. Rhizosphere effect was very obvious.

(4) Differences in the fractionated enzyme activity between rhizosphere and bulk were quite obvious. 50.73% and 45.67% of the  $\alpha$ -D-glucosidase activity in the rhizosphere water existed in the dissolved (<0.22  $\mu\text{m}$ ) and particle (>3  $\mu\text{m}$ ) forms; 65.87% of the  $\alpha$ -D-glucosidase activity in the bulk water existed in the particle form. 65.02% of the phosphatase activity in the rhizosphere attached on the particle; the phosphatase activity on the surface of bacteria accounted for 68.75% in the non-rhizosphere. The ratio of bacterial lipase activity in the rhizosphere was 93.02%; all of the lipase activity in the bulk water existed in the particle form; the proportion of the particle and bacterial leucin-aminopeptidase activity in the rhizosphere and bulk was equivalent to 44.06% and 40.23%, respectively.

(5) For the degradation of the organic nitrogen compound, the coalition of the root and rhizosphere bacteria had higher degradation efficiency. In the experiment, protein was added three times, and decomposed in the 36 h in the experiment group of root and rhizospheric microbial; degradation efficiency of the bacterial group was lower than the former. After antibiotic was added, individual root could not play role in the

protein degradation; therefore, the decomposition efficiency of the bacteria depended on the root. Protein degradation associate with the increasing the aminopeptidase and bacterial abundance, EEA of the plant group was higher than that of the bacterial group in the procession. In the later experiment, EEA decreased follow the protein reducing. In addition, microbial community changed from coccus-dominated community to the bacillus dominated community in the degradation procedure.

**Key Words:** Extracellular enzymes; *Sesuvium portulacastrum*; Rhizosphere; Microbe

厦门大学博硕士学位论文摘要

## 第 1 章 绪 论

随着我国经济的快速发展和城市化进程的不断加快,水污染日趋严重、水体水质日益恶化。全国有监测的 1200 多条河流中有 850 条受到不同程度的污染,并且有不断加重的趋势。工业较发达的城镇河段污染突出,城市河段中 78%的不适合作为饮用水源。七大水系以及太湖、滇池和巢湖中,只有 36%的河段达到或优于地表水环境质量 3 类标准,超 5 类标准的河段达到了 37%,大型淡水湖泊和城市湖泊均为中度污染,75%以上的湖泊富营养化加剧<sup>[1]</sup>。

海洋作为地球上最大的水体,随着沿海地区人口的急剧增加、工农业和海水养殖业的迅速发展、大量人工合成污染物的不合理排放,使得海洋也面临着严重的污染问题。近年来近海污染范围不断扩大,海域污染事件频繁发生,海洋环境质量严重恶化。虽然国家和各相关地方政府采取了众多的防治海洋环境污染的措施,但我国海洋环境质量现状仍然不容乐观。

根据国家海洋局公布的 2008 年中国海洋环境质量公报,在 2008 年,虽然我国未达到清洁海域水质标准的面积较 2007 年有所减少,近岸局部海域水质略有好转,但是仍有 88%的入海排污口超标排放污染物,而且部分排污口邻近海域环境污染严重,由大气输入海洋的部分污染物总量仍呈上升趋势。主要海湾、河口及滨海湿地生态系统健康状况不容乐观,大多数监控区都处于亚健康或不健康状态,海洋环境污染程度依然较高<sup>[2]</sup>。

我国近海污染普遍以氮、磷和油类为主,局部海区以有机氯农药、重金属为主。富营养化是我国近岸海域面临的主要环境问题。由于营养盐和有机污染逐年加重,20 世纪 90 年代以来我国近海赤潮发生频率、面积、区域和损失都大为增加,其中 2003 年共发生赤潮 119 起。近年来,我国沿海因赤潮灾害造成的直接经济损失已达上百亿元。

### 1.1 植物修复概述

为了控制、治理环境污染,植物修复、微生物修复、化学修复、物理修复以



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