藻类对垃圾填埋场渗滤液的净化

黄立南[1,2]，林 里[1]，陈月琴[1]，蓝崇钰[1*]，屈良鹄[1]
(1. 中山大学生命科学学院 生物防治国家重点实验室，广州 510275；2. 中山大学环境科学系，广州 510275)

摘要：采用 PCR 及序列测定的方法，对两个分别分离自广州市李坑垃圾填埋场的渗滤液收集塘以及广州市郊的一个普通池塘的藻类种群的 rDNA ITS 区进行了序列的测定和分析，结果证实两者均为蛋白核小球藻，分别记作 *Chlorella pyrenoidosa* (LK)和 *Chlorella pyrenoidosa* (P)。将上述两个藻类种群的纯培养液分别接种至一系列不同浓度的垃圾渗滤液中，以研究它们在渗滤液中的生长、对渗滤液的耐性以及对渗滤液中污染物的去除等差异。结果表明，藻类的生长在10%的渗滤液中都得到了一定的促进，而在更高浓度的渗滤液中则受到抑制。但 *C. pyrenoidosa* (LK)在经过一段时间的适应后，对 30%的渗滤液表现出较强的耐性。藻类的生长使垃圾渗滤液中的 NH_3-N、PO_4-P 和 COD 等污染物的含量显著下降，而 NO_3-N 含量下降不明显。

关键词：蛋白核小球藻；垃圾渗滤液；营养物去除

Molecular Identification of Algae and Their Use in Landfill Leachate Purification

HUANG Li-Nan[1,2], LIN Li[1], CHEN Yue-Qin[1], LAN Chong-Yu[1], QU Liang-Hu[1] (1. *School of Life Sciences, Zhongshan University, Guangzhou* 510275, *China*; 2. *Department of Environmental Sciences, Zhongshan University, Guangzhou* 510275, *China*). ***Acta Ecologica Sinica*, 2002, 22(2): 253～258.**

Abstract: Many studies demonstrated the success of employing algal cultures to remove nutrients from wastewater rich in nitrogenous and phosphorus compounds. Until recently, very little specific work has been focused on landfill leachate treatment using microalgae. The main objective of this study was to identify two microalgal strains of different origins by applying molecular techniques, and examine their growth in landfill leachate and ability to remove the nutrients.

Two algal strains were isolated respectively from the leachate in the surface recirculation pond at Likeng Landfill, Guangzhou, and an ordinary, less polluted pond in the same city. After DNA extraction, sequences of internal transcribed spacers (ITS) of their rDNA were successfully amplified, and the amplification products were used directly for sequencing. They were both identified as *Chlorella pyrenoidosa* by comparative analysis of the ITS sequences, and named *Chlorella pyrenoidosa* (LK) and *Chlorella pyrenoidosa* (P), respectively.

Leachate samples were collected from the surface recirculation pond at Likeng Landfill, and immediately filtered with 0.45 μm membrane filters. They were subsequently diluted to a series of concentrations using sterile deionized water, and inoculated aseptically with stock cultures of *Chlorella pyrenoidosa* (LK) and *Chlorella pyrenoidosa* (P) previously grown in Bold's Medium and in exponential phase. Meanwhile, a series of the same concentrations of leachate without any algal inoculations were used as the controls for nutrient removal test. All treatments were in triplicates. At every 1～2 days interval, the algal growths were monitored and compared with those of the same strain grown in Bold's Medium, in terms of increase

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* 通讯联系人
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作者简介：黄立南(1971～)，男，硕士，讲师。从事环境生态学的教学与研究工作。

in cell density counted by a haemocytometer. Aliquots of leachate were sampled every 3 days from each treatment, and analyzed for NH_4^+-N, PO_4^{3-}-P, COD and NO_3^--N.

Enhanced growths were found for both *Chlorella pyrenoidosa* (LK) and *Chlorella pyrenoidosa* (P) in 10% leachate, and their cell densities went up to 81 and 105 times over its initial values. Higher leachate concentrations were roughly toxic to algae. However, *Chlorella pyrenoidosa* (LK) showed an obvious tolerance to 30% leachate, after a 10-day period of adapting. Under this leachate condition, it had a better growth and was more effective in nutrient removal than *Chlorella pyrenoidosa* (P).

Removal rates of NH_4^+-N, PO_4^{3-}-P and COD under all treatments, especially in lower dilution series, were obviously higher than those in the control groups. For example, after a 12-day growth, *Chlorella pyrenoidosa* (LK) could remove up to 76.1, 75.8 and 71.6% of NH_4^+-N, PO_4^{3-}-P and COD from 10% leachate, respectively. While the values for *Chlorella pyrenoidosa* (P) were 66.2, 64.9 and 61.6%, respectively. Meanwhile, no obvious removal was found for NO_3^--N.

The preliminary. results demonstrated the feasibility of using green algae for landfill leachate treatment. Tolerant strains were preferable. Appropriate dilution of pre-treatment of the leachate might be necessary. In combination with traditional techniques in classification, the emerging molecular approaches should play an important role in identifying and screening algal species tolerant to different landfill leachates.

Key words: *Chlorella pyrenoidosa*; landfill leachate; nutrient removal
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垃圾渗滤液常常含有高浓度的总氮、难降解 COD、复杂有机物以及诸如重金属、氨、脂溶性有机物等有毒物质[1~4],而且不同的垃圾填埋场其渗滤液的理化性质差异甚大[5,6],其产量及化学组成随当地的气候条件(主要是降雨)、填埋场的年龄等不同而变化,从而使它们的净化处理面临很大的困难。很早以前,微型藻类就被建议作为取代污水处理中活性污泥的生物系统[7]。虽然国内外大量的研究证实了微型藻类用于污水净化的可行性[8~13],但迄今为止极少有应用于垃圾渗滤液净化的报道。要利用藻类对垃圾渗滤液进行有效净化,必须筛选或驯化出对垃圾渗滤液具有较强耐性的藻种,并调节和评估耐性藻的种类的最佳组合。

然而,由于藻类大多形体微小,其形态特征常常随生长环境及生长阶段的不同而改变,实验室培养种与野生种存在较大差异,从而给常规的分类鉴定带来相当的困难。由于 rRNA 基因的转录间隔区(Internal transcribed sequences, ITS)的异质性,使得它在各类生物属种间表现出明显的差异,从而被公认为是生物各类群属下种间水平比较研究的一个很好的分子指标,并且已在动物、被子植物、真菌以及藻类等得到广泛应用[14~17]。

本研究通过 ITS 序列分析的方法鉴定两个分离自不同生境——垃圾渗滤液收集塘及普通池塘的微型藻类种群,比较不同浓度垃圾渗滤液对其生长的影响,评估它们对垃圾渗滤液的净化效果,探讨利用微型藻类净化垃圾填埋场渗滤液的可行性。

1 材料与方法

1.1 藻种的分离与鉴定

试验用的渗滤液采自广州市北郊的李坑垃圾填埋场的渗滤液收集塘(其中生长有丰富的藻类)。该填埋场自 1992 年开始使用,现场建有渗滤液回灌循环系统,目前每天接受城市生活垃圾约 1500t,计划到 2002 年封闭。将渗滤液中的一种优势绿藻分离出来,经初步鉴定为蛋白核小球藻,记作 *Chlorella cf. pyrenoidosa* (LK)。暨南大学水生生物研究所提供了分离自本市市郊一普通池塘的蛋白核小球藻种群,记作 *Chlorella pyrenoidosa* (P)。

对藻种 rDNA ITS 区的 PCR 扩增及其序列测定[18],结果表明,扩增得到约 700 碱基长的片断,与预期扩增产物一致。PCR 扩增产物经 DNA 纯化系统纯化后,直接用自动测序仪进行序列测定并获得了两个株系的 rDNA ITS 区序列。该序列共 613 碱基对,其中包括 ITS1 的 218 对(部分)、ITS2 的 238 对以及 5.8S rDNA 的 157 对。采用计算机分析软件包对所获得的序列进行分析比较,并将所得到的序列输入 Genbank,以 Blast 程序进行比较分析,发现分离自广州市李坑垃圾填埋场的藻种与分离自普通池塘的对照株蛋白核小球藻的序列基本一致(仅有 3 个核苷酸的差异),而与小球藻属的其它种差异较大。序列分析的结果证实了从渗滤液中新分离到的藻种为蛋白核小球藻(*Chlorella pyrenoidosa*)。

1.2 渗滤液的理化分析

渗滤液经 0.45μm 滤膜过滤后,测定其 pH 值(pH 计)、NH_3-N(滴定法)、NO_3-N(戴氏合金还原法)、PO_4-P(钼蓝比色法)和 COD(高锰酸钾消化法)等理化参数[19]。

1.3 藻类的生长试验及其对渗滤液的净化

所有试验用渗滤液均经 0.45μm 滤膜过滤以消除其它微生物的干扰,之后用灭菌的去离子水稀释成 10%、30%和 50%共 3 个等级(分别记作 L10、L30 和 L50),加上渗滤液原液(记作 L100)和用作对照的 Bold 培养液,总共设 5 个处理组,每组 3 个重复。各处理组分别取 300ml 并转移至已灭菌的、具脱脂棉塞的 500ml 三角烧瓶中。将在 Bold 培养液中生长至指数期的藻类接种至各三角瓶内的培养液中并获得每毫升约 5×10^4 个细胞的初始浓度。将所有三角瓶装上恒温摇床并调节转速至 100rpm,然后移入到设定温度为 25±2℃、光周期为 L∶D = 16h∶8h 的生化培养箱中连续培养。培养液中藻类细胞的密度每 1 到 2d 用血球计数器统计 1 次,整个实验持续约 12d(在 30%渗滤液中生长的 *Chlorella cf. pyrenoidosa* (LK),由于其细胞密度在 12d 后仍持续增长,所以适当延长其细胞计数时间直至它开始出现下降为止)。渗滤液的化学参数每 3d 测定 1 次(方法见 1.2)。与此同时,没有接种藻类的各浓度梯度的渗滤液也在同样的条件下培养作为空白对照,以修正试验期间其理化参数变化所带来的影响。

2 结果与分析

2.1 渗滤液的理化特征

表 1 给出了试验用的各浓度梯度渗滤液的主要理化参数。李坑垃圾填埋场的渗滤液(原液)呈弱碱性(pH 7.80),含有相当高浓度的 NH_3-N (1345 mg·L^{-1}),但其 NO_3-N 及 PO_4-P 的含量却处于较低的水平(分别约为 68.44 和 5.13 mg·L^{-1})。

表 1 渗滤液稀释系列的主要理化参数

Table 1 Main physiochemical parameters of dilution series of the leachate

参数 Parameters	10% (L10)	30% (L30)	50% (L50)	100% (L100)
pH	7.46	7.56	7.58	7.80
EC (ms·cm^{-1})	2.00	3.10	4.80	7.70
COD (mg·L^{-1})	128.04	384.11	640.18	1280.36
NH_3-N (mg·L^{-1})	134.52	403.55	672.59	1345.18
NO_3-N (mg·L^{-1})	6.84	20.53	34.22	68.44
PO_4-P (mg·L^{-1})	0.51	1.54	2.56	5.13

2.2 藻类在不同浓度梯度的渗滤液中的生长

总体而言,两个藻类种群在各种浓度渗滤液中的生长(以细胞密度衡量)远远不如它们各自在 Bold 培养液中的生长,而且在所有渗滤液中的增长均表现出明显的滞后效应(图 1),表明它们对渗滤液需要一定时间的调整与适应。其中,两者在 10%的垃圾渗滤液中均有可观的生长,*C. pyrenoidosa* (LK)和 *C. pyrenoidosa* (P)的细胞密度最大可分别达到每毫升 41.3×10^6 和 53.5×10^6 个(分别在第 11 和第 9 天,图 1),比其初始细胞密度分别大幅增长了约 82 和 106 倍。另外,接种至 30%渗滤液中的 *C. pyrenoidosa* (LK)种群,虽然其生长在最初的 10d 内基本处于停滞状态,但之后便开始迅速增长,直至第 23 天时达到最大值每毫升 243.2×10^6 个(有关数据在图 1 中未给出),比其初始密度增长了 485 倍;而 *C. pyrenoidoda* (P)种群在同样浓度渗滤液中的生长则始终受阻,在整个实验过程中其最大细胞密度仅比其初始值增长约 1.7 倍。初步的结果表明,源自渗滤液收集塘的 *C. pyrenoidosa* (LK)种群,在经过相对较长时间的适应后,对 30%的垃圾渗滤液表现出较强的耐性。本研究中,在 30%、50%及 100%渗滤液中 *C. pyrenoidosa* (P)的生长均受到抑制,实验期间藻细胞密度比接种时的初始密度有所下降,且呈无规律状波动。

2.3 藻类对渗滤液的净化效率

接种藻类的各渗滤液中的 NH_3-N、PO_4-P 和 COD 等污染物的去除率明显高于它们相应的对照组(未接种藻类的各渗滤液);同一藻类种群对这些污染物的去除在低浓度渗滤液(L10 和 L30)中的效率明显高

于在高浓度渗滤液(L50 和 L100)中的效率(图 2)。例如,接种 *C. pyrenoidosa* (LK)的各组垃圾渗滤液中,在实验结束时其 NH_3-N、PO_4-P 以及 COD 的去除率分别是 76.1、75.8 和 71.6%(L10);74.0、67.3 和 73.9%(L30);48.4、51.8 和 48.1%(L50);15.6、16.6 和 18.5%(L100)。

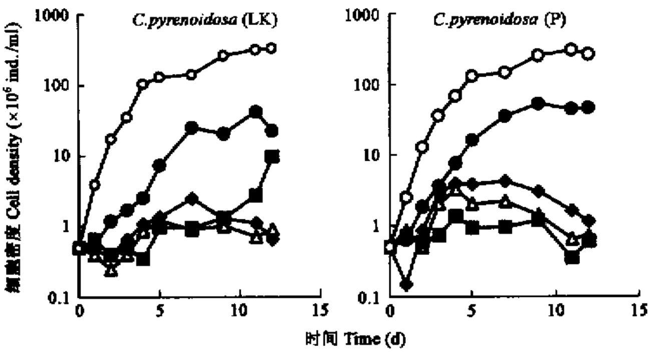

图 1 藻类在不同浓度的垃圾渗滤液中的种群对数增长曲线

○:Bold's ●:10% ■:30% △:50% ◆:100%

Fig. 1 Algal logarithmic growth curves in different concentrations of landfill leachate

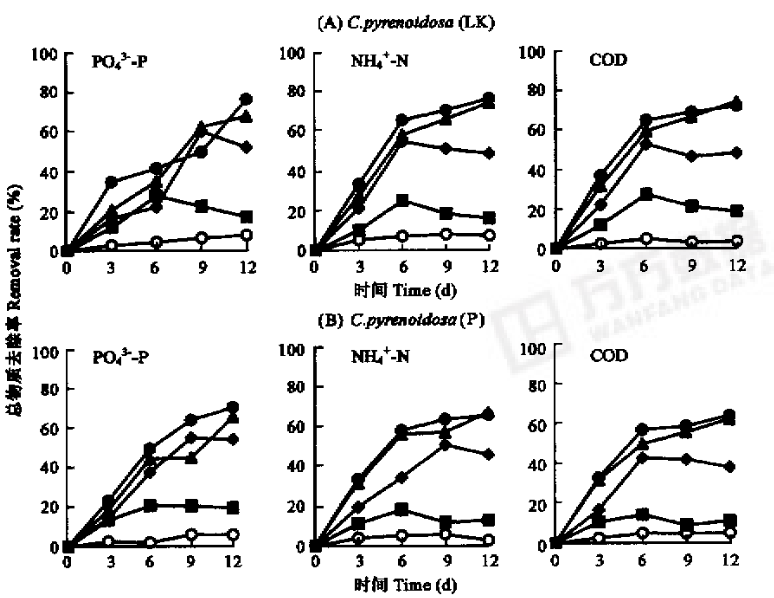

图 2 不同浓度的垃圾渗滤液中藻类对 NH_4^+-N、PO_4^{3-}-P 和 COD 去除率的影响

Fig. 2 Effects of algae on removal rate of NH_4^+-N, PO_4^{3-}-P and COD in different concentrations of landfill leachate

用作对照而未接种藻类的各种渗滤液,由于其污染物的去除率很低,因而图中仅显示了 10%的稀释系列 Because removal rates of the control treatments (leachates without any algal inoculation) were all very low, only those of 10% leachates were shown. ●:TL10,○:CL10,▲:TL30,◆:TL50,■:TL100. T:**接种藻类** Inoculated with algae, C:**未接种藻类** Inoculated without algae

在各种浓度的渗滤液处理中,两藻类种群对污染物去除的效率之间并无明显的差异。虽然 *C. pyrenoidosa*(LK)对 30%的渗滤液具有较强的耐性而 *C. pyrenoidoda*(P)较敏感,但 *C. pyrenoidosa*(LK)在 30%渗滤液中的迅速增殖基本上是在第 11 天以后才开始的,在此之前它也与 *C. pyrenoidoda*(P)一样处于一种受抑的状态,因此,两者对 30%渗滤液中污染物去除的效率在试验期间相差不大,到实验结束(第 12 天)时 *C. pyrenoidoda*(LK)和 *C. pyrenoidoda*(P)对 NH_3-N、PO_4-P 以及 COD 的去除率分别是 74.0、67.3 和 73.9%以及 66.2、64.9 和 61.6%。

各种渗滤液中 NO_3-N 的浓度在整个试验过程中均无明显变化,在接种藻类与未接种藻类(对照)之间也无显著差异(图 3)。

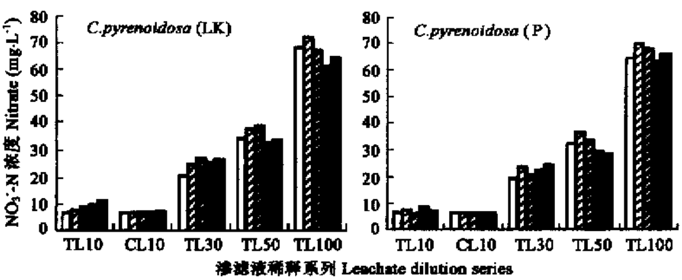

图 3　接种与未接种藻类的不同浓度垃圾渗滤液中的 NH_3^--N 在试验期间的变化

Fig. 3　Nitrate dynamics in different leachates with and without algae growing

每一处理组从左到右 5 个立柱分别代表第 0,3,6,9 和 12 天渗滤液中 NH_3^--N 的浓度 For each treatment, five columns from left to right stands for the level of nitrate analyzed on day 0,3,6,9 and 12, respectively. T:接种藻类 Inoculated with algae, C:未接种藻类 Inoculated without algae

3　讨论

生物过程用于垃圾渗滤液的净化已被许多研究与实践证明是切实可行的[20],而且在大多数情况下,生物处理要比物理/化学处理便宜得多。利用藻类进行污水净化的研究已在国内外得到广泛开展,其范围涉及从各种污水中富集和去除氮、磷、硝酸盐、重金属以及各种有机物等[8~13]。虽然本试验初步证实了利用绿色藻类能有效去除垃圾渗滤液中的 NH_3-N、PO_4-P 和 COD 等污染物,但由于迄今为止有关利用藻类净化垃圾渗滤液的研究极少,因而它在实际应用上的可行性仍有待作进一步的研究及评估。另外,藻类一般会优先利用污水中的 NH_3-N 或其它还原态的氮[21],这可能是导致本实验中各垃圾渗滤液的 NO_3-N 去除不明显的主要原因。垃圾渗滤液产量的不稳定性、高浓度的有机与无机物质、有机物中可生物降解部分的变动(随垃圾填埋场年龄的不同而变化)以及通常很低的磷含量等特征都可能使藻类的生长受到很大的影响,而且渗滤液的毒性也会严重影响生物处理过程的净化效率。本研究的结果表明,对垃圾渗滤液进行适当的稀释或预处理是有必要的。当然,更有必要对不同垃圾填埋场渗滤液中的藻类的种类组成、分布及其动态开展广泛的调查,在此基础上筛选出对垃圾渗滤液具有较强耐性的藻种[如本试验分离出的 *C. pyrenoidosa* (LK)],然后再对它们进行有目的的驯化,优化不同耐性藻种的组合并评估其净化效率。

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