投加酞酸酯的构建湿地基质微生物活性的研究

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摘要: 在复合垂直流构建湿地系统的源水中连续投加酞酸酯达一年之久, 并对该系统基质中的微生物数量、酶活性(脲酶、磷酸酶、脱氢酶)以及呼吸作用强度等各项指标进行了测定。结果表明下行流池微生物活性明显高于上行流池; 实验系统中酞酸酯对微生物的生长没有表现出抑制作用, 相反促进真菌类微生物的生长, 其中真菌数量是对照系统的4.6—5.5倍; 实验系统中酶活与呼吸作用强度也比对照系统高出1.0—5.9倍; 分别投加长链和短链酞酸酯的实验系统之间微生物数量差别不显著。从湿地生物膜的研究结果来看, 生物膜量为1—6mg/g, 生物膜的酶活性在基质酶活中所占比重很大, 达到70%以上, 生物膜的脱氢酶甚至比基质酶活高出10倍以上, 说明生物膜是湿地基质微生物的主要活性部分。

关键词: 构建湿地; 微生物; 酶活; 呼吸作用; 生物膜
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构建湿地是由基质和生长在其上的植物组成并用以净化污水的土壤-植物-微生物生态系统。这种独特的生态系统, 将微生物和植物的净化能力结合在一起, 成为一个高效的净化系统。传统的构建湿地有单一的表面流、潜流和垂直流三种类型, 本研究中的复合垂直流构建湿地(Integrated Vertical-flow Constructed Wetlands, IVCWs)则是一种新型的具有下行流—上行流串联水流方式的人工湿地。这种水流形态能充分利用污水处理面积, 提高对污水的处理效率[1]。

构建湿地不仅可以净化城市污水、地表径流和农业溢流, 还可有效地去除污水中的特殊污染物, 如酚、苯系物、酞酸酯、藻毒素及重金属等[2—5]。研究表明, IVCWs 对酞酸酯(即邻苯二甲酸酯, Phthalic Acid Esters, PAEs)这种优先控制污染物有很好的去除效果[6], 有报道证明生物降解是酞酸酯降解的主要途径[7]。微生物作为构建湿地中的重要成员, 有必要对其中微生物的活性进行研究, 为人工湿地处理含酞酸酯等特殊污染物的废水提供理论依据。

1 材料与方法

1.1 复合垂直流构建湿地小试系统的构造 温室中复合垂直流构建湿地小试系统共三套, 分别标号为1#、2#、3#, 其中1#、2#两套为实验系统(在进水中周期性的投加酞酸酯), 3#为对照系统(进水中不投加酞酸酯)。其结构都是由一个下行流池和一个上行流池串联而成。具体管网构造参照文献[6]。系统中采用的基质有细沙(上层, 粒径为0—4mm, 深度为35cm)、砾石(中层, 粒径为4—10mm, 深度为20cm)、碎石(下层, 粒径为40—55mm, 深度为15cm)。在下行流池中栽种陆生植物美人蕉(*Canna generalis*), 上行流池中为水生植物石菖蒲(*Acorus tartarinowii*), 种植密度为(3×3)株/m^3。小试系统的水流方式为下行流-上行流, 水力负荷为420mm/d, 间歇进水, 每8h进水一次, 每次进水量为140L, 水力停留时间(HRT)约为18h。进水采自武汉东湖茶港排污口, 经蓄水池初步沉淀, 在1#和2#配水池中再人为施加酞酸酯。1#系统为邻苯二甲酸二辛酯(DOP), 浓度为3.0mg/L, 2#系统为邻苯二甲酸二

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丁酯(DBP),浓度为9.84mg/L。

1.2 试验材料 材料取自中国科学院水生生物研究所人工湿地温室基地中1#、2#、3#系统中土壤,采样点依次为1#下行流池、1#上行流池、2#下行流池、2#上行流池、3#下行流池、3#上行流池,分别对应编号为1、2、3、4、5、6。

1.3 好氧微生物数量的测定 细菌、真菌、放线菌培养基分别为土壤浸出液琼脂、查氏培养基、改良高氏一号,细菌、真菌、放线菌计数采用稀释平板法,28℃培养,分别在培养后的第2d、5d、7d计数。

1.4 基质酶活性及生物膜酶活的测定 基质磷酸酶采用磷酸苯二钠法,基质脲酶采用奈氏比色法[8],脱氢酶采用三苯基四氮唑氯化物(TTC)比色法(以土壤中H^+的微升数表示)[9]。具体方法:采用梅花点阵法,在上下行流池中用酒精消毒的小铲取表层(7—8cm)土样并充分混匀,准确称取5.00g新鲜土样(生物膜酶活性的测定同此,样品为洗净的土样),分别经处理后在37℃培养12h,48h和12h,再显色后分别在波长为660nm(磷酸酶)、460nm(脲酶)和492nm(脱氢酶)时测量其光吸收值,并与标准曲线对照,计算出酶活性。

1.5 土壤微生物呼吸强度的测定 采用碱吸收法[10]。按每消耗1mL 0.1mol/L NaOH相当于2.2mg二氧化碳,可得出二氧化碳的释放量。

1.6 生物膜量的测定 具体步骤为:用去离子水轻轻洗净新鲜土样中的悬浮物质,称取一定重量的洗净土放入已称重的铝箔(w_1)中,于105℃烘箱中烘干,再称重(w_2);加1mol/L NaOH 20mL于烘干的土样中,80℃水浴1h,加以搅拌。弃去脱膜碱液,再用去离子水洗净,于105℃烘箱中烘干,再称重(w_3)。通过w_1、w_2、w_3可以得出生物膜量。

1.7 理化指标的测定 人工湿地系统进水的常规理化指标参照文献[11]。

2 结果

2.1 构建湿地系统中进水的理化参数(表1)

表1 构建湿地系统中进水的理化参数(平均值)

Tab.1 The physical and chemical parameters of the influent in the IVCWs(mean)

温度 ℃	pH	电导 μs/cm	电位 (mV)	溶解氧 (mg/L)	CODcr (mg/L)	BOD_5 (mg/L)	KN (mg/L)	TP (mg/L)
17.7	7.9	424	17.5	4.5	86.7	18.5	5.5	0.2

2.2 基质好氧微生物的数量(cfu/g)变化

基质中的微生物是人工湿地的重要组成部分,微生物的数量在很大程度上代表了复合垂直流构建湿地系统净化污水的能力[12]。温室构建湿地小试系统于2001年2月底初步建好,正常运行达一年后,于2002年4—5月采样,对温室系统基质中好氧微生物数量进行了测定。由表2可知构建湿地下行流池中各微生物类群数量均明显高于上行流池的数量,其数量比在3.6—10.0之间。从各微生物类群来看,1#、2#、3#系统之间总微生物数量相差不大,但微生物各类群所占比重不同。1#、2#系统中真菌数量是3#对照系统的4.6—5.5倍,在微生物类群中所占比重较高,与细菌只差一个数量级。

表2 温室构建湿地微生物数量[cfu/g(dry soil)]

Tab.2 Amounts of microorganism of the IVCWs in the greenhouse [cfu/g(dry soil)]

采样点	细菌($\times 10^6$)	真菌($\times 10^5$)	放线菌($\times 10^4$)
1	36(7.2)	99(7.9)	80(5)
2	6(0.36)	21(5)	10(2)
3	14(4)	96(8.2)	20(4.6)
4	3(0.4)	14(3)	5(0.31)
5(CK)	44(8)	18(4.1)	220(31.3)
6(CK)	12(2.5)	3(1)	20(4.3)

注:括号内为标准差,下同。

2.3　构建湿地基质的酶活性

从三套系统的基质酶活性来看, 基本上都是下行流池酶活性比上行流池酶活性高, 其数值比为1.3—12.0之间。1# 、2# 实验系统的酶活性高于3# 系统, 脲酶、磷酸酶、脱氢酶的数值比依次为1.3—1.7, 1.00—5.97, 2.2—2.9之间。当然, 也有个别实验系统中酶活性低于对照系统(表3) 。

表 **3**　温室构建湿地酶活性(37℃)

Tab.3　Enzymatic Activity of the IVCWs in the Greenhouse

采样点	脲酶(μg(NH_3^-N) / g, 48h)	磷酸酶(μg(酚) / g, 5h)	脱氢酶(μL H^+ / g, 12h)
1	75.69(12.5)	134.75(20.5)	380.05(36.6)
2	33.97(6.1)	63.29(10.2)	122.09(12.1)
3	54.83(8)	93.92(15)	380.05(36.6)
4	39.00(6)	53.08(11)	31.52(3.1)
5(CK)	56.99(7.2)	22.46(5.2)	128.65(10.1)
6(CK)	21.74(4.2)	53.08(10.2)	55.24(4.1)

2.4　构建湿地基质的呼吸作用

土壤的呼吸作用是土壤微生物生命活动中释放二氧化碳的过程, 通常以微生物的呼吸作用来衡量土壤中微生物的总活性, 进而反映土壤代谢的旺盛度。图1反映温室基质新鲜土样与对应基质生物膜的呼吸作用。由图1可知, 除3# 系统上行流与下行流池差别不明显外, 其余两套系统下行流池呼吸作用明显高于上行流池, 原土比对应生物膜的呼吸作用大。1# 、2# 实验系统中释放的二氧化碳的量比对照池高, 其数值比有的高达5.3。

2.5　生物膜酶活性的比较

对基质中的生物膜量进行了测定, 并且比较了生物膜与原土的酶活性(表4) 。从生物膜量来看, 1# 、2# 系统生物膜没有3# 系统成熟。各系统中生物膜量约为1—6mg/g。从生物膜和鲜土的酶活性来看, 生物膜的酶活性在基质酶活中所占比重很大, 达到70% 以上, 生物膜的脱氢酶甚至比基质酶活高出10倍以上。

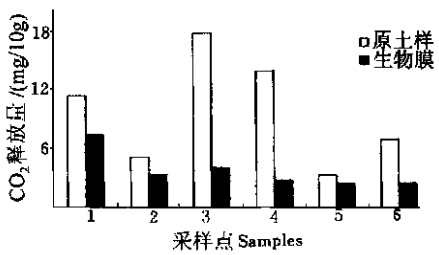

图 1　温室构建湿地基质的呼吸作用

Fig.1　Respiration of the substrate of the IVCWs in the Greenhouse

表 **4**　温室构建湿地生物膜及其酶活(平均值)

Tab.4　Biofilm and eneyme activity of the IVCWs in the greenhouse

采样点	生物膜量(mg/20g)	脲酶活% (生物膜/ 原土)	脱氢酶活% (生物膜/ 原土)
1	61.464	75.89	111.55
2	28.25	34.68	1186.96
3	76.116	571.73	438.40
4	20.686	74.04	1038.38
5(CK)	100.246	92.95	2290.22
6(CK)	62.516	70.33	100

3　讨论

从微生物数量、酶活性、呼吸作用强度等指标可以看出下行流池均强于上行流池, 这是由于上行流池中水位较高, 处于水淹状态, 氧气含量低, 并且由于水流方向是由下行流到上行流, 上行流池中营养物含量较下行流池少, 所以微生物类群数量相比下行流池也较少。这与成水平、陈博谦[13—14] 等人的结论相似, 说明酶活性、呼吸作用强度与微生物数量三者之间具有一定的相关性。

施加酞酸酯后,实验系统的各微生物类群数量与对照系统的差别不大。这可能是由于微生物适应性强,系统在长时间运行后,酞酸酯对微生物的生长抑制作用表现不明显。实验系统的真菌数量反而比对照要高,推测在系统运行一年中,投加的酞酸酯改变了基质微生物类群,诱导产生了更多的真菌来降解酞酸酯,使得酞酸酯在湿地中有效地得以去除。当比较两种不同酞酸酯对好氧微生物各类群的影响时可以发现其差别不显著,尽管 1# 系统中施加的是低水溶性的难降解 DOP,2# 系统中施加的是水溶性大的较易降解的 DBP。而赵文玉等的研究[6]中表明系统运行初期,在下行流池中 2# 系统好氧微生物数量比 1# 系统高出一个数量级。其中主要原因是在系统运行初期,较易降解的酞酸酯比较受"欢迎",并且对微生物毒性较小,而在系统运行一年以后,基质微生物适应了环境,数量差别不明显,对长链(DOP)和短链(DBP)酞酸酯都有降解。

脲酶是一种酰胺酶,能酶促水解有机质分子中的肽键,磷酸酶能酶促水解有机磷化物,二者在物质转化过程中起着非常重要的作用,它们是表征土壤肥力的重要指标;脱氢酶是一种氧化酶,它往往作为检测微生物毒性的一种指标,通过检测其酶活性来从细胞分子水平上研究环境化合物的毒性。实验池中脲酶、磷酸酶、脱氢酶的活性比对照池高,其主要原因是有机污染物在短时期内可对微生物的活性表现出一定的抑制作用,但微生物适应性强,变异快,对于 IVCWs 这种稳定并且营养丰富的人工生态系统,在经过长时间的运行后,微生物能够很快恢复且提高其活性。从呼吸作用强度的结果来看,实验系统强于对照系统,在国内外的一些报道中可见类似的结果,即在施加有机污染物的基质中,在一定时期内剂量高的基质中呼吸产生的二氧化碳反而高[15]。在本研究中,实验系统中微生物酶活性高,微生物活动加强,并且以酞酸酯为生长代谢的碳源,这些因素都是导致实验系统基质呼吸作用增强的重要原因。

在赵庆良等人的研究[16]中,以轮胎颗粒为载体的复合生物膜反应器在运行 60d 后生物膜含量达到了50—65mg/g。本系统中生物膜量为1—6mg/g,相比而言,整个湿地系统生物膜还不成熟。因为生物膜量大小与反应器构型、基质种类与浓度、水力剪切力大小、微生物种类等因素都有关。本研究中进水水源为生活污水(水质情况如表 1),CODcr 为80mg/L左右,赵庆良等人在研究中采用人工合成污水,CODcr 达1000mg/L,进水量为 180L/min,污染负荷高,从而生物膜相对成熟。从生物膜方面来考虑,构建湿地可以从提高污染负荷等方面来挖掘其处理污水的潜力。

综上所述,投加酞酸酯的人工湿地运行一段时间后,酞酸酯对微生物的生长并没有表现出抑制作用,实验系统中酶活性、呼吸作用强度等指标相比对照系统还有所增强,这些都为酞酸酯的生物降解提供了有利的环境。人工湿地系统中酞酸酯的生物降解机理还有待于进一步研究。

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MICROBIAL ACTIVITIES OF THE SUBSTRATE IN THE INTEGRATED VERTICAL-FLOW CONSTRUCTED WETLANDS TREATED WITH PHTHALATE ACID ESTERS

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Abstract: In this paper, a new-typed constructed wetland(IVCWs) consisting of downflow-upflow is described. Substrates were sampled using a column sampler in subsurface (7—8cm). The amounts of bacteria, actinomyces and fungi were determined using serial dilution plate method; substrate enzymatic activities, such as dehydrogenase, urease, phosphatase were measured by colorimetry; soil respirations were determined using alkali absorption method.

This research was done after the source water of IVCWs had been treated continuously with Phthalate Acid Esters for more than one year. The results showed that the microbial activities in the down-flow of both the control systems and to treated systems were obviously stronger than those in the up-flow. As the effects of phthalate acid eaters on microorganism activities were concerned, the enzymatic activities and respirations in the treated systems were stronger than the control systems, and which in the former system were 2.0—6.9 times of that in the latter. The total microbial amounts showed no obvious difference between two systems, but the community structure existed variation, especially, the fungal amounts in the treated systems were 4.6—5.5 times of which in the control systems, amount to 10^5cfu/g; Adding different phthalate acid eaters to two treated systems, the results showed that there was no obvious difference between treated systems added to different long-chain (DOP) and short-chain (DBP) Phthalate Acid Esters after the systems had been running for one year, which were different from the results of incipience, and incipient results showed that the microorganism activities of treated system added with DBP were higher than that added with DOP. The research results of wetland biofilm showed that the mass of biofilm were 1—6mg/g, the enzymatic activities of biofilm amounted to 70% of the total medium enzymatic activities, the activities of dehydrogenase were even 10 times of that of the medium, which showed that biofilm was the main component of the wetland microorganisms and contributed more to the microorganism activities.

All of the parameters such as microorganism quantities, enzymatic activities and soil respirations in downflow chamber are stronger than that in upflow. The substrate in downflow is 10cm higher than that in upflow, then the substrate surface in upflow is prone to being submerged, and oxygen content there is lower; dissolved wastewater first passes through downflow, then through upflow and nutrient contents in upflow chamber is also lower than that in downflow chamber. The difference of oxygen and nutrient contents can directly influence microorganism population size. Enzymatic activities, respiration, and microorganism quantities show consistency to some degree, which shows relativity exists among

them.

As microorganism quantity is concerned, there is little difference between treated systems (added PAEs for more than one year) and control systems. Results show that Fungi amount in treated system is higher than that in control system. We also compare long-chain PAEs(DOP) with short-chain PAEs(DBP), despite of the difference of water-solubility and degradation, results show that there are little difference, which is different from former research results (added PAEs for only one month). The reason is that in early days, short-chain PAEs is "popular" with indigenous microorganism and it can be taken as one carbon source, so former research results showed that microorganism quantities in system treated with DBP is higher than that treated with DOP, but when systems work well for more than one year, microorganisms in substrate adapt to environment, so the quantities difference is unconspicuous.

Urease and phatase have important effects during substance transformation and they are important indexes of soil fertility. Dehydrogenase can be taken as one index of microorganism toxicity. We can do some research work on environmental compound toxicity from cell molecular level through determination of dehydrogenase activities. Urease, phosphatase and dehydrogenase activities in treated systems is higher than that in control system, highly adaptation of microorganism is the main reason, for IVCWs is a stable and nutrition enrichment man-made ecological system, microorganism can quickly rehabilitate their activities. As respiration is concerned, carbon dioxide produced by respiration in treated system is higher than that in control system, all these factors such as high enzymatic activities and added carbon source can lead to higher respiration.

In this system, biofilm weight is 1—6mg/g, compared with biofilm weight of 50—65mg/g in hybrid biological reactor (working for 60 days), which shows that the biofilm in whole CW system is not mature. Because biofilm weight is related with reactor type, substrate, water quality, microorganism, et al. In this research, the COD_{cr} of influent is about 80mg/L, while in hybrid biological reactor, the COD_{cr} is about 1000mg/L and pollutant load is higher than that in CW, so the biofilm is relative mature. We can increase pollutant load to open out CW potential to treat wastewater.

From all above, we can conclude that after treated with PAEs for a long time, PAEs shows no inhibiting effect on microorganism; Some indexes such as enzymatic activities, respiration in treated systems is higher than that in control system. All these can supply biodegradation of PAEs with advantaged condition.

Key words: Constructed wetland; Microorganism; Enzymatic activity; Respiration; Biofilm