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Nitrogen mass balance and microbial analysis of constructed wetlands treating municipal landfill leachate

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Abstract

Experiments were conducted to investigate the feasibility of applying constructed wetlands to treat a sanitary landfill leachate containing high nitrogen and bacterial contents. Under a tropical condition (temperature of about 30 °C), the constructed wetland units operating at the hydraulic retention time of 8 d yielded the best treatment efficiencies with BOD_5, TN and fecal coliforms removal of 91%, 96% and more than 99%, respectively. Cadmium removal in the SFCW bed was 99.7%. Mass balance analysis, based on total nitrogen contents of the plant biomass and dissolved oxygen and oxidation–reduction potential values, suggested that 88% of the input total nitrogen were uptaken by the plant biomass. Fluorescence in situ hybridization results revealed the predominance of bacteria, including heterotrophic and autotrophic, responsible for BOD_5 removal. Nitrifying bacteria was not present in the constructed wetland beds.

Keywords: Subsurface-flow constructed wetland; Landfill leachate; Nitrogen mass balance; Bacterial species and fluorescence in situ hybridization

1. Introduction

Landfill leachate is wastewater emanated from sanitary landfills treating a variety of municipal and industrial solid wastes. Due to anaerobic conditions and long retention time prevailing in sanitary landfills, landfill leachate normally contains high concentrations of organic matters, nutrients, pathogens and heavy metals which, if not properly collected and treated, can cause serious pollution to nearby surface and groundwater sources. The presence of heavy metals at high concentrations in landfill leachate usually causes toxic effects to microbes, making it difficult to be treated biologically. Although several physical, chemical and biological processes can be employed to treat landfill leachate, for developing countries they can be expensive in construction, operation, and requiring high-skilled labor in operation. On the other hand, where land is available at low-cost, natural systems, such as waste stabilization ponds and constructed wetlands are attractive alternatives for landfill leachate treatment.

Fluorescence in situ hybridization (FISH) is a molecular technology which can be used to identify a number of bacterial species or strains and their relative distribution in the environment. This technology thus offers insights into the composition of microbial communities and the role a specific organism or related group of organisms play in ecosystem (Madigan et al., 2003). FISH of whole cells using 16S rRNA targeted oligonucleotide probes is a powerful technique with which to evaluate the phylogenetic identity, morphology, number, and spatial arrangements of microorganisms in environmental settings (Amann et al., 1995). To date, FISH using oligonucleotide probes targeting signature regions of the 16S rRNA of ammonia- and nitrite-oxidizing bacteria has been successfully applied for

Abbreviations: SFCW, subsurface-flow constructed wetland; FISH, fluorescence in situ hybridization; TC, total coliform bacteria; FC, fecal coliform bacteria; TN, total nitrogen; d, day.

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phylogenetical identification and quantification in environmental and engineered systems (Wagner et al., 1995).

Because constructed wetlands (CW) are known to be effective in removing nitrogen from wastewaters through nitrification/denitrification, plant uptake and volatilization (Koottatep and Polprasert, 1997), it would be of interest to employ FISH technology to determine the predominant species of bacteria growing in CW beds.

The objectives of this research were to: (1) investigate the feasibility of applying a CW system to treat landfill leachate containing high total nitrogen (TN) and bacterial contents; (2) analyze mass balance of TN removal in CW beds; and (3) identify the major bacterial species, through FISH technology, present in the CW beds.

2. Methods

The pilot-scale experiments on CW treatment of landfill leachate were conducted under ambient condition with an average temperature of about 30 °C. Sanitary landfill leachate samples, collected from the Pathumthani municipal landfill site, located about 30 km north-east of Bangkok city, Thailand, had characteristics as shown in Table 1. These data were obtained from analyses of leachate samples collected six times during the period of November 2003 and February 2005. The leachate characteristics showed a wide variation depending on tropical climatic changes such as monsoon and dry periods, similar to those found in other places (Tchobanoglous et al., 1993).

2.1. Experimental set up and operating conditions of CW

To avoid odor problems, CW units were operated in subsurface-flow mode in which all the influent wastewater was made to flow through the CW beds and no wastewater flowing above the CW beds. This type of CW is called subsurface-flow CW or SFCW.

Two pilot-scale SFCW units, made of reinforce concrete, were built at the Asian Institute of Technology, Thailand, each with a dimension of $0.5 \times 4.0 \times 0.5$ m (width × length × depth), and a bed slop of 1%. The support media of these units consisted of large gravel (2–3 cm in diameter), medium gravel (1–2 cm) and sand (0.1 cm) at depths 15, 20, and 15 cm, from the bottom, respectively. Cattail plants (*Typha angustifolia* L.) were planted at a density of 40 no. m^{-2}. The campus domestic wastewater was fed continuously to the SFCW units to acclimatize the soil microbes and to support growth of the cattail plants.

Table 1
Characteristics of landfill leachate used in SFCW experiments

Parameters	Unit	Range	Average	SD
pH	–	7.7–8.1	8.73	±0.3
Conductivity	mS	8.1–8.5	6.61	±2.6
Salinity	ppt	4.7–4.9	4.53	±0.3
COD	mg l^{-1}	1820–4100	2950	±811
BOD	mg l^{-1}	370–950	775	±212
TKN	mg l^{-1}	140–1260	389	±490
Total phosphorus	mg l^{-1}	19.0–26.8	24.7	±3.0
Fecal coliforms	no. (100 ml)$^{-1}$	1600–4940	2236	±1336
Apparent color	ADMI unit	300–1000	642	±223
Cd	μg l^{-1}	0.13–3.00	2.41	±1.10
Mn	μg l^{-1}	2500–6370	4746	±2008

SD = standard deviation.

After the cattails were fully grown to an average height 3 m, the SFCW units were continuously fed with the campus wastewater at an initial organic loading rate (OLR) of 100 kg BOD_5 ha^{-1} d^{-1} until a steady state condition, based on relatively constant BOD_5 concentrations in the effluent for at least three times of hydraulic retention time (HRT), was reached. After that, the landfill leachate diluted with tap water (to maintain the influent BOD_5 concentrations of 110–130 mg l^{-1}) but spiked with Cd to a concentration of 1000 μg l^{-1} was fed to the experimental SFCW unit, while the campus wastewater was still fed to the control SFCW unit. The effects of HRT on the treatment performance of SFCW were studied by varying the HRT at 1, 3, 5 and 8 d. Harvesting of the cattails plants was conducted once every three months by cutting the plant stems at about 50 cm above the SFCW beds. About 50% of the cattails plants were harvested each time to allow for the SFCW beds to maintain treatment efficiencies. The harvested plants were then analyzed for biomass production, TN and Cd contents. All the physical, chemical and biological parameters of the wastewater and plant biomass samples were analyzed according to the methods described in Standard Methods for the examination of water and wastewater (APHA-AWWA-WEF, 1998).

2.2. Fluorescence in situ hybridization (FISH) analysis

The FISH method involves application of oligonucleotide probes to permeablized whole microbial cells and specifically hybridize the cells to their complementary target sequence in the ribosomes. In this study, only samples of the cattail roots and soil of the SFCW units operating at HRT of 8 d were collected to determine the presence of nitrifying bacteria. The samplings, done randomly at three locations in each of the SFCW units, were conducted three times during steady state conditions. The collected samples were put in sterile plastic bags and transported to the laboratory for FISH analysis.

2.2.1. Sample preparation

About 20 g of the collected samples were put in 20 ml of 0.85% NaCl solution, centrifuged at 150 rpm for 60 min to extract the bacterial cells from the cattail roots and soil, and again centrifuged at 3000 rpm for 10 min to separate the bacterial cells from other contaminants.

2.2.2. Cell fixation

The supernatant from above was centrifuged again at 700 rpm for 3 min to homogenize the bacterial cells and make them settle. To fix the bacteria cells, the settled cells were added with 1 ml of paraformaldehyde, washed with