



Investigating microbial activities of constructed wetlands with respect to nitrate and sulfate reduction

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ABSTRACT

The biological reduction of both nitrate and sulfate in constructed wetlands, connected to a wastewater treatment plant, was investigated. Three different macrophytes, including *Acorus*, *Nuphar*, and *Typha* plants, were dominant in the free surface flow constructed wetlands; wastewater effluent flows through the *Acorus*, *Nuphar* and *Typha* plants ponds in order. Nitrate was substantially reduced throughout the wetlands, while sulfate was significantly reduced only in the *Typha* wetland, under anoxic conditions. A real-time polymerase chain reaction (RT-PCR) technique was performed to identify and quantify the denitrifying bacteria (DNB) and sulfate reducing bacteria (SRB) in both the wetland effluent and soils, and DNA bands obtained from 16S RNA based PCR experiments were also compared to DNA ladders, which were provided by the corresponding manufacturers. The numbers of the total bacteria in the *Typha* wetland effluent (i.e., a stagnant pond) were higher than those in the other ponds. However, the numbers of total and DNB bacteria extracted from the wetland soils was lower in the *Typha* than in the *Acorus* wetlands. RT-PCR and acetylene-blocking methods confirmed that the first (NO_3^- to NO) and second (NO to N_2) half denitrification procedures were dominant in the *Acorus* and *Typha* wetlands, respectively. The fractions of both DNB and SRB extracted from the wetlands effluent and soils, against total bacteria, were 2 and 40%, respectively. This work suggests that both nitrate and sulfate ions were effectively reduced by the biological activity of the DNB and SRB present in the wetlands, especially in the *Typha* wetland. The RT-PCR experimental results were shown to be in good agreement with those of the 16S rDNA PCR performed using gel electrophoresis.

Keywords: Denitrifying bacteria; Sulfate reducing bacteria; Total bacteria; Real-time polymerase chain reaction; Constructed wetlands

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