



Review

Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens, and real-time process control

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Abstract

Traditionally, the detection of pathogens in water, wastewater, and other environmental samples is restricted by the ability to culture such organisms from complex environmental samples. During the last decade the use of molecular methods have supplied the means for examining microbial diversity and detecting specific organisms without the need for cultivation. The application of molecular techniques to the study of natural and engineered environmental systems has increased our insight into the vast diversity and interaction of microorganisms present in complex environments. In this paper, we will review the current and emerging molecular approaches for characterizing microbial community composition and structure in wastewater processes. Recent studies show that advances in microarray assays are increasing our capability of detecting hundreds and even thousands of DNA sequences simultaneously and rapidly. With the current progress in microfluidics and optoelectronics, the ability to automate a detection/identification system is now being realized. The status of such a system for wastewater monitoring is discussed.

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1. Introduction

Secondary treatment is one of the key components of a wastewater treatment plant (WWTP). It involves the biological reduction of biochemical oxygen demand (BOD), suspended solids (SS) and toxicity of industrial wastewaters and the production of low nutrient, environmentally benign outgoing effluent. These functions are carried out by the resident microbial community which is considered the foundation of the secondary treatment process. Processing of the effluent also has been shown to reduce the incidence of bacteria and in particular the number of pathogenic organisms that may be present (Sahlstrom et al., 2004; Betancourt and Rose, 2004). Over many decades, engineers have designed these systems based mainly on chemical and physical parameter information and have been fairly successful in utilizing the advantages of microbial community's amazing metabolic potential without detailed knowledge about the organisms involved. However, the prolific growth of certain microorganisms can contribute to process detriments such as bulking, foaming and settling problems and the presence of pathogens in wastewater effluent can be a potential threat to public and environmental health. In order to monitor and control such phenomena it is important to better understand the biological agents associated with these systems. Therefore, it is essential that information about the composition, structure and activity of the microbial community within water and wastewater treatment systems is collected and examined.

In recent years, the application of molecular techniques to the study of natural and engineered environmental systems has increased our insight into the vast diversity and interaction of microorganisms present in complex environments. These innovative techniques have also been applied to the detection of microbial pathogens and biological warfare agents (BWAs) and to the identification and enumeration of organisms from water, food, clinical, and environmental samples (Bull et al., 2000). Molecular methods have increased our ability to circumvent lengthy culture methods and provide direct detection of pathogenic organisms. Techniques

such as amplified ribosomal DNA restriction analysis (ARDRA and also referred to as 16S-RFLP), ribosomal spacer analysis (RISA), terminal restriction fragment length polymorphism (t-RFLP) and denaturing gradient gel electrophoresis (DGGE) have been used in many areas of microbial research to advance our ability to illustrate and monitor mixed populations and distinguish between virulent and non-virulent stains of the same species. Full-cycle 16S rRNA analysis has allowed microbiologists to describe the diversity of individuals within populations and identify novel organisms (Amann et al., 1998). Also, the use of fluorescent in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM) has provided a means to study microbial populations in a more quantitative way (Wagner et al., 1993).

The development and use of high throughput technologies that have exploited the use of PCR amplification and DNA microarrays has recently provided a way to rapidly examine and screen the composition as well as the activities of microorganisms (Wilson et al., 2002; Dennis et al., 2003; Gupta et al., 2003). This technology, however, has yet to be applied directly to the operation of water and sewage treatment facilities. The recent advances in lab-on-chip technology have the potential to perform molecular testing on-line (Barry and Ivanov, 2004). This new nanotechnology could be the bridge that unites the fundamental knowledge about the microbial community with the water and wastewater treatment process. Designing microchips based on microfluidics with biosensors that can be used in real-time monitoring is one step forward in the quest to directly monitor the level of pathogenic organisms, and observe the diversity and activities of the microbial community associated with specific operational parameters. The difficulty in marrying molecular methods to process operations, however, lies in not solely establishing a correlation between measurements and microbiological analysis but a question of structure–function. Ultimately, understanding the microbial consortium would allow engineers to harness the microbial functions to model and improve the design and operation of the systems (Wilderer et al., 2002).

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