

Analysis of the functional diversity of the microbial communities in a paper-mill water system

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Abstract

Most microbiological studies of wastewater treatment systems rely on methods that are dependent on culturing. However, due to the limitation of conventional microbiological methods, alternative assays, such as the analysis of the functional diversity of the microbial communities using Biolog substrate utilisation profiles, have been proposed. The Biolog method was thus evaluated in a paper-mill water system. The influence of the production of various paper grades, biocide combinations and monthly maintenance shut-downs on the functional diversity of the microbial communities were determined using the Biolog technique. The communities in the planktonic as well as the sessile phases were analysed approximately every second week for a period of one year. The average well colour development (AWCD) technique was used to transform the data obtained from the Biolog plates prior to multivariate statistical analysis. Our results indicated that different microbial communities developed during the production of the different paper grades. A difference in substrate utilisation was evident after a single day of production of fluting or linerboard in the planktonic communities, while differences in the sessile communities only became significant after an extended period of production. The effect of the application of different biocides was more distinct in the planktonic than in the sessile populations. No clear trends concerning the influence of maintenance shut-downs and cleaning of machines could be observed in the sessile phase, while a shift in the microbial community could be observed in the planktonic phase. Biodiversity indices indicated that a high functional diversity existed in both the planktonic and sessile phases. It was concluded that the substrate utilisation profiles obtained with Biolog plates were a sensitive measure, which enabled the detection of shifts in the microbial community function and possibly structure within the same water system.

Introduction

Most microbiological studies of wastewater treatment systems rely on cultivation, which quantifies a limited percentage of the microbial populations present (Schneider et al., 1998). Incorrect conclusions concerning the function and structure of the microbial community *in situ* are consequently made. Due to the limitation of conventional microbiological methods, numerous alternative techniques that circumvent these limitations have been developed to characterise microbial communities *in situ*.

One such a technique is the analysis of the functional diversity of the microbial community (Buyer and Drinkwater, 1997; Garland and Mills, 1991; Zak et al., 1994). Zak et al. (1994) defined the functional diversity of a microbial community as the numbers, types, activities and rates at which a suite of substrates is utilised. Garland and Mills (1991) proposed that substrate utilisation, using commercially available Biolog plates, could be used to characterise the functional differences among microbial communities. The application of the substrate utilisation assay has been studied extensively in various environments such as soil, water (Garland and Mills, 1991) and wastewater systems (Victorio et al., 1996).

Due to the success of the application of the substrate utilisation approach, it was decided to test this system in an industrial water system. The aim of this study was, therefore, to monitor the functional diversity of the microbial community in the water system of the Sappi Cape Kraft paper-mill, South Africa, over a period of one year. The influence of the production of various paper

grades, types of biocide used and monthly maintenance shut-downs on the substrate utilisation profiles for both the planktonic and sessile microbial communities was studied during this period.

The Sappi Cape Kraft paper-mill, Cape Town, South Africa manufactures both fluting and linerboard from recycled fibre and reuses a large percentage of the process water. Fluting is produced at pH values between 6.5 and pH 7.5 while linerboard is produced at pH values between 4.5 and pH 5.5. Aluminium sulphate and rosin size are added during the production of linerboard to improve the printing quality and to make storage under conditions of high humidity possible. Fluting is produced without any additives.

Different combinations of biocide formulations were added to the clarifier to control microbial fouling. The first combination consisted of a formulation with organosulphur (sulphone) and quaternary ammonium compounds and a formulation of organosulphur (carbamate). The organosulphur (sulphone) and quaternary ammonium compounds are effective against bacteria and fungi (Boiven, 1995; McCoy, 1980). It has also been reported that organosulphur (carbamate) is very effective against sulphate reducing bacteria (Boiven, 1995). The second combination consisted of a formulation with quaternary ammonium compounds and organotin that is more effective against fungi than bacteria (Drew Industrial Division, 1994).

Materials and methods

Sample preparation and incubation

Duplicate planktonic and sessile samples (~50 ml) were collected fortnightly from the Cape Kraft mill for a period of one year. Approximately 5 ml of biofilm or 10 ml of the planktonic phase

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