Analysis of the structural diversity of the microbial community in a paper-mill water system

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Abstract

Microbial populations in paper-mill water systems are usually enumerated using microbiological techniques such as plate counts and the most probable number technique. These conventional methods can only quantify a limited percentage of the microbial populations and the microbial numbers are, therefore, generally underestimated. One possible alternative to these methods involves the analysis of signature lipid biomarkers to study the structural diversity of microbial populations. The aim of this study was, therefore, to evaluate the applicability of signature lipid biomarker analysis in a paper-mill water system. Samples from the sessile and planktonic phases were collected over a period of one year at the Sappi Cape Kraft paper-mill. The samples were subjected to analysis of signature lipid biomarkers as well as conventional culturing. Analysis of the phospholipid fatty acids revealed the presence of a large diversity of micro-organisms. The same trends in the number of cultured cells and the counts obtained with signature lipid biomarker analysis were observed, although the numbers obtained with signature lipid biomarker analysis were at least 1 000 times higher. Profiles of signature lipid biomarkers reflected changes in production and water management practices, which could not be detected with culturing techniques. The analysis of signature lipid biomarkers provided more information for the characterisation of microbial communities in paper-mill water systems and has potential for application in other industrial water systems.

Introduction

Many problems are experienced with conventional microbiological techniques such as the standard plate count procedure, and studies have indicated that only 0.01% to 1% of all microbes are culturable on artificial media (Vestal and White, 1989; Palojarvi et al., 1997). Culturing methods are selective due to the media used and the interactions that exist between micro-organisms (Kerster et al., 1997). Furthermore, most sampling techniques applied in industrial and environmental microbiology result in microbial stress. The micro-organisms may subsequently become dehydrated and damaged, which result in their non-culturability (Macnaughton et al., 1997). In order to overcome the difficulties of cultivation, alternative assays have been developed. These assays include the application of molecular techniques, the evaluation of substrate utilisation profiles (Biolog) and the evaluation of signature lipid biomarkers (SLBs) (White and Macnaughton, 1997).

A major disadvantage of the application of substrate utilisation profiles and molecular techniques is the non-quantitative recovery of microorganisms from the environment. White and Macnaughton (1997) stressed that although several methods for DNA extraction have been developed, there is no guarantee that all the DNA is extracted. Most of the molecular techniques are also very time-consuming and complicated to perform. The Biolog assay is a relatively simple and rapid technique compared to other community level approaches such as DNA analysis, but does not provide as much information as SLB analysis (Buyer and Drinkwater, 1997).

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The analysis of SLBs provide a method that is quantitative, independent of cell culturability and allows the identification of micro-organisms that have distinctive phospholipid fatty acid (PLFA) profiles (Macnaughton et al., 1997). Petersen and Klug (1994) proposed that phospholipids could be a fingerprint of the microbial community and could, therefore, provide a means to determine overall changes in the composition of the microbial community (Frostegard et al., 1997). It has also been shown that specific patterns of PLFAs are indicative of physiological stress, nutritional status as well as the viable biomass of the microbial population, none of which are reflected by any of the alternative techniques (Mandelbaum et al., 1997; Steward et al., 1996).

Valuable information can, therefore, be obtained using the SLB technique, which could assist in the development of successful microbial control programmes in paper mills. Microbial control is very important in the paper industry, since biofilm formation can lead to breakages, spotting, holes and discolouration of the paper, resulting in a loss of production and product quality (Robertson, 1993). Signature lipid biomarker analysis was, therefore, used to study the microbial populations in the water system of a paper-mill.

The Sappi Cape Kraft paper-mill, Cape Town, South Africa produces both fluting and linerboard from recycled fibre. Fluting is produced from pulp at pH 6.5 to pH 7.5 while linerboard is produced at pH 4.5 to pH 5.5. Fluting is produced without additives, while additives are included during the production of linerboard to improve the printing quality and to make storage of boxes under conditions of high humidity possible. A large percentage of the water at the mill is reused and the mill shuts down monthly for routine maintenance operations. The influence of these operations on microbial populations was evaluated using SLB profiles. To our knowledge no similar study has been conducted previously in paper mill water systems.