Comparison of the direct enzyme assay method with the membrane filtration technique in the quantification and monitoring of microbial indicator organisms – seasonal variations in the activities of coliforms and *E. coli*, temperature and pH

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Abstract

The aim of this project was to monitor variations and relationships between coliform and *E. coli* counts, the activities of their marker enzymes GAL and GUD, and temperature and pH over a period of 12 months in river samples obtained from the Eastern Cape, South Africa. Several polluted water samples were collected for direct coliform β-D-galactosidase (B-GAL) and *Escherichia coli* β-D-glucuronidase (B-GUD) assays and the membrane filtration technique. While all the samples showed enzyme activities, not all exhibited growth on CM1046 media. Variation in B-GAL activity (40%) was observed between November (highest activity month) and May (lowest activity month). The highest and lowest B-GUD activities were observed in the months of September and May/June, respectively. The sensitivity of the spectrophotometric assay method was indicated by a limit of detection (LOD) of 1 coliform forming unit (CFU)/100 mℓ and 2 CFU/100 mℓ for coliforms and *E. coli*, respectively. There was a significant ($P < 0.05$) positive correlation between E. coli counts and GUD activity ($R^2 = 0.8909$). A correlation of $R^2 = 0.9151$ was also observed between total coliforms and B-GAL activity, even though the CFUs were not evenly distributed. Direct enzyme assays were also shown to be more sensitive than the membrane filtration (MF) technique.

Keywords: chlorophenol red β-D-galactopyranoside (CPRG), chlorophenol red (CPR), coliforms, *E. coli*, *p*-nitrophenyl-β-D-glucuronide (PNPG), *p*-nitrophenyl (PNP)

Introduction

Coliform bacteria have the ability to produce the enzyme β-D-galactosidase (B-GAL) while *Escherichia coli* produce β-D-glucuronidase (B-GUD). These two enzymes have been used as selective markers for coliforms and *E. coli* respectively in the water quality industry for over 100 years. The enzymes hydrolyse a wide range of chromogenic and fluorogenic substrates yielding products which can be monitored spectrophotometrically or fluorometrically.

The traditional techniques of detecting and enumerating these micro-organisms require up to 72 h to perform. This required time is too long to provide useful information about a decrease in water quality that may require immediate attention to protect the public (Berg and Fiksdal, 1988). One other major limitation with the traditional methods is the inability to detect viable but non-culturable bacterial cells (VBNC). As a result, direct enzyme assays, which are faster and not limited by VBNC cells, have become popular amongst water quality scientists.

Each enzyme has a characteristic optimum pH and temperature requirement. Similar enzymes, but from different environments, can have different pH and temperature optima. pH plays an important role in the activity of enzymes. It affects the structural stability and solubility of, as well as the charge and charge distribution on both enzymes and substrates (Holme and Peck, 1998). These factors, in turn, alter bonds and bonding patterns, ultimately determining the rate of the enzyme-catalysed reaction.

Temperature also influences the rate of molecular collision and bond vibrations. An increase in temperature (usually up to 40°C) increases the number of collisions between substrates and enzymes, hence increasing the reaction rates. Increased bond vibrations due to an increase in temperature leads to bond disruptions that can facilitate easy and rapid substrate breakdown. However, this bond disruption will also cause denaturation of the enzymes themselves. As a result, temperature optimum will be a compromise between maximum activity and enzyme denaturation (Holme and Peck, 1998).

The use of the direct enzyme assay technique on environmental water samples thus presents a very complex situation as there may be compounds in the environment which may react to either increase or inhibit the activities of the enzymes. The effect of temperature and pH variations as a result of seasonal changes may also influence enzyme activities.

The two major objectives of this study were to compare the direct enzyme assay with the MF technique in quantifying microbial indicator organisms and to monitor variations in the activities of coliforms and *E. coli* throughout a 12-month cycle. Temperature and pH changes were also studied due to their effects on enzyme activity.

Materials and methods

Materials

Chlorophenol red β-D-galactopyranoside (CPRG), disodium hydrogen phosphate, dihydrogen sodium phosphate were all obtained from Merck (Darmstadt, Germany). The buffers were prepared using water purified with a Milli-Q system (Millipore, Milford, CT, USA). All reagents were of the highest analytical grade available.

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