

EXECUTIVE SUMMARY

Management of the microbiological quality of water requires a good understanding of the behaviour of microorganisms at an ecosystem level. The behaviour, which is linked to the microbial population structure and diversity, consequently influences the effectiveness of catchment level mitigation strategies that contribute to sustainable management of our surface water resources. Managing microbial loads at catchment levels could provide both financial and health benefits for downstream users.

Accordingly two distinct, but somewhat related, topics were focussed on during the tenure of this project: Firstly, a literature survey was conducted to summarize the current mitigation approaches and practices applicable at catchment level, and secondly the applicability of novel molecular approaches for the determination of true microbial diversity in surface waters was investigated.

The objectives were to:

- Provide an overview of current mitigation approaches and practices applied at catchment level;
- Assess the effectiveness of each mitigation method in the production of a better quality water based on published studies;
- Evaluate the applicability of new molecular approaches for the true diversity of the microbial communities in surface waters; and
- Determine future research needs and priorities.

Mitigation strategies for the management of microbial water quality in catchments

The literature on vegetated buffer strips and wetlands as mitigation measures and their effectiveness for reducing faecal bacteria loads at catchment level were perused. To gain insight into the behaviour of bacteria, other than faecal bacteria, two molecular approaches were used to determine the true diversity of bacterial communities of surface water as opposed to measuring only faecal indicators.

The performance of vegetated buffer strips and wetlands in terms of reducing contaminating organisms has been determined using faecal indicators. The efficiency of vegetated buffer strips is dependent on local rainfall intensity, vegetation types, soil types, length and slope of the strips. Shallow and slow flow enhances their efficiency. At high flow *E. coli* recovery varied between 16 and 62% while at low flow recovery was as low as <1% to 5%.

Wetlands are intermediary wet areas between water and land. They occur naturally but can also be constructed. They are characterised by wet soils and plants adapted to the wet conditions.

Inorganic chemicals are converted to organic chemicals by wetlands, which in turn provide nutrients for growth of the wetland vegetation. The effectiveness of wetlands is dependent on the rate of effluent flow and its quality that enters the wetland, die-off rate of organisms, rate of removal by filtration and sedimentation, rate of addition of transient or resident animal sources and the rate of predation. They have been shown to remove various bacteria and viruses at rates that range from 41% to 99%.

Pre-treatment processes, for example waste stabilisation ponds, soil infiltration areas and settling basins are well known methods for removal of solids, nutrients and pathogens, before discharging to a secondary technology. Water stabilisation ponds are similar to vegetation buffer strips and wetlands in respect of attenuation and retention of faecal bacteria. They effectively reduce pathogenic bacteria through ultra violet irradiation from the sun, high pH and predation in the ponds. Soil infiltration has also been shown as an effective method for improving contaminated water. Feedlot runoff treatment is often achieved by soil infiltration areas. The microbial quality of the water is improved by infiltration of the

contaminated water into the soil. An additional pretreatment process is settling of water in settling basins. The latter improves water quality by holding or storing, undisturbed water without mixing for long enough periods to allow particles to settle out with gravity.

Land management engineering is another option to prevent the transfer of microbial contamination to surface waters. Flow connection modelling using GIS and field observations provides information for flow directions due to contours land management features and subsequently allow for landscape engineering to disconnect and redirect flows away from sensitive areas and water bodies.

Applicability of new molecular approaches to determine microbial diversity

Limited information exists on the actual composition of the microbes in the water and how these could perhaps affect the efficiency of the mitigation methods.

New developments in the molecular field provide alternative sequencing and microarray technologies that enable a more holistic approach when determining the microbial quality of water resources. Assessment of the microbial quality of surface waters can now be based upon the bacterial community composition rather than the presence of indicator organisms alone. Accurate information on the microbial diversity of surface waters could help in assessing and modelling the potential health risks to water users in the catchment.

Two methods, pyrosequencing and phylochip analysis, were applied to a limited number of samples to gain information on possible mitigation procedures for wetlands through the management of the microbiological quality of surface waters.

Findings showed that both pyrosequencing and phylochip analysis reveal a much greater diversity of the bacterial population in the water samples when compared to Denaturing Gradient Gel Electrophoresis (DGGE) analysis. DGGE only revealed the most dominant phyla and is only useful for determining the most dominant members of a bacterial community. Pyrosequencing or phylochips were also able to reveal taxa that are present at low levels.

Primary phylochip data only provided an indication of presence/absence and not of abundance. However, using the fluorescence intensity data of the individual hybridizations, the phylochip data also provided an indication of abundance which could be correlated with the pyrosequencing results.

Although a detailed analysis of the bacterial species diversity and abundance could be obtained from these new molecular methods it was evident that species and genera representing waterborne pathogens were seldom detected and if detected, were present at very low levels. The impact and response of natural freshwater ecosystems to contamination with human wastes could thus be accurately analysed for bacterial species diversity and dynamics using these methods. The water-borne pathogens typically only represent a small fraction of the total bacterial community and are not easily detected when doing whole community diversity analyses. As a result health risk assessments cannot be performed using these methods.

For risk assessments, direct analysis of the water-borne pathogens or their indicators is preferable. Molecular approaches targeting water-borne pathogens directly such as q-PCR are at present still the methods of choice. This situation may change in future, but it does not seem that the current developments in sequencing technologies and microarrays will provide a viable alternative. This study has shown that pyrosequencing and phylochips can provide insight into the bacterial community composition in contaminated waters. However, even in highly contaminated water sources the indicator organisms and pathogens are present as a low percentage of the total bacterial population. As the techniques have difficulty in detecting

taxa present as a low percentage, the data generated by pyrosequencing and phylochips are not suitable for use in microbial risk assessment methods. For now the main application of these techniques will be to study the overall microbial diversity in aquatic ecosystems; providing pertinent information on the microbial composition and dynamics of these systems.