EXECUTIVE SUMMARY

Introduction

Cyanobacterial blooms are a commonly occurring phenomenon in water bodies due to increasing nutrient levels from industrial effluent and agricultural soil leachates. This increases the potential of blooms that produce toxic secondary metabolites, which are harmful to human and animal consumers. These toxins include neurotoxins as well as hepatotoxins such as microcystins. It is important to develop suitable laboratory tools to study biofilms of Cyanobacteria, so that a better understanding can be obtained of the environmental factors that affect their occurrence and functioning. In addition, microcystin standards for analytical use are extremely expensive, difficult to obtain and there is a growing demand for standards of the more common variants. This project was therefore undertaken to develop a membrane photo-bioreactor as a tool for the study of algal biofilms as well as a potential tool for production of toxins.

By immobilizing bacteria on membranes it was found to be possible to manipulate their environment such that a particular nutrient concentration can be achieved at extra-capillary membrane surfaces, allowing for a nutrient gradient that decreases from the interior to the exterior of the biofilm. With *Microcystis aeruginosa* PCC7806 secondary metabolite (microcystin LR and Asp-microcystin LR) production was obtained, which appeared to be modulated by temperature, nutrient ratios and concentrations and light intensities. Synthetic membrane modules would allow for fine-tuning of such ratio's and concentrations within the biofilm thus, theoretically, allowing analysis and fine control of toxin production in biofilms.

Methodology

The first stage of the project involved acquisition of relevant information of the factors that would affect toxin production, bacterial immobilization and methods of toxin extraction from the cyanobacterial biofilm.

The second stage of the project involved development of a suitable membrane module and a method to immobilize the bacteria. Externally skinless polyethersulfone capillary membranes were evaluated in this study. Adsorption, pressure and cell slurry immobilization techniques were tested for the establishment of a biofilm on the

membranes. Each module and immobilization technique had inherent advantages and disadvantages. The variability with bacterial adherence was also investigated.

The third stage of the project was to assess toxin production in the biofilm. Toxin production was measured as a function of time, biofilm thickness, nitrate concentration and light intensity. Toxin production was then established with a 4 X 2 factorial experiment using time, light intensity, nitrate concentration and biofilm thickness as primary factors. An immunolabeling technique was developed in order to establish where in the biofilm toxin production was greatest.

Results

The externally skinless polyethersulfone membranes showed good potential for biofilm establishment. Of the techniques tested for immobilization the best result was achieved with pressure filtration inoculation. The cell slurry adsorption technique also showed promise but had the disadvantage of contaminants being easily introduced. Adsorption was not a feasible method for rapid biofilm development.

Buoyant cells in a rapid state of division, having less developed cell walls, did not adhere well to the membranes. Non-buoyant cells with a well-developed extracellular polysaccharide layer adhered to one another and the membranes well.

Toxin production appeared to be rapid with the immobilized cells, but the yields were low. According to the experiments a high nitrate concentration in the lumen and a moderate light intensity were optimal for toxin production at 25°C. Toxin was produced optimally above the pore openings, where there was a higher light and nutrient concentration. The production of toxin occurred in veins, due to quicker diffusion of nutrients through channels formed during the immobilization procedure.

Conclusion

Although toxin production is rapid using this system, the biomass development and total toxin produced is low at this stage of development of the project. Further optimisation needs to be done once a better understanding of the physiology of the biofilm has been obtained. The cells also have to pass through their most productive stage of toxin production before they immobilize well. Toxin production occurred in clusters around the

pore openings, where the nitrate concentration and light intensity was higher. Veins of microcystin production occurred in the biofilm as channels formed during immobilization allowed for nutrients to diffuse to the outer biofilm more rapidly than the lateral movement (parallel to the lumen) of nutrients. Thus considerable new information was obtained on the physiology of microcystin production using a membrane bioreactor as a research tool. However, as a tool for production of microcystin as analytical standards, further optimisation would be required, particularly with regard to product extraction.