EXECUTIVE SUMMARY

BACKGROUND:

The need for nutrient removal from wastewater is due to the worldwide problem of eutrophication. Eutrophication occurs when water bodies receive large volumes of water which contain excessive quantities of nutrients such as nitrates and more specifically phosphates. This leads to the growth of aquatic photosynthetic plants, notably algae. The large population of algae depletes oxygen in the water and the algae release toxins into the water. The decay of the algae following die-off results in the lowering of the dissolved oxygen content of the water and consequent death of other aquatic organisms. To prevent eutrophication, phosphate removal from effluents is necessary whether it is by chemical and/or biological means.

Until now it has not been possible to isolate a pure culture of bacteria that could be responsible for biological phosphorus removal. Due to the problems deriving from culture-dependent methods other methods were considered to determine the difference between P removing and non-P removing activated sludge systems. Therefore, there is a need for techniques that do not necessarily identify individual species, but that can differentiate bacterial communities in terms of their constituents. These methods alleviated the need for culturing and samples were analyzed in a more direct manner which prevent the selection of specific organisms. There is a need for better understanding the microbial community structure and function, in order to manage wastewater treatment systems to control bulking or to improve biological phosphate removal capacity.

Analysis of total proteins extracted from an environmental sample can be employed as a "fingerprint" to type the diversity in the sample, in a way similar to grouping of bacteria according to enzyme polymorphisms and immunological reactions. Such fingerprints may eventually be used to monitor the deterioration or enrichment of species diversity in microbial communities.

OBJECTIVES:

The objectives of this study were therefore to use the whole cell protein extraction and analysis of the proteins with the SDS-PAGE technique to study the microbial community structure in activated sludge in order to determine the differences between different activated sludge systems. This study will enable us to have a better understanding of activated sludge systems. It will also help to identify factors responsible for non performance, by comparing management systems, system parameters, chemical analysis and biomass with our bacterial community analysis findings.

The objectives were:

1. Monitoring a specific activated sludge plant over time (e.g. every week) using PAGE analysis of total cell protein extracts.
2. The comparison of different zones within the same system using PAGE analysis of total protein extracts.
3. Comparing different systems according to their design and phosphorus removal using PAGE analysis of total cell protein extracts.
4. The comparison of systems which remove phosphorus to those which do not remove phosphorus using PAGE analysis of total cell protein extracts.
5. Comparing the same system when removing phosphorus and when it's not removing phosphorus, using PAGE analysis.

SUMMARY OF RESULTS:

Whole cell protein extractions and SDS-PAGE

The results obtained with SDS-PAGE indicated that there were no differences amongst the bacterial community structures of the different activated sludge zones (anaerobic, anoxic and aerobic). The bacterial communities of phosphorus-, and nitrogen-removing laboratory scale systems indicated no difference amongst their protein profiles according to SDS-PAGE. System design and the type of waste treated by the plant did not result in altered protein patterns. This indicated that the microbial community structure of activated sludge plants were

closely related. Seasonal changes did not have an affect on the protein profiles of the bacterial community of the Daspoort activated sludge plant. The protein profiles of a phosphorus-removing and non-phosphorus-removing system indicated a high percentage of correlation indicating little variation in their bacterial communities.

Activated sludge systems were not dominated by one or a few specific bacterial species but consisted of a combination of different bacterial species which co-exist and function together in a complex community according to SDS-PAGE.

SDS-PAGE was a sensitive method to determine the similarities and differences between the protein profiles of the bacterial community structure of activated sludge samples. Resulting protein profiles, after SDS-PAGE were normalized and analyzed with the Gelcompar 4.0 programme. This programme calculated the % similarities and differences between each protein profile, with the Pearsons product moment correlation coefficient (r) between samples to construct a matrix. The samples were then clustered using the unweighted pair group method of arithmetic average (UPGMA) which resulted in a dendrogram.

Researchers tend to construct dendrograms consisting of only a few samples and then base the identification of a new genus or species on their findings. When samples are added to smaller dendrograms the dendrogram is more likely to vary. However, the larger the dendrogram, the more value can be attached to the results. When new samples are added, the groups will stay the same and only a small variation in the % correlation might appear. Each dendrogram must be evaluated on its own and not be compared with other dendrograms. These are the main reasons why no definite value of > 80 % for the same species and > 60 % for the same genus can be attached to a dendrogram. Percentage correlation between the samples must only be an indication of similarity.

One disadvantage of the SDS-PAGE method is that it needs to be standardized. Results between different laboratories may differ if standard methods are not followed. An exact value can not be attached to the % similarity or correlation of the resulting dendrogram after SDS-

PAGE. The % similarity can rather be used as an guideline. SDS-PAGE can therefore, not discriminate between the bacterial populations of the different activated sludge samples, it can only indicate samples with a high % similarity or not.

Immunochemical investigation

Antigen preparation from the anoxic and aerobic zones contained intact and lysed bacterial cells. Besides conventional immunization, subtractive immunization* using cyclophosphoamide was also used to focus the immune response on unique epitopes in the zones. *{Subtractive immunization, is a term referring to the homology to the process of "subtractive hybridization". To obtain messenger RNA's of an abundance, is used here to describe how antigen of low immunogenicity and absorbance can be made immunologically prominent by first immunizing with a crude immunogen containing the abundance of irrelevant antigens, followed by chemical paralysis of this immune response by cyclo-phosphamide treatment, and finally immunization of these animals with the complex antigen which then also contains the sought for antigenic determinant (Matthew & Patterson, 1983). This results in an immune response where the minor antigen acts prominently to elicit specific antibodies}. Neither strategy provided antibodies capable of distinguishing phenotypic diversity between the two zones, emphasizing the homogeneity of the microbial populations in the different zones of phosphate removing activated sludge systems. Nine stable hybridoma lines were established, all secreting IgM cross reactive to both antigen preparations but differing in the antigen specificity. Monoclonal antibody 7B9, putatively protein-directed, could clearly distinguish between the aerobic zones of the two activated sludge systems differing only in phosphate removal ability: immunoblot showed five discrete bands in the system successful at phosphate removal. The molecular weights of the bands appeared to be multiples of 18 kDa, indicating possible involvement of an 18 kDa proteinaceous monomer in phosphate uptake. A significant finding emerging from this study was based on the observation that antigenic differences were clearly detected between the aerobic zones of two activated sludge systems with differing phosphate removal ability. A monoclonal antibody was found that was capable of distinguishing between the two systems both by ELISA and immunoblot. Characterization of the antigen recognised suggested a protein nature.

Conclusions

* The main conclusion from SDS-PAGE is that the protein profiles do not differ significantly in any of the systems, suggesting that the bacterial community remains constant.

* Monoclonal antibodies can be used as diagnostic tools in phosphorus removing and non-removing activated sludge plants.

RECOMMENDATIONS FOR FURTHER RESEARCH:

A thorough knowledge of the bacterial populations responsible for a functioning activated sludge process can only originate from the combination of different approaches. Therefore, there is a need for techniques that do not necessarily identify individual species, but that can differentiate bacterial communities in terms of their constituents. These methods alleviated the need for culturing and samples were analyzed in a more direct manner which prevents the selection for specific organisms. These methods include SDS-PAGE, molecular techniques and monoclonal and polyclonal antibodies. Population shifts could serve as early indicators of malfunctions (e.g. filamentous bacteria as indicators for sludge bulking) so that corrective actions could be taken in time. Keeping in mind the biases caused by cultivation, future studies should rely on in situ identification of individual cells with immuno- or nucleic acid probes. There is a need to better understand community structure and function, in order to manage wastewater treatment systems to control bulking or to improve biological phosphate removal capacity.

Previous studies also indicated that biomass was related to phosphorus removal. The higher the "biomass" the better the P-removal. This suggested that the main difference between P-removing and non P-removing systems is biomass related and not due to the microbial community structure. The aims for future studies are therefore: i) To determine the relationship between biomass and P removing and non-P removing systems ii) To determine the P removal capacity of a system based on biomass iii) To determine the effect of

bioaugmentation on phosphorus removal in a conventional activated sludge system by adding biosupplements and/or anaerobic sludge in order to increase the biomass.