Direct extractions of proteins to monitor an activated sludge system on a weekly basis for 34 weeks using SDS-page

Marthie M Ehlers* and TE Cloete
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0001, South Africa

Abstract

The diversity and dynamics of microbial communities of phosphorus-removing and non-phosphorus-removing activated sludge systems have mostly been analysed by culture-dependent methods. A more direct method is the isolation of the total protein content of samples of activated sludge systems and separating the proteins with SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Total proteins were analysed and used as fingerprints to type and compare the diversity of the bacterial community. The objectives of this study were to determine if there were any differences between the anaerobic, anoxic and aerobic zones of an activated sludge plant as well as the effect of seasonal changes on the bacterial community structure of an activated sludge plant over a 34-week period. The protein profiles, over this study period, indicated a relatively high (> 63%) similarity between the samples. The results indicated no specific protein pattern in the different zones or due to seasonal changes. This implicated that a stable bacterial community was present throughout the study period.

Abbreviations

BDM	2-β-mercaptoethanol
COD	Chemical oxygen demand
EBPR	Enhanced biological phosphate removal
LMG	Laboratorium voor Microbiologie Ghent Culture Collection, State University Ghent, Belgium
N	Nitrogen
P	Phosphorus
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
STB	Sample treatment buffer
UPGMA	Unweighted pair group method of arithmetic averages

Introduction

In terms of wastewater treatment, the activated sludge process is probably today's most important biotechnological process (Wagner et al., 1993). Nutrients such N and P can be removed from wastewater under specific conditions. The present design for P removal, namely EBPR, requires wastewater to pass through an initial anaerobic treatment process and thereafter an aerobic stage, during which P removal takes place (Bond et al., 1995).

The need for nutrient removal from effluents 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 (Slim, 1987; Toerien et al., 1990). This leads to the growth of aquatic photosynthetic plants, notably algae. To prevent eutrophication, phosphate removal from effluents is necessary, whether it is by chemical and/or biological means (Toerien et al., 1990). Substantial savings are also achieved through biological rather than chemical P removal (Toerien et al., 1990).

* To whom all correspondence should be addressed.
☎ (012) 420-2995; fax (012) 420 -3266; e-mail mehlers@nsnper1.up.ac.za
Received 9 April1998; accepted in revised form 3 July 1998.

Although a considerable amount of work has been done on system design and process engineering, the knowledge and understanding of the microbial community structure-function and consequently the microbiology behind the activated sludge process is still very limited. Diversity and dynamics of the microbial consortia in activated sludge have mostly been analysed by culture-dependent methods (Wagner et al., 1993). The literature indicates that there is a large discrepancy between the total direct microscopic counts and viable plate counts (usually less than 1% of the former) for many ecosystems (Cloete and Steyn, 1987, Wagner et al., 1993). EBPR from activated sludge has been well documented, but attempts to elucidate the exact mechanism have not been successful as a result of these inadequate microbiological techniques (Srinath et al., 1959; Shapiro, 1967; Shapiro et al., 1967).

The need excists to better understand the EBPR process, since it is not optimised and routinely fails (Bond et al., 1995). To achieve this, a more complete knowledge of microbial phosphate metabolism in activated sludge is required. Since conventional techniques offer limited possibilities, an alternative method was investigated in this study. Protein electrophoresis is a sensitive technique, yielding valuable information on the similarity or dissimilarity amongst bacterial cultures during taxonomical studies. Until now no direct method has been developed to analyse the protein products in gene expressions of environmental samples (Ogunseitan, 1993). This method could therefore, possibly also be used to determine the similarity or dissimilarity between different environmental samples containing micro-organisms. SDS-PAGE of whole-cell soluble proteins, prepared under standard conditions, produced a complex banding pattern (termed a protein electrophore gram or electrophoretic protein pattern), which is reproducible and can be considered as a "fingerprint" of the sample investigated (Kersters, 1990). The resulting protein profiles after SDS-PAGE could possibly lead to the better understanding of the diversity and dynamics of P- and non-P-removing microbial communities present in activated sludge systems, since these profiles would indicate similarity or dissimilarity in samples obtained from this system.

The total bacterial protein content of activated sludge samples was therefore, used as a fingerprint to give insight into the