Protein profiles of phosphorus- and nitrate-removing activated sludge systems

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Abstract

Samples from laboratory-scale activated sludge systems operating under specific conditions which differ in phosphate-removing capabilities were obtained from the University of Cape Town. The total protein content of samples of these systems was used and the proteins separated with SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Analysis of the total proteins was employed as a fingerprint to type and compare the diversity of the bacterial communities of P-and N-removing systems. Samples of six activated sludge systems (three N- and P-removing and three N-removing) were used in this study. Protein profiles indicated a high (>70%) correlation for all the systems. No difference was observed in the protein profiles of the bacterial communities of N- and P-removing or N-removing systems. We can therefore conclude that the same bacterial communities were present in P- and N-removing systems.

Abbreviations

BPR	biological phosphorus removal
EBPR	enhanced biological phosphate removal
LMG	Laboratorium voor Microbiologie Gent Culture Collection, State UniversityGent, Belgium
N	nitrogen
P	phosphorus
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
UCT	University of Cape Town
UPGMA	unweighted pair group method of arithmetic averages

Introduction

Biological phosphorus removal in activated sludge is of interest due to low sludge production and the fertiliser value of the sludge (Henze, 1996). Substantial savings are also achieved through biological, rather than chemical P-removal (Toerien et al., 1990).

Biological phosphorus removal can be performed by a rather broad group of micro-organisms referred to as the phosphate-accumulating organisms (PAOs or polyP-organisms) (Henze, 1996). The micro-organisms responsible for biological phosphorus removal are still partly unknown with respect to taxonomy, but we know that they are present in all biological wastewater treatment processes, although not necessarily showing any biological P-removal activity, until being activated (Henze, 1996). Optimisation of enhanced P-removal processes therefore depends on a complete understanding of the ecophysiology of poly-P organisms.

The mechanism of enhanced P-removal in activated sludge systems must therefore depend on a group of organisms (the poly-P organisms) which in nature are favoured by fluctuating conditions of aerobiosis-anaerobiosis. Their selective advantages require: their presence; alternating aerobiosis-anaerobiosis; degradable organic matter which can be fermented by acidogenic bacteria; and the presence of a sufficient quantity of P to allow uptake of fatty acids during anaerobiosis and P-uptake during aerobiosis (Toerien et al., 1990). However, the precise role of the consortia of micro-organisms of enhanced P-removal in activated sludge systems has not yet been clarified.

The bacterial genome contains information involved in the production of some 2 000 proteins, which function either enzymatically or structurally (Kersters, 1990; Priest and Austin, 1993). Electrophoresis of the total cellular proteins in polyacrylamide gels (PAGE) provides a partial separation in which individual bands mostly represent several proteins (Kersters, 1990). This complex pattern represents a "fingerprint" of a specific strain (Priest and Austin, 1993; Van Damme et al., 1996). Previously this technique has been used for comparative taxonomical studies. More recently sodium dodecyl sulphate (SDS) PAGE has found application for use in comparative studies of environmental samples such as activated sludge (Ogunseitan, 1993).

Expression of genes in micro-organisms correlates with a variety of environmental stimuli, ranging from the presence of particular nutrients to changes in the physical-chemical conditions (Ogunseitan, 1993). Until now no direct method was available to analyse the protein products of gene expressions of environmental samples (Ogunseitan, 1993).

Protein electrophoresis is a sensitive technique, yielding valuable information on the similarity or dissimilarity amongst bacterial cultures. 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 standardised conditions, produces a complex banding pattern (called 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 the microbial communities of P-removing and non-P-removing activated sludge systems, since this would indicate similarity or dissimilarity in those samples. This would indicate whether a difference existed in the microbial community structure between P-removing, non-P-removing and N-removing systems.

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Received 17 August 1998; accepted in revised form 3 December 1998.