Microbial community profile of a biological excess phosphorus removal (BEPR) activated sludge system using a cultivation-independent approach

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Abstract

It is generally accepted that biological release of phosphorus in the anaerobic zone of a nutrient removal system and phosphorus accumulation in the subsequent aerobic zone is directly proportional to the quantity of volatile fatty acid or the readily biodegradable COD fraction (f_{bs}) entering the system. This will enrich for polyphosphate accumulating organisms (PAOs) in the system and an increase in biological phosphorus removal will be observed. Enrichment for PAOs during the present study was essentially achieved by increasing both the phosphorus and f_{bs} concentrations (maintaining constant total COD loads) in the influent to the system. Fluorescence *in situ* hybridisation (FISH) using kingdom-, subdivision- and genus-level probes was used to identify and enumerate the bacterial community implicated in biological excess phosphorus removal (BEPR). Hybridisation of up to 78% of the cells (in relation to DAPI staining) with probe EUB338 indicated that a high proportion of the sludge comprised metabolically active bacteria. Bacterial predominance in the BEPR sludge appeared, in descending order, as such: β Proteobacteria (22%); α Proteobacteria (19%); γ Proteobacteria (17%); and, Actinobacteria (11%). Incidence of *Acinetobacter* spp. appeared to be relatively low with counts amounting to < 9% of the total bacterial count. The results indicate that the β and α Proteobacteria are metabolically functional (either directly or synergistically) in BEPR processes and reiterate the functional misconception of *Acinetobacter* spp. in these same systems.

Nomenclature

AE1/2	aerobic reactor/s 1 and/or 2
AN	anaerobic reactor
API	Analytical Profile Index
AX	anoxic reactor
BEPR	biological excess phosphorus removal
BNR	biological nutrient removal
CGYA	Casitone Glycerol Yeast Autolysate Agar
COD	chemical oxygen demand
DAPI	4',6'-diamidino-2-phenylindole
DGGE	denaturing gradient gel electrophoresis
DO	dissolved oxygen
DSVI	dilute sludge volume index
EDTA	ethylenediaminetetra-acetic acid
FISH	fluorescence *in situ* hybridisation
FSA	free and saline ammonia
f_{bs}	biodegradable, soluble COD fraction
HAc	acetic acid
MLSS	mixed liquor suspended solids
NaAc	sodium acetate
OUR	oxygen utilisation rate
PAO	polyphosphate accumulating organism
PBS	phosphate buffered saline
Q_i	influent flow rate
R_s	sludge age
RBCOD	readily biodegradable COD (synonymous with f_{bs})
SRP	soluble reactive phosphorus
S_{bsi}	influent biodegradable, soluble COD
S_{ti}	influent total COD
TKN	total Kjeldahl nitrogen
TP	total phosphorus
VSS	volatile suspended solids
WWTP	wastewater treatment plant
WWW	wastewater works

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Received 29 July 1999; accepted in revised form 19 May 2000.

Introduction

Although BEPR plant operations have been successfully and widely applied, they still experience irregularities with regards to biological phosphorus removal. Mathematical descriptions of the BEPR mechanism have resulted in the construction of a number of simulation models (Dold et al., 1991; IAWQ, 1995). However, these models have usually been constructed via observations under controlled, laboratory conditions. Variability in real operational conditions (influent flow, load and composition) indicates the difficulty in directly applying these models when developing new or upgrading existing WWTP to incorporate BEPR. The biological mechanism, therefore, has to often be supplemented with chemical dosage (with its negative impacts) to ensure the WWTP concerned complies with regulatory P concentrations in their effluents. Advances in process and sanitary engineering during the past three decades have ensured that advanced wastewater treatment processes such as BEPR and nitrogen removal could indeed be constructed. Yet it remains disconcerting to note that a technologically advanced biological process such as BEPR still lacks fundamental understanding from a biochemical and microbiological point of view (Mino et al., 1998a).

Molecular based analysis has emerged as a powerful tool to facilitate a better understanding of the structure and function of the microbial population comprising various ecosystems (Stahl et al., 1988; Gersdorf et al., 1993; Lim et al., 1993; Raskin et al., 1994).