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A review and update of the microbiology of enhanced biological phosphorus removal in wastewater treatment plants

Linda L. Blackall[1,*], Gregory R. Crocetti[1], Aaron M. Saunders[1] & Philip L. Bond[2]
[1]*Advanced Wastewater Management Centre, The University of Queensland, St. Lucia, 4072, Queensland, Australia;* [2]*School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK (*Author for correspondence; Tel.: +61-7-3365-4645; Fax: +61-7-3365-4620; E-mail: blackall@biosci.uq.edu.au)*

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Abstract

Enhanced biological phosphorus removal (EBPR) from wastewater can be more-or-less practically achieved but the microbiological and biochemical components are not completely understood. EBPR involves cycling microbial biomass and influent wastewater through anaerobic and aerobic zones to achieve a selection of microorganisms with high capacity to accumulate polyphosphate intracellularly in the aerobic period. Biochemical or metabolic modelling of the process has been used to explain the types of carbon and phosphorus transformations in sludge biomass. There are essentially two broad-groupings of microorganisms involved in EBPR. They are polyphosphate accumulating organisms (PAOs) and their supposed carbon-competitors called glycogen accumulating organisms (GAOs). The morphological appearance of microorganisms in EBPR sludges has attracted attention. For example, GAOs as tetrad-arranged cocci and clusters of coccobacillus-shaped PAOs have been much commented upon and the use of simple cellular staining methods has contributed to EBPR knowledge. *Acinetobacter* and other bacteria were regularly isolated in pure culture from EBPR sludges and were initially thought to be PAOs. However, when contemporary molecular microbial ecology methods in concert with detailed process performance data and simple intracellular polymer staining methods were used, a *betaproteobacteria* called '*Candidatus* Accumulibacter phosphatis' was confirmed as a PAO and organisms from a novel *gammaproteobacteria* lineage were GAOs. To preclude making the mistakes of previous researchers, it is recommended that the sludge 'biography' be well understood – i.e. details of phenotype (process performance and biochemistry) and microbial community structure should be linked.

Abbreviations: EBPR – enhanced biological phosphorus removal; GAO – glycogen accumulating organism; PAO – polyphosphate accumulating organism

Phosphorus is a problem

Effluent from wastewater treatment plants is a major contributor of phosphorus (P) to receiving waters with elevated levels of P leading to environmentally detrimental eutrophication. Recognition of this has lead to the initiation of extensive projects, for example, in the Baltic Sea and the Great Lakes of North America to reduce the P loading on these receiving waters (Harremoës 1994; Vallentyne 1994). In Australia, P is a limiting nutrient which stimulates toxic cyanobacterial (blue-green algal) outbreaks in the Murray Darling and other river systems and it is recommended in the management of such inland waters that nutrient loadings from sewage treatment plants be carefully considered for each site (Cullen 1994).

The fact that excess P causes environmental damage demands that something be done to prevent or slow the introduction of this nutrient to the environment. This is therefore the justification for the development of technologies for removing P from sewage effluents.

In municipal activated sludge wastewater treatment systems the two main approaches utilised for P removal are biological processes, known as enhanced biological phosphorus removal (EBPR), and chem-

ical removal processes. This paper focuses on EBPR where microorganisms accumulate unusually large amounts of P in the form of intracellular polyphosphate (polyP). The accumulated P is then removed from the system by wasting P-rich sludge. Sludge wastage is a conventional component of wastewater treatment, necessary to remove the microbial biomass which has grown on the sewage influent nutrients. In the chemical process, the phosphate is precipitated from the wastewater and this provides a simple and reliable method for P removal. While both processes can produce adequately low levels of P in treated wastewater, advantages of EBPR include:

- significantly lower operating costs,
- reduced sludge production,
- obviation of effluent salinity problems experienced with the chemical process, and easier management and significantly higher reuse potential of produced sludges.

Therefore, in the design of new and upgraded treatment plants, EBPR is clearly the method of choice. However, most municipal sewage treatment plants are activated sludge systems with little or no capacity for P removal, but construction of new activated sludge plants, or retrofitting of existing ones, for biological P removal is happening at a rapid pace.

Typically, domestic sewage treatment plants have effluent discharge licences dictating their legal discharge of P to receiving waters. Influent total-P concentrations can fluctuate dramatically but many domestic wastewaters are typically in the range 10–15 mg/l. Cellular growth of activated sludge microorganisms in the treatment process usually removes 1–2 mg/l of influent P, thus leaving more than 10 mg/l in the effluent if no specific phosphorus removal measures are implemented. EBPR processes can achieve effluent total-P levels as low as 0.1–0.2 mg/l. However, problems with the stability of biological P removal have been highlighted (Hartley & Sickerdick 1994), but these problems are generally not well publicised. Among some in the wastewater industry, the notion is that P removal works very well most of the time (e.g. van Loosdrecht et al. 1997a). The fact that most EBPR plants have chemical removal capacity as a backup, indicates problems with the EBPR performance. There are clear gaps in P removal efficiency between what is achievable and what is typically achieved with EBPR. A deeper understanding of the process is required to bridge these gaps which include:

- improved performance and reliability of P removal from wastewater;
- better prediction and management of P removal failures; and
- refined design of EBPR plants leading to savings in construction and operating costs.

Aspects of the biology of EBPR have been reviewed in the past (Ramadori 1987; Jenkins & Tandoi 1991; Kortstee et al. 1994; van Loosdrecht et al. 1997a, b; Mino et al. 1998; Bond & Rees 1999) but this review partially summarises material of these earlier ones and then brings the reader up to date since the last review.

The EBPR process in activated sludge

The conventional activated sludge plant was designed for the removal of carbon (Ardern & Lockett 1914). The influent wastewater mixes with return activated sludge and the carbon is oxidised in an aerobic stage called the aeration tank. The sludge is then separated from the treated wastewater by gravity clarification and returned to mix with influent in the aeration tank. In conventional full-scale activated sludge plants, a small amount of P removal is achieved due to growth of microorganisms. The P content typically only reaches 1.5–2% of the sludge dry weight, which is the P requirement for normal bacterial growth (Schlegel 1993). To achieve EBPR, an activated sludge plant requires the influent wastewater to mix with returned sludge (biomass) which then passes through an anaerobic stage followed by an aerobic stage (Figure 1). The sludge P content in full-scale EBPR processes can reach levels of 4–5% of the sludge dry weight, while some lab-scale EBPR enrichment processes have reported up to 15% P (Crocetti et al. 2000). The anaerobic zone is thought to provide the selective pressure for particular bacteria that accumulate large amounts of phosphorus as polyP (Osborn & Nicholls 1978). These bacteria are known as polyphosphate accumulating organisms (PAOs) and are responsible for the high P content of EBPR sludge.

In the anaerobic and aerobic stages of EBPR certain biochemical transformations have been observed to occur. In the anaerobic stage significant release of phosphate is observed, while in the following aerobic stage an even greater amount of phosphate is taken up by the organisms thereby also removing the phosphate in the incoming wastewater. The removal of P enriched sludge after or during the aerobic stage (waste sludge) results in the removal of P from the wastewater (Figure 1). EBPR processes were first employed in 1974 (Barnard 1974) and since then, extensive empir-

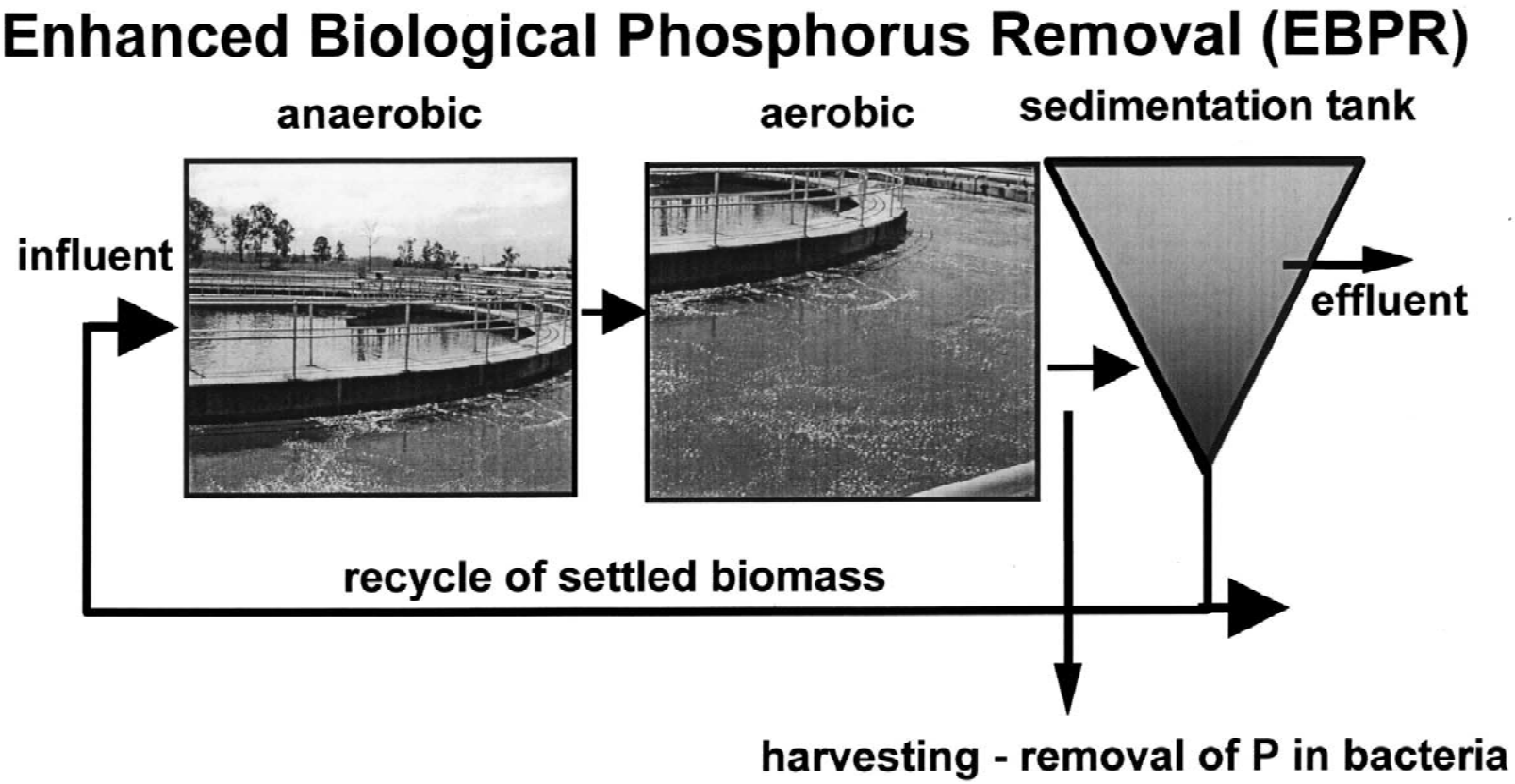

Figure 1. Diagram of the layout of an enhanced biological phosphorus removal process showing the anaerobic and aerobic cycling of the sludge and its recycle to be mixed with incoming wastewater.

ical knowledge has developed by correlating chemical measurements with process performance.

The P-removal and the non-P-removal phenotypes

The transformations relevant to EBPR are diagramatically depicted in Figure 2. In P-removal, P and carbon (C) transformations occur in both the anaerobic and the aerobic zones and currently, all EBPR transformations (to be summarised here) are presumed to occur in the same organisms – the PAOs. Return activated sludge is mixed with influent wastewater under anaerobic conditions. PAOs present in the return activated sludge contain high amounts of stored polyP, which is degraded in the anaerobic conditions to produce energy, and orthophosphate is released to the mixed liquor. Glycogen stored in the returned PAOs is metabolised by glycolysis to produce ATP and reducing equivalents (NADH). C, typically in the form of volatile fatty acids (VFAs) is taken up by PAOs and converted to intracellular poly-β-hydroxy alkanoates (PHAs).

Aerobically, the P from the surrounding liquor is taken up by PAOs and stored as polyP, and intracellular stored PHA is used for growth and replenishment of glycogen reserves. Other microorganisms known as glycogen non-polyphosphate accumulating organisms (GAOs) (Mino et al. 1995) were discovered in the early 1990s (Cech & Hartman 1993) but their phenotype was known of since the mid 1980s (Fukase et al. 1985). GAO transformations of C are the same as those of PAOs, but GAOs do not release or accumulate P with anaerobic-aerobic cycling as the PAOs do. It is hypothesised that all the energy for GAOs in the anaerobic period comes from glycolysis of stored glycogen. The conundrum for wastewater operators is how to preclude GAOs and promote PAOs as the same external conditions (anaerobic-aerobic cycling of biomass with nutrients) select both types but GAOs are hypothesised to be detrimental to the P removal process.

Microbiology of EBPR. Polyphosphate accumulating organisms

The first microscopic observations of the organisms relevant to EBPR were of clusters of coccobacillus-shaped microorganisms containing polyphosphate according to chemical staining methods like the Neisser stain (Jenkins et al. 1993). Their abundance in EBPR reactors and their chemical staining profile lead to them being called PAOs. EBPR sample dilution and spread-plate inoculation to laboratory media lead to the ready isolation of coccobacillus-shaped *Acinetobacter* spp. which were concluded to be the PAOs (Fuhs & Chen 1975; Deinema et al. 1980, 1985; Buchan 1983; Lötter 1985; Wentzel et al. 1988; Beacham et al. 1992). However, their pure-culture phenotype has never matched that of EBPR and, over the years a series of carefully executed studies by different researchers has demonstrated that *Acinetobacter* spp. are not PAOs (Cloete & Steyn 1987; Hiraishi & Morishima 1990; Auling et al. 1991; Wagner et al. 1994; Bond et al. 1995; Kämpfer et al. 1996).

Pure cultures of bacteria other than *Acinetobacter* spp. have been obtained from EBPR reactors and the organisms associated with phosphorus removal. The list of cultures includes *Lampropedia* (Stante et al. 1997), *Microlunatus phosphovorus* (Nakamura et al. 1995), *Micropruina glycogenica* (Shintani et al. 2000) and *Tetrasphaera* spp. (Maszenan et al. 2000). The studies attempting to prove these organisms can carry out P removal according to the currently-accepted anaerobic-aerobic sludge cycling EBPR have been extensive (where *M. phosphovorus* was found to not be a PAO as reported by Santos et al. 1999) to non-existent (e.g. *Tetrasphaera* spp.).

In the search for the real PAOs, fluorescence *in situ* hybridisation (FISH) was used to investigate the microbial community in a full scale plant showing some degree of EBPR behaviour (Wagner et al. 1994). The bacterial population of the plant comprised 36% *Actinobacteria*, 36% *betaproteobacteria*, 10% *gammaproteobacteria*, but *Acinetobacter* accounted for only 3–6% of the total bacterial community. It was claimed *Actinobacteria* matched the morphology and arrangement of cells containing polyphosphate, however, no evidence was produced. Kämpfer et al. (1996) reported a similar microbial community structure for an EBPR sludge and found *Acinetobacter* represented between 0 and 5% of the bacterial cells.

Bacterial community structures of phosphate removing and non-phosphate removing sludges were compared by 16S rDNA clone library analysis (Bond et al. 1995). Two laboratory scale reactors were operated with different phosphate removing capabilities, and in both sludges, the predominant bacterial group represented in the clones was the *betaproteobacteria* at 28%. The *Rhodocyclus* group within the *betaproteobacteria*, was represented more in the reactor with

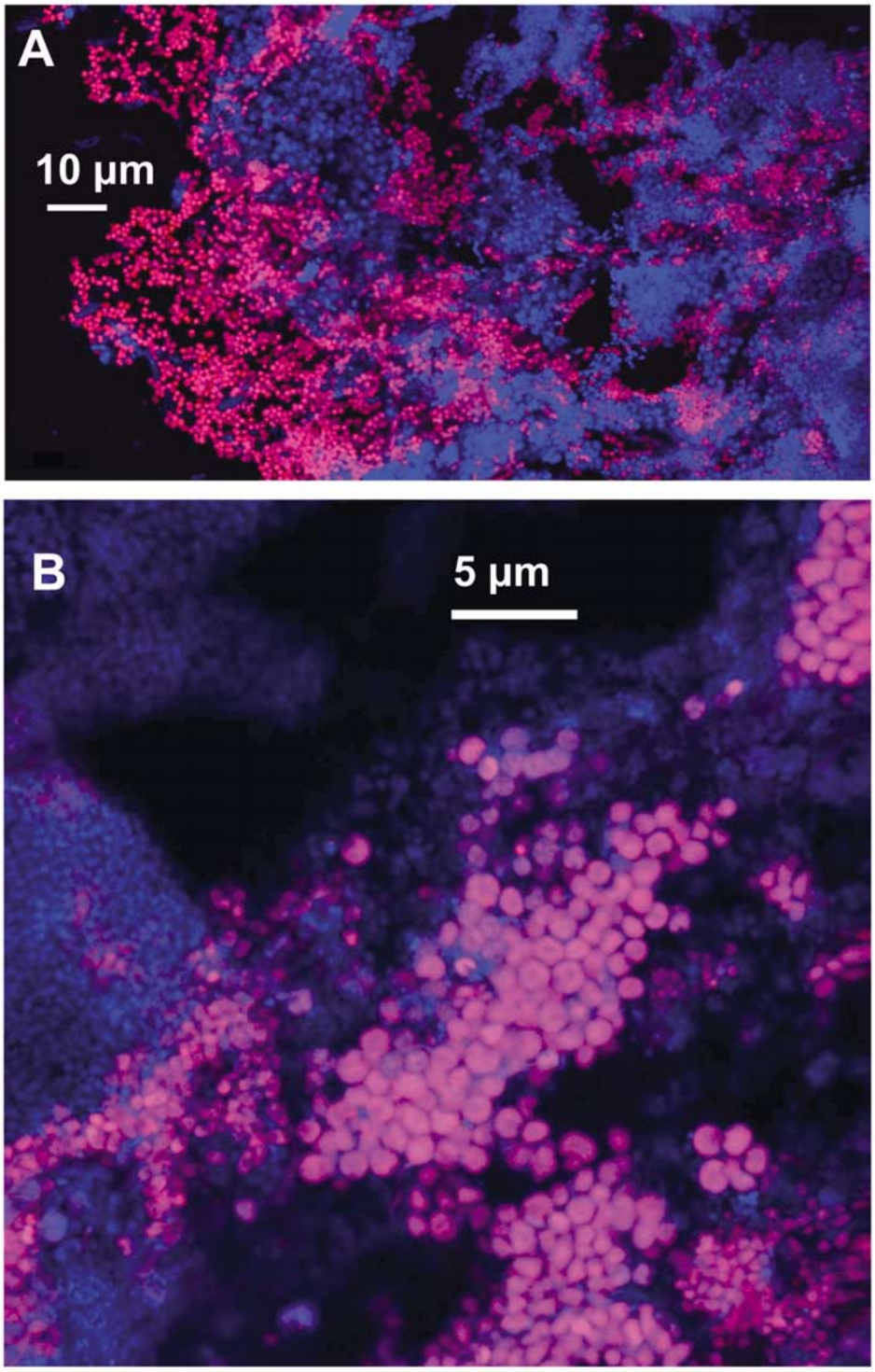

Figure 2. Confocal laser scanning micrographs of FISH images. (A) Biomass from a laboratory-scale sequencing batch reactor for efficient EBPR showing magenta cells of *Accumulibacter* due to binding PAO651–Cy3 and EUBMIX–Cy5. Other bacteria are blue due to binding EUBMIX–Cy5. (B) Biomass from a laboratory-scale sequencing batch reactor operating for EBPR but where phosphorus removal was severely reduced. Probes used were GAOQ431–Cy3 and EUBMIX–Cy5. Bacteria from the novel *gammaproteobacteria* cluster targeted with GAOQ431 are magenta and all other bacteria are blue. Sludges for both these images were kindly supplied by Raymond Zeng, Advanced Wastewater Management Centre, The University of Queensland.

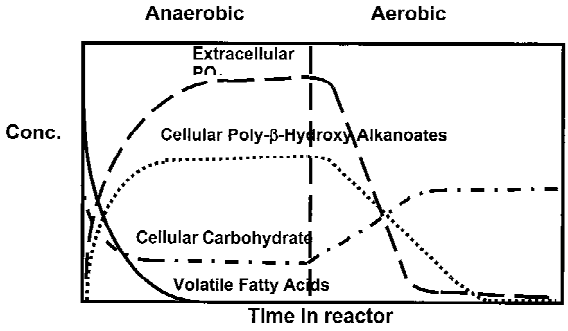

Figure 3. Diagram of the important phosphorus and carbon transformations involved in enhanced biological phosphorus removal.

greater phosphate removal. However, determining the microbial community structure from this method of analysis is imprudent, since a relatively low number of clones (189 in this study) clearly not representing the full species abundance of the sludge were examined and the method is not quantitative due to methodological biases.

Since 1993 (Wagner et al. 1993), *betaproteobacteria* were reported to be the most abundant bacteria in activated sludges of many configurations when FISH was used as the method of analysis (Wagner et al. 1994; Kämpfer et al. 1996; Snaidr et al. 1997; Sudiana et al. 1998; Liu et al. 2001). Bond et al. (1999a) used FISH to determine that within, the *betaproteobacteria*, the *betaproteobacteria*-2 subgroup comprised 55% of all bacteria in an efficiently operating laboratory-scale EBPR reactor. For the first time, more detail of the specific *betaproteobacteria* comprising the system was available (Bond et al. 1999a). PAO-enriched, high-performing EBPR cultures were generated in the laboratory and subjected to the full-cycle rRNA analysis as initially described by Olsen et al. (1986). Hesselmann et al. (1999) were the first to report the definitive phylogenetic placement of the *betaproteobacteria*-2 subgroup PAO as a close relative of *Rhodocyclus* spp. and called the organism '*Candidatus* Accumulibacter phosphatis' (henceforth called *Accumulibacter*). Crocetti et al. (2000) supported this finding and extended the knowledge by using FISH (Figure 3A) and post-FISH chemical staining to demonstrate that the *Accumulibacter* cells cycled polyP according to EBPR. A range of FISH probes for *Accumulibacter* is listed in Table 1.

Dabert et al. (2001a, b) studied the microbial ecology of efficient and deteriorated EBPR by the use of single strand conformational polymorphism (SSCP) and 16S rDNA clone library analysis. During good EBPR, SSCP showed an abundance of *Accumulibacter* in the microbial ecosystem but also the prominent appearance of other bacteria notably some *gammaproteobacteria* and organisms closely related to *Haliscomenobacter* in the *Bacteroidetes* phylum (Dabert et al. 2001b).

A recent conference of the International Water Association on 'Microorganisms in Activated Sludge and Biofilm Processes' heard from numerous presenters that *Accumulibacter* was a PAO in laboratory-scale (Kawaharasaki et al. 2001; Onuki et al. 2001) and full-scale EBPR (Lee et al. 2001; Zilles et al. 2001). These papers will be published in Water, Science & Technology during 2002.

Numerous reviews of EBPR microbiology suggest there is probably a diversity of organisms involved in polyP accumulation (Mino et al. 1998; Mino 2000) so *Accumulibacter* is presumed to be the first of many confirmed PAOs (Kawaharasaki et al. 2001; Lee et al. 2001; Zilles et al. 2001). Additional PAO candidates include *Actinobacteria* (Wagner et al. 1994; Christensson et al. 1998; Kawaharasaki et al. 1999; Gieseke et al. 2001; Liu et al. 2001), *alphaproteobacteria* (Kawaharasaki et al. 1999), and *gammaproteobacteria* (Liu et al. 2001).

Microbiology of EBPR. Non-polyphosphate glycogen accumulating organisms

In the early 1990s, tetrad-arranged cocci were observed in glucose fed reactors and they were called 'G bacteria' but these organisms can also grow quite well when acetate is the carbon source in anaerobic-aerobic reactors (Cech & Hartman 1993). 'G bacteria' were described as large oval cells (2–3 μm in diameter) forming compact aggregates, apparently attached together with extracellular slime (Cech & Hartman 1993). Sometimes, these tetrads contained lipophilic inclusions, possibly PHAs, according to chemical staining methods like Sudan Black B and Nile Blue A (e.g. Sudiana et al. 1998). Tetrads have not been reported to contain intracellular polyphosphate granules but sometimes the cell exteriors stain with Neisser, possibly indicating polyphosphate in this location (e.g. Cech & Hartman 1990; Blackall et al. 1997; Sudiana et al. 1998). Tetrad-arranged cocci were isolated in pure culture by Cech & Hartmann (1993) and were named *Amaricoccus kaplicensis* (Maszenan et al. 1997). Additional phenotypic data on this organism can be found in the literature (Cech & Hartman

Table 1. Sequences of oligonucleotide probes designed for polyphosphate accumulating and glycogen accumulating organisms

Target organism	Probe name	Probe sequence (5′–3′)	Reference
Accumulibacter phosphatis	RHC439	CNATTTCTTCCCGCCGA	(Hesselmann et al. 1999)
Accumulibacter phosphatis	RHC175	TGCTCACAGAATATGCGG	(Hesselmann et al. 1999)
Accumulibacter phosphatis	PAO462	CCGTCATCTACWCAGGGTATTAAC	(Crocetti et al. 2000)
Accumulibacter phosphatis	PAO651	CCCTCTGCCAAACTCCAG	(Crocetti et al. 2000)
Accumulibacter phosphatis	PAO846	GTTAGCTACGGCACTAAAAGG	(Crocetti et al. 2000)
Novel *gammaproteobacteria*	Gam1019	GGTTCCTTGCGGCACCTC	(Nielsen et al. 1999)
Novel *gammaproteobacteria*	Gam1278	ACGAGCGGCTTTTTGGGATT	(Nielsen et al. 1999)
Novel *gammaproteobacteria*	GAOQ431	TCCCCGCCTAAAGGGCTT	(Crocetti et al. pers. comm.)
Novel *gammaproteobacteria*	GAOQ989	TTCCCCGGATGTCAAGGC	(Crocetti et al. pers. comm.)

1993; Blackall et al. 1997). In many cases, the terms 'G bacteria' and GAO are used interchangeably, but over time, 'G bacteria' has come to mean the tetrad-arranged cocci in sludges, often without determination or mention of the GAO phenotype. Many different tetrads have now been isolated in pure culture from sludges, but the pure-culture C transformations, which would clarify the GAO-status of the tetrads, have not been well investigated (Seviour et al. 2000). In a recent study, *Amaricoccus kaplicensis* was studied in pure culture and found to not be a GAO (Falvo et al. 2001).

Nielsen et al. (1999) used denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rDNA fragments from sludge biomass in a deteriorated EBPR reactor to find a novel cluster of *gammaproteobacteria* were the likely PAO-competitors. The morphology and staining features (Neisser for polyP) of the bacteria in the sludge were studied and large (3–4 μm diameter) coccoid cells not containing polyP comprised 35% of all bacteria and bound FISH probes designed to the novel *gammaproteobacteria* (Gam1019 or Gam1278, Table 1, Nielsen et al. 1999). An interesting observation was that cells hybridising with Gam1019 did not bind the Gam42a probe for *gammaproteobacteria*. This observation was similar to a later one by Liu et al. (2001) where only ca. 7% of bacteria in an efficient EBPR sludge bound the GAM42a probe (for *gammaproteobacteria*) but ca. 24% of cells bound Gam1019 and Gam1278 for the novel cluster in the *gammaproteobacteria*.

Bond et al. (1998, 1999a, b) reported on two deteriorated EBPR reactors called the Q (Bond et al. 1998, 1999a) and T reactors (Bond et al. 1999b). The Q sludge was highly dominated by large coccobacilli which carried out EBPR cycling of PHA but not polyP (according to chemical staining procedures). These cells were identified as *betaproteobacteria* (92% bound probe BET42a) not belonging to the *betaproteobacteria* subgroups 1 (detectable with BONE23a) or 2 (detectable with BTWO23a) (Bond et al. 1999a). The T sludge was complex but the dominant groups were *alphaproteobacteria* tetrad-arranged cocci (40% of bacteria) and *Actinobacteria* (42% of bacteria). Large coccobacilli of indeterminate identity, but not tetrad-arranged cocci contained and cycled PHA. From these studies, the identity of the GAOs was not unequivocally resolved, however, in the Q sludge it was known they were *betaproteobacteria* not in subgroups 1 or 2.

Although Liu et al. (1996, 1998, 2000a, b, 2001) have studied EBPR sludge microbial communities with a focus on the identification of the GAOs, no definitive conclusion on the identity of a GAO could be made. However, there was some overlap with the results of Nielsen et al. (1999) in that the most likely GAO was the large coccobacilli phylogenetically placed as a novel cluster in the *gammaproteobacteria*.

Dabert et al. (2001b) studied the microbial community of an SBR during its transition from good to poor P removal by SSCP. Initially, *Accumulibacter* dominated the community. As EBPR deteriorated, periodic SSCP analysis showed a dramatic increase in the novel *gammaproteobacteria* of Nielsen et al. (1999) and a dramatic decline in *Accumulibacter*. The conclusion was that the *gammaproteobacteria* were the GAOs.

Many of the studies into GAOs were reported from comprehensively operated and analysed laboratory-scale reactors (e.g. Nielsen et al. 1999; Bond et

al. 1999a; Liu et al. 2000a; Dabert et al. 2001b). Nielsen et al. (1999) demonstrated that bacteria from a novel *gammaproteobacteria* cluster were abundant in a deteriorated EBPR reactor but did not directly link these organisms with the GAO phenotype. Crocetti et al. (2001) prepared and analysed 16S rDNA clone libraries from the well-characterised GAO sludges called Q (Bond et al. 1999a) and T (Bond et al. 1998). FISH probes (Table 1) designed from the near-complete 16S rDNA clones were optimised and used in conjunction with post-FISH chemical staining on anaerobic and aerobic sludge from laboratory-scale and full-scale deteriorated EBPR processes. The same novel *gammaproteobacteria* GAO cluster reported by Nielsen et al. (1999) was found in the Q and T sludges and these organisms were identified by probes GAOQ431 and GAOQ989 (Table 1). In laboratory- scale, deteriorated EBPR processes (e.g. Figure 3B) and in full-scale EBPR processes, the novel *gammaproteobacteria* group were found to be GAOs according to FISH (with GAOQ431 and GAOQ989) and post-FISH chemical staining for PHA (Crocetti et al. pers. comm.).

The two FISH probes designed to the novel *gammaproteobacteria* cluster (GAOQ431 and GAOQ989 labelled with Cy3) were used simultaneously with Cy5-labelled BET42a (for *betaproteobacteria*) and fluorescein-labelled GAM42a (for *gammaproteobacteria*) on the Q sludge. The two GAOQ probes (for the novel *gammaproteobacteria* cluster) bound the same cells and comprised 92% of the Q sludge. A total of 11% of the GAOQ-probe-binding cells bound GAM42a and 88% of GAOQ-probe-binding cells bound BET42a. Although this result is confusing, Nielsen et al. (1999) and Liu et al. (2001) also found anomalies in their FISH where more cells bound Gam1019 and Gam1278 than bound GAM42a (for *gammaproteobacteria*). However, neither Nielsen et al. (1999) nor Liu et al. (2001) used Gam1019 and/or Gam1278 in dual probing experiments with BET42a.

The BET42a and GAM42a probe targets are in the 23S rRNA and only differ from each other by one central nucleotide. The use of competitor probes in the BET42a and GAM42a probe solutions should adequately facilitate distinction between *betaproteobacteria* and *gammaproteobacteria* cells in FISH (Manz et al. 1992). Pure cultures of the organisms from the novel *gammaproteobacteria* cluster are not available and there is no information on their 23S rDNA sequences. Therefore, the reason why there is a higher abundance of bacteria binding probes to subgroups of *gammaproteobacteria* than binding the probe for all *gammaproteobacteria* awaits information on the BET42a-GAM42a probe target region of the 23S rDNA for these organisms.

Conclusion and future perspectives

The analysis of EBPR activated sludge microbial communities is complex. Results from the literature are difficult to compare due to different approaches used by the different researchers in particular attention to detailed process performance monitoring, and method of microbial investigation. The approach of comparing the results of bacterial communities between different sludges to deduce what bacteria may be important to EBPR is challenging due to the wide bacterial diversity in these systems. Pure culture methods have repeatedly suggested that *Acinetobacter* species are abundant in EBPR sludges. However the selection of certain bacteria from sludge by pure culturing raises doubts over the validity of this suggestion. Recently, a variety of non-culture dependent methods in combination with detailed process performance data and biochemical knowledge as determined by storage polymer staining, have been used to analyse EBPR sludges. The results from these methods suggest that EBPR sludge bacterial communities are diverse. These methods have highlighted at least one PAO (*Accumulibacter* as a close relative of the *betaproteobacteria Rhodocyclus*) and one GAO (an organism in the *gammaproteobacteria* radiation but with no close organismal relatives), but the search for others is being actively pursued. Knowledge of the biodiversity of PAOs and GAOs is needed to be able to optimise EBPR in differing conditions since different PAOs and GAOs might be selected by these conditions.

Future studies should strive to clearly demonstrate that GAOs are present in full- scale EBPR processes and that in these environments, they do compete for sparingly-available VFAs with PAOs. This would prove GAOs adversely affect EBPR, and a method for eliminating GAOs and promoting PAOs in EBPR would then be highly desirable. However, as far as we are currently aware, the same conditions (anaerobic-aerobic cycling of biomass) select both GAOs and PAOs. So studies into the competitive mechanisms employed by these organisms are needed. Other studies should explore the biodiversity of PAOs and GAOs and experimental evidence for the biochemical mech-

anisms employed by PAOs and GAOs should also be determined.

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