Identification and occurrence of tetrad-forming \textit{Alphaproteobacteria} in anaerobic–aerobic activated sludge processes

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In an acetate-fed anaerobic–aerobic membrane bioreactor, a deteriorated enhanced biological phosphorus removal (EBPR) community was developed (as determined based on the chemical profiles of organic substrate, soluble phosphate, and intracellular carbohydrate and polyhydroxyalkanoate (PHA) concentrations). Microscopic observations revealed the dominance of tetrad-forming organisms (TFOs), of which the majority stained positively for PHA under anaerobic conditions. Fluorescence \textit{in situ} hybridization (FISH) confirmed that the \textit{Alphaproteobacteria} (85 ± 7\% of total cells) were the most dominant group. A 16S rRNA gene clone library specific for the \textit{Alphaproteobacteria} indicated that most 16S rRNA gene clones (61 \% of total clones) were closely affiliated with ‘\textit{Defluvicoccus vanus}’, forming a cluster within subgroup 1 of the \textit{Alphaproteobacteria}. Combined PHA staining and FISH with specific probes designed for the members of the ‘\textit{Defluvicoccus}’ cluster suggested diversity within this TFO cluster, and that these TFOs were newly identified glycogen-accumulating organisms in EBPR systems. However, these ‘\textit{Defluvicoccus}’-related TFOs were only seen in low abundance in 12 different EBPR and non-EBPR systems, suggesting that they were not the key populations responsible for the deterioration of full-scale EBPR processes.

\textbf{INTRODUCTION}

Enhanced biological phosphorus removal (EBPR) processes employ a cyclic anaerobic and aerobic configuration to enrich microbial populations capable of removing phosphate from the bulk solution by intracellular polyphosphate accumulation (Seviour et al., 2003). These polyphosphate-accumulating organisms (PAOs) utilize internally stored polyphosphate as an energy source for the uptake and accumulation of carbon substrates as intracellular storage reserves, for example polyhydroxyalkanoate (PHA), during the anaerobic feeding stage. This carbon sequestering mechanism enables PAOs to grow under subsequent aerobic conditions where a carbon source is not available to them, providing them with a competitive advantage over strictly aerobic heterotrophs (Seviour et al., 2003). Glycogen-accumulating organisms (GAOs) share similar carbon assimilation and storage features to PAOs under anaerobic conditions, and have been reported to compete with PAOs for carbon substrate by utilizing glycogen as an internally stored energy source (Liu et al., 1996). Although GAOs are often more dominant than PAOs in deteriorated EBPR systems (Liu et al., 1996), the mechanism of EBPR deterioration is still not well understood. One possible reason for this is the poor understanding of the microbial diversity and physiological function of GAOs in various EBPR systems.

Culture-dependent and culture-independent molecular techniques have been used to identify GAOs. One postulated GAO that has been described is the coccobacillus-shaped organism seen in an acetate-fed deteriorated EBPR reactor (Liu et al., 1996), and phylogenetically placed in the bacterial lineage GB consisting of at least seven phylogenetic subgroups (Nielsen et al., 1999; Kong et al., 2002b), or ‘\textit{Candidatus Competibacter phosphatis}’ (Crocetti et al., 2002) in the \textit{Gammaproteobacteria}. This group exhibits several metabolic traits similar to those proposed for GAOs (Nielsen et al., 1999; Crocetti et al., 2002), and appears to be widely distributed in laboratory- and full-scale EBPR processes (Crocetti et al., 2002; Kong et al., 2002b).
The tetrad-forming organisms (TFOs), which occur as clusters of four or more cells, are another hypothesized GAO (Cech & Hartman, 1993; Liu et al., 1996; Tsai & Liu, 2002). So far, TFOs dominate mainly in laboratory-scale EBPR systems fed with synthetic carbon sources. Culture-independent studies have obtained several bacterial isolates with morphological traits resembling TFOs observed in EBPR systems. These isolates have been shown phylogenetically to be members of the Alphaproteobacteria (Amaricoccus spp., and ‘Defluviococcus vanus’), Betaproteobacteria (Quadraecoccus sp.), Gammaproteobacteria, and Actinobacteria (Tetrasphaera spp., Micropruna glycogenica and Kineosphaera limosa) (Maszenan et al., 1997; Kong et al., 2001; Shintani et al., 2000; Hanada et al., 2002; Liu et al., 2002a). However, except for M. glycogenica, none of those isolates have been seen in abundance in either full-scale laboratory-scale EBPR processes (Kong et al., 2001; Sevinour et al., 2003). While the absence of these TFO isolates in EBPR systems remains unexplained (Sevinour et al., 2003), molecular approaches have detected several as-yet-identified TFOs from different major phylogenetic lineages dominating in laboratory-scale EBPR systems (Kong et al., 2001, 2002a; Levantesi et al., 2002; Tsai & Liu, 2002). These findings suggest that using morphological traits to identify members of a physiologically functional group like the GAOs is inappropriate. There needs to be an improvement in the current understanding of the microbial diversity and metabolic functions of TFOs, and their distribution and possible role as GAOs in systems designed to perform EBPR.

Membrane bioreactors (MBRs) have emerged as important wastewater treatment technologies because of their small footprint, high mass/liquid separation efficiencies and low sludge production and operational costs (i.e. high biomass concentration) (Stephenson et al., 2000). Applying MBRs to biological phosphorus removal is also promising (Adam et al., 2002). Here, a sequencing MBR with anaerobic, aerobic and liquid-solid separation stages was established, but it failed to perform EBPR over an operation period of 260 days. Microscopic observation revealed that the microbial community was dominated by cells with a TFO morphology. This paper describes the in situ physiological traits of these TFOs, resolves their phylogenetic affiliation and diversity, and examines their occurrence in laboratory- and full-scale EBPR and non-EBPR systems.

METHODS

Reactor operation. A laboratory-scale sequencing MBR (working volume 20 l) was operated under cyclic anaerobic and aerobic conditions to mimic EBPR processes. It was inoculated with sludge taken from a local conventional wastewater treatment plant (capacity 1400000 population equivalent). The sequencing MBR was operated with four 6 h cycles per day for 260 days. Each cycle consisted of a feeding phase (10 min), an anaerobic phase (140 min), and an aerobic phase (210 min). During the feeding phase, a synthetic medium (Kong et al., 2002a) with acetate as the sole carbon source was delivered into the reactor at an organic loading rate of 0.8–0.9 g l−1 day−1. The total organic carbon (TOC) and phosphorus (P) concentrations in the bulk solution after feeding were 200 mg l−1 and 20 mg l−1, respectively (P:TOC ratio, 1:10). Mechanical mixing was used during each cycle. Aeration was carried out during the aerobic phase only, to maintain a minimum dissolved oxygen level of 1 mg l−1. In the final 60 min of the aerobic phase clarified liquor was obtained from the mixed liquor using a submerged membrane (effective filtration area 0.1 m2; pore size 0.4 μm) purchased from Kubota. The suction pressure was maintained at 50 kPa with a peristaltic pump. The sludge retention time (SRT) and hydraulic retention time (HRT) were maintained at 15 days and 5 days, respectively, and pH was regulated at 8.0±1.0 using a pH controller through acid and base addition (Chemitreat pH/REDOX 800). The pH, COD, orthophosphate (P), total phosphate, TOC, mixed liquor suspended solids (MLSS), and cellular carbohydrate, glycogen and PHA contents were monitored as described previously (Liu et al., 1994, 1996).

Microscopy. Epifluorescence microscopy and confocal laser scanning microscopy (CLSM) were both used for microscopic observations and staining methods. The epifluorescence microscope (model BX51, Olympus) was equipped with a cooled CCD camera SPOT-RT Slider (Diagnostic Instruments), a 100 W HBO bulb, and three different fluorescence filter sets (U-MWU2, U-MWB2 and U-MF2). The CLSM model LSM 5 Pascal (Carl Zeiss) was equipped with an inverted microscope, an argon-ion laser (458–514 nm), two helium/ neon lasers (543 nm and 633 nm), three Zeiss filter sets (01, 09 and 15), and different objective lenses (x20, x40, x63 and x100 oilimmersion). Image processing and analysis were performed with the software package provided by Zeiss, Metamorph (Universal Imagine) and Adobe Photoshop software (Adobe).

Chemical staining. Neisser and Sudan black B staining procedures were used to confirm the presence of intracellular polyphosphate and PHA granules, respectively (Jenkins et al., 1993). Due to equipment setup, Nile blue A staining (Ostle & Holt, 1982) combined with FISH was used in CLSM to detect accumulated intracellular PHA in microbial cells of interest.

DNA extraction and construction of the 16S rRNA gene clone library. DNA from biomass samples was obtained by the protocol of Liu et al. (1997). Bacterial primers ALF1b, targeting the Alphaproteobacteria (Manz et al., 1992), and EUB1512R, for the domain Bacteria (Kane et al., 1993), were used to selectively amplify the 16S rRNA gene of the Alphaproteobacteria in the community DNA. PCR was performed with a Hybaid thermal cycler (Hybaid) as follows: an initial denaturation at 94 °C for 5 min; 30 cycles of denaturation (45 s at 94 °C), annealing (45 s at 55 °C) and extension (1 min at 72 °C); and a final extension at 72 °C for 5 min. After confirmation with electrophoresis on a 0.8% (w/v) agarose gel, PCR products were used in the construction of the 16S rRNA gene clone libraries as reported previously (Liu et al., 2002b). 16S rRNA gene sequences were analysed using an ABI model 377 automated sequencer (Applied Biosystems) and the Taq Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems).

Phylogenetic analysis of 16S rRNA gene clones. The 16S rRNA gene sequences obtained were compared to GenBank using the NCBI BLAST program (Altschul et al., 1990), and also checked for chimeric artifacts with the CHECK_CHIMERA tool in the Ribosomal Database Project (RDP) (Maidak et al., 2001). For phylogenetic analysis, sequences of those selected clones and closely related bacterial species were aligned with the CLUSTAL W version 1.4 program (Thompson et al., 1994) available in the BioEdit software package (Hall, 1999). For phylogenetic analysis of cloned
nucleotide sequences, a neighbour-joining tree (Saitou & Nei, 1987) with the Jukes–Cantor method was constructed (1000 replicate bootstraps) with the MEGA2 program (Kumar et al., 2001).

**FISH.** Fresh sludge samples were fixed in both paraformaldehyde and ethanol solutions for Gram-negative bacteria and Gram-positive bacteria, respectively, then washed with PBS, and stored in PBS/ethanol solution at −20 °C prior to further analysis (Manz et al., 1999; Roller et al., 1994). Probes were commercially synthesized and 5' labelled with FITC, Cy3 or Cy5 (MWG Biotech). Sludge samples were initially hybridized with the probe NON338 labelled with Cy3 to exclude nonspecific-probe-binding (Wallner et al., 1993), and then analysed with the domain- and group-specific oligonucleotide probes listed in Table 1 to provide information on microbial community structure.

Semi-quantitative FISH analysis was performed on the CLSM-captured or epifluorescence-microscope-captured images according to protocols described previously (Bouché et al., 2000; Schmid et al., 2000; Liu et al., 2001). Cells hybridized to a given probe in each field were statistically expressed as a percentage of the total area of bacteria hybridizing to the EUBmix with the functions provided in MetaMorph (Universal Imagine). The ratio of Cy3-labelled EUBmix-binding cells to 4,6-diamidino-2-phenylindole (DAPI) (1 μg ml⁻¹ for 10 min) or SYTO 9 (Molecular Probes) -binding cells was also determined. Results were corrected for nonspecific binding with the results of the negative control using probe NON338 (Gieseke et al., 1999).

**RESULTS AND DISCUSSION**

**Performance of an acetate-fed MBR exhibiting deteriorated EBPR activity**

The bioreactor was operated continuously for approximately 260 days. During this period, the MLSS concentration gradually increased from 3000 mg l⁻¹ at day 1, and levelled off at around 12 860 ± 2524 mg l⁻¹ from day 41 onwards. The carbon removal efficiencies at the end of the aerobic phase after day 41 were 85 ± 6·4 % of the feed concentration. The sludge phosphorus content at the end of the aerobic phase ranged from 1 to 2 % of biomass dry

![Fig. 1. Chemical changes that occurred during an aerobic–aerobic cycle in the acetate-fed MBR. △, PHA (mg (g TSS)⁻¹); □, glycogen (mg (g TSS)⁻¹); ○, TOC (mg l⁻¹); ●, Pi (mg l⁻¹).](http://mic.sgmjournals.org)
weight during bioreactor operation. Fig. 1 shows the typical chemical changes that occurred during the sequencing batch reactor cycles which were measured on three occasions between days 55 and 260. The carbon source (acetate) expressed as TOC was rapidly consumed; its concentration decreased from $190 \pm 36$ mg $l^{-1}$ initially to less than $23 \pm 2.7$ mg $l^{-1}$ within the first hour of the anaerobic stage. The remaining TOC was possibly soluble microbial products, which are often reported in MBR systems (Urbain et al., 1998). The decrease in carbon concentration was accompanied by a decrease in biomass glycogen content from $253 \pm 35$ to $154 \pm 14$ mg (g VSS)$^{-1}$ and an increase in biomass PHA content from $13 \pm 8$ to $57 \pm 8$ mg (g VSS)$^{-1}$. The PHA produced consisted of mainly 3-hydroxybutyrate (~80% of total PHA detected) and 3-hydroxyvalerate (~20%). No obvious $P_i$ release and $P_i$ uptake were observed during the anaerobic phase and the aerobic phase, respectively. At the aerobic stage, the biomass carbohydrate level increased gradually while the PHA level decreased to almost zero. This metabolic behaviour suggested a deteriorated EBPR activity occurring in the reactor.

**Microscopic observations of the community**

Activated sludge samples taken at the end of anaerobic phase were frequently observed by light microscopy. After the enrichment period (day 55 and onwards) the predominant morphotype was bacterial cells occurring in clusters of four or more cells (the so-called TFOs) (Fig. 2a). Different morphotypes of TFO, based on size and cluster formation patterns, were observed and recognized. For example, one (Fig. 2a, circle 4) was a large cluster which typically contained at least 16 cells (resembling sarcina-type). Another image of this pattern from another sludge sample is shown in the inset of Fig. 2(a). In addition, most TFOs stained positively with Sudan black B, with granule inclusions or whole cells stained at different intensities (Fig. 2b), which suggested the accumulation of biomass PHA. This was further confirmed using Nile blue A staining, which is reported to be a more specific and sensitive PHA staining method than Sudan black B staining (Ostle & Holt, 1982). As shown in Fig. 2(c), more cells stained positively using Nile blue A stain than Sudan black B stain, and different degrees of fluorescence intensity were observed among different cell morphotypes and among cells from a single cluster.

**Preliminary phylogenetic profiling of the microbial community structure**

FISH with oligonucleotide probes targeting different phylogenetic groups was performed to provide information on the microbial community structure in the reactor. Over 80% of DAPI-stained or SYTO 9-stained cells were detected with the domain Bacteria probes (EUBmix). Based on data

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Fig. 2. Micrographs of activated sludge samples from the acetate-fed MBR. (a) Phase-contrast image of different morphotypes of TFO. (b) Sudan black B-stained image of the same field as in (a) with two insets providing close-up views on the PHA accumulations in two different types of TFO. (c) Nile blue A-stained image. (d) CLSM micrograph of FISH of the same field as in (c) showing the TFOs in yellow, which were simultaneously hybridized by probe ALF968 (Cy3-labelled, red) targeting the alphaproteobacterial group and probe EUBmix (FITC-labelled, green) targeting total bacterial cells.
from epifluorescence microscopy and CLSM, the biomass sample taken at day 55 was dominated by Alphaproteobacteria (82.7 ± 3.0/85.0 ± 7.0 % of EUBmix-stained cells, epifluorescence microscopy/CLSM), followed by Gammaproteobacteria (10.1 ± 4.4/4.5 ± 8.8 %), Betaproteobacteria (5.3 ± 1.7/4.8 ± 1.5 %), Cytophaga–Flexibacterium–Bacteroides (3.8 ± 3.3/8.1 ± 4.5 %) and Actinobacteria (1.9 ± 1.2/＜1 %). Differences between the data obtained by the two methods were not statistically significant.

Figure 2(d) shows the CLSM-FISH image of the bacterial cells simultaneously hybridized by probes EUBmix (FITC-labelled, green) and ALF968 (Cy3-labelled, red). The bacterial cells that appear yellow (due to superimposition of red and green) represent members of the Alphaproteobacteria. From the Alphaproteobacteria at least two morphotypes of TFO by size were observed. The first, which resembled the one in Fig. 2(a) (circle 4), contained large coccoid cells in clusters of 16 or more. The second, which resembled the TFO observed in Fig. 2(a) (circle 2), appeared after FISH as ‘flower buds’, and differed from the first in cell size and in FISH staining response. Since the Alphaproteobacteria appeared to dominate the community, a 16S rRNA gene clone library was constructed for this subdivision to phylogenetically place the TFOs.

**Phylogenetic diversity of TFOs in the Alphaproteobacteria**

A total of 51 clones were selected from the clone library, screened, and classified into 11 different operational taxonomic units (OTUs) after full sequencing (~1400 bp). Phylogeny analysis (Fig. 3) showed that the majority of these clones (eight OTUs, 67 % of the total clones) were related to the Alphaproteobacteria. Five of those eight OTUs (61 % of the total clones) formed a lineage within the family Rhodospirillales of the Alphaproteobacteria. Of these five OTUs, four formed a tight cluster with 'Defluvicoccus vanus', isolated from a full-scale EBPR process (Kong et al., 2002a, b), with a sequence similarity of 92.6–97.2 %. Within this ‘Defluvicoccus’ cluster, OTU TFOa28 (47 % of the total clones) represented the most dominant OTU or population in the reactor. The remaining fifth OTU TFOa27, with low clone abundance in the lineage, was related to three environmental clones previously retrieved from a laboratory-scale EBPR reactor (McMaholm et al., 2002). The other three Alphaproteobacteria-related OTUs not within this lineage were related to Paracoccus and Rhodobacter species, and Hyphomonas species (Fig. 3). Finally, of the non-Alphaproteobacteria-related OTUs, one was identified as a chimeric sequence and the other two were related to the genus Prosthecobacter from the phylum Verrucomicrobia. It should be noted that the primer ALF1b used in clone construction is not very specific for Alphaproteobacteria, and the successful construction of the clones was attributed to the high abundance of the Alphaproteobacteria organisms as revealed through FISH analysis.

**Design and optimization of probes for the ‘Defluvicoccus’-related TFOs**

Kong et al. (2001) designed a probe DEF438 targeting ‘D. vanus’. However, sequence comparison revealed that the probe sequence was identical instead of complementary to the ‘Defluvicoccus’-related OTUs (Fig. 3), and had one mismatched nucleotide to four environmental clone sequences (i.e. LPB46, TFOa27, LPB60 and LPU04) closely related to ‘D. vanus’. These two issues could possibly lead to a failure in detecting the ‘Defluvicoccus’-related cells or reduce the probe specificity for detecting target cells. Therefore, three further oligonucleotide probes (TFO_DF218, TFO_DF618 and TFO_DF862) that targeted the OTUs found in the ‘Defluvicoccus’ cluster were designed.
(Fig. 3), and were used in FISH analysis to clarify whether they represented the different morphotypes of TFOs (Fig. 2a) observed in the bioreactor. These probes (listed in Table 1) had at least two mismatched sequences to any non-target sequences. The multiple probe strategy could also be used to avoid any failure of probe design associated with poor accessibility of the 16S rRNA sequence (Fuchs et al., 1998), and to determine if inter-group diversity existed within the ‘Defluvicoccus’ cluster. Based on the probe-target dissociation curves (data not shown) obtained at different formamide concentrations with ‘D. vanus’ as a perfect-matched reference strain, the optimized formamide concentrations for probes TFO_DF218, TFO_DF618, and TFO_DF862 were determined to be 25–35 %, 25–35 % and 35 %, respectively.

Phylogenetic confirmation and PHA-accumulating traits of the ‘Defluvicoccus’-related TFOs

The CLSM-FISH results indicated that probes TFO_DF218 and TFO_DF618 could bind to approximately 86·8–93·2 % and 64·1–78·6 %, respectively, of all alphaproteobacterial cells in three biomass samples taken each 2–3 weeks over a period of two months. This suggests that the probe TFO_DF218 has a broader specificity than probe TFO_DF618 toward these TFOs. Probe TFO_DF862 gave no hybridization signals to any cells in these biomass samples, suggesting that ‘D. vanus’ was not present in the reactor.

Fig. 4(a) shows a FISH image of a biomass sample taken at the end of anaerobic phase hybridized simultaneously with probes TFO_DF218 and TFO_DF618. At least two different FISH-positive TFOs were observed. One, in clusters of 4–6 cells, fluoresced with both probe TFO_DF618 (red) and probe TFO_DF218 (green) as yellow-coloured cells. The other type, which fluoresced only with probe TFO_DF218 (green) and usually formed clusters of more than 16 cells, represented about 8–20 % of the bacterial cells in the reactor community. These differences in probe responses suggested the existence of other as-yet-uncultured populations within this ‘Defluvicoccus’ cluster. Fig. 4(b) shows the corresponding Nile blue A-stained image of the FISH-positive cells as shown in Fig. 4(a). All the ‘Defluvicoccus’-related TFOs reacted positively to Nile blue A stain but with different levels of fluorescence intensity, for example, among the TFO_DF218-positive, TFO_DF618-negative cells (indicated by arrows in Fig. 4b). These observations suggested variation among the TFOs in their ability to accumulate PHA.

Currently, no methods are available to directly link glycogen consumption with PHA production for microbial populations of interest following anaerobic substrate uptake. Thus, the involvement of glycogen in the metabolism of substrates and production of PHA in microbial cells was indirectly suggested by the concurrent anaerobic consumption and aerobic production of cellular glycogen (Crocetti et al., 2002; Levantesi et al., 2002). Using this approach, probable glycogen-accumulation-ability of cells that were hybridized by probes TFO_DF218 and TFO_DF618 could be indirectly supported by combining the metabolic profiles in Fig. 1 and the FISH results in Fig. 4. In biomass samples dominated by cells positive for the probes TFO_DF218 and TFO_DF618 (>70 % of the total cells), the PHA content increased from 0 % to ~5 % of sludge dry weight after anaerobic uptake of acetate. These levels subsequently decreased to almost 0 % by the end of the aerobic phase. Thus, these ‘Defluvicoccus’-related TFOs may be tentatively described as putative GAOs, since their key phenotypic traits are similar to those proposed for other GAOs in EBPR processes (Nielsen et al., 1999; Crocetti et al., 2002; Kong et al., 2002b).

Occurrence of ‘Defluvicoccus’-related TFOs in activated sludge treatment processes

The occurrence of these ‘Defluvicoccus’-related TFOs in biomass samples from the laboratory-scale and full-scale systems with or without EBPR activity that were studied in Kong et al. (2002b) was examined using FISH and the

Fig. 4. (a) CLSM micrographs of FISH showing different types of TFO hybridized by probe TFO_DF618 (Cy5-labelled, red) and/or probe TFO_DF218 (Cy3-labelled, green) targeting the ‘Defluvicoccus’ cluster at different levels of specificity. (b) Nile blue A-stained image of the same field as in (a) showing the accumulation of PHA inside some of the TFO cells.
probes designed here. With the exception of the biomass sample taken from the bioreactor operated in this study, none of the other biomass samples contained a high percentage of their total cells as ‘Defluvicoccus’-related TFOs. Unlike the gammaproteobacterial lineage GB (Kong et al., 2002b), this survey suggested that ‘Defluvicoccus’-related TFOs were not the dominant populations in laboratory- and full-scale EBPR systems or in conventional activated sludge processes. This difference could be due to the MBR system used here, where the biomass concentration and organic loading were much higher and lower, respectively, than other conventional gravity settling systems.

In fact, dominance of TFOs in EBPR processes has been reported mainly in laboratory-scale systems. Using light microscopy, Cech & Hartman (1993) and Liu et al. (1994) observed the proliferation of TFOs in deteriorated laboratory-scale EBPR systems. Kong et al. (2001, 2002a) reported that diverse unidentified alphaproteobacterial TFOs represented more than 50% of the total bacterial cells in a deteriorated EBPR reactor fed with acetate or a mixture of acetate and glucose. They did not detect any TFOs related to the genera ‘Defluvicoccus’ and ‘Amaricoccus’ in their systems, and suspected the existence of other novel TFOs in the Alphaproteobacteria. Likewise, Levantesi et al. (2002) reported that TFOs other than ‘Amaricoccus’ species from the Alphaproteobacteria were dominating (25% of total bacterial cells) in an acetate-fed EBPR reactor, but whether the reported TFOs were related to the ‘Defluvicoccus’ cluster revealed here remains to be validated. Thus, the role of the ‘Defluvicoccus’-related TFOs in the deterioration of full-scale EBPR processes was less clear than the role for members of the gammaproteobacterial lineage GB reported previously (Kong et al., 2002b).

REFERENCES


