Sulfur bacteria in wastewater stabilization ponds periodically affected by the ‘red-water’ phenomenon

Abdelaziz Belila · Ben Abbas · Imed Fazaa · Neila Saidi · Mejdi Snoussi · Abdennaceur Hassen · Gerard Muyzer

Received: 13 December 2011 / Revised: 27 January 2012 / Accepted: 28 January 2012 / Published online: 23 February 2012
© The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract Several wastewater stabilization ponds (WSP) in Tunisia suffer periodically from the ‘red-water’ phenomenon due to blooming of purple sulfur bacteria, indicating that sulfur cycle is one of the main element cycles in these ponds. In this study, we investigated the microbial diversity of the El Menzeh WSP and focused in particular on the different functional groups of sulfur bacteria. For this purpose, we used denaturing gradient gel electrophoresis of PCR-amplified fragments of the 16S rRNA gene and of different functional genes involved in microbial sulfur metabolism (dsrB, aprA, and pufM). Analyses of the 16S rRNA revealed a relatively high microbial diversity where Proteobacteria, Chlorobi, Bacteroidetes, and Cyanobacteria constitute the major bacterial groups. The dsrB and aprA gene analysis revealed the presence of deltaproteobacterial sulfate-reducing bacteria (i.e., Desulfovibrio and Desulfoviblobus), while the analysis of 16S rRNA, aprA, and pufM genes assigned the sulfur-oxidizing bacteria community to the photosynthetic representatives belonging to the Chlorobi (green sulfur bacteria) and the Proteobacteria (purple sulfur and non sulfur bacteria) phyla. These results point on the diversity of the metabolic processes within this wastewater plant and/or the availability of sulfate and diverse electron donors.

Keywords Sulfur bacteria · PCR–DGGE · Functional diversity · Red-water phenomenon · Wastewater stabilization ponds

Introduction

Wastewater stabilization ponds (WSPs) are an extremely effective, natural form of wastewater treatment. They combine simplicity, robustness, and low cost with a very high degree of purification. They rely upon the natural ability of a shallow water body to achieve self-purification, whereby light penetration is of fundamental importance (Curtis et al. 1994). WSPs are usually designed as one or several series of anoxic, facultative, and maturation ponds, with the first two being mainly responsible for the removal of suspended solids and organic matter (biological oxygen demand) and the last one for the removal of pathogens and nutrients. Their low operation and maintenance costs have made them a popular choice for wastewater treatment, particularly in developing countries, since there is little need for specialized skills to operate these systems. WSP systems are widely used in Mediterranean countries (Mara 2008); in Tunisia, they account for more than 25% of the wastewater treatment plants (ONAS 2009). However, although these systems are very effective in the treatment of wastewater, they sometimes suffer from severe problems, such as the ‘red-water’ phenomenon.

The ‘red-water’ phenomenon is a temporal change in water color resulting from the massive growth (‘blooming’)
of phototrophic anoxygenic purple bacteria. This bacterial phenomenon has been observed in several different wastewater stabilization ponds (Veenstra et al. 1995). The ‘red-water’ phenomenon is a sign of process failure causing malfunction of the wastewater treatment (Belila et al. 2009). The phenomenon is caused when the WSP system is overloaded with organic material, which stimulates sulfate reduction in the anoxic and facultative ponds. Consequently, the rising sulfide concentration is toxic for the algae but stimulates the growth of phototrophic sulfur bacteria that flourish under these anoxic conditions (Villanueva et al. 1994). The red-water phenomenon causes a deterioration of the effluent quality, i.e., red-colored water, a strong hydrogen sulfide smell, and high concentrations of suspended solid (Nair 1992).

The sulfate-reducing and the sulfur-oxidizing bacteria (SOB) encompass phylogenetically and physiologically diverse groups. The first group falls into three major branches: the delta-subclass of Proteobacteria, the Gram-positive bacteria, and branches formed by the thermophilic Archaeal sulfate-reducing bacteria (SRB) (Tang et al. 2008), while the taxonomic affiliation of sulfur-oxidizing bacteria has a broad range, from α-, β-, γ-, ε-proteobacteria, and Chlorobia to Chloroflexi (Ghosh and Dam 2009; Vannini et al. 2008). Both bacterial guilds are of immense importance from the industrial and environmental points of view and thrive in a wide variety of natural and engineered ecosystems (Asano et al. 2007; Ben-Dov et al. 2007). Their ecological and biogeochemical importance was recognized early due to their key role in the nitrogen, carbon, and sulfur cycles (Madigan 1995).

The anoxygenic phototrophic bacteria (APB) are responsible for the oxidation of sulfide as a major biogeochemical activity in aquatic ecosystems (Pierson and Olson 1987) since most known groups of APB, such as the purple sulfur, the purple non-sulfur, the green sulfur, and green non-sulfur bacteria, are able to use reduced sulfur compounds as electron donors for anoxygenic photosynthesis (Dahl and Prange 2006; Sander and Dahl 2008).

A wide variety of molecular tools have been applied to assess the diversity of bacteria involved in the sulfur cycle by targeting the 16S RNA gene (Dar et al. 2007; Lücke et al. 2007). However, the lack of phylogenetic coherence among both bacterial guilds and the different metabolic pathways involved in the oxidative and reductive processes of the sulfur cycle limit the use of 16S rRNA genes for the detection and the ecophysiological assignment of these bacteria. Targeting functional genes implicated in the microbial sulfate reduction and sulfur oxidation processes is a better strategy to analyze the phylogenetic complexity of bacteria of the sulfur cycle, specifically the sulfate-reducing and the sulfur-oxidizing bacteria (Kubo et al. 2011; Miletto et al. 2007). Functional genes such as dsrB (Geets et al. 2006), Sox (Meyer et al. 2007), aprA (Meyer and Kuever 2008), and pufM (Ranchou-Peyruse et al. 2006) have been successfully applied to resolve the genetic diversity of both sulfate-reducing and sulfur-oxidizing bacteria.

The dissimilatory sulfite reductase (DSR) and adenosine-5'-phosphosulfate (APS) reductase are two keys enzymes in microbial sulfate reduction and sulfur oxidation processes, highly conserved among the sulfate-reducing and the sulfur-oxidizing bacteria, and consequently dsrAB and aprAB genes constitute the most suitable targets for molecular profiling of the microbial community structure of the sulfur cycle in the environment (Meyer and Kuever 2007a). In addition, the pufM gene encoding the M subunit of the photosynthetic reaction center in purple sulfur and purple non-sulfur bacteria (Corson et al. 1999) has been successfully applied to the phylogenetic characterization of phototrophic bacterial communities in aquatic environments.

So far, most studies on WSP systems have concentrated on the fate and removal of pathogenic microorganisms, such as fecal enterococci, Cryptosporidium, Giardia (Anceno et al. 2007; Reinoso and Bécares 2008), and helminth eggs (e.g., Taenia, Ascaris, and Trichuris) (Tyagi et al. 2008). However, here we present for the first time a detailed study on the microbial communities present in WSP systems. We focused on the bacterial diversity during ‘red-water’ phenomenon occurrence and especially on the diversity of the sulfate-reducing and the sulfur-oxidizing bacteria.

Materials and methods

Wastewater stabilization ponds

Our study was carried out in a wastewater stabilization pond (WSP) system located in the city Mutuelleville in north-east of Tunisia (36°49′ N, 10°10′ E). The WSP system consists of four inter-connected ponds: an anaerobic, a facultative, and two maturation ponds. The geometric characteristics of these ponds are summarized in Table 1. The system mainly receives wastewater of domestic origin, i.e., ‘black’ water (water from toilets) and ‘grey’ water (domestic sewage). The wastewater fills up the first, anoxic (A) pond, then...
enters the secondary facultative (F) pond through an out-
flow, and subsequently enters the maturation (M) pond.
Finally, the treated water is released in a small river.

Environmental sampling

For molecular analysis, 16 samples were collected in April
2009. Five sediment samples each were collected from the
anaerobic (S4a to S45) and from the facultative pond (S1a to
S45) with a Plexiglas core tube (5 cm in diameter and 20 cm
in length). Sediment samples were taken from the middle
each corner of the basins. The sediment layers within
the maturation ponds were very thin and could not be
collected. Six water samples (W4, W5, WMI, WM2, WM3,
and WM4) were collected and pre-filtered through polyacryl-
monomer filters (0.8 μm in pore size) to exclude debris.

Physical and chemical parameters

The in situ temperature and pH were measured with a
handheld pH and temperature meter (WTW 340i model,
WTW, Weilheim, Germany). The dissolved oxygen (DO)
concentration was determined using a Multiline F/set P4
universal meter (WTW, Weilheim, Germany). The 5-day
biochemical oxygen demand (BOD5), chemical oxygen
demand (COD), and total suspended solids (TSS) as well
as the sulfate and sulfide concentrations were determined
according to the analytical methods described in “Standard
Methods for the Examination of Water and Wastewater”
(APHA 1995). Chlorophyll a concentration was estimated
by the methanol extraction method (Pearson 1986).

Nucleic acid extraction

Prior to DNA extraction, water samples (250 ml) were
centrifuged at 13,500 rpm at 4 °C. A total of 0.5 g of
concentrated biomass and the same quantity of sediments
were used for DNA extraction by using the UltraClean Soil
DNA Extraction Kit (MOBIO Laboratories, Inc., CA, USA)
according to the manufacturer’s instructions.

PCR amplification

Amplification of 16S rRNA and dsrB gene fragments was
performed using the primer pairs 341F-GC and 907R
(Muyzer et al. 1995) and DSRp2060F-GC and DSR4R
(Wagner et al. 1998), respectively. For the amplification of
the dsrB gene, a “touchdown” protocol was used, wherein
the annealing temperature was decreased from 65 to 55 °C
in 20 cycles. Thermal cycling was carried out as follows:
5 min of initial denaturation at 95 °C, followed by 20 cycles
of denaturation at 95 °C for 40 s, a “touchdown” annealing
step for 40 s, and elongation at 72 °C for 1 min. This was
followed by another 30 cycles of denaturation at 95 °C for
40 s, annealing at 55 °C for 40 s, and elongation at 72 °C for
1 min (Dar et al. 2007). Amplification was completed by a
final elongation step at 72 °C for 10 min. DNA from
Desulfobulbus propionicus was used as a positive control
and deionized water as a negative control in all PCR ampli-
fications. A ‘touchdown’ PCR protocol was used to amplify
aprA gene fragments using primers AprA-1-FW and AprA-
5-RV-GC (Meyer and Kuever 2007c). Thermal cycling was
carried out as follows: 5 min of initial denaturation of DNA
at 95 °C, followed by 35 cycles of denaturation at 95 °C for
60 s, a ‘touchdown’ annealing step for 90 s (annealing
temperature was decreased in the first 20 cycles by 0.5 °C
until reaching 50 °C in every cycle, while the subsequent 15
cycles were carried out at constant temperature), and
elongation at 72 °C for 120 s. Amplification was completed by
a final elongation step at 72 °C for 10 min. Primer set
pufM557F and pufM750R was used to amplify the photosynthetic
unit-fragment gene (puf/M) of purple phototrophic
bacteria (Achenbach et al. 2001). PCR was performed by
using an initial denaturation step at 95 °C for 15 s, followed
by 35 amplification cycles of denaturation (95 °C for 1 min),
annealing (54 °C for 30 s), elongation (72 °C for 1 min), and
a final extension step at 72 °C for 10 min. Amplifications
were performed in Biometra T Gradient Thermocycler
(Biometra GmbH, Germany), and PCR products were verified
on 1% or 2% (w/v) agarose gel in 1× Tris/acetate/EDTA
(TAE) buffer.

Denaturing gradient gel electrophoresis

All DGGE experiments were performed with the D-Code
system (Bio-Rad Laboratories, CA, USA). For the 16S
rRNA, electrophoresis was performed with 6% polyacryl-
amide gels (ratio of acrylamide to bisacrylamide, 40:1)
submerged in 1× TAE buffer (40 mM Tris, 40 mM acetic
acid, 1 mM EDTA, pH 7.5) at a constant temperature of 60 °C.
The electrophoresis conditions for gene fragments were: 16 h
at 100 V in a linear 20% to 80% denaturant gradient (100%
denaturant was a mixture of 7 M urea and 40% [v/v] formam-
ide) (Schäfer and Muyzer 2001). However, for
dsrB gene fragments, a gradient of 35–80% denaturant (the 100%
denaturant solution contained 7 M urea and 40% [v/v] formam-
ide) was constructed in a 1.5-mm-thick 8% polyacrylamide
gel. The gel was initially run at 150 V for 5 min to facilitate the
access of PCR products into the gel and then at a constant
voltage of 75 V for 30 h in a 0.5× TAE buffer at a stable
temperature of 60 °C (Miletto et al. 2007). For pufM gene
fragments, samples were electrophoresed on a 10% polyacryl-
amide gel with 20% to 80% denaturant (100% denaturant was
7 M urea and 40% [v/v] formamide) at 130 V for 8 h at 60 °C
(Karr et al. 2003). For aprA, a double-gradient DGGE was
used with a linear polyacrylamide gradient of 6–8% and a
linear denaturant gradient of 30–60% (100% denaturant was 7 M urea and 40% [v/v] formamide. Electrophoresis was performed at 60 °C for 2 h at 150 V and subsequently for 2 h at 200 V. All DGGE gels were stained with SYBR Green I (Sigma-Aldrich Corporation, St. Louis, MO, USA) and were visualized on a UV transilluminator. Individual bands were excised, re-suspended in 20 μl of Milli-Q water, and stored overnight at 4 °C. A volume of 3 to 5 μl of the supernatant was used for re-amplification using the original PCR conditions and primer pair without a GC clamp and then photographed using a G:BoxiChemi 2D gel image analyzer using Genesnap software 7.03 (SYNGENE, Synoptics, Ltd, Cambridge, UK).

Comparative sequence analysis
Sequences obtained from the excised DGGE bands were first compared to sequences stored in the publicly accessible database GenBank using the NCBI BLAST search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the sequences were imported into the ARB software package (Ludwig et al. 2004) and aligned using the automatic aligner function. The phylogenetic trees were constructed on the basis of long (more than 1,300 bp) sequences using different methods integrated within the ARB software. Partial sequences obtained in this study were then inserted into the pre-established tree using the ARB parsimony tool.

GenBank accession numbers
The nucleotide sequence data are available under the GenBank accession numbers, HQ222639 to HQ222674 (dsrB), HQ222675 to HQ222729 (pufM), HQ222613 to HQ222638 (aprA), and HQ222730 to HQ222810 (16S rDNA).

Results
Performance of the wastewater stabilization pond system
The performances of the wastewater stabilization pond system investigated in April 2009 were unsatisfactory regarding to the BOD and COD removal efficiency (Table 2). The chemical and biological parameters within the stabilization ponds gave evidence of a eutrophic state. The system performance decreased during this period of the year and the percentage removal of the TSS as well as the biological and chemical oxygen demands (BOD and COD) were unsatisfactory (50%, 40%, and 43%, respectively). The proliferation of the purple red color throughout the water column, reaching up to the surface of the four ponds, supports the prevalence of anoxic conditions within the whole system. Wastewater treatment successfully enhanced SO4\(^{2-}\) removal, accounting for greater than 83% reduction in SO4\(^{2-}\) concentrations during the sampling campaign. Maximum sulfate removal (86%) was reached within the first maturation pond (M1), showing a distinctive development of sulfates reducing conditions within this pond.

DGGE analysis of 16S rRNA gene fragments
DGGE analysis of PCR-amplified 16S rRNA gene fragments showed many different bands, between 12 and 23 per lane, indicating a high microbial diversity in the WSP system (figures of the DGGE gels are provided as “Electronic supplementary material”). Largely reproducible patterns were obtained with the five sediment samples from the anaerobic pond (lanes 1–5) and the five samples from the facultative pond (lanes 6–10). Differences within the DGGE profiles appeared more pronounced among the water samples (lanes 11–16) and between the water and the sediment samples than among the sediment samples (lanes 1–10). Several bands were detected concomitantly within different samples, although with varying intensities (i.e., bands A2, G2, H2, C2, and D2). In total, 90 bands were excised from the denaturing gel and sequenced in order to identify the predominant community members. A total of 15 DGGE bands gave ambiguous sequences and were not included in the phylogenetic analysis.

The phylogenetic affiliation of the different community members present in the WSP system is presented in Fig. 1a, b. A total of 26 sequences (28.8%) were attributed to different classes within the Proteobacteria (Fig. 1a), i.e., the Betaproteobacteria, the Gamma-, the Alpha-, and the Deltaproteobacteria. The other 64 sequences showed similarity with sequences from other phyla; 14 sequences

### Table 2 Physical and chemical parameters of the wastewater stabilization pond system

|        | T (°C) | pH   | DO (mg/L) | TSS (mg/L) | BOD\(_5\) (mg/L) | COD (mg/L) | Chl a (μg/L) | SO4\(^{2-}\) (mg/L) | S\(^2\) (mg/L) | Salinity (mg/L) |
|--------|--------|------|-----------|------------|----------------|------------|-------------|-------------------|---------------|-----------------|
| A      | 14.7±2 | 6.9±0.2 | 0.20±0.2  | 533±18     | 473±19.3      | 908±13     | 156±28      | 320±33            | 40.8±0.7     | 2±0.4          |
| F      | 13.5±1 | 7.6±0.2 | 2.50±0.4  | 342±38     | 360±19.6      | 720±17     | 2,634±268   | 245±43            | 25±0.9       | 1.4±0.6        |
| M1     | 12.2±2 | 7.5±0.1 | 3.10±0.3  | 247±18     | 283.3±11.6    | 510±26     | 3,456±265   | 45±15             | 9±3.2        | 1.1±0.5        |
| M2     | 12.9±2 | 7.4±0.1 | 3.10±0.9  | 262±0.9    | 262±18        | 505±11     | 3,288±345   | 52±17             | 10±0.7       | 1.1±0.3        |

A anaerobic ponds, F facultative pond, M1 maturation pond 1, M2 maturation pond 2
(15.5%) grouped with sequences in the phylum *Chlorobi*, 18 other sequences (20%) with the phylum *Bacteroidetes*, 13 (14.4%) with the phylum *Cyanobacteria*, 4 (4.4%) with *Bacteroidetes/Chlorobi* group, 4 (4.4%) with the phylum *Spirochaeta*, 3 (3.3%) with the phylum *Synergistetes*, 4 (4.4%) with the phylum *Acidobacteria*, 1 (1%) with the phylum *Thermotoga*, and 3 (3.3%) with uncultured bacterium partial 16S rRNA gene.

The sequences corresponding to bands C5, C3, and E2 showed 99% and 100% similarity with uncultured *Aminoaerobia* bacterium 16S rRNA gene clones. The closest relative for DGGE band sequences F3 and D3 was uncultured *Acidobacteria* bacterium 16S rRNA gene with 99% similarity. Sequences of bands B10, C10, and A5 had 93% and 95% similarity with uncultured cyanobacterium clone 16S ribosomal RNA gene and those related to bands F3, G2, E6, and F6 showed 99% similarity with uncultured *Bacteroidetes/Chlorobi* group bacterium clone. The closest relatives for bands D11 and B12 was an uncultured *Burkholderiaceae* bacterium with similarity percentage of 95% and 99%, respectively. DGGE band E4 was closely related to an uncultured *Chlorobiaceae* bacterium with 99% similarity. DGGE band sequence C12 showed 93% similarity with an uncultured *Chromatiaceae*. Band F8 sequence was closely related to uncultured *Bacteroidetes* bacterium 16S rRNA gene with 99% similarity. Notably, most of the 16S rRNA gene sequences were affiliated to uncluttered bacteria (Fig. 1a, b); thus, the use of this phylogenetic marker could not conclude on the physiological properties and specific biochemical pathways acting in the targeted communities.

Bacteria involved in the sulfur cycle identified within the WSP were represented by the SRB of the *Deltaproteobacteria* (Desulfovibrio spp.), the gammaproteobacterial purple sulfur bacteria (PSB) (Thiocapsa spp.), the alphaproteobacterial purple non-sulfur bacteria (PNSB) (Rhodobacter spp.), and the green sulfur bacteria (GSB) (Chlorobium spp.). The sequence of band D2 was closely related (96% similarity) to a *Desulfovibrio* sp. Sequences corresponding to bands C7 and D6 had 95% and 99% similarity with a sequence of *Thiocapsa* sp., respectively. The closest relative for bands A11 and G12 was *Thiocapsa pendens* (99% similarity). The sequence of band C12 showed 93% similarity to the sequence of an uncultured bacterium within *Chromatiaceae*. Members belonging to the green sulfur bacteria were, however, the most prominent group within the WSP with 11 sequences. Sequences of bands C1 and H3 had 100% and 99% similarity with the 16S rRNA sequence of *Chlorobium phaseobacteroides*, respectively, while the sequence of band C4 had 98% similarity with the sequence of *Chlorobium limicola*. The sequences of band E4 showed 99% similarity to the sequence of an uncultured bacterium within the *Chlorobiaceae*.

DGGE analysis of *dsrB* gene fragments

DGGE analysis of the *dsrB* gene fragments showed the highest diversity in sediments of the anaerobic and facultative ponds (“Electronic supplementary material”). Some of the DGGE bands (i.e., bands 1 and 2) were detected only in the sediments and not in the water phase. The SRB community composition varied significantly within all sediment samples as shown in “Electronic supplementary material”, except that DGGE bands 5 and 6 were detected in all anaerobic pond sediment samples. The SRB community diversity decreased within the sediment of the facultative pond (lanes 9 and 10, “Electronic supplementary material”) and then increased in the maturation pond water samples (lanes 15 and 16, “Electronic supplementary material”). The number of bands per lane varied between 4 (lane 11) and 25 (lane 7).

A total of 36 bands were sequenced and used for phylogenetic analysis. All sequences were assigned to sulfate-reducing bacteria within the *Deltaproteobacteria*. Band 48 had a 97% similarity to the *dsr* sequence of *Desulfomicrobium macestii*. The closest relative for band 1 was Desulfofotobacterium (*dsr* similarity (93%) similarity). The sequence corresponding to band 30 showed 87% similarity to *Desulfotignum balticum*. Five *dsrB* sequences were assigned to the *Desulfobulbus* genus with similarity levels varying from 84% to 90%. *DsrB* sequences of bands 40, 41, and 44 had 84% and 90% similarity with *D. propionicus*, respectively, while sequence of band 45 had 87% similarity with *Desulfobulbus rhabdoformis*. The sequence corresponding to band 24 had a similarity of 87% with uncultured *Desulfobulbus* sp. The sequences corresponding to bands 2, 19, 46, and 50 were affiliated to members of the genus *Desulfomicrobium*. The closest relative for bands 2 and 46 was *Desulfomicrobium escambiense* with 97% similarity. Band 19 had 93% similarity with *Desulfomicrobium* sp., while band 50 showed 97% level of similarity with *Desulfomicrobium* sp. ADR28. Several *dsrB*-related sequences were assigned to uncultured sulfate-reducing bacteria, such as band 3 and 14, which had 99% and 97% levels of similarity with *dsrB* gene sequences of uncultured sulfate-reducing bacteria, respectively. The sequence corresponding to band 15 had a similarity of 94% with *Desulfococcus oleovorans* Hxd3. The sequences corresponding to bands 34, 35, 36, and 37 were affiliated to *Desulfobulbus sapovorans*, a Gram-negative fatty acid-oxidizing species (Devereux et al. 1989) which is a member of the *Desulfofotobacterium* family. The *dsrB* sequences of DGGE bands 5, 6, and 8 were affiliated with an uncultured sulfate-reducing bacterium clone LGW106 with a percentage similarity of 84%, whereas DGGE bands 17 and 20 sequences were affiliated with *Desulfitibacillus aliphaticivorans*, an *n*-alkane- and *n*-alkene-degrading, sulfate-reducing bacterium (Cravo-Laureau et al. 2004).
The dsrB sequences of bands 7 and 13 were assigned to the Desulfosarcina/Desulfococcus cluster. The dsrB band 51 showed 94% similarity with Desulfostis glycolicus. No Gram-positive spore-forming SRB were detected.

DGGE analysis of aprA gene fragments

Because sulfate-reducing as well as sulfur-oxidizing prokaryotes use APS reductase, the genes of this reversible
Fig. 1 (continued)

sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulbus genus. Sequence analysis showed that bands A4, B8, and F10 had 95% similarity with Desulfobulbus sp. DSM 2033, while band C sequence had 89% similarity with D. postgatei. The closest relative for the sequences D3 and A8 was Desulfonema ishimotonii (95%). The sequences corresponding to DGGE bands A1, F10, C1, B8, and C8 belonged to representatives of the Desulfobulb...
genus *Rubrivivax* and *Rhodoferax* of Betaproteobacteria. Sequences of bands (A2, A3, C1, G3, and D6) were affiliated to the phototrophic purple non-sulfur *Phaeospirillum* genus of the *Rhodospirillales* family, while those of bands (D11, H11, B12, E12, B8, and D5) were affiliated to the photosynthetic purple non-sulfur bacteria *Rhodobaca bogoriensis* of Alphaproteobacteria. The PNSB were represented also by three different families: *Bradyrhizobiaceae*,
Fig. 1 (continued)
Rhodobacteraceae, and Rhodospirillaceae. Three DGGE bands, D5, A4, and A11, had similarities of 98%, 88%, and 87% with the sequences of Rhodobacter sphaeroides and Rhodobacter blasticus, respectively, while the closest relatives for the sequences of bands H10, A2, and G1 were Rhodopseudomonas sp. (88%), Rhodospirillum centenum (87%), and Rhodobacteraceae bacterium (90%), respectively.

Representatives of the aerobic photosynthetic bacteria were identified and were assigned to three different families within Alphaproteobacteria: Erythrobacteraceae, Rhodobacteraceae, and Acetobacteraceae. The sequence corresponding to band F3 showed 96% similarity to the sequence of Erythromicrobium sp. Sequences corresponding to bands H3 and B8 had similarities of 90% and 94% with pufM sequences of a Roseobacter sp. and Roseococcus thiosulfatophilus, respectively.

Discussion

Performance of the wastewater stabilization pond system

Wastewater stabilization ponds are without doubt the most important method of wastewater treatment in developing countries where sufficient space is available and where the temperature is most favorable for their operation. The deepest ponds (the anaerobic and facultative ponds) generally stratify between March and September due to their small size and as a result of warm climatic conditions and the absence of artificial aeration (Abis and Mara 2006). During this period, the sulfate reduction process becomes the dominant process in the sediments of these ponds and is stimulated by the increase in temperature. All ponds become fully anoxic and this was indicated by the black coloration of elemental sulfur deposits from the oxidation of hydrogen sulfide (Table 2). The stabilization ponds failure is reflected by the changes in the pond biology through the reduction of the wastewater treatment efficiency and the change of the WSP pigmentation. The hydrogen sulfide produced spontaneously reduces oxygen expanding the anaerobic zone throughout the water column within the facultative pond and may even diffuse into the maturation ponds. Sulfide toxicity affects algal growth (Konig et al. 1987) and stimulates the prevalence of anoxic conditions and the purple photosynthetic bacteria become visible at the surface (Lai and Lam 1997).

Analysis of the total bacterial community

So far, most studies have focused on the removal of pathogens and only a few studies have focused on the characterization of microbial communities in wastewater stabilization ponds (Shammas et al. 2009). The major bacterial groups (i.e., Proteobacteria, Chlorobi, Bacteroidetes, and Acidobacteria) found in this study have been previously found in other wastewater treatment systems, such as activated sludge, aerated lagoons, and sewage treatment plants (Malik et al. 2008; Moura et al. 2009). The bacteria detected were related to known organisms involved in the degradation of diverse pollutants, suggesting a similar role of these microorganisms within the wastewater stabilization pond system. The 16S rRNA gene sequences affiliated with the Proteobacteria, Chlorobi, Bacteroidetes, and Cyanobacteria phyla were the most frequently retrieved. A similar bacterial community composition has been previously described from a wastewater treatment plant (Wagner et al. 1993; Wagner and Loy 2002). The predominance of the Proteobacteria was in accordance with previous results obtained by Boon et al. (2002) and Ding et al. (2010).

Representatives of the Proteobacteria classes were most abundantly present within the WSP; this result was in accordance with those obtained by Xia et al. (2010) who reported the predominance of the Proteobacteria within five biological wastewater treatment reactors. This bacterial phylum is known to flourish in eutrophic ponds and is responsible for the removal of the organic matters from municipal wastewater (Wagner et al. 2002). The distribution of the different classes of Proteobacteria varies according to the type of wastewater treated (Arroyo et al. 2010). In contrast, while the Alphaproteobacteria tend to dominate within activated sludge (LaPara et al. 2000), the proteobacterial community in our system was dominated by members of Betaproteobacteria and Gammaproteobacteria.

SRB in wastewater treatment systems

SRB are present in wastewater treatment plants (Muyzer and Stams 2008) treating domestic (Baena et al. 1998) and industrial (Ben-Dov et al. 2007) wastewater. Sulfate reducers play a significant role in the anaerobic biomineralization pathways, especially in wastewater treatment systems (Oude Elferink et al. 1994) where sulfate reduction can be the dominant terminal electron-accepting process and can even account for up to 50% of mineralization of organic matter in wastewater.

16S rRNA, aprA-, and dsrB- genes analysis revealed the presence of the sulfate reducers within all water and sediment samples. The SRB community was phylogenetically diverse and all representatives are Gram-negative mesophilic SRB of the Deltaproteobacteria class. Representatives of this bacterial guild belonged to the family of Desulfobacteraceae (Desulfobacter, Desulfotignum, Desulfobotulus, and Desulforcoccus), the family of Desulfobulbaceae (Desulfobulbus and Desulfofustis), the family of Desulfomicrobiaceae (Desulfomicrobium), the family of...
Synthrophaceae (Desulfoomonile), and the family of Syntrophobacteraceae (Syntrophobacter). Most of these bacterial genera have been previously isolated and/or identified within wastewater treatment systems (Mohanakrishnan et al. 2011; Raskin et al. 1995). Similar sulfate-reducing bacteria community composition has been previously reported from wastewater environments, such as upflow anaerobic sludge bed wastewater treatment reactors (UASB) (Dar et al. 2005) or wastewater biofilm (Okabe et al. 1999).

The SRB are divided into two broad subdivisions that belie physiological and ecological roles of the two groups: complete and incomplete oxidizers. Complete oxidizers typically utilize a broader range of substrates than incomplete oxidizers and may be considered as generalists compared with the more specialist incomplete oxidizers. Representatives of both groups were identified within the WSP, complete oxidizers were represented by species of the genera Desulfobacter, Desulfococcus, Desulfofurna, and Desulfomonile (Rabus et al. 2006), while the incomplete oxidizers include representatives of the genera Desulfovibrio, Desulfomicrobium, and Desulfobulbus (Madigan and Martinko 2006). Incomplete oxidizers are present in the anoxic pond, while the complete oxidizers may be located in the following facultative and maturation ponds. Indeed the growth kinetics for incomplete oxidizers is generally faster than the complete oxidizers. However, the former are less versatile regarding their nutritional requirements (Widdel 1988); in addition, complete oxidizers have the ability to oxidize the organic compound to carbon dioxide, and incomplete oxidizers carry out the incomplete oxidation of the organic compound to acetate and CO₂ which subsequently can be used by complete oxidizers further in the WSP system.

Analysis of SOB-like sequences

Based on the 16S rRNA, apnA, and pufM gene analysis, the phylogenetic complexity of SOPs in the WSP consisted of putative strains of the GSB (Chlorobi), the Gammaproteobacterial PSB and the Alphaproteobacterial PNSB; all these bacteria have been shown to be able to oxidize reduced sulfur compounds (Brune 1995).

Many pufM sequences were related to the purple non-sulfur bacteria; these bacteria preferentially grow photolithoautotrophically under anaerobic conditions in the light by using various organic substrates. Nevertheless and contrary to the misleading nomenclature, many of these bacteria are also able to use sulfur compounds as a source of electrons (Imhoff et al. 2005). It is now well established that a number of purple non-sulfur bacteria are able to grow photolithoautotrophically with reduced sulfur compounds such as Rhodobacter, Rhodopseudomonas, Rhodofex, and Rubrivivax, which can use hydrogen, sulfide, thiosulfate, or ferrous iron as electron donors to support their anoxic, phototrophic growth (Kopriva et al. 2008).

In contrast to results generated by the pufM DGGE-based approach, only two aprA sequences were affiliated to Thiodyctyon, a sulfur-oxidizing bacterium which is an obligate and strictly anaerobic phototroph. This difference between the pufM- and the aprA-based approaches may be explained by the limited phylogenetic distribution of APS reductase-encoding genes among phototrophic bacteria, unlike the sox gene which is found in all Chromatiaceae (Meyer et al. 2007). Indeed aprAB gene distribution is restricted in Chlorobiaceae to members of sub-clusters 3 and 4b, to some species of Chromatiaceae, while absent throughout the Rhodospirillaceae and Ectothiorhodospiraceae families (Meyer and Kuever 2007b), thus limiting the utility of using apr genes to the survey of anoxygenic phototrophic SOB. The pufM gene universally distributed among all purple anoxygenic photosynthetic (Tank et al. 2009), which may explain the high diversity of the purple anoxygenic phototrophic bacteria revealed in comparison with the former genes (dsrB and aprA). This gene may constitute a better target to circumvent this limitation but this gene is not directly linked to the sulfide oxidation process and this may explain why many pufM-related sequences belonged to bacteria which were not able to use reduced sulfur compounds (i.e., PNSB). The primer set pufM557F-pufM750R used within this study allows the detection of both PSB and PNSB. It should be remembered that this primer set might also amplify pufM gene fragment of aerobic phototrophs (Shimada 1995) and the green non-sulfur bacterium Chloroflexus aurantiacus, which performs a purple bacterial-type reaction (Achenbach et al. 2001), but in our case C. aurantiacus was not detected within the investigated wastewater stabilization ponds.

Another important bacterial sulfur-oxidizing community was identified within the stabilization plant. A total of 15.5% of the retrieved 16S rRNA gene sequences were assigned to the GSB phylum. These bacteria carry out anoxygenic photosynthesis with reduced sulfur compounds such as sulfide and elemental sulfur and, for some species, thiosulfate as the electron donor for photautotrophic growth (Frigaard and Dahl 2009). Some GSB also use hydrogen and ferrous iron as the electron donors. GSB are obligately anaerobic and obligately photautotrophic, and they form a phylogenetically and physiologically distinct group (Imhoff 2008). They are commonly found in anoxic and sulfide-rich freshwater and estuarine environments as well as in wastewater treatment plants (Siefert et al. 1978) where they may form green or brown bloom depending on their light-harvesting pigments (bacteriochlorophyll c or d or e).

While the ecology of GSB and PSB is to some extent similar (Overmann 2008), their oxidative sulfur metabolism probably shares many characteristics as well (Brune 1995).
GSB have a high affinity for sulfide, which is usually the preferred substrate even if other sulfur substrates are available (Brune 1995). The GSB affinity for sulfide is one order of magnitude higher than that of Chromatiaceae (Van Gemerden and Mas 1995). In addition, GSB are capable of using significantly lower light intensities for photosynthetic growth. Consequently, green sulfur bacteria thrive below layers of Chromatiaceae in close association with the sulfate-reducing bacteria.

Congruency of phylogenetic results

Congruent phylogenetic results were obtained by applying aprA and dsrB genes for the characterization of the SRB community with exception concerning D. tiedjei. Both functional marker genes allowed the detection of representatives belonging to the Desulfobulbaceae, Desulfo bacteraceae, and Desul fomicrobiaceae families. SRB representatives of the Desulfococcus/Desulfosarcina cluster, Desulfobacter, Desulfobulbus, and Desulfomicrobium were found in both dsrB and aprA data.

The sulfate-reducing bacterium D. tiedjei was only detected by aprA-based DGGE approach. This bacterial species is a lateral gene transfer-affected member of the Syntrophobacteraceae family, the reclassification of which through Deltaproteobacteria is proposed based on the phylogenetic relationship of the xenologous aprBA genes (Meyer and Kuever 2007a). While all sequences retrieved with the dsr and apr primers were dsr and apr sequences, only one of the 16S rRNA sequences was clearly related to sulfate-reducing bacteria; this difference may be attributed to the fact that these functional genes are directly linked to the dissipatory sulfate reduction and sulfur oxidation processes whereas the ecophysiology and the metabolic function of the microorganisms characterized only by the 16S rRNA sequence remain largely unknown. Furthermore, the phylogenetic specificity of the dsrB and aprA primer sets was superior to the specificity of the 16S rRNA primer set used herein for revealing the SRB community, while both primers used in this study target all known groups of sulfate-reducing bacteria (Wagner et al. 1998). The differences observed in resolving the diversity of the phototrophic SOB community between aprA and pufM genes may be attributed to the limited distribution of aprA gene among the photosynthetic sulfur-oxidizing bacterial community. So, the use of different genes to characterize microbial communities as has been performed in this study nicely complements the limitations and biases of the different individual genes and will hence give a good overview of the different members that are present in our WSP system.

The data recovered from the 16S rRNA, dsrB, aprA, and pufM genes were complementary. The 16S rRNA genes allowed the determination of the major bacterial groups independently on their physiological or metabolic capacities, while dsrB and aprA genes were used to get insight on the sulfur metabolism pathways. The aprA gene allowed the identification of the SOB and SRB communities concomitantly since the primer used targets the same conserved gene region (Meyer and Kuever 2007c). In contrary, the primer designed on dsrB is not specific for SOB analysis and all dsrB sequences belonged to SRB. Despite that the core molecular unit dsrABCNMKJOP is common to both sulfur oxidizers and sulfate reducers, only dsrEFH and dsrL are specific for SOB (Grimm et al. 2008). The pufM gene—while not directly involved in sulfur oxidation process—allowed the detection of all anoxygenic purple phototrophic bacteria, most representatives of which are able to oxidize sulfide. The use of functional genes of SOB such as sox and sqr genes will offer a better view of the sulfide oxidation pathways among this photosynthetic bacterial community.

The PCR–DGGE approach has proven efficient in studying the microbial ecology of wastewater treatment systems as reviewed by Sanz and Köchling (2007). Although the approach provides new insights into the genetic and metabolic composition of these ecosystems, limitations may exist, such as the difficulty in band isolation and the overestimation of sequence heterogeneity in single DGGE bands (Zhang et al. 2005). In addition, the limited DNA sequence information obtained from these relatively short fragments (i.e., pufM, 229 bp; aprA, 400 bp) can lessen the specificity of the phylogenetic identification. Apart from limitations attributed to DGGE, general biases, such as DNA extraction efficiency, inhibition of PCR, differential amplification, and the incidence of artifact bands due to excessive amplification cycles (Moura et al. 2009), have to be taken into account as well.

Ecological significance of sulfur bacteria in WSP systems

In such aquatic ecosystems as WSP, a complex microbial consortium with interacting and complementary metabolic processes often exist where major metabolic bacterial groups, such as methanogens, nitrifiers, SRB, sulfide-oxidizing bacteria, and fermenters, can coexist and dominate when the conditions favor their metabolic processes. Based on the 16S rRNA gene database, the dominant metabolism is not linked to sulfate reduction and neither to sulfide oxidation; consequently, fermentation and syntrophic pathways might be important but this cannot be discerned with the 16S rRNA gene results. Chlorobi (15.5%) was one of the most dominant bacterial groups identified in this study. Representatives of this phylum share a large set of orthologues to accomplish the demanding task of photosynthesis and sulfur oxidation. They utilize various combinations of sulfide, elemental sulfur, and thiosulfate and sometimes also ferrous iron and hydrogen (Goh et al. 2009).
Since the sulfur cycle involves the presence of SRB and sulfide-oxidizing bacteria, the identification of Chlorobi, PSB, and PNSB in the WSP suggest the presence of sulfate-reducing bacteria. This was confirmed by the detection of both dsrB and aprA sequences related to SRB. Due to their strict anaerobic character, SRB identified herein are likely to dominate within the anoxic and the facultative ponds (i.e., in sediments and the deeper water layers) or within anoxic micro-niches rather than in the maturation ponds where they may constitute only a minor component of bacterial community.

The SRB reduce sulfate to sulfide using either hydrogen as an energy source or CO₂, acetate, lactate, and other short-chain carboxylic acids as carbon and energy sources. In this study, two SRB communities were distinguished: the SRB with respiratory type of metabolism such as Desulfobacter, Desulfomonile, and Desulforumine and SRB community with both respiratory and fermentative type of metabolism like Desulfotignum, Desulfomicrobium, and Desulfojobulus. The sulfide generated by the SRB activity constitutes a major electron donor for the phototrophic purple sulfur bacteria and may be also used by purple non-sulfur bacteria identified herein such as Rhodopseudomonas, Rhodobacter, and Rhodospirillum. Sulfide oxidation to sulfate would prevent accumulation of sulfide in the wastewater stabilization ponds. The sulfur-oxidizing microbial community within the wastewater stabilization plant is complex and it was suggested to consist of the phototrophic Gammaproteobacteria sulfur-oxidizing representatives, the phototrophic Alphaproteobacteria and Betaproteobacteria (PNSB), and the photoautotrophic sulfur-oxidizing green sulfur bacteria.

The collective data obtained in this study provided insights regarding the composition and the structure of the sulfur microbial community within a wastewater stabilization plant; this may allow a better understanding of the seasonal changes that may affect the microbial community structure especially during the spring and the summer seasons and thus the wastewater treatment's efficiency. Certainly, in pond design, both effluent characteristics and bacterial community should be taken into account and each pond provides the proper environmental conditions needed for bacterial growth, that is why complex bacterial communities should be monitored in order to guarantee the efficiency of the WSP. The relatively high phylogenetic diversity of the anoxygenic photosynthetic purple and green bacteria reflects their predominance among the total bacterial community within the wastewater stabilization plant since they constitute the most prominent group among the sulfur-oxidizing bacterial community. The diversity of the purple anoxygenic phototrophic bacteria traduces their ecological role in the wastewater treatment process. These bacteria, stimulated by the degradation of environmental parameters during the warm seasons (spring and summer), flourish by forming red-water and may contribute also to biological balance restoration. Further studies on these bacteria can contribute to a better understanding of their roles in these ecosystems.

Acknowledgments We are grateful to the Tunisian Ministry of High Education Scientific Research and Technology for financial support.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

Abis KL, Mara DD (2006) Temperature measurement and stratification in facultative waste stabilization ponds in the UK climate. Environ Monit Assess 114:35–47
Achenbach LA, Carey J, Madigan MT (2001) Photosynthetic and phylogenetic primers for detection of anoxygenic phototrophs in natural environments. Appl Environ Microbiol 6:2922–2926
Anceno AJ, Ozaki M, Dang YND, Chuluun B, Shipin OV (2007) Canal networks as extended waste stabilization ponds: fate of pathogens in constructed waterways in Pathumthani province, Thailand. Water Sci Technol 55:143–156
APHA—American Public Health Association (1995) Standard methods for the examination of water and wastewater, 19th edn. American Public Health Association, Washington, DC
Arroyo P, Genna A, Ivan B, Patricia M, de Estanislao LC, Luis E, de Saene M (2010) Comparative analysis of the composition of bacterial communities from two constructed wetlands for municipal and swine wastewater treatment. J Water Health 8:147–157
Asano R, Sasaki T, Nakai Y (2007) Isolation and characterization of sulphur oxidizing bacteria from cattle manure compost. Anim Sci J 78:330–333
Baena S, Fardeau ML, Labat M, Ollivier B, Garcia JL, Patel BKC (1998) Desulfovibrio aminophilus sp. nov., a novel amino acid degrading and sulphate reducing bacterium from an anaerobic dairy wastewater lagoon. J. Syst Appl Microbiol 21:498–504
Belila A, Gtari M, Ghrabi A, Hassen A (2009) Purple anoxygenic phototrophic bacteria distribution in Tunisian wastewater stabilization plant exhibiting red water phenomenon. Ann Microbiol 59:399–408
Ben-Dov E, Brenner A, Kushmaro A (2007) Quantification of sulfate-reducing bacteria in industrial wastewater, by real-time polymerase chain reaction (PCR) using dnrA and apsA genes. Microb Ecol 54:439–451
Boon N, De Windt W, Verstraete W, Top EM (2002) Evaluation of nested PCR–DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rDNA primers for the analysis of bacterial communities from different wastewater treatment plants. FEMS Microbiol Ecol 39:101–112
Brune DC (1995) Sulfur compounds as photosynthetic electron donors. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer Academic, Dordrecht, The Netherlands, pp 847–870
Corson GE, Nagashima KVP, Matsuura K, Sakuragi Y, Wettasinghe R, Qin H, Allen R, Knaff DB (1999) Genes encoding light harvesting and reaction center proteins from Chromatium vinosum. Photosynth Res 59:39–52
Okabe S, Itoh T, Satoh H, Watanabe Y (1999) Analyses of spatial distributions of sulfate-reducing bacteria and their activity in aerobic wastewater biofilms. Appl Environ Microbiol 65:5107–5116

ONAS (2009) Annual report of the National Office of Sanitation. National Office of Sanitation, Tunisia

Oude Elferink SJWH, Visser A, Hulshoff PLW, Stams AJM (1994) Sulfate reduction in methanogenic bioreactors. FEMS Microbiol Rev 15:119–136

Overmann J (2008) Ecology of phototrophic sulfur bacteria. In: Hell R, Dahl C, Knaff DB, Leustek T (eds) Advances in photosynthesis and respiration. Springer, New York, pp 375–396

Pearson HW (1986) Estimation of chlorophyll a as a measure of algal biomass in waste stabilization ponds. Regional Seminar on Waste Stabilization Pond Research, CEPIS, Lima

Pierson BK, Olson JM (1987) Photosynthetic bacteria. In: Amesz J (ed) Photosynthesis. Elsevier Science, BV, Amsterdam, pp 21–42

Rabus R, Hansen TA, Widdel F (2006) Dissimilatory sulfate and sulfur-reducing. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes, vol. 2, 3rd edn. Springer, New York, pp 659–768

Reinoso R, Bécares E (2008) Environmental inactivation of Cryptosporidiu parvum oocysts in waste stabilization ponds. Microb Ecol 56:585–592

Ranchou-Peyruse A, Herbert R, Caumette P, Guyoneaud R (2006) Comparison of cultivation dependent and molecular methods for studying the diversity of anaerogenic purple photrophs in sediments of a eutrophic brackish lagoon. Environ Microbiol 8:1590–1599

Raskin L, Zheng D, Griffin ME, Stroop PG, Misra P (1995) Characterization of microbial communities in anaerobic bioreactors using molecular probes. Antonie Van Leeuwenhoek 68:297–308

Rabinow R, Bécares E (2008) Environmental inactivation of Crypto sporidium parvum oocysts in waste stabilization ponds. Microb Ecol 56:585–592

Sander J, Dahl C (2008) Metabolism of inorganic sulfur compounds in purple bacteria. In: Hunter CN, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria. Kluwer Academic, Dordrecht, pp 595–622

Sanz JL, Köchling T (2007) Molecular biology techniques used in wastewater treatment: an overview. Process Biochem 42:119–133

Schäfer H, Muyzer G (2001) Denaturing gradient gel electrophoresis in marine microbial ecology. In: Paul J (ed) Methods in Microbiology, Vol 30, Academic Press, London, pp 425–468

Shammam NK, Wang LK, Wu Z (2009) Wastewater stabilization ponds and lagoons. Biological treatment processes. In: Wang LK, Pereira NC, Hung YT (eds) Biological treatment processes, vol 8. Humana, Totowa, NJ, pp 315–370

Shimada K (1995) Aerobic anoxygenic phototrophs. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anaerogenic photosynthetic bacteria. Kluwer Academic, Dordrecht, pp 105–122

Siefert E, Irgens RL, Pfennig N (1978) Phototrophic purple and green bacteria in a sewage treatment plant. Appl Environ Microbiol 35:38–44

Tang K, Baskaran V, Nemati M (2008) Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries. Biochem Eng J 44:73–94

Tank M, Thiel V, Imhoff JF (2009) Phylogenetic relationship of phototrophic purple sulfur bacteria according to pufL and pufM genes. Int Microbiol 12:175–185

Tyagi VK, Kazmi AA, Chopra AK (2008) Removal of fecal indicators and pathogens in a waste stabilization pond system treating municipal wastewater in India. Water Environ Res 80:2111–2117

Van Gemerden H, Mas J (1995) Ecology of phototrophic sulfur bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anaerogenic photosynthetic bacteria. Advances in photosynthesis, vol 2. Springer, Dordrecht, pp 49–85

Vannini C, Munz G, Mori G, Lubello C, Venni F, Petroni G (2008) Sulphide oxidation to elemental sulfur in a membrane bioreactor: performance and characterization of the selected microbial sulphur-oxidizing community. Syst Appl Microbiol 31:461–473

Veenstra S, Al-Nozaily FA, Alaerts GJ (1995) Purple non-sulfur bacteria and their influence on waste stabilization pond performance in the Yemen Republic. Water Sci Technol 31:141–149

Villanueva J, Grimalt JO, Wit RD, Brendan JK, Maxwell JR (1994) Sources and transformations of chlorophylls and carotenoids in a monomeric sulphate-rich karstic lake environment. Org Geochem 22:739–757

Wagner M, Loy A (2002) Bacterial community composition and function in sewage treatment systems. Curr Opin Biotechnol 13:218–227

Wagner M, Amann R, Lemmer H, Schleifer KH (1993) Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. Appl Environ Microbiol 59:1520–1525

Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998) Phylogeny of dissimilatory sulfite reductase supports an early origin of sulfate respiration. J Bacteriol 180:2975–2982

Wagner A, Loy R, Nogueira U, Purkhold LN, Daims H (2002) Microbial community composition and function in wastewater treatment plants. Antonie Van Leeuwenhoek 81:665–680

Widdel F (1988) Microbiology and ecology of sulphate and sulphur reducing bacteria. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley Interscience, New York, pp 469–586

Xia S, Duan L, Song Y, Li J, Piceno YM, Andersen GL, Alvarez-Cohen L, Moreno-Andrade I, Huang CL, Hermanowicz SW (2010) Bacterial community structure in geographically distributed biological wastewater treatment reactors. Environ Sci Technol 44:7391–7396

Zhang X, Yan X, Gao P, Wang L, Zhou Z, Zhao L (2005) Optimized sequence retrieval from single bands of temperature gradient gel electrophoresis profiles of the amplified 16S rDNA fragments from an activated sludge system. J Microbiol Methods 60:1–11