Characterization of Microbial Communities Removing Nitrogen Oxides from Flue Gas: the BioDeNOx Process

Rajkumari Kumarswamy,1 Udo van Dongen,1 J. Gijs Kuenen,1 Wiebe Abma,2 Mark C. M. van Loosdrecht,1 and Gerard Muyzer1*

Environmental Biotechnology Group, Department of Biotechnology, Delft University of Technology, NL-2628 BC Delft, The Netherlands,1 and Paques B.V. NL-8561 EL Balk, The Netherlands2

Received 18 February 2005/Accepted 20 May 2005

BioDeNOx is an integrated physicochemical and biological process for the removal of nitrogen oxides (NOx) from flue gas. In this process, the flue gas is purged through a scrubber containing a solution of Fe(II)EDTA2−, which binds the NOx to form an Fe(II)EDTA·NO2− complex. Subsequently, this complex is reduced in the bioreactor to dinitrogen by microbial denitrification. Fe(II)EDTA2−, which is oxidized to Fe(III)EDTA− by oxygen in the flue gas, is regenerated by microbial iron reduction. In this study, the microbial communities of both lab- and pilot-scale reactors were studied using culture-dependent and -independent approaches. A pure bacterial strain, KT-1, closely affiliated by 16S rRNA analysis to the gram-positive denitrifying bacterium Bacillus azotoformans, was obtained. DNA-DNA homology of the isolate with the type strain was 89%, indicating that strain KT-1 belongs to the species B. azotoformans. Strain KT-1 reduces Fe(II)EDTA·NO2− complex to N2 using ethanol, acetate, and Fe(II)EDTA2− as electron donors. It does not reduce Fe(III)EDTA−. Denaturing gradient gel electrophoresis analysis of PCR-amplified 16S rRNA gene fragments showed the presence of bacteria closely affiliated with members of the phylum Deferribacteres, an Fe(III)-reducing group of bacteria. Fluorescent in situ hybridization with oligonucleotide probes designed for strain KT-1 and members of the phylum Deferribacteres showed that the latter were more dominant in both reactors.

Nitrogen oxides (NOx), i.e., nitric oxide (NO) and nitrogen dioxide (NO2), are important greenhouse gases causing air pollution. The NOx act as indirect greenhouse gases by producing the tropospheric greenhouse gas ozone during their breakdown in the atmosphere. Over the long time scale, they contribute to climate changes (36). Moreover, nitric oxide is highly toxic. It affects neurons and plays a role in the development of several diseases, such as Parkinson’s disease and asthma (27). Therefore, removal of NOx from flue gas, which are responsible for 17 to 20% of the anthropogenic NOx emission (29), is of paramount importance for a clean and healthy environment.

To remove NOx from flue gas, several chemical and biological processes have been developed (9, 18, 28). However, a promising process, in which the advantages of chemical and biological processes are combined, is the BioDeNOx process (6). In this process, the NOx is absorbed by the Fe(II)EDTA2−-chelating complex in a scrubber and subsequently removed by microbial denitrification in a reactor. Due to the high temperature of flue gases, the process operates at a temperature of between 50 to 55°C.

The BioDeNOx process comprises four reactions, i.e., two chemical reactions in the scrubber and two microbiological reactions in the bioreactor (Fig. 1). The first reaction is the absorption and complexation of NOx with Fe(II)EDTA2−. This chemical reaction is fast and leads to an increased mass transfer rate of NOx (9). The second reaction is the oxidation of Fe(II)EDTA2− to Fe(III)EDTA− by oxygen in the flue gas. This reaction is an unwanted chemical reaction as it lowers the availability of Fe(II)EDTA2− for NO absorption. In the bio-reactor, NOx are reduced to N2 by denitrifying bacteria by using an organic substrate, e.g., ethanol, as electron donor. Subsequently, iron reducers reduce Fe(III)EDTA− to Fe(II)EDTA2−, using, e.g., ethanol as an electron donor.

So far, nothing is known about the species composition of the microbial communities and their involvement in the BioDeNOx process. Therefore, the aim of this research was to study the structure and dynamics of the microbial community involved in the BioDeNOx process. For this purpose, we used a combination of culture-dependent and -independent approaches (5, 19). In the first approach, we isolated and characterized microorganisms from the BioDeNOx process. In addition, denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments was used to study the species composition of the reactors removing NOx and to monitor population shifts over time. The phylogenetic affiliations of predominant microorganisms were inferred by sequencing of separated DGGE bands. Fluorescence in situ hybridization (FISH) with specific oligonucleotide probes was used to determine the abundances of the particular populations in the reactors.

MATERIALS AND METHODS

Bioreactor characteristics. Biomass samples for enrichment and molecular analysis were obtained from lab- and pilot-scale BioDeNOx reactors operated by Paques B.V. The media in the reactors were not completely cellular, as there were some iron precipitates. The lab-scale reactor was run with an artificial flue gas containing an NO concentration of between 1,200 and 2,000 ppm and an oxygen percentage of 2.5 to 3.0%. The rest of the gas was N2. The pilot-scale
FIG. 1. Schematic drawing of the BioDeNOx process. NOx is a mixture of NO and NO2 (6).

Purified gas

Fe(II)EDTA

Scrubber

N2

Flue gas

(NO + O2)

Fe(II)EDTA-NO2

Reactor

Microbiological Denitrification & Fe(III)EDTA reduction

Ethanol

TABLE 1. Operational characteristics of lab- and pilot-scale reactors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-scale reactor</td>
<td>Pilot-scale reactor</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>41–55</td>
</tr>
<tr>
<td>pH</td>
<td>6.8–7.5</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>−200 to −50</td>
</tr>
<tr>
<td>Hydrolytic retention time (days)</td>
<td>2–3.5</td>
</tr>
<tr>
<td>Overall Fe-EDTA (mM)</td>
<td>25</td>
</tr>
<tr>
<td>Gas flow rate (m3/h)</td>
<td>0.09</td>
</tr>
<tr>
<td>NOx (NO + NO2) (ppm)</td>
<td>1,200–1,500</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>2.5–3</td>
</tr>
<tr>
<td>NOx removal (%) (at the time of sampling)</td>
<td>99</td>
</tr>
<tr>
<td>Ethanol dissolved (mol/h)</td>
<td>0.065</td>
</tr>
<tr>
<td>% Ethanol used for NO reduction</td>
<td>30–40</td>
</tr>
<tr>
<td>% Ethanol used for Fe(II)EDTA reduction</td>
<td>60–70</td>
</tr>
</tbody>
</table>
Aligner function. Subsequently, a neighbor-joining tree was created using the Jukes-Cantor correction mode. The statistical significance of the phylogenetic groups in the tree was tested using bootstrap analysis (1,000 replicates), which was performed with the software program PAUP.

Oligonucleotide probes used in this study. Specific probes for the isolated strain KT-1 and for members of the phylum Deferribacteres were designed using the Probe Design function in the ARB software (21). The complete 16S rRNA sequence of strain KT-1 was used for designing probes. In case of the Deferribacteres probe, sequences obtained from excised DGGE bands were used. Fixed cells of type strains (see above) were used as reference strains for testing the specificities of the probes. Table 2 gives an overview of the probes used in the study.

FISH. Samples from the reactors and from pure cultures were washed with 10 mM phosphate buffer (pH 7.2) containing 130 mM NaCl and were resuspended in the same buffer. Subsequently, the cells were fixed with paraformaldehyde for the fixation of gram-negative bacteria or with ethanol for the fixation of gram-positive bacteria. Hybridization was carried out according to the protocol described by Pernthaler et al. (31), using formamide concentrations as specified in Table 2. For gram-positive bacteria, an intermediate lysozyme incubation step of 30 min on ice was carried out, followed by another dehydration step through 50%, 80%, and 96% (vol/vol) ethanol as described previously (11, 25). The slides were embedded in Vectashield (Vector Laboratories, Burlingame, CA) and observed with a Zeiss Axioskop 2 epifluorescence microscope. Images were acquired with Leica FW4000 software.

Cell counts of strain KT-1, Deferribacter, and other bacteria were done by semiquantitative evaluation as described by Neef et al. (26). The hybridized samples were analyzed by two observers for determining the fraction of probe-positive cells relative to all cells visualized with the general bacterial probe Eub338 or DAPI (4′,6-diamidino-2-phenylindole) stain. The hybridization experiments were repeated three-times to confirm the results, and different microscopic fields of the slides were photographed and analyzed.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in the GenBank database under accession numbers AY920317 to AY920325.

**RESULTS**

Reactor operation. The nitrogen oxide removal efficiency in both lab- and pilot-scale reactors was between 90 and 99% within a temperature range from 45°C to 60°C (see Table 1 for the operational details of the reactors). In the pilot-scale system, the sulfur dioxide removal efficiency in the gypsum scrubber was 99% (data not shown). Sulfate concentrations of 20 to 70 mg/liter were measured in the pilot-scale reactor. Calculation of the stoichiometry of ethanol oxidation for NO reduction and Fe(III)EDTA\(^{-}\) reduction (based on the concentration of NO removed, the oxygen concentration in the influent gas, and the amount of ethanol consumed) showed that 60 to 80% of the ethanol was used for Fe(III)EDTA\(^{-}\) reduction and 20 to 40% was used for NO reduction (Table 1). In both reactors an approximate biomass concentrations of 1 g/liter was measured by the industrial operators.

**Enrichment and isolation of NO-reducing bacteria.** Enrichment of microorganisms reducing Fe(II)EDTA-NO\(^{-}\) was performed with inocula from both the lab-scale and pilot-scale reactors. The cultures showed a change in medium color from green to reddish brown after 2 to 3 days of incubation. The chemical analysis of controls and inoculated bottles confirmed the reduction of Fe(II)EDTA NO\(^{-}\) complex to \(\text{N}_2\) with Fe(II) EDTA\(^-\) as the electron donor. After six successive transfers (with a 1:10 dilution in every transfer), the enrichment culture obtained from the lab-scale reactor was used for isolation of pure cultures. Pure cultures were obtained in 2% (wt/vol) agar incubated at 55°C. Colonies appeared after 3 to 4 days and showed a similar morphology. Five of the colonies were picked and inoculated in different bottles with the same culture medium used for the enrichment. One of the bottles showed growth and was taken for indefinite serial liquid dilution to obtain a pure culture. The purity of the culture was checked by microscopic observation. In addition, PCR-DGGE analysis showed one band (Fig. 2, lane 7), indicating a pure culture.

**Characterization of strain KT-1.** After the purity was confirmed, the nearly complete 16S rRNA gene sequence of the isolate, named strain KT-1, was determined. Comparative analysis with sequences stored in the GenBank database showed a similarity of 95% with the 16S rRNA sequences of *Bacillus azotoformans* (33, 39). A neighbor-joining tree was created to infer the phylogenetic affiliation of strain KT-1 (Fig. 3). Strain KT-1 clustered with *B. azotoformans* (DSM 1046), as confirmed by a bootstrap value of 90%. DNA-DNA homology between strain KT-1 and *B. azotoformans* was 89%. Strain KT-1 and *Bacillus azotoformans* are gram-positive sporeformers and were checked for growth at different temperatures and pHs. Both strains showed optimum growth at pH 7 to 7.5. Strain KT-1 can grow within a temperature range of 40°C to 60°C, with an optimal temperature of 50°C to 55°C. *B. azotoformans* (DSM 1046) can grow between 30°C and 40°C, with 30°C as the optimal temperature for growth. Ethanol, acetate, succinate, and yeast extract were used by both strains as organic electron donors. Fe(II)EDTA\(^-\) can be used as an inorganic electron donor by both strains, with the supplement of an organic substrate (i.e., 100 mg/liter yeast extract) as a carbon source. The electron acceptors nitrate, nitrite, Fe(II)EDTA-NO\(^{-}\), and oxygen can be used by both strains, whereas Fe(III)EDTA\(^-\) was not used as an electron acceptor. Growth was observed for both strains when NO was flushed in

### TABLE 2. FISH probes used in this study

<table>
<thead>
<tr>
<th>Probe name</th>
<th>Target organism(s)</th>
<th>Probe sequence (5’ to 3’)</th>
<th>Length (nucleotides)</th>
<th>Formamide concen (%)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB 338</td>
<td>Eubacteria</td>
<td>GCT GCC TCC CGT AGG AGT</td>
<td>18</td>
<td>80–80</td>
<td>1, 7</td>
</tr>
<tr>
<td>ALF968</td>
<td>Alpha Proteobacteria</td>
<td>GGT AAG GTT CTC CGC GTT</td>
<td>18</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>BET42A</td>
<td>Beta Proteobacteria</td>
<td>GCC TTC CCA CTT CGT TT</td>
<td>17</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>GAM42A</td>
<td>Gamma Proteobacteria</td>
<td>GCC TTC CCA CAT CGT TT</td>
<td>17</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>Deferribacteres</td>
<td></td>
<td>CGG GGT CGC TGC GTC AGG</td>
<td>18</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>CF319a</td>
<td>Cytophaga</td>
<td>TGG TCC GTG TCT CAG TAC</td>
<td>18</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>HGC69a</td>
<td>Gram-positive, high-G+C bacteria</td>
<td>TAT AGT TAC CAC CGC CGT</td>
<td>20</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>ARCH915</td>
<td>Archaea</td>
<td>GTG CTC CCC CGC CAA TTC CT</td>
<td>20</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>BDN 584</td>
<td>Deferribacteres group</td>
<td>GCG GUG CGG UAA GUC AGG G</td>
<td>19</td>
<td>30</td>
<td>This study</td>
</tr>
<tr>
<td>BDN 174</td>
<td>KT-1</td>
<td>CGG GAU AAC UCU UUG GAU CG</td>
<td>20</td>
<td>35</td>
<td>This study</td>
</tr>
</tbody>
</table>
the headspace and ethanol was used as an electron donor [the medium did not have Fe(II)EDTA\textsuperscript{2−}].

Batch experiments were conducted with strain KT-1 in two different culture media, and kinetic data were obtained. In the first experiment (Fig. 4A), 5 mM nitrosyl complex, with Fe(II)EDTA\textsuperscript{2−} (25 mM) and ethanol (1 mM), were provided. The Fe(II)EDTA\textsuperscript{2−} oxidation to Fe(III)EDTA\textsuperscript{−} with the oxidation of ethanol and reduction of nitrosyl complex showed that Fe(II)EDTA\textsuperscript{2−} was used as the electron donor and ethanol as the carbon source. The biomass yield obtained was 4 mg protein/mol of nitrite. In the second experiment, ethanol was provided in surplus (i.e., 3 mM) with the same concentrations of nitrosyl complex and Fe(II)EDTA\textsuperscript{2−} (Fig. 4B). The results showed ethanol oxidation and nitrosyl complex reduction without significant iron oxidation, indicating that ethanol was used both as an electron donor and as a carbon source. The yield obtained was 5.6 mg protein/mol of nitrite.

PCR-DGGE analysis of the BioDeNOx microbial community. The microbial communities of the bioreactors from the lab- and pilot-scale reactor were studied by DGGE analysis of PCR-amplified 16S rRNA gene fragments (Fig. 2). All reactor samples showed a relatively low complexity, with a maximum of five bands. The microbial community in the lab-scale reactor changed over time and with temperature, and the communities of the lab- and pilot-scale reactors were only slightly different (Fig. 2). There were DNA bands with melting behavior similar to that for strain KT-1 (Fig. 2, bands PS1 and LS1). To identify the microorganisms in the reactors and to substantiate the presence of strain KT-1, the bands were excised and sequenced.

Identification of bacteria in the BioDeNOx process. A neighbor-joining tree was generated using the sequences of the excised DNA fragments, showing the phylogenetic affiliation of BioDeNOx community members (Fig. 3). Both the lab- and pilot-scale reactors showed the presence of DNA fragments at the same position in the gel as for strain KT-1. Nevertheless, comparative analysis of the sequences of these fragments (Fig. 2, bands PS1 and LS1) showed that they were more closely related to members of the Deferribacteres phylum, i.e., Deferribacter thermophilus (16) and Flexistipes sinusarabici (12).

The sequence from another DNA fragment of the pilot-scale reactor (Fig. 2, band PS2) was closely related to the genus Desulfitobacterium, a thermophilic sulfite- and Fe(III)-reducing bacterium (13). Furthermore, sequences of bacteria affiliated with members of the β subdivision of the Proteobacteria, such as Hydrogenophilus spp. and Alcaligenes spp., were identified in the lab-scale reactor sample (Fig. 2, band LS2). Sequences affiliated with the phylum Bacteroidetes, which includes members of the Cytophaga, Flavobacteria, and Bacteroides (Fig. 2, bands LS5, LS6, and LS7), and with the phylum Spirochaetes (Fig. 2, band LS4) were also identified in the lab-scale reactor samples.

In situ detection of members of the Deferribacteres group. Probes specific for the phylum Deferribacteres were designed from the sequences of the DGGE-separated DNA fragments. Probe BDN_584, which belongs to accessibility class IV (3), was tested with the type species Deferribacter thermophilus, Denitrovibrio acetophilus, and KT-1 as reference strains. The probe gave positive signals with D. thermophilus and D. acetophilus but not with strain KT-1. The probe was tested on samples from both the lab-scale (May 2002, 40°C) and pilot-scale (May 2002, 50°C) reactors. Approximately 65% of all cells were stained with this probe, using Eub338 as a general probe targeting all bacteria (Fig. 5A).

Of the other probes tested, only the probe specific for members of the β subdivision of the Proteobacteria gave a positive signal with some of the bacterial cells in the reactor samples (Fig. 5A).

In situ detection of the strain KT-1. Specific oligonucleotide probes were designed from the complete 16S rRNA sequence of strain KT-1 and used to study the relative abundance of this strain in the lab- and pilot-scale reactors. Probe BDN_174, which belongs to accessibility class III (3), was tested at different formamide concentrations with strain KT-1, Bacillus thermodenitrificans, Bacillus simplex, and Bacillus infernus. The probe gave a positive result with strain KT-1 and no results with the other bacteria. Subsequently, the probe was tested on different samples of the lab-scale (May 2002, 40°C) and pilot-scale (May 2002, 50°C) bioreactors. Approximately 20% of the total number of bacterial cells, as determined with DAPI, were stained with the probe in both samples, indicating a substantial presence of strain KT-1 (Fig. 5B).
Characterization of strain KT-1. KT-1 is a moderately thermophilic bacterium, which grows at a broad range of temperatures. The batch experiments show that strain KT-1 could reduce nitrosyl complex at a concentration of 5 mM with both Fe(II)EDTA$^{2-}$ and ethanol as electron donors. However, the results showed that ethanol is the preferred electron donor over Fe(II)EDTA$^{2-}$, and Fe(II)EDTA$^{2-}$ oxidation occurs only when ethanol is limited in concentration. The electron donors and acceptors tested showed that the strain is a facultative anaerobe.

The initial results of 16S rRNA sequencing of strain KT-1 showed only 95% similarity with the type species Bacillus azotoformans. This suggested that strain KT-1 could be a new Bacillus species belonging to the phylum Firmicutes of the low-G+C gram-positive bacteria, since the similarity percentage was lower than 97% (standard threshold percentage for new species description). However, DNA-DNA hybridization analysis of both strains gave a result of 89% homology. Clearly, this is far above the species common boundary (50 to 70%) described by Rosello-Mora and Amann (35) in their article discussing the comparison of DNA-DNA homology and 16S
rRNA similarities of 180 strains. The same article also mentioned that around 10% of these microorganisms, which fall inside the species common boundary, have a 16S rRNA similarity lower than 97%. In addition, high similarity of the whole-cell protein profile (results not shown) and physiological characteristics (except the optimum temperature for growth) of strain KT-1 and *B. azotoformans* (in this study) suggested that strain KT-1 is a new strain of the species *B. azotoformans*.

**Analysis of diversity and dynamics in BioDeNOx reactors.** DGGE analysis showed that the microbial community in the BioDeNOx reactors was changing with time, temperature, and flue gas composition (Fig. 2). In the pilot-scale reactor, apart from oxygen, some traces of untreated SO₂ in the flue gas could also have been the reason for the difference in the banding pattern. The detection of SO₄²⁻ (20 to 70 mg/liter) in the bioreactor can be explained because of SO₂ oxidation.

The low microbial diversity observed in DGGE may be due to the high temperature of 50 to 55°C at which the process is carried out. The high temperature might have eliminated mesophilic bacteria, which are commonly found in other processes and environments.

**Microbial community composition in BioDeNOx reactors.** Both the lab- and pilot-scale reactors showed sequences closely affiliated to type strains of the phylum *Deferribacteres*. The literature indicated that the members of the phylum *Deferribacteres* are moderately thermophilic bacteria that can reduce Fe(III), nitrate, arsenate, and sulfur by using organic substrates as electron donors (12, 16). *Deferribacter thermophilus* is a thermophilic dissimilatory iron-reducing bacterium isolated from a petroleum reservoir (16), and *Flexistipes sinusarabici* is a thermophilic bacterium isolated from hot brine water of the Red Sea (12). In addition to these bacteria, the pilot-scale reactor biomass showed sequences affiliated with the genus *Desulfotobacterium* (13), which can reduce sulfate as well as Fe(III). The remaining amount of SO₂ in the flue gas of the pilot-scale reactor could be the reason for this bacterium to appear in the reactor.

Hybridization analysis with probe BDN_584, designed for the *Deferribacteres* group, confirmed their presence in large numbers both in the lab- and pilot-scale reactors (Fig. 5A). This is consistent with the DGGE results. FISH results with probe BDN_174, specific for strain KT-1, showed that the isolate is a representative bacterium in the samples from both reactors (approximately) but cannot be considered the most dominant species in either reactor (Fig. 5B). This is in contrast to the DGGE results, where sequences of strain KT-1 were not found. The reason for this difference could be a bias in the PCR or the relatively low number of cells. Also, the gram-positive cell wall structure would have been highly resistant to the cell wall disruption step of the DNA extraction methodology.

FISH experiments using general probes gave positive signals with bacteria belonging to the β subclass of the *Proteobacteria*. This is also consistent with PCR-DGGE results (Fig. 3). The positive result obtained with β subclass-specific probes (20 to 25% of the biomass [Fig. 5A]) indicates that these bacteria might play a role in the BioDeNOx process.

The oligonucleotide probe for members of the phylum *Bacteroidetes* did not show a positive signal, in contrast to the DGGE sequencing results. This may be because these microorganisms were low in number or because the general probe for this phylum does not target them. However, *Cytophaga*-like bacteria cannot be directly related to the process function, as it is known that they are common in anaerobic microbial communities as fermentative chemoorganoheterotrophs (15). In addition, DGGE sequences closely related to *Spirochaeta* were detected in only one sample of the lab-scale reactor, indicating that this group does not play an important role in the process.

**Conclusions.** The combination of culture-dependent and -independent approaches gave important information on the
structure and function of the microorganisms involved in the BioDeNOx process. Strain KT-1, isolated from the BioDeNOx reactor, is a moderately thermophilic strain of the species *Bacillus azotoformans*. It can reduce Fe(II)EDTA/NO₂⁻ to N₂ but does not reduce Fe(III)EDTA⁻. DGGE analysis of PCR-amplified 16S rRNA gene fragments showed sequences of community members closely affiliated with *Deferribacter thermophilus*, which is a moderately thermophilic iron-reducing bacterium. Moreover, fluorescence in situ hybridization with KT-1-specific and *Deferribacter*-specific probes showed that the former covered between 15 and 20% and the later around 65% of the overall microbial community. The process data showed that ethanol as an electron donor was used predominantly (60 to 70%) for the reduction of Fe(III)EDTA⁻ and only 20 to 30% for the reduction of the nitrosyl complex. This indicates that members of the phylum *Deferribacteres* are important players in the reduction of Fe(III)EDTA⁻. For a better insight into the dynamics of the microbial community, however, replicate samples from the bioreactors need to be taken more frequently. Future work should concentrate on this and on the isolation of community members involved in the reduction of Fe(III)EDTA⁻.

ACKNOWLEDGMENTS

Esenigli Yildirim and Paul Roosken are acknowledged for their technical assistance.

The Dutch Science Foundation for Applied Research (STW) and Biorstat Development C.V. financially supported this research (project WMK 4963).

Collaborative research was carried out by scientists of the Department of Environmental Technology at Wageningen University and the Department of Chemical Engineering at the University of Groningen.

REFERENCES


