Molecular characterisation of the microbial community of a full-scale bioreactor treating Bayer liquor organic waste

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ABSTRACT

Sodium oxalate is an organic impurity produced during the Bayer refining of bauxite as a result of the degradation of humic materials associated with the ore. Physico-chemical oxalate destruction techniques, such as combustion are often expensive and often pose greater environmental risks than the storage of solid oxalate waste. Biodegradation is an economical and environmentally friendly way to degrade oxalate, but the microbial communities responsible for degradation have remained largely uncharacterised. In the present work the microbial community of a full-scale bioreactor achieving complete degradation of sodium oxalate was characterised using 16S rRNA gene clone libraries followed by phylogenetic analysis of the near full length gene clone sequences. The community was dominated by species belonging to the α-, β- and γ-*Proteobacteria* groups. Novel oxalate-degrading bacteria belonging to the genus *Halomonas* and the β-*Proteobacteria* group were isolated from the microbial community and are currently being characterised.

***Keywords**: bacteria, biotechnology, non-ferrous metallic ores, waste processing*

INTRODUCTION

The digestion of bauxite with hot caustic soda results in the co-digestion of humic and fulvic materials associated with the host rock, and produces soluble sodium salts of organic acids including acetate, formate, malonate, succinate and oxalate. The majority of these remain in solution and pose no problem to the Bayer process. Sodium oxalate remains soluble in the process liquor but co-precipitates with the agglomeration of alumina, resulting in the production of fine, non-uniform crystals. This affects the purity and particle size of the final alumina product making it unsuitable for the production of aluminium and resulting in the loss of alumina and caustic soda as dust. Alumina and caustic losses represent a significant increase in operating costs to alumina refineries. Sodium oxalate can be removed from the Bayer liquor by crystallisation in a diverted portion of the process stream, which enables its separation from the liquor by filtration. Sodium oxalate has traditionally been destroyed by combustion, which results in the release of carbon dioxide and other gases, or contained in large storage facilities, creating a further significant cost to refineries.

Oxalate-degrading microorganisms are ubiquitous in nature, and the biological destruction of oxalate in the rhizosphere of oxalate-producing plants and the gastrointestinal tracts of animals has been described (Şahin, 2003). Bioreactors for the treatment of sodium oxalate produced during the Bayer processing of bauxite are used at various alumina refineries (Brassinga *et al.*, 1990; Chinloy *et al.*, 1993; Morton *et*

al., 1991; Thè *et al.*, 1990), however, the identity and ecology of microorganisms responsible for sodium oxalate oxidation have been poorly described.

Less than 1% of environmental microorganisms have been described using culture-dependent microbiological methods (Kehrmeyer *et al.*, 1996; Lindahl and Bakken, 1995). The use of culture independent techniques, such as molecular biology has facilitated the detection and identification of a wealth of non-cultivable microorganisms from environmental samples. Targeted 16S rRNA gene surveys of the microbial populations in pilot- and full-scale bioreactors treating Bayer liquor organic wastes, using the polymerase chain reaction in combination with denaturing gradient gel electrophoresis (PCR-DGGE), have indicated that microorganisms belonging to the α, β and γ subgroups of the *Proteobacteria* dominate these processes (McSweeney *et al.*, 2009). In this study 16S rRNA gene clone libraries were constructed to estimate the abundance and distribution of these microbial groups in the attached and suspended biomass of a full-scale moving bed biofilm reactor (MBBR) achieving complete degradation of oxalate.

MATERIALS AND METHODS

Bioreactor operating conditions and sampling

The oxalate-degrading bioreactor consisted of 2 trains (BU1 and BU2), each comprising three MBBRs (150 m^3) (Fig. 1). Each train was supplied with carbonated process liquor (pH 9.5) containing approximately 10 g/L oxalate at a flow rate of 40 m^3/h, with the feed split equally to MBBRs 1 and 2. MBBRs 1 and 2 were considered to be replicates of each other so sampling was only performed on MBBR 1 of each

bioreactor unit. MBBR 3 was operated in sequence to MBBRs 1 and 2 and as all oxalate destruction was observed in these two MBBRs, samples were not obtained from MBBR 3 for this study.

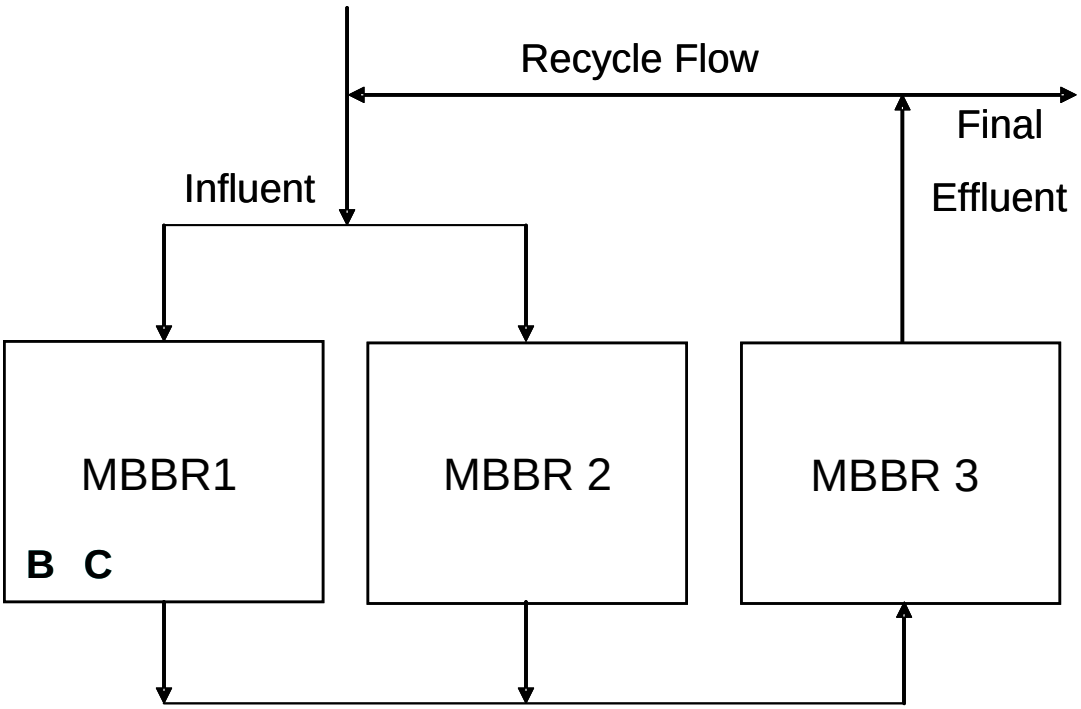


Figure 1 Schematic flow diagram of the bioreactor units (BU). Samples were obtained from the tank liquor (B) and biofilm beads (C) of MBBR 1 of each BU.

Each MBBR contained high density polyethylene beads (50% w/v; AnoxKaldnes, Natrix model O), which provide a protected surface area of 300 m^2/m^3 for microbial growth and biofilm formation. Steady-state operation of the full-scale bioreactor was considered to be achieved when both units had been operating stably and consistently for more than 3 months, and achieving complete degradation of oxalate (9.6 T/day) in the influent feed. Liquid samples and beads were collected from the first MBBR (MBBR 1) of each of BU1 and BU2, and transported to the laboratory within 4 h of sampling for measurement of pH and determination of cell numbers. The samples were stored at −80°C for subsequent molecular analysis.

DNA extraction and amplification

Alumina and other particulates were separated from the liquid samples by low speed centrifugation (3000 × *g*, 1 min). Cells in the supernatant were pelleted (10, 000 × *g*, 8 min) and used for DNA extraction. The biofilm was physically removed from beads using a sterile spatula. The cells from the liquid samples and the detached biofilm were lysed with lysozyme and low speed bead beating (Plumb *et al.*, 2002). Total genomic DNA was extracted using phenol:chloroform:isoamyl alcohol (24:1:1, pH 8.0) and precipitated with sodium acetate (3 M, pH 5.2) and isopropanol, as described previously (Plumb *et al.*, 2002). DNA was extracted from the liquid and bead biofilms from MBBR 1 of each of BU1 and BU2.

The 16S rRNA genes were amplified (HotStar Taq Kit; Qiagen) using bacterial 27F forward primer (5′-GAG TTT GAT CCT GGC TCA G-3′; 25 pM) and universal 1492R modified reverse primer (5′-ACG GdIT ACC TTG TTA CGA CTT-3′; dI = 2′-deoxyInosine modification; 25 pM) and a thermal cycling program of: 94°C for 15 min, 35 cycles of 95°C for 1 min, 48°C for 1 min, 72°C for 2 min, and 10 min at 72°C. PCR products were purified using UltraClean PCR CleanUp Kit (MO BIO Laboratories Inc. USA).

Clone library construction

Ligations were carried out using the pGEM-T Easy Vector Systems (Promega) kit with vectors carrying a *lacZ* gene and an ampicillin resistance gene (*Amp*^{r}). Ligated products were transformed into XL2-Blue MRF′ Ultracompetent cells (Genotype: Δ(*mcrA*)*183* Δ(*mcrCB-hsdSMR-mrr*)*173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^r) Amy Cam^r]; Stratagene, Agilent Technologies).

Transformed cells were diluted and spread-inoculated onto Luria Broth agar containing ampicillin and X-gal. Clones were screened by colour, based on the successful ligation of the 16S rRNA gene insert into the *lacZ* gene. Colonies that were blue in colour had a functional *lacZ* gene, indicating that there was no 16S rRNA gene fragment inserted into the transformed vector. Colonies that were white had a non-functional *lacZ* gene, indicating that ligation of the insert into the vector had been successful. White colonies were picked and subcultured aseptically onto another LB plate containing ampicillin, X-Gal and isopropyl β-D-1-thiogalactopyranoside (IPTG) to confirm the presence of the insert. A blue clone was also picked from each plate as a negative control. A total of 150 transformed white colonies were picked for each bioreactor sample analysed. Picked colonies were lysed and inserts were amplified as described above using the primers T7 (5′-TAATACGACTCACTATAGGG-3′; 25 pM) and SP6 (5′-CGATTTAGGTGACACTATAG-3′; 25 pM). Amplified inserts were run on a 1% (w/v) agarose gel with Hyperladder I molecular weight marker to determine the length of the amplified fragment. Clones that contained the correct insert size were sequenced.

Phylogenetic analysis

Amplified 16S rRNA gene fragments were sequenced at Macrogen Inc. (Korea) using BigDye Terminator cycle sequencing with 518F primer (5′-CCAGCAGCCGCGGTAATACG-3′; 10 pM). Sequences were analysed using ChromasPro (www.technelysium.com.au/ChromasPro.html), and phylogenetic affiliations were initially determined using BLAST (Basic Local Alignment Search Tool; www.ncbi.nlm.nih.gov/BLAST/). Inserts that did not have readable sequences were discarded prior to alignment. Partial 16S rRNA gene fragments were aligned

using the ClustalW function of the Mega4 software, using reference sequences obtained from the NCBI GenBank database. Sequence similarity was calculated using a complete deletion distance matrix and neighbour-joining method with Jukes and Cantor single parameter correction in the Mega4 software. Partial sequences that were 99% similar were grouped into the same operational taxonomic unit (OTU).

Representative near full-length insert fragments were sequenced at Macrogen Inc. (Korea) using the forward primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′), 357F (5′-CCTACGGGAGGCAGCAG-3′) and 518F (5′-GWATTACCGCGGCKGCTG-3′) and reverse primers 800R (5′-TACCAGGGTATCTAATCC-3′ 907R (5′-GTGCTCCCCCGCCAATTCCT-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Primer concentrations were 10 pM. Near full-length 16S rRNA gene sequences were constructed using ChromasPro and aligned using the ClustalW function of Mega4. A phylogenetic tree of representative clone 16S rRNA gene fragments was constructed in Mega4 using the complete deletion distance matrix and neighbour-joining method with Jukes and Cantor single parameter correction.

Estimation of biodiversity

The microbial diversity in the suspended and attached biomass in MBBR 1 of each of BU1 and BU2 was estimated by plotting the cumulative number of different OTUs in each clone library against the number of clones analysed. A curve was fitted using the equation $y = x/(ax+b)$, where y is the cumulative OTU number, x is the number of clones analysed, and a and b are constants (Sekiguchi *et al.*, 1998). The constants were solved using an ordinary least squares method in Microsoft Excel 2003. The

theoretical maximum number of different phylotypes in each sample was estimated from the equation where *y* becomes *1/a* if *x* = infinity.

RESULTS

Bioreactor operation

The process performance of BU1 and BU2 at the time of sampling is outlined in Table 1, which shows that the two trains were effectively removing all the oxalate added in the influent stream.

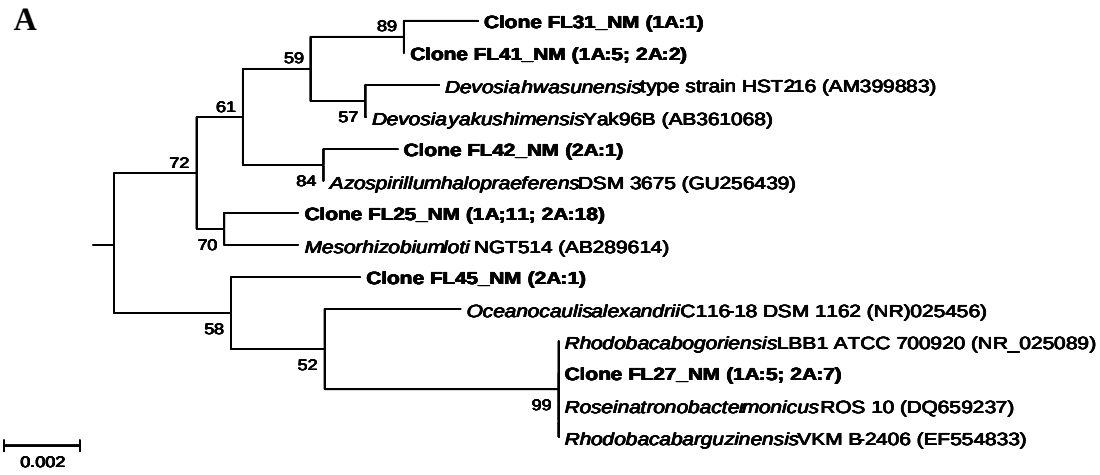
Table 1. Performance characteristics of bioreactor units BU1 and BU2 at the time of sampling for construction of clone libraries.

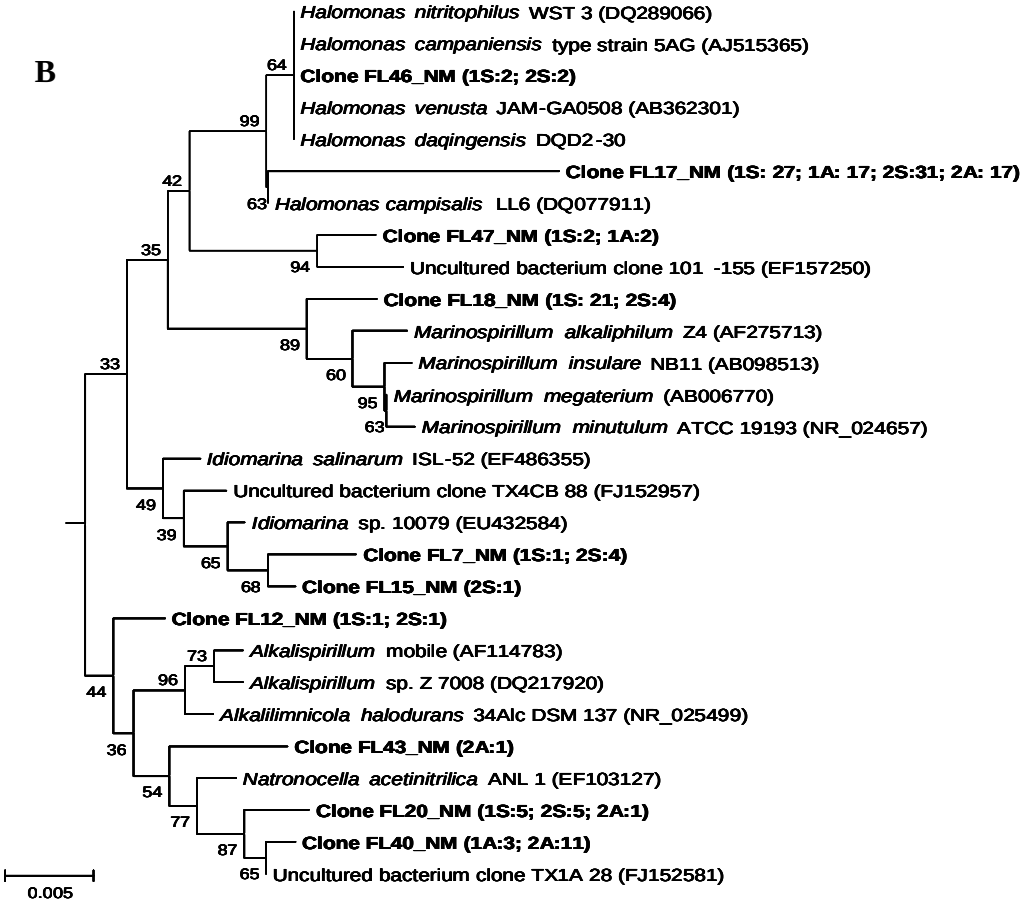
	BU1	BU2
Flow Rate (m^3/h)	16.2	30.5
Hydraulic retention time (h)	27.7	14.7
Average temperature (°C)	38.6	37.7
Average pH	9.7	9.7
Influent oxalate concentration (g/L)	22.5	31.3
Effluent oxalate concentration (g/L)	0.04	0
MBBR 1 Degradation Rates (kg/m3.d)	19.46	51.12
Cell count in MBBR 1 (cells/mL)	3.9×10^8	5.3×10^8
MBBR 1 Bead Cell Count (cells/bead)	9.7×10^{10}	7.2×10^{10}

Phylogenetic analysis of 16S rRNA gene clones

Four clone libraries constructed from the suspended and attached bacterial biomass from MBBR 1 of both bioreactor units resulted in 150 clones in each of the libraries. One hundred clones from each library were randomly selected for analysis; clones with the incorrect insert size were discarded. Analysis of initial sequencing of the 16S

rRNA gene inserts with primer 530F revealed 13 distinct phylogenetic groups in both the suspended and attached biomass of both MBBRs. The sequence similarity between the members within each group was 99%. A phylogenetic tree was constructed after obtaining the near-complete 16S rRNA gene sequence of representative clones from each group (Fig. 2).


A
89
Clone FL31_NM (1A:1)
59
Clone FL41_NM (1A:5; 2A:2)
Devosia hwasunensis type strain HST216 (AM399883)
61
57
Devosia yakushimensis Yak96B (AB361068)
Clone FL42_NM (2A:1)
72
84
Azospirillum halopraeferens DSM 3675 (GU256439)
Clone FL25_NM (1A;11; 2A:18)
70
Mesorhizobium loti NGT514 (AB289614)
Clone FL45_NM (2A:1)
Oceanocaulis alexandrii C116-18 DSM 1162 (NR)025456)
58
Rhodobacabogoriensis LBB1 ATCC 700920 (NR_025089)
52
Clone FL27_NM (1A:5; 2A:7)
99
Roseinatronobacter monicus ROS 10 (DQ659237)
Rhodobacabarguzinensis VKM B-2406 (EF554833)
0.002


B
Halomonas nitritophilus WST 3 (DQ289066)
Halomonas campaniensis type strain 5AG (AJ515365)
64
Clone FL46_NM (1S:2; 2S:2)
Halomonas venusta JAM-GA0508 (AB362301)
99
Halomonas daqingensis DQD2-30
Clone FL17_NM (1S: 27; 1A: 17; 2S:31; 2A: 17)
42
63
Halomonas campisalis LL6 (DQ077911)
Clone FL47_NM (1S:2; 1A:2)
35
94
Uncultured bacterium clone 101 -155 (EF157250)
Clone FL18_NM (1S: 21; 2S:4)
Marinospirillum alkaliphilum Z4 (AF275713)
89
Marinospirillum insulare NB11 (AB098513)
33
60
Marinospirillum megaterium (AB006770)
95
63
Marinospirillum minutulum ATCC 19193 (NR_024657)
Idiomarina salinarum ISL-52 (EF486355)
Uncultured bacterium clone TX4CB 88 (FJ152957)
49
Idiomarina sp. 10079 (EU432584)
39
Clone FL7_NM (1S:1; 2S:4)
65
68
Clone FL15_NM (2S:1)
Clone FL12_NM (1S:1; 2S:1)
Alkalispirillum mobile (AF114783)
73
96
Alkalispirillum sp. Z 7008 (DQ217920)
44
Alkalilimnicola halodurans 34Alc DSM 137 (NR_025499)
36
Clone FL43_NM (2A:1)
Natronocella acetinitrilica ANL 1 (EF103127)
54
Clone FL20_NM (1S:5; 2S:5; 2A:1)
77
Clone FL40_NM (1A:3; 2A:11)
87
65
Uncultured bacterium clone TX1A 28 (FJ152581)
0.005

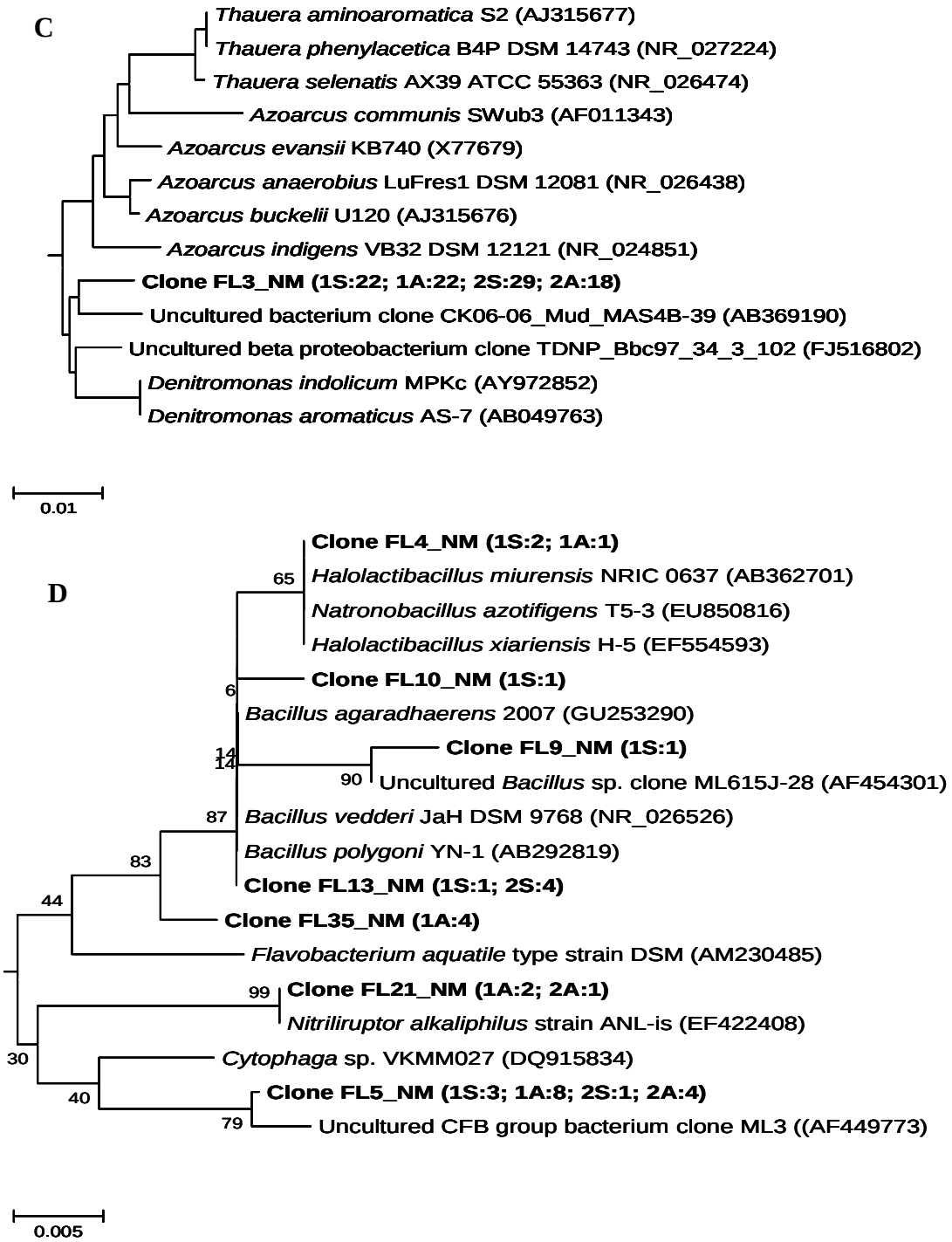


Figure 2. Phylogenetic trees generated using distance matrix and neighbour-joining methods based on 16S rRNA gene sequences of clones obtained from the suspended (1S and 2S, respectively) and attached (1A and 2A, respectively) biomass (bold) of bioreactor units BU1 and BU2, and reference sequences obtained from the NCBI database. The total number of similar clones retrieved from each biomass sample is shown in parentheses. A: Matches to the α-*Proteobacteria* subgroup. B: Matches to the γ-*Proteobacteria* subgroup. C: Matches to the β-

***Proteobacteria* subgroup. D: Matches to the classes *Firmicutes, Actinobacteria* and *Bacteroidetes*. Numbers at nodes represent bootstrap values based on 1000 iterations. *Flavobacterium aquatile* (AM230485) was used as an outgroup for trees A, B and C. *Archeoglobus veneficus* (AF418181) was used as an outgroup for tree D. The scale bars in A–D represent changes per nucleotide.**

Among the analysed clones, 13 and 15 different OTUs were detected in the suspended and attached biomass of MBBR 1 of BU1, respectively, and 13 and 16 different OTUs were detected in the suspended and attached biomass of MBBR 1 of BU2, respectively. The distributions of the different OTUs in the suspended and attached biomass of the MBBRs are shown in Tables 2 and 3.

Table 2. Distribution of operational taxonomic units (OTUs) in 16S rRNA gene clone libraries constructed from the DNA extracted from suspended and attached biomass of MBBR 1 of BU1.

Group	Suspended Biomass			Attached Biomass		
	OTUs	Number of clones	Percentage of total clones	OTUs	Number of clones	Percentage of total clones
Proteobacteria						
α-Proteobacteria	-	-	-	3	22	27
β-Proteobacteria	2	22	24	2	22	27
γ-Proteobacteria						
Halomonas	3	32	36	3	18	22
Marinospirillum	1	21	23	-	-	-
Alkalispirillum	1	1	1	-	-	-
Idiomarina	1	1	1	-	-	-
Natronocella	1	5	6	1	3	4
Firmicutes						
Bacillus	1	2	2	1	4	5
Natronobacillus	1	2	2	1	1	1
Other	1	1	1	1	1	1
Actinobacteria	-	-	-	2	2	2
CFB Group	1	3	3	1	8	10
Total	13	90	100	15	81	100

Table 3. Distribution of operational taxonomic units (OTUs) in 16S rRNA gene clone libraries constructed from the DNA extracted from suspended and attached biomass of MBBR 1 of BU2.

Group	Suspended Biomass			Attached Biomass		
	OTUs	Number of clones	Percentage of total clones	OTUs	Number of clones	Percentage of total clones
Proteobacteria						
α-Proteobacteria		-	-	5	29	34
β-Proteobacteria	2	29	34	3	19	22
γ-Proteobacteria						
Halomonas	3	34	40	1	19	22
Marinospirillum	2	4	5		-	-
Alkalispirillum	1	1	1		-	-
Idiomarina	2	5	6		-	-
Natronocella	1	5	6	1	12	14
Other		-	-	1	2	2
Firmicutes						
Bacillus	1	4	5		-	-
Other	1	3	3		-	-
Actinobacteria		-	-	1	1	1
CFB Group	1	1	1	4	4	5
Total	14	86	100	16	86	100

Phylogenetic analyses of the clone libraries constructed from the attached and suspended biomass in MBBR 1 of both bioreactor trains showed that the bacterial biodiversity was low but similar in both. The dominant microorganisms by percentage composition belonged to the γ-, β- and α-Proteobacteria groups. Species belonging to the genus *Halomonas* represented approximately 36–40% of the suspended biomass

and 22% of the attached biomass in each MBBR. Matches to an unknown species of β-*Proteobacteria* represented approximately 25–35%of the attached and suspended biomass in each MBBR. Matches to members of the α-*Proteobacteria* represented approximately 27–34% of the attached biomass, but was not detected in the suspended biomass of either MBBR. Matches to less dominant groups including *Bacillus* spp., an uncultured *Actinobacteria*, and the *Cytophaga-Flexibacter-Bacteroidetes* group were also detected, but based on their percentage composition within the attached and suspended biomass of each MBBR, these microorganisms were probable derived from soil and water in the environment adjacent to the refinery.

Estimation of bacterial diversity

The number of different phylotypes was estimated theoretically by analysing the cumulative OTU distributions of the clone libraries constructed for both the suspended and attached biomass of MBBR 1 of both bioreactor units. The cumulative OTU distributions showed that the theoretical maximum number of different phylotypes in the suspended and attached biomass of MBBR 1 of BU1 was 16 and 20, respectively (Fig. 3). The theoretical maximum number of different phylotypes in the suspended and attached biomass of MBBR 1 of BU2 was 18 and 16, respectively (Fig. 4).

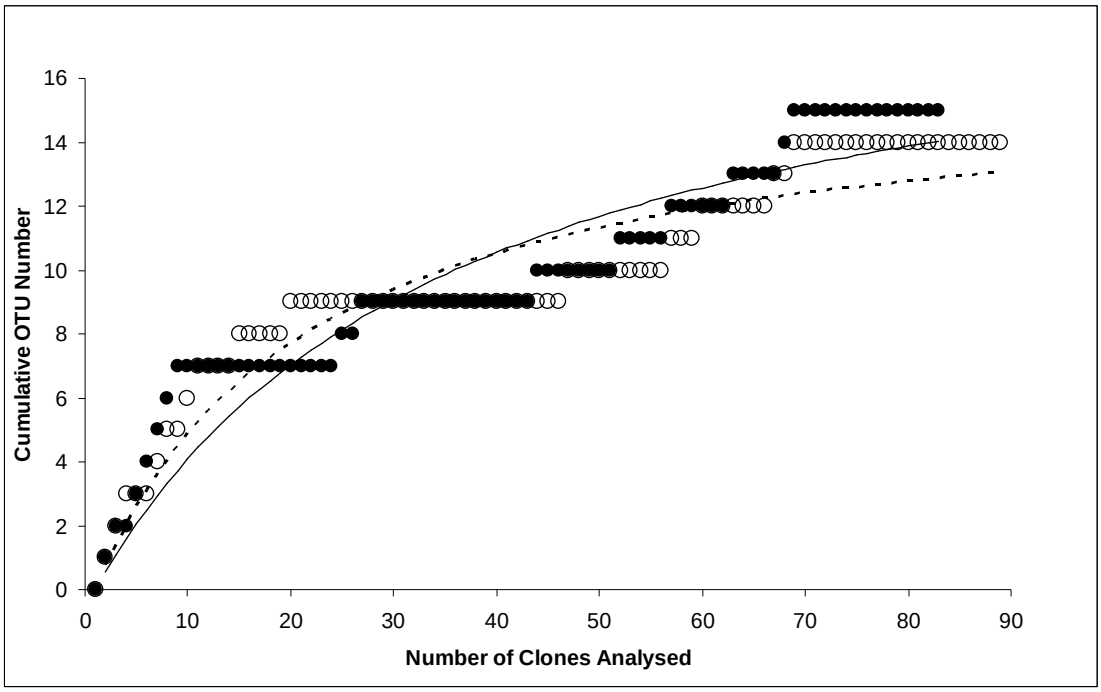


Figure 3. Estimation of the bacterial diversity in the suspended (○, ----) and attached (●, ▬) biomass of MBBR 1 of BU1 based on cumulative number of operational taxonomic units (OTUs). The sequential detection of cumulative OTUs reflects the order of detection, which was assumed to be stochastic relative to the distribution of clones generated from the sample library. The prediction of cumulative lines was performed using the curve from the equation $y = x/(ax+b)$, where y is the cumulative number of OTUs, x is the number of clones analysed, and a and b are constants.

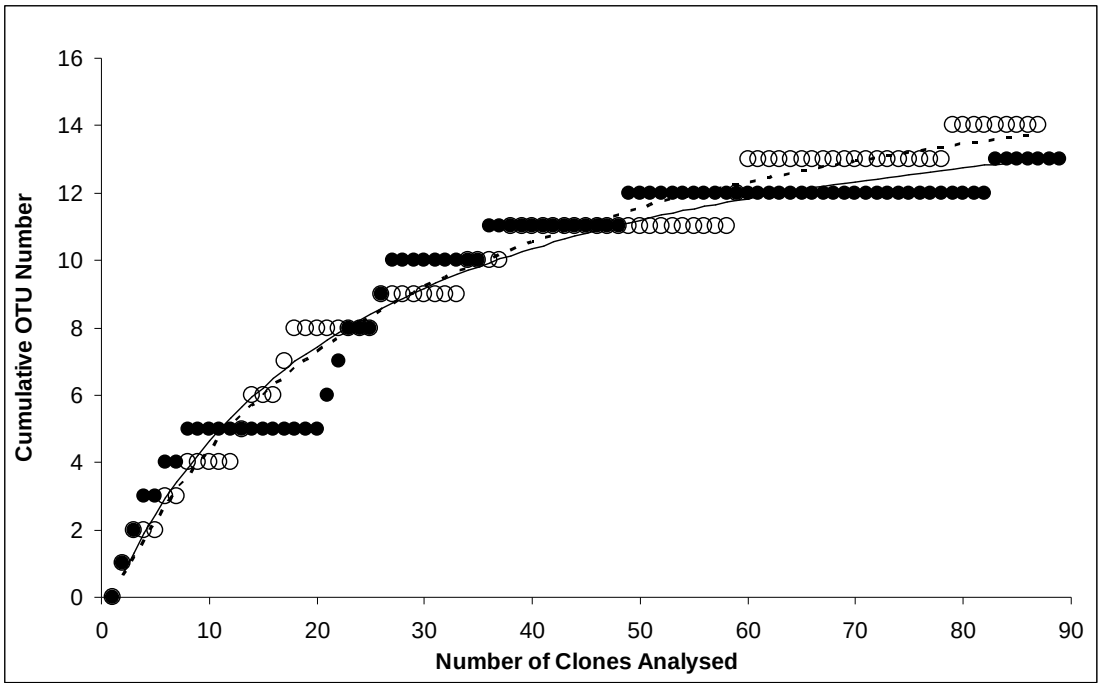


Figure 4. Estimation of bacterial diversity in the suspended (○, ----) and attached (●, ▬) biomass of MBBR 1 of BU2 based on cumulative number of operational taxonomic units (OTUs). The sequential detection of cumulative OTUs reflects the order of detection, which was assumed to be stochastic relative to the distribution of clones generated from the sample library. The prediction of cumulative lines was performed using the curve from the equation $y = x/(ax+b)$, where y is the cumulative number of OTUs, x is the number of clones analysed, and a and b are constants.

DISCUSSION

16S rRNA gene clone libraries were used to estimate the abundance and diversity of the microbial community in both the suspended and attached biomass in two MBBR units of a full-scale oxalate-degrading bioreactor. Analysis of the clones in each library indicated that the dominant members of the community belonged to the β- and γ-*Proteobacteria* subgroups. The detection of these groups in the libraries is consistent with those detected in a pilot-scale bioreactor treating sodium oxalate, using PCR-DGGE (McSweeney *et al.*, 2009). The 16S rRNA gene clone libraries also

show that these microorganisms were numerically dominant, which indicates that they are the likely major contributors to the biological degradation of oxalate.

All microorganisms detected in the clone library analysis have been described as organotrophic, halophilic and neutrophilic or alkaliphilic bacteria (Anders *et al.*, 1995; Boldareva *et al.*, 2008; Boldareva *et al.*, 2007; Mormile *et al.*, 1999; Zhou *et al.*, 1995). Cloned sequences grouping with the genus *Halomonas* and an uncultured species of β-*Proteobacteria* constituted a large proportion of the microbial community in both the suspended and attached biomass of each MBBR. Members of the genus *Halomonas* are typically organotrophic and have the ability to degrade most organic acids (including acetate and formate), but have previously been thought to be unable to utilise oxalate as a sole source of carbon and energy (Caton *et al.*, 2004; Joshi *et al.*, 2008; Mormile *et al.*, 1999; Romano *et al.*, 1996). Many microorganisms belonging to the subgroup β-*Proteobacteria* are involved in the cycling of nitrogen through denitrification or nitrogen fixation process, and are also well known for their ability to degrade environmental pollutants including toluene and other hydrocarbons (Anders *et al.*, 1995; Zhou *et al.*, 1995). It is possible that this group plays a key role in nitrogen cycling in the bioreactor in addition to its involvement in the degradation of oxalate. Oxalate-degrading microorganisms most closely related to *Halomonas* and β-*Proteobacteria* detected in the bioreactor are currently being isolated and characterised. Research into their roles in the biological destruction of oxalate is also underway.

Sequences representing microorganisms belonging to the α subgroup of the *Proteobacteria* were only detected in the attached biomass of the MBBRs, and not in

the suspended biomass. This finding is consistent with the results of the PCR-DGGE analysis of the pilot-scale bioreactors used in the development of the full-scale process (McSweeney *et al.*, 2009). The microorganisms with sequences most closely related to the α subgroup members found in this study are *Roseinatronobacter monicus* ROS 10, *Rhodobaca* (*Rca*.) *barguzinensis* and *Rca. bogoriensis*. These are all obligately aerobic, heterotrophic and haloalkaliphilic, and can produce pigments under reduced light conditions or grow photosynthetically (Boldareva *et al.*, 2008; Boldareva *et al.*, 2007; Milford *et al.*, 2000). These microorganisms may be slow growing under the operating conditions of the bioreactor, and attached growth may prevent washout of their biomass from the bioreactor.

Estimation of the bacterial diversity based on the cumulative OTU distribution indicated that the theoretical maximum number of different phylotypes in the suspended and attached biomass in MBBR 1 of BU1 was 16 and 20, respectively, and in MBBR 1 of BU2 was 18 and 16, respectively. The total number of OTUs detected in analysis of the suspended and attached biomass in MBBR 1 of BU1 was 13 and 15, respectively, and in MBBR1 of BU2 was 14 and 16, respectively, which in both cases was relatively close to the theoretical maximum numbers determined. These results highlight that in each of the MBBR units the biodiversity was low and the number of groups of microorganisms was similar. This suggests that a specialised biomass is required for the biological removal of oxalate, and that the operating conditions are only suitable for a narrow range of microbial groups.

Based on analysis of the microbial ecology in pilot- and full-scale bioreactors it is evident that the microbial community responsible for the biological oxidation of

oxalate are conserved in both processes and not highly diverse. This, and the detection of some common soil and water microorganisms in the PCR-DGGE analysis of the pilot-scale bioreactor (McSweeney *et al.*, 2009) and in the clone library analysis of the full-scale process (this study) also indicate that the microorganisms in the oxalate-degrading bioreactors are probably indigenous to the water and soil environments in the vicinity of the bioreactor site.

The use of 16S rRNA gene clone libraries in this study enabled the estimation of the abundance and distribution of the dominant groups of bacteria in a full scale bioreactor which had been previously detected (by not quantified) by PCR-DGGE of pilot-scale bioreactors (McSweeney *et al.*, 2009). Full characterisation of the microbial communities involved in the biological destruction of oxalate in bioreactors will provide insights into the optimum growth conditions of these microorganisms, and aid the establishment of full-scale bioreactor processes at refineries worldwide. The identification and characterisation of isolated oxalate-degrading bacteria from the full-scale bioreactor is currently underway. This is the first step in developing a greater understanding of the role of individual members of the bioreactor microbial community in the oxidation of oxalate, with the objective of maximising the efficiency of oxalate degradation.

Acknowledgements

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