Biodegradation (2009) 20:559–567
DOI 10.1007/s10532-008-9245-8

ORIGINAL PAPER

Biological sulphate reduction using food industry wastes as carbon sources

Mónica Martins · Maria Leonor Faleiro · Raúl Jorge Barros · Ana Raquel Veríssimo · Maria Clara Costa

Received: 6 March 2008 / Accepted: 29 December 2008 / Published online: 11 January 2009

Abstract Biological treatment with dissimilatory sulphate-reducing bacteria has been considered the most promising alternative for decontamination of sulphate rich effluents. These wastewaters are usually deficient in electron donors and require their external addition to achieve complete sulphate reduction. The aim of the present study was to investigate the possibility of using food industry wastes (a waste from the wine industry and cheese whey) as carbon sources for dissimilatory sulphate-reducing bacteria. The results show that these wastes can be efficiently used by these bacteria provided that calcite tailing is present as a neutralizing and buffer material. A 95 and 50 % sulphate reduction was achieved within 20 days of experiment by a consortium of dissimilatory sulphate-reducing bacteria grown on media containing waste from the wine industry or cheese whey respectively. Identification of the dissimilatory sulphate-reducing bacteria community using the *dsr* gene revealed the presence of the species *Desulfovibrio fructosovorans, Desulfovibrio aminophilus* and *Desulfovibrio desulfuricans*. The findings of the present study emphasise the potential of using wastes from the wine industry as carbon source for dissimilatory sulphate-reducing bacteria, combined with calcite tailing, in the development of cost effective and environmentally friendly bioremediation processes.

Keywords Calcite tailing · Carbon sources · Cheese whey · Dissimilatory sulphate-reducing bacteria · Wine industry waste

M. Martins · M. C. Costa
Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

M. L. Faleiro · A. R. Veríssimo
Centro de Biomedicina Molecular e Estrutural (CBME), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

R. J. Barros
Centro de Investigação de Química do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

M. C. Costa (✉)
FERN, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
e-mail: mcorada@ualg.pt

Introduction

In recent years several bioremediation processes based on the use of dissimilatory sulphate-reducing bacteria (DSRB) have been developed for the treatment of acid mine drainage (AMD) (Neculita et al. 2007; Johnson and Hallberg 2005; Tabak and Govind 2003; Steed et al. 2000) or other sulphate-rich effluents (Burgess and Stuetz 2002; Lima et al. 2001). DSRB use sulphate as electron acceptor and an energy rich carbon source as electron donor (Pfenning et al. 1981), generating sulphide. This sulphide reacts additionally with certain

metals dissolved in contaminated waters, such as copper, iron or zinc, forming insoluble precipitates (Vega-López et al. 2007; Costa and Duarte 2005; White et al. 2003) and, as a result, the concentrations of sulphates and dissolved metals are reduced.

Considering that sulphate reduction is an energy intensive process (Barnes 1998), a considerable amount of an energy-rich reductant is required. Consequently, the choice of the carbon source has an important effect on the efficiency and economical viability of the bioremediation technologies based on the use of these bacteria.

Dissimilatory sulphate-reducing bacteria are known to utilise simple organic compounds such as carboxylic acids or alcohols (Widdel and Bak 1992; White 1995) as carbon and energy sources. Lactate is the carbon source most widely used by DSRB in laboratory culture conditions (Barnes 1998; Postgate 1984; El Bayoumy et al. 1999). However lactate would be too expensive for a large scale process.

Hydrogen can also be used as an energy source by some DSRB (Lens et al. 2003; Fedorovich et al. 2000; Nagpal et al. 2000). Although hydrogen would be a relatively inexpensive substrate, this was deemed not to be an acceptable energy source because of engineering and safety requirements at a commercial scale (Huisman et al. 2006). According to the literature, ethanol seems to be the most cost effective substrate (Huisman et al. 2006; Tsukamoto et al. 2004).

Several natural sources of organic materials serving as electron donors and carbon sources have been already investigated: molasses, bagasse, sewage sludge, leaf mulch, wood chips, animal manure, vegetal compost, sawdust, mushroom compost, whey, and other agricultural wastes (Coetser et al. 2006; Costa and Duarte 2005; Annachhatre and Suktrakoolvait 2001; Waybrant et al. 1998; Christensen et al. 1996; Hammack et al. 1994; Dvorak et al. 1992). The selection of the carbon source depends to a great extent on the degradability of the organic substrate.

The purpose of this work was to investigate the possibility of using two wastes from food industry as sources of carbon compounds to promote sulphate reduction by DSRB. The two wastes were selected from wine and cheese industries, since they are produced in Portugal in large amounts and widespread geographic locations and consequently are easily available at zero or negative cost. Tens of thousands of cubic metre of both wine industry wastes and cheese whey are produced yearly in the continental Portuguese territory.

The search for efficient, low cost and largely available carbon sources (preferably wastes) for DSRB to be used in bioremediation processes for the treatment of sulphate rich effluents is of outmost importance. In addition, to use wastes in such processes is relevant from an environmental point of view, since it reduces the problematic of their disposal and promoting their biodegradation contributes to decrease pollutant release to the environment.

Materials and methods

Source and cultivation of DSRB community

The community of DSRB utilised in these experiments was obtained from a sludge sample from a wastewater treatment plant, located in Montenegro, Faro, in southern Portugal. The bacterial community was grown and maintained in Postgate B medium (Postgate 1984), at room temperature in anaerobic conditions. The bacteria were harvested by centrifugation, washed with Postgate B medium, and transferred to the batch solutions.

Composition of industrial food wastes used as carbon source

The wastes used in the experiments as carbon sources came from the cheese and wine Portuguese industries. Cheese whey was analysed using a MILKOSCAN spectrophotometer and is mainly composed by fat (1.75%), proteins (0.30%), lactose (4.52%) and total solids (6.37%). Lactate was not detected by HPLC analysis. The waste from the wine industry was collected prior to the bottling stage and contains 53.5 g/l ethanol, measured by HPLC. Both wastes were stored at 4°C.

Composition of calcite tailing

The calcite tailing used in the experiments as a neutralizing and buffer material is the residue from a marble stone cutting and polishing industry. The presence of crystalline phases was assessed by X-ray diffraction, using a Bruker AXS-D8 Advance diffractometer with Cu Kα radiation and step of 0.02°/s.

The EVA code was used for the identification of the peaks and phase analysis. This material is mainly composed of magnesium calcite (~89%), quartz (~11%) and traces of illite mineral. Previous studies (data not shown) show that no biological sulphate reduction occurs in the presence of calcite tailing without addition of a suitable electron source.

Batch experiments

The growth experiments were carried out in duplicate using 120 ml glass bottles containing 100 ml of Postgate B medium (Postgate 1984) with the following modifications: 6 g/l of a carbon and energy source compound, 2 g/l sulphates and resazurin as a redox indicator (0.03 g/l). Oxygen diffusion was eliminated by adding 10 ml of sterile liquid paraffin. The inoculum size used was 5% (v/v), with most probable number (MPN) of DSRB of 1.8×10^6 CFU/ml. After inoculation, the bottles were sealed with butyl rubber stoppers and aluminium crimp seals and incubated at room temperature (±21°C), in anaerobic conditions.

Several carbon sources were tested: cheese whey, waste from the wine industry, lactate, lactose and ethanol. A test without carbon source was carried out as control. Batch tests were also carried as described, but the media were supplemented with 2 g of calcite tailing.

Statistical analysis

Each set of experiments was carried out in duplicate. The data were subject to one-way ANOVA. All differences were considered to be statistically significant if $P < 0.05$.vv

Sampling and analytic methods

A 5 ml of samples were periodically collected, using a syringe, and filtered using a 0.2 µm hydrophilic polyestersulfone membrane (Machererey-Nagel). Redox potencial and pH were determined using a pH/E Meter GLP 21, Crison. High performance liquid chromatograph (Beckman) equipped with a polyspher® OAHY column (30 × 0.65 cm, Merck) and a refractive index detector, was used for soluble lactate, lactose, ethanol and acetate analysis. The analysis was performed with sulphuric acid (H_2SO_4 1.4 mM) as eluent, at a flow rate of 0.5 ml/min. The compounds were identified by their retention times in comparison to standards: lactate (11.347 min) as sodium lactate, ethanol (17.123 min), acetate (13.636 min) as sodium acetate 3-hydrate and lactose (6.859 min) monohydrate. Sulphate concentration was quantified by UV/visible spectrophotometry at 450 nm (Hach-Lange DR2800 spectrometer) using the method of sulfaVer4 (Hach-Lange), (Susuki et al. 2003).

Molecular characterization of DSRB community

Extraction of DNA

Total genomic DNA was extracted from cell cultures grown on Postgate B medium. The cells were harvested from 20 ml of cell culture by centrifugation at 4,000 rpm for 10 min and twice washed with chilled deionised water. DNA extraction was carried out by the following method: 300 µl of sodium dodecyl sulphate (SDS) lysis mixture [500 mM Tris–HCl pH 8, 3% (w/v) SDS, 100 mM NaCl] and 300 µl of phosphate buffer pH 8 were added to the washed pellet, followed by a freeze-thaw treatment (three cycles consisting of 1 min in liquid N_2 followed by 5 min in a 37°C water bath). After cellular lysis, 300 µl of chloroform-isoamyl alcohol (24:1) were added. The solution obtained was centrifuged at 16,000*g*, for 10 min. After precipitation with isopropanol at −20°C, for 20 min, DNA was resuspended in 35 µl H_2O. Nucleic acid extraction was evaluated on a 1% (w/v) agarose gel electrophoresis in Tris–Acetate–EDTA (TAE) buffer.

PCR amplification of dsr gene

PCR was conducted in a total volume of 50 µl. Community dissimilatory sulphite reductase (*dsr*) genes were amplified using the primers DSR1F (5′-ACC CAC TGG AAG CAC G-3′) and DSR4R (5′-GTG TAG CAG TTA CCG CA-3′) (Wagner et al. 1998; Chang et al. 2001; Castro et al. 2002), which amplify a 1.9 kb fragment. The primers were purchased from Thermo Ficher Scientific. The reaction mixture used for PCR amplification contained 30.75 µl of nuclease-free water, 1 µl of each primer (10 pmol/µl), 1 µl of dNTP's (10 mM), 5 µl of $MgCl_2$ (25 mM), 10 µl of 5× Go Taq® buffer (Promega, Madison,

USA), 0.25 µl of GoTaq®DNA polymerase (Promega, Madison, USA), and 1 µl of DNA. The DNA of a strain of *Desulfovibrio* subsp. was used as positive control (that contains the *dsr* gene in its genome) and of *Escherichia coli* as a negative control (that does not contain the *dsr* gene in its genome). PCR amplification was carried out in a thermocycler (T1, Biometra). Thermal cycling was carried out by using an initial denaturation step of 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 2 min and completed with an extension period of 5 min at 72°C. The PCR products were separated in a 1% (w/v) agarose gel in TAE Buffer.

Cloning of dsr gene and restriction fragment length polymorphisms (RFLP) analysis

PCR products were purified (E.Z.N.A.™ Gel Extration Kit, Omega) and ligated into the cloning vector pGEM®-T Easy (an insert vector ratio of 3:1) with T4 ligase enzyme followed by transformation into *E. coli* DH5-alpha competent host cells, according to the manufacturer's instructions (Promega, Madison, USA). All the white colonies were amplified by direct PCR with the DSR1F and DSR4R primers according to the conditions described above.

Restriction fragment length (RFLP) analyses were done using the restriction enzymes HhaI and HaeIII (Promega). Fragments of the digested PCR products were separated in a 2% (w/v) TAE agarose gel.

Sequencing and phylogenetic analysis

Representative plasmids from each digestion pattern were selected for sequencing at CCMAR (Centro de Ciências do Mar, Universidade do Algarve). The *dsr* gene inserted in plasmids was amplified using the primers DSR1F and DSR4R, according to the conditions described above. PCR products were purified (E.Z.N.A.™ Gel Extration Kit, Omega) and sequenced. Sequence identification was performed by using the BLASTN facility of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Cladograms were constructed using MEGA version 4 (Tamura et al. 2007) and the Neighbour-Joining algorithm was applied (Saitou and Nei 1987; Studier and Keppler 1988).

Results

Lactate as carbon source

The profile of sulphate reduction by DSRB in the presence of lactate as carbon source is shown in Fig. 1. In the first 7 days of the experiment sulphate was completely reduced and lactate was totally consumed. At the same time acetate production by DSRB was observed. Without carbon source sulphate concentration was always near 2 g/l. The pH values were close to 7 during all the experiment.

Lactose and cheese whey as carbon source

Sulphate reduction was not detected when lactose was utilised as carbon source (Fig. 2a). A similar behaviour was observed when cheese whey was utilised (Fig. 2b). However, lactose consumption was verified in both cases (around 33%). The production of lactate (0.96 g/l) was observed in the presence of cheese whey.

When the media containing lactose and cheese whey were supplemented with calcite tailing, efficient sulphate reduction was achieved (Fig. 2c, d): 80 and 94% sulphate reduction was obtained, respectively, at the end of the experiment. In addition, higher consumption of lactose and higher production of lactate and acetate were observed in both cases, in comparison with the experiments performed without

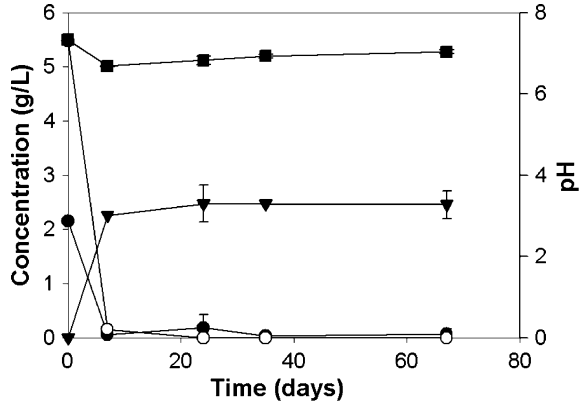

Fig. 1 Profile of sulphate reduction and consumption of carbon source by bacterial consortium using lactate as carbon source without calcite tailing. Data are the average of duplicates and *error bars* indicate the standard deviations of the average values. Symbols: (●) sulphates, (○) lactate, (▼) acetate and (■) pH

calcite tailing. It was observed that the pH of the media containing lactose and cheese whey in the absence of calcite tailing decreased from 7 to values below 4 during the experiments. When these carbon sources were supplemented with calcite tailing the pH increased from 4 to 6 and thereafter it was maintained near this value until the end of the experiments.

Ethanol and waste from the wine industry as carbon source

The sulphate reduction profile with ethanol as carbon source is shown in Fig. 3a. The pH of the solution when ethanol was used as carbon source does not decrease to values lower than 5, not compromising DSRB growth and activity. This behaviour, already observed in previous studies, justified that no calcite tailing needed to be added.

High sulphate reduction was observed when pure ethanol was used as carbon source.

A 98.5% reduction of sulphates was observed in the first 20 days. For this reduction the consortium of DSRB used 1.8 mmol ethanol per mmol sulphate reduced and 1.6 mmol acetate was produced.

When the waste from the wine industry was used by itself as carbon source sulphates were not reduced and ethanol was not consumed by DSRB (Fig. 3b). However, in the presence of calcite tailing (Fig. 3c) efficient sulphate reduction was achieved. After 20 days 95% sulphate reduction was observed. Ethanol consumption and acetate production were also observed. For each mmol sulphate reduced, 0.94 mmol ethanol was consumed and 1.6 mmol acetate was produced. When the waste from the wine industry was used in the absence of calcite tailing the pH of the medium presented values near 4.7 during all the experiment. On the other hand, when calcite tailing was present the pH increased to values close to 6, similar to what was observed in the experiment with ethanol.

Phylogenetic analysis of DSRB community

The dissimilatory sulphite reductase gene (*dsr*) was used to elucidate the composition of the DSRB consortium from the experiment. This gene has revealed to be a powerful tool on DSRB diversity studies where the phylogenetic analyses were based either on restriction analysis of the cloned fragment or on sequencing of the cloned 1.9 kb *dsr* fragment (Baker et al. 2003; Liu et al. 2003; Bahr et al. 2005).

A total of 16 clones was obtained and all of these clones were subjected to RFLP analysis. Eight different patterns were obtained. The representative clones from each pattern were selected for sequencing

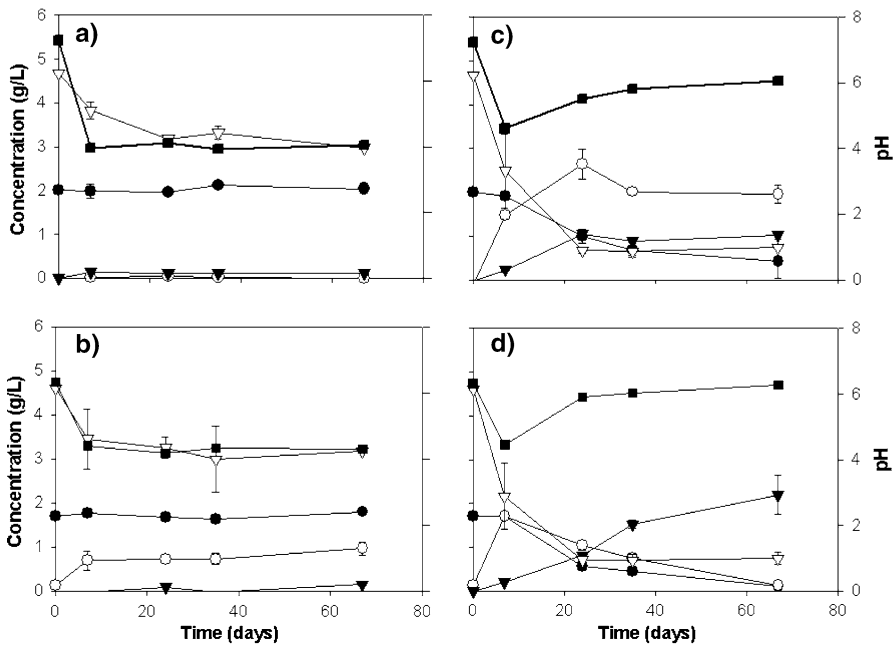

Fig. 2 Profile of sulphate reduction and consumption of carbon source by bacterial consortium using two lactose-based carbon sources: **a** lactose, **b** cheese whey, **c** lactose with calcite tailing and **d** cheese whey with calcite tailing. Data are the average of duplicates and *error bars* indicate the standard deviations of the average values. Symbols: (●) sulphates, (○) lactate, (▼) acetate, (▽) lactose and (■) pH

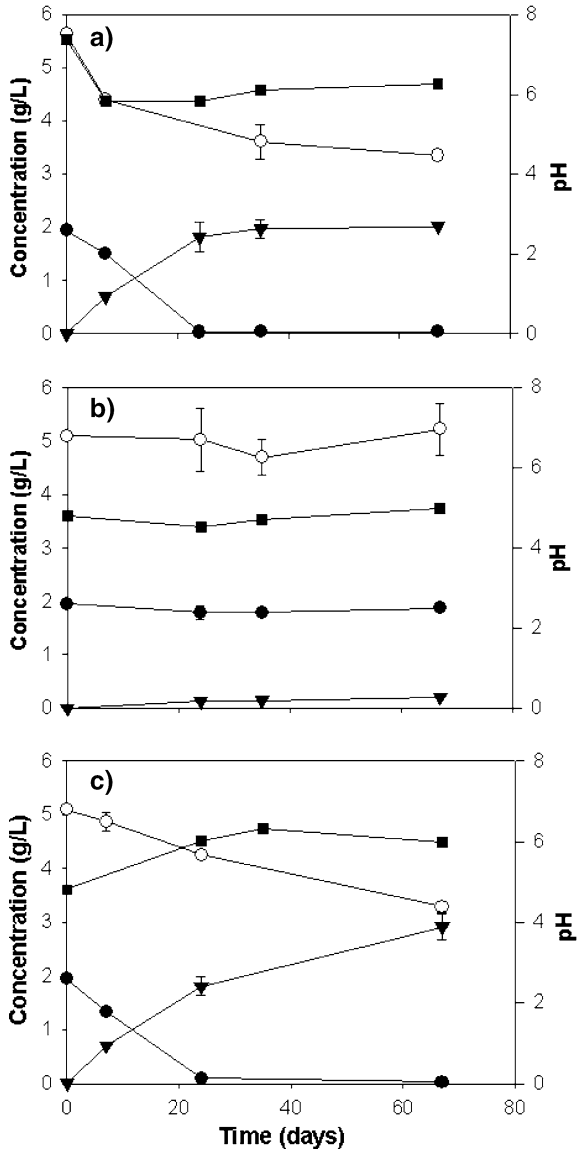

Fig. 3 Profile of sulphate reduction and consumption of carbon source by bacterial consortium using two ethanol-based carbon sources: **a** ethanol, **b** wastes of winery industry and **c** wastes of winery industry with calcite tailing. Data are the average of duplicates and *error bars* indicate the standard deviations of the average values. Symbols: (●) sulphates, (○) ethanol (▼) acetate and (■) pH

and these sequences were submitted to the GenBank (accession numbers: EU552471 to EU552486).

In the cladogram obtained for the *dsr* gene sequence of the selected clones three major clusters were identified (Fig. 4). The first one is composed of two clones affiliated to *Desulfovibrio desulfuricans*: *Desulfovibrio desulfuricans* isolate SRDQC (accession number DQ450464.1) and *Desulfovibrio desulfuricans* strain F28-1 (accession number DQ092635.1). The second group contains clones similar to *Desulfovibrio fructosovorans* (accession number AB061538.1). The third assemblage consists of clones with affiliation to *Desulfovibrio aminophilus* strain DSM 12254 (accession number AY626029.1).

Discussion

This study shows, for the first time, that a waste from the wine industry can be used by DSRB present in a bacterial consortium, provided that a neutralising and buffer material is present. In this case calcite tailing, another waste material, was used. According to *dsr* gene analysis, the DSRB consortium is constituted by members of the species *Desulfovibrio fructosovorans*, *Desulfovibrio aminophilus* and *Desulfovibrio desulfuricans*. The dominance of the *Desulfovibrio* genus was observed, which is consistent with literature data mentioning its predominance in wastewater treatment plants (Santegoeds et al. 1998; Baena et al. 1998; Dar et al. 2005). The genus *Desulfovibrio* represents a group of Gram-negative sulphate reducers in which all species oxidise their substrates incompletely to acetate (Widdel and Bak 1992).

The increased efficiency of sulphate reduction by DSRB observed when the food industry wastes were supplemented with calcite tailing can be explained by the increased pH. The difficulty to grow DSRB in media with low pH has already been mentioned previously (Garcia et al. 2001; Benedetto et al. 2005). According to the literature, specific conditions such as an anaerobic environment, a redox potential around −200 mV and pH values above 5 must be met to enable sulphate-reducing activity (Cohen 2006). When the medium was supplemented with calcite tailing, due to its neutralising and buffer capacities, the pH was close to 6 or higher during all the experiment. Therefore, good conditions for DSRB activity were achieved, allowing sulphate reduction and consumption of the carbon source.

Sulphate reduction observed in the presence of lactose and in the presence of cheese whey (both supplemented with calcite tailing) was slower compared to sulphate reduction in the presence of lactate, ethanol or waste from the wine industry, the last one

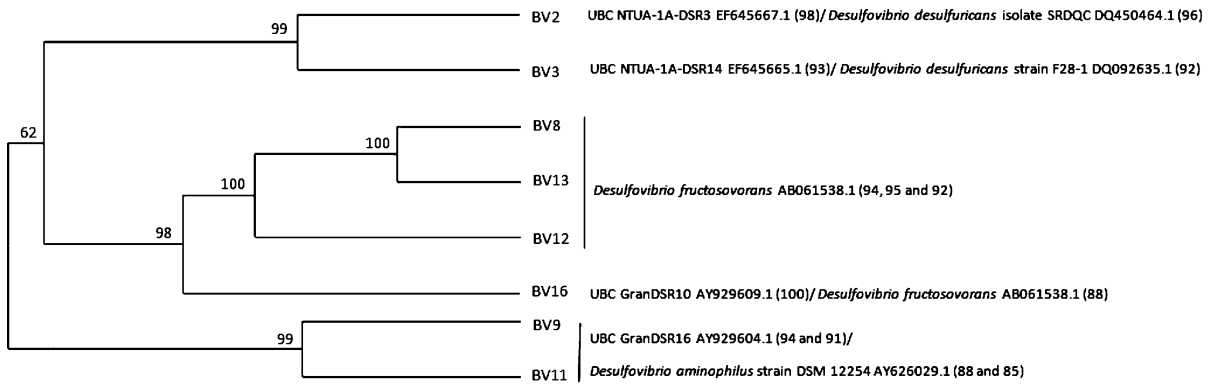

Fig. 4 Cladogram constructed for the selected clones using *dsr* gene sequence Partial weighted combined-data cladogram of relationships of *dsr* gene sequences for the selected dissimulatory sulphate-reducing bacteria clones. The Neighbour-Joining algorithm was used. *Bootstrap values* are indicated on branches. Following the clone name the most closely related species and/or the most related cultured species are indicated. The percentage of similarity is indicated in *brackets*. *UBC* uncultured bacterium clone

supplemented with calcite tailing. When lactose was supplemented with calcite tailing, approximately 20 days were needed to consume half of the added sulphate concentration, while with lactate and ethanol near complete sulphate reduction occurred within 7 and 20 days, respectively. The complexity of lactose molecule, in comparison with ethanol or lactate, may explain this result. In fact, few bacterial species, and among them no DSRB, are able to metabolize lactose, thus it required more time for its degradation.

When lactose or cheese whey was provided as electron donors for sulphate reduction the pH of the medium decreased and lactose was consumed (Fig. 2a, b). However, no sulphate reduction was observed. These results can be explained by the presence in the bacterial consortium of bacteria other than DSRB with ability to use lactose in their metabolism. The production of lactate observed in Fig. 2b can result from the activity of lactic bacteria present in the cheese whey.

When lactose and cheese whey were supplemented with calcite tailing, production of lactate was observed suggesting that lactose can be used by other bacteria present in the consortium, such as lactic bacteria, producing lactate that may be further utilized by DSRB and converted to acetate. This indirect path was already reported when molasses were used as carbon source (Maree et al. 1986).

Consumption of the acetate produced, by DSRB, mentioned by several authors (Barnes 1998; Dar et al. 2007), was not observed. This probably indicates the absence of acetate utilising bacteria in this particular community.

When waste from the wine industry was used as carbon source in the presence of calcite tailing, lower ethanol consumption (0.94 mmol/mmol sulphate reduced) was observed compared with pure ethanol (1.8 mmol/mmol sulphate reduced). This is probably due to the fact that the waste from the wine industry may contain other nutrients that may be used as carbon source. *Desulfovibrio fructosovorans* is known to differ from all other described *Desulfovibrio* species by its ability to use fructose (Olliver et al. 1988) and *Desulfovibrio aminophilus* has been reported to be able to use amino acids as carbon and energy sources (Hernandez-Eugenio et al. 2000). The waste from the wine industry usually contains amino acids and fructose in significant amounts, so it is possible that these can be consumed by the DSRB consortium. This in turn explains the reduced amount of ethanol consumed per mmol sulphate. According to the literature (Waybrant et al. 1998; Zagury et al. 2006), materials containing multiple organic substrates or mixtures are most effective in promoting sulphate reduction compared to those containing a single organic substrate.

The use of waste from the wine industry as carbon source for sulphate reduction by DSRB is promising in contrast to other wastes. When conifer sawdust and composted spruce chips were used no sulphate reduction was observed (Zagury et al. 2006). Waybrant et al. (1998) achieved sulphate reduction only

when sheep manure plus calcite and sand were mixed with other organic constituents.

Conclusions

Taking into account the results achieved the waste from the wine industry in the presence of calcite tailing seems to be promising as carbon source to promote DSRB activity. Cheese whey in the presence of calcite tailing can also be used as carbon source for biological sulphate reduction, but the process is considerably slower.

Their efficiency as carbon sources is only revealed when those wastes are supplemented with calcite tailing that acts as a neutralizing and buffer material, achieving suitable pH conditions for DSRB activity.

The possibility of using food industry wastes, particularly the wastes from wine industry, to promote an efficient sulphate reduction is an important finding. By this way these wastes can be reutilised in bioremediation processes based on DSRB for the treatment of sulphate rich effluents, with both environmental and economical benefits.

Acknowledgments This research was funded by the Fundação para a Ciência e a Tecnologia (FCT) through Project ECOTEC (POCI/AMB/58512/2004). The authors would like to thank Centro de Desenvolvimento de Ciências e Tecnologias de Produção Vegetal (CDCTPV), from the Universidade do Algarve, for the utilization of HPLC and to Câmara Municipal de Faro for the authorization to collect sludge samples. We are grateful to Rogério Tenreiro and Sandra Chaves from Universidade de Lisboa, Faculdade de Ciências for providing the *Desulfovibrio* sp. DNA used in this study.

References

Annachhatre AP, Suktrakoolvait S (2001) Biological sulfate reducing using molasses as a carbon source. Water Environ Res 73:118–126. doi:10.2175/106143001X138778

Baena S, Fardeau ML, Labat M, Olliver B, Garcia JL, Patel BKC (1998) *Desulfovibrio aminophilus* sp. nov., a novel amino acid degrading and sulfate reducing bacterium from an anaerobic dairy lagoon. Syst Appl Microbiol 21:498–504

Bahr M, Crump BC, Klepac-Ceraj V, Teske A, Sogin ML, Hobbie JE (2005) Molecular characterization of sulfate-reducing bacteria in a New England salt marsh. Environ Microbiol 7:1175–1195. doi:10.1111/j.1462-2920.2005.00796.x

Baker BJ, Mose DP, Macgregor BJ, Fishbain S, Wagner M, Fry NK, Jackson B, Speolstra N, Loos S, Takai K, Lollar BS, Fredrickson J, Balkwill D, Onstott TC, Wimpee CF, Stahl DA (2003) Related assemblages of sulphate-reducing bacteria associated with ultradeep gold mines of South Africa and deep basalt aquifers of Washington State. Environ Microbiol 5:267–2677. doi:10.1046/j.1462-2920.2003.00408.x

Barnes LJ (1998) Removal of heavy metals and sulphate from contaminated grounwater using sulphate-reducing bacteria: development of a commercial process. In: Sikdar SK, Irvine RL (eds) Bioremediation technologies, vol 3. Publishing Company Inc., Lancaster, USA, pp 577–619

Benedetto JS, De Almeida SK, Gomes HA, Vazoller RF, Ladeira ACQ (2005) Monitoring of sulfate-reducing bacteria in acid water from uranium mines. Min Eng 18:1341–1343. doi:10.1016/j.mineng.2005.08.012

Burgess JE, Stuetz RM (2002) Activated sludge for the treatment of sulphur-rich wastewaterss. Min Eng 14:839–846. doi:10.1016/S0892-6875(02)00049-3

Castro H, Reddy KR, Ogram A (2002) Composition and function of sulphate-reducing prokaryotes in eutrophic and pristine areas of the Florida Everglades. Appl Environ Microbiol 68:6129–6137. doi:10.1128/AEM.68.12.6129-6137.2002

Chang Y, Peacock AD, Long P, Stephen JR, McKinley JP, Macnaughton SJ, Hussain AKM, Saxton AM, White DC (2001) Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. Appl Environ Microbiol 67:3149–3160. doi:10.1128/AEM.67.7.3149-3160.2001

Christensen B, Laake M, Lien T (1996) Treatment of acid mine water by sulphate-reducing bacteria; results from a bench scale experiment. Water Res 30:167–177

Coetser S, Pulles W, Heath R, Cloete T (2006) Chemical characterisation of organic electron donors for sulfate reduction for potential use in acid mine drainage treatment. Biodegradation 17:67–77. doi:10.1007/s10532-005-7567-3

Cohen RH (2006) Use of microbes for cost reduction of metal removal from metals and mining industry waste streams. J Clean Prod 14:1146–1157. doi:10.1016/j.jclepro.2004.10.009

Costa MC, Duarte JC (2005) Bioremediation of acid mine drainage using acidic soil and organic wastes for promoting sulphate-reducing bacteria activity on a column reactor. Water Air Soil Pollut 165:325–345. doi:10.1007/s11270-005-6914-7

Dar SA, Kuenen JG, Muyzer G (2005) Nested PCR-denaturing gradient gel electrophoresis approach to determine the diversity of sulfate-reducing bacteria in complex microbial communities. Appl Environ Microbiol 71:2325–2330. doi:10.1128/AEM.71.5.2325-2330.2005

Dar SA, Alfons JM, Stams J, Kuenen G, Muyzer G (2007) Coexistence of physiologically similar sulfate-reducing bacteria in a full-scale sulfidogenic bioreactor fed with a single organic electron donor. Appl Microbiol Biotechnol 75:1463–1472. doi:10.1007/s00253-007-0968-y

Dvorak DH, Hedin RS, Edenborn HM, Mclntire PE (1992) Treatment of metal-contaminated water using bacterial sulfate reduction: results from pilot-scale reactors. Biotechnol Bioeng 40:609–616. doi:10.1002/bit.260400508

El Bayoumy MA, Bewtra JK, Ali HI, Biswas N (1999) Sulfide production by sulfate reducing bacteria with lactate as

feed in an upflow anaerobic fixed film reactor. Water Air Soil Pollut 112:67–84. doi:10.1023/A:1005016406707

Fedorovich V, Greben M, Kalyuzhnyi S, Lens P, Pol LH (2000) Use of hydrophobic membranes to supply hydrogen to sulphate reducing bioreactors. Biodegradation 11:295–303. doi:10.1023/A:1011100120121

Garcia C, Moreno DA, Ballester A, Bláquez ML, González F (2001) Bioremediation of an industrial acid mine water by metal-tolerant sulphate-reducing bacteria. Min Eng 14:997–1008. doi:10.1016/S0892-6875(01)00107-8

Hammack TW, Edenborn HM, Dvorak DH (1994) Treatment of water from an open-pit copper mine using biogenic sulfide and lime stone: a feasibility study. Water Res 28:2321–2329. doi:10.1016/0043-1354(94)90047-7

Hernandez-Eugenio G, Fardeau ML, Patel BKC, Macarie H, Garcia JL, Ollivier B (2000) *Desulfovibriomexicanus* sp. nov. a sulfate-reducing bacterium isolated from an upfow anaerobic sludge blanket (UASB) reactor treating cheese wastewaters. Anaerobe 6:305–312. doi:10.1006/anae.2000.0354

Huisman JL, Schouten G, Dijkman H (2006) Biotechnological solutions for the treatment of pickle liquors. In: Dutrizac J, Riveros PA (eds) Iron control tecnologies. Metallurgical Society, Montreal, pp 805–814

Johnson DB, Hallberg KB (2005) Biogeochemistry of the compost bioreactor components of a composite acid mine drainage passive remediation system. Sci Total Environ 338:81–93. doi:10.1016/j.scitotenv.2004.09.008

Lens PNL, Gastesi R, Lettinga G (2003) Use of sulfate reducing cell suspension bioreactors for the treatment of SO_2 rich flue gases. Biodegradation 14:229–240. doi:10.1023/A:1024222020924

Lima ACF, Gonçalves MM, Granato M, Leite GF (2001) Anaerobic sulphate-reducing microbial process using UASB reactor for heavy metals decontamination. Environ Technol 22:261–270. doi:10.1080/09593332208618286

Liu X, Bagwell CE, Wu L, Devol AH, Zhou J (2003) Molecular diversity of sulphate-reducing bacteria from different continental margin habitats. Appl Environ Microbiol 69:6073–6081. doi:10.1128/AEM.69.10.6073-6081.2003

Maree JP, Gerber A, Strydom WF (1986) A biological process for sulphate removal from industrial effluents. Water SA 12:139–144

Nagpal S, Chuichulcherm S, Livingstone A, Peeva L (2000) Ethanol utilization by sulfate-reducing bacteria: an experimental and modeling study. Biotechnol Bioeng 16:533–543. doi:10.1002/1097-0290(20001205)70:5<533::AID-BIT8>3.0.CO;2-C

Neculita CM, Zagury GJ, Bussière B (2007) Passive treatment of acid mine drainage in bioreactors using sulphate-reducing bacteria. J Environ Qual 36:1–16. doi:10.2134/jeq2006.0066

Ollivier B, Cord-Ruwisch R, Hatchikian EC, Garcia JL (1988) Characterization of *Desulfovibrio fructosovorans* sp. nov. Arch Microbiol 149:447–450. doi:10.1007/BF00425586

Pfennig N, Widdel F, Truper H (1981) The dissimilatory sulfate-reducing bacteria. In: Mortimer PS, Heinz S, Truper HG, Balows A, Schelegel GH (eds) The prokaryotes: a handbook on habitats, isolation and identification of bacteria. Springer, Berlin, p 926

Postgate JR (ed) (1984) The sulphate-reducing bacteria. Cambridge University Press, New York

Saitou N, Neil M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–525

Santegoeds CM, Ferdelman TG, Muyzer G, Beer D (1998) Structural and functional dynamics of sulfate-reducing populations in bacterial biofilms. Appl Environ Microbiol 64:3731–3739

Steed VS, Suidan MT, Gupta M, Miyahara T, Acheson CM, Sayles GD (2000) Development of a sulfate-reducing biological process to remove heavy metals from acid mine drainage. Water Environ Res 72:530–535. doi:10.2175/106143000X138102

Studier JA, Keppler KJ (1988) A note on the neighbor-joining algorithm of saitou and neil. Mol Biol Evol 5(6):729–731

Susuki Y, Kelly SD, Kemmer KM, Banfield JF (2003) Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. Appl Environ Microbiol 69:1337–1346. doi:10.1128/AEM.69.3.1337-1346.2003

Tabak HH, Govind R (2003) Advances in biotreatment of acid mine drainage and biorecovery of metals: 2. Membrane bioreactor system for sulfate reduction. Biodegradation 14:437–452. doi:10.1023/A:1027332918844

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599. doi:10.1093/molbev/msm092

Tsukamoto TK, Killion HA, Miller GC (2004) Column experiments for microbiological treatment of acid mine drainage: low-temperature, low pH and matrix investigations. Water Res 38:1405–1418. doi:10.1016/j.watres.2003.12.012

Vega-López A, Amora-Lazcano E, López-López E, Terrón O, Proal-Nágera JB (2007) Toxic effects of zinc on anaerobic microbiota from Zimapán Reservoir (Mexico). Anaerobe 13:65–73. doi:10.1016/j.anaerobe.2007.01.001

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

Waybrant KR, Blowes DW, Ptacek CJ (1998) Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. Environ Sci Technol 32:1972–1979. doi:10.1021/es9703335

White D (1995) The physiology and biochemistry of prokaryotes. Oxford University Press, USA

White C, Dennis JS, Gadd GM (2003) A mathematical process model for cadmium precipitation by sulfate-reducing bacterial biofilms. Biodegradation 14:139–151. doi:10.1023/A:1024026319479

Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes. Springer, New York, pp 3352–3378

Zagury GJ, Kulnieks VI, Neculite CM (2006) Characterization and reactivity assessment of organic substrates for sulphate-reducing bacteria in acid mine drainage treatment. Chemosphere 69:944–954. doi:10.1016/j.chemosphere.2006.01.001