African Journal of Microbiology Research Vol. 6(50), pp. 7595-7602, 28 December, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.2296
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Morphologial, physiological and phylogenetic characterization and functional verification of an anaerobic strain SN22-2 with simultaneous capabilities of denitrification and sulfate reduction

Li Wei[1]*, Zhen Zhao[1], Fang Ma[1], Chun-ying Li[1,2], Qiu Shi Zhao[2] and Wen-ge Gu[2]

[1]State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China.
[2]Daqing Oil-field Design and Research Institute, Daqing, 163712 , China.

Accepted 29 November, 2012

Anaerobic bacterium (strain SN22-2) with simultaneous capabilities of denitrification and sulfate reduction was enriched and isolated in a specially designed medium by anaerobic Hungate technology. The characterisation of SN22-2 was conducted from the morphological, physiological and phylogenetic aspects. It was a rod-shaped, gram-negative, 0.2 to 0.7 µm in width and 3.0 to 5.5 µm in length. Most of the fatty acids of SN22-2 were distributed among $C_{12:0}$–$C_{19\text{-}CYC\text{-}9}$ and the main fatty acids consisted of $C_{16:1\text{-}CIS\text{-}9\text{-}FAME}$, $C_{16:0\text{-}FAME}$, $C_{18:0\text{-}FAME}$ and $C_{18:0\ DMA}$. Based on the morphological and physiological characteristics as well as the phylogenetic analysis, SN22-2 was identified as *Bacillus coagulans* since its 16S rDNA bore 99% resemblance to that of *B. coagulans* (Accession No. AB240205). The dissimilatory sulfite reductase (*Dsr*) and the nitrite reductase (*nirS*) genes were successfully amplified and cloned from strain SN22-2. The removal efficiencies of NO_3^- and SO_4^{2-} were up to 96.48 and 97.98% when the initial concentrations of NO_3^- and SO_4^{2-} were 4368.45 and 586.70 mg/L, respectively. High activities of nitrite reductase and sulfate reductase were detected in the culture medium of SN22-2. The analysis of gene sequences and the enzyme activities as well as the nitrate and sulfate removal efficiency data confirmed that strain SN22-2 had simultaneous denitrifying and sulfate reducing capabilities. The characterization of the present isolate had important ecological implications in treating organic wastewater containing high concentration of sulfate and nitrogen.

Key words: Strain with simultaneous denitrifying and sulfate reducing capabilities, characterization, function verification.

INTRODUCTION

The sulfate reducing bacteria (SRB) were characterized by their ability to reduce sulfate to sulfide with the simultaneous oxidation of the organic substrates (Jenneman et al., 1986). Sulfide production is a major concern to the petroleum industry since it is toxic and corrosive and causes plugging due to the formation of insoluble iron sulfides (Reinsel et al.,1996). Inhibition of SRB by nitrate (NO_3^-) injection had been widely investigated. Sulfide production was temporarily inhibited due to the preferential use of nitrate as an electron acceptor. The prolonged decrease of SRB as a result of prolonged exposure to an oxidzing envrionment was due to the buildup of N_2O or NO or both by nitrate reduction. Similarly, other studies (Senez and Pichinoty,1958) also concluded that the production of intermediate metabolites (N_2O and NO) by denitrification led to the raise of redox potential and thus long-term inhibition sulfate reduction was realized.

It was reported that the genera of *Desulfovibrio*, *Desulfobulbus* and *Desulfomonas* could utilize nitrate as

*Corresponding author. E-mail: weilihit@126.com.

electron acceptor and obtain energy for their growth. Successful dissimilatory nitrate reduction to ammonia was achieved by a strain of *Desulfovibrio desulfuricans* (a strict anaerobic SRB), which confirmed that dissimilatory nitrate reduction to ammonia was not confined to facultatively anaerobic bacteria (Keith and Herbert,1983). Marietou et al. (2005) found that the nitrate reduction by strain *D. desulfuricans* ATCC 27774 was catalyzed by a periplasmic nitrate reductase system including a unique tetraheme *c*-type cytochrome, NapM. Liu et al. (1994) detected the hexaheme nitrite reductase for the first time in nitrate-respiring *D. desulfuricans* ATCC 27774. It demonstrated that the nitrate reductase isolated from *D. desulfuricans* ATCC 27774 was a periplasmic enzyme and seemed to belong to a class of monomeric enzymes (Moura et al.,1997). K. Mori isolated a novel type of moderate thermophilic autotrophic SRB from a hot spring, which could grow with nitrate in place of sulfate (Mori et al., 2003). Medium applied for the isolation of SRB contained $NaNO_3$ as nitrogen source, which indicated that the isolated strain could utilize nitrate (Hong et al.,2010).

In an anaerobic baffled reactor (ABR) process operated in our lab for the inhibition of SRB with the dosage of nitrate, the presence of bacteria with simultaneous capabilities of denitrification and sulfate reduction was proposed. In the present study, a special medium for the isolation of strains with simultaneous denitrifying and sulfate reducing capablities was designed. An anaerobic strain with simultaneous denitrifying and sulfate-reducing capablities, named as SN22-2, was isolated. Morphological observation, physiological tests, fatty acids analysis, phylogenetic analysis of 16S rDNA, cloning and sequencing of *Dsr* and *nirS* genes and detection of enzyme activities were conducted on SN22-2 for the function verification of its simultaneous denitrifying and sulfate reducing capablities. The characterization of the present isolate had important ecological implications in treating organic wastewater containing high concentration sulfate and nitrogen generating from light chemical engineering industries, food processing and pharmaceutical factories.

MATERIALS AND METHODS

Strain source

Sample for strain isolation was the activated sludge collected from a denitrification-based SRB inhibition bioreactor. The reactor was fed with a synthetic wastewater, which contained 600 mg/L SO_4^{2-}, 600 mg/L NO_3^-, 2350 mg/L COD (pH, 8.0).

Medium and isolation

Techniques of Hungate, the most probable number (MPN) and the roll tube were applied for the isolation of the bacterial strain. A special medium was designed for the isolation of strains with simultaneous denitrifying and sulfate-reducing capabilities. The liquid medium was composed of 1750 mL distilled water and the following salts: Na_2SO_4, 4 g; KNO_3, 2.0 g; $NaNO_3$, 2.0 g; $MgSO_4 \cdot 7H_2O$ 1g; K_2HPO_4 0.5 g; $KNaC_4H_4O_6 \cdot 4H_2O$ 5 g; KH_2PO_4 1.0 g; $CaCl_2 \cdot 2H_2O$ 0.2 g (final pH, 7.5). Resazurin (0.2%, wt/vol) was added as redox indicator to the medium. Then, the medium was boiled for complete dissolution and 0.5 g L-cysteine was added. After that, high purity nitrogen was introduced to drive away oxygen for 30 min. The medium was autoclaved for 20 min at 121°C. The sterilized medium was cooled and 0.1 mL 3% $FeSO_4 \cdot (NH_4)_2SO_4$ was added. Powdered agar (1.6%, wt/vol) was added when solid medium was prepared before sterilization.

Characterization of the isolated strain

Gram-stained strains were observed by electron microscope (CX31, Olympus, Japan). Morphological observations of the cells were also carried out through atomic force microscope (AFM) (Di BioScope, Veeco, USA). The physiochemical tests of the isolate were conducted according to the guidance of “Bergey’s Manual of Determinative Bacteriology”. The microbial fatty acids of the isolated strain were identified using the Sherlock MIS (MIDI Sherlock, MIDI, USA) equipped with the gas chromatograph (6890N, Agilent, USA).

Identification of the isolate by 16S rDNA-based phylogenetic analysis

The DNA extraction of the isolate was conducted with a bacterial genomic DNA extraction kit (TaKaRa, Dalian, China). The 16S rDNA genes were amplified by using universal primers (TaKaRa, Dalian, China). The 50 µL PCR mixture contained 2 µL template DNA, 0.5 µL *rTaq* polymerase (TaKaRa, Dalian, China, 5 U/µL), 5 µL 10×PCR buffer, 4 µL of dNTPs (2.5 mM of each dNTP), 1 µL of each primer (50 mM) and 37.5 µL deionized water. The thermal cycling included: 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30s, annealing at 58 °C for 45 s, extension at 72°C for 90 s, and a final extension at 72°C for 10 min. PCR product was subjected to agarose gel electrophoresis. Target fragment was purified using an agarose gel DNA purification kit (TaKaRa, Dalian, China) and ligated to a TA type vector pGEM-T (Promega, Madison, WI, USA). Ligated product was transformed to an *E. coli* chemical competent cell TOP10 (Tiangen Biotech, Beijing, China). Transformed *E. coli* was selected on Lysogeny Broth (LB) solid culture medium with Amp (50 µg/mL) and X-gal (200 µg/mL). The transformed *E. coli* was analyzed using the universal primers T7 and SP6 and sequenced by the Shanghai Sangon Biological Engineering Co., Ltd, China. The sequenced 16S rDNA were compared with a non-redundancy nucleotides database by using the basic local alignment search tool (BLAST). Multiple sequence alignment was conducted using BioEdit Version 5.06, and a phylogenetic tree was constructed by MEGA Software Version 4.1 with a neighbor-joining method.

The cloning and sequencing of *nirS* gene

The *nirS* gene of the isolate was PCR amplified from its DNA extract by using the primer pair 832F (5’-TCACACCCCGAGCCGCGCGT-3’) and 1606R (5’-AGKCGTTGAACTT KCCGGTCGG-3’) (Kutyavin et al., 2000), and otherwise the same PCR reaction mixture and thermal cycling procedure as described above. The cloning and sequencing of the *nirS gene* were identical to that of the 16S rDNA.

The cloning and the sequencing of *Dsr* gene

The *Dsr* gene was PCR amplified from the DNA extract of the isolate using the primer pair DSR1F (5'- AC[C/G]CACTGGAAGCACG

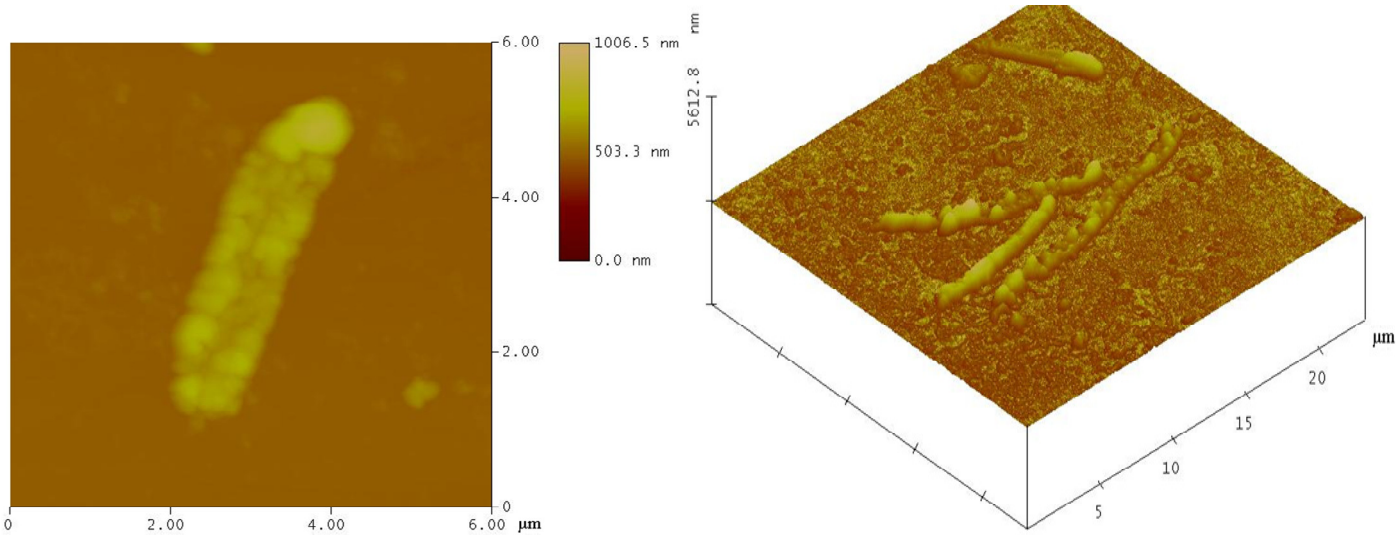

Figure 1. AFM images of strain SN22-2.

-3') and DSR5R (5'-TGCCGAGGAGA ACGATGTC-3'). The 20 µL PCR mixture contained 2 µL template DNA, 0.5 µL *rTaq* polymerase (TaKaRa, Dalian, China, 5 U/µL), 2 µL 10×PCR buffer, 0.5 µL *rTaq* polymerase (TaKaRa, Dalian, China, 5 U/µL), 2uL of dNTPs (2.5 mM of each dNTP), 1 µL of each primer, and 12 µL deionized water. The thermal cycling procedure, the cloning and sequencing of the *Dsr* gene were identical as described above.

Analytical methods

The concentrations of NO_3^-, NO_2^- and SO_4^- were detected by ion chromatography (ICS-3000, Dionex, USA). The activity of nitrite reductase was determined according to the method of Cole et al. (1974). The determination of sulfite reductase followed the method described by Ostrowski et al. (1989).

RESULTS AND DISCUSSION

Morphological and physiochemical characterization of the isolated strain SN22-2

An anaerobic strain, named as SN22-2, was isolated for its simultaneous denitrification and sulfate-reducing capabilities. Figure 1 shows that strain SN22-2 was long rod-shaped, 0.2 to 0.7 µm in width and 3.0 to 5.5 µm in length. It was Gram-positive with polar flagella. Its colony on the agar plate was white and round in moderate size with convex surface. The results of physiochemical tests are listed in Table 1. It shows that strain SN22-2 was mesophilic and grew at temperatures ranging from 20 to 40°C with an optimum growth temperature of 36°C. Optimal growth occurred at pH 7.5. Growth was observed on the following substrates: glucose, citrate and grease. Strain SN22-2 was not able to grow using fructose, lactose, sucrose, ethanol, starch, urea and gelatin as electron donors. The nitrate reduction and H_2S production tests confirmed that strain SN22-2 had denitrification and sulfate reduction capabilities. Besides, the analysis of fatty acids analysis indicated that most of the fatty acids were distributed around $C_{12:0}$–$C_{19\text{-}CYC\text{-}9}$. The main fatty acids consisted of $C_{16:1\text{-}CIS\text{-}9\text{-}FAME}$, $C_{16:0\text{-}FAME}$, $C_{18:0\text{-}FAME}$ and $C_{18:0\ DMA}$, which accounted for 62.76% of the total fatty acids with 6.35, 21.42, 28.95 and 6.04%, respectively.

Phylogenetic analysis based on the 16S rDNA sequences

A 1468 bp sequence of 16S rDNA gene from SN22-2 was obtained. The nucleotide sequences of 16S rDNA of SN22-2 had been deposited in the GenBank database under accession numbers DQ450463. Representative strains that had high homology with SN22-2 were selected and the phylogenetic tree was constructed. As shown in Figure 2, the average genetic distances of the 16 strains was 0.043. The resemblance between strain SN22-2 and *B. coagulans* (Accession No. AB240205) was 99%. Consequently, based on the results of morphological observation, physiological tests, fatty acids and 16S rDNA analysis, strain SN22-2 was identified as *B. coagulans* and named as *B. coagulans* SN22-2.

Analysis of *nirS* sequence

Nitrite reductase reduces nitrite to NO and is encoded by two structurally different genes: *nir*K, encoding a copper-containing enzyme, and *nirS* encoding cytochrome *cd1* (Garbeva et al., 2007). These genes are functionally and physiologically equivalent and appear to be mutually exclusive. Figure 3 shows an 893 bp fragment *of nirS* gene obtained and the gene sequences were deposited in the GenBank database under accession number

Table 1. The results of the physiochemical tests.

Parameter	Result
Indole test	+
Methyl red test	+
Citrate utilization	+
Glucose fermentation	Acid production through fermentation
Fructose	-
Lactose	-
Sucrose	-
Ethanol	-
Starch hydrolysis	-
Grease hydrolysis	+
Urea hydrolysis	-
Gelatin hydrolysis	-
Gas production from glucose	-
Nitrate reduction	+
H2S production	+
Catalase test	-
Vogers-Proskauer test	+
Ammonia production	+
Litmus milk test	Organic acid production and solidification
Oxygen demand	Anaerobic

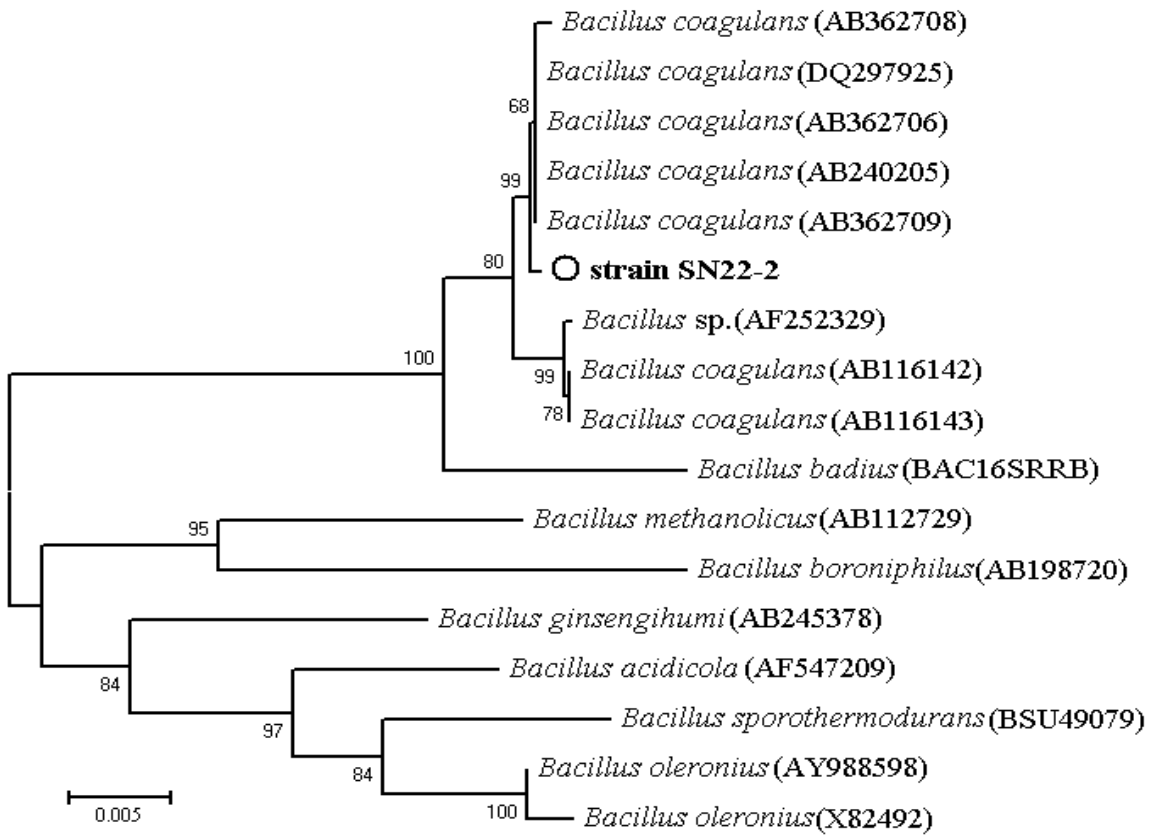

Figure 2. Phylogenetic tree based on the 16S rDNA gene sequences of strain SN22-2 and the selected species from GenBank Database using the neighbour-joining method.

```
1   CACACCCCGA GCCGCGCGTG GCCGCCATCG TGGCCTCGCA CGAGCACCCG GAGTTCATCG TCAACGTCAA GGAAACCGGC AAGATCATGC TGGTGAACTA  100
101 CGAGGACATC GAGAACCTCA AGACCACCAC GATCAATGCC GCACGCTTCC TGCACGACGG CGGCTGGGAT TCGACCAAGC GCTATTTCCT GACCGCGGCC  200
201 AACCAGAGCG ACAAGATCGC GGTGGTGGAC TCGCGCGACC AGAAGCTGGC CGCGCTGATC GATGTCGACA AGATCCCGCA CCCGGGCCGC GGCGCCAACT  300
301 TCATGCATCC CAAGTGCGGG CCGGTGTGGG CGACCTCGGC GCTGGGCAAC GAGAAGATCA CCCTGATCGC CACCGATCCG GTCAACCACA AGGACTATGC  400
401 CTGGAAGGTC TGCGAAGTGC TGAAGGGCCA GGGCGGCGGC TCGCTGTTCG TGAAGACCCA CCCGAAGTCG CGGAACCTGT GGGTCGACAC CACCCTCAAC  500
501 CCCGACCCGA AGATCAGCCA GTCGATCGCG GTGTTCGACA CCACCGACCT CGCCAAGGGG TACCAGGTGC TGCCGATCGC GGAATGGGCG AACCTGGGCG  600
601 AAGGCCCCAA GCGCGTGGTG CAGCCGGAAT ACAACGCCAA GGGCGACGAG GTCTGGTTCT CGGTGTGGAC GGCAGACCAG CGCTCGGCGA TCGTGGTGGT  700
701 GGACGACAGG ACCCGCAGCT CAAGCCGTCT GGACGACAGC GCTGGTCACC CGACGGCAGT CACGCTATCA CTAGTGCGCG CTGCAGTCGA CATATGGAAG  800
801 TCCACGGTGA GAACTGGATC AATGCCCAAA GTGCGACTGC TACGTCTGTG ATGTCGTCAT CCCAAACACA GACGGAGGAC TATGTCTCTA TCG 893
```

Figure 3. Sequences of the *nirS* gene amplified by primer pair 832 F and 1606R.

```
1   CCCACTGGAA GCACGGCGGC ATCGTGGGTG TGTTCGGTTA CGGCGGCGGC GTTATCGGCC  60
61  GTTACTGCGA CCAGCCCGAA ATGCTCCCCG GCGTGGCGCA CTTCCACACC ATGCGTGTGG  120
121 CCCAGCCTTC CGGCAAGTAC TACCACAGCA AGTTCCTGCG CGACCTGTGC GACATTTGGG  180
181 ATCTGCGTGG TTCTGGTCTG ACCAACATGC ACGGCTCCAC CGGCGACATC GTTCTCCTCG  240
241 GCA 243
```

Figure 4. Sequences of the Dir gene amplified by primer pair DSR1F and DSR5R.

DQ450463. A 97% resemblance was obtained when the sequenced *nir*S gene was compared with reference sequence of gene under accession number of AF549053, which were the conserved sequences of *nir* gene.

Analysis of *Dsr* sequence

For SRB, the *Dsr* gene, which encodes the dissimilatory sulfite reductase , was a key enzyme in sulfate reduction that catalyzes the reduction of sulfite to sulfide and hence is required by all sulfate reducers. As shown in Figure 4, a 243 bp fragment *of Dsr* gene was obtained. A 98% similarity was obtained when the sequenced *Dsr* gene was compared with the sequence of α-subunit *Dsr* gene of *D. desulfuricans* (AF273034), which were the conserved sequences of *nir* gene.

The denitrification and sulfate-reduction capabilities of strain SN22-2

The bacterial solution of SN22-2 was inoculated in the specially designed medium with a 5% volume ratio to the medium. The concentrations of SO_4^{2-}, NO_3^- and NO_2^- were detected at daily intervals for the assessment of its denitrification and sulfate-reduction capabilities. As shown in Figure 5, the concentration of NO_3^- declined from 4368.45 mg/L in the beginning to 88.12 mg/L at the end. The removal efficiency of NO_3^- was 82.75% on the 4^{th} day and the highest was 97.98%. The greatest concentration of NO_2^- was detected on the 4^{th} day (1493.60 mg/L), which was produced as the intermediate product when the concentration of NO_3^- dropped dramatically. Along with the cultivation of strain SN22-2, the NO_2^- concentration was gradually declined without

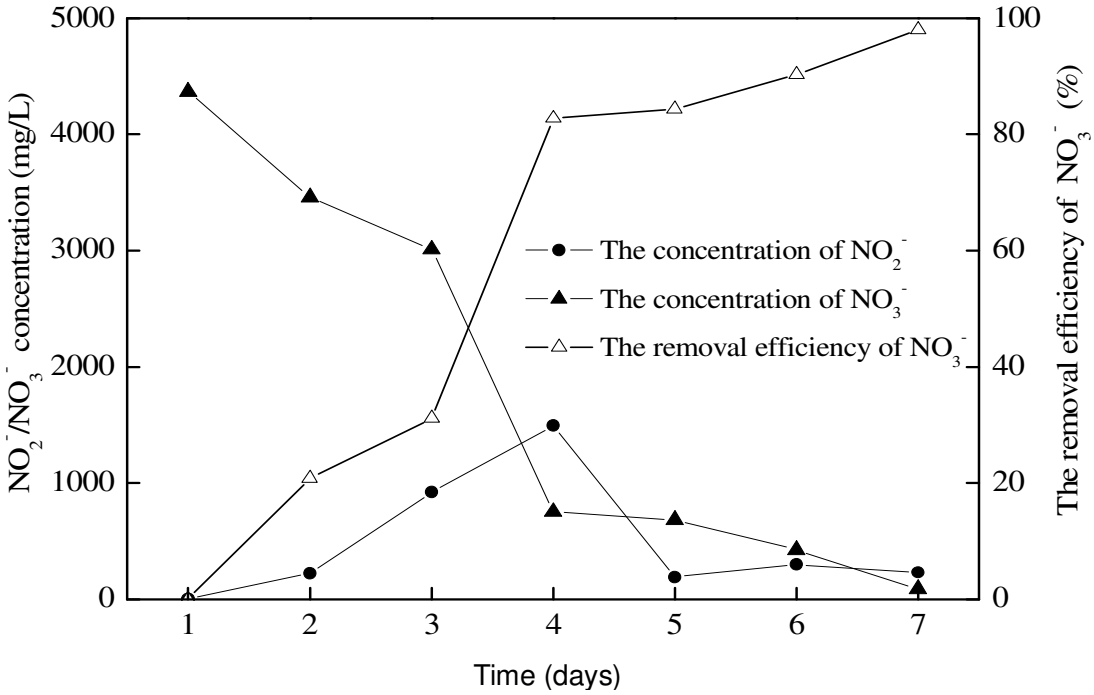

Figure 5. The daily variations of NO_3^- and NO_2^- concentrations and the removal efficiency of NO_3^-.

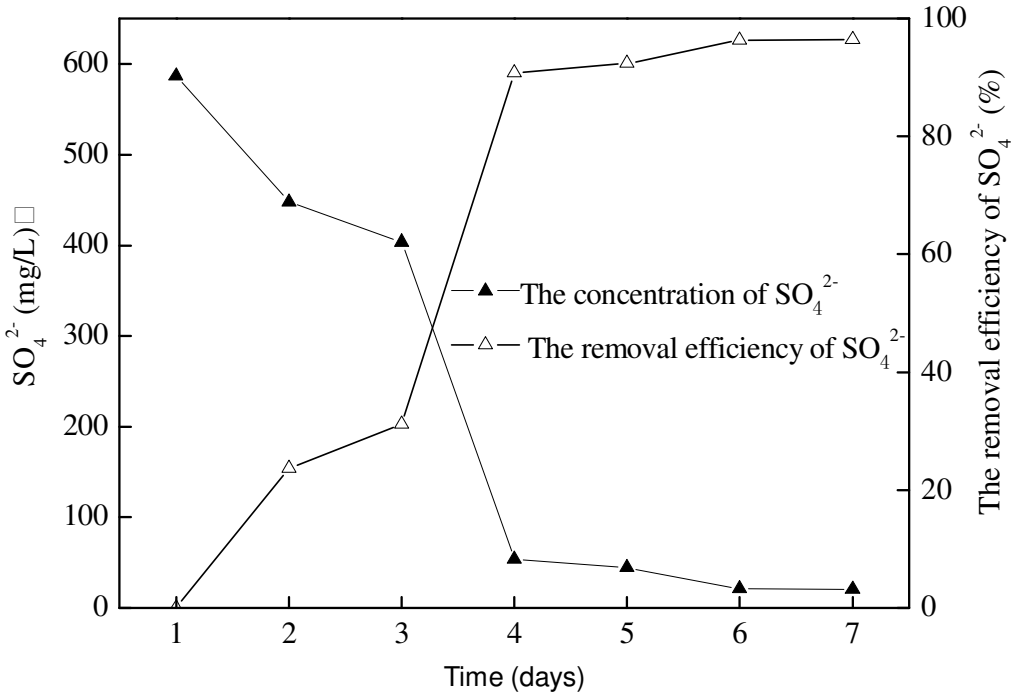

Figure 6. The daily variations of SO_4^{2-} concentrations and the removal efficiency of SO_4^{2-}.

accumulation. Thus, strain SN22-2 had great denitrification capability. Figure 6 shows that the concentration of SO_4^{2-} dropped from 586.71 to 20.63 mg/L during the denitrification process of strain SN22-2. The removal efficiency of SO_4^{2-} on the 4th day was up to 90.84% and the highest was 96.48%. It demonstrated that strain SN22-2 was efficient for sulfate reduction. Consequently, SN22-2 was a strain with simultaneous capabilities of denitrification and sulfate reduction.

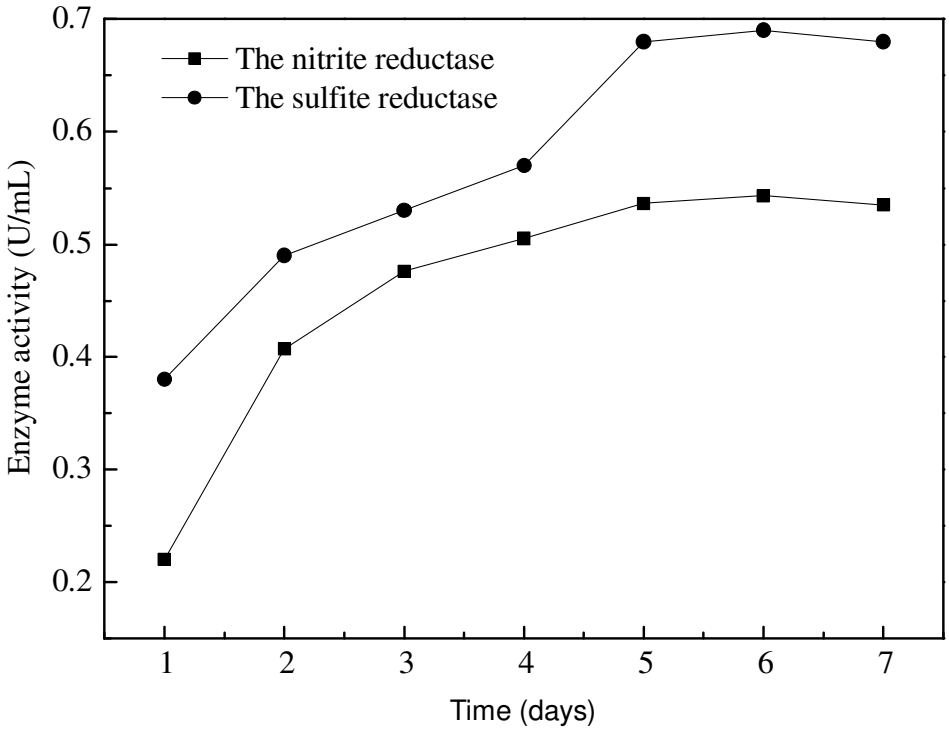

Figure 7. The nitrite and sulfite reductase activities of SN22-2.

The detection of enzyme activities

Nitrite reductases are a group of enzymes that catalyze the reduction of nitrite. Dissimilatory sulfite reductase was a key enzyme in sulfate reduction that catalyzes the reduction of sulfite to sulfide. Figure 7 shows that the activity of the nitrite reductase enhanced from 0.22 U/mL on the 1st day to 0.543 U/mL on the 6th day. For the sulfite reductase, its activity increased from 0.38 U/mL on the 1st day to 0.69 U/mL on the 7th day. It gave further evidence that SN22-2 was a strain with denitrification and sulfate reduction capabilities.

Evidence had been presented that SRB could utilize various types of refractory organics (such as polycyclic aromatic hydrocarbons and *n*-alkenes) as carbon sources (Tsai et al., 2009). The use of SRB for metal and radioactive waste remediation was also widely studied (Zhengji, 2010). However, the application of SRB in the treatment of wastewater containing high concentration of sulfate and nitrogen was rare. Generally, the transformations of sulfate and nitrogen were performed in individual reactors or with different technology (Wang et al., 2009). The isolated SN22-2 with simultaneous capabilities of denitrification and sulfate-reduction had great potential in the treatment of wastewaters produced from light chemical engineering industries, food processing and pharmaceutical factories. These wastewaters were rich in sulfate and nitrogen and the isolated strain could be applied without the consideration of interspecific competition. Further studies are necessary to explore the feasibility and the characteristics of the isolate in practical wastewater treatment.

Conclusions

An anaerobic strain, named as SN22-2 with simultaneous denitrifying and sulfate-reducing ablities was isolated. It was a rod-shaped, Gram-negative, 0.20 to 0.70 µm in width and 3.0 to 5.5 µm in length. Most of the fatty acids of SN22-2 were distributed among $C_{12:0}$–$C_{19\text{-}CYC\text{-}9}$ and the main fatty acids consisted of $C_{16:1\text{-}CIS\text{-}9\text{-}FAME}$, $C_{16:0\text{-}FAME}$, $C_{18:0\text{-}FAME}$ and $C_{18:0\ DMA}$. Strain SN22-2 was identified as *Bacillus coagulans* as its 16S rDNA bored 99% resemblance to that of *Bacillus coagulans* (Accession No. AB240205). The dissimilatory sulfite reductase (*Dsr*) and the nitrite reductase (*nirS*) genes were successfully amplified and cloned from strain S22-2. The removal efficiencies of NO_3^- and SO_4^{2-} were up to 96.48 and 97.98% when the initial concentrations of NO_3^- and SO_4^{2-} were 4368.45 and 586.70 mg/L. High activities of nitrite reductase and sulfate reductase were detected in the culture medium of SN22-2. In conclusion, strain SN22-2 had simultaneous denitrifying and sulfate reducing capablities. The characterization of the present isolate had important ecological implications in treating organic wastewater containing high concentraions of sulfate and nitrogen.

ACKNOWLEDGEMENT

This project was supported by the National Natural Science Foundation of China (50908063), the Science Fund for Creative Research Groups of China (Grant No. 51121062).

REFERENCES

Cole JA, Coleman KJ, Compton BE, Kavenagh BM, Keevil CW (1974). Nitrite and ammonia assimilation by anaerobic cultures of Escherichia coli., J. Gen. Microbiol. 85:11–22.

Garbeva P, Baggs EM, Prosser JI (2007). Phylogenyof nitrite reductase (nirK) and nitricoxide reductase (norB) genes from Nitrosospira species isolated from soil, FEMS Microbiol. Lett. 266:83–89.

Hong X, Zhang XJ, Liu BB, Mao YJ, Liu YD, Zhao LP (2010). Structural differentiation of bacterial communities in indole-degrading bioreactors under denitrifying and sulfate-reducing conditions, Res. Microbiol. 161:6873–693.

Jenneman GE, Mcinerney MJ, Knapp RM (1986). Effect of nitrate on biogenic sulfide production, Appl. Environ. Microbiol. 51:1205–1211.

Keith SM, Herbert RA (1983). Dissimilatory nitrate reduction by a strain of Desulfovibrio desulfuricans, FEMS Microbiol. Lett. 18:55–59.

Kutyavin IV, Afonina IA, Mill A (2000). Minor groove binder-DNA probes increase sequence specificity at PCR extension temperatures, Nucleic Acids Res. 28:655–661.

Liu MC, Costa C, Moura I (1994). Hexaheme nitrite reductase from Desulfovibrio desulfuricans (ATCC27774), Meth. Enzymol. 243:303–319.

Marietou A, Richardson D, Cole J (2005). Nitrate reduction by Desulfovibrio desulfuricans: a periplasmic nitrate reductase system that lacks NapB, but includes a unique tetraheme c-type cytochrome, NapM, FEMS Microbiol. Lett. 248:217–225.

Mori K, Kim H, Kakegawa T, S Hanada (2003). A novel lineage of sulfate-reducing microorganisms: Thermodesulfobiaceae fam. nov., Thermodesulfobium narugense, gen. nov., sp. nov., a new thermophilic isolate from a hot spring, Extremophiles,7:283–290.

Moura I, Bursakov S, Costa C, Moura JJ (1997). Nitrate and nitrite utilization in sulfate-reducing bacteria, Anaerobe, 3:279–290.

Ostrowski J, Wu JY, Rueger DC, Miller BE, Siegle LM, Kredich NM, (1989). Characterization of the cysJIH regions of Salmonella typhimurium and Escherichia coli B. DNA sequences of cysI and cysH and a model for the siroheme-Fe4S4 active center of sulfite reductase hemoprotein based on amino acid homology with spinach nitrite reductase, J. Bio. Chem. 264:15726–15737.

Reinsel MA, Sears JT, Stewart PS, McInerney MJ (1996). Control of microbial souring by nitrate, nitrite or glutaraldehyde injection in a sandstone column, J. Ind. Microbiol. 17:128–136.

Senez JC, Pichinoty F (1958). Reduction of nitrite at the expense of molecular hydrogen by Desulfovibrio desulfuricans and other bacterial species, Bull. Soc. Chim. Bio. Paris, 40:2099–2117.

Tsai JC, Kumar M, Lin JG (2009). Anaerobic biotransformation of fluorene and phenanthrene by sulfate-reducing bacteria and identification of biotransformation pathway, J. Hazard. Mater., 164: 847–855.

Wang J, Lu H, Chen GH, Lau GN, Tsang WL, Van Loosdrecht MCM, (2009). A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment, Water Res. 43:2363–2372.

Zhengji Y (2010). Microbial removal of uranyl by sulfate reducing bacteria in the presence of Fe (III) (hydr)oxides, J. Environ. Radioact. 101:700–705.