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The Research of HPAM Degradation Efficiency of an Anaerobic Strain SN10 with Simultaneous Capabilities of Denitrification and Sulfate Reduction

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Keywords: Strain with simultaneous denitrifying and sulfate reducing capabilities, Characterization, Identification of fatty acids, Function verification, Hydrolyzed Polyacrylamide (HPAM) Degrading.

Abstract. Anaerobic bacterium (strain SN10) with simultaneous capabilities of denitrification and sulfate reduction was enriched and isolated by anaerobic Hungate technology. The characterisation of SN10 was conducted from the morphologial, physiological, phylogenetic aspects and HPAM degradation efficiency. It was a rod-shaped, gram-negative, 0.40~1.0μm in width and 1.5~2.5 μm in length. Most of the fatty acids of SN10 was distributed among C12:0~C19-CYC-FAME and the main of them was consisted of C14:0 FAME, C16:0-FAME, C18:1c-FAME, C18:0-FAME and C19-CYC-FAME. Based on the morphologial, physiological characteristics and the phylogenetic analysis, SN10 was identified as Paenibacillus sp. (EU124565), Because its 16S rDNA can reach 97% of the similarity to that of Paenibacillus lautus SN10 (Accession No.DQ450463). The removal efficiencies of NO_3^- and SO_4^{2-} were up to 98.52% and 96.49 % when the initial concentrations of NO_3^- and SO_4^{2-} were 5830.92 mg/L and 682.83 mg/L, HPAM solution density was 2400mg/L, three days strain biodegradation rate was 68.27%, which can result in obviously decreased viscosity. The SN10 strain isolated would have provided the new microorganism resources for the polyacrylamide biodegradation.

Introduction

HPAM is extensively applied in the thrice oil recovery in Daqing Oil Field, northeast China, which reached its later period and whose oils pumped out contain high concentration of water, because HPAM can evidently increase viscosity and viscoelasticity [1]. Up to 2010, the demand for HPAM will reach 10~11t/a in Chinese petroleum industries, and 80% of the amount will be used by Daqing Oil Field. The discharge of wastewater containing high concentration HPAM polymer will result in serious environmental problem [2]. The sewage water containing HPAM in oil-field is quite complex and special, oil and viscous. How to effectively decompose HPAM is important when HPAM is used as driver for oil exploitation. The key problem is to seek microorganism which can produce the reducible material, promote HPAM chain-like oxidative degradation, then biodegrade, and to treat sewage water containing HPAM. At present, there are few reports about the microorganism which can degrade HPAM effectively[3~5].

It was reported that the genera of Desulfovibrio, Desulfobulbus and Desulfomonas could utilize nitrate as 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[6~8].

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 capabilities was designed. A anaerobic strain with simultaneous denitrifying and sulfate-reducing capabilities, named as SN10, was isolated. Morphological observation, physiological tests, fatty acids analysis, phylogenetic analysis of 16S rDNA. 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

The strain isolation sample was the activated sludge, which was collected from a denitrification-based SRB inhibition bioreactor. Oil field wastewater was treated in the reactor. The concentrations of HPAM, Oil and COD were 420 mg/L, 125 mg/L and 840mg/L respectively when pH was 8.0. In the same time, the same concentration of SO_4^{2-} and NO_3^- which were 600 mg/L was added .

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: HPAM 2.5 g, Na_2SO_4 4 g, KNO_3 2.0 g, $NaNO_3$ 4.0 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 0.5g 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 ^oC. The sterilized medium was cooled and 0.1 mL 3% $FeSO_4 \cdot (NH_4)_2SO_4$ was added. Powdered agar (1.5%, wt/vol) was added when solid medium was prepared before sterilization.

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 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.

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. The determination of sulfite reductase followed the method described by Ostrowski et al, The concentration of HPAM was detected by starch-cadmium iodide method, The measurement of Polymer viscosity was determined by Hakke viscometer Bush.

Results and Discussion

Morphological and Physiochemical Characterization of the Isolated Strain SN10

An anaerobic strain, named as SN10, was isolated for its simultaneous denitrification and sulfate-reducing capabilities.

Figure 1 showed that strain SN10 was long rod-shaped, 0.40~1.0 μm in width and 1.5~2.5 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 were listed in Table 1. It showed that strain SN10 was mesophilic growing at temperatures ranging from 20 to 45^oC with an optimum growth temperature of 36 ^oC. Optimal growth occurred at pH 7.5. Growth was observed on the following substrates: glucose, citrate and grease. Strain SN10 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 SN10 had denitrification and sulfate reduction

capabilities. Besides, the analysis of fatty acids analysis indicated that most of the fatty acids distributed around $C_{12:0}$~$C_{19\text{-CYC-FAME}}$.

Table 1. The results of the physiochemical tests.

Items	Results	Items	Results
Ethanol	-	Litmus milk test	Organic acid production and solidification
Indole test	+	Gelatin hydrolysis	-
Fructose	-	Nitrate reduction	+
Lactose	-	Vogers-Proskauer test	+
Sucrose	-	Ammonia production	+
Citrate utilization	+	H_2S production	+
Glucose fermentation	Acid production through fermentation	Catalase test	-
Starch hydrolysis	-	Oxygen demand	Anaerobic
Grease hydrolysis	+	Gas production from glucose	-
Methyl red test	+	Urea hydrolysis	-

The main fatty acids consisted of $C_{14:0\ FAME}$, $C_{16:0\text{-FAME}}$, $C_{18:1c\text{-FAME}}$, $C_{18:0\text{-FAME}}$ and $C_{19\text{-CYC-FAME}}$, which accounted for 80.59% of the total fatty acids with 7.56%, 48.23%, 9.15%,8.67% and 6.98%, respectively.

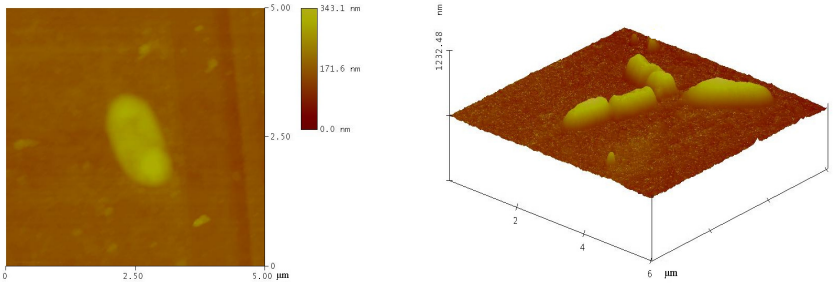

Figure 1. AFM images of Strain SN10.

Phylogenetic Analysis Based on the 16S rDNA Sequences

A 1420 bp sequence of 16S rDNA gene from of SN10 were obtained. The nucleotide sequences of 16S rDNA of SN10 had been deposited in the GenBank database under accession numbers DQ450464. Representative strains had high homology with SN10 were selected and the phylogenetic tree was constructed. As shown in Figure 2, the average genetic distances of the 16 strains was 0.042. The resemblance between strain SN10 and *Paenibacillus* sp.(Accession No.EU124565) was 97%. Consequently, based on the results of morphological observation, physiological tests, fatty acids and 16S rDNA analysis, strain SN10 was identified as *Paenibacillus lautus* and named as *Paenibacillus* sp. SN10.

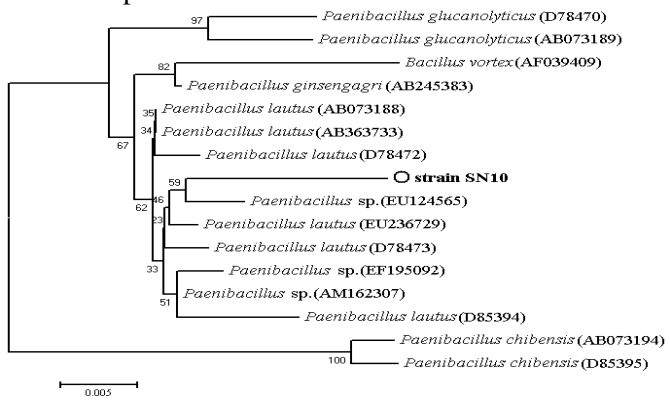

Figure 2. Phylogenetic tree based on the 16S rDNA gene sequences of strain SN10 and the selected species from GenBank Database by using the neighbor-joining method.

The Denitrification and Sulfate-Reduction Capabilities of Strain SN10

The bacterial solution of SN10 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 3, the concentration of NO_3^- declined from 5830.92 mg/L in the beginning to 86.41 mg/L at the end. The removal efficiency of NO_3^- on the highest was 98.52%. The greatest concentration of NO_2^- was detected on the 4th day (686.07 mg/L), which was produced as the intermediate product when the concentration of NO_3^- dropped dramatically.

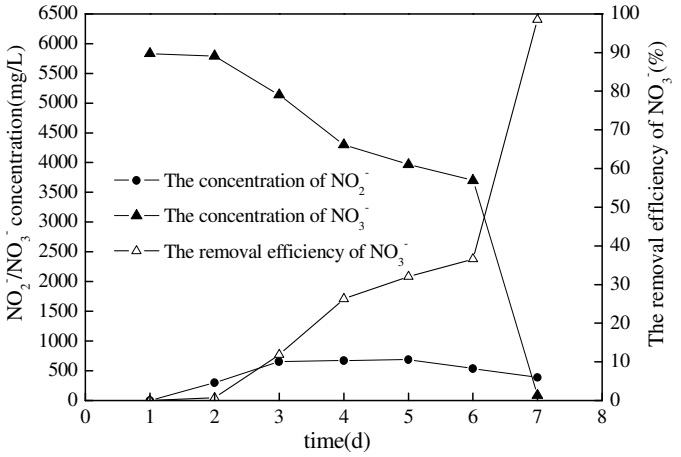

Figure 3. The daily variations of NO_3^- and NO_2^- concentrations and the removal efficiency of NO_3^-.

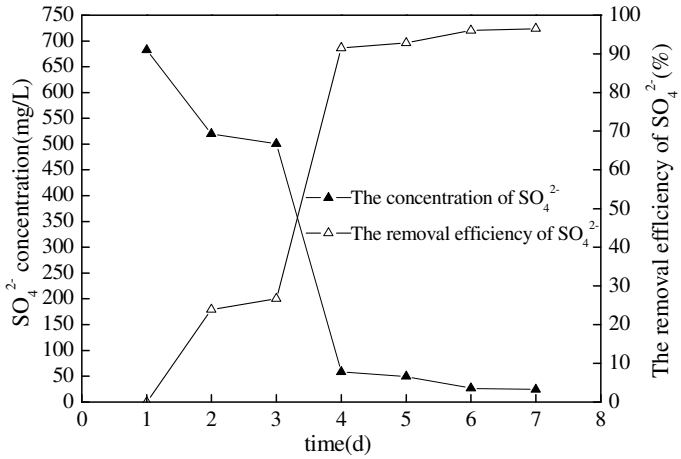

Figure 4. The daily variations of SO_4^{2-} concentrations and the removal efficiency of SO_4^{2-}.

Along with the cultivation of strain SN10, the NO_2^- concentration was gradually declined without accumulation. Thus, strain SN10 had great denitrification capability. Figure 4 showed that the concentration of SO_4^{2-} dropped from 682.83 mg/L to 23.94 mg/L during the denitrification process of strain SN10. The removal efficiency of SO_4^{2-} on the highest was 96.49%. It demonstrated that strain SN10 was efficient for sulfate reduction. Consequently, SN10 was a strain with simultaneous capabilities of denitrification and sulfate reduction.

Conclusions

An anaerobic strain, named as SN10 with simultaneous denitrifying and sulfate-reducing ablities was isolated. It was a rod-shaped, gram-negative. Most of the fatty acids of SN10 distributed among C12:0~C19-CYC-FAME and the main of them was consisted of C14:0 FAME, C16:0-FAME, C18:1c-FAME, C18:0-FAME and C19-CYC-FAME. Strain SN10 was identified as Paenibacillus

sp. because its 16S rDNA can reach 97% of the similarity to that of Paenibacillus sp.(EU124565). The removal efficiencies of NO^{3-} and SO_4^{2-} were up to 80.78% and 96.22 %. High activities of nitrite reductase and sulfate reductase were detected in the culture medium of SN10，the HPAM solution density was 2400mg/ L, three days strain biodegradation rate was 68.27%, which resulted in obviously decreased viscosity. In conclusion, strain SN10 had simultaneous denitrifying and sulfate reducing capablities, in the same time, it had the nice degrading properties to HPAM. Strain SN10 can afford perfect microbial resources to the high concentration sulfate, Nitrogenous chemical wastewater and oil field wastewater.

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