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Anaerobic degradation of sodium dodecyl sulfate (SDS) by denitrifying bacteria

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ABSTRACT

Two denitrifying bacteria were isolated using sodium dodecyl sulfate (SDS) as substrate. Strains SN1 and SN2 were isolated from an activated sludge reactor of a wastewater treatment plant (WWTP) with Anaerobic–Anoxic–Oxic (A^2/O) steps. Based on 16S rRNA gene analysis strain SN1 is 99% similar to *Pseudomonas stutzeri* (CCUG 11256^T), while strain SN2 is 99% similar to *Pseudomonas nitroreducens* (DSM 14399^T). The two novel strains are able to grow with a variety of organic compounds, including intermediates of SDS degradation. Alkylsulfatase activity was induced by SDS in strain SN1 and was enhanced by SDS in strain SN2. The type strain *P. stutzeri* (CCUG 11256^T) is not able to grow with SDS. However, *P. nitroreducens* (DSM 14399^T) is able to degrade and grow with SDS and nitrate or oxygen as electron acceptors, adding new information to the physiological abilities of this type strain.

1. Introduction

Surfactants are surface-active agents used in the formulation of detergents, personal care products, paints, polymers and other products. Surfactant molecules typically consist of a polar head group (charged or not charged) and a nonpolar hydrocarbon tail. There are three main classes of surfactants: anionic, nonionic and cationic. The hydrophilic and hydrophobic properties make these compounds useful for cleaning/solubilization purposes (Ying, 2006). Surfactants are widely used for domestic and industrial applications and after use they are discharged to wastewater treatment plants (WWTP's) (Gonzalez et al., 2007).

Aerobic degradation of surfactants has been described in several studies (Swisher, 1987; Scott and Jones, 2000). Less is known about anaerobic degradation of surfactants but many surfactants, especially linear alkyl benzene sulfonates, secondary alkane sulfonates and cationic surfactants are known to be persistent under anaerobic conditions (Ying, 2006).

Sodium dodecyl sulfate (SDS) is present in many cosmetics and personal hygiene products (Sirisattha et al., 2004) and is often used as model compound for aliphatic alkylsulfates degradation (Abboud et al., 2007). SDS degradation in the presence of oxygen is known (Swisher, 1987; Scott and Jones, 2000). Initial studies were focused on aspects associated to the alkylsulfate degradation in polluted rivers sites, such as SDS-degraders occurrence and distribution, alkylsulfatase activity and attachment of alkylsulfate-degraders to river sediments (Anderson et al., 1988; White et al., 1989; Marchesi et al., 1994). However, SDS degradation by denitrifying bacteria is only scarcely documented. Thus far, SDS degradation coupled to nitrate reduction was only described for unidentified bacteria isolated from river sediments (Dodgson et al., 1984). In that study, alkylsulfatase activity of an isolate able to degrade SDS and other alkylsulfates, in anoxic and oxic conditions, was investigated. The isolate degraded SDS coupled to nitrate reduction to N_2, not accumulating N_2O.

Nowadays, most of the developed countries have wastewater collection connected to municipal WWTP's. In a WWTP elimination of organic matter and nitrogen removal together with phosphorus accumulation can be performed using an A^2/O biological process. In this biological process, anaerobic treatment occurs first, followed by the anoxic and the oxic processes. In this way, most of the organic matter is degraded in the first two steps (anaerobic and anoxic), with no need for extra addition of carbon source to the

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anoxic reactor. Nitrate, resulting from nitrification, is recirculated from the oxic reactor to the anoxic reactor, where denitrifying bacteria reduce nitrate to dinitrogen gas (Metcalf and Eddy, 1991).

A degradation pathway for SDS has been proposed for *Pseudomonas* C12B (Payne and Feisal, 1963; Thomas and White, 1989). In this pathway, the initial cleavage occurs via hydrolysis of the ester that bonds the alkyl chain to the sulfate. Since degradation of SDS does not require oxygenases, it is expected that in an A^2/O process such surfactants will be degraded in the anaerobic or anoxic compartment before reaching the oxic step. The aim of the present study was to get insight into occurrence and activity of microorganisms capable of SDS degradation coupled to denitrification in a WWTP with the A^2/O concept.

2. Materials and methods

2.1. Enrichment, isolation and identification

Activated sludge from a wastewater treatment plant (Valladolid, Spain) was used as inoculum. The initial enrichment was done in batch culture. The batches were prepared in 120-mL serum bottles and contained 50 mL of medium and a gas phase of helium (1.4 atm). Bottles were sealed with butyl-rubber stoppers and crimp seals. The standard mineral salts medium contained (per liter distilled water): 1 g KH_2PO_4, 3.48 g $Na_2HPO_4 \cdot 2H_2O$, 1 g $(NH_4)_2SO_4$, 0.033 g $MgCl_2 \cdot 6H_2O$, 0.0090 g $CaCl_2 \cdot 2H_2O$, 0.01 g Fe(NH_4) citrate. Vitamins and trace elements were added in the final concentrations as described by Holliger et al. (1993). SDS (0.35 mM) was used as sole carbon and energy source and added from a filter sterilized anoxic stock solution. KNO_3 (4.3 mM) was added as electron acceptor from a sterilized stock solution. Initial inoculum concentration in the bottles was about 215 mg VSS L^{-1}. Batch enrichments were incubated at 30 °C and the pH was 7.3 ± 0.1. After several transfers of 5% (v/v) of the enriched culture to fresh medium, dilutions of the last enriched culture were streaked on agar plates to isolate the microorganisms. Agar plates contained standard mineral salts medium, 0.4 mM of SDS and 20 g L^{-1} of agar. The plates were incubated at 30 °C, under air. Colonies with different morphology were selected and streaked on new plates until single colonies were obtained. The ability of each of the pure cultures to degrade SDS in anoxic conditions was tested by transferring the single colonies back to anoxic conditions in batch liquid cultures.

Purity and morphology of the isolated microorganisms was routinely observed by phase contrast microscopy. Gram staining was performed using standard techniques. For phylogenetic analysis DNA was extracted with the FastDNA® Spin kit for soil (MP Biomedicals, USA) according to the manufacturer protocol. The bacterial 16S rRNA genes were amplified by PCR (Polymerase Chain Reaction) using the universal bacterial primers 7f and 1510r (Lane, 1991) and the GoTaq DNA Polymerase Kit (Promega, USA). Each PCR mixture contained: 1 μL of DNA template, 0.25 μL of Taq DNA polymerase, 1 μL dNTPs, 1 μL primer 7f, 1 μL primer 1510r, 10 μL PCR buffer and 35.75 μL PCR water. The settings for PCR were: initial denaturation for 2 min at 95 °C, followed by 25 cycles of 30 s denaturation at 95 °C, 40 s annealing at 52 °C and 1.5 min elongation at 72 °C. Post-elongation was 5 min at 72 °C. PCR products were purified with DNA Clean & Concentrator™-5 kit (Zymo Research, USA). Sequencing was performed by Baseclear (The Netherlands). The sequences were checked with the alignment programs of BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Closely related 16S rRNA gene sequences were identified using BLAST for homology searches (Altschul et al., 1990). The 16S rRNA gene sequences of strains SN1 and SN2 have been deposited in the GenBank database under accession numbers JF461537 and JF461538, respectively. Both strains SN1 and SN2 have been deposited in the open collection of DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) under the DSM numbers 26095 and 26096, respectively.

2.2. Growth with SDS

Anoxic biodegradation of SDS with nitrate as electron acceptor was studied with the enriched culture and the obtained isolates. For this, 1.1 L glass bottles were used containing 750 mL of standard mineral medium with SDS (0.35 mM) and KNO_3 (4.3 mM). The incubations were performed in triplicate while appropriate duplicate controls were included, lasting around 50 h. All experiments were conducted at 30 °C, during approximately 50 h. In time, liquid and gas samples were taken. Biomass increase was monitored using optical density (OD) measurements at 600 nm. NO_3^-, NO_2^- and N_2 were also analyzed. SDS degradation was indirectly monitored by quantifying the dissolved organic carbon (DOC) in the medium. At the beginning of the growth experiments 2% (v/v) of inoculum of enriched culture, strain SN1 or strain SN2 was added. In all growth experiments the initial concentration of inoculum was similar. Carbon mass balances were calculated at the beginning of incubation and at the end of the exponential growth phase. Total organic carbon (TOC), inorganic carbon (IC), dissolved organic carbon (DOC) and CO_2 were measured for this purpose. The carbon present as biomass was determined by subtracting DOC from TOC values. The amount of carbon present as biomass at the beginning of the tests was disregarded. Total inorganic carbon in the bottles was calculated as the sum of CO_2 (g) + CO_2 (aq) + HCO_3^- (aq). The Henry's law was used to calculate the expected dissolved CO_2 in the medium. Growth of isolated strains was studied with concentrations of SDS up to 40 g L^{-1} (139 mM) with NO_3^- (20 mM). Growth was determined by measuring increase in OD at 600 nm, NO_3^- reduction and NO_2^- and SO_4^{2-} accumulation, after a period of incubation of 5 days.

2.3. Optimal temperature and pH

Temperature and pH ranges were tested in 120-mL bottles with 40 mL standard medium, acetate (10 mM) and NO_3^- (10 mM). Isolates were incubated at a pH of 7.3 ± 0.1 from 4 °C to 55 °C and at a pH range between 4 and 10.5, at 30 °C, during two weeks. The optimal temperature and pH were determined by measuring the OD at 600 nm and nitrate depletion over the exponential growth phase, in duplicate.

2.4. Other electron donors

Different organic carbon sources such as sugars, alcohols, amino acids, volatile fatty acids and intermediates of the citric acid cycle were tested as growth substrates. Substrates were added to the medium from sterilized anoxic stock solutions to a final concentration of 10 mM. Peptone and yeast extract (final concentration 1 g L^{-1}) were also tested as growth substrates. Possible intermediates of SDS degradation (dodecanol, dodecanal and lauric acid) were directly added to the bottles before sterilization and tested at a final concentration of 0.2 mM.

Other anionic surfactants were also tested, including sodium dodecyl sulfonate and linear alkyl benzene sulfonate. Triton X-100, a nonionic surfactant, was tested as well. All the surfactants were applied in two concentrations (25 and 50 mg L^{-1}) and added to the bottles from filter sterilized stock solutions. Alkylamine degradation, such as hexylamine (0.25 and 0.5 mM) and dodecylamine (0.15 and 0.3 mM), was also evaluated. Hexylamine was added from a filter sterilized anoxic stock solution, while dodecylamine was added directly to the bottles before sterilization. Alkane degradation was tested by adding decane (0.6 mM) and dodecane