Vanadium is a transition metal which, at neutral pH, can exist in two oxidation states, $\text{V}^{IV}$ (vanadyl ion, cationic species $\text{VO}^{2+}$), and $\text{V}^{V}$ (vanadate ion, anionic species, $\text{H}_2\text{VO}_4^-$) (10, 11).

The environmental chemistry of vanadium is complex. Vanadium is an abundant element that has proven to be a valuable resource for different industrial applications such as vanadium alloys, oxidation catalysis in sulfuric acid manufacturing and automobile catalytic converters, photographic development, textile dying, and ceramic coloring. A number of bacteria are able to reduce metal compounds, most commonly iron and manganese, through anaerobic reduction. Some organisms are known to be able to reduce other metals such as arsenic, mercury, selenium, uranium, technetium, chromium, molybdenum, gold, silver, and copper (4, 8, 13). The microbial reduction of vanadium has also been reported (1, 14). To date, a number of microorganisms are known to be able to reduce other metals such as iron and manganese, through anaerobic reduction. Some or-
rpm for 15 min, washed and resuspended in Tris-EDTA buffer, and lysed by sonication. The vanadate concentration was determined with the DPC assay. Of the total vanadate initially present in the medium, 2% (standard error of the mean 6%) or 9% (standard error of the mean 5%) was detected in the cells grown with 2 and 10 mM V_{2}O_{5}, respectively.

**Effect of different buffers.** The effect of different buffers on anaerobic vanadium reduction was tested. Previous to all anaerobic experiments, sterile solutions were placed overnight in an anaerobic glove box (Coy Laboratories). Cultures were grown aerobically on a rotary shaker (150 rpm) at 28°C in LB medium to the late exponential growth phase (2 × 10^8 cells/ml) before being washed in appropriate buffer in anaerobic conditions and resuspended in equal volumes of anaerobic bicarbonate buffer containing 5 mM V_{2}O_{5} and 10 mM lactic acid, formic, pyruvic, fumaric, or citric acid. Vanadate reduction rates were monitored over time. While only a limited reduction of vanadate was measured in cultures containing fumaric and citric acid compared to cultures containing no electron donor, the cultures containing formic, lactic, and pyruvic acid significantly reduced vanadate (Fig. 3). Citric and fumaric acid cannot be used as electron donors by the organism and hence they do not support vanadate reduction.

**Vanadium reduction is a biochemical process.** Proof was found to eliminate the possibility that vanadate reduction was the result of a chemical or spontaneous process catalyzed by a biological component or product of the cell. In each experiment, 50 ml of an overnight-grown culture (2 × 10^8 cells/ml) was centrifuged; the cells were washed, weighed, and resuspended in equal volumes of anaerobic bicarbonate buffer containing 10 mM lactate and 5 mM V_{2}O_{5}. To demonstrate that the reduction is a biological process, one set of cells was heat killed prior to inoculation. Over a period of 5 h, no reduction in vanadate concentration could be measured. The reference culture had an initial reduction rate of 12.4 mmol of V^V per h per g of cells (wet weight). By heat killing the cells and subsequently assaying their vanadate reduction, we demonstrated

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**FIG. 1.** Electrophoretic determination of vanadium(V) and vanadium(IV). (A) Reference sample with 10 mM V^V (right peak) and 10 mM V^{IV} (left peak) (a) and analysis of precipitate composition (b). (B) Vanadium speciation during anaerobic reduction at different times: 0 min (a), 30 min (b), 45 min (c), 60 min (d), 90 min (e), 150 min (f), 1 day (g), and 1 week (h).

**FIG. 2.** Change in vanadate concentrations over time in the non-growth mode for anaerobic cultures containing 5 mM V_{2}O_{5} and 10 mM lactic acid in phosphate (●), Tris (▼), HEPES (■), or carbonate (▲) buffer.
tein synthesis, the ribosome inhibitor tetracycline was added at 5 mM V$_2$O$_5$ and 10 mM formic acid. This reduction required intact and metabolically active S. oneidensis cells.

To assess whether vanadate reduction requires de novo protein synthesis, the ribosome inhibitor tetracycline was added at 10 mM tetracycline per ml. After 5 min of incubation, 10 mM lactate and 5 mM V$_2$O$_5$ were added. Vanadate concentration was monitored over time with the DPC assay. No significant de-crease in the reduction rate could be measured, indicating that the activity does not depend on the induction of specific proteins (results not shown). The vanadate reduction pathway thus seems to be constitutively present in the bacterium.

Vanadium-containing granular precipitate. In the course of the experiments with anaerobic vanadate-reducing cultures, a significant formation of precipitate was detected. In an attempt to rule out precipitation of a compound from the medium, different media were tested. Growth on vanadate in Luria Bertani broth, carbonate buffer, HEPES buffer, Tris buffer, and to a limited extent, phosphate buffer resulted in a vanadate-containing precipitate. The granules were revealed to be insoluble at neutral pH, insoluble in 10 N NaOH, and soluble in 2 M H$_2$SO$_4$. V$^V$ is known to be soluble in basic and acidic solutions (2), while V$^{IV}$ is soluble in acid solutions and is not oxidized to V$^V$ below pH 2 (9). The sediment was washed three times with water. The pellet was solubilized in 2 M H$_2$SO$_4$. The sample was centrifuged to remove cell debris and subsequently analyzed by capillary electrophoresis. Only V$^{IV}$ and no vanadate could be detected (Fig. 1A).

Further examination of the precipitate revealed large granules with a diameter of up to 100 μm after 5 days of growth. Precipitate formed by cultures grown with 10 mM lactic acid and 5 mM V$_2$O$_5$ was examined with differential interference contrast (DIC) microscopy at a magnification factor of 320× (Leica DMLB, Wetzlar, Germany). Microscopic examination combined with crystal violet staining showed that the granules were completely colonized with S. oneidensis (Fig. 4C). By confocal microscopy with a combination of DIC and HeNe laser excitation at 543 nm (Zeiss Axiovert 100 M; Carl Zeiss), an autofluorescence of the bacteria was detected (Fig. 4D and E), which allowed us to unambiguously localize the bacteria in the granule matrix. Only a small number of motile free-living cells were seen compared to the dense accumulations of sessile cells contained in the granule matrix (Fig. 4A and B).

In summary, we present evidence for a dissimilatory vanadate reduction process in S. oneidensis. The bacterium is able to reduce V$^V$ (vanadate ion) to V$^{IV}$ (vanadyl ion) under anaerobic conditions. We evaluated different electron donor compounds for their ability to sustain vanadium pentoxide reduction and showed that significant reduction can be attained with formic, lactic, and pyruvic acids under these conditions. A small but measurable accumulation of vanadium pentoxide was present inside the bacterial cells. Anaerobic reduction resulted in a granular precipitate containing predominantly V$^{IV}$ (vanadyl ion), which was revealed to be completely colonized by sessile S. oneidensis. Further studies will focus on identification and isolation of the components of the vanadate reduction pathway.

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