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Effect of Nitrate on Reduction of Selenite by a Bacterium Isolated From a Selenium Contaminated Site

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Abstract. The possible inhibitory effect of nitrate on the reduction of selenite was examined on a bacterium isolated from a selenium contaminated site. Nitrate and selenite were reduced simultaneously by this bacterium. There was no inhibitory effect of nitrate on selenite reduction by this bacterium which suggests it can be used to treat selenite in wastewater containing nitrate.

INTRODUCTION

Selenium oxyanions are toxicants in the environment and commonly found in wastewaters associated with fossil fuel combustion ash disposal, oil refining and drainage water from irrigated areas and agricultural soils (1). Our previous study showed that *Shewanella putrefaciens* reduced selenite completely to elemental selenium which is an insoluble form of selenium and is not toxic (2). However, the ability of other electron acceptors such as nitrate and nitrite to inhibit selenate or selenite reduction by selenate-respiring bacteria in freshwater has been reported (3). The wastewater samples which were contaminated with selenium oxyanions also contained nitrate with the highest concentration observed being 5 mg/l. The ability of bacteria to use nitrate as an alternative electron acceptor is widely distributed amongst different genera. Hence, it is possible that *S. putrefaciens* could use nitrate as electron acceptor which may cause nitrate to be a competitive electron acceptor to selenite.

MATERIALS AND METHODS

Bacterial strain and growth conditions. *S. putrefaciens* was isolated from a coal combustion disposal site in N.S.W., Australia. The growth medium consisted of yeast extract 2.0 g/l, peptone 5.0 g/l, lab lemco 1.0 g/l and NaCl 5.0 g/l. The electron acceptor competition experiments described below were conducted with batch cultures of *S. putrefaciens* grown in a 1 l glass reactor. A 10 % log phase culture was inoculated into the reactor containing sterile media (500 ml). The temperature was held constant at 27°C by a thermostat. The pH of medium was maintained at 6.8-7.4 by addition of 1 M NaOH or 1 M HCl using pH controller. The growth of bacteria was followed by measuring optical density at 660 nm.

Chemical analyses. Nitrate was determined with a U.V. spectrophotometer at 515 nm using spectroquant 14773 from E.Merck. Nitrite was determined using a method developed by Montgomery and Dymock (4). Ammonium was determined using an enzyme kit from Boehringer Manheim. Selenite was determined by flow-

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through hydride generation atomic absorption spectrophotometer (196 nm) (5). The detection limit for selenium was 0.3 μg/l.

RESULTS AND DISCUSSION

Selenite reduction was not affected by the presence of nitrate as illustrated in Fig.1, suggesting that *S.putrefaciens* may express a set of at least two physiologically distinct terminal reductases which serve as electron donors to nitrate and selenite respectively. It has been reported that the terminal reductases for selenate and nitrate respiration in *Thauera selenatis* which reduced selenate to selenite and nitrate to nitrite are two distinct enzymes with the possibility that the nitrite reductase may catalyse the reduction of selenite to elemental selenium, because elemental selenium was formed when denitrification occured (6,7). However, selenite was not completely reduced to elemental selenium which implied that reduction of selenite via nitrite reductase was inefficient in the presence of nitrate or nitrite (7). Nitrate reductase has been studied in detail (8,9). However, the reductase associated with selenite reduction (referred to as selenite reductase) has not been studied in detail and hence may or may not be physiologically unique in different bacterial species. In our work with *S. putrefaciens*, nitrate reductase is associated only with nitrate (NO_3^-) reduction and hence serves as an electron donor for nitrate (NO_3^-) but not for selenite (SeO_3^{2-}). Hence, nitrate reductase could transfer electrons only to nitrate but not to selenite whereas selenite reductase transfers only to selenite but not to nitrate. In the presence of selenite and nitrate, this bacterium reduces selenite and nitrate simultaneously. The selenite reduction activity in the presence of nitrate was approximately equal to the selenite reduction activity in the absence of nitrate as indicated in Fig.1 and Fig.2. Moreover, the nitrate reduction activity in the absence selenite was also similar to the nitrate reduction activity in the presence of selenite as can be seen in Fig.2 and Fig.3. These results suggest that selenite or nitrate was not responsible for inhibiting electron transport to nitrate or selenite respectively. Therefore, it is proposed that electron transport of selenite and nitrate possibly proceeds via at least two physiologically independent pathways. It would

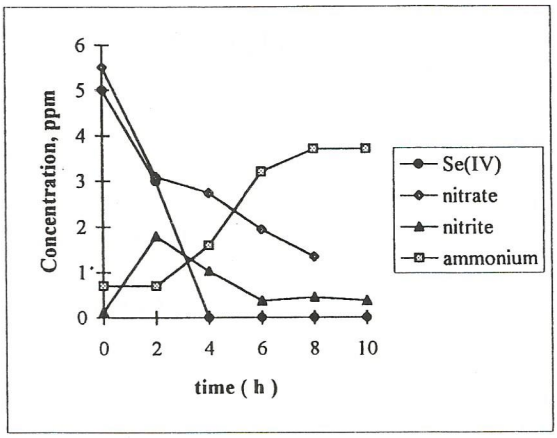

Figure 1. Consumption of selenite, nitrate, production and consumption of nitrite, production of ammonium in *S. putrefaciens*.

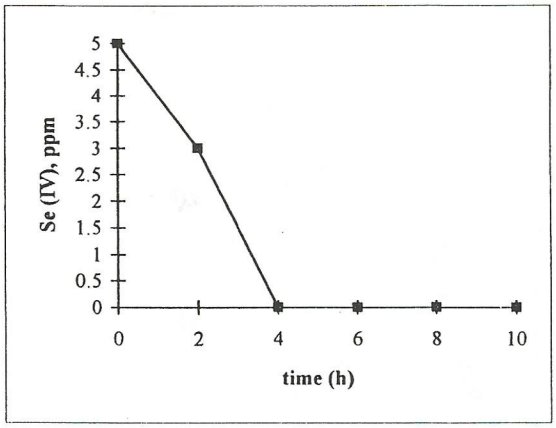

Figure 2. Selenite reduction in *S. putrefaciens*.

be necessary to measure enzyme activity for each reductase when the bacterium is grown either in nitrate or selenite to further elucidate the mechanisms.

DiChristina *et al.*(10) previously showed that nitrate and nitrite partially inhibited the reduction of Fe^{3+} by *Shewanella putrefaciens* 200 when grown in aerobic condition. Nitrate did not impair the growth in medium containing selenite as illustrated in Fig. 4.

Rapid accumulation of nitrite occured to a maximum after 2 hours and then decreased after which a period of ammonium production followed. There is a large production of

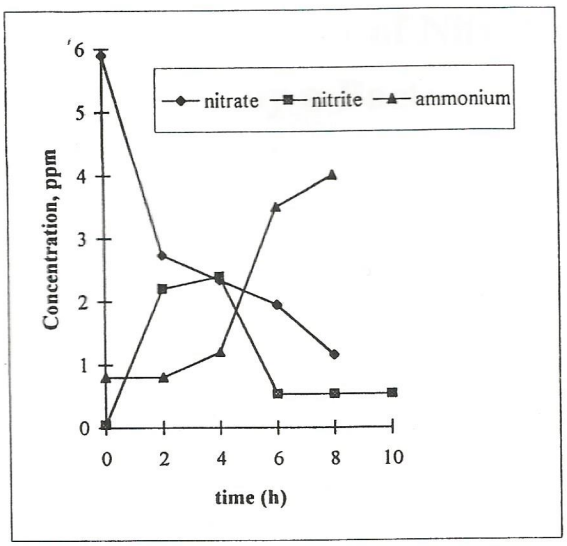

Figure 3. Consumption of nitrate, production and consumption of nitrite, production of ammonium in *S. putrefaciens*.

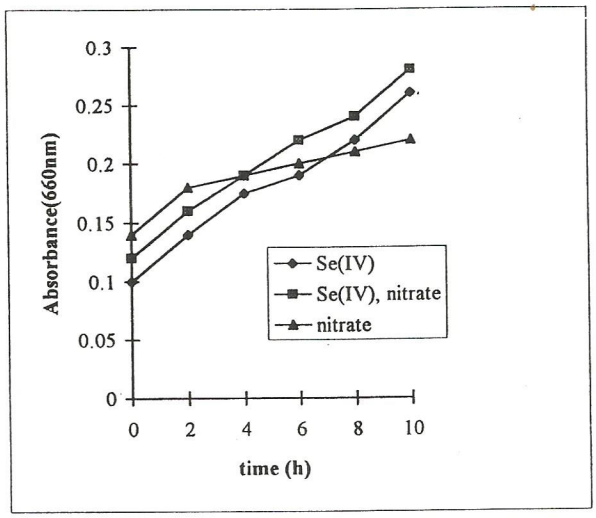

Figure 4. Growth of *S. putrefaciens* in a medium with nitrate, selenite or nitrate plus selenite.

ammonium when the amount of nitrate was limited. The final levels of ammonium and nitrite accounted for 96 % of the nitrate consumed, with the missing quantity presumably retained for cellular nitrogen biosynthesis.

It is clear that *S. putrefaciens* can reduce nitrate and selenite concomitantly which is beneficial in treating wastewater which contains both selenite and nitrate. This finding offers a possible process option for simultaneous nitrate and selenite removal.

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