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VANADIUM(V) REDUCTION IN *THIOBACILLUS THIOOXIDANS* CULTURES ON ELEMENTAL SULFUR

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SUMMARY

We describe the reduction of vanadium (V) to vanadium (IV) in cultures of *Thiobacillus thiooxidans* on elemental sulfur, for initial vanadium (V) concentrations up to 5 mM. The vanadium (V) is reduced by intermediate compounds generated by bacterial oxidation of elemental sulfur. The limit of initial vanadium (V) allowing bacterial action seems to be related to the inhibition caused by such vanadium species, rather than connected to the vanadium (IV) species, which did not show inhibitory effects up to concentrations of about 0.1 M. This reduction mechanism of vanadium (V) is potentially applicable in the recovery of vanadium from spent solid catalysts, by a low-cost methodology.

INTRODUCTION

The chemi-autotroph microorganism *Thiobacillus thiooxidans* is able to obtain energy from the catalysed oxidation of partially-oxidized sulfur compounds using oxygen as last acceptor of electrons (Imai K., 1978). The oxidation of such compounds, particularly that of elemental sulfur, generates a series of sulfur oxoanions (sulfite and thiosulfate, among others) with high reducer power. This activity has been utilized in previous work to catalyze the reduction of manganese IV compounds (Porro et al., 1990) and iron III (Donati et al., 1995).
In the present work, we attempted to use the capacity of *Thiobacillus thiooxidans* to generate such intermediate sulfur compounds so as to reduce "in-situ" the vanadium (V).
Such a low-cost procedure might be of potential use in the recovery of vanadium from spent solid catalysts of the vanadium-titanium and vanadium-phosphorus type. Traditionally, these catalysts were disposed in landfill but, owing to strict environmental regulations, this practice is mostly no longer used. Therefore, two alternative ways for the treatment of spent catalysts are being developed: the first involves, as long as possible, catalysts regeneration and the second, the recovery and re-use of the metals that make up them (Lassner et al., 1994). The more widespread recovery techniques involve either pyrometallurgical or hydrometallurgical processes which use dangerous chemical products and comprise high energy costs (Lassner et al., 1994).

MATERIALS AND METHODS

Microorganism: we used a strain of *Thiobacillus thiooxidans (T.t.)* normally grown in modified Imai medium ($CaCl_2$: 0.25 g/l; KH_2PO_4: 1 g/l; $MgSO_4.7H_2O$: 0.3 g/l; $(NH_4)_2SO_4$: 2 g/l) on elemental sulfur (10 g/l) at an initial pH of 2.0. When the culture reached the exponential growth stage (which was followed by the decrease of pH), it was filtered to eliminate any unoxidized elemental sulfur and then centrifuged to prepare the inoculate to be used in the experiments. The total population of the inoculate was of 5×10^8 bact/ml.

Experiments: were done at 30 °C in containers (the total culture volume was 100 ml) stirred at 140 rpm. The material and the medium were sterilized in autoclave.

Experiment on toxicity of vanadium (IV) over T.t.: from suitable mixtures of the above-mentioned modified Imai medium and of the same medium added with vanadyl sulfate up to final vanadium concentrations of 100.0 mM, we prepared systems with final vanadium concentrations of 0.1, 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 mM. An additional system was prepared in the same way, but without vanadium. All systems were inoculated with 1 ml of the previously-described inoculate plus 1 g of elemental sulfur in powder form.

Experiment on vanadium (V) reduction: systems with vanadium(V) concentrations of 2.25, 4.50 and 10.0 mM were formulated by mixing, in the adequate proportions, the modified Imai medium and the same medium added with sodium metavanadate up to final concentrations of 50.0 mM. This last solution had to be filtered (to eliminate the precipitate of V_2O_5) before being sterilized. As in the previous case, all systems were inoculated with 1 ml of the previously-described inoculate plus 1 g of elemental sulfur in powder form. As controls, we prepared sterile systems with the same initial concentrations of vanadium (V) but, instead of placing 1 ml of inoculate on them, 1 ml of medium (without inoculation) was added.

Analytical determinations: vanadium (IV) and vanadium (V) were determined by potentiometric titrations of the corresponding fractions, which were periodically extracted (titrations were done with standard solutions of potassium permanganate for vanadium (IV) and iron (II) for vanadium (V)).

In some cases, we determined total vanadium by atomic absorption spectrophotometry. Besides, the acid medium production was measured by titration with standard NaOH solution. Total counts were performed in a Petroff-Häuser chamber by means of a microscope fitted with a phase-contrasting device.

RESULTS AND DISCUSSION

Figure 1 shows the production of acid medium in the T. t. cultures in the presence of vanadium (IV). At each time, the acid medium production was calculate as the difference of the titrations with standard NaOH solutions at time zero and those at that time.

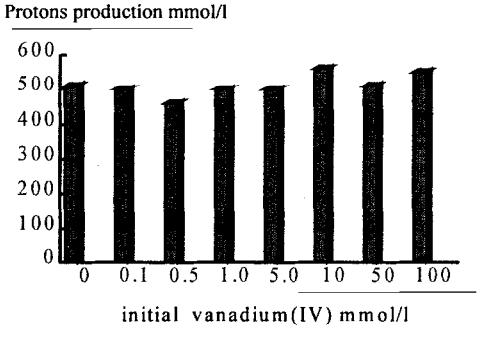

FIGURE 1: Production of sulfuric acid in *Thiobacillus thiooxidans* cultures on elemental sulfur in the presence of vanadium(IV).

No significant differences of acid production over the whole range of V(IV) concentration were observed, so the conclusion is that V(IV) does not inhibit the growth of *T.t.*, at least up to concentrations of about 100.0 mM.

On the other hand, figure 2 shows the evolution of V(IV) and the production of acid medium as a function of time for the *T.t.* cultures on sulfur with (or without) initial addition of V(V). Values of the sterile systems are not shown since they did not vary during the experiment. As in the cultures with vanadium (IV), the production of acid medium was calculated as the

difference of titrations performed with standard NaOH solutions at a given time and the initial one.

For the system with an initial vanadium (V) concentration of 10.0 mM, there was no reduction of vanadium (V) nor production of acid medium, and, no bacterial growth. The same situation was observed for systems having higher initial concentration of vanadium (V) (data not shown).

In systems having initial vanadium (V) concentrations of 2.25 mM and 4.50 mM, a total vanadium reduction was observed after 400 h, the reduction being slightly slower in the second system. In these systems the production of acid became faster after the complete reduction of vanadium(V).

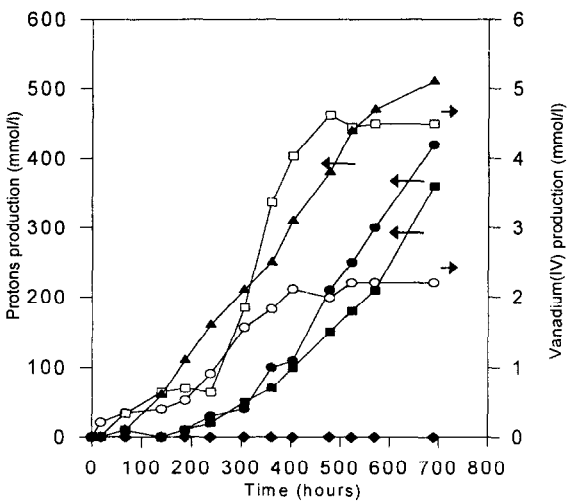

FIGURE 2: Reduction of Vanadium (V) in cultures of *Thiobacillus thiooxidans* on elemental sulfur. Symbols: Protons (●) and Vanadium(IV) production (○) for initial Vanadium(V) concentration of 2.25 mM; (■), (□) Idem for initial vanadium (V) concentration of 4.50 mM; (◆) Idem for initial vanadium (V) concentration of 10.0 mM; (Δ) protons production for the system without vanadium.

The oxidation of elemental sulfur catalyzed by *T.t.* generates, as previously indicated, a series of intermediates among which the sulfite ion is the most important:

$$S + O_2 + H_2O \longrightarrow SO_3^{2-} + 2\,H^+ \qquad [1]$$

the sulfite so formed can be oxidized either by an exclusively chemical action or by oxygen action catalyzed by bacteria. In the former, the oxidation is caused by the presence of vanadium (V) (represented here as metavanadate, though the most probable vanadium (V) species at this pH values is the dioxovanadium (V) (VO_2^+). Both oxidation mechanisms are represented as follows:

$$SO_3^{2-} + 6\,H^+ + 2\,VO_3^- \longrightarrow 2\,VO^{2+} + SO_4^{2-} + H_2O \qquad [2]$$

$$SO_3^{2-} + \tfrac{1}{2}\,O_2 \longrightarrow SO_4^{2-} \qquad [3]$$

The proposed mechanism corroborates the increased production of acid as the vanadium (V) runs out, since the production of acid by oxidation of sulfur in the reaction [1] is compensated by the consumption of acid during the reduction of vanadium (V) in the reaction [2]. The production of acid in the "vanadium-less" system is clearly higher and faster because of

absence of reaction [2] and also because of an evident inhibitory action of vanadium (V) on the growth rate of *T.t.* on elemental sulfur.

According to the last observation and noting the clear inhibitory effect taking place in the system of 10.0 mM initial vanadium (V), we attempted a bacterial adaptation from the system that had reduced 4.50 mM initial vanadium (V). To this end, the system was filtered to eliminate any residual elemental sulfur and the medium was centrifuged at 4500 rpm to separate the bacteria. The pellet so obtained was resuspended and inoculated again in a system with elemental sulfur and 10.0 mM of vanadium (V). However after a period of more than 30 days, neither bacterial growth, nor vanadium (V) reduction, nor production of acid were observed.

In order to study the inhibitory action of vanadium (V), a culture with T.t. growing on elemental sulfur in the early exponential phase was kept at 30 °C under stirring and forced aeration. Under such conditions, we measured the consumption rate of dissolved oxygen as 0.23 mg/l.h; to do this, the system was degassed by interrupting the aeration (Bandyopadhtyay and Humprey, 1967); the concentration of dissolved oxygen at the moment the experiment was carried out, was 83% of the equilibrium value (7 mg/l) (Liu et al., 1973). After this, we added vanadium (V) until a final concentration of 10 mM. After 5 minutes of the addition, the consumption rate of dissolved oxygen did not show noticeable changes with respect to the vanadium-less system; however, the dissolved oxygen concentration gradually increased until reaching, after 4 hours, the equilibrium value; therefore, there was no appreciable consumption of dissolved oxygen. This confirms that, unlike vanadium (IV) species, the species of vanadium (V) shows a clear inhibitory action. Hence, the reduction of vanadium (V) should be done by continuous addition of this species keeping it below the critical concentration of 10.0 mM.

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