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# Interaction of Amino Acids with Metal and Sulphate Reduction by *Desulfotomaculum Ruminis* Bacteria

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**Abstract**

A simultaneous effect of some amino acids and metals on the activity of *Desulfotomaculum ruminis* bacteria in the process of microbiological sulphate reduction is studied. It has been established that the simultaneous presence of valine and one of the metals tested (cadmium, chromium or molybdenum) produces a synergetic effect of enhanced inhibitory effect relative to the effect at the presence of only one of these components. Moreover, serine, aspargine and isoleucine have insignificant effect on the desulfurication of sulphates, but the efficiency of this process significantly depends on the presence of metal ions in the medium.

**Keywords:**

## Introduction

One of the most important recent environmental problems is increasing pollution of air, soil and water with heavy metals. From the chemical point of view they can be divided into those inevitable for correct metabolism [1] (Fe, Cu, Cr, Mo, Zn) and those exerting a harmful effect on living organisms (Pb, Hg, Cd). However, it should be regarded that the amount of a given metal necessary for correct metabolism is idiosyncratic. The presence of toxic metals or an excess of the basic metals can lead to disturbances in cell metabolism and thus to pathological changes. Thus, it is important to determine the toxic levels of particular metals for specific micro-organisms. The first micro-organisms affected by the heavy metal ions released into the environment are bacteria. The bioavailability of metals to bacteria depends on the form of the metal occurrence (free ion, organic complex, inorganic complex) and physical and chemical properties of the environment [2 ]. The toxicity of a given metal in pure bacteria culture is significantly different from that in natural conditions. Metal toxicity can also be influenced through interactions with other components of the medium such as proteins and products of their hydrolysis, e.g. amino acids. These components of the medium can be used by micro-organisms as elements of the structure of their specific cell proteins or can undergo further transformations. The catabolic activity of sulphate reducing bacteria (SRB) in media containing the amino acids studied in this work has been assessed by Szymańska et al. [3]. The results of the study have shown that the amino acids studied did not have a significant effect on inhibition of sulphides contained in the Starkey medium. It has also been established that serine meets the requirements of the so-called minimum medium, being a source of assimilable nitrogen and carbon. The interactions of metals with proteins influence the activity of micro-

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-organisms. Only some ions of the metals not occurring naturally in living organisms can replace the active metal in a protein complex, and usually such a replacement has a deteriorating effect on metabolism, sometimes leading to total inactivation of such a complex [4].

Increasing pollution of the natural environment can disturb natural cycles of transformations of many elements, e.g. the geochemical cycle of sulphur. Although the content of sulphur in living organisms is low, it plays a significant role in life-substaining processes and biogeochemical transformations. Depending on the environmental conditions and the type of microorganisms sulphur compounds can be reduced or oxidised. In this cycle the sulphur reducing bacteria (SRB) enable direct desulfurication.

SRBs are common in the natural environment. They occur in bottom sediments of fresh water and marine water reservoirs and in underground water. SRBs have also been found in the stomachs of the ruminants. They interact with many different chemical compounds and play an important role in ecology. Processes occurring with involvement of the microorganisms of this type have been widely used in waste purification and waste product utilisation [5].

Recognition of the effect of some chemical compounds on the efficiency of desulfurication seems very important, especially when analysing the effect of heavy metals on SRB catabolism.

This paper presents results of the study on the interaction of cadmium, chromium and molybdenum as well as valine, serine and isoleucine on the process of desulfurication taking place with involvement of the bacteria species *Desulfotomaculum ruminis.*

## Materials and Methods

The amino acids tested (L-serine, L-izoleucine, L-valine) were purchased from Merck and D-asparagine from Labo Feinchemie; cadmium and chromium sulphates and sodium molybdate come from the Polskie Odczynniki Chemiczne in Gliwice.

Sulphate reducing bacteria were isolated and identified as *Desulfotomaculum ruminis* by the method described earlier [6].

Kinetic study was performed at 37°C, in anaerobic conditions (helium), at pH 6.8-7.2, in tightly sealed glass reactors containing 50cm$^3$ of a modified Starkey medium composed of [g/dm$^3$]: $MgSO_4 \times 7H_2O$ = 2.00; $Na_2SO_4$ = 2.42; $NH_4Cl$ = 1.00; $K_2HPO_4$ = 5.00; $CaCl_2$ = 0.13; Mohr salt = 0.5; sodium lactate = 10 and microelements [6]. Appropriate amounts of the amino acids and metals tested were added. To this medium cadmium and chromium were introduced in the form of sulphates, while molybdenum as sodium molybdate. When cadmium or chromium were added, the content of sodium sulphate was reduced accordingly in order to to preserve a constant C/S ratio. After deoxygenation the medium was inoculated with 4% vol of the inoculum collected in the phase of the logarithmic growth. The rate of the reaction was determined from the degree of sulphate reduction measured at certain time intervals. In order to establish the degree of sulphate reduction the reactor was flown with helium and then the concentration of sulphites obtained as a result of bioreduction of sulphates was measured.

The instruments and media used in the study were sterilised for 20 min at 120°C. In parallel analogous experiments were carried out for the control samples without the compounds tested. In our earlier work on the kinetic model of desulfurication [6], the correlation coefficient for sulphate reduction was determined as 0.98. Therefore, in this paper no statistical analysis is given and the results are averages of at least three independent measurements.

Analytical methods: changes in concentrations of sulphides were determined by the iodometric method in the samples after precipitation of CdS [7].

## Results and Discussion

The effect of cadmium and the amino acids tested is illustrated in Fig. 1. The effect of serine or asparagine introduced into a standard Starkey medium in the concentration of 10 g/dm$^3$ on the process of desulfurication is insignificant and the degree of sulphates reduction reaches over 80%. The presence of cadmium, a metal toxic for SRB, introduced in the concentration of 0.175 g/dm$^3$, causes a decrease in the yield of desulfurication to a level of ~50% after 30 hours. In the presence of serine or asparagine and cadmium the final degree of desulfurication is almost the same as in the sample containing only cadmium in the medium, therefore, the presence of cadmium seems to determine the course of the reaction.

The presence of isoleucine has a somewhat greater inhibitory effect on the activity of the *Desulfotomaculum ruminis* bacteria than the above amino acids. Introduction of cadmium along with isoleucine resulted in a further decrease in the degree of desulfurication. In this experiment the effect of cadmium was also prevalent, except the samples with low concentrations of cadmium and high concentrations of isoleucine (Fig. 1).

The addition of valine (a compound with a much developed side chain) to a standard Starkey medium results in a decrease in the degree of desulfurication and an increase in the time of the reaction. Introduction of cadmium to the medium with valine causes a significant decrease in the reaction rate due to a small synergetic effect. The activity of SRB is lower than in the presence of valine or cadmium alone.

The influence of chromium and particular amino acids on the activity of the *Desulfotomaculum ruminis* bacteria is illustrated in Fig. 2. Chromium is less toxic to the bacteria than cadmium [8]. Introduction of chromium to the medium with valine does not produce a marked effect on the course of desulfurication. Only at low concentration of chromium and valine is a small synergetic effect noted. Therefore, the effect of valine seems prevalent.

The situation is different in the presence of chromium

along with serine or asparagine. The kinetic curves obtained for the samples with 0.436 g Cr/dm$^3$ and 7 or 10 g of serine/dm$^3$ and the samples with 0.726 g Cr/dm$^3$ and 7 or 10 g of asparagine are very similar. Thus, the final degree of desulfurication depends mainly on the concentration of the metal.

In the samples with low concentrations of chromium and isoleucine, the effect of the amino acid is dominant, but with increasing metal concentration its effect on the activity of the bacteria *Desulfotomaculum ruminis* becomes increasingly important.

The effect of molybdenum and the amino acids tested on the course of desulfurication is shown in Fig. 3. The presence of molybdenum alone in the form of sodium molybdate in the sample results mainly in the increasing

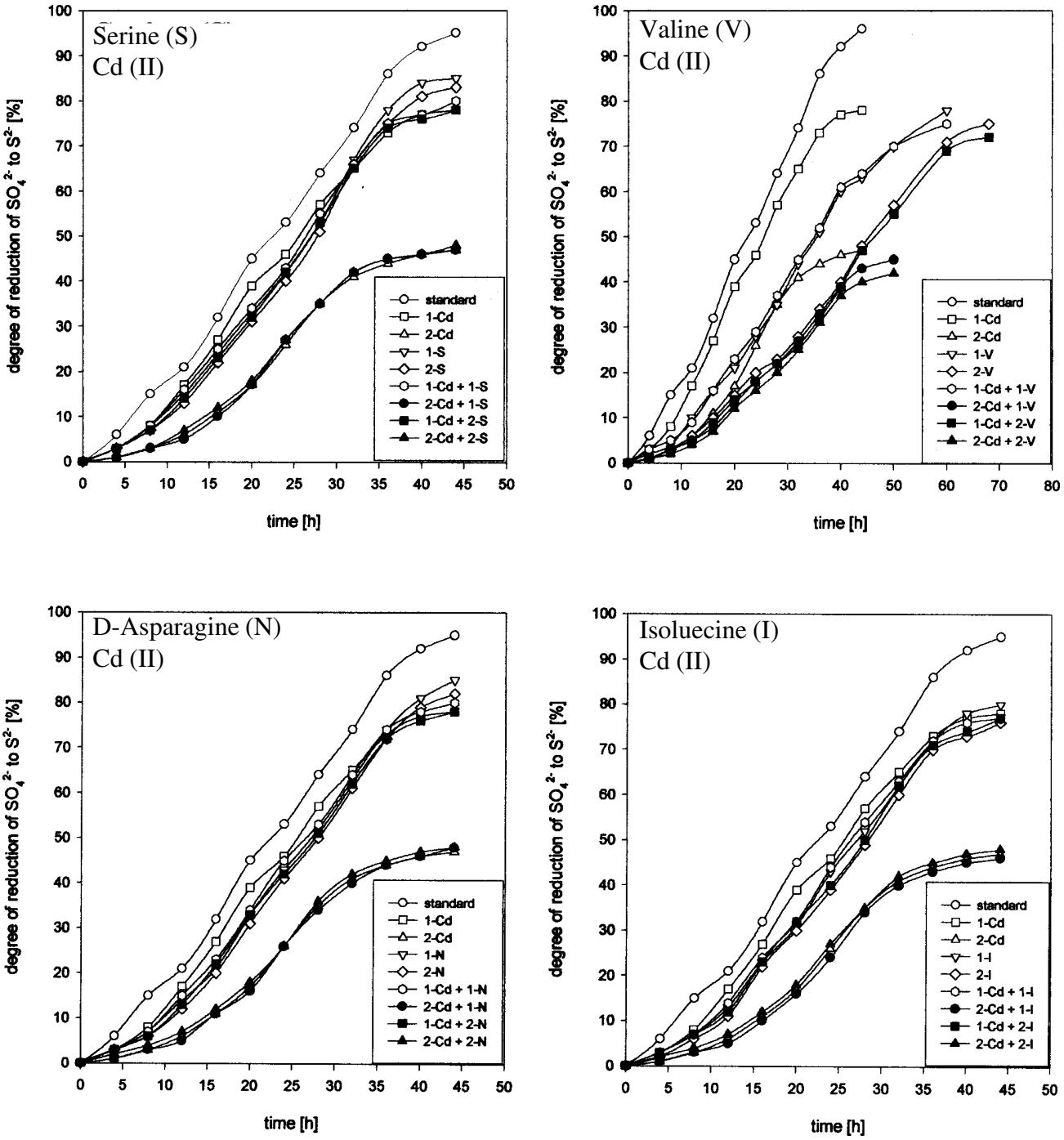


Fig. 1. The effect of cadmium ions (1 - Cd(II) = 0.088g/dm$^3$; 2 - Cd(II) = 0.175g/dm$^3$) introduced into medium containing serine (S), valine (V), D-asparagine (N), and isoleucine (I) (in concentration 1 = 7g/dm$^3$; 2 = 10g/dm$^3$), on the process of dissimilatory sulphate reduction taking place with the involvement of *Desulfotomaculum ruminis* bacteria( temp. 37$^o$C, pH = 6.8 - 7.2).

time of the reaction. After the addition of valine the inhibitory effect is significantly enhanced (synergetic effect). In the samples with molybdenum and asparagine, izoleucine or serine, the effect of the metal is definitely prevalent.

The above-discussed results have shown that the presence of valine with each of the metals studied in the medium results in a small synergetic effect, which means that the inhibitory effect of the two compounds on the course of desulfurication is greater than the effect of each compound present alone in the medium. When the medium contains chromium or cadmium in a low concentration and isoleucine in a high concentration (Fig. 1 and 2) the course of the desulfurication is determined mainly by the amino acid. The effect of these two metals becomes more important with

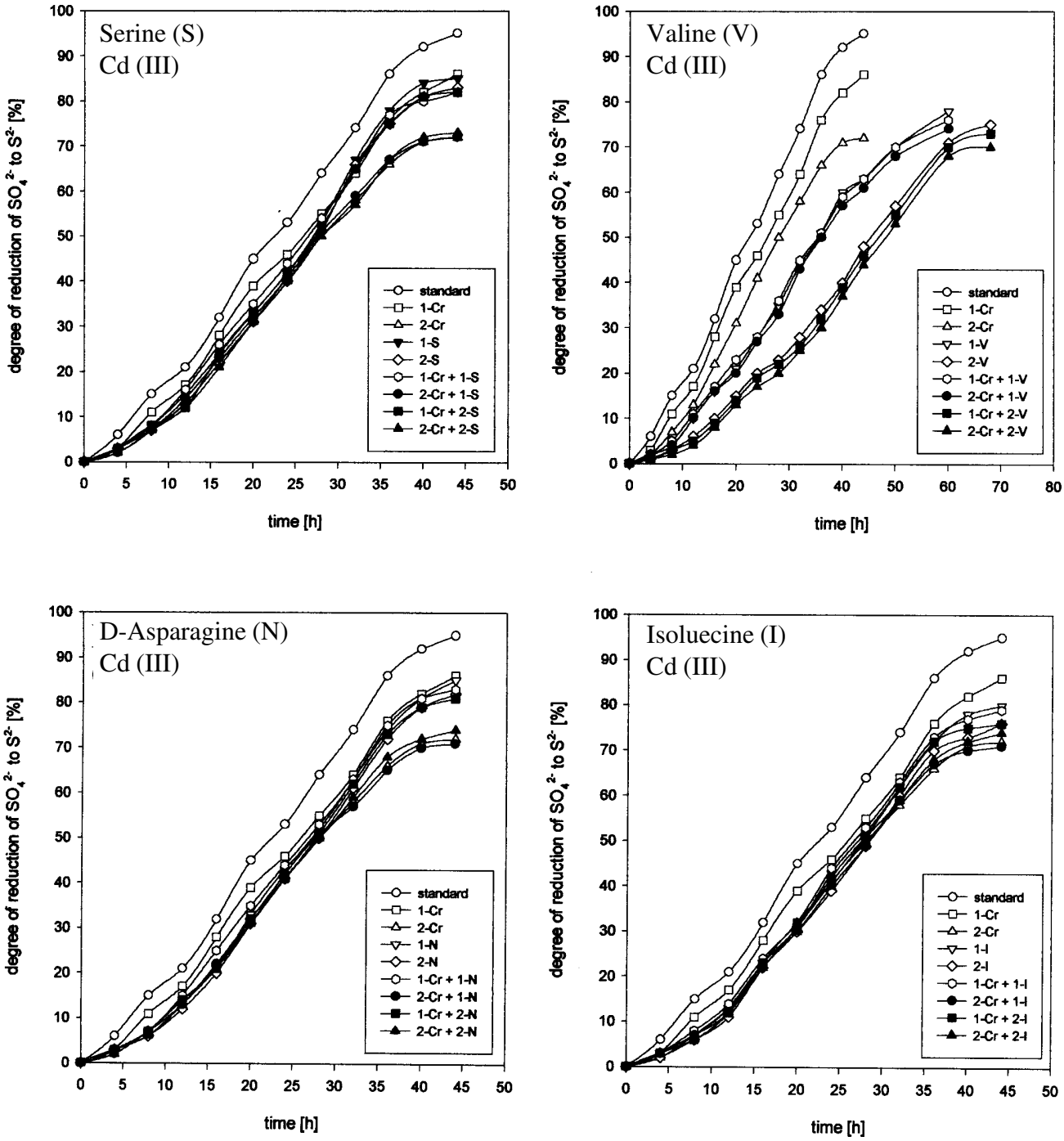


Fig. 2. The effect of cadmium ions (1 - Cd(III) = 0.436g/dm$^3$; 2 - Cd(III) = 0.726g/dm$^3$) introduced into medium containing serine (S), valine (V), D-asparagine (N), and isoleucine (I) (in concentration 1 = 7g/dm$^3$; 2 = 10g/dm$^3$), on the process of dissimilatory sulphate reduction taking place with the involvement of *Desulfotomaculum ruminis* bacteria( temp. 37°C, pH = 6.8 - 7.2).

their increasing concentration. The results obtained for molybdenum and the amino acids studied are similar (Fig. 3). The effect of serine and asparagine on the course of desulfurication in the presence of the metals tested is insignificant.

The contribution of metals in metabolic processes is well known to be inevitable to sustain correct functioning of living cells. The main compounds involving metals are metal-protein complexes and in particular metal enzymes. The ions of heavy metals introduced into the medium containing the amino acids tested disturb metabolism and lead to inhibition of catabolic activity of SRB. The presence of cadmium, chromium and molybdenum along with valine causes a synergetic effect enhancing the effect of each of these components added separately to the medium. This

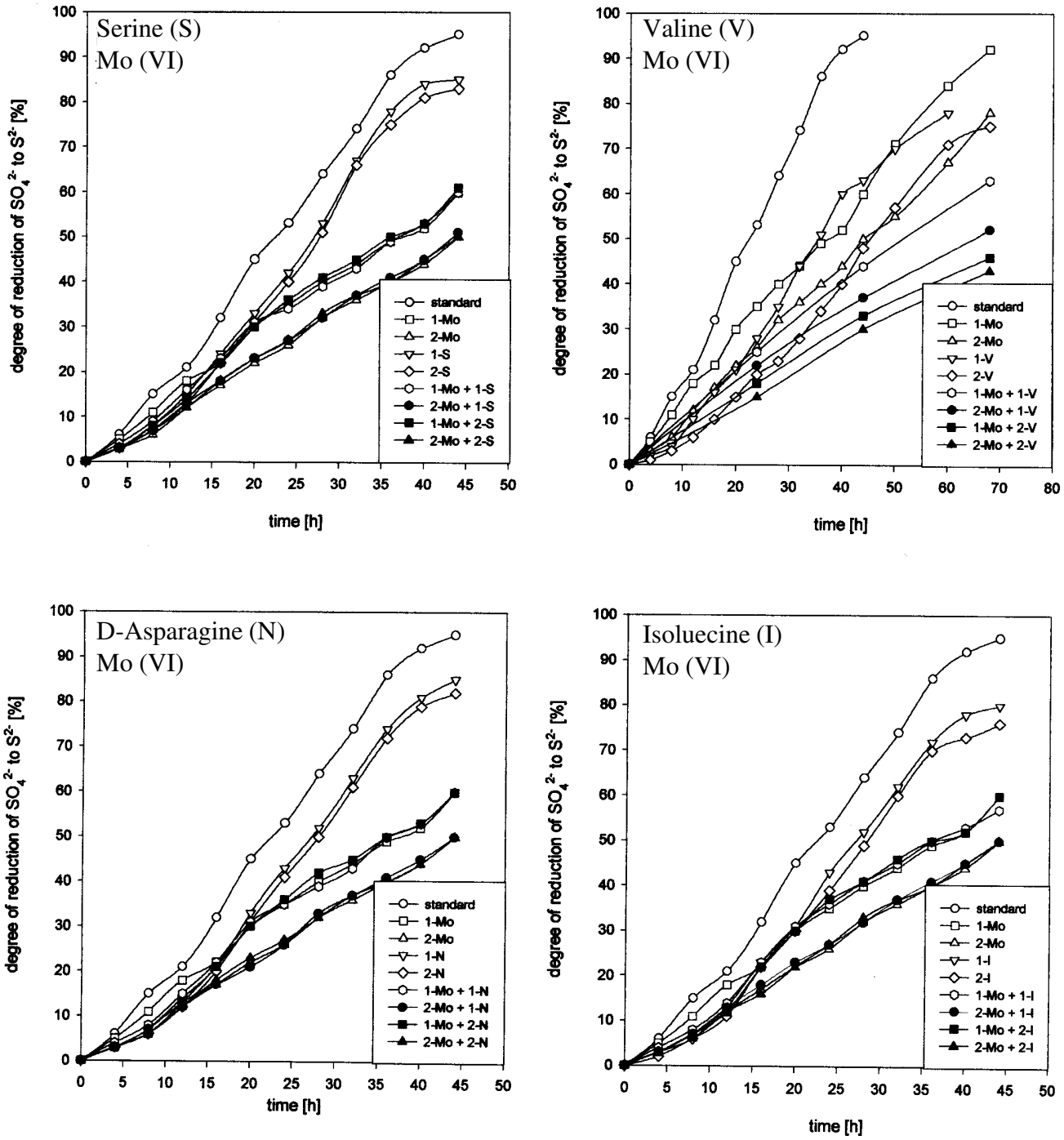


Fig. 3. The effect of cadmium ions (1 - Mo(VI) = 0.024g/dm$^3$; 2 - Mo(VI) = 0.032g/dm$^3$) introduced into medium containing serine (S), valine (V), D-asparagine (N), and isoleucine (I) (in concentration 1 = 7g/dm$^3$; 2 = 10g/dm$^3$), on the process of dissimilatory sulphate reduction taking place with the involvement of *Desulfotomaculum ruminis* bacteria( temp. 37°C, pH = 6.8 - 7.2).

synergetic effect is interpreted as related to the developed side chain of valine, which, according to Szymańska et al. [3], can be used as an effective source of nitrogen in the process of SRB proliferation. In the other systems studied the effect of the metals tested depends on their concentration.

The results of the study can be of interest for cattle breeders. The process of desulfurication of sulphates eaten in fodder is used by the ruminants for synthesis of proteins. If fodder components are polluted with heavy metal ions which are able to connect to the functional groups of proteins (-SH, =NH, -OH), the catabolic activity of SRB can be seriously inhibited. The results of this study allow estimation of the ranges of concentrations of the amino acids and metals studied tolerated by SRB as well as those causing significant or even total inhibition of their activity.

In conclusion, the effect of amino acids and metals studied on the activity of SRB depends on the concentration of these components and the chemical composition of the medium, and should be established individually for particular microorganisms. When two or more compounds are introduced into the medium, the results also depend on their concentration ratios.

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