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Catabolic Activity of *Desulfotomaculum ruminis* Bacteria in Media Containing Amino Acids

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Abstract

The influence of the presence of certain amino acids at different concentrations on the catabolic activity of the bacteria *Desulfotomaculum ruminis* was studied. Introduction of amino acids of the simple chain molecule in concentrations up to 10 g/dm^3 in the Starkey media leads to a small decrease of the sulphate conversion degree. A more significant decrease in the reaction rate is observed for amino acids with branched side chains such as valine. Moreover, it was shown that except for the D-asparagine isomer, which is not assimilable by the bacteria *Desulfotomaculum ruminis,* the other amino acids are a source of assimilable nitrogen. Serine meets the demands of the minimum substrates and is a source of assimilable carbon and nitrogen in the process of bacteria proliferation.

Keywords: *Desulfotomaculum ruminis* bacteria, catabolic activity, amino acids

Introduction

Apart from providing metabolic energy, the catabolic processes are a. source of fundamental intermediate metabolites used in cell synthesis. They play a very important role in the cycles of many organic substances, including pollutants of the natural environment [1, 2]. For instance, the sulphate reducing bacteria (SRB), the most common in the natural environment are crucial not only for the sulphur cycle and its biotransformation [3,4], but because of their interactions with many chemical compounds they play a very important role in the economy [5]. The processes with the involvement of SRB have been studied in connection with their use in waste purification [6], waste utilisation [7], detoxication of water and soil [8], etc.

An important component of the growth medium of the bacteria are proteins, products of their hydrolysis, including amino acids, which can be used in metabolical processes as a source of carbon and nitrogen. For example, sodium glutaminate proved a very effective medium for reduction of nitrates [9].

Amino acids can be used by microorganisms for construction of specific cell proteins or can undergo transformations leading to production of different metabolic substances. The processes of amino acid conversions in protein growth have been relatively well recognised. Fewer works have been devoted to the role of amino acids as the only source of organic carbon and nitrogen in microbiological processes taking place in natural conditions with the involvement of SRB.

A diversity of the enzymatic properties of SRB and their ability to adapt to an environment rich in different substrates are the reasons why the microbiological conversions of amino acids are still poorly recognised. The aim of this study was to test the influence of selected amino acids on the process of desulfurisation taking place with the involvement of the bacteria *Desulfotomaculum ruminis,* known for their ability to use different organic compounds in the processes of sulphate breathing [10].

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Materials and Methods

The amino acids tested were purchased from Merck (L-glutamine, L-serine, L-izoleucine, L-valine, L-alanine, L-tirozine) or Labo Feinchemie (D-asparagine).

Sulphate reducing bacteria (SRB) were isolated and identified as *Desulfotomaculum ruminis* by the method described earlier [11].

Kinetic studies were conducted at 37°C, in anaerobic conditions (helium) at pH = 6.8 - 7.2, in tightly closed glass reactors containing 50 cm^3 of the modified Starkey medium composed of [g/dm^3]: $MgSO_4 \cdot 7H_2O$ = 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 = 10.00 and microelements [12]. The medium obtained was then supplemented with chosen amounts of the amino acids studied (3, 5, 7, 10 g/dm^3) and after deoxidisation it was inoculated with 4% vol of the inoculum collected at the phase of logarithmic growth (after 24h). The reaction rate was determined as a degree of sulphates reduction measured at certain time intervals. In the tests checking the possibility of using amino acids as a source of carbon, sodium lactate in the medium was replaced by a given

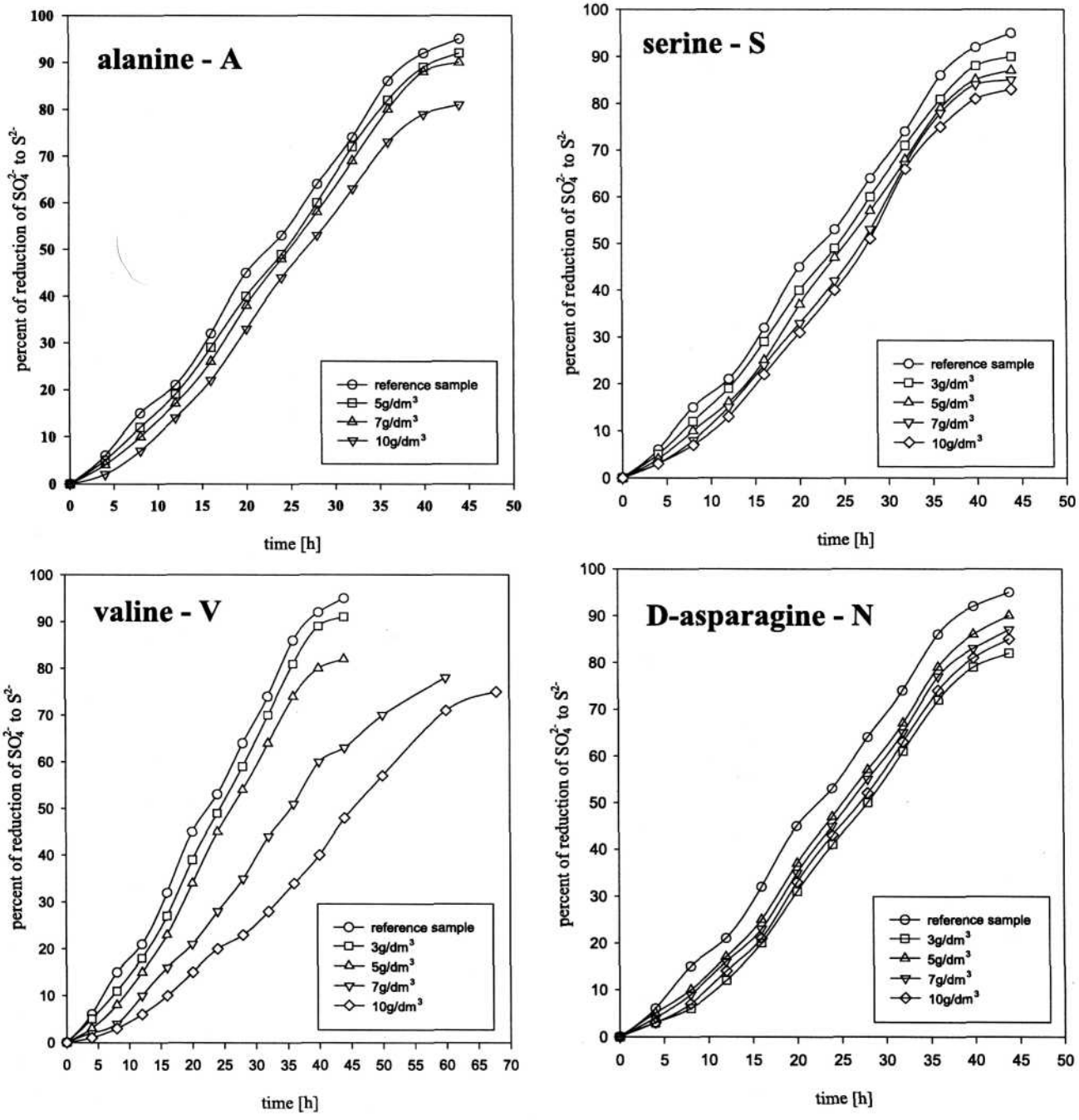

Fig. 1. The influence of alanine (A), serine (S), valine (V) and D-asparagine (N) in different concentrations on the process of desulfurication conducted with the involvement of the bacteria *Desulfotomaculum ruminis* (temp. 37°C, pH = 6.8-7.2).

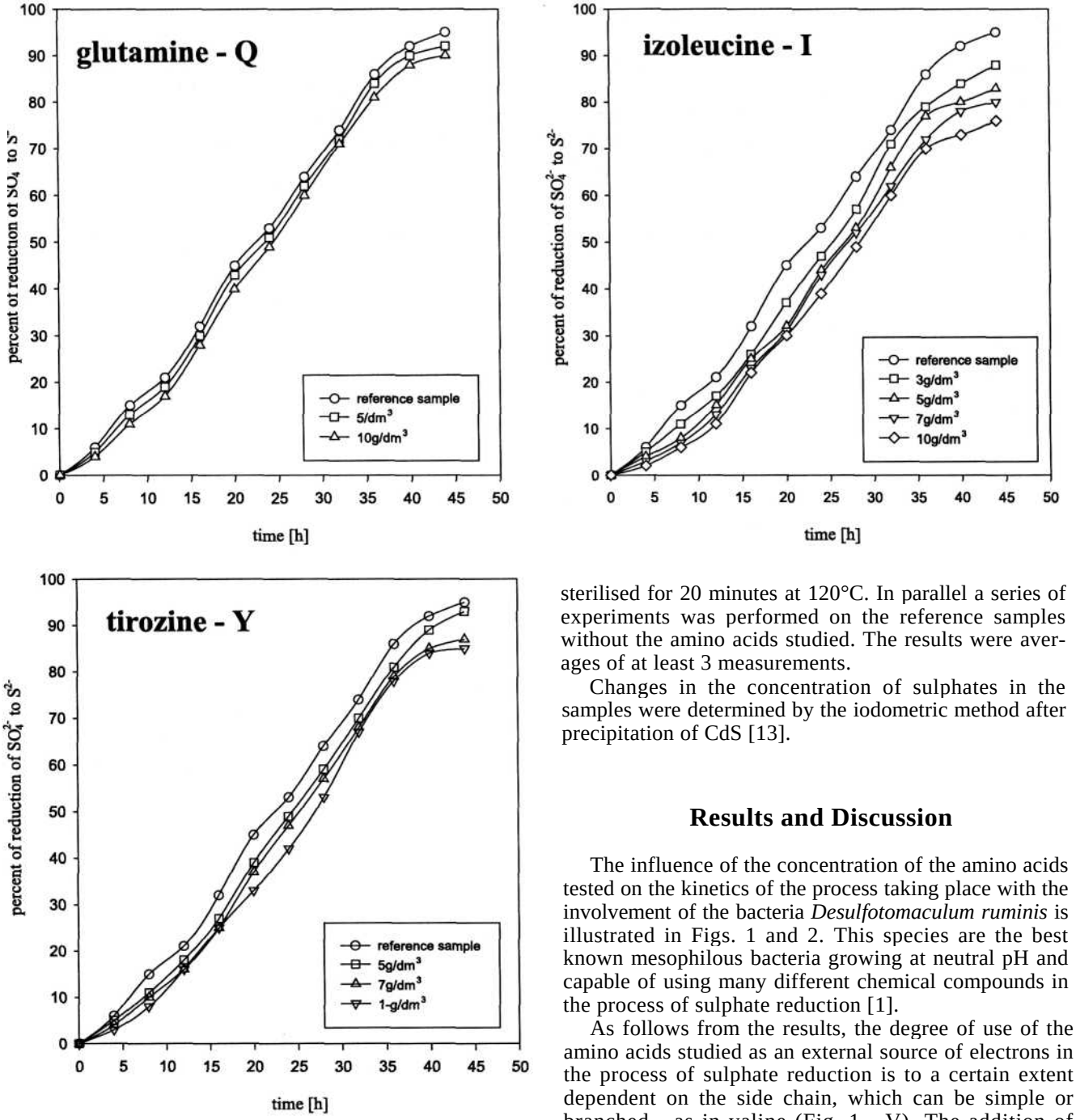

Fig. 2. The influence of glutamine (Q), izoleucine (I) and tizine (Y) in different concentrations on the process of desulfurication conducted with the involvement of the bacteria *Desulfotomaculum ruminis* (temp. 37°C, pH = 6.8-7.2).

amino acid containing 3.2 g C/dm^3 (close to that in the standard Starkey medium).

Analogously, when testing amino acids as a source of nitrogen, NH_4Cl and Mohr's salt were replaced by chosen amino acids of a concentration of nitrogen atoms close to that in the standard Starkey medium.

For the sake of comparison a series of tests was also performed in the minimum medium containing only the amino acid tested.

The apparatus and media used in the study were sterilised for 20 minutes at 120°C. In parallel a series of experiments was performed on the reference samples without the amino acids studied. The results were averages of at least 3 measurements.

Changes in the concentration of sulphates in the samples were determined by the iodometric method after precipitation of CdS [13].

Results and Discussion

The influence of the concentration of the amino acids tested on the kinetics of the process taking place with the involvement of the bacteria *Desulfotomaculum ruminis* is illustrated in Figs. 1 and 2. This species are the best known mesophilous bacteria growing at neutral pH and capable of using many different chemical compounds in the process of sulphate reduction [1].

As follows from the results, the degree of use of the amino acids studied as an external source of electrons in the process of sulphate reduction is to a certain extent dependent on the side chain, which can be simple or branched - as in valine (Fig. 1 - V). The addition of valine at a concentration of 7 g/dm^3 or higher to the Starkey medium results in a decrease of the rate of sulphate reduction (the reaction time increases by about 68 hours) and a decrease of the degree of the sulphate conversion to about 75% (Fig. 1). The kinetic curves determined for the conversions of the other amino acids studied added in concentrations up to 10 g/dm^3, have very similar course corresponding to the three phases of the process: the phase of multiplication, logarithmic growth and stabilisation. In the concentration range studied the character of the phase of bacteria multiplication - or the induction period is very similar for each of the amino acids studied, and this phase lasts about 5 hours. The curves corresponding to the phase of logarithmic growth are much flattened only for the samples

with valine present in the medium in concentrations above 7 g/dm^3.

The bacteria activity and the degree of sulphate reduction has been estimated at 80-90%.

A small decrease in the degree of the reduction of $SO4^{2-}$ to S^{2-} can also be observed for the sample containing isoleucine in concentrations greater than 7 g/dm^3 (Fig. 2 - I). This compound, like valine, has a branched carbon skeleton.

The degree of assimilability of the amino acids depends not only on the environmental conditions and type of microorganisms but surprisingly also on the structure of the amino acids themselves. It has been reported that the side chain of amino acids can affect the ability to bond formation, chemical reactivity and assimilability by bacteria [14]. As follows from our results the presence of the amino acids tested in concentrations up to 7 g/dm^3, except valine and isoleucine, does not influence significantly the catabolic activity of the bacteria species studied. The degree of reduction of sulphates to sulphides is maintained at a level of 80%.

At the next stage of the study, the performance of the amino acids was tested in the so-called minimum media, containing only a given amino acid, in order to assess the degree of its assimilability by the bacteria and thus its influence on bacterial activity. In such media bacteria multiplication, and thus biomass increase, is not so fast but it is possible to check the usefulness of the media (particular amino acids) as the source of carbon and nitrogen in the bacteria metabolism.

The results of the study have shown that only serine (Fig. 1 - S) can be an effective source of carbon and nitrogen for the bacteria *Desulfotomaculum ruminis.* In the medium containing up to 10 g serine/dm^3 the yield of the sulphates reduction to sulphites was ~ 70%. Thus, serine meets the requirements of a minimum medium (without the Starkey medium). The explanation is that serine undergoes dehydration and dehydrogentaion forming pirogronian, being an intermediate metabolite of the path of the known cycle of fatty acids [14].

Total inhibition of sulphate reduction and the complete lack of bacterial activity was observed in the medium containing the D-asparagine isomer. The lack of assimilability of D-asparagine by the bacteria *Desulfotomaculum ruminis* is related to the selective activity of the asparginase enzyme, which enables the cleavage of the L-aspargine isomer, but is not active towards D-aspargine. The lack of splitting of D-izomer under enzymatic effect has been observed for many enzymes [15].

The replacement of ammonium nitrogen in the Starkey medium by amino acids containing nitrogen at appropriate concentrations does not inhibit bacterial activity. It means that such amino acids like alanine, valine, serine, glutamine, izoleucine and tyrozine can be effective sources of nitrogen in the process of multiplication of the bacteria *Desulfotomaculum ruminis.*

The results obtained have shown that the amino acids tested do not have a significant effect on the rate of sulphate conversions in the Starkey medium. The amino acids studied, except the D-asparagine isomer, in concentrations up to 10g/dm^3, can be a source of assimilable nitrogen in the process of desulfurication taking place with the involvement of the bacteria *Desulfotomaculum ruminis.* Serine meets the requirements of the so-called minimum medium as a source of assimilable nitrogen and carbon. Only the D-asparagine isomer is neutral towards the bacteria *Desulfotomaculum ruminis* and up to 10 g/dm^3 does not act as a source of assimilable carbon and nitrogen.

Estimation of the catabolic activity of the bacteria *Desulfotomaculum ruminis* in the medium containing the amino acids tested is also interesting from the point of view of the symbiotic relations between the bacteria and certain animals (ruminant such as sheeps or cows), which use SRB instead of digestive enzymes. The animals use the process of reduction of the sulphates contained in the fodder for synthesis of proteins; the bacteria have constant access to a rich source of nutrients.

High nutritive value of amino acids introduced into the fodder and some other food products makes them attractive for the food industry and stimulates efforts to give their comprehensive characterisation, including the processes of biotransformation with SRB.

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