Polish Journal of Environmental Studies Vol. 13, No. 2 (2004), 209-217

Biotransformation of Phosphogypsum by Sulphate-Reducing Bacteria in Media Containing Different Zinc Salts

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Received: 26 May, 2003
Accepted 11 July, 2003

Abstract

The effect of zinc on the biotransformation of phosphogypsum, COD reduction and growth rate (μ_{max} day^{-1}) of an SRB community and *Desulfotomaculum ruminis* in media with sodium lactate or ethanol was examined. Depending on the form of zinc ($Zn_3(PO_4)_2$ x $4H_2O$, $ZnSO_4$ x$7H_2O$, $ZnCl_2$, $Zn(NO_3)_2$ x $6H_2O$) and its initial concentration (0-80 mg Zn^{2+}/l) lower sulphate reduction and COD reduction was observed. The effect of Zn^{2+} also depended on the composition of the studied populations and carbon source in the medium. The lowest inhibition of specific growth rate was determined in cultures of the pure strain and in medium with zinc phosphate (with lactate or ethanol IC_{50}=63 or 75 mg Zn^{2+}/l, respectively) and the highest in cultures of sulphate-reducing bacterial communities in medium with zinc nitrate (with lactate or ethanol IC_{50}= 35 or 20 mg Zn^{2+}/l, respectively).

Keywords: zinc, biotransformation of phosphogypsum, COD reduction, SRB community, *Desulfotomaculum ruminis*

Introduction

Sulphate-reducing bacteria (SRB) are ubiquitous in anaerobic environments where organic substrates and sulphates are available. They can be encountered in aqueous and soil environments: hot springs, crude oil, sulphur deposits, natural gas outlets, estuary sludges, salt water reservoirs, corroding iron, the alimentary tracts of animals and humans, industrial wastewaters rich in sulphates (e.g. from the chemical, metallurgical or paper industries), mining waters as well as in bioreactors [1, 2, 3, 4, 5, 6].

SRB are obligatory anaerobes that obtain the energy they require from the oxidation of organic substrates, with sulphates being the terminal electron acceptors:

$$SO_4^{2-} + \text{organic matter} = HS^- + H_2O + HCO_3^-$$

SRB can also use thiosulphates or sulphites, and even elementary sulphur as electron acceptors. The preferred carbon source for this group of microorganisms are compounds that are derived from fermentation processes, which are formed during the anaerobic degradation of organic matter: organic acids (e.g. lactate, pyruvate, formate and acetate) as well as alcohols (ethanol, propanol and butanol) [1, 3, 4, 6].

SRB, similar to methanogenic archeons and acetogenic bacteria, constitute an important link in the anaerobic degradation of organic matter. The similar requirements of these groups of microorganisms for organic compounds cause them to occur in various mutual relationships (syntrophy, commensalism, competition). Consequently, the relationships between sulphate-reducing bacteria and methanogenic archeons are affected, amongst others, by COD/SO_4^{2-} ratio, concentrations of acetate, sulphates, sulphites and sulphide as well as by heavy metals [6].

Knowledge regarding the effect of metals on bacteria and particularly on sulphate-reducing bacteria is currently very scant. For instance, data on the concentrations of

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Table 1. Chemical composition of phosphogypsum from Wizów [19] (major components, data as weight %).

Component	CaO	SO_3	Fe_2O_3	Al_2O_3	MgO	SrO	BaO	Na_2O	K_2O	P_2O_5	Ln_2O_3	H_2O	F_2
%	29.23	41.95	0.13	0.20	0.05	1.53	0.04	0.31	0.10	2.20	0.61	20.40	0.50

various metals inhibiting the growth of these bacteria are very divergent [6, 7, 8, 9, 10]. The values given sometimes differ several-fold since the toxicity of a given metal depends not only on its concentration and physical and chemical properties, but also on the type of salt it forms and environmental conditions [11, 12].

The use of sulphate-reducing bacteria to purify industrial wastewaters, mining waters, drainage waters from the metallurgical industry, wastewater sediments and drainage from communal and industrial dumps [9, 13, 14, 15, 16, 17, 18] demands in-depth knowledge of the toxic activity of heavy metals on these microorganisms.

In recent years studies have also been made [19, 20, 21, 22, 23, 24] on the use of sulphate-reducing bacteria for the biodegradation of organic matter, combined with the transformation of phosphogypsum. Phosphogypsum is a waste product that is formed in the production of phosphoric acid. The main components of phosphogypsum are hydrated calcium sulphates (gypsum-$CaSO_4$ x $2H_2O$; halfhydrate - $CaSO_4$ x $0{,}5H_2O$ and anhydryte - $CaSO_4$), which account for approx. 95% of its mass. This research was initiated with the optimisation of the course of the process, the possibility of the use of various electron donors, the acquisition of strains and communities of bacteria characterized by high activity as well as tolerance to the presence of heavy metals, in mind.

The aim of the current study was to examine the effect of zinc on the biotransformation of phosphogypsum and COD reduction by SRB community and a strain of *Desulfotomaculum ruminis* in media containing sodium lactate or ethanol.

Materials and Methods

Microorganisms:

1 - community of sulphate-reducing bacteria (SRB community) formed by mixing equal volumes of eight cultures of bacterial communities originating from various environments [21].

2 - strain of *Desulfotomaculum ruminis* isolated from the above community of sulphate-reducing bacteria and identified as described [25, 26, 27].

Media:

Cultures of SRB community and of *Desulfotomaculum ruminis* strain were maintained in minimal medium supplemented with (g/l): phosphogypsum (5.0); sodium lactate (4.68) or ethanol (2.40) and NH_4Cl (1.0). Phosphogypsum (Table 1) was from a dump in Wizów near Bolesławiec. The effect of zinc on the growth of sulphate-reducing bacteria was studied by adding $Zn(NO_3)_2$ x $6H_2O$; $ZnCl_2$, $ZnSO_4$ x $7H_2O$ or $Zn_3(PO_4)_2$ x $4H_2O$ to the medium in concentration 10, 20, 40, 60 or 80 mg Zn^{2+}/l. All media were sterilized by autoclaving for 30 min at 121°C and the pH of the medium was adjusted with 1M NaOH and 1M HCl. The initial pH

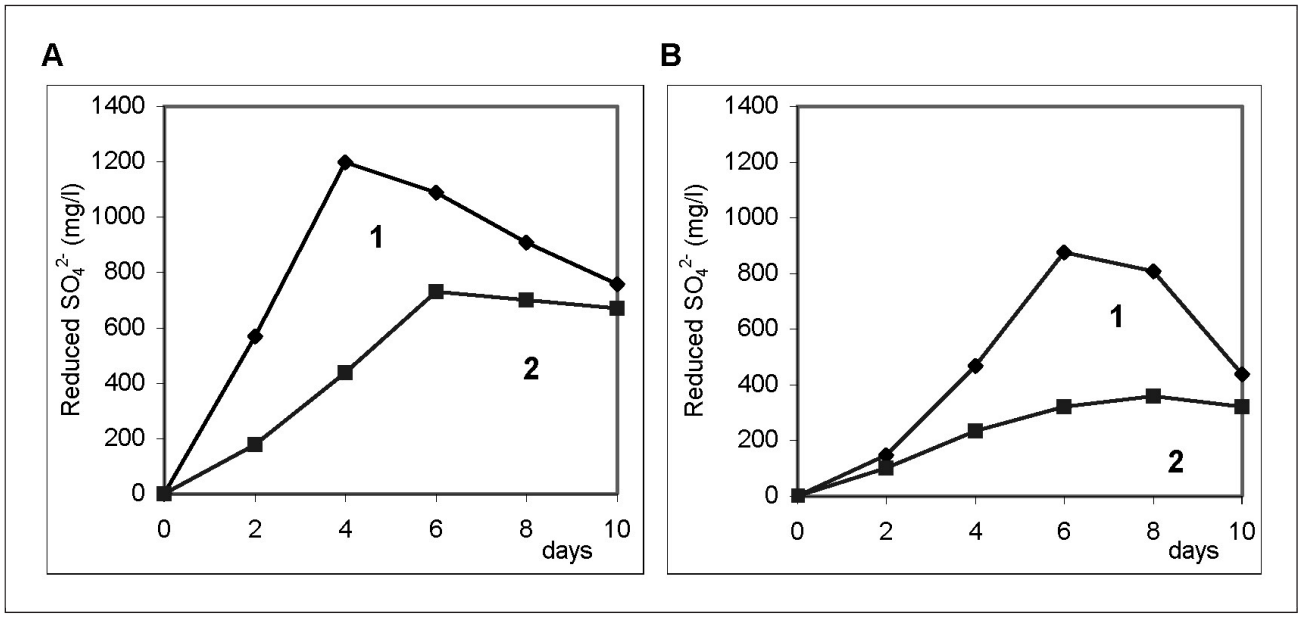

Fig. 1. Sulfate reduction in cultures of SRB communities (1) and *D. ruminis* (2) in medium with phosphogypsum and sodium lactate (A) or ethanol (B).

Table 2. Growth of SRB community and *D. ruminis* in medium with phosphogypsum and sodium lactate (a) or ethanol (b).

Parameter	a. sodium lactate		b. ethanol	
	SRB community	D. ruminis	SRB community	*D. ruminis*
Reduction of sulfate (mg/l)	1197	730	882	350
COD reduction (mg O_2/l)	2350	460	2350	220
COD/SO_4^{2-}	1.96	0.63	2.66	0.63
μ day^{-1}	0.27	0.20	0.18	0.12
V_{max} SO_4^{2-} (mg/l x day)	315	146	160	90
COD/SO_4^{2-} in the medium ($t_o \rightarrow t_{max}$)	1.40 → 0.91	1.40 → 0.98	1.40 → 1.26	1.40 → 1.94
pH ($t_o \rightarrow t_{max}$)	7.4 → 7.0	7.4 → 7.2	7.4 → 6.6	7.4 → 6.8

of the medium was set at 7.4 and was not adjusted in the course of the experiment.

Culture conditions*:*

The cultures were set up (in three repetitions) in 0.33 l bottles with rubber stoppers. Thirty cm^3 of an active mother culture were introduced with the use of a syringe into 270 cm^3 of medium, to obtain an initial concentration of sulphides in the range 80-100 mg HS^-/dm^3. The cultures were incubated in a thermostat for 15 days at 30°C.

Chemical determinations:

1 - sulphides were determined by the iodometric method with the use of Lugol's solution (0.05M) and sodium thiosulphate (0.05M) against starch (0.5%) as an indicator.

2 - chemical oxygen demand (COD) was determined according to [28].

Calculations:

1 - reduction of sulphates was calculated according to the formula: $R_{SO4} = (HS^-t_{max} - HS^-t_o) \times 2.91 / t_{max}-t_o$, where: R - maximum reduction of sulphates (mg SO_4^{2-}/l), HS^-t_{max} - concentration of sulphites at time t_{max}, HS^- - concentration of sulphites at time t_o;

2 - specific growth rate (μ day^{-1}) calculated on the basis of amount of sulphates reduced;

3 - coefficient COD/SO_4^{2-} calculated as the quotient of a decrease in COD value and amount of sulphates reduced;

4 - COD/SO_4^{2-} ratio in the medium was calculated as the quotient of COD and concentration of sulphates in the medium;

5 - maximum rate of sulphate reduction (V_{max}) was calculated on the basis of maximum rate of sulphate reduction (mg SO_4^{2-}/l x day) at time $t_{max} - t_o$.

Results and Discussion

Growth of SRB Community and Strain of *D. ruminis* in Media with Phosphogypsum and Sodium Lactate or Ethanol

The biotransformation of phosphogypsum in cultures of SRB community and strain of *D. ruminis* in media with phosphogypsum and sodium lactate or ethanol was studied.

Phosphogypsum is an equally good electron acceptor for sulphate-reducing bacteria [19, 21] as the commonly used Na_2SO_4. The amount of phosphogypsum that can be dissolved in the medium at 30°C is 3.9 g/l, which corresponds to the release of 2020 mg SO_4^{2-}/l. Such a high amount of sulphate ions allows for the high activity of sulphate-reducing bacteria (ca. 600 mg SO_4^{2-}/l x day) [24].

Sodium lactate is considered the best source of carbon for most species of sulphate-reducing bacteria, whereas ethanol is mentioned (after lactate and pyruvate) among other carbon sources used by these bacteria [1, 29]. However, the available literature contains few positions describing the growth of sulphate-reducing bacteria in stationary cultures set up in medium with ethanol and sulphates [3, 4, 6, 30, 31, 32, 33, 34].

In our studies the growth of bacteria was estimated on the basis of the amount of sulphates reduced. Maximum COD reduction, sulphate reduction rate (V_{max}), specific growth rate (μ day^{-1}) and value of so-called coefficient COD/SO_4^{2-}, being the quotient of decreased organic carbon and amount of sulphates reduced were also calculated.

Maximum reduction of sulphates (1197 mg/l and 730 mg/l) in cultures with sodium lactate (Fig. 1A) was observed after 4 and 6 days (i.e. t_{max}) for cultures of SRB community and strain of *D. ruminis*, respectively. In cultures with ethanol (Fig. 1B) maximum reduction of sulphates was by about 300 mg SO_4^{2-}/l lower than in media with lactate and was 882 for the SRB community and 350 mg SO_4^{2-}/l for *D. ruminis* after 6 and 8 days of cultivation, respectively.

It is worth noting that in monospecies cultures the maximum reduction of sulphates was always lower than in cultures of the microbial community. Moreover, the sulphate reduction rate ($V_{max}SO_4^{2-}$) as well as growth rate (μ day^{-1}) were in this case lower (Table 2). In SRB community cultures COD reduction in medium with lactate or ethanol was similar at 2350 mg O_2/l, whereas in cultures of *D. ruminis* these values did not exceed 460 and 220 mg O_2/l, respectively. Therefore,

the growth of sulphate-reducing bacteria in the studied cultures depended not only on the carbon source used and availability of sulphates but also on the type of inoculum used.

It is known that the bacteria in cultures of SRB community compete for available carbon sources. Sulphate--reducing bacteria dominate when the COD/SO_4^{2-} ratio in the medium is less than 1.7, with COD/SO_4^{2-} between 1.7 and 2.7 they have to compete with other organisms for available sources of carbon [3, 4, 6]. The COD/SO_4^{2-} ratio in the studied control cultures (Table 2) at the beginning of the experiment (time t_o) was 1.4. During the course of the experiment (time t_{max}) this ratio decreased in cultures of SRB community to approx. 0.91 (in media with lactate) and to 1.26 (in media with ethanol). Therefore, the COD/SO_4^{2-} ratio in the control cultures inoculated with SRB community did not exceed the value of 1.7, which created good conditions for the growth of sulphate-reducing bacteria.

The reason for competition for a substrate, besides its availability, may also be the growth rate of sulphate--reducing bacteria. Studies [33] on the growth of *Desulfobulbus propionicus* and *Paleobacter propionicus* in stationary cultures in media with ethanol and sulphates demonstrated that because of their slower growth sulphate--reducing bacteria utilized only part of the ethanol available in the medium (ca. 30%).

In the studied cultures of SRB community containing lactate and ethanol approx. 60 and 30%, respectively, of the available source of carbon were used. The coefficient of oxidized carbon (COD/SO_4^{2-}) in the case of the use of lactate and ethanol was 1.96 and 2.66, respectively, and was far above the theoretical value of 0.67 [6]. An increase in the above coefficient can be brought about by the greater use of organic matter by fermenting bacteria and/or methanogenic archeons which compete with sulphate-reducing bacteria for organic substrates [31, 33, 35]. In all cultures of *D. ruminis* the reduction of sulphates and the use of carbon source was far lower than in mixed cultures. The so-called oxidized carbon coefficient was also lower, averaging 0.63 in the cultures supplemented

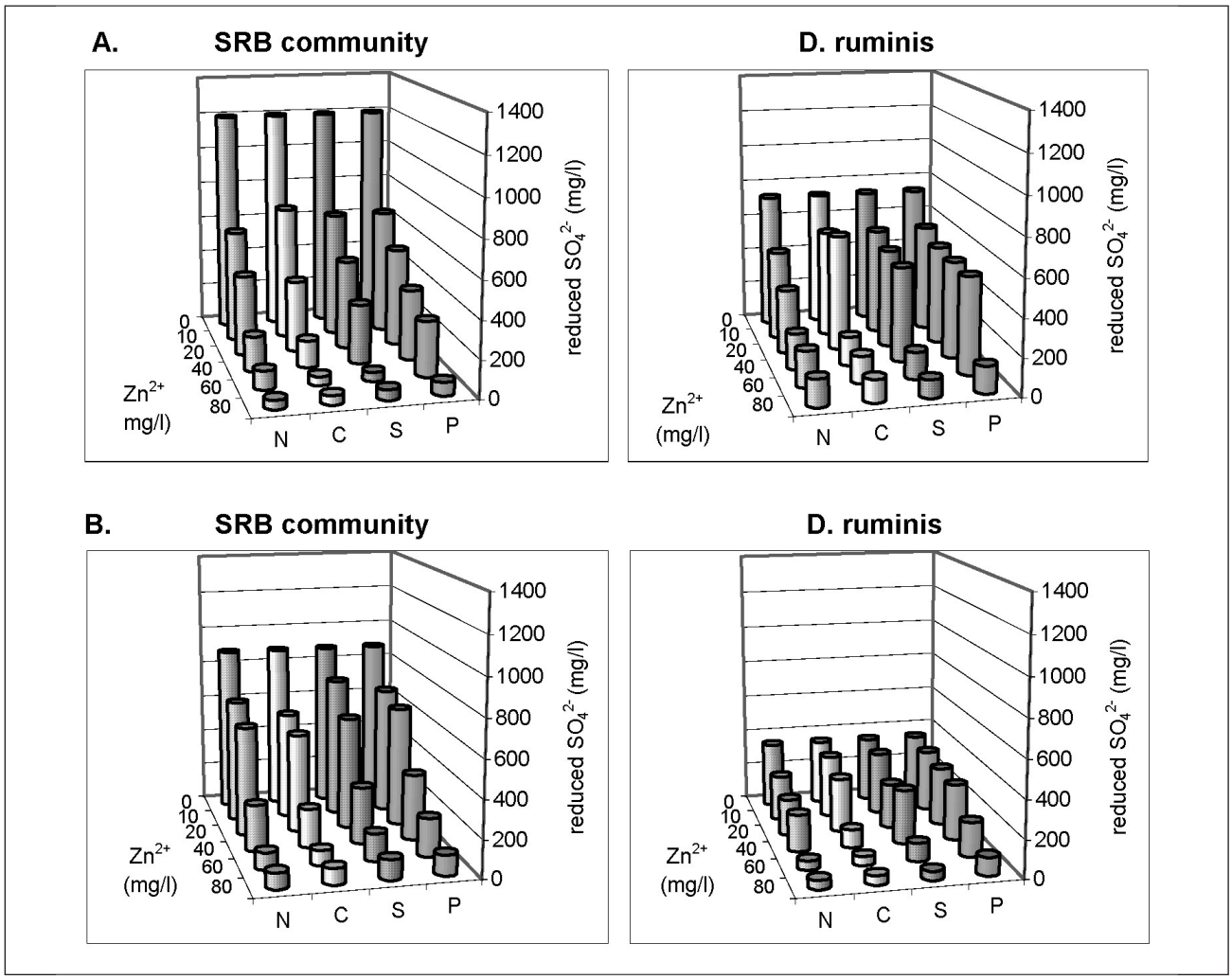

Fig. 2. Reduction of sulfates in cultures of SRB communities and pure cultures of *D. ruminis* in medium with phosphogypsum and sodium lactate (A) or ethanol (B) and different concentrations (0-80 mg Zn^{2+}/l) zinc salts: N-$Zn(NO_3)_2$ x $6H_2O$; C-$ZnCl_2$; S-$ZnSO_4$ x $7H_2O$; P- $Zn_3(PO_4)_2$ x $4H_2O$.

with lactate and ethanol. It is commonly known that theoretically, the entire COD can be degraded by sulphate--reducing bacteria when this ratio is less than 0.67 [35].

Effect of Zinc on the Biotransformation of Phosphogypsum by SRB Community and Strain of *D. ruminis* in Media with Sodium Lactate or Ethanol

The presence of zinc salts in all the cultures supplemented with lactate and ethanol resulted in a decrease in the maximum reduction of sulphates (Fig. 2), this being in proportion to the initial concentration of the metal. Reduction of sulphates in cultures of both SRB community and *D. ruminis* was the lowest in the presence of $Zn(NO_3)_2$ x $6H_2O$ and the highest when $Zn_3(PO_4)_2$ x $4H_2O$ was present. A reduction in the level of sulphate-reduction in the presence of nitrates was observed by several authors [24, 36, 37, 38, 39]. However, phosphates may react with zinc to form highly insoluble complex compounds that cannot be utilized by bacteria [40].

Similarly as in the case of the control cultures mentioned above, also in cultures containing different concentrations of zinc (Fig. 3), COD reduction was several times higher in cultures of SRB community than in cultures of *D. ruminis*. The uptake of organic matter was always considerably lower in the presence of zinc than in the control cultures. Zinc (especially in the lower concentrations of 10 and 20 mg Zn^{2+}/l) inhibited COD reduction (Fig. 3) to a lesser degree than the reduction of SO_4^{2-} (Fig. 2). This is understandable, because sulphate-reducing bacteria are more sensitive to the presence of heavy metals than the remaining bacteria. As shown by previous studies [6, 7, 23] zinc causes inhibition of sulphate-reduction already at 10 mg Zn^{2+}/l and at 40 mg Zn^{2+}/l inhibits the process by 50%.

An additional factor enabling the good development of accompanying microflora (and subsequently high COD reduction) could be the lower amount of toxic hydrogen sulphide being formed, this being due to growth inhibition of the sulphate-reducing bacteria.

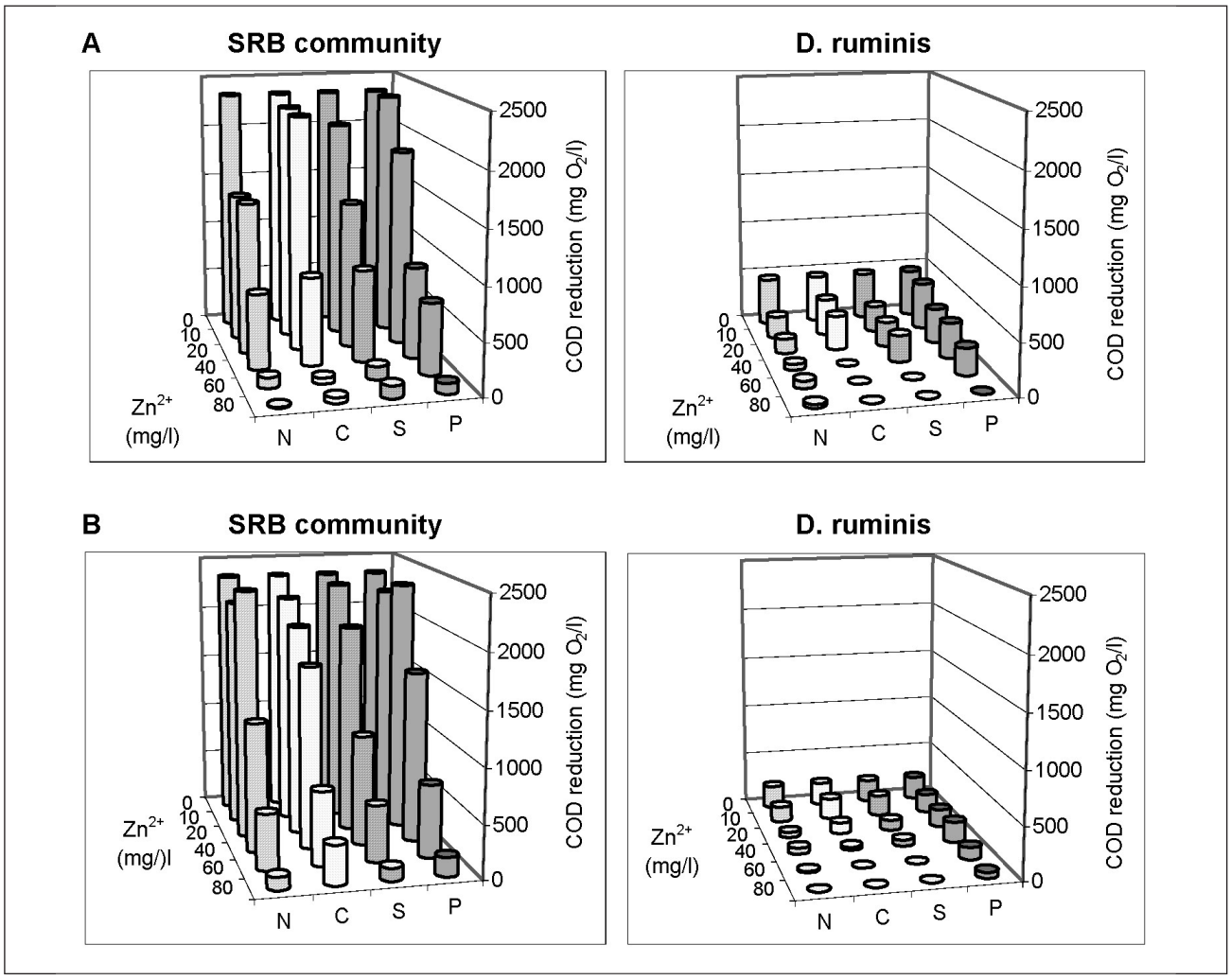

Fig. 3. COD reduction in cultures of SRB communities and pure cultures *D. ruminis* in medium with phosphogypsum and sodium lactate (A) or ethanol (B) and different concentrations (0-80 mg Zn^{2+}) zinc salts: N-$Zn(NO_3)_2$ x $6H_2O$; C-$ZnCl_2$; S-$ZnSO_4$ x $7H_2O$; P-$Zn_3(PO_4)_2$ x $4H_2O$.

Table 3. Coefficients COD/SO_4^{2-} in cultures of SRB communities and pure cultures *D. ruminis* in medium with phosphogypsum and sodium lactate (a) or ethanol (b) and different concentrations (0-80 mg Zn^{2+}/l) and zinc salts: $Zn(NO_3)_2$ x $6H_2O$; $ZnCl_2$; $ZnSO_4$ x $7H_2O$; $Zn_3(PO_4)_2$ x $4H_2O$.

salt	Zn^{2+} (mg/l)	a. sodium lactate		b. ethanol	
		SRB community	*D. ruminis*	SRB community	*D. ruminis*
$Zn(NO_3)_2$ x $6H_2O$	0	1.96	0.63	2.65	0.63
	10	2.35	0.45	3.27	0.63
	20	3.38	0.41	4.00	0.26
	40	3.84	0.29	4.92	0.30
	60	1.00	0.36	5.80	0.20
	80	0.21	0.24	1.43	0.00
$ZnCl_2$	0	1.96	0.64	2.66	0.63
	10	3.16	0.61	3.82	0.59
	20	5.89	0.54	3.77	0.38
	40	5.89	0.00	8.44	0.31
	60	1.18	0.00	8.78	0.00
	80	1.18	0.00	4.34	0.00
$ZnSO_4$ x $7H_2O$	0	1.96	0.64	2.65	0.63
	10	3.10	0.44	2.80	0.58
	20	2.94	0.45	3.26	0.42
	40	2.77	0.51	3.32	0.21
	60	2.65	0.00	3.68	0.00
	80	0.47	0.00	1.14	0.00
$Zn_3(PO_4)_2$ x $4H_2O$	0	1.96	0.63	2.65	0.63
	10	3.50	0.79	3.26	0.54
	20	3.58	0.63	3.66	0.55
	40	2.33	0.66	4.55	0.67
	60	2.32	0.51	3.34	0.61
	80	1.42	0.00	1.67	0.58

Table 4. Specific growth rate (μ day^{-1}) in cultures of SRB communities and pure cultures *D. ruminis* in medium with phosphogypsum and sodium lactate (a) or ethanol (b) and different concentrations (0-80 mg Zn^{2+}/l) and zinc salts: $Zn(NO_3)_2$ x $6H_2O$; $ZnCl_2$; $ZnSO_4$ x $7H_2O$; $Zn_3(PO_4)_2$ x $4H_2O$.

salt	Zn^{2+} (mg/l)	a. sodium lactate		b. ethanol	
		SRB community	*D. ruminis*	SRB community	*D. ruminis*
$Zn(NO_3)_2$ x $6H_2O$	0	0.27	0.20	0.18	0.12
	10	0.23	0.19	0.15	0.10
	20	0.17	0.17	0.07	0.09
	40	0.12	0.11	0.05	0.06
	60	0.08	0.06	0.04	0.04
	80	0.04	0.06	0.03	0.04
$ZnCl_2$	0	0.27	0.20	0.18	0.12
	10	0.25	0.18	0.18	0.10
	20	0.19	0.17	0.11	0.09
	40	0.12	0.10	0.05	0.06
	60	0.04	0.06	0.04	0.04
	80	0.03	0.06	0.03	0.04
$ZnSO_4$ x $7H_2O$	0	0.27	0.20	0.18	0.12
	10	0.24	0.17	0.17	0.11
	20	0.20	0.16	0.13	0.08
	40	0.17	0.10	0.09	0.07
	60	0.09	0.04	0.06	0.03
	80	0.02	0.04	0.02	0.01
$Zn_3(PO_4)_2$ x $4H_2O$	0	0.27	0.20	0.18	0.12
	10	0.25	0.19	0.18	0.11
	20	0.20	0.18	0.15	0.10
	40	0.15	0.16	0.10	0.08
	60	0.06	0.13	0.03	0.07
	80	0.04	0.09	0.01	0.03

Table 5. Concentrations (IC_{50}) of different zinc salts (mg Zn^{2+}/l) at which 50% inhibition of specific rate of sulfate reduction occurred in cultures of SRB communities and pure cultures *D. ruminis* in medium with phosphogypsum and sodium lactate or ethanol.

salt	a. sodium lactate		b. ethanol	
	SRB community	*D. ruminis*	SRB community	*D. ruminis*
$Zn(NO_3)_2$ x $6H_2O$	35	45	20	32
$ZnCl_2$	36	40	33	40
$ZnSO_4$ x $7H_2O$	47	50	35	45
$Zn_3(PO_4)_2$ x $4H_2O$	43	63	42	75

The dependence between the utilization of organic matter and amount of reduced sulphates is illustrated by the so-called coefficient of oxidized carbon COD/SO_4^{2-}, whose theoretical value, as mentioned above, is 0.67. In control cultures of *D. ruminis* this coefficient was 0.63, whereas in cultures containing different zinc salts it successively dropped with increasing zinc concentration in the medium (Table 3).

In theory, the whole amount of COD can be degraded by sulphate-reducing bacteria, as long as the COD/SO_4^{2-} ratio is less than 0.67 [35]. Any increase in its value points to the competitive utilization of organic matter by the microflora accompanying sulphate-reducing bacteria. Such a phenomenon probably occurred in cultures of SRB community, in which the addition of zinc in the form of different salts, such as ($Zn(NO_3)_2$ x $6H_2O$, $ZnCl_2$, $ZnSO_4$ x $7H_2O$ or $Zn_3(PO_4)_2$ x $4H_2O$) at the low concentration of 10 mg Zn^{2+}/l brought about an increase in the magnitude of the coefficient COD/SO_4^{2-} from 1.96 (in medium with lactate) or 2.66 (in medium with ethanol) to 2.35; 3.16; 3.10 and 3.50 as well as 3.27; 3.82; 2.80 and 3.26, respectively.

It thus seems that the presence of zinc in cultures of SRB community brings about a change in the quantitative relationships between sulphate-reducing bacteria and other groups of bacteria present in the inoculum. In these cultures growth inhibition of sulphate-reducing bacteria and the multiplication of accompanying heterotrophic bacteria, which demonstrate higher resistance to the presence of heavy metals, takes place. In cultures of *D. ruminis*, because of the absence of any coexisting microflora, the coefficient of oxidized carbon is always lower.

Another parameter describing the growth of a culture of SRB community and *D. ruminis* in media containing various concentrations of four different zinc salts is the comparison of specific growth rate (μ day^{-1}). In all the cultures the specific growth rate was inversely correlated with concentration of zinc in the medium (Table 4). Based on the results obtained, the percent inhibition of specific growth rate (IC_{50}) in the presence of zinc was calculated (Table 5). In culture of SRB community in medium containing sodium lactate and: zinc phosphate, sulphate, chloride or nitrate an inhibition of specific growth rate by 50% was observed at the concentrations of 43; 47; 36 and 35 mg Zn^{2+}/l, respectively, and in culture of *D. ruminis* at the concentrations of 63; 50; 40 and 45 mg Zn^{2+}/l, respectively. In medium with ethanol the IC_{50} in the presence of zinc phosphate, sulphate, chloride or nitrate was 42; 35; 33 and 20 mg Zn^{2+}/l, respectively, for the SRB community and analogously 75; 45; 40 and 32 mg Zn^{2+}/l for *D. ruminis*. It is evident that irrespective of the source of carbon employed, somewhat lower concentrations of zinc caused a 50% inhibition of the specific growth rate of the SRB community than in the case of *D. ruminis*.

The effect of zinc on the growth of sulphate-reducing bacteria also depends on the zinc salt used in the experiment. In cultures of SRB community set up in medium with sodium lactate and phosphogypsum it was found [24] that the IC_{50} in the presence of $ZnCl_2$ was 34 mg Zn^{2+}/l whereas in the presence of $ZnSO_4$ x $7H_2O$ it occurred at a higher concentration, that is 54 mg Zn^{2+}/l.

In this study the lowest value of inhibition of specific growth rate was observed in *D. ruminis* cultures in medium with lactate or ethanol and zinc phosphate (IC_{50}=63 or IC_{50}=75 mg Zn^{2+}/l, respectively) and the highest in cultures of SRB community in medium containing lactate or ethanol and zinc nitrate (IC_{50}=35 or IC_{50}=20 mg Zn^{2+}/l, respectively).

In cultures of SRB community inhibition of the growth of sulphate-reducing bacteria was greater than in cultures of *D. ruminis*. In the case of cultures containing ethanol (particularly in the presence of zinc nitrate) inhibition of the specific growth rate was greater than in media with lactate. It seems that one of the reasons for this could be acidification of the medium, which is caused by the activity of accompanying heterotrophic bacteria. The high value of the coefficient COD/SO_4^{2-} (Table 3) may also be taken as indirect proof of their presence in the described cultures.

Therefore, sulphate-reducing bacteria seem more sensitive in a microbial community to the presence of zinc than *D. ruminis*. In cultures in which stronger acidification of the medium occurs, (e.g. in the presence of ethanol) the inhibitory action of zinc is stronger. A decrease in pH is known to enhance the toxicity of heavy metals [6]. At low pH the concentration of H_2S also increases, which inhibits the growth of sulphate-reducing bacteria [41, 42].

The results obtained in this study allow us to conclude that it seems feasible to use the SRB community for the biodegradation of organic matter and the transformation of phosphogypsum in the presence of heavy metals (e.g. zinc). In culture of SRB community the quotient of

reduced organic carbon and amount of sulphate reduced (COD/SO_4^{2-}) is far higher than in cultures of *D. ruminis*. The presence in the cultures of other groups of bacteria (besides SRB) is thus an advantageous factor since it allows the removal of larger amounts of organic matter. It should, however, be remembered that transient acidification of the medium, resulting in increased toxicity of H_2S and heavy metals, may not only occur in a microbial community but be stronger than in a monospecies culture.

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