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Catabolic Activity of *Desulfotomaculum ruminis* Bacteria in a Medium Containing Fluorides

K. Szymańska[1], F. Domka[1]*, T.Wójtowicz[2]

[1]Department of Kinetics and Catalysis, Faculty of Chemistry, Adam Mickiewicz University,
60-780 Poznań, ul. Grunwaldzka 6, Poland
[2]Faculty of Commodity Science, Economic Academy,
Aleja Niepodległości 10, 60-967 Poznań, Poland

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Abstract

The effect of soluble fluorine species in different concentrations on the catabolic activity of *Desulfotomaculum ruminis* bacteria has been studied. The fluoride concentrations tolerated by and toxic to the bacteria have been determined. The degree of fluorine species toxicity was found to depend on the form in which fluorine is introduced into the medium and increases in the following order: $NaF<NH_4F<ZnF_2<$ $<Na_2SiF_6<ZnSiF_6<CdF_2$. The results indicate that the difference between the concentrations tolerated by and toxic to the bacteria was very small.

Keywords: *Desulfotomaculum ruminis* bacteria, fluorides, catabolic activity, dissimilatory sulphate reduction

Introduction

Although environmental pollution with fluorine compounds has mainly local character, their considerable toxicity means that they pose a serious threat to the ecosystem [1,2].

Fluorine compounds are relatively common in the natural environment. Their presence in drinking water in contents up to 1.5mg/dm^3 is recommended as protection against tooth decay. In the USA 60% of drinking water contains fluorine in the optimum concentration of 1mg/dm^3, either from natural sources or as a result of fluorination [3]. Organic species of fluorine are common in aerosols, cosmetics and some pharmaceuticals. Moreover, fluorine as an impurity is found in certain food products, e.g. tea leaves.

In regions where natural water contains elevated levels of fluorine (higher than 1.5mg/dm^3) people often develop spotted teeth. Intake of an excessive amount of fluorine (e.g. with water containing more than 5mg/dm^3) can lead to deformation of extremities [4,5].

The main sources of local pollution with fluorine compounds such as HF, SiF_4 or H_2SiF_6 are industrial plants processing apatite and phospherite, or using cryolite, white clay or fluosite. Such plants are aluminium works, plants producing phosphoric acid or phosphate fertilisers, glass works, ceramic works, enamel producing plants, steelworks, brickyards and coal power plants [5,6].

In the vicinity of such plants fluorine compounds pose a serious threat to the natural environment. In humans, when taken with food or inhaled, they can lead to undesirable processes of bone mineralisation [3]. They are taken from water and soil by plants, which they also fall in the form of dust [7]. Fluorine compounds are accumulated in the aboveground parts and pose a serious treat to grazing animals. In regions polluted by fluorine compounds, their effect is synergistically enhanced by SO_2 from precipitation [7].

*Corresponding author

Disturbances in the ecological equilibrium caused by fluorine compounds also affect the cycles of many biogenic elements [8]. Because of the considerable importance of still unresolved problems related to the effect of fluorine compounds on microbiological processes, the study of their influence on living organisms and ecosystems seems to be the of particular interest. In this study we are interested in the effect of fluorine on the catabolic processes taking place with involvement of sulphate-reducing bacteria (SRB), which play a significant role in the migration of pollutants in the natural environment [9]. Sulphate-reducing micro-organisms are common in natural environment. They occur in bottom sediments of marine and fresh water reservoirs, in underground waters and have been found in the stomachs of ruminants. They are used in processes of self-purification of sewage and in utilization of wastes [8,10]. In view of the above it seems important to recognise the effect of fluorine on the effectiveness of desulfurication with the involvement of micro-organisms.

This paper presents results of a study on the effect of concentration of the fluoride ions on the process of microbiological sulphate reduction taking place with involvement of the *Desulfotomaculum ruminis* bacteria.

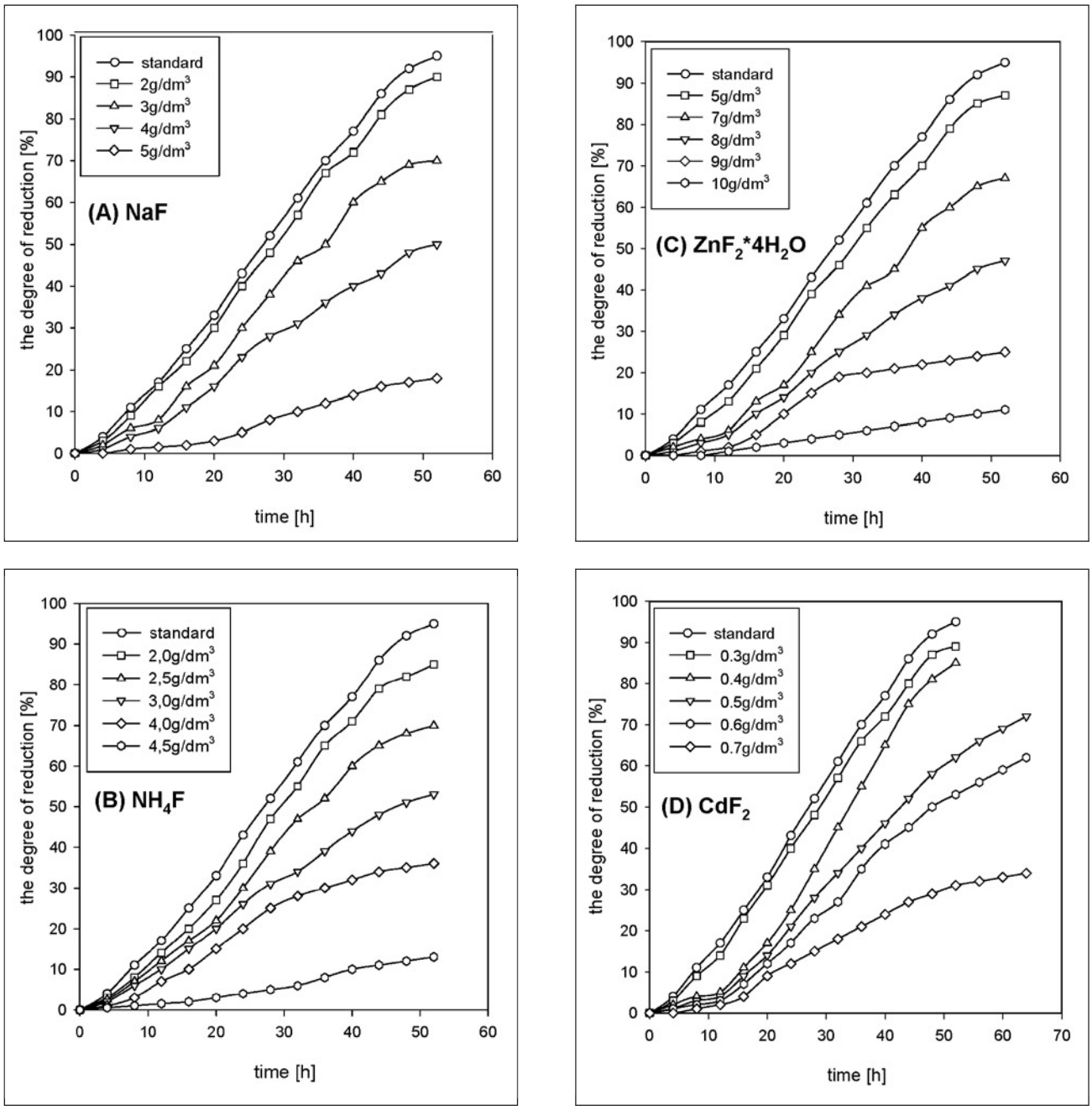

Fig.1. The effect of the concentrations of sodium fluoride (A), ammonium fluoride (B), zinc fluoride (C) and cadmium fluoride (D) on the dissimilatory sulphate reduction taking place with the involvement of *Desulfotomaculum ruminis* bacteria, pH 6.8-7.2, at 37°C.

Materials and Methods

The fluorine species tested were introduced into the medium in the form of the following compounds: NaF, NH_4F, ZnF_2*4H_2O, CdF_2, $ZnSiF_6*6H_2O$, and Na_2SiF_6 in concentrations in which they were fully soluble in the conditions of the experiment.

The sulphate-reducing bacteria were isolated and identified as *Desulfotomaculum ruminis* by the earlier described method [11].

Kinetic studies were conducted at 37^0C, in anaerobic conditions (under helium), at pH 6.8-7.2, in tightly closed glass reactors containing 50cm^3 of the modified Starkey medium of the composition [g/dm^3]: $MgSO_4.7H_2O$=2.00, Na_2SO_4=2.42, NH_4Cl=1.00, K_2HPO_4=5.00, $CaCl_2$= 0.13, Mohr salt 5.0, sodium lactate=10 and microelements [12]. Then, appropriate amounts (see results) of fluorine species tested were added to the medium and after deoxidisation the medium was inoculated with 4% vol. of the inoculum taken from the phase of the logarithmic growth (after 24h of growth). The rate of the reaction was determined as a degree of reduction of sulphates to sulphides, measured at certain time intervals.

The laboratory equipment and the media were sterilized before use for 20 min. at 120^0C. In parallel, control experiments were performed on reference samples, without the fluorine species tested, in the same conditions. The final results are mean values from at least three measurements.

Analytical methods: changes in the sulphide concentrations were measured by the iodometric method after precipitation of CdS. [13].

Results and Discussion

The kinetic curves illustrating the catabolic activity of the *Desulfotomaculum ruminis* bacteria, expressed as a degree of sulphate reduction in the media containing different concentrations of the fluorine species studied, (Fig. 1 and 2), are similar in shape, which means that the reaction took place according to similar mechanisms. In general, with increasing concentration of the fluorine species tested the degree of sulphate reduction decreases, and the differences between the concentration tolerated by the bacteria and toxic to them are very small.

The shape of the kinetic curves corresponds to the typical phases of proliferation of microorganisms: the induction period, phase of the logarithmic growth and phase of equilibrium and stabilization of growth.

The process of sulphate reduction taking place in the medium containing approximately 1gF$^-$/dm^3, with sodium, ammonium or zinc fluorides added (Figs. 1A, B, C) runs without the induction period and with a yield not much different than that in the reference samples, without those fluorine species. With increasing concentration of the fluorides in the reaction medium, the decreasing rate of desulfurication is accompanied by the appearance of clearly marked induction period of about 12h, undoubtedly related to the adaptation of the bacteria to the environment. Total inhibition of the catabolic activity of the bacteria occurs at the fluoride concentrations of 2.71gF$^-$/dm^3 (corresponding to 6gNaF/dm^3), 2.57 gF$^-$/dm^3 (corresponding to 5gNH_4F/dm^3) and 2.39 gF$^-$/dm^3 (corresponding 11gZnF_2*4H_2O/dm^3). Thus, it can be concluded that the fluoride concentration of 2.3-2.7 gF$^-$/dm^3 causes total inhibition of the reaction

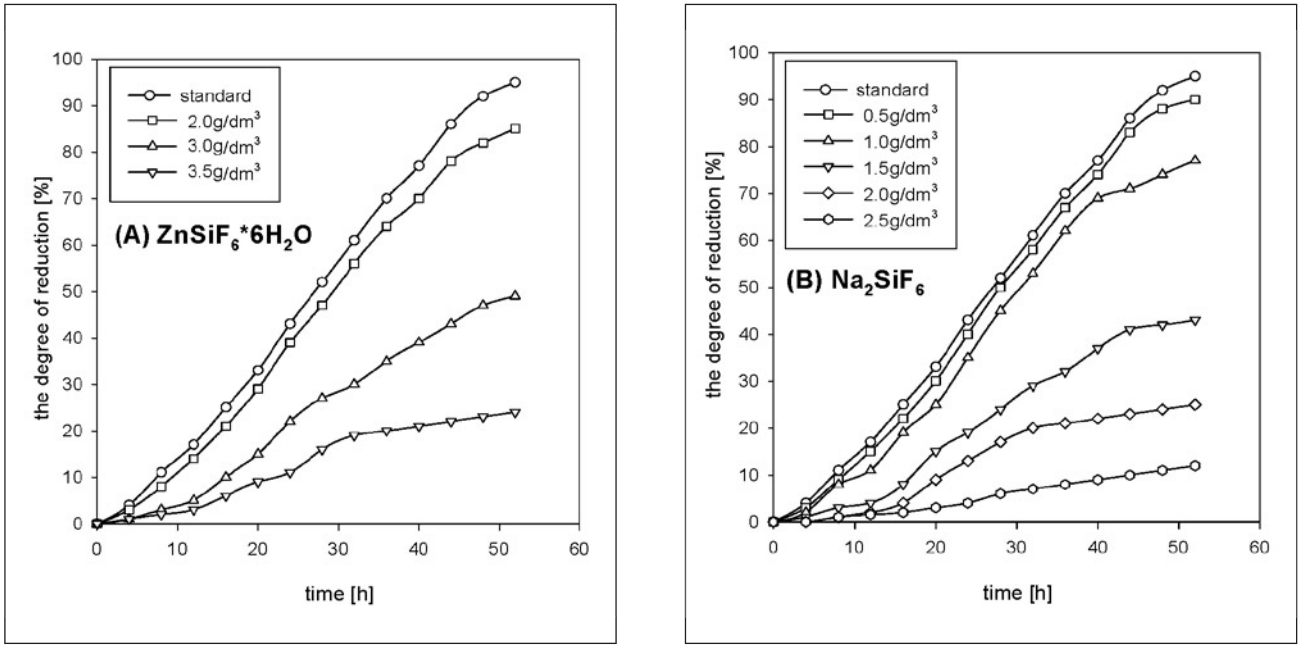

Fig.2. The effect of the concentrations of zinc fluorosilicate (A) and sodium fluorosilicate (B) on the dissimilatory sulphate reduction taking place with the involvement of *Desulfotomaculum ruminis* bacteria, pH 6.8-7.2, at 37°C.

Table 1. The effect of fluorine (g F^-/dm^3) contained in the compounds tested on the yield of desulfurication taking place with involvement of the *Desulfotomaculum ruminis* bacteria (37^0C, pH 6.8-7.2, C/S=3.6).

No.	Compound	Concentration of fluorine [g/dm^3]	
		tolerated	toxic
1	NaF	0.91-2.71	>2.71
2	NH_4F	1.02-2.57	>2.57
3	ZnF_2*4H_2O	1.09-2.39	>2.39
4	CdF_2	0.075-0.202	>0.202
5	Na_2SiF_6	0.30-1.82	>1.81
6	$ZnSiF_6*6H_2O$	0.72-1.45	>1.45

of desulfurication taking place in the lactate medium (Fig. 3).

The observed small differences between the concentrations of fluoride ions coming from the tested sodium, ammonium and zinc fluorides inhibiting the catabolic activity of the bacteria have been interpreted as a result of the effect of the accompanying metal ions [14-17].

A typical illustration of their effect is the inhibition of dissimilatory sulphate reduction by cadmium ions described in our earlier work [18]. According to the results of this work, the presence of cadmium ions in concentrations above 0.25g/dm^3 causes total and irreversible inhibition of the activity of the *Desulfotomaculum ruminis* bacteria. Such a concentration of cadmium ions occurs in the medium containing 0.5g CdF_2/dm^3 (0.126g/F^-/dm^3). However, to our great surprise in such a medium the yield of desulfurication is close to 60%. This result indicates

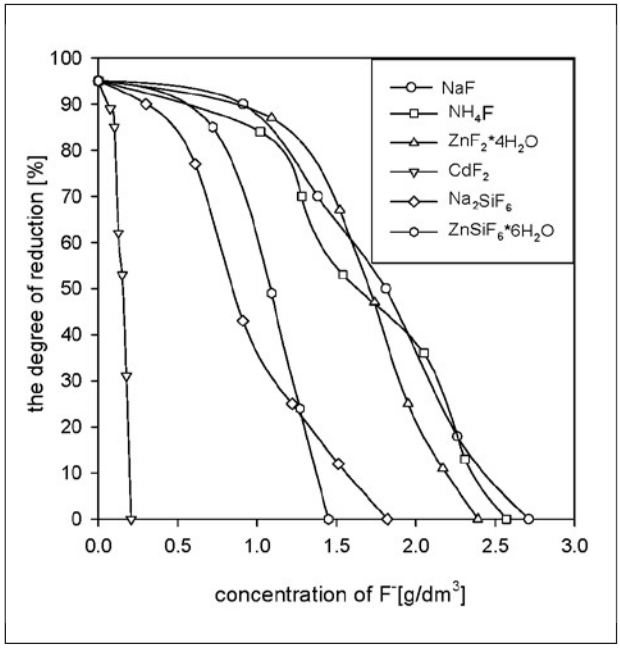

Fig.3. The yield of dissimilatory sulphate reduction versus the concentration of fluorine in the reaction medium.

that fluorine weakens the toxic effect of cadmium by stimulating individual resistance of the bacteria studied to its influence. This observation is consistent with the earlier observed differences in the admissible dose of fluorine dependent on the individual sensitivity and external factors [19]. In our experiment the toxic effect of CdF_2 on the catabolic activity of the bacteria begins from the concentration of 0.2 gF^-/dm^3 after the addition of more than 0.8g CdF_2/dm^3 (Fig.3).

Fig. 2 presents the kinetic curves describing the process of desulfurication in the medium containing sodium fluorosilicate or zinc fluorosilicate. According to earlier reports the complex fluorosilicate anions are highly toxic [20]. In the media containing 3.0g/dm^3 of zinc fluorosilicate or 1.5g/dm^3 sodium fluorosilicate, the process of sulphate reduction takes place with the induction period of about 12h. In these two media the yield of reduction is close to 50%, and the content of fluorine ions is ~1g/dm^3. The total inhibition of the process occurs at zinc fluorosilicate concentrations higher than 1.45 gF^-/dm^3 (4g $ZnSiF_6*6H_2O$/ dm^3) (toxic concentration) and at sodium fluorosilicate concentrations higher than 1.82 gF^-/dm^3 (3g Na_2SiF_6/dm^3) (toxic concentration) (Fig. 3). As follows from our results, the differences between the tolerated and toxic concentrations of fluorine ions are very small. Because at present there are no unambiguous relations allowing prediction of the fluorine compounds toxicity on the basis of their structure or species, each mineral compound containing fluorine should be treated as strongly toxic until it is proven otherwise.

Although the cations introduced into a medium containing fluorides cause some disturbances in the catabolic activity of the bacteria studied, it is reasonable to assume that the presence of fluorides in concentrations not higher than 2.4 gF^-/dm^3 has no inhibitory effect on the process of dissimilatory sulphate reduction taking place in the bottom sediments of marine or fresh water reservoirs, in underground waters [8], or in the stomachs of ruminants. The determined levels of admissible concentrations of fluorine not inhibiting the activity of sulphate-reducing bacteria should be taken into regard in designing processes of sewage or waste treatment with the use of this bacteria type [21].

Table 1 presents data illustrating the effect of fluorides in the compounds tested (present in different concentrations) on the catabolic activity of the *Desulfotomaculum ruminis* bacteria, specifying the tolerated and toxic levels of a given compound. In general, the toxicity of the fluorine compounds studied increases in the sequence: $NaF < NH_4F < ZnF_2 < Na_2SiF_6 < ZnSiF_6 < CdF_2$.

The results obtained in our study indicate that the fluorides occurring in surface and underground waters (above 2mg/dm^3 – 3rd class waters), or in mineral water (3-12mg/dm^3) are present at concentrations not inhibiting the process of proliferation of the *Desulfotomaculum ruminis* bacteria [4]. Elevated levels of fluorine have been noted in the soil near the sources emitting fluorine to the atmosphere, but even there the concentrations of fluorides do not always affect the proliferation of sulphate reducing bacteria (SRB). Only the content of fluorine in the soil near aluminium works varying from 1.5 to 5 gF^-/kg. d.w.[22] can be toxic to bacteria.

In conclusion, the effect of the fluoride compounds studied on the activity of SRB depends not only on their concentration but also on the type of species in which they occur, and the interaction between the effect of fluorides and metal ions depends on the concentration and toxicity of the metals. The results of the study permit a determination of concentrations of the fluorine compounds tested tolerated by SRB, weakening and inhibiting the process of desulfurication in which these bacteria are involved.

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