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Attempts at Microbiological Utilization of Rubber Wastes

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Abstract

This paper reports results of studies on the possibility of microbiological utilization of rubber waste products with the use of *Thiobacillus ferrooxidans* bacteria. Both raw and chemically-modified rubber vulcanizate were found to be toxic towards bacteria. The toxicity of particular components of the rubber was determined and the most toxic agent was found to be the anti-ageing Dusantox 6PPD (N-(l,3-dimethyl-butyl)-N-phenyl-p-phenylenediamine).

Keywords: *Thiobacillus ferrooxidans,* rubber wastes, biodegradation, toxicity, antioxidant

Introduction

Progress in technology in various branches of the economy stimulates the demand for rubber products, chief among them motor vehicle tyres. As their durability is limited, the amount of rubber waste increases and the problem of their utilization becomes of increasing importance. Tyres for motor vehicles and different machines make over 61% of all rubber industry production, which means that the used tyres pose a considerable threat to the natural environment.

Rubber products deteriorate mostly due to mechanical damage. The structure of rubber does not show significant changes upon exploitation, therefore rubber products can be secondary raw materials containing well-preserved caoutchouc hydrocarbon, fillers, plasticizers (softeners) and reinforcing substances. Recycling of rubber products would lead to reclamation of valuable and deficit materials, at the same time being a measure towards protection of environment [2]. The need for such the recycling was realized already at the beginning of the 19th century, soon after the discovery of natural rubber vulcanization. Already at that time attempts were made to restore original properties of used rubber, first of all its plasticity. Studies carried out for over 150 years have not brought a general solution to the problem and the majority of waste still ends up at dumping grounds. The recommendation of the European Community authorities is that until the year 2000 the dominant methods of utilization of rubber products would be recapping of tyres, production of surface-active granulate, rubber reclamation, pyrolysis, thermal utilization (combustion of tyres in cement production plants, combustion for energy) [3]. According to available data, at the present stage of technology the utilization of rubber wastes in the world is as follows: recapping of tyres 20%, material recycling 10-15%, combustion for energy 45%, others and the amount that goes to dumping grounds 20% [4].

New methods for utilization of rubber wastes are constantly sought. The most promising are those based on the cleavage of sulphate bridges by selectively working reagents such as: thiol-amines, HO- ions, radiation beams of appropriate frequency and energy, and thiophilic bacteria species. Although these methods are at present at the stage of laboratory testing, they may find practical use in the near future [3].

From the beginning of studies on microbiological degradation of natural rubber vulcanizates the main question has been whether microorganisms can use rubber as a source of carbon or live on some other substances included in the rubber mixtures. The results of the study by Cundell and Mulcock prove that degradation of rubber in a vulcanizate by microorganism is possible. The effect of microorganisms activity can be surface decomposition of rubber or vulcanizate, degradation by penetration of microorganisms into the rubber structure, decomposition of fillers which lose their properties of structure stabilization, deco-

mposition of certain components of rubber or vulcanizate. Each of the effects of microorganisms on rubber or its vulcanizate is related to changes in their physical, chemical and mechanical properties and leads to the degradation of the products, but on the other hand, can prove to be an excellent method of rubber waste disposal. Despite a significant interest in microbiological degradation of rubber vulcanizates, the microorganisms which can develop in them have been relatively poorly recognized. It has been established that only a few species of bacteria can cause degradation of rubber, mainly *Pseudomonas species* and the *Actinomycetales* species from the genus *Streptomyces* [5].

This paper presents results of the studies on the possibility of microbiological utilization of rubber wastes with the use of *Thiobacillus ferrooxidans.* These bacteria obtain the energy they need to maintain their life functions from oxidation of Fe (II) ions or reduced inorganic sulfur compounds. These studies are the continuation of those describing the process of microbiological desulfurication of coal [6].

Materials and Methods

The *Thiobacillus ferrooxidans* bacteria were isolated from the mine waters of the Siersza Colliery. The standard culture was grown in the Silverman medium 9K in 350 ml Erlenmayer flasks containing 100 ml of the medium, at 37°C, pH 2.2. The flasks were placed in thermostated shakers Elpan 357. The bacteria were inoculated every 48 hours. The inoculum introduced into reactors made 10% (vol./vol.) of the medium and the initial number of the bacteria was of the order of 10^7 cells/ml, which is typical of *Thiobacillus ferrooxidans.*

Trials of biological degradation of rubber were performed in 350 ml Erlenmayer flasks containing 100 ml of the medium and 2% (wt/vol.) of the rubber tested. The raw vulcanizate was found toxic towards the bacteria, it was subjected to preliminary physical and chemical modification. The raw rubber vulcanizate was modified with 1M HC1, 1M H_2SO_4, 1M $KMnO_4$ acidified with H_2SO_4, Dreumex-multi cleaner detergent. Toxicity of particular components of rubber on the *Thiobacillus ferrooxidans* bacteria was checked by analysis of their effect on the kinetic parameters of microbiological oxidation of iron (II) ions. The experiment was performed in 350 ml Erlenmayer flasks containing 100 ml of the medium Silverman 9K and different amounts of the rubber component tested. The changes in the concentration of iron (II) ions in these systems were monitored. The loss of the substrate was spectrophotometrically analyzed (Beckman DU 640) by measurements of iron (II) ions concentration by the o-phenentroline method. The iron (III) ions concentration was measured by the rhodanate method [7].

Table 1. Size of production of rubber products in Poland in the years 1990-95 **[1],**

Product	Quanlity	1990	1991	1992	1993	1994	1995
Total production of rubber products	In thousand ton	204	161	171	184	210	249
Including tyres for motor vehicles and other machines	In thousand ton	106	84.8	99.4	106	128	152
Car tyres	In thousands	4704	4516	5607	6479	7612	9502
Radial car tyres	In thousands	4246	4125	5204	6150	7346	9317
Passenger car tyres	In thousands	3575	3625	4542	5278	6223	7753
Lorry tyres and machine tyres	In thousands	1129	891	1056	1201	1389	1729
Tractor tyres	In thousands	502	351	485	505	634	712
Tyres for agricultural machines	In thousands	493	218	310	369	514	474
Bicycle tyres	In thousands	5723	3940	4498	4212	4830	5748

Table 2. Management of used tyres (in %) in UE countries in the years 1992-94 [3].

Ways of management	France	Germany	Italy	Spain	Belgium	Holland	UK
Total amount (tons)	363	545	320	139	71	65	402
Recapping	23	17	30	25	11	25	24
Reclaimed material	8	7	8	8	8	9	12
Combustion for energy	10	33	11	10	39	13	29
Pyrolysis	0	1	0	0	0	0	0
Other	0	11	0	0	0	0	6
Dumping grounds	59	31	51	57	42	53	29

The Kinetic Model

The process of microbiological oxidation of iron (II) ions is best described by the model of autocatalytic reaction of the 1st order with respect to the substrate (Fe^{2+}) and the product (Fe^{3+}):

$$\frac{d[S]}{dt} = k[S] \cdot [X]$$

And hence:

$$S = \frac{S_0(S_0 + X_0)e^{-k(X_0 + S_0)(t - t_0)}}{X_0 + S_0\, e^{-k(X_0 + S_0)(t - t_0)}},$$

where $[S]_0$ - the initial concentration of iron (II) ions,

$[X]_0$ - the initial concentration of iron (III) ions,
[S] - the concentration of iron (II) ions at time t,
t - time,
t_0 - induction period.

This equation was used for determination of the kinetic parameters such as the reaction rate constant (k) and the induction period (t_0). The fit of the model curve to experimental data was carried out by non-linear regression - the least square method - using the computer program Scientist.

Results and Discussion

The Effect of Zinc White

The effect of zinc white at concentrations of from 0 to 2000 ppm on the kinetics of oxidation of iron (II) ions from $FeSO_4$ x $7H_2O$ was studied at 37°C, pH 2.2 in the Silverman 9K medium. With increasing concentration of zinc white the rate constant of the reaction decreased from 1.93 to 0.73 x 10^{-3} [g/dm^{3}h^{-1}], and the induction period decreased. The shortening of the induction period to was accounted for by chemical oxidation of iron (II) by zinc (II).

Table 3. The effect of zinc white on the kinetics of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria (pH = 2.20, 37°C).

Zinc white [ppm]	Kinetic parameters of the process		
	Rate constant k [dm^{3}g^{-1}h^{-1}] x 10^{-3}	Induction time t_0 [h]	Process duration [h]
0	1.93 ± 0.16	9.1 ± 1.3	44
250	1.68 ± 0.17	10.5 ± 1.9	50
500	1.31 ± 0.16	8.4 ± 1.9	53
1000	0.88 ± 0.09	4.4 ± 3.1	63
1500	0.73 ± 0.06	0.2 ± 3.2	67
2000	TOXIC		

The maximum amount of zinc white in the system studied (2% wt./vol. of rubber) was 1000 ppm. This amount caused almost twice reduction of the reaction rate constant and elongation of the induction period by about 5 hours when compared to the reaction without zinc white. The total oxidation of iron (II) ions occurred at 63 hours; 19 hours more than in the standard medium. A further increase in zinc white concentration by another 500 ppm resulted in a decrease in the reaction rate by 20%, reduction of the induction period and elongation of the time needed for the whole iron to get oxidized by another 4 hours. The concentration of 2000 ppm proved toxic as no microbiological oxidation of iron (II) ions was observed even after 70 hours. For zinc white concentrations from 0 to 500 ppm, the rate constant of the process decreased by 30%, the induction period was slightly reduced and the total time of the oxidation increased by 53 hours. The results are presented in Fig. 1 and Table 3.

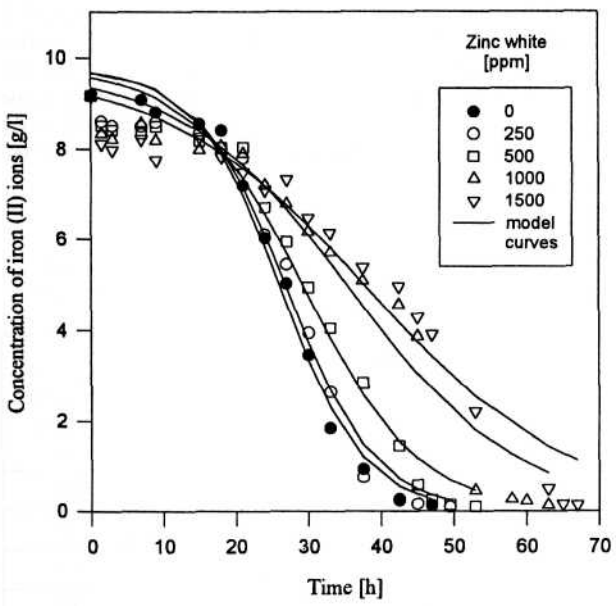

Fig. 1. The effect of zinc white on the kinetics of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria (Silverman 9K medium, 37°C, pH = 2.20).

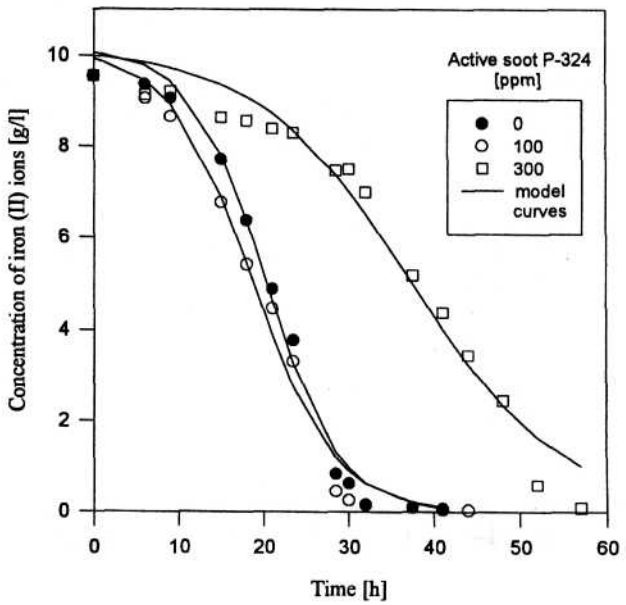

Fig. 2. The effect of active soot P-324 on the kinetics of oxidation of iron (II) ions by *Thiobacillus ferrooxidans* bacteria (Silverman 9K medium, 37°C, pH = 2.20).

The Effect of Active Soot P-324

A series of experiments was performed to check the effect of active soot P-324 on the concentration from 100 to 6000 ppm on the kinetics of microbiological oxidation of iron (II). The maximum soot concentration which could be present in the system with 2% wt./vol. rubber was 6000 ppm, which was the toxic threshold. Moreover, toxic properties of soot were already observed at a concentration 10 times lower than this maximum, bacteria growth was undisturbed only in the range 0-300 ppm, whereas the concentration of 100 ppm did not cause changes in the process, i.e. it was similar to the standard variant. An increase in the active soot concentration to 300 ppm resulted in twice reduction of the rate constant of the process, elongation of the induction period by 4 hours, and oxidation of the total amount of iron after 57 hours. The results are presented in Fig. 2 and Table 4.

Table 4. The effect of active soot P-324 on the kinetics of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria (pH = 2.20, 37°C).

Active soot P-324 [ppm]	Kinetic parameters		
	Rate constant k $[dm^3g^{-1}h^{-1}] \times 10^{-3}$	Induction time t_0 [h]	Process duration [h]
0	1.93 ± 0.16	9.1 ± 1.3	44
100	2.02 ± 0.19	10.5 ± 1.9	41
200	1.09 ± 0.09	8.4 ± 1.9	57
600	TOXIC		

The Effect of Stearin

In another series of experiments the effect of stearin in the concentration from 0 to 1500 ppm on the course of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria was studied. When the concentration of stearin was of 1000 (the maximum concentration) and 1500 ppm the process of iron (II) ions oxidation was not observed, which means that at these concentrations stearin was toxic to the bacteria. The concentration of stearin tolerated by the bacteria was 500 ppm at which the reaction rate constant decreased only by 13% and the induction period increased by over 7 hours. The process was complete after 56 hours. As follows from the results, stearin at the concentration at which it is present in rubber is toxic to the *Thiobacillus ferrooxidans* bacteria. The results are shown in Fig. 3 and Table 5.

Table 5. The effect of stearin on the kinetics of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria (pH = 2.20, 37°C).

Stearin [ppm]	Kinetic parameters		
	Rate constant k $[dm^3g^{-1}h^{-1}] \times 10^{-3}$	Induction time t_0 [h]	Process duration [h]
0	1.93 ± 0.16	9.1 ± 1.3	44
500	1.68 ± 0.12	16.3 ± 1.4	56
1000	TOXIC		

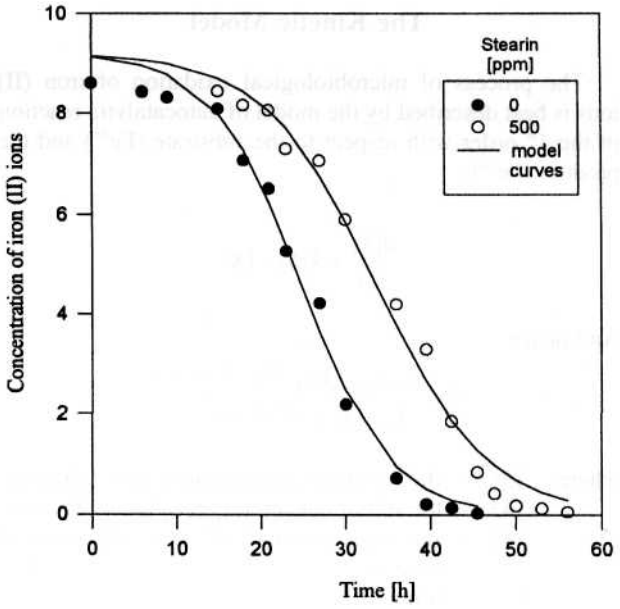

Fig. 3. The effect of stearin on the kinetics of oxidation of iron (II) ions by *Thiobacillus ferrooxidans* bacteria (Silverman 9K medium, 37°C, pH = 2.20).

The Effect of Dusantox 6PPD

The effect of Dusantox 6PPD on the kinetics of the process of microbiological oxidation of iron (II) ions was studied in the concentration range from 10 ppb to 500 ppm. The maximum concentration of these compounds in the systems studied was 300 ppm. The toxic effects of this compound were noted already at the concentration of 30 ppb, so 10,000 times lower than the maximum one. For the concentrations from 10 to 25 ppb, no changes in the process were observed, its rate constant was 2.4×10^{-3} [$g/dm^3\ h^{-1}$], the induction period 7.6 hours and the process was completed in 37 hours. No gradual inhibition of the process with increasing concentration of Dusantox was detected. It is also difficult to explain the fact that in the presence of Dusantox the process was about 20% faster and the induction period was shorter when compared with that in the standard culture. Dusantox proved to be the most toxic component of rubber. The results are collected in Fig. 4 and Table 6.

Table 6. The effect of Dusantox 6PPD on the kinetics of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria (pH = 2.20, 37°C).

Dusantox 6PPD [ppb]	Kinetic parameters		
	Rate constant k $[dm^3g^{-1}h^{-1}] \times 10^{-3}$	Induction time t_0 [h]	Process duration [h]
0	1.93 ± 0.16	9.1 ± 1.3	44
10	2.44 ± 0.25	8.1 ± 1.2	37
15	2.41 ± 0.26	7.5 ± 1.3	37
20	2.30 ± 0.23	7.0 ± 1.3	37
25	2.38 ± 0.22	7.7 ± 1.2	37
30	TOXIC		

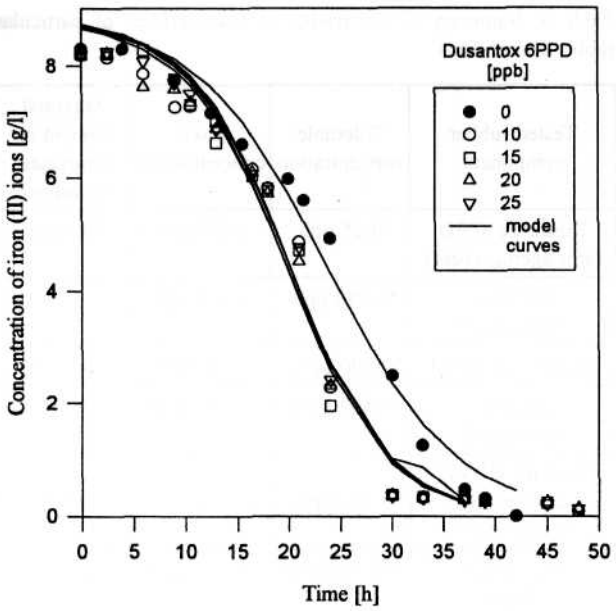

Fig. 4. The effect of Dusantox 6PPD on the kinetics of oxidation of iron (II) ions by *Thiobacillus ferrooxidans* bacteria (Silverman 9K medium, 37°C, pH = 2.20).

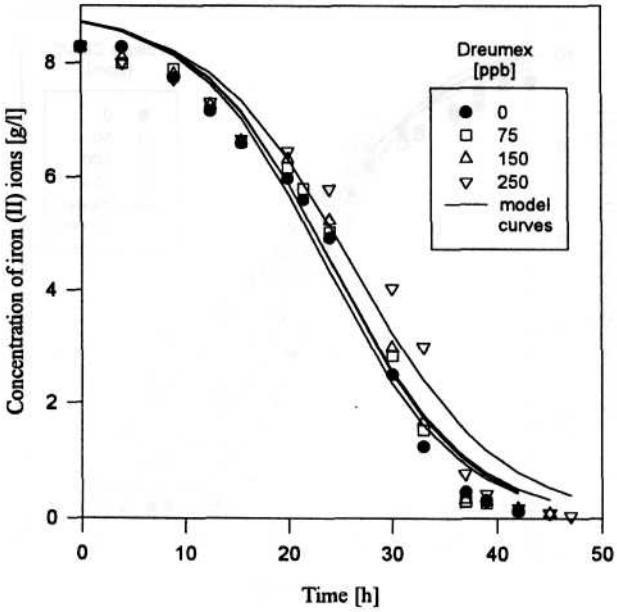

Fig. 5. The effect of Dreumex on the kinetics of oxidation of iron (II) ions by *Thiobacillus ferrooxidans* bacteria (Silverman 9K medium, 37°C, pH = 2.20).

The Effect of Dreumex

The effect of Dreumex on the kinetics of oxidation of iron (II) ions by *Thiobacillus ferrooxidans* bacteria was tested at concentrations of from 75 ppb to 10 ppm. With increasing concentration of the detergent the reaction rate constant decreased. The addition of Dreumex at a concentration of 250 ppb caused a 16% reduction of the process rate and a slight elongation (by 3h) of the time of its completion. No significant influence of Dreumex on the induction period was detected. It was of similar duration for all concentrations of the surfactant studied, although its values are slightly shorter from that in the standard variant, taking into regard the errors in their determination the differences cannot be interpreted. The concentration of 375 ppm of Dreumex proved toxic for the bacteria. Dreumex is not a component of rubber and has been used as a modifier. Its high toxicity to the bacteria indicates that it can be a very good detergent of disinfectant properties. The results are collected in Fig. 5 and Table 7.

Table 7. The effect of Dreumex on the kinetics of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria (pH = 2.20, 37°C).

Dreumex [ppb]	Kinetic parameters		
	Rate constant k $[dm^3g^{-1}h^{-1}] \times 10^{-3}$	Induction time t_0 [h]	Process duration [h]
0	1.93 ± 0.16	9.1 ± 1.3	44
75	1.81 ± 0.15	8.2 ± 1.5	42
150	1.77 ± 0.16	8.0 ± 1.6	45
250	1.63 ± 0.18	8.4 ± 2.2	47
375	TOXIC		

The Effect of Vulcafil CBS/C

The effect of sulphenoamide accelerator Vulcafil CBS/C on the kinetics of oxidation iron (II) ions from $FeSO_4 \times 7H_2O$ was studied in the concentration range from 0 to 150 ppm, at 37°C, pH 2.2 and in the Silverman 9K medium. With increasing concentration of Vulcafil the rate constant of the process decreased and was the lowest (twice lower than in the system without Vulcafil) for the Vulcafil concentration of 150 ppm. For the concentration of 100 ppm - the maximum in rubber - the rate constant was only 30% lower than that in the standard system, the induction period was slightly reduced, and the process was completed in 50h. For Vulcafil concentration from 0 to 100 ppm the induction period decreased slightly by about 6%, but for its concentration increased to 150 ppm the induction period was 40% reduced.

Since Vulcafil is not able to oxidize iron (II) ions, the shortening of the induction period (and hence the earlier start of iron (II) oxidation by bacteria) may be an error of measurement caused by the interference of Vulcafil in the reaction between iron (II) ions and o-fenantroline. The results are given in Fig. 6 and Table 8.

Table 8. The effect of Vulkafil CBS/C on the kinetics of iron (II) ion oxidation by *Thiobacillus ferrooxidans* bacteria (pH = 2.20, 37°C).

Vulkafil CBS/C [ppm]	Kinetic parameters		
	Rate constant k $[dm^3g^{-1}h^{-1}] \times 10^{-3}$	Induction time t_0 [h]	Process duration [h]
0	1.93 ± 0.16	9.1 ± 1.3	44
50	1.36 ± 0.11	7.9 ± 1.5	48
100	1.30 ± 0.11	8.5 ± 1.8	50
150	0.95 ± 0.08	5.4 ± 2.2	53

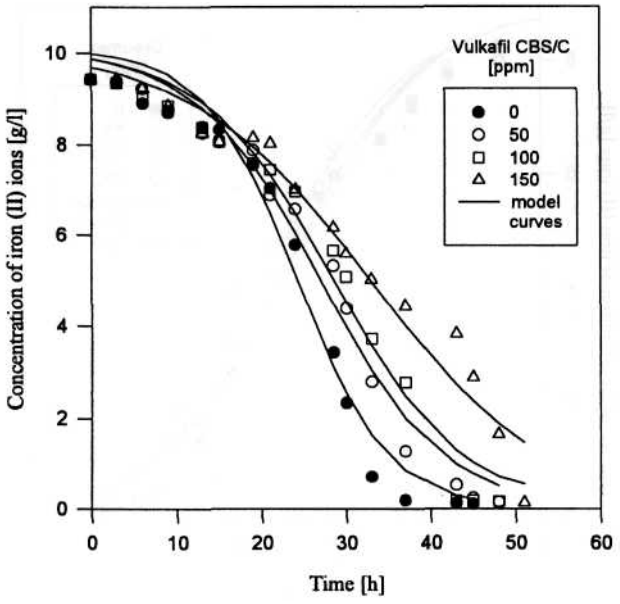

Fig. 6. The effect of Vulkafil CBS/C on the kinetics of oxidation of iron (II) ions by *Thiobacillus ferrooxidans* bacteria (Silverman 9K medium, 37°C, pH = 2.20).

Conclusion

The effects of five components of rubber and one surfactant used as a rubber modifying factor on the kinetics of iron (II) oxidation with participation of *Thiobacillus ferrooxidans* bacteria was tested. The most toxic component of rubber proved to be the anti-ageing agent Dusantox 6PPD (N-(l,3-dimethylbutyl)-N-phenyl-p-phenylenediamine). The surfactant Dreumex (which was not a component of rubber) also showed high toxicity towards the bacteria studied, so can be used as a bacteriostatic. The active soot P-324 proved toxic to the bacteria but at a concentration 10 times lower than the maximum in the systems studied. Stearin (the activator) was shown to be toxic only when at concentrations equal to or higher than the maximum in the systems studied. No toxic effect of Vulcafil CBS/C (vulcanization accelerator) was observed in the concentration range studied. Zinc white (activator) proved toxic only when at a concentration twice higher than the maximum in the rubber in the systems studied, so it was not the component of rubber showing toxic effects.

In the course of the studies reported we have identified the components of rubber responsible for its toxicity towards the bacteria *Thiobacillus ferrooxidans.* However, the next question to be solved appears to be the problem of their interaction which can either enhance or weaken the toxic effect. For the next stage of the study we plan to investigate of the synergistic or antagonistic relations among the rubber components with a further goal of eliminating or neutralizing of their toxic influence.

Table 9. Summary of the results of toxic effects of particular rubber components.

Tested rubber component	Tolerable concentration	Toxic concentration	Maximal content of component of rubber
Dusantox 6PPD (anti-ageing agent)	0-25 ppb	> 30 ppb	300 ppm
Dreumex (surfactant)	0-250 ppb	> 375 ppb	–
Active soot P-324	0-300 ppm	> 600 ppm	600 ppm
Stearin (activator)	0-500 ppm	> 1000 ppm	1000 ppm
Vulkafil CBS/C (sulphenamide accelerator)	0-150 ppm	–	100 ppm
Zinc white (activator)	0-1500 ppm	> 2000 ppm	1000 ppm

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