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USE OF DISSIMILATORY REDUCTION OF SULFATES FOR THE SIMULTANEOUS REMOVAL OF ORGANIC CONTAMINANTS, SULFATES AND HEAVY METALS FROM WASTEWATER

There was investigated the potentiality of utilising *Desulfovibrio desulfuricans* for the simultaneous removal of organic pollutants, sulfates and ions of Cd(II), Co(II), Cu(II), Ni(II) and Zn(II), which were added to an industrial pig farm effluent in the form of sulfates. Sulfate concentration after dissimilatory reduction did not exceed $65 \text{ mg} \cdot \text{dm}^{-3}$ and COD removal was higher than 79.2%. The efficiency of heavy metal removal varied from 99.8% to 99.9% when the sulfates were added to the reactors batchwise, and the successive doses were added each time the concentration of the sulfate entering the medium with the previous dose was reduced to the level of $\leq 150 \text{ mg SO}_4 \cdot \text{dm}^{-3}$.
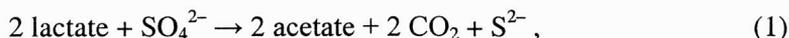
1. INTRODUCTION

In the past decade, many investigators have analysed potential applications of sulfate-reducing bacteria (SRB), in the treatment of sulfate-contaminated effluents [4], [13]–[20]. Among pollutants, which are particularly hazardous to the environment, are not only organic substances, but also numerous heavy metals (and specifically their ionic forms). Heavy metals generally enter the environment together with wastewater, sludge or solid wastes, and their influence on the self-purification process is defined as an inhibiting or a destructive one. That is why the removal of heavy metals from wastewater in general and by microorganisms in particular has attracted the attention of environment-conscious scientists for many years. Of the microorganisms that might be utilised for this purpose, SRB deserve special attention. They grow under anaerobic conditions and interact with the natural environment (e.g., participating

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in biocorrosion, fuel production, geochemical transformations, food spoilage, biore-
mediation, environmental nutrient cycles and wastewater treatment) [2]. In the process
referred to in specialised literature as dissimilatory sulfate reduction (DSR) or sulfate
respiration, SRB degrade organic substances and oxidised sulfur forms, which are their
carbon and energy source, and the hydrogen sulfide released via this route is ready to
form sparingly soluble sulfides with heavy metals according to the following equations:

$$2 \text{ lactate} + SO_4^{2-} \rightarrow 2 \text{ acetate} + 2 CO_2 + S^{2-} , \tag{1}$$

$$Me^{2-} + S^{2-} \rightarrow MeS \downarrow . \tag{2}$$

SRB are also able to utilise heavy metals as electron acceptors, and this occurs in
the process referred to as dissimilatory metal reduction [11], [12]. The solubility of
many heavy metals is higher at an increased degree of oxidation, so it is possible to
make use of the microbiological reduction process to detoxify wastewater and solid
wastes. Three capabilities of SRB – decomposition of organic substances, degradation
of sulfates and removal of metals – play an important role in environmental pollution
control, and the results, which have been reported so far, are promising [9], [22]–[25].

The objective of this study was to utilise *Desulfovibrio desulfuricans* with the aim to
remove simultaneously organic pollutants, sulfates and ions of Cd(II), Co(II), Cu(II), Ni(II)
and Zn(II) from wastewater. To achieve this goal the effluent from industrial pig farming
was chosen because it contained many of readily available organic substrates and nutrients,
and was not contaminated by sulfates or heavy metals. These pollutants (sulfates and
heavy metals) were added into the effluent as the sulfates of the metals investigated. In this
way, they were both a source of sulfates and a source of ions of heavy metals.

2. MATERIALS AND METHODS

Microorganisms. The inoculum was a mixed anaerobic culture dominated by the
Desulfovibrio desulfuricans species (isolated from the hydrogen sulfide-containing
curative waters of the spa of Busko Zdrój in the south-east of Poland) [5] and had a vol-
ume of 0.5 dm^3 (44.2 g dry wt. dm^{-3}, dry organic solids accounting for 47.5%; COD,
SO_4, pH and alkalinity totalled 81 mg $O_2 \cdot dm^{-3}$, 13 mg $SO_4 \cdot dm^{-3}$, 8.2 and 2250 mg
$CaCO_3 \cdot dm^{-3}$, respectively). The method of inoculum production is shown in figure 1.

Substrates. The culture media were prepared using non-sterile effluents from in-
dustrial pig farm. The effluents were supplemented with Cd(II), Co(II), Cu(II), Ni(II)
and Zn(II) sulfates. The compounds ($CdSO_4 \cdot 8 H_2O$, $CoSO_4 \cdot 7 H_2O$, $CuSO_4 \cdot 5 H_2O$,
$NiSO_4 \cdot 7 H_2O$ and $ZnSO_4 \cdot 7 H_2O$) were added in appropriate portions in such a way as to
enable the sulfates introduced via this route to maintain the COD/SO_4 ratio values close
to the range of 0.8–1.2 (which was found to be optimal for the wastewater and bacteria
investigated [10]). The control medium contained ferric sulfate ($FeSO_4 \cdot 7 H_2O$). The
chemical parameters of the experimental media are listed in table 1.

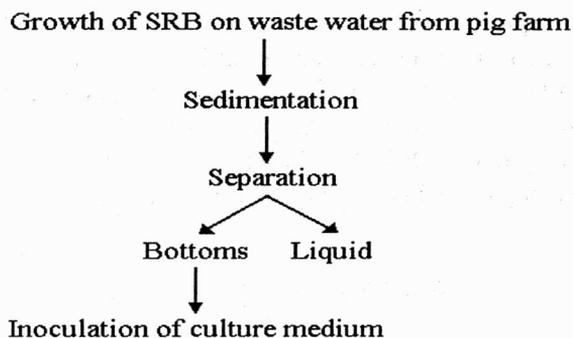**Growth of SRB on waste water from pig farm**

↓

Sedimentation

↓

Separation

⤡ ⤢

Bottoms **Liquid**

↓

Inoculation of culture medium

Fig. 1. A schematic diagram of inoculum production

Table 1

Chemical parameters of experimental media

Metal	COD (mg $O_2 \cdot dm^{-3}$)*	SO_4 (mg $SO_4 \cdot dm^{-3}$)*	Metal concentration (mg $\cdot dm^{-3}$)		pH	COD/SO_4
			Total**	In liquid phase*		
Cd(II)	3670	3340	2850	2375	6.85	1.1
Co(II)	3900	4100	2520	2118	7.8	0.95
Cu(II)	3730	3360	2224	460	6.55	1.1
Ni(II)	3300	2750	1682	1608	6.67	1.2
Zn(II)	3370	4400	2996	1223	6.4	0.8
Fe(II)						
Control sample	4180	4296	2523	2410	6.58	0.97

* Filtered samples.
** Unfiltered samples.

The total concentrations of heavy metals were higher than their concentrations in liquid phase. This might be attributed to the physicochemical properties of the pig farm effluent investigated, as well as to the presence of a certain amount of hydrogen sulfide ions, which formed in the inoculum in the course of its preparation.

Methods of bacterial growth. The experiments were run in five air-tight 2 dm^3 effective volume reactors, each filled up with a medium (1.5 dm^3 in volume) containing one of the metal sulfates investigated. The reactors were then inoculated with 0.5 dm^3 portions of microorganisms, and bacterial growth took place at 38 °C for 264 hours.

Analytical methods. 50 cm^3 samples were taken from the reactors once a day by means of a peristaltic pump, after the reactors had been mechanically mixed. Metal concentrations were determined (in unfiltered samples and in the supernatant) by

means of atomic absorption spectrophotometry. The metal content in the solid phase was calculated based on the metal concentration in the unfiltered samples and in the supernatant. Alkalinity and pH were measured by the potentiometric method. Sulphate concentration and chemical oxygen demand (COD) were determined gravimetrically as a precipitate with $BaCl_2$ [26] and by the dichromate method [27], respectively, after the samples had been filtered through a filter paper.

3. RESULTS AND DISCUSSION

The experimental results for the metal sulfates investigated were similar. No considerable variations of pH, COD or SO_4 were observed in the samples. Metal content did not increase in the sludge and did not decrease in the liquid phase. The experimental DSR process (exemplified by zinc sulfate in figure 2) proceeded in almost similar way in all of the reactors. The experiments revealed that a single dose of the heavy metal applied at the beginning of the culture growth exerted an inhibiting effect on the metabolism of *D. desulfuricans* and on the DSR process, which took a totally different course in the control medium (with ferric sulfate; figure 3). Thus, in the control, after the same reaction time, sulfate reduction amounted to 98%, with a final SO_4 concentration of 110 mg $\cdot$ dm^{-3}. COD decreased by approximately 75% (from 4180 mg $O_2 \cdot$ dm^{-3} to about 1000 mg $O_2 \cdot$ dm^{-3}) and almost all Fe(II) ions were removed; their concentration, which totalled 2410 mg Fe(II) $\cdot$ dm^{-3} at the beginning of DSR, approached 55 mg Fe(II) $\cdot$ dm^{-3} after termination of the process. pH increased in the first hours of the DSR process to stabilise at the end of it.
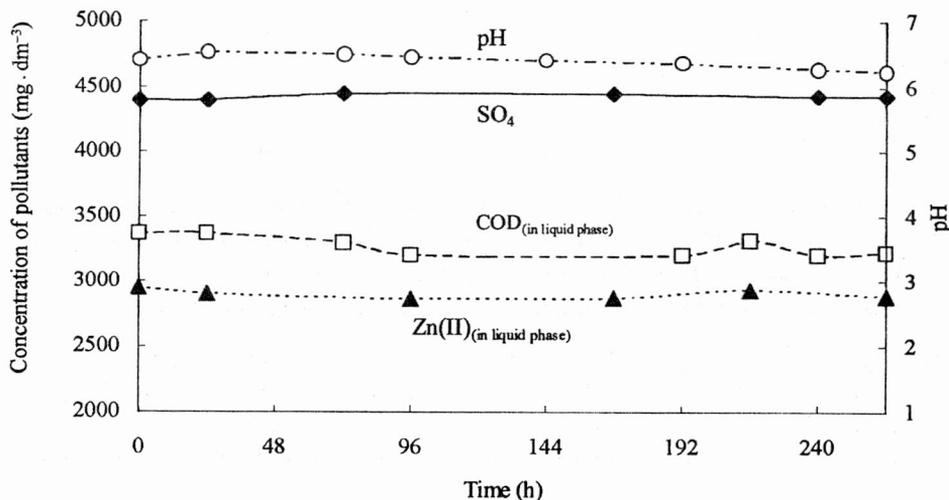

Fig. 2. DSR process after single application of the whole zinc sulfate dose
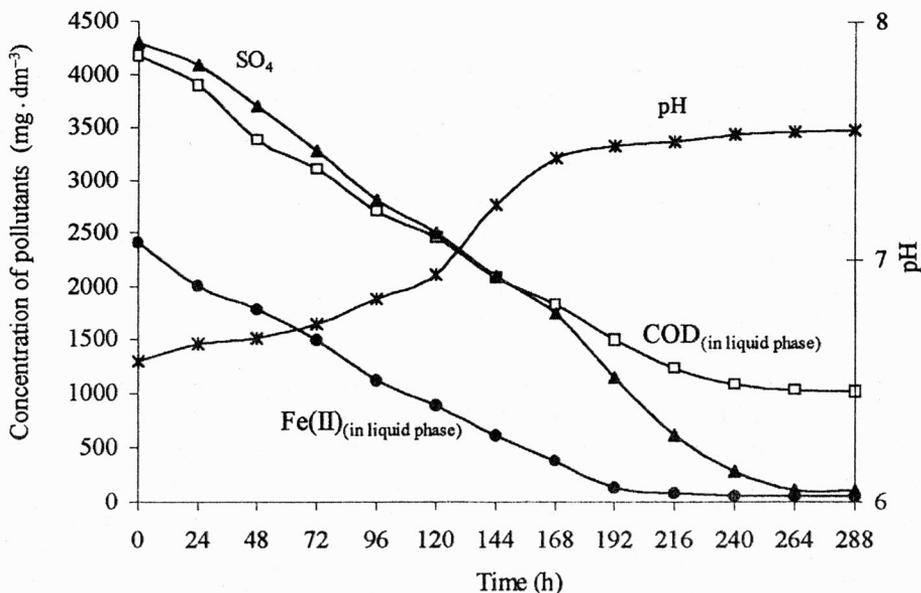at the beginning of culture growth

Fig. 3. DSR process after single application of the whole iron sulfate dose
at the beginning of culture growth

The portions of heavy metal ions added to the medium as single doses of their sulphates were found to be too high, so it became necessary to modify the method of their portioning. For this purpose, further tests were carried out, in which single doses of the metal sulfates investigated were successively decreased. They were added to the reactors in portions, and each successive portion was dosed only after the concentration of the sulfate entering the medium with the previous dose had been reduced to the level of ≤ 150 mg $SO_4 \cdot dm^{-3}$. The quantity of sulfates, which had to be divided into portions, was calculated in such a way as to maintain the COD/SO_4 ratio close to the range of 0.8–1.2. The size of a single portion was determined in such a way that the heavy metal content would not inhibit the DSR process. Additional tests (unpublished results) showed that the modified method of applying metal sulfate doses did not inhibit the process. Thus, COD removal efficiency amounted to 79.2%; 83.4%; 84.4%; 80.7% and 84.7% for Cd, Co, Cu, Ni and Zn, respectively. The efficiency of sulfate reduction was even higher, totalling 97%; 96.9%; 95.7%; 96.7% and 98.9%, respectively. The proportion of heavy metals (added in sulfate form) that entered the solid phase varied from 99.8 to 99.9%. The DSR process is shown in figures 4 and 5 for the zinc sulfate-containing medium and cadmium sulfate-containing medium, respectively. The DSR process for the other metals followed similar way. More details can be found in table 2.

Concentrations of pollutants before and after modification of the DSR process (filtered samples)

Metal	Me (mg·dm^{-3})		Removal (%)	COD (mg O$_2$·dm^{-3})		Removal (%)	SO$_4$ (mg SO$_4$·dm^{-3})		Removal (%)	COD/SO$_4$ total
	Before	After		Before	After		Before	After		
Cd(II)	2135	0.63	99.9	2390	498	79.2	1880	56	97	1.27
Co(II)	1153	1.85	99.8	2880	478	83.4	1880	58	96.9	1.53
Cu(II)	1863	1.35	99.9	4580	713	84.4	2800	120	95.7	1.63
Ni(II)	1200	1.51	99.8	2520	485	80.7	1960	65	96.7	1.28
Zn(II)	2220	1.78	99.9	3730	570	84.7	3260	35	98.9	1.14

Total amounts of successively added metal ions were similar to those of the single doses (table 1), but the effects of the DSR process were different. COD removal and SO$_4$ reduction obtained with the modified method of heavy metal dosing were similar to those obtained in the control sample containing ferric sulfate (figure 3). As shown by the experimental results, DSR was an effective method of removing heavy metals that had been introduced to the pig farm effluent in the form of sulfates. On modifying the way of heavy metal inflow into the wastewater, it was possible to eliminate the toxic effect of the metal ions on the SBR biomass involved in the DSR process. Sulfate concentration can be regarded as a sufficiently good parameter for the control of heavy metal inflow.
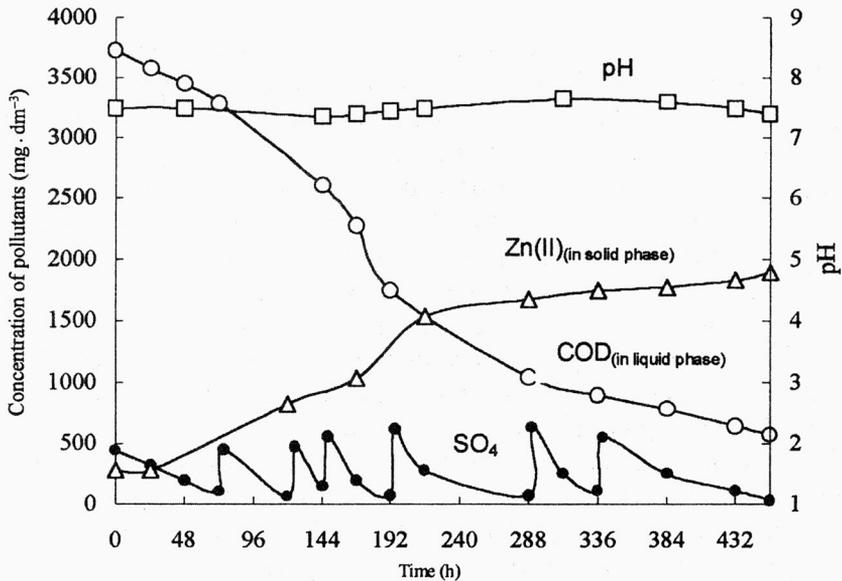

Fig. 4. DSR process where ZnSO$_4$·7H$_2$O was added in seven consecutive portions, yielding zinc concentration in the reactor amounting to 303, 305, 286, 279, 368, 374 and 307 mg Zn(II)·dm^{-3}, respectively. The second, third, fourth, fifth, sixth and seventh portion was dosed at a sulfate concentration in the reactor of 103, 60, 150, 80, 80 and 100 mg SO$_4$·dm^{-3}, respectively
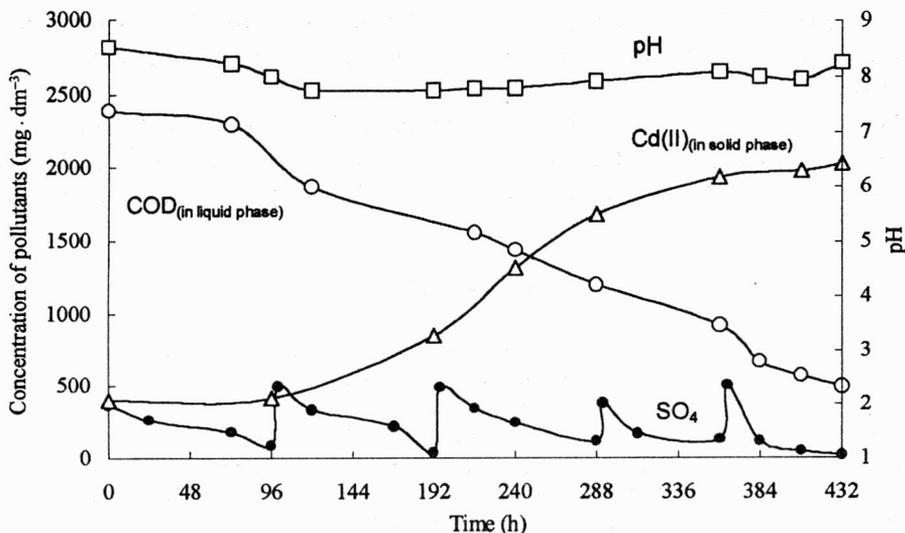
Fig. 5. DSR process, where CdSO$_4$·8H$_2$O was added in five consecutive portions,
yielding cadmium concentration in the reactor amounting to 433, 480, 526, 310 and
386 mg Cd (II)·dm^{-3}, respectively. The second, third, fourth and fifth portion was dosed
at a sulfate concentration in the reactor of 90, 40, 120 and 150 mg SO$_4$·dm^{-3}, respectively

In the past decades, many investigators have reported on the utility of the DSR process in the treatment of metal-contaminated mine water or detoxication of groundwater in the vicinity of smelters [1], [3], [6]–[9], [21], [28]. In the Netherlands, for example, the effluent from zinc refining in the Budelco system is treated using DSR with acetates, lactates, propionates, ethanol and methanol as a carbon source [1]. In this method, the efficiency of Zn(II) removal is very high – from the initial value of 916 mg·dm^{-3} Zn(II) concentration decreases to 0.05 mg·dm^{-3}. From the results of our present study it may be inferred that the organic compounds utilised in the Budelco system as carbon sources can be replaced by the organic pollutants from the pig farm effluent which are biodegradable via SRB. Thus, SRB might be of utility in an integrated process for biological treatment of a mixture of wastewater, which leads to simultaneous microbial removal of sulfates and heavy metals. Such utilisation of the versatility of SRB is very promising in that it provides removal of organic pollutants, reduction of salinity and detoxication of metal-contaminated effluents.

The investigations carried out allow the following conclusions:

1. DSR was an efficient method of removing heavy metals being introduced in portions of sulfates to the effluent from industrial pig farm using SO$_4$ as a control parameter. The efficiency of Cd(II), Co(II), Cu(II), Ni(II) and Zn(II) removal varied from 99.8 to 99.9%.

2. Elimination of heavy metals was accompanied by SO$_4$ removal as well as degradation of organic pollutants. The concentration of sulfates in the effluents that were

subjected to the DSR process did not exceed 65 mg $SO_4 \cdot dm^{-3}$ (the experiment with copper sulfate was the only exception), and the efficiency of COD removal was higher than 79.2%.

3. What needs further studies is the optimisation of the metal sulfate portions, since those used for the purpose of the present study might not have been optimal. If changed appropriately, they are likely to improve the rate and efficiency of heavy metal removal.

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WYKORZYSTANIE DYSYMILACYJNEJ REDUKCJI SIARCZANÓW
DO JEDNOCZESNEGO USUWANIA ZE ŚCIEKÓW ZANIECZYSZCZEŃ ORGANICZNYCH,
SIARCZANÓW I METALI CIĘŻKICH

Badano możliwość wykorzystania bakterii *Desulfovibrio desulfuricans* do jednoczesnego usuwania zanieczyszczeń organicznych, siarczanów oraz jonów Cd(II), Co(II), Cu(II), Zn(II) oraz Ni(II), które wprowadzono w formie siarczanów do ścieków pochodzących z przemysłowej hodowli trzody chlewnej. Stężenie SO_4 po dysymilacyjnej redukcji siarczanów nie przekraczało 65 mg·dm^{-3}, a poziom redukcji COD był wyższy od 79,2%. Stopień eliminacji metali wynosił od 99,8% do 99,9%, gdy siarczany dozowano sukcesywnie porcjami, przy czym następną porcję dozowano wtedy, gdy poziom siarczanów wprowadzonych z poprzednią dawką spadał poniżej 150 mg SO_4·dm^{-3}.