Original Research

Sulphate-Reducing Bacteria, Their Properties and Methods of Elimination from Groundwater

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Abstract

In the Gdańsk region urban development and intensive take off have caused an increase in water exploitation. As a consequence of the changes of hydrogeological conditions, a deterioration of chemical and bacteriological quality of water has been observed. Routine bacteriological quality control does not include some specific bacteria which are characteristic for the groundwater environment and can have negative influence on the physical and chemical properties of water. Among such bacteria there are sulphate-reducing bacteria (SRB). In the paper frequency of SRB occurrence in raw water from Cretaceous and Quaternary formations and in treated water has been described. It has been proved that bacterial consortium isolated from groundwater show preference to sodium lactate as a main source of carbon and energy. SRB have a negative influence on organoleptic properties of water quality and the technical condition of distribution net, the water should be disinfected. It has been proved that non-reagent methods, like UV radiation and microfiltration, are effective for SRB elimination from groundwater.

Keywords: sulphate-reducing bacteria, UV radiation, membrane filtration, groundwater

Introduction

In the Gdańsk region some of the richest groundwater resources among hydrogeological units in Poland are found. This fact is due to the aquifers present in well-developed and widely spread Cretaceous and Quaternary formations, morphological diversity of alimentation area and influence of the sea, which is the basic draining reservoir of all aquifers, establishing flow directions.

Intensive take off as well as urbanization and industrialization of the area are the major reasons for substantial transformations of hydrogeological regime and deterioration of all aquifer quality.

In the Gdańsk region, some ground water is characterized by specific properties which limit their usefulness to consumption as follows: relatively high concentrations of iron, manganese, nitrogen ammonium and periodically appearing unpleasant hydrogen sulphide odour.

Routine bacteriological control of water quality includes only evaluation of pollution with faecal bacteria, while specific groups of bacteria, as manganese bacteria and iron bacteria and also sulphate-reducing bacteria, characteristic for groundwater environment and in many cases responsible for deterioration of physical and chemical parameters of water, are not examined.

In the study occurrence of sulphate-reducing bacteria (SRB) in groundwater from Cretaceous and Quaternary formations in Gdańsk region was investigated. These bacteria use sulphate ions as acceptor of electrons in the process of organic matter oxidation. SRB are considered the oldest organisms detected in this environment [1]. Their presence was observed in quaternary aquifer at depths of

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15-50 m and 230 m [2], and also in the Cretaceous aquifer at depths of 360-410 m [3].

SRB belong to the archaebacteria group, which are characterized by specific features: their cell wall does not contain peptidoglycan, cell membrane has specific composition of lipids, and ribosomes are characterized by specific sequence of nucleotides in RNA chain [4]. SRB are morphologically diverse; cell forms include cocci, rods, curved (vibrioid) types, cell aggregates (sarcina-like) and multicellular gliding filaments [5]. SRB cell size is in the range of 0.5 – 1.3 x 0.8 – 5 µm (*Desulfovibrio*) and 0.5 – 2 x 2 – 9 µm (*Desulfotomaculum*) [6]. SRB are anaerobes, but also can survive under aerobic conditions [7-9]. They commonly occurr in soils, sediments and in non-polluted water-bearing layers [10].

The production of H_2S often indicates the activity and presence of sulphate-reducing microorganisms in natural habitats. The presence of H_2S is obvious by its characteristic smell, black precipitation of ferrous sulfide when iron minerals are present. As result of SRB metabolism, sulphur-reduced compounds can decrease organoleptic features of water, making for unpleasant odour, and also can contribute to biological colmatation of well filters.

Electron donors are usually low-molecular organic compounds for SRB [11]. These compounds are most often products of fermentation, such as volatile fatty acids [12].

The Study Area

Investigations were carried out at eight Cretaceous water intakes, located at the Marine Terrace ("Czarny Dwor" and "Zaspa"), Zulawy ("Lipce," "Krakowiec," "Pruszcz Gdański," "Sobieszewo"), at the edge of Kaszubian Lake District Upland ("Chelm") and at Quaternary-Tertiary water intake ("Reda II") located in the central part of Kaszubian Proglacial Stream Valley. Locations of investigated water intakes are presented in Fig. 1.

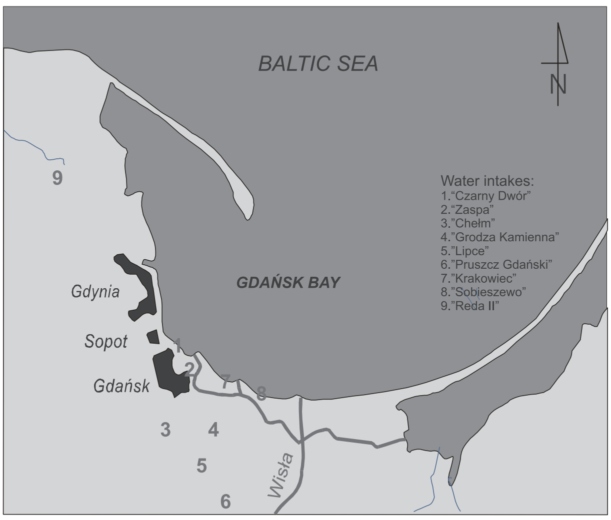

Fig.1. Location of investigated groundwater intakes.

Additionally, water outflowing from water treatment plant processing Quaternary-Tertiary groundwater from the intake "Reda II" was analyzed. In the treatment plant traditional technological processes of aeration and filtration at filters filled with quartz sand are conducted. Cretaceous water do not undergo treatment and are mixed with treated Quaternary water.

Methods

Samples of Cretaceous groundwater from 8 intakes were collected; eighty five samples form the following wells: K1 at "Chelm", K1 at "Czarny Dwor", K2 at "Grodza Kamienna", K4 at "Krakowiec", K2 at "Lipce", K1 at "Pruszcz Gdański", K2 at "Sobieszewo" and K2 at "Zaspa" were examined.

Samples of Quaternary groundwater were collected from 16 wells at the intake "Reda II" (no. A-1A.1B, P-2A, 2A, 3B, 4A, 5A, 6B, 7B, 8B, 9B, 10B, 11B, 12C, 14C, 16B). Altogether eighty samples of Quaternary water were examined.

Samples of treated water were collected from the pipeline of the water treatment plant "Reda II." Ninety four samples of treated water were collected.

Odour of water samples was identified directly after the sample was collected.

Bacteriological analyses included determination of the SRB occurrence in Cretaceous, Quaternary and treated water. Moreover, most probable number (MPN) of SRB in Cretaceous formation was examined. Starkey liquid nutrient medium (0.5 g K_2HPO_4, 1.0 g NH_4Cl, 1.0 g Na_2SO_4, 0.1 g $CaCl_2*2H_2O$, 2.5 g $MgSO_4*7H_2O$, 6.0 g of sodium lactate (70%), 1000 ml of distilled water, 10 ml of $FeSO_4(NH_4)2SO_4*6H_2O$ (10%), pH=7.0-7.5) was used. Incubation at 22°C lasted for 4 weeks.

From the tested groundwater 12 consortium (8 consortium from Cretaceous formations: no 1, 2, 3, 4, 5, 6, 7, 8 and 4 consortium from Quaternary formations: no 9, 10, 11, 12) of the sulphate-reducing bacteria have been isolated and some of their properties have been specified in periodic anaerobic cultivation. Bacterial consortium were incubated on Starkey medium, in which sodium lactate or acetate (interchangeably) as a main source of carbon was used.

In water samples optical density was analyzed – in order to indicate SRB growth. The optical density measurement (with wave length 420 nm) was done by means of spectrophotometer Cadas 30S Dr Lange production. A sterile Starkey medium was the control sample in every measurement. Analyses of hydrogen sulphide concentration were done with spectrophotometer DR/2000 using wavelength 665 nm by dilution method according to Hach.

SRB spore-forming ability was investigated on Starkey medium after pasteurisation of water sample at 80°C for 15 minutes.

Concentrations of volatile fatty acids analysis were performed by means of gas chromatography technique coupled with flame-ionization detector. A Hewlett Packard gas chromatograph 5890 II was used [13]

Survival rate of SRB under UV radiation was investigated at the water treatment plant "Reda II" using an E-2 device (Wedeco) equipped with 4 low-pressure Spectrotherm radiators and an AME-7 device equipped with 1 low-pressure radiator. Effectiveness of radiation doses in the range from 13 mJ/cm^3 to 88 mJ/cm^3 was analyzed.

Investigations of effectiveness of SRB removal from water by means of filtration were carried out at the pilot station type "Zee Weed" using hydrophilous membranes with pore size from 0.08 to 0.1 μm. Fifteen series of investigations including raw water and treated water were conducted.

Results

Occurrence of Sulphate-Reducing Bacteria in Water

Water from Cretaceous Formations

Sulphate-reducing bacteria were present in water samples of all 8 investigated Cretaceous groundwater intakes. Occurrence and MPN of SRB are shown in Fig. 2. The highest number of samples containing SRB (67%) were collected at the Chelm intake (well K1), where the maximum MPN of these bacteria was 18. In four intakes: Czarny Dwor (well K1), Zaspa (well K2), Grodza Kamienna (well K2) and Pruszcz Gdański (well K1), from 38 to 66% of water samples contained SRB and maximal MPN ranged from 6 to 16. In cases of other intakes – Lipce (well K2), Sobieszewo (well K2) and Krakowiec (well K4), SRB were detected in 25-38% of analyzed samples and MPN ranged from 2 to 3.

In all analyzed water samples from Cretaceous formations the presence of SRB was accompanied by unacceptable sulphur hydrogen odour.

Water from Quaternary Formations

Sulphate-reducing bacteria were detected in all of 16 investigated Quaternary wells at the intake "Reda II" (Fig. 3).

SRB were present in 100% of samples from three wells: no. P-2A, no. 3B and no. 6B, in 80% of samples from wells no. A-1A and 4A and in 60% of samples from wells no. 1B, 2A, 5A, 7B and 16B. In the case of six other investigated wells (no. 8B, 9B, 10B, 11B, 12C and 14C) SRB were detected in 50% of analyzed samples or less.

In all analyzed water samples the presence of SRB was accompanied by an unacceptable sulphur hydrogen odour.

Treated Water

In 73 (86%) samples of treated water from the water treatment plant "Reda II" sulphate-reducing bacteria were detected. This result indicates that SRB were almost constantly present in treated water. Since the number of these bacteria is relatively low (1/100 ml), there are some periods when they are undetectable in this volume.

Selected Properties of Investigated SRB Consortium

It has been proved that consortium of the sulphate-reducing bacteria display preference for growing and producing H_2S on sodium lactate as the main source of carbon and energy. Table 1 shows the values of optical density and H_2S concentration in consortiums incubated on medium with sodium lactate and sodium acetate. In all consortiums incubated on medium with sodium lactate the optical density was higher than in the same consortiums with sodium acetate. Maximal value of optical density in consortiums with sodium acetate ranged from 0.070 to 0.167, whereas in consortiums with sodium lactate they ranged from 0.199 to 0.612. Also, in all consortiums incubated on medium with sodium acetate concentration of H_2S was vestigial (0.3 – 1.6 mgH_2S/dm^3), whereas in consortiums on sodium lactate production of H_2S was indicated (except consortiums no.6, no.7, no.12) and maximal values of H_2S concentration ranged from 17.5 to 92.7 mgH_2S/dm^3.

Bacteria which were in the composition of consortium only partly oxidized the lactate; however, they accumulated the acetate and some amount of propionate and butyrate (exception was one consortium in which bacteria almost entirely mineralized the lactate into carbon dioxide and water). Table 2 shows concentration of volatile fatty acids in consortiums incubated on medium with sodium lactate.

Apart from accumulation of acetate, the ability to form spores has been detected in six consortium of SRB (no 3, no. 4, no. 5, no. 6, no. 7 and no. 10). Non spore-forming species occurring in the other consortium accumulating acetate (no. 1, no. 2, no. 8, no. 9 and no. 11).

It has been proved that all the consortium have the ability to grow and to reduce sulphate at 9-11°C, characteristic for water-bearing stratum.

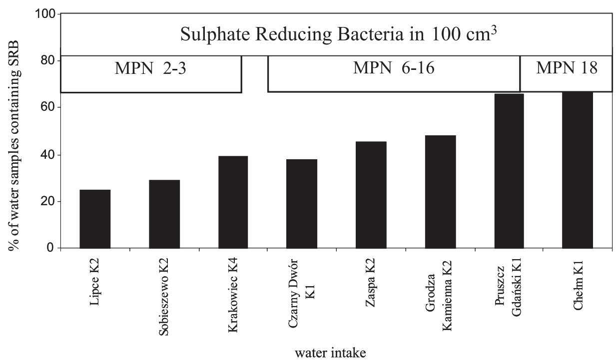

Fig. 2 Percentage share of Cretaceous water samples containing SRB; MPN of SRB.

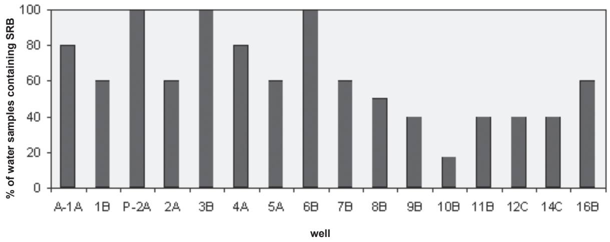

Fig. 3 Percentage share of Quaternary water samples containing SRB.

Elimination of SRB from Water

Results of investigations of SRB elimination from treated Quaternary groundwater by means of UV disinfection are presented in Fig. 4.

Effectiveness of UV doses in the range from 13 to 88 mJ/cm^2 was analyzed. It was found that doses below 40 mJ/cm^2 were ineffective in SRB elimination. In doses ranging from 40 to 75 mJ/cm^2 the effectiveness of SRB removal varied from 55 to 86%. The highest removal effectiveness (about 100%) was observed when UV doses above 77 mJ/cm^2 were applied.

Results of investigations of SRB elimination from treated Quaternary groundwater by means of membrane filtration were as follows: SRB were present in 66.7% of raw water samples and the samples of water after microfiltration (permeat) did not contain SRB.

Discussion and Conclusions

Sulphate-reducing bacteria occur in Cretaceous and Quaternary water in the region of Gdańsk. The most probable number of SRB was low and did not exceed 20 in 100 ml. Johnson and Wood [2] also proved SRB occurrence in sandy sediments in the region of London. Unpleasant sulphur hydrogen odour usually accompanied the presence of SRB in water. Formation of sulphur hydrogen in groundwater is characteristic of anaerobic environments where suitable conditions for growth of sulphate-reducing bacteria occur[14]. SRB growth in the aquifer results in negative changes of water quality – deterioration of organoleptic properties [15, 16].

Moreover, these bacteria are present in treated water after aeration and filtration processes. Owing to the fact that SRB has a negative influence on organoleptic properties of this water quality and the technical condition of distribution net, the water should be disinfected.

Lactate, acetate and higher fatty acids can be oxidized by sulphate-reducing bacteria[17]. Mineralization of fermentation products is determined by the availability of suitable electron acceptor in groundwater environment. In the case of low quantity of Mn (IV) and Fe (III) coumpounds, the most frequent acceptors of electrons are sulphates, which are used by SRB. According to Laanbroek and Pfennig [12], acetate in natural ecosystems belongs to the main substrates used by SRB bacteria. Different results were characterized in this article. SRB consortium described in the paper (isolated from ground water) preferentially used lactate rather than acetate as the main source of organic carbon and energy [11, 18]. These studies have been confirmed by results of volatile fatty acids analysis[13]. A similar observation has been made by Mudryk et al.[19].

The oxidation of organic substrates in sulphate-reducing bacteria may be complete, leading entirely to CO_2, or incomplete with acetate being the end product [5]. According to Widdel [17], sulphate-reducing bacteria, from the requirement nutrients, can be divided into two subgroups.

Table1. Maximal values of H_2S and optical density in consortiums with sodium lactate and sodium acetate as a source of carbon.

Consortium	H_2S [mg/dm^3]		optical density	
	sodium lactate	sodium acetate	sodium lactate	sodium acetate
1	92.7	0.3	0.496	0.167
2	42.5	0.8	0.277	0.083
3	88.2	1.2	0.269	0.113
4	17.5	0.7	0.291	0.110
5	38.5	0.4	0.612	0.148
6	0.6	0.4	0.260	0.147
7	1.8	0.4	0.270	0.141
8	48.9	0.8	0.591	0.160
9	64.0	0.3	0.504	0.070
10	68.0	1.3	0.199	0.118
11	65.6	1.6	0.238	0.087
12	0.3	0.9	0.475	0.127

Table 2. Volatile fatty acid concentrations in BRS consortiums incubated on medium with sodium lactate.

Consortium	Volatile fatty acid concentration [mM/dm^3]			
	acetate	propionate	butyrate	capronate
1	17.75	0.57	0.01	0.002
2	26.83	3.65	0.03	<0.001
3	15.12	0.09	0.00	0.002
4	9.17	6.76	0.22	0.003
5	8.17	0.45	0.93	0.021
6	8.83	11.24	0.85	<0.002
7	9.03	<0.01	0.87	<0.002
8	14.73	7.78	0.89	<0.002
9	11.68	0.77	0.93	<0.002
10	12.25	0.34	0.00	0.003
11	10.73	0.23	0.91	<0.002
12	0.00	0.19	0.92	<0.002

To the first group belong genera (*Desulfotomaculum* and *Desulfovibrio*) which oxidize suitable organic substrate and acetate is the final product of their activity. Voordouw [20] had obtained similar results. According to that research, *Desulfovibrio* bacteria in their metabolism use lactate as a source of energy and produce acetate.

To the second one belong genera (*Desulfobacter, Desulfococcus, Desulfosarcina, Desulfonema, Desulfobacterium*) which completely oxidize substrates, also acetate.

Most often SRB originating from groundwater environment belong to *Desulfotomaculum* (spore forming) and *Desulfovibrio* genera [12, 15, 17]. The properties of SRB consortium isolated from groundwater in the region of Gdańsk, allow us to suppose that consortiums could be involved to *Desulfovibrio* and *Desulfotomaculum* genera, considered in literature as typical for groundwater environments. To confirm that statement further investigations should be done, specially RNA analyses.

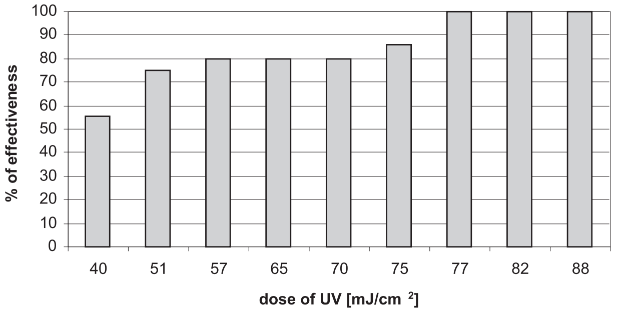

Fig. 4 Effectiveness of SRB elimination with various doses of UV radiation.

Disinfection technologies in common use have their strengths and weaknesses in treating municipal drinking water. It has been proven that SRB are present in treated water after aeration and filtration on quartz sand beds. Seeing that SRB causes deterioration of distributed water quality and has a negative effect on the water-pipe network, treated waters ought to be constantly or periodically disinfected. In the study two non-reagent methods of SRB elimination were applied: UV radiation and membrane filtration. Recently, disinfection with ultraviolet has been successfully used in municipal wastewater treatment and also has potential for drinking water applications. Its most likely application seems to be for groundwater, where water clarity and other factors are most frequently required[21]. Usage of UV technology for water treatment has several inherent advantages over most traditional technologies. UV radiation is a non-reagent technology. It only imparts energy to water stream in the form of UV radiation to accomplish the process of disinfection. It is fast, effective and environmentally friendly[22]. Dose is a function of the UV intensity reaching the organism multiplied by exposure time. Factors affecting dose include energy of the UV lamp, clarity or transmission of water flow rates and residence time in the treatment chamber. Typically, UV systems are rated at different flow rates based on fluid UV transmission to deliver doses of 30 mJ/cm^2 [23]. In the paper it has been proved that UV radiation is an effective method of SRB elimination if the UV dose is higher than 77 mJ/cm^2. This dose is much higher than the minimum recommended by the U.S. Public Health Service for disinfection of water with ultraviolet irradiation[24]. Conventional filtration has been used for many years in water treatment to remove solids by passing the water through beds of sand or other inert porous media. The usage of more costly membrane processes for finer filtration of drinking water is now becoming more attractive because of the increasingly stringent regulations[21, 25, 26]. In this research, membrane filters were used to stop particles larger than 0.08 μm (size of membrane pores 0.08 – 0.1 μm). SRB cell size is in the range of 0.5 – 2 x 0.8 – 9 μm and consequently high effectiveness of membrane filtration in SRB elimination has been indicated.

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