



**Dorota Wawrzak, Wojciech Kustal**

*Akademia im. Jana Długosza w Częstochowie*

*al. Armii Krajowej 13/15, PL42-200 Częstochowa, Poland,*

*e-mail: d.wawrzak@ajd.czest.pl*

## DAIRY WASTEWATER TREATMENT IN ANAEROBIC DISSIMILATION REDUCTION OF SULFATES

**Abstract.** The study has been devoted to testing the impact of sulfides to optimize the treatment of domestic waste, the proceeds of eco-tourism, anaerobic bacteria using desulfurization (specifically *Desulfotomaculum ruminis*). The most important and fundamental process, that has been discussed in this work is the treatment of wastewater. The main representatives of prokaryotes are bacteria of *Desulfotomaculum ruminis* genus. Bacteria induce sulfur-breathing process known as dissimilative sulfate reduction. Sulfate ions are the final acceptors of hydrogen and electrons. The end product is a hydrogen sulfide. Its occurrence is evidence of a decrease in the concentration of organic substrates- decrease of COD.

**Keywords:** sewage treatment SRB sulphur reducing bacteria, biological methods, *Desulfotomaculum ruminis*.

## OCZYSZCZANIE ŚCIEKÓW MLECZARSKICH W BEZTLENOWEJ DYSYMIŁACYJNEJ REDUKCJI SIARCZANÓW

**Streszczenie.** Celem niniejszej pracy jest zbadanie możliwości zastosowania procesu oddychania siarkowego do usunięcia zanieczyszczeń organicznych w przypadku wycieku ścieków przemysłowych, jak również w oczyszczalniach ścieków. Prezentowane wyniki są kontynuacją programu badawczego dotyczącego wykorzystania różnych rodzajów bakterii w uzdatnianiu wody ścieków przemysłowych. Ogólnym celem tego badania było sprawdzenie możliwości wykorzystania BRS (bakterii redukujących siarczany), szczególnie *Desulfotomaculum ruminis*, w procesie oczyszczania ścieków przemysłu mleczarskiego. Ten rodzaj ścieków zawiera węglowodany, głównie laktozę (30,9%), białka (23,6%, w tym 80% kazeiny) oraz tłuszcze (41,4%) pochodzące z mleka i jego przetworów. Przeprowadzone badania dotyczyły redukcji

siarczanów w ściekach przemysłu mleczarskiego za pomocą szczepu bakterii *Desulfotomaculum ruminis*. Proces oddychania siarkowego prowadzono w warunkach laboratoryjnych za pomocą BRS po namnożeniu kultury bakteryjnej na podłożu Starkeya wzbogaconym ściekami mleczarskimi stanowiącymi jedyne źródła węgla i energii. Prowadzone badania oceniają wpływ bakterii na zmniejszenie zawartości siarki w zależności od stężenia ścieków. Badano zmiany ChZT, poziom zawartości siarczków, zmiany pH w badanych próbkach. Porównywane próbki zawierały 2%, 5%, 7.5% i 10% ścieków mleczarskich. Wyniki wykazały, że konieczne jest znalezienie optymalnego stosunku zawartości ścieków w zawieszynie poddanej działaniu BRS, jak również prowadzenie procesu w optymalnym czasie, w celu uzyskania maksymalnych wyników.

**Słowa kluczowe:** oczyszczanie ścieków, BRS – bakterie redukujące siarkę, metody biologiczne, *Desulfotomaculum ruminis*.

## Introduction

Sulfur as a typical representative of the chalcogenides and is a widespread element in nature. This may occur both in free forms (known. Native sulfur) and in many organic and inorganic compounds. It is present in minerals and their deposits, sedimentary and igneous in coal, crude oil, soil, water and atmosphere, as well as in all living organisms. Under natural conditions, sulfur is not very reactive. May be associated with certain halogen (especially chlorine and fluorine) and certain metals (copper, silver), reacts with them at room temperature. At increased temperatures, the sulfur is more reactive and can be connected to almost all elements except for nitrogen platinum, gold, and noble gases. Sulfur compounds, widely distributed, are playing a special role in nature. There are hydrogen sulfide ( $H_2S$ ) and derivatives thereof, sulfur dioxide ( $SO_2$ ), sulfur trioxide ( $SO_3$ ), sulfuric acid ( $H_2SO_3$ ) ( $H_2SO_4$ ), and organic sulfur compounds [1]. Sulfur is known to be a group of elements that are involved in a number of reversible chemical reactions. The course of these reactions, as well as the result of the final products is closely linked to environmental conditions.. Sulfur is an essential element of life in its transformation involving all living organisms. The largest amounts are processed by micro-organisms, and a relatively small plants and animals [1]. Living environment of sulfate reducing bacteria are strictly anaerobic conditions. Oxygen inhibit the process of dissimilative sulfate reduction, because the presence of BRS in such conditions is excluded. They occupy a habitat rich in sulphates. Therefore, there are numerous estuaries, such as on anaerobic soil areas, wetlands and coastal and marine sediments. Extremely rich in sulfates habitat is the water of the oceans. Their concentration significantly exceeds that found in freshwater sediments [2, 3]. Because BRS may use other electron acceptors than sulfates, they can also colonize other habitats such as the human gastrointestinal tract. It has been found that in nearly 82% of the human population BRS present in the oral cavity, however, were the microorganisms belonging to the genera

*Desulfovibrio*. Sulfate-reducing bacteria are the most numerous in habitats where they have unlimited resources sulphate [2, 4]. Occurrence of BRS in diverse environments, is the result of the ability of these microorganisms to the use of a variety of organic compounds as carbon sources. They can oxidize lactate, pyruvate, malate, acetate, ethanol, fumarate or succinate. The presence of these compounds in the culture medium resulted in a rapid growth of bacteria, and colonies are apparent after 2-3 days. If the medium is present propionate, butyrate, valerate, methanol, glutamate, glycine, et al. BRS growth takes longer and takes about seven days. If the medium is glucose, fructose, starch, phenol, benzene, p-cresol, indole bacterial growth was not observed even after 14 days of incubation [4,5,6]. Remediation of industrial wastewater using microbiological methods, namely: bacteria, is nowadays part of the technology used in waste water treatment plant. There are many species of bacteria in a wastewater treatment plant. In a recent study, over 300 species were identified in an aeration basin. However, they can all be categorized by the way that they obtain oxygen as aerobic, anaerobic or facultative. Aerobic bacteria are used in most new treatment plants in an aerated environment. Anaerobic bacteria are normally used in an anaerobic digester to reduce the volume of sludge to be disposed of and to produce methane gas. This process is completed in anaerobic conditions, without any dissolved oxygen in the water. The anaerobic bacteria normally get the oxygen needed for their respiration from their food source and the process is also called fermentation. Another use of anaerobic bacteria is in the biological removal of phosphorus and sulphur. During this process, a part of the aeration section of the treatment plant may be made into an anaerobic zone to facilitate the growth of phosphorus and sulphur accumulating organisms, which in turn lowers the amount of phosphorus as well as sulphur in the effluent. Facultative bacteria are able to change their mode of respiration from aerobic to anaerobic and back again. These bacteria are able to adapt to either condition, although they prefer the aerobic condition. The aim of this study is to investigate the possibility of applying sulphate respiration process to remove sulphur from wastewater being a result of eco-tourism, namely from domestic wastewater in rural areas.

## Materials and method

Research programme covered by this study is a part of a wider project covering use of different types of bacteria in water treatment of domestic wastewater [7]. The general purpose of this study was to check the possibility of using SRB for the process of eco-tourism wastewater purification.

The study was focused on evaluation of the catabolic activity and dynamics of growth of SRB culture in the process of COD reduction, accompanied by reduction of sulphates to sulphides, taking place in a modified Starkey medium con-

taining dairy industry wastewater as the only source of energy needed for the bacteria metabolism. Sulphur reducing bacteria (SRB) used in the study were isolated from the marshy soil from the vicinity of Poznan city and identified as *Desulfotomaculum ruminis* [9]. The isolated culture of these bacteria was stored and grown on liquid Starkey medium [10] containing [g/dm<sup>3</sup>]:

MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.00
Na <sub>2</sub> SO <sub>4</sub>	2.42
NH <sub>4</sub> Cl	1.00
K <sub>2</sub> HPO <sub>4</sub>	5.00
CaCl <sub>2</sub> ·6H <sub>2</sub> O	0.25
FeSO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ·6H <sub>2</sub> O	0.50

The media studied were domestic wastewater from the Krzepice Water Treatment Plant [11]. Laboratory equipment as well as bed were sterilized in 120°C. Sulphides were indicated in precipitated cadmium sulphide – iodometric method [12]. Kinetic studies were carried out at 37°C, pH =7.0 - 7.5 in anaerobic conditions (helium) in tightly closed reactors of 50cm<sup>3</sup> capacity, filled with the modified Starkey medium without lactate and the wastewater which was the only source of carbon and energy for SRB. The amounts of the wastewater are specified in the results section. After blowing helium to ensure anaerobic conditions, the samples were inoculated with a 4% inoculum collected from the culture in the phase of logarithmic growth (after 24 hours). The wastewater samples of pH close to 6.5, were stored in a refrigerator. Reference (blank) sample was conducted on Starkey medium with lactate. The samples to be used in experiments were heated to room temperature. Prior to the study they also had to increase their pH to about 7.0, which was made by adding a diluted NaOH solution. The rate of the microbiological process of sulphate decomposition was evaluated from the degree of SO<sub>4</sub><sup>2-</sup> reduction to S<sup>2-</sup> and the rate of reduction in chemical oxygen demand, measured at certain time intervals. To make the measurements the reactors were blown with helium and the blown out H<sub>2</sub>S was absorbed in washer containing 0.02 mol/dm<sup>3</sup> solution of cadmium acetate. The sulphides precipitated were quantified by the iodometric method [11]. The effectiveness of desulphurisation (reduction in COD - indicator of organic matter content) was measured by the amount of oxygen consumed in the reactions upon heating the sample with an oxidising reagent (potassium dichromate) according to the method described in [12].

## Results and discussion

The kinetic curve of dissimilative sulphate reduction in the standard Starkey medium has a specific shape corresponding to the three typical phases of microorganisms growth, i.e. to the induction growth lasting for the bacteria studied for about 15 hours, the phase of growth - disturbed by a temporary decrease in the rate of transformation, and the phase of equilibrium and stabilisation. Alkaline reaction increased pH of samples as a result of bacteria activities from 6.8 – 7.2 to 7.5 – 8. The higher intake of sewage the higher pH and more alkaline reaction (Fig. 1.).

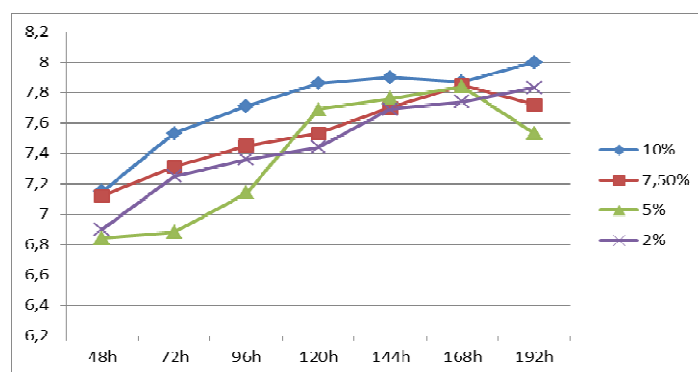


Fig 1. Changes of pH in the Starkey medium supplemented with 2%, 5%, 7.5% and 10% content of wastewater

COD (Chemical Oxygen Demand) was also examined, being an indicator determining the content of organic substance, with amount of oxygen consumed in reactions while warming up the sample with oxidizing reagent. Potassium dichromate was used as oxidant applied in the acid environment in the presence of ions of silver acting as a catalyst and mercury sulphate masking influence of ions of chlorine.

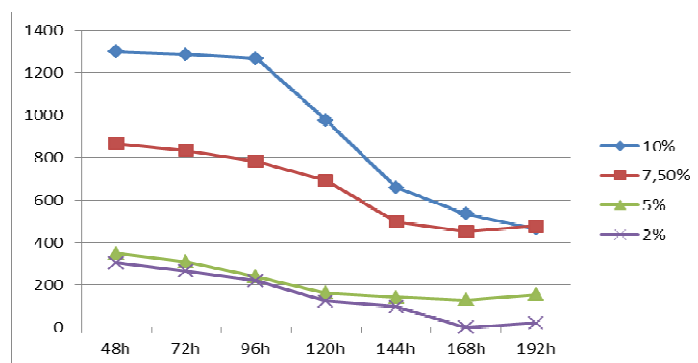


Fig 2. Changes of COD in the Starkey medium supplemented with 2%, 5%, 7.5% and 10% content of wastewater

The process of changing sulphates into sulphides is accompanied by a reduction in the content of organic pollutants measured by COD. Measurement of COD was done comparing results of modified medium with 2%, 5%, 7,5% and 10% content of wastewater. Decrease of COD depends on wastewater content. The higher level of wastewater the lower decrease of COD rate (Fig. 2.).

The process is completed in about 192h hours and after the concentration of sulphides studied in the Starkey medium is 100 mg of sulphides in 1 litre. The kinetic curves characterising microbiological reduction of sulphates in the presence of these samples showed roughly the same diversity obtaining the same level averagely of around 1400 (Fig.3.). The amount of sulphides obtained as a result of microbiological reduction of sulphates after 8 days in the medium containing 2%, 5%, 7,5% and 10% of wastewater from "Krzepice" is close to 1400 mg/dm<sup>3</sup>, while at the initiation stage the sulphates content was diverse between 800 to 1200 mg/dm<sup>3</sup>.

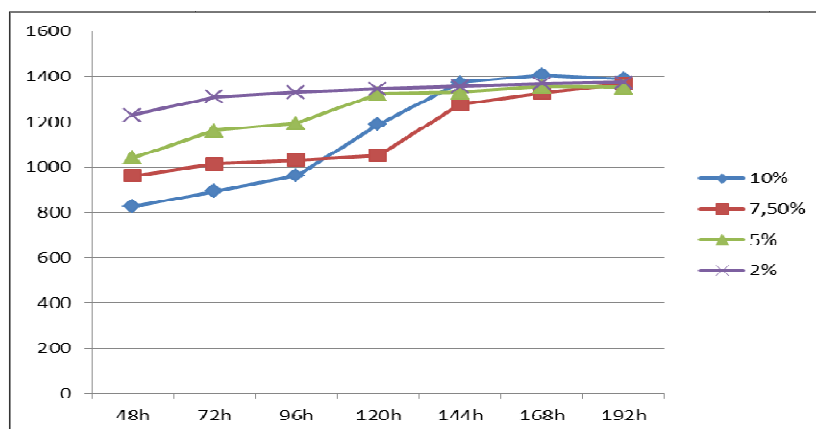


Fig 3. Changes of FeS in the Starkey medium supplemented with 2%, 5%, 7,5% and 10% content of wastewater

In the anaerobic environment sulphates are reduced to sulphides, with secreting hydrogen sulphide as incidental compound. This reduction is possible as a result of sulphate bacteria. Inoculation of *Desulfotomaculum ruminis* into inspected samples resulted in considerable decrease of sulphides in the sewage. Simultaneously bacteria are contributing to the rise in the number amount of simpler post reduction substances.

Large numbers of the desulphurisation bacteria breed considerably during the first three days. It proves that minimal volumes of oxygen are needed for oxidizing organic compounds contained in slurry. COD value was diminishing with time. It is a result of consuming oxygen acquired from oxidant, for oxidizing organic compounds. The pollutants present in the wastewater were proved to

be nontoxic to the sulphur reducing bacteria and did not inhibit their growth. The bacteria strain tested can be used for the removal of soluble mineral (metal ions) and organic pollutants.

## **Conclusions**

The results of research conducted using effluent from Krzepice Sewage Treatment Plant indicated that a particular concentration of SRB determine their activity by measuring content of sulphides in the test samples.

The longer time the higher sulphides content in examined samples. Increased level of sulphides reduced from sulphates showed impact of BRS. In all samples this content is growing at the different pace. Samples with the highest content of slurry are achieving large amounts of sulphides, in this case FeS.

In the eight day of the research process there was the highest number of BRS in each of the tested samples. Due to the increased amount of sulfate reducing bacteria (SRB) occurs significant reduction of organic substances contained in wastewater which determines good purification.

The use of a dissimilation process in sewage treatment plant to sulfate reduction is beneficial for the oxidation of organic substances contained in wastewater.

The pH value increased in time. During the first three days the pH value was maintained at a similar level, while in seventh day its value was roughly the same in brackets between 7.7 to 7.9. During the study, these values were dropped down in eight day by 0.1 but only in two samples with 7.5 and 5% of wastewater. Intake.

Research findings proved that the content of sulphides, with the specific concentration in samples, determines the activity of bacteria reducing sulphates. The volume of BRS bacteria grew in time and the highest number of bacteria was the eight day after inoculation in all inspected samples. As a result of the increased number of bacteria reducing sulphates a considerable reduction in organic substances contained by sewage is taking place what decides about good cleaning.

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