



# Microbiological Reduction of Sulfates to Sulphides Used in Dairy Wastewater Treatment

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## Summary

The study presents comparison of effectiveness in using microbial desulphurification of dairy industry. Comparative study covers two factors describing performance and effectiveness depending on medium and wastewater content: COD and sulphites. Authoress proves that usage of SRB can be successfully exploited in significant reduction of sulphates and their immediate change into sulphites, which can be separated from the wastewater in form of sluge.

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

## Introduction

The aim of this study is to investigate the possibility of applying sulphate respiration process to remove organic impurities in case of wastewater from the dairy industry with sulfates content decomposition to determine the effectiveness of the cleaning process using bacteria *Desulfotomaculum ruminis*. Microbial sulfate reduction (sulphur breathing), which is a part of the natural cycle of sulphur in nature, plays an increasingly important role in environmental protection methods. Removal of sulphates by chemical treatment is very costly, and therefore the biological method of sulphate reduction in wastewater and sludge can be very effective and economically viable. Microorganisms responsible for the implementation and exploitation of the sulphate respiration are sulfate-reducing bacteria (SRB), commonly found in sediments and groundwater, especially in the vicinity of organic pollutants. Their effect on the ecosystem is limited to anaerobic conditions, the activity of these microorganisms conditioned supply of organic substrates and sulphates depends mainly on the quantitative relationship between organic contaminants (e.g., COD content) are prone to degradation in the process of metabolism, and sulphates (or sulfites, tiosulphites) reduced to hydrogen sulphide or sulphide. Due to these properties, these bacteria are used in wastewater and sludge treatment, industrial waste treatment, bioremediation of soil, and in other activities used in the environmental protection of the environment.

## Bioremediation of dairy industry wastewater

Metabolism is the process of enzymatic conversion of low-value substrates into more valuable products. Thanks biotransformation we are able to get organic products found in plants in small amounts, and synthesize unknown substances. Biotransformation processes do not provide energy, nor precursors

for the cellular synthesis and proceed with the participation of a limited number of enzymes (most often changes are single enzyme). This process accompany normal metabolic activity of cells, those growing and fixed as well as resting forms of microorganisms. This is possible, due to the many enzymes low selectiveness towards substrate individual nature (specificity), that enzymes are involved and active not only in the transformation of their respective metabolic substrates, but also in a structurally similar compounds (such as xenobiotics), which do not occur in normal or regular cellular metabolism [1]. Microbiological reduction of sulphates has been thoroughly studied [2, 3, 4, 5] and applied in technologies of natural environment protection and restoration, in cleaning of environments polluted with organic compounds, sulphates and soluble heavy metals [2, 6]. Within the process of microbiological reduction of sulphates SRB (Sulphur Reducing Bacteria) use sulphates as the final acceptors of electrons in the breathing chain. SRB gain energy from oxidation of organic compounds, for instance lactates and simple organic compounds contained in wastewater of post-wastewater sediments [7, 5].

Dairy sewage induce deficit in oxygen content of receiving ponds as a result of high BOT<sub>5</sub>. This leads to fast concrement expansion of *Filamentous bacteriae* and *Sphaerotilusnatans*. Prompt decay processes in bottom sediments cause exhaust of reek gases, which contaminate atmospheric air in surroundings. Influence of diary sewage on receiving ponds is seen in: sedimentation of slurry, fungi expansion, strong oxygen absorption, distribution of hydrogen sulphide and others accompanying undesirable and harmful phenomena [9]. Bedrock aim of the biological sewage treatment is removal from the sewage all biologically decomposed pollutants. Decomposition of organic pollutants comes from natural colonies of micro-

organisms suspended in fluid, which initiate and carry out the process naturally (method of active sediment) or microorganism creating harden biomass (trickling filter method). Organic pollutants are exploited by microorganisms due to biochemical changes as pasture inducing increase of bacterial biomass. Remaining part of decomposed pollutants are discharged in oxygen conditions as carbon dioxide and water. In case of anaerobic processes gaseous products of organic mass decomposition are methane and carbon dioxide [7, 8]. Sewage treatment, in which active sediment was applied, is based upon creation in fluid flocks with 50–100 mm diameter and extremely well developed surface. Flocks are built of mineral, brown, grainy nucleus placed in mucosal envelope and consisting of numerous heterofites bacteria species such as *Acinetobacterium*, *Pseudomonas*, *Zoogloea*, *Enterobacteriae*, *Aeromonas*, *Flavobacterium*, *Achromobacter* and *Micrococcus*. Metabolism of microorganism results in mineralization of organic pollutants and absorption on outer surface of flocks. Active sediment method need to deliver oxygen as bio oxidation substrate of organic impurities [9]. Advantage of sewage treatment using active sediment method is high effectiveness of disposal sedimentation up to 95% and liquidation of pathogenic microbes up to 98% with relatively low demand for oxygen (BOT<sub>5</sub>). In turn disadvantage is high vulnerability of microorganisms to toxic complexes and other factors affecting their expansion. High performance of active sediment method in brewing industry is proved by decreasing BOT<sub>5</sub> between 85–95% [10].

## 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 industrial wastewater [11]. The sulphate general purpose of this study was to check the possibility of using SRB for the process of dairy industry wastewater purification. This type of wastewater contain mainly carbohydrates, mainly lactose (30.9%), proteins (23.6%, including 80% of casein) and fats (41.4%) coming from milk and its products [10, 11].

The main aim of the study was to evaluate 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 containing 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* [12]. The isolated culture of these bacteria was stored and

grown on liquid Starkey medium [13] 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 only source of carbon and energy was 10.16 cm<sup>3</sup> of sodium lactate. The medium also contained microelements in the following amounts [g/cm<sup>3</sup>]:

MnSO <sub>4</sub> ·4H <sub>2</sub> O	6.2·10 <sup>-4</sup>
CuSO <sub>4</sub> ·5H <sub>2</sub> O	2.4·10 <sup>-4</sup>
ZnNO <sub>3</sub> ·6H <sub>2</sub> O	2.0·10 <sup>-5</sup>
(NH <sub>4</sub> ) <sub>2</sub> MoO <sub>4</sub>	2.0·10 <sup>-5</sup>
NaHSeO <sub>3</sub>	2·10 <sup>-11</sup>
H <sub>3</sub> BO <sub>3</sub>	1.7·10 <sup>-4</sup>

The media studied were industrial wastewater from the Regional Dairy Cooperatives in Krzepice and Radomsko, containing organic and inorganic pollutants including sulphates and metals [11]. Laboratory equipment as well as bed were sterilized in 120°C. Sulphides were indicated in precipitated cadmium sulphide – iodometric method [12] local literature number.

Kinetic studies were carried out at 37°C, pH = 7.0–7.5 in anaerobic conditions (helium) in tightly closed reactors of 50 cm<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 [17].

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 [13].

## 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 logarithmic growth – disturbed by a temporary decrease in the rate of transformation, and the phase of equilibrium and stabilisation.

The process is completed in about 240 hours and after the concentration of sulphides studied in the

Starkey medium is 100 mg of sulphides in 1 litre. Simultaneously series of measurements were made for the wastewater from the water treatment plant “Radomsko”. The kinetic curves characterising microbiological reduction of sulphates in the presence of these samples showed roughly the same diversity, (Fig.2). The amount of sulphides obtained as a result of microbiological reduction of sulphates after 8 days in the medium containing 70% of wastewater from “Radomsko” is close to 800 mg/dm<sup>3</sup>, while in the medium containing 50% and 30% of this wastewater is near 700 mg S per litre. For the samples from “Radomsko” the influence of concentration of the wastewater sample on the course of sulphate breathing is almost the same.

The longer time the higher sulphites content in examined samples. Increased level of sulphites re-

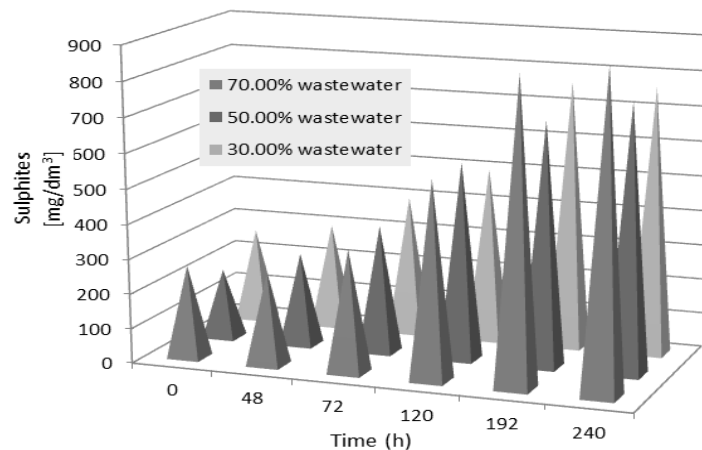


Fig. 1. The influence of the initial content of wastewater from “Krzepice” in modified Starkey medium on the effectiveness of microbiological reduction of sulphates to sulphites

Rys. 1. Wpływ zawartości początkowej ścieków z Krzepic w zmodyfikowanym czynniku Starkey’ a na efektywność redukcji mikrobiologicznej siarczanów do siarczynów

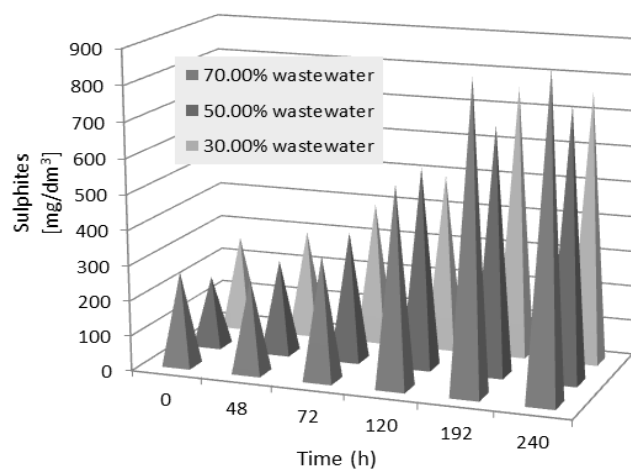


Fig. 2. The influence of the initial content of wastewater from “Radomsko” in modified Starkey medium on the effectiveness of microbiological reduction of sulphates to sulphites

Rys. 2. Wpływ zawartości początkowej ścieków z Radomska w zmodyfikowanym czynniku Starkey’ a na efektywność redukcji mikrobiologicznej siarczanów do siarczynów

duced from sulphates showed impact of BRS. In all samples this content is growing at the similar pace. Samples with the lowest content of slurry are achieving large amounts of sulphites. In order to maintain continuity of the process, the wastewater should be supplemented with additional portions of sulphates to keep the best proportion between the content of sulphates and the level of wastewater pollutants.

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.

The process of changing sulphates into sulphites is accompanied by a reduction in the content of organic pollutants measured by COD. There were comparative study carried out between two above mentioned dairies wastewater. Measurement of COD was done comparing results of modified medium with 1%, 5% and 10% content of wastewater. Decrease of COD depends on wastewater content. The higher level of wastewater in the lower decrease of COD rate.

In “Krzepice“ case COD was reduced by about 60 mg O<sub>2</sub>/dm<sup>3</sup> in the media containing 5% or 10% of

the wastewater, while in the medium containing 1% - by about 40 mg/dm<sup>3</sup>. The reduction in COD for the medium containing 10% wastewater after 11 days of reaction was 44 mg O<sub>2</sub>/dm<sup>3</sup>, so COD decreased from 98 mg O<sub>2</sub>/dm<sup>3</sup> to 54 mg O<sub>2</sub>/dm<sup>3</sup> in (Fig.3).

The linear ratio of the decrease in COD for samples of different initial content of the wastewater was the same in compared samples. For the sample containing initially 70% of the wastewater, besides the linear dependence, an increase in the content of sulphides coming from microbiological decomposition of sulphates can be noted at a temporary stabilisation of COD whose reduction was inhibited at COD for “Krzepice” between 31–45 mg O<sub>2</sub>/dm<sup>3</sup> and for “Radomsko” between 25–54 mg O<sub>2</sub>/dm<sup>3</sup> (Fig 3 and 4).

Examination of COD intensity showed its decrease in time. The higher concentration of sewage the later drop is occurring, but is most visible and evident. Final COD levels are considerably lower than initial and in all samples stay at the similar level.

*Desulfotomaculum ruminis* bacteria strain was used for conducting examinations on the fluent standard Starkey's medium in glass reactors, providing anaerobic conditions for growth. Kinetic examinations were being conducted on bacteria in their logarithmic phase of growth making transplants of every 48 hours. Samples, containing the Starkey's medium and different content of dairy sewage, were inoculated with BRS bacteria in the most intense

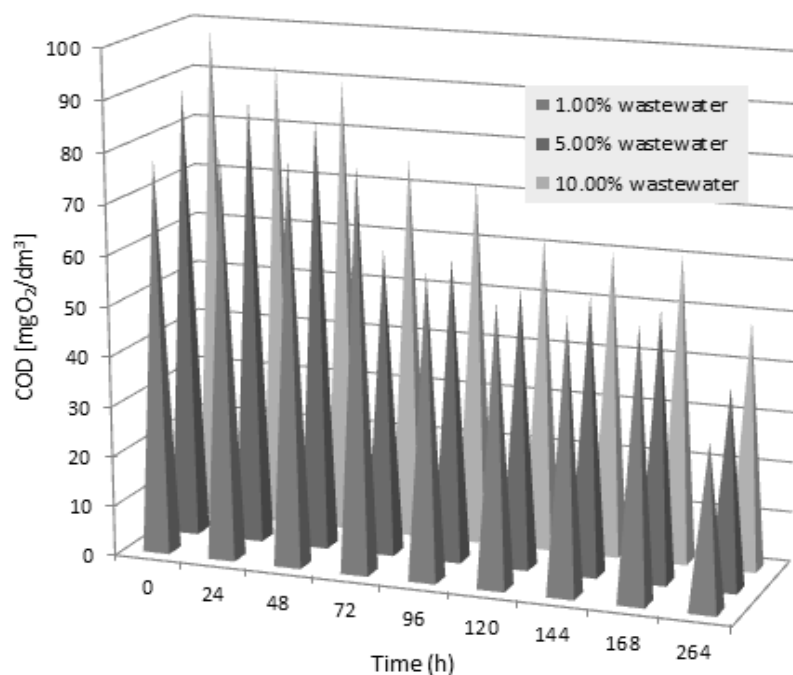


Fig. 3. The influence of the initial content of wastewater from “Krzepice” in modified Starkey medium on the reduction in COD

Rys. 3. Wpływ zawartości początkowej ścieków z Krzepic w zmodyfikowanym czynniku Starkey’a na redukcję w COD

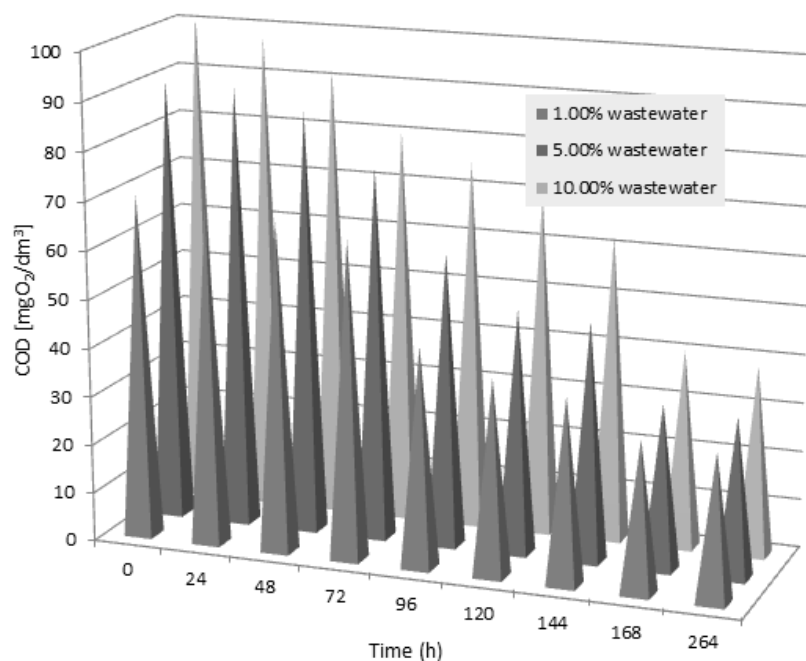


Fig. 4. The influence of the initial content of the wastewater from “Radomsko” in modified Starkey medium on microbiological reduction in COD

Rys. 4. Wpływ zawartości początkowej ścieków z Radomska w zmodyfikowanym czynniku Starkey’ a na mikrobiologiczną redukcję w COD

phase of growth and degasified with helium. Temporal appearance of FeS traces were observed on the bottom of reactors already after 24 hours from the moment of inoculation.

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 fifth 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. Application of sulphate dissimilative reduction in the process of sewage treatment plant is beneficial for oxidizing organic substance included in sewage.

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 desulphurization 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.

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### **Mikrobiologiczna redukcja siarczanów do siarczynów w oczyszczalni ścieków w mleczarni**

Badanie przedstawia porównanie efektywności w użyciu mikrobiologicznego odsiarczania w przemyśle mleczarskim. Badanie porównawcze obejmuje dwa aspekty opisujące wydajność i skuteczność w zależności od zawartości czynnika i ścieków: COD (chemical oxygen demand – chemiczne zapotrzebowanie tlenu) i siarczyny. Autor udowadnia, że użycie SRB może być pomyślnie wykorzystywane w znaczącej redukcji siarczanów i ich natychmiastową przemianę na siarczyny, które z kolei mogą być oddzielone od ścieków w formie szlamu.

Słowa kluczowe: bakterie redukujące siarkę (SRB), metody biologiczne, *Desulfotomaculum ruminis*, przeróbka osadów ściekowych, przemysł spożywczy