Microbiological Decomposition of Sulphates in Reduction of COD in Diary Industry Wastewater

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Summary

One of the most heavily laden pollutants is dairy wastewater with excessive volume of sulphates. Biological methods use sulphate-reducing bacteria Desulfotomaculum ruminis in dairy wastewater treatment. They carry out dissimilatory reduction of sulphates, which uses sulphate ions (IV and VI) as the final acceptors of electrons and hydrogen. Laboratory studies have shown that sulfides (mostly FeS) precipitated during dissimilatory reduction having deterrent effect on the decrease of ions SO_4^{2-} to S2- when concentration of Fe is over 400 mg/dm³. Diary wastewater treatment process with the participation of SRB results in secretion of hydrogen sulfide and a decreasing COD, i.e. reducing the concentration of organic substrates.

Keywords: Sulphate-reducing bacteria (SRB), Desulfotomaculum ruminis, dissimilatory sulfate reduction, diary wastewater treatment

Introduction

Microbiological reduction of sulphates (sulphate breathing) has been increasingly often used in biotechnological processes [1, 2, 3]. It involves the use of sulphate-reducing bacteria (SRB) whose activity is particularly attractive in the processes of heavy metal removal from wastewater and underground water, in bioremediation of soil [4], utilisation of wastewaters [5, 6, 7, 8] and in biodegradation of organic compounds especially in systems of highly charged wastewaters, where aerobic processes are preceded by those of anaerobic biodegradation [9, 10]. Particular biotechnologies are adapted to specific needs and conditions, e.g. removal of metal ions from wastewater in which they occur in low concentrations [11] or reduction in chemical oxygen demand COD in municipal wastewater [8]. Bioprocesses of this type are promoted because of low cost, easy control, nontoxic wastewater, and first of because they are economically and ecologically beneficial [12, 13].

Microbiological reduction of sulphates has been thoroughly studied [4, 14, 15, 16] and applied in technologies of natural environment protection and restoration, in cleaning of environments polluted with organic compounds, sulphates and soluble heavy metals [4, 11]. In 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 [1, 16]. In general the process can be described as:

$$(C, O, N, P, H) + FeSO_4\ H_2O \text{ --}^{BRS}\text{-- } FeS + COO^- + CO_2 \quad (1)$$

In these processes, bio-treatment of wastewater reduces the level of organic compounds content (COD) and simultaneously removes heavy metal ions in the form of nontoxic insoluble sulphides which are easy to separate and allow recovery of metals [1]. Usually in technologies of treatment of wastewater heavily charged with organic compounds, aerobic processes are preceded by anaerobic ones [18] in which the activity of SRB, occurring in bottom sediments and underground water, especially in the regions polluted with organic compounds [19], is determined by the supply of organic substances and sulphates. In the case of mixed bacteria cultures with domination of SRB, competing e.g. with methane bacteria, it is determined by the ratio of the initial concentrations of COD to $[SO_4^{2-}]$[18]. The process of sulphate breathing will not proceed if the environment conditions such as temperature, pH, salinity or organic compounds content, do not meet the demands of SRB, so the use of these bacteria must be preceded by checking the possibility of their application in each specific case.

The general purpose of this study is to check the possibility of using SRB for the process of dairy industry wastewater purification. This type of wastewater contain mainly carbohydrates, dominated by lactose (30.9%), proteins (23.6%, including 80% of casein) and fats (41.4%) coming from milk and its products [17]. 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 which take place in a modified Starkey medium containing dairy industry wastewater as the only source of energy needed for the bacteria metabolism.

Materials and methods

The sulphate reducing bacteria (SRB) used in the study were isolated from the marshy soil from the vicinity of Poznan city and identified as *Desulfotomaculum ruminis* [18]. The isolated culture of these bacteria was stored and grown on liquid Starkey medium [18] containing [g/dm³]:

$MgSO_4 \cdot 7H_2O$ – 2.00; Na2SO4 - 2.42; NH_4Cl - 1.00;
K_2HPO_4 - 5.00; $CaCl_2 \cdot 6H_2O$ - 0.25;
$FeSO_4(NH_4)2SO_4 \cdot 6H_2O$ - 0.50.

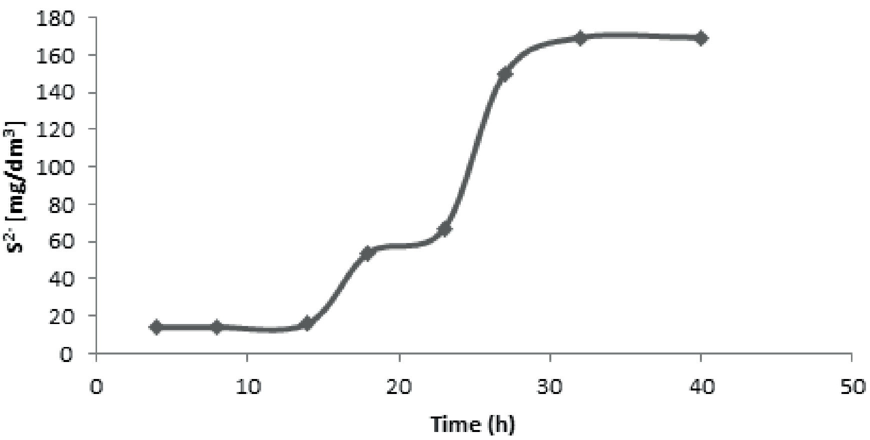

Fig. 1 Changes in the rate of SO_4^{2-} reduction to S^{2-} taking place in the process of microbiological reduction in a standard Starkey medium, (37°C, *Desulfotomaculum ruminis*)

Rys. 1 Stopień redukcji SO_4^{2-} do S^{2-} zachodzącej podczas mikrobiologicznej redukcji zachodzącej w pożywce standardowej Starkey'a (temp. 37°C, *Desulfotomaculum ruminis*)

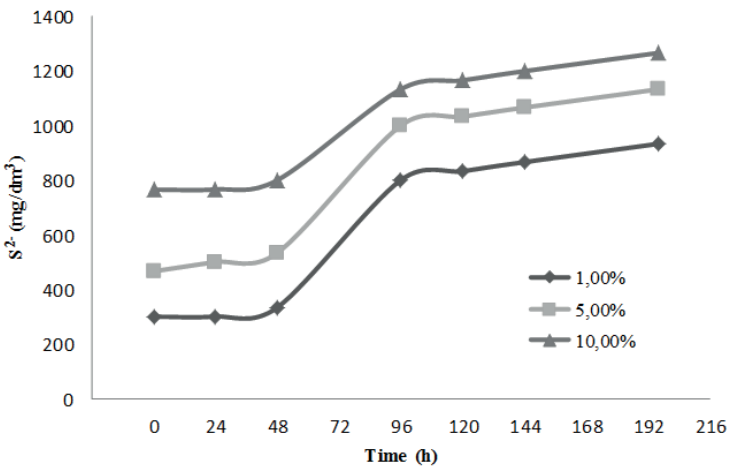

Fig. 2 The influence of the initial content of wastewater from "Krzepice" in modified Starkey medium on the effectiveness of microbiological reduction of sulphates to sulphides

Rys. 2 Wpływ początkowej zawartości ścieków z Krzepic w zmodyfikowanej średniej Starkey'a na efektywność redukcji mikrobiologicznej siarczanów do siarczków

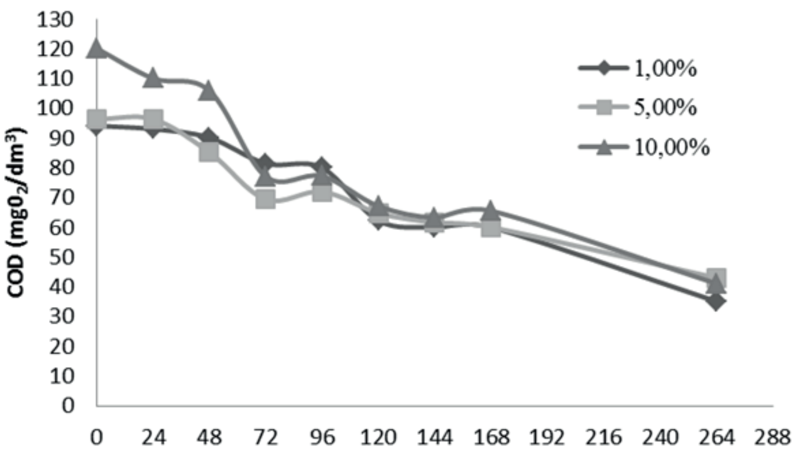

Fig. 3 The influence of the initial content of wastewater from "Krzepice" in modified Starkey medium on the reduction in COD

Rys. 3 Wpływ początkowej zawartości ścieków z Krzepic w zmodyfikowanej średniej Starkey'a na redukcję w COD

The only source of carbon and energy was 10.16 cm^3 of sodium lactate. The medium also contained microelements in the following amounts [g/cm^3]:

$MnSO_4 \cdot 4H_2O$ - 6.2·10-4; $CuSO_4 \cdot 5H_2O$ - 2.4·10-4; $ZnNO_3 \cdot 6H_2O$ - 2.0·10-5; $(NH_4)_2MoO_4$ - 2.0·10-5; $NaHSeO_3$ - 2·10-11; H_3BO_3 - 1.7·10-4.

The media studied were industrial wastewater the Regional Diary Cooperatives in Krzepice and Radomsko, containing organic and inorganic pollutants including sulphates and metals [17]. Kinetic studies were carried out at 37°C, pH=7.0 - 7.5 in anaerobic conditions (helium) in tightly closed reactors of 50cm^3 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. 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_4^{2-} reduction to S^{2-} 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_2S was absorbed in washer containing 0.02 mole/dm^3 solution of cadmium acetate. The sulphides precipitated were quantified by the iodometric method [20]. The effectiveness of desulphurisation (reduction in COD - indicator

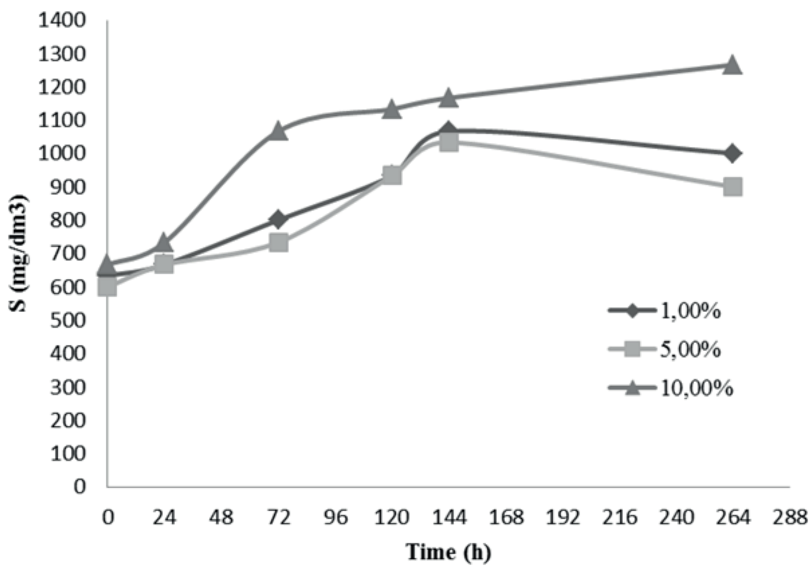

Fig. 4 The influence of the initial content of wastewater from "Radomsko" in modified Starkey medium on the effectiveness of microbiological reduction of sulphates to sulphides

Rys. 4 Wpływ początkowej zawartości ścieków z Radomska w zmodyfikowanej średniej Starkey'a na efektywność redukcji mikrobiologicznej siarczanów do siarczków

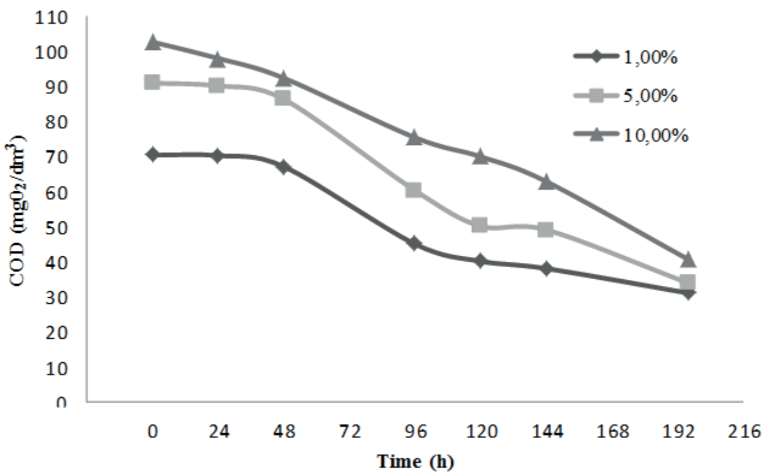

Fig. 5 The influence of the initial content of the wastewater from "Radomsko" in modified Starkey medium on microbiological reduction in COD

Rys. 5 Wpływ początkowej zawartości ścieków z Radomska w zmodyfikowanej średniej Starkey'a na redukcję w COD 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 [21].

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 (Fig. 1).

The process is completed in about 30 hours and after the concentration of sulphides studied in the Starkey medium is 100 mg of sulphides in 1 litre (Fig. 1). In the modified Starkey media lactate has been replaced by a given wastewater sample from the water treatment plant "Krzepice" in the amounts of 1%, 5% and 10%. After 6 days of reaction the greatest amount of sulphates was reduced in the medium containing 10% of the wastewater (Fig. 2). For instance, after 11 days of reaction, in the medium containing 10% of the wastewater the presence of about 1200 mg of sulphides formed as a result of microbiological reduction of sulphates, was found. This result indicates that in these conditions the molar ratio of organic carbon introduced with the wastewater to the amount of reduced sulphates was the optimum.

The decrease COD in the medium containing 10% wastewater after 11 days of reaction attained 85 mg O_2/dm^3, and the COD values decreased from 120 mg O_2/dm^3 to 35 mg O_2/dm^3.

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 greater diversity (Fig. 4).

The amount of sulphides obtained as a result of microbiological reduction of sulphates after 8 days in the medium containing 1% or 5% of wastewater from "Radomsko" are lower than those of "Krzepice", however kept in the same range and are close to 700 mg/dm^3 and 1000 mg/dm^3 respectively, while in the medium containing 10% of this wastewater it is 1200 mg/dm^3.

The reduction in COD observed for this series of experiments is illustrated in Fig. 5. In the media containing 5% or 10% of the wastewater, COD was reduced by about 60 mg O_2/dm^3, while in the medium containing 1% of the wastewater - by about 40 mg/dm^3.

According to the above presented results, in the modified Starkey medium in which lactate has been replaced by diary industry wastewater, acting as the only source of carbon and energy for SRB, after about 10 days the nontoxic sulphides are obtained as a result of microbiological decomposition of sulphates in the amounts from 400 mg S^{2-}/dm^3 to 700 mg S^{2-}/dm^3, in the form of precipitate. The above process is accompanied by a reduction in the content of organic pollutants measured by COD (chemical oxygen demand) which in the optimum conditions was 1000 mg O_2/dm^3. 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. 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.

Acknowledgments
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Zastosowanie mikrobiologicznego rozkładu siarczanów w procesie redukcji ChZT w ściekach przemysłu mleczarskiego

Jednym z zanieczyszczeń najbardziej obciążających środowisko naturalne są ścieki mleczarskie z nadmierną ilością siarczanów. Metody biologiczne pozwalają użyć bakterii redukujących siarczany szczepu Desulfotomaculum ruminis *w oczyszczaniu ścieków mleczarskich. Baterie te dokonują dysymilacyjnej redukcji siarczanów, podczas której wykorzystują jony siarczanowe (IV i VI) jako ostateczne akceptory elektronów i wodoru. Badania laboratoryjne wykazały, że siarczki (głównie FeS) wytrącane podczas dysymilacyjnej redukcji ograniczają zmniejszenie jonów* SO_4^{2-} *do* S^{2-}*, gdy stężenie Fe wynosi ponad 400 mg/dm*^{3}*. Proces oczyszczania ścieków mleczarskich z użyciem SRB powoduje wydzielania się siarkowodoru i doprowadza do zmniejszenia ChZT, czyli zmniejszenie stężenia substratów organicznych.*

Słowa kluczowe: bakterie redukujące siarkę (SRB), Desulfotomaculum ruminis*, rozkład związków organicznych, redukcja siarczanów, oczyszczanie ścieków mleczrskich*