Diary Wastewater Treatment by Microbial Reduction of Sulfates by *Desulfotomaculum Ruminis*

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Summary

Presented results are a continuation of a research programme on use of different types of bacteria in water treatment of industrial wastewater. The general purpose of this study was to check the possibility of using SRB (Sulphate-Reducing Bacteria) 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 [2, 3]. The aim of this study is to investigate the possibility of applying sulphate respiration process to remove organic impurities in case of leakage of industrial wastewater. Conducted research concerns the reduction of sulphate to sulphide in the dairy industry wastewater using Desulfotomaculum ruminis strain of bacteria. Reported study evaluate the effect of reducing the bacterial content of the sulfur in the effluent, depending on the concentration of wastewater. The sulphate breathing process was carried at in laboratory conditions using the SRB on standing culture with modified Starkey medium containing dairy wastewaters as the unique source of carbon and energy. The study examines changes in the COD, level of sulphide content and the pH changes in the test samples. Compared samples contained 30%, 50% and 70% of the dairy effluent from the dairy. The results show the need to find the optimal ratio between the content of the effluent in the suspension being subjected to the SRB, as well as lead the process at the optimum time in order to maintain maximum results. The pH value increased in time. During the first three days the pH value was maintained at a similar level, while in fifth day its value suddenly increased. During the research reaction of tested wastewater samples pH ranged between 6.9-8.0.

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

Introduction

The aim of the study was to research the reduction of sulfides to optimize dairy wastewater treatment under anaerobic conditions involving sulphur reducing bacteria (SRB). As the only carbon and energy source there were used sodium lactate and the pre-treated wastewater from OSM Radomsko Dairy (Poland). These substances were added in suitable proportions to the standard Starkey medium, used during cultivation of SRB. Additionally the study was focused on evaluation of the catabolic activity and dynamics of growth of SRB culture in the process of COD (Chemical Oxygen Demand) reduction, accompanied by reduction of sulphates to sulphides. 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* [7, 8].

Microbiological reduction of sulphates has been thoroughly studied [9, 10, 11] and applied in technologies of natural environment protection and restoration, in cleaning of environments polluted with organic compounds, sulphates and soluble heavy metals [12]. Within the process of microbiological reduction of sulphates SRB 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 [13].

Dairy sewage induce deficit in oxygen content of receiving ponds as a result of high BOD5. 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 dairy sewage on receiving ponds is seen in: sedimentation of slurry, fungi expansion, strong oxygen absorption, distribution of hydrogen sulphite and others accompanying undesirable and harmful phenomena [9]. Bedrock aim of the biological sewage treatment is removal from the sewage all biologically decomposed pollutants [20, 21, 22].

Materials and method

The first step in carrying out research is preparation of working solutions. Trace elements were ultimately placed in measuring cylinder and mixed all together. Then, the 1 dm^3 container was poured with 100 cm^3 of each of the base components, 16 cm^3 of sodium lactate and 100 cm^3 of micronutrients. The solution was supplemented with distilled water, mixed and then prepared medium was poured into a glass bottle, pH was measured and container was sterilized for 20 min. After sterilization, the medium should has cream colour [13]. The medium is cooled down and the pH was re-checked and should range between 6.8. to 7.2. [14]. The isolated culture of bacteria was stored and grown on liquid Starkey medium where the only source of carbon and energy was 10.16 cm^3 of sodium lactate. The medium has also contained microelements (Table 1.).

The media studied were industrial wastewater from the Regional Diary Cooperative Radomsko, containing organic and inorganic pollutants including sulphates and metals [11]. Laboratory equipment as well as bed were sterilized in 120°C. Sulphites were indicated in precipitated cadmium sulphite – iodometric method [12].

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 inter-

Tab. 1 The Starkey medium composition and microelements contents
Tab. 1 Średnia Starkey'a zawartości składu mikroelementów

Composition (g/dm^3)		Microelements (g/cm^3)	
$MgSO_4 \cdot 7H_2O$	2.00	$MnSO_4 \cdot 4H_2O$	$6.2 \cdot 10^{-4}$
Na_2SO_4	2.42	$CuSO_4 \cdot 5H_2O$	$2.4 \cdot 10^{-4}$
NH_4Cl	1.00	$ZnNO_3 \cdot 6H_2O$	$2.0 \cdot 10^{-5}$
$K2HPO_4$	5.00	$(NH_4)_2MoO_4$	$2.0 \cdot 10^{-5}$
$CaCl_2 \cdot 6H_2O$	0.25	$NaHSeO_3$	$2 \cdot 10^{-11}$
$FeSO_4(NH_4)2SO_4 \cdot 6H_2O$	0.50	H_3BO_3	$1.7 \cdot 10^{-4}$
$MgSO_4 \cdot 7H_2O$	2.00		
Na_2SO_4	2.42		

vals. To make the measurements the reactors were blown with helium and the blown out H_2S was absorbed in washer containing 0.02 mol/dm^3 solution of cadmium acetate. The sulphides precipitated were quantified by the iodometric method [15, 16, 23].

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].

Propagation of bacteria was carried out in a sterile glass reactor with volume of 15 cm^3 tightly sealed with a rubber stopper. Anaerobic conditions were obtained by 10 minutes ventilation using helium.

The content was inoculated with 4% volume of which medium was 14.4 cm^3 and 0.6 cm^3 of active bacteria). Bacterial activity was previously tested what resulted in a black precipitate of iron sulfide and a turbidity of solution. The reaction was run at a constant temperature of 37°C in a laboratory incubator for 48 hours. Intensive bacteria growth was obtained by multiple grafts (transplantations were held every 48h). Reactors and rubber stoppers should be wrapped in aluminum foil and sterilized, the same way as the medium for 20 minutes. In case of reactor opening during the process, e.g. for pH adjustment, each sample should be ventilated with helium for 10 minutes, in order to restore anaerobic conditions.

Methodology of kinetic studies

During the desulfurization process, the each sample was degassed for 10 minutes with helium. Degassing may vary in intensity, for example the process can be conducted once a day, twice a day, or may also take place for every four hours. The more research was conducted, the more accurate picture of the process was obtained. Hydrogen sulfide separated during the process is then passed through displaced the 150 cm^3 scrubber containing 0.2M solution of cadmium acetate. The absorbed hydrogen sulfide is precipitated as a yellow solid cadmium sulfide (CdS), which is then analyzed. There is also a research carried out of precipitated amount of sulphide in the substrate (FeS), which results from the reduction of sulphate. Samples for determining of sulphites are collected using syringes after degassing after one day of process termination. Sulphites content is determined by iodometric titration [17].

Determination of sulphide iodometrically

Sulfide contained in the sample is oxidized with iodine to the sulfur, and then the excess of iodine is back-titrated with sodium thiosulphate solution.

Reagents and solutions are the following:
* 0.05mol/dm^3 iodine solution,
* HCl (1:1),
* 0.01 mol/dm^3 solution of $Na_2S_2O_3$ * $5H_2O$,
* 5% starch solution.

Well shaken sample was collected using syringe with 2cm^3 capacity and poured into Erlenmeyer flask. Next 100cm^3 of distilled water and 5cm^3 of HCl were added and mixed with H_2O in 1:1 proportion. Then precisely measured amount of 0.05M iodine solution was added. The flask was sealed and left in the dark place for about 30 minutes. Excess amount of iodine was titrated using 0.01M solution of $Na_2S_2O_3$ * $5H_2O$ in the presence of starch.

Determination of the chemical oxygen demand (COD)

Chemical oxygen demand is a conventional index of organic substance content, expressed as the amount of oxygen consumed in the reaction of these substances during the heating of the sample with the oxidizing agent. Oxidant is potassium dichromate in an acidic medium in the presence of silver ions as the catalyst and mercury sulfate, which masks the effect of chloride ions. Determination is carried out by adding to the sample a known number of moles of potassium dichromate and titration of the excess with the Mohr salt solution. Hach's vial was filled with 2.5ml of the centrifuged sample, 1.5ml of $K_2Cr_2O_7$ and 3.5ml of H_2SO_4 + Ag^+ was added. The vial was capped and the content was well mixed. Vials were heated up to 150°C for 2 hours. Samples stained in green have to be rejected. Three assays were performed for each concentration. The samples were then titrated with the Mohr salt in presence of ferroin sulphate as indicator [19, 20].

Results

Study presents comparison of effectiveness of SRB through assessment of pH before and after the whole process, sulphates reduction by measuring increase of sulphite content and COD decrease as a result of bacteria activity. The first researched parameter were changes in pH of three different tests with Starkey medium supplemented with different content of waste water from the dairy industry. pH

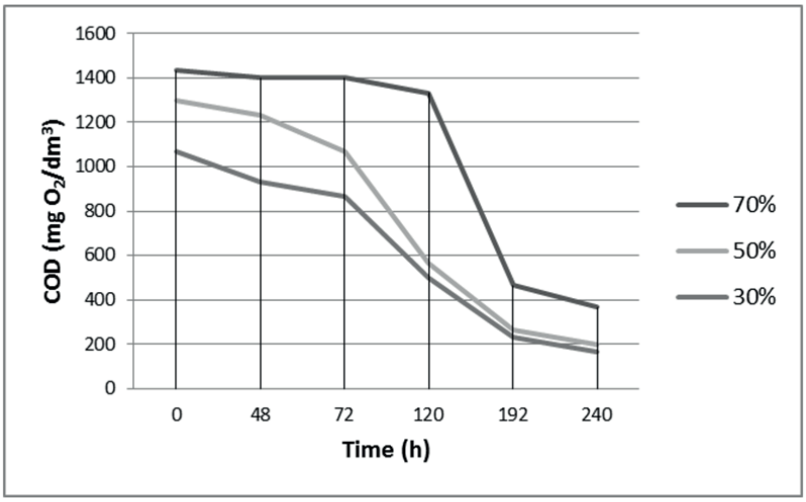

Fig. 1 Changes in the pH values measured in time comprising Starkey substrate with 30%, 50% and 70% content of dairy wastewater

Rys. 1 Zmiany wartości pH mierzone w czasie dla zawartości substrat Starkey'a 30%, 50% i 70% ścieków mleczarskich

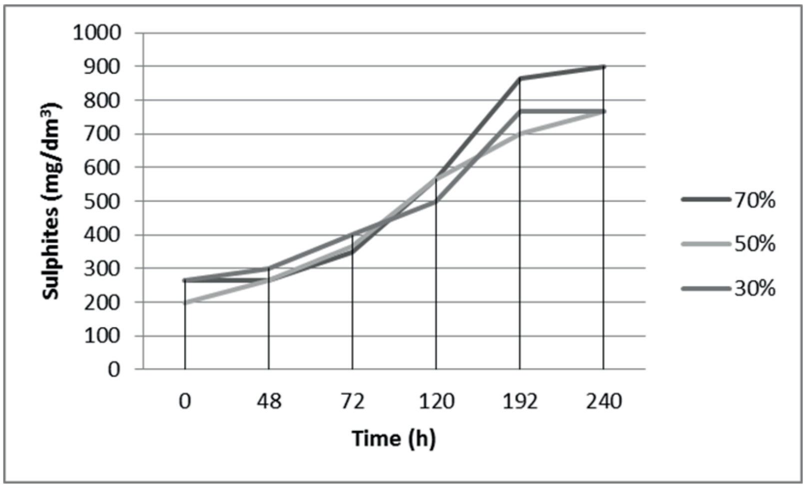

Fig. 2 The influence of the initial content of the dairy effluent (30%, 50%, 70%) on the effect of microbial reduction of sulphate to sulphites contained by the substrate

Rys. 2 Wpływ początkowego stężenia ścieków mleczarskich (30%, 50%, 70%) na skutek redukcji mikrobiologicznej siarczanów do siarczynów zawartych w podłożu

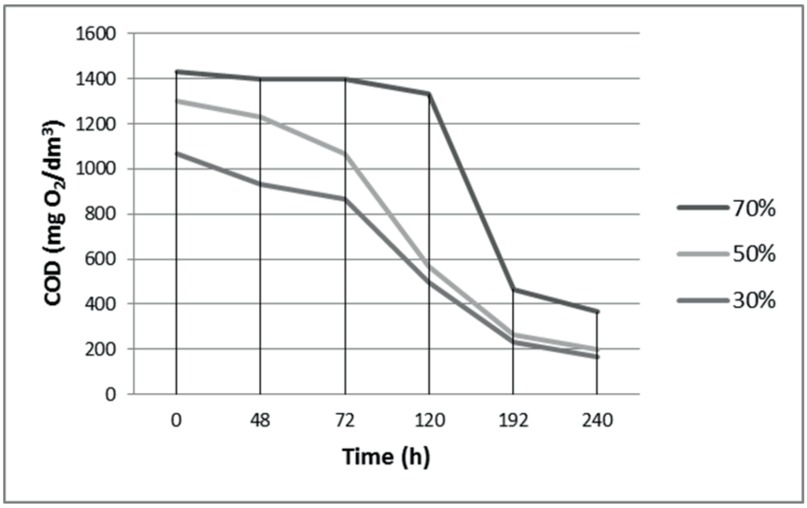

Fig. 3 The influence of the initial content of the dairy effluent (30%, 50% and 70%) on the effect of the reduction process in COD of the medium

Rys. 3 Wpływ początkowego stężenia ścieków mleczarskich (30%, 50%, 70%) na skutek procesu redukcji pożywki COD output was set to: * pH = 6.94 for 30% of wastewater in the sample, * pH = 6.98 for 50% of wastewater in the sample, * pH = 6.88 for 70% of the plants in the sample.

The more wastewater content the higher acidity of sample, however the differences are not considerable (Fig. 1).

The process is completed in about 240 hours and after the concentration of sulphides in the Starkey medium is 100mg of sulphides in 1 litre. The kinetic curves characterising microbiological reduction of sulphates showed roughly the same diversity (Tab. 2, 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 diary is close to 800 mg/dm3, while in the medium containing 50% and 30% of this wastewater is near 700mg S per litre.

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

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 noticed at a temporary stabilisation of COD whose reduction was inhibited at COD between 25 – 54 mg O_2/dm^3 (Tab. 3, Fig. 3). 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.

Summary and conclusion

Bacteria of *Desulfotomaculum ruminis* strain were research on the affect of sodium lactate and organic substances contained in the diary wastewater on their growth as well as their activity reducing sulphates to sulphites. Results of research conducted using effluent from Radomsko dairy indicated that a particular concentration of SRB determine their activity by measuring content of sulphites in the test samples.

In the fifth 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.

COD was also investigated in the effluent, which is an indicator determining the content of organic matter indicated as the amount of oxygen consumed in reactions during heating of the sample material with an oxidizing reagent. Potassium dichromate was used as the oxidant in an acidic medium in the presence of silver ions being the catalyst and the sulfate ions masking effect of mercury chloride impact. The largest amount of desulfurization bacteria occurred in the first 3 days of the process, and on the fifth day their number has fallen sharply. This indicates that there is the need to use small quantities of oxygen in oxidation of organic compounds contained in the wastewater. The last parameter that was studied was the pH of the effluent. The pH value increased in time. During the first three days the pH value was maintained at a similar level, while in fifth day its value suddenly increased. During the research reaction of tested wastewater samples ranged between 6.9-8.0. Suitable pH value in the effluent is ranging between 6.5 to 8.0. During the study, these values were reproducible, which indicates that the purification of wastewater was effective.

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Oczyszczanie ścieków z przemysłu mlecznego za pomocą redukcji mikrobiologicznej z użyciem Desulfotomaculum ruminis

Zaprezentowane wyniki są kontynuacją program badań na temat użycia różnych typów bakterii w oczyszczaniu ścieków przemysłowych. Głównym celem badań było zbadanie możliwości użycia bakterii redukujących siarczany – SRB w procesie oczyszczania ścieków z przemysłu mlecznego. Ten typ ścieków zawiera głównie węglowodany, głównie laktozę (30,9%), białka (23,6%, w tym 80% kazeiny) oraz tłuszcze (41,4%) pochodzące z mleka i jego produktów [2, 3]. Celem badań jest znalezienie możliwości zastosowania procesu oddychania siarczanów aby usunąć organiczne nieczystości w przypadku wycieku ścieków przemysłowych. Przeprowadzone badania skupiały się na redukcji siarczanów do siarczków w ściekach z przemysłu mleczarskiego przy użyciu szczepu bakterii Desulfotomaculum ruminis. Badania przedstawione w raporcie oceniają efekt redukcji bakteryjnej zawartości siarki w wypływie w zależności od stężenia ścieków. Proces oddychania siarczanów został przeprowadzony w warunkach laboratoryjnych z użyciem SRB na stojącą kulturę bakterii ze zmodyfikowanym nośnikiem Starkeya zawierającym ścieki mleczarskie jako unikalne źródło węgla i energii. W pracy zbadano zmiany w COD, poziom zawartości siarczków i zmiany pH w badanych próbkach. Porównywane próbki zawierały 30%, 50% i 70% ścieku mleczarskiego. Wyniki wskazują na istnienie potrzeby znalezienia optymalnego stosunku między zawartością ścieku w zawiesinie będącej skierowaną do SRB, jak również sposobu przeprowadzania procesu w optymalnym czasie w celu uzyskania maksymalnych wyników. Wartość pH zwiększa się w czasie. Podczas pierwszych trzech dni wartości pH utrzymywały się na podobnym poziomie, podczas gdy piątego dnia wartość nagle wzrosła. Podczas badanej reakcji pH próbek przyjmowało wartości z zakresu 6,9-8,0.

Słowa kluczowe: Desulfotomaculum ruminis*, SRB, dysymilatorowa redukcja siarczanu, oczyszczanie ścieków mleczarskich*