The Optimization of Food Effluent Treatment Using *Desulfotomaculum Ruminis* in Anaerobic Conditions

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Abstract

One of the most heavily laden pollutants is dairy wastewater with excessive volume of sulphates. Biological methods use sulphate-reducing bacteria Desulfotomaculum ruminis in diary wastewater treatment. 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. An application of the sulfate reducing bacteria in the treatment of dairy wastewater was investigated. The wastewater impurities formed in the milk and dairy product processing consist of carbohydrates – (mainly lactose), proteins, including important part of casein), and fats. The activity of catabolic growth of the sulfate reducing bacteria cultures was studied. SRB 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 the growth environment during dissimilatory reduction having deterrent effect on the decrease of ions SO_4^{2-} to S^{2-} In this article there are presented results of treating wastewater containing different concentration of affluent. The sulphate breathing process was carried out in laboratory conditions using the SRB culture on modified Starkey medium containing dairy wastewaters as the unique source of carbon and energy. The study examines and determines the optimum pH level for SRB which ranges between 6.84 to 8.0. There were four samples with different waste water content: 2,5%, 5%, 7,5% and 10%. Dependence of affluent content in waste water was research through measuring the reduction of sulphates to sulfites by increasing the last as well as COD changes.

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

Introduction

The main goal of this research is wastewater treatment in the food industry using anaerobic sulfate reduction process to eliminate sulfur content. Tests were performed using the analytical methods, e.g. the designation of cadmium sulfide and iron (II) by iodometric titration. It can be observed SRB bacterial activity in the dairy industry effluents based upon designated sulfides produced in the sulfate dissimilation reduction present in the medium.

SRB used in the study

Desulfotomaculum ruminis strain of bacteria belongs to the family Petococcaceae and come from the genus Desulfotomaculum, which has 30 species of microbes. These bacteria have incomparable versatility and capabilities in processing different compounds. During dissimilative sulfate reduction process many elements or substances can provide the needed ion can be [1]:

– hydrogen,
– spirits,
– alanine
– hexose,
– fatty acids and other mono- and di carboxylic acids.

Desulfotomaculum ruminis are bacteria in the form of Mycobacterium, with length of 2–6 microns and a width of 0.5 to 0.7 microns. They are absolute Anaerobes and their development occurs in temperature of 37°C. The pH range in which they can live is in the range between 6.0 to 8.5 [2,3].

The SRB bacteria were isolated from wetland soils. Growth was conducted on the liquid Starkey medium. Properly prepared medium [Tab. 1. and Tab. 2.] was sterilized for 20 minutes. Correct pH measured before sterilization should be approx. 7.5, after sterilization between 7.0 to 7.2. The bacterial culture was performed in a sterile glass reactor of 15 cm3 volume tightly sealed with rubber stoppers. To maintain anaerobic conditions, helium was used to blow out all the air from the reactors. Bacteria activity was previously tested through a black iron sulfide precipitate and turbidity.

The reaction was carried in a laboratory oven at 37° C for 48 hours. After two days from the reactors' preparation bacteria were transplanted every 48 hours by an automatic pipette. Viability of the bacteria were observed when pH tolerance was ranging between 6.8–7.2. The measurement was performed by pH meter with silver chloride electrode and a temperature sensor. Solutions of HCl or NaOH were used to adjust the pH. The isolated culture of Desulfotomaculum ruminis grown on

Tab. 1. Micro-elements added to the standard Starkey liquid medium

Tab. 1. Mikroelementy dodane do standardowego roztworu Starkleya

Content	g/dm^3
$MnSO_4 * 4H_2O$	$6.2*10^{-4}$
H_3BO_3	$1.7*10^{-4}$
$CuSO_4 * 5H_2O$	$2.4*10^{-4}$
$Co(NO_3)_2 * 6H_2O$	$1.0*10^{-4}$
$Zn(NO_3)_2 * 6H_2O$	$2.0*10^{-5}$
$(NH_4)_2MoO_4$	$2.0*10^{-5}$
$NaHSeO_3$	$2.0*10^{-11}$
$Ni(NO_3)_2 * 6H_2O$	$3.0*10^{-6}$

Tab. 2. Macro-elements added to the standard Starkey liquid medium

Tab. 2. Makroelementy dodane do standardowego roztworu Starkleya

Content	g/dm^3
$MgSO_4 * 7H_2O$	2.00
$NaSO_4$	2.42
NH_4Cl	1.00
K_2HPO_4	5.00
$CaCl_2 * 6H_2O$	0.25
$FeSO_4(NH_4)_2SO_4 * 6H_2O$	0.50
Sodium lactate	10.16

Tab. 3. Changes in pH values for the various contents of wastewater in the test samples

Tab. 3. Zmiana wartości pH dla różnych składów ścieków

Time (h)	pH depending from waste water content in the analyzed samples			
	2%	5%	7.5%	10%
48	6.90	6.84	7.12	7.15
72	7.25	6.88	7.31	7.53
96	7.36	7.14	7.45	7.71
120	7.44	7.69	7.53	7.86
144	7.69	7.76	7.70	7.90
168	7.74	7.84	7.85	7.87
192	7.83	7.53	7.72	8.00

liquid Starkey medium [4] containing micro- and macro-elements to increase the rate of propagation.

Methodology of kinetic studies

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 [5,6].

Determination of iron sulfides (II) by iodometric method

The activity SRB bacteria was assessed by determining the sulfides content present in the medium with the food industry waste produced during the sulfate dissimilative reduction process [7,8,9].

Reagents were:

- 0.05M solution of iodine
- 5% starch solution
- solution of HCl:H_2O (1:1)
- 0.1M solution of $Na_2S_2O_3 * 5H_2O$

Sulfides of iron were taken from the reactor without precipitation, because FeS precipitate spontaneously sulphate breathing process. 2ml shaken the sample was taken from the reactor. The transferred to Erlenmeyer flask, and then diluted with 100 cm^3 of distilled water [10]. There were 2cm^3 of the prepared solution of HCl: H_2O (1:1) and 1cm^3 iodine added. Next the flask was sealed with glass stopper and shaken for a few minutes. Then solution is set aside for 30 minutes in dark place to allow reaction carried:

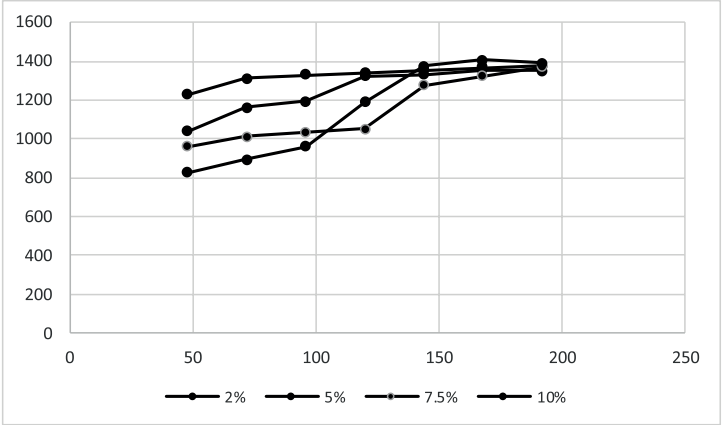

Fig. 1. The dependence of pH from time for the 2% , 5%, 7.5% and 10% wastewater content samples
Fig. 1. Zależność pH od czasu dla próbek zawierających 2%, 5%, 7.5% and 10% ścieków

Tab. 4. The content of iron sulfides (II) in the test samples
Tab. 4. Zawartość siarczków żelaza (II) w próbkach testowych

Time	The content of iron sulfides (II) in the test samples			
	2%	**5%**	**7,5%**	**10%**
48	1230	1041	963	827
72	1311	1161	1014	895
96	1331	1195	1031	963
120	1345	1324	1052	1188
144	1355	1331	1277	1373
168	1369	1355	1328	1407
192	1373	1352	1369	1389.6

Tab. 5. The degree of reduction of COD
Tab. 5. Stopień redukcji COD

Time (h)	COD values during the desulphurization			
	2%	**5%**	**7.5%**	**10%**
48	303.66	348.88	865.75	1298.62
72	264.89	310.12	833.44	1285.70
96	219.67	239.05	781.76	1266.32
120	122.75	161.52	684.84	975.58
144	96.91	142.14	497.48	659.00
168	71.07	129.22	452.26	594.39
192	19.38	155.06	478.10	465.18

$$S^{2-} + I_2 \rightarrow S^0 + 2I- \quad (1)$$

After 30 minutes the iodine excess was titrated with 0.1M solution $Na_2S_2O_3 * 5H_2O$ in the presence of starch, which is an indicator to the compete decoloration. The reaction proceeds quantitatively, oxidized iodine takes the form tetrathionate:

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-} \quad (2)$$

Determination of the chemical oxygen demand (COD) by dichromates

COD is the conventional rate of the organic matter content expressed in the amount of oxygen which is consumed in the reactions of these compounds during heating of the sample with an oxidizing agent.

Potassium dichromate in an acidic medium, in the presence of silver ions as a catalyst, and mercuric sulphate, which is aimed at masking the effect of chloride ions, was used as the oxidant.

The reaction of potassium dichromate with organic compounds is as following [11]:

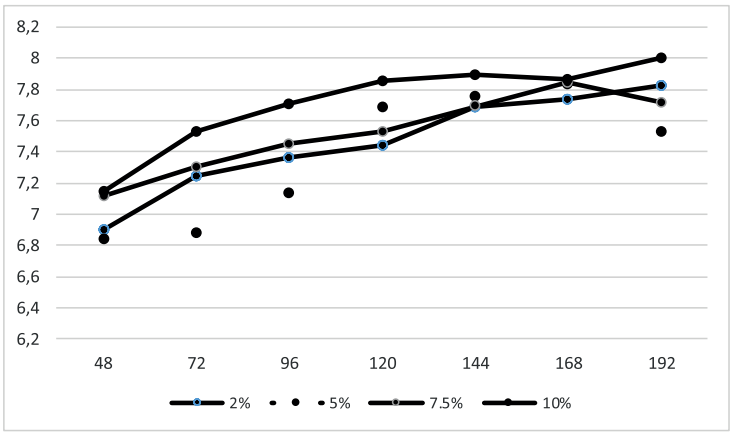

Fig. 2. The dependence of FeS(II) from time for the 2%, 5%, 7.5% and 10% wastewater content samples
Rys. 2. Zeleżnosc FeS(II) od czasu dla zawartości ścieków 2%, 5%, 7.5% i 10%

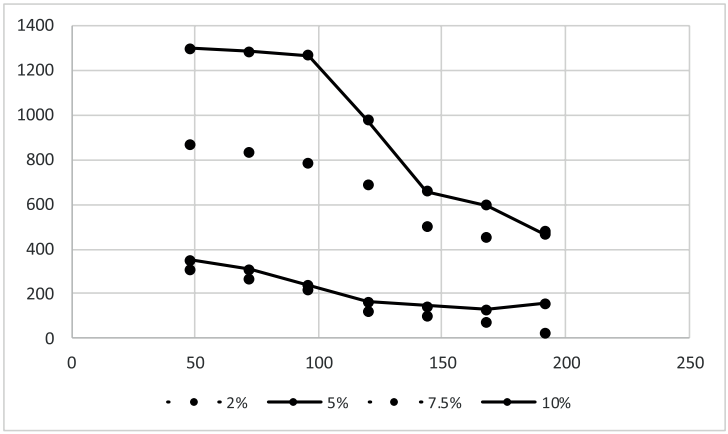

Fig. 3. The dependence of COD from time for the 2%, 5%, 7.5% and 10% wastewater content samples
Rys. 3. Zalezność COD od czasu dla zawartości ścieków 2%, 5%, 7.5% i 10%

$$C_nH_aO_bN_c + dCr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + (a + 8d - 3c)/2\ H_2O + cNH_4^+ + 2dCr^{3+} \quad (3)$$

The test samples were taken from the reactor and transferred to the centrifuge and centrifuged for 15 minutes. From the centrifuged samples 0.25 cm^3 was taken and put into Hach's vials. Two solutions were added: 1.5 cm^3 of $K_2Cr_2O_7$ and 3.5 cm^3 H_2SO_4/Ag +. The prepared test-tube was capped and mixed well, then heated for 2 hours at 150°C. The heated samples after getting rotten green color were poured into Erlenmeyer flasks and titrated with a standard solution of Mohr's salt with ferroin sulfate as an indicator [12,13].

Results and discussion

The study was conducted on dairy wastewater. The parameter that was investigated firstly was the pH of a wastewater. The pH increased in time, and depending on waste water content in samples, varied between 6.84 to 8.0. This is appropriate and suitable alkaline environs for SRB since optimum range of pH in the effluent is between 6.5 to 8.0 [14].

For obvious reasons there are different levels of sulfides in samples with different concentration of waste water. FeS precipitate spontaneously during sulphate breathing process. Iodine excess, present in the tested samples, was titrated with 0.01 M sodium thiosulfate, in the presence of starch as an indicator, which causes oxidation of the iodine to tetrathionate [15,16]. RSB activity is observed based upon sulfides determination resulting from the process of dissimilative sulfate reduction. The data show that the sample of the effluent concentration of 2% for 7 days reached sulfides level in the range of 1230 to 1373 mg/dm^3. In the case of a sample having a concentration of 5% sulfides reach of 1041 to 1355 mg/dm^3. For the sample with 7.5% waste water content the sul-

fides were between 963 to 1369 mg/dm^3. And for a sample with 10% content represented the widest range from 827 to 1407 mg/dm^3. With increasing content of waste water in the solution the widest range of sulfides is observed [17,18].

COD is the conventional rate of the organic matter content expressed in the amount of oxygen which is consumed in the reactions of these compounds during heating of the sample with an oxidizing agent. Potassium dichromate in an acidic medium in the presence of silver ions as a catalyst and mercuric sulphate was used as the oxidant, which is aimed on masking the effect of chloride ions [19]. The results show direct dependence of the COD value of the elapsed time for the tested samples with various concentration of wastewater. Evidently the value of the COD [mg O_2/dm^3] decreases in time[20]. Thus, for a sample with 2% content of waste water COD value ranges from 303.66 to 19.38 mg O_2/dm^3. And respectively: for 5% from 348.88 to 129.22 mg O_2/dm^3, for 7.5% between 865.75 to 452.26 mg O_2/dm^3, and for the last 10% from 1298.62 to 465.18 mg O_2/dm^3.

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Optymalizacja oczyszczania ścieków żywnościowych za pomocą Desulfotomaculum Ruminis *w warunkach anaerobowych*

Jednym z największych istniejących zanieczyszczeń wód są ścieki spożywcze zawierająca ogromne ilości siarczanów. Biometody uwzględniają użycie bakterii redukującej siarczany (ang. skrót SRB) Desulfotomaculumruminis w procesach przetwórczych ścieków. Proces przetwórczy ścieków spożywczych z udziałem SRB powoduje wydzielanie siarkowodoru i spadek chemicznego zapotrzebowania na tlen, czyli redukcję stężenia substratów organicznych. Zbadano efekty po dodaniu bakterii redukującej siarczany w procesie przetwórstwa ścieków spożywczych. Zanieczyszczenia wody ściekowej powstałej z mleka i przetworzonych produktów nabiałowych składają się z węglowodorów-(głównie laktozy), protein (w dużej mierze kazein) i tłuszczy. Zbadano również aktywność wzrostu katabolitycznego kultury bakterii redukującej siarczany. SRB przeprowadza dysymilacyjną redukcję siarczanów, które wykorzystują jony siarczanów (IV) oraz (VI), jako główne akceptory elektronów i wodoru. Badania laboratoryjne wykazały, że siarczki (głównie FeS) wzrost osadów w środowisku podczas dysymilacyjnej redukcji, tym samym wstrzymując redukcję jonów z SO_4^{2-} na S^{2-}. W artykule przedstawiono wyniki przetwórstwa ścieków zawierających różnego rodzaju składniki. W warunkach laboratoryjnych przeprowadzono proces utleniania siarczanowego, przy użyciu kultur bakterii redukującej siarczany na zmodyfikowanym medium Starkey'a zawierającym ścieki spozywcze, które są wyjątkowym źrodłem węgla i energii. W pracy sprawdzono i określono optymalny poziom pH dla SRB, które zamknęło się w zakresie od 6,84 do 8,0. Wzięto pod uwagę 4 próbki ze zróżnicowaną zawartością ścieków: 2,5%, 5%, 7,5% oraz 10%. Zależność zawartości istotnych składników w ściekach zbadano przez pomiar redukcji siarczanów do siarczynów i zmniejszenie chemicznego zapotrzebowania na tlen.

Słowa kluczowe: oczyszczanie ścieków mleczarskich, bakterie redukujące siarczany (SRB), Desulfotomaculum ruminis*, dissimilatory redukcji siarczanu*