Environment Protection Engineering

Vol. 17 1991 No. 3–4

ZOFIA DOMAGAŁA*, FLORIAN DOMKA*

ESTIMATION OF THE EFFECT OF *DESULFOTOMACULUM RUMINIS* BACTERIA ON THE PROCESS OF DEGRADATION OF SIMPLE ORGANIC SUBSTRATES

Isolated bacteria of *Desulfotomaculum ruminis* were tested for the degree of assimilation of simple organic substrates, used as the only source of carbon. Kinetic assays proved that such organics as sodium pirogronate, glucose, sodium lactate, starch and sodium glutamate are well assimilated by an isolated culture of bacteria. The presence of *L*-ascorbic acid, sodium formate, glycerine, sodium succinate and sodium citrate was found to bring about insignificant growth of bacteria, whereas *L*-cystine, sodium acetate, sodium malate, ethanol and sodium benzoate ihibit the process of desulfurication.

1. INTRODUCTION

The cycle of transformations of nitrogen and sulfur in biosphere relies on the activity of bacteria and the products obtained are used by other microorganisms and plants in biosynthesis. Increasing pollution may significantly disturb the balance between the processes of synthesis, degradation and formation of various species in natural environment. The factors affecting this transformation require detailed investigation with the objective of reducing the harmful effect of pollution on environment.

Microbiological transformation of elements treated as model systems developed by nature in the process of its evolution can be adapted and used in water and sewage treatment [1]–[3], in industry [4], in the processes of protection against biological corrosion [5]–[9], desulfurization of coal [10] and others.

Dissimilational reduction of sulfate carried out by the bacteria of *Desulfovibrio* and *Desulfotomaculum* species enables anaerobic respiration with inorganic sulfates

* Department of Kinetics and Catalysis, Faculty of Chemistry, Adam Mickiewicz University, 60-780 Poznań, Grunwaldzka 6, Poland.

as a final electron acceptor and the organic compounds as an electron donor, in other words, energetic substrates are various organic compounds present in sewage. The activity of these two species of bacteria depends on the components of the sewage, the conditions of the process and the species of microorganisms involved.

In the biotechnological processes connected with environment protection, imitation of transformations developed by nature may prove to be extremely beneficial. The use of microorganisms permits us to reduce the temperature of the relevant process and to increase its efficiency and selectivity as well as to overcome the waste problems.

This paper reports the results of investigations of *Desulfotomaculum ruminis* bacteria activity in degradation of various organic substrates found frequently as pollutants in industrial wastes, water and soil.

2. EXPERIMENTAL PROCEDURES

Bacteria to be studied were isolated from the soil samples taken from Zielona Góra area. The samples were collected from underneath manure at a depth of about 20 cm and put into 50 cm^3 bottles containing the nutrient medium of the composition specified in table 1 (nutrient medium B). Bacteria were grown under anaerobic conditions (helium blown through the reactors) at a constant temperature of 37°C in a KC-65 thermal chamber. After noticeable growth of bacteria, evidenced by black deposit of ferric sulfide, samples of 2 cm^3 each were taken to bottles with fresh nutrient medium. Intense growth of bacteria was achieved by multiple grafting. In the next step, samples of the bacteria were transferred under sterile conditions to a nutrient medium which had been previously sterilized for half an hour at 120°C. The bacteria were grown under anaerobic conditions at 37°C. To obtain a pure culture, the process of isolation was continued in vials, blown with helium, on agar medium (2.5% of agar) [11]. Prior to transferring onto solid nutrient media, the inoculum was diluted at a ratio of 1:100 by phosphate buffer of pH = 7.2 (0.2 M solution of Na_2HPO_4 and 0.2 M solution of NaH_2PO_4 were mixed at a ratio of 1:25) [12], then 0.1 cm^3 samples were transferred to vials with solid medium and incubated under anaerobic conditions at 37°C. The obtained individual colonies of bacteria were transferred again into liquid media.

The isolated culture of bacteria was identified under the electron microscope, and then subject to morphological and physiological tests. Kinetics of desulfurication was investigated in the media of a few types in order to find the optimum conditions of bacterial growth [13]. The compositions of the examined media are given in table 1.

Identification of microorganisms was based on the following tests: the Grama test, the test of bacteria resistance to high temperature (spore formation test), the desulfoviridin test, as well as physiological examination. Electron pictures were taken under a JEM-6B microscope (Japan). In the test of bacteria resistance to high

Table 1

Chemical composition of nutrient media (g/dm^3) used in the process of microbiological sulfates reduction carried out by the bacteria of *Desulfotomaculum ruminis*

Macroelements

Components	Type of nutrient media and C/S (g/g)				
	A C/S=3.6	B C/S=11.4	C C/S=2.1	D C/S=3.6	E C/S=3.6
$Na_2SO_4 \cdot 10H_2O$	5.5	6.31	4.5	4.5	5.5
$MgSO_4 \cdot 7H_2O$	2.0	2.0	0.06	0.06	2.0
NH_4Cl	1.0	1.0	1.0	1.0	1.0
$CaCl_2$	0.13	0.13	0.03	0.03	0.13
K_2HPO_4	5.0	5.0	–	5.0	–
KH_2PO_4	–	–	0.5	–	5.0
Mohr's salt	0.5	0.006	–	–	0.5
$FeSO_4 \cdot 7H_2O$	–	–	0.004	0.004	–
sodium lactate	10.0	31.4	6.0	10.0	10.0
yeast extract	–	–	1.0	1.0	–
sodium citrate	–	–	0.3	0.3	–

Microelements (mg/dm^3) were prepared by their dissolution in distilled water: $Co(NO_3)_2 \cdot 6H_2O$ – 5.14, $Ni(NO_3)_2$ – 0.15, Na_2SeO_3 – $1 \cdot 10^{-6}$, $CuSO_4 \cdot 5H_2O$ – 12.0, $(NH_4)_2MoO_4$ – 0.99, $Zn(NO_3)_2 \cdot 6H_2O$ – 1.0, H_3BO_3 – 8.6, $MnSO_4 \cdot 4H_2O$ – 30.8.

temperature, the bacterial culture was heated at 100°C for 10 min. The Grama test was performed following the procedure given by KOCWOWA [11]. In the desulfoviridin test, red fluorescence due to the release of the chromophore of the pigment desulfoviridin is the evidence of the presence of *Desulfovibrio* species [13], [14]. In order to obtain some information about the physiology of the isolated bacteria, their abilities to oxidize different sources of organic carbon were also tested (table 2).

Kinetics of bacteria growth was tested in the best available nutrient media (table 1, type B). Advancement of the reaction was followed by checking the current concentration of hydrogen sulfide coming from biocatalytic reduction of sulfates. The isolated hydrogen sulfide was absorbed in washers containing 0.02 M solution of cadmium acetate, and the content of sulfides was determined iodometrically. The same method was applied to identify sulfides in the reaction media, by sampling 1 cm^3 of the nutrient.

3. RESULTS AND DISCUSSION

The physiological and morphological studies (the Gram test, and the desulfoviridin and temperature tests as well as the electron-microscope pictures) and

Bergey's suggestions [15] and J.R. Postgate's description [13] proved that in the isolated culture *Desulfotomaculum ruminis* bacteria occur. They are found to occur in fresh water, marsh, as well as in the stomachs of ruminates and in an environment containing sulfates and organic substances. The rods of the bacteria are Gram-negative; they are either straight or curved in shape and they produce spores (figure 1).

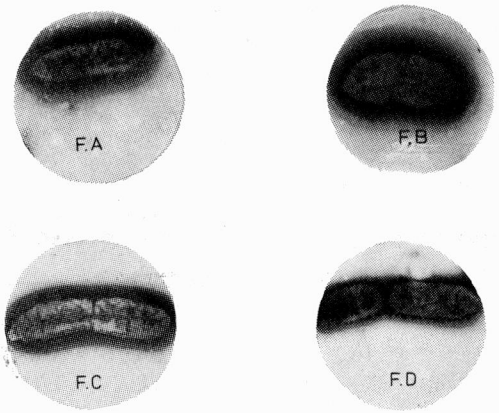

Fig. 1. *Desulfotomaculum ruminis*
A – enlargement 14000×, B – enlargement 22000×,
C – bacteria upon division (enlargement 14000×),
D – final moment of bacteria division (enlargement 16000×)

In a cycle of biological transformations of sulfur, these microorganisms permit, via indirect stages, reduction of SO_4^{2-} to S^{2-} [6], [14], [16]. For their growth, they used inorganic sulfates and various organic compounds contained in the media. The amount of the released hydrogen sulfide is proportional to the amount of the organic substrate used. The activity of microorganisms in desulfurication is considerably affected by the composition of nutrient media. This issue has been illustrated in figure 2 showing the dependence of the degree of sulfates reduction on the type of the media used which differ mainly in the C/S ratio, i.e. the ratio of the concentration of organic carbon to that of sulfate sulfur. The data presented show that the optimum medium, whose composition is listed in table 1, is at C/S ratio equal to 11.4, labelled as type B.

In other types of the media, the activities of the bacteria can be arranged in the following ascending order: B>A>E>D>C, which signifies the order of a decreasing C/S ratio in the medium.

Table 2 shows that after introduction of such electron donors as *L*-cystine, sodium acetate, sodium malate, ethanol and sodium benzoate, the bacteria remain totally inactive in a very wide range of organic substrate concentration or, in other words, at varying C/S ratio.

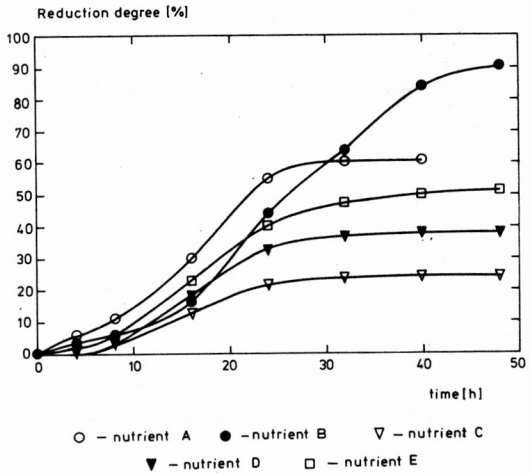

Fig. 2. Degree of microbiological reduction of sulfates in different types of nutrient media (temperature of 37°C, bacteria of *Desulfotomaculum ruminis* species)

Table 2

Activity of bacteria depending on the type of organic substrate in the nutrient medium

Organic substrate	Bacteria growth
sodium formate	+
sodium acetate	–
sodium succinate	+
sodium malate	–
sodium citrate	+
sodium malate	++
L-ascorbic acid	+
sodium pyruvate	++
sodium *L*-glutamate	++
L-cystine	–
glucose	++
starch	++
ethanol	–
glycerine	+
sodium benzoate	–

– lack of growth, + growth, ++ intensive growth.

The group of effective donors comprises sodium pyruvate, sodium lactate, starch, glucose and sodium *L*-glutamate. On the other hand, a low activity of bacteria was observed in a nutrient containing *L*-ascorbic acid, sodium formate, glycerine, sodium succinate and sodium cistrate.

The sulfate respiration system investigated is a closed cycle of transformation, which is an important element of sulfur circulation in nature.

Figure 3 illustrates the effect of sodium lactate, as the only source of carbon, on the process of desulfurication. Moreover, figure 3 shows changes in the degree of

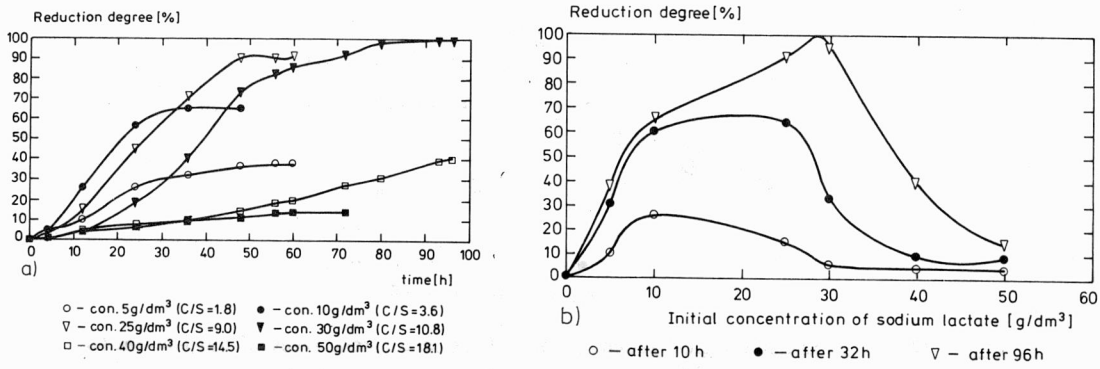

Fig. 3. Degree of microbiological reduction of sulfates in a nutrient containing sodium lactate as the only source of carbon versus time (a) and versus initial concentration of sodium lactate (b) (temperature of 37°C, bacteria of *Desulfotomaculum ruminis* species)

sulfate reduction with respect to the initial concentration of this substrate. Thus, the bacteria reach an optimum activity when they are introduced to a medium containing ca 30 g of sodium lactate in 1 dm^3. Further increase in the concentration of this substrate results in a substantial decrease in their activity in the process of desulfurication, while at lower concentration of sodium lactate the reduction degree decreases correspondingly.

Figure 4 presents the effect of sodium pyruvate applied as the only source of carbon on the reduction process. As in the previous cases, the optimum activity is

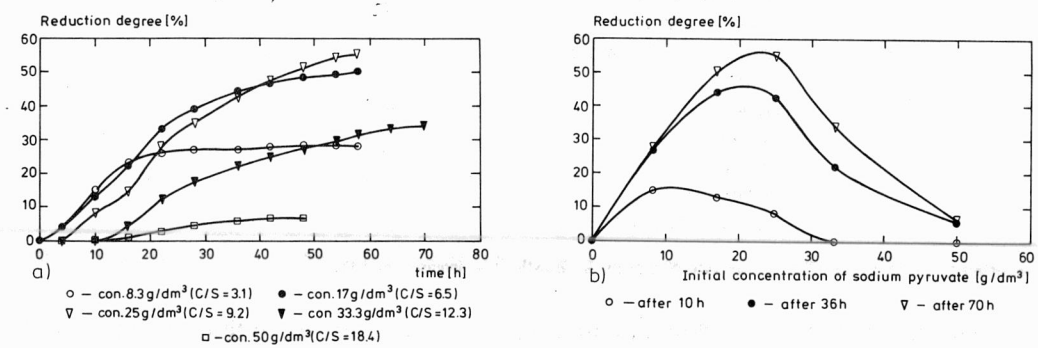

Fig. 4. Degree of microbiological reduction of sulfates in a nutrient containing sodium pyruvate as the only source of carbon versus time (a) and versus initial concentration of sodium pyruvate (b) (temperature of 37°C, bacteria of *Desulfotomaculum ruminis* species)

reached for the concentration of 25 g/dm^3. Both higher and lower concentrations of this substrate bring about a decrease in the degree of sulfate transformation. As the concentration of substrate is higher, the maximum of bacteria activity is more shifted in time, thus the reaction induction period increases.

The maximum degree of glucose transformation was observed for its concentration of 10 g/dm^3 and degree of sulfate reduction reached 82%. One can also notice that for glucose introduced at the concentration of up to 16 g/dm^3 sulfate reduction reaches 67% after 68 hours of the reaction. The effect of glucose on this transformation is illustrated by the results compiled in figure 5.

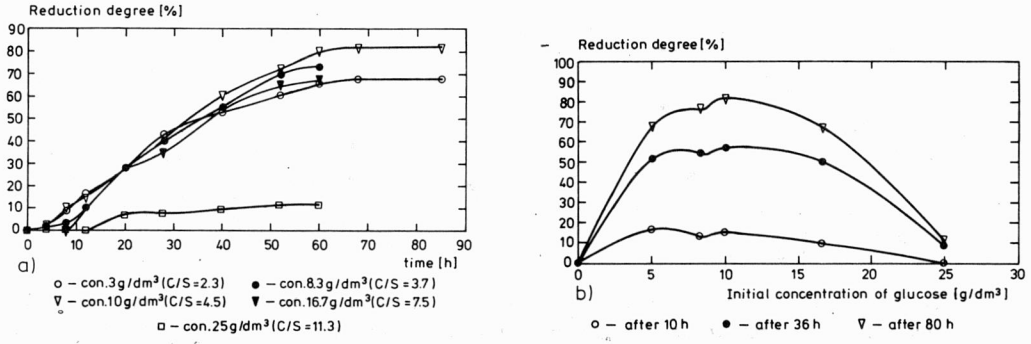

Fig. 5. Degree of microbiological reduction of sulfates in a nutrient containing glucose as the only source of carbon versus time (a) and versus initial concentration of glucose (b) (temperature of 37°C, bacteria of *Desulfotomaculum ruminis* species)

Kinetic curves illustrating the process of desulfurication occurring in the nutrient medium containing starch as the only source of carbon are shown in figure 6a, while the influence of starch concentration on desulfurication is presented in figure 6b.

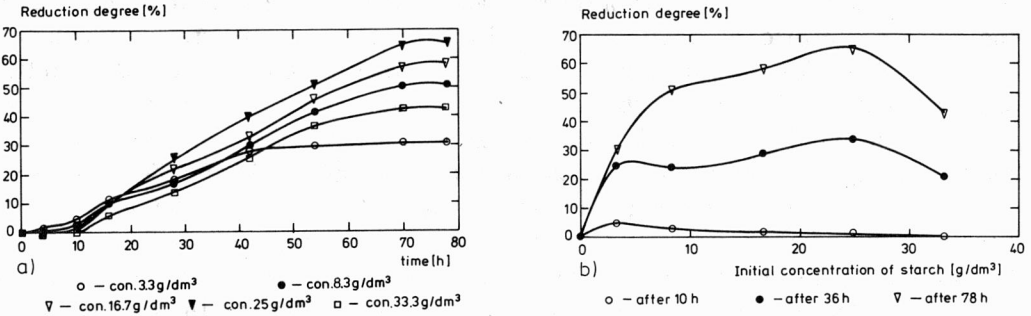

Fig. 6. Degree of microbiological reduction of sulfates in a nutrient containing starch as the only source of carbon versus time (a) and versus initial concentration of starch (b) (temperature of 37°C, bacteria of *Desulfotomaculum ruminis* species)

The optimum activity is obtained when starch at the concentration of 25 g/dm^3 is introduced to the medium. Higher and lower concentration cause a decrease in the reduction degree. After about 70 hours of this reaction proceeding under optimum conditions, about 65% transformation of sulfates into sulfides takes place.

Sodium *L*-glutamate proved to be also assimilated by the microorganisms under study (figure 7). In this case, the optimum activity of microorganisms is observed at the concentration of sodium glutamate ranging from 25 to 30 g/dm^3.

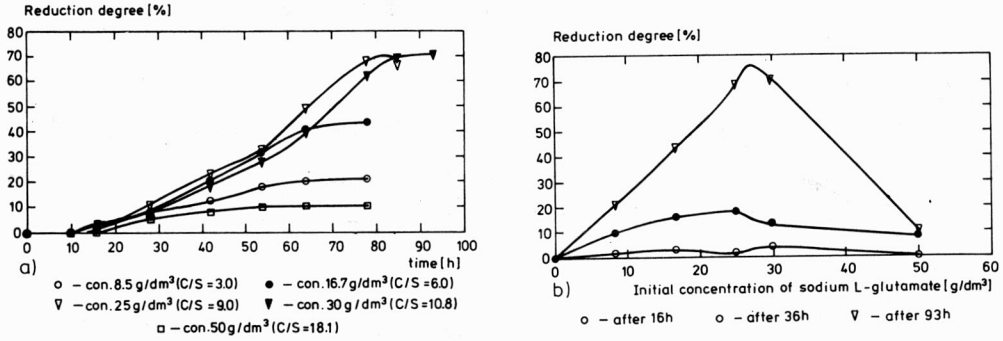

Fig. 7. Degree of microbiological reduction of sulfates in a nutrient containing sodium *L*-glutamate as the only source of carbon versus time (a) and versus initial concentration of sodium *L*-glutamate (b) (temperature of 37°C, bacteria of *Desulfotomaculum ruminis* species)

Further studies have indicated that in the presence of such organic substrates as *L*-ascorbic acid, sodium formate, glycerine, sodium succinate and sodium citrate applied as the only source of carbon the bacteria are kept alive, yet the degree of sulfate reduction is very insignificant, whereas the reduction of sulfates is inhibited completely in nutrient medium containing *L*-cystine, sodium acetate, sodium malate, ethanol and sodium benzoate. These results suggest that the latter compounds are not assimilated by bacteria of the *Desulfotomaculum ruminis* species.

As follows from the analysis of the kinetic curves, sodium *L*-glutamate applied as the only source of carbon brings about the longest induction period, though the shapes of all the kinetic curves prove that all the processes proceed according to the same mechanism. Moreover, the kinetic assays prove that sodium lactate is the substrate most effectively assimilated by *Desulfotomaculum ruminis*. In this case, under optimum conditions, desulfurication reaches a 100% yield after 80 h reaction.

For a nutrient containing glucose as the only source of carbon, at the concentration of 5–16 g/dm^3, the kinetic curves have similar shapes and they run asymptotically to the point in which glucose rearches the maximun degree of conversion. The degree of sulfate reduction brought about by *Desulfotomaculum ruminis* bacteria in the precense of the other, effectively assimilated substrates is observed to increase with an increasing initial sulfate concentration, until it reaches a given maximum, after which any further increase in initial concentration causes

inhibition of the reduction. This limiting (optimum) value of the initial concentration of the assimilated organic substrate, after which the reduction degree decreases, is the following for given substrates: 30 g/dm^3 for sodium lactate and sodium *L*-glutamate, 25 g/dm^3 for starch and sodium pyruvate and 10 g/dm^3 for glucose. These data permit us to make a generalization that except for glucose the maximum sulfate reduction occurs in a medium with the C/S ratio equal to 10. This value appears to be very close to the optimum C/S ratio observed for a medium of the B type (table 1).

The studies on activity of bacteria in a given organic medium have brought results which can be applied in order to decrease concentration of organics in various types of industrial sewage and, in particular, in wastes containing sodium lactate, sodium pyruvate, sodium *L*-glutamate as well as glucose and starch. The rates of transformation of these compounds depend heavily on the concentration of both organic substrates and sulfates. In the above cases, oxidation of the assimilated organic compounds takes place simultaneously with sulfate reduction. Hydrogen sulfide continuously yielded during this process may be subject to further microbiological transformation into elementary sulfur [6], [18], while the periodically collected deposit may be used as mineral-organic fertilizer [17]. Moreover, in anaerobic respiration, the waste sulfates may be used as the final electron acceptors, and in this way they are utilized [19], [20].

REFERENCES

[1] GĄSIOREK J., DOMKA F., *Badania laboratoryjne nad zastosowaniem biologicznej redukcji siarczanów do oczyszczania ścieków z produkcji kwasu cytrynowego*, Przem. Spożywczy, 4 (1988), 108–110.

[2] DOMKA F., GĄSIOREK J., *Wpływ mikrobiologicznej redukcji siarczanów na efektywność oczyszczania ścieków garbarskich*, Ochrona Środowiska, 1/38 (1989), 23–26.

[3] SZULCZYŃSKI M., DOMKA F., *Utilization des certains composes organiques par les bacteries Desulfovibrio vulgaris*, Bull. Acad. Polon. Sci., Ser. Sci. Biol., 28 (1980), 8–9, 479–484.

[4] DOMKA F., GĄSIOREK J., *Mikrobiologiczna metoda przerobu odpadowego siarczanu żelazawego z równoczesnym oczyszczaniem ścieków przemysłowych*, Gaz, Woda, Technika Sanitarna, 9 (1983), 260–263.

[5] GĄSIOREK J., DOMKA F., *Mikrobiologiczna korozja betonowych składowisk w przemyśle siarkowym*, Cement, Wapno, Gips, 9 (1983), 241–243.

[6] DOMKA F., *Studia nad procesem mikrobiologicznej redukcji siarczanów*, UAM, Poznań, Seria Chemia, Nr 23, 1977.

[7] CWOLINA B., DZIERŻEWICZ Z., ZASADA Z., *Mikrobiologiczna korozja metali*, Postępy Mikrobiologii, 1989.

[8] KENNETT A.C., *A corrosion investigation using the microorganisms Desulfovibrio desulfuricans*, Corrosion Australosia, 3 (4), 1978.

[9] ORTON M.D., *Sulphate Reducing Bacteria Corrosion Control*, The American Gas Association Transmission Conference, Montreal, Quebec, Canada, May 1978.

[10] WILCZOK T., BUSZMAN E., CWALINA B., Przegląd Górniczy, 11, 12 (1983), 457–459.

[11] KOCWOWA E., *Ćwiczenia z mikrobiologii ogólnej*, PWN, Warszawa 1977.

[12] KŁYSZEJKO-STEFANOWICZ L., *Ćwiczenia z biochemii*, PWN, Warszawa 1980.

[13] POSTGATE J.R., *The Sulphate-Reducing Bacteria*, Second edition, 208 pp., Cambridge University Press, London 1984.

[14] AKAGI J.M., *Dissimilatory Sulfate Reduction, Mechanistic Aspect, Biology of Inorganic Nitrogen and Sulfur*, edited by H. Bothe and A. Trebst, Springer Verlag, Berlin, Heidelberg, New York 1981, p. 178.
[15] BERGEY D., *The short Bergey's Manual of Determinative Bacteriology*, 1988.
[16] PECK H.D., Jr., Le GALL J., *Biochemistry of dissimilatory sulphate reduction*, Phil. Trans. R. Soc. Lond., B 298 (1982), 443–466.
[17] GĄSIOREK J., DOMKA F., *Ocena wartości nawozowej ścieków krochmalniczych surowych po biologicznym oczyszczaniu*, Nowe Rolnictwo, 11–12 (1988), 28–30.
[18] GĄSIOREK J., DOMKA F., *Utlenianie zredukowanych, nieorganicznych związków siarki przez bakterie Thiobacillus hiioparus w świetle badań laboratoryjnych*, Cement, Wapno, Gips, 4 (1984), 101–104.
[19] SZULCZYŃSKI M., GĄSIOREK J., DOMKA F., *Niektóre aspekty skutecznego oczyszczania ścieków z równoczesnym przetwarzaniem siarczanowych odpadów przemysłowych*, Gaz, Woda, Technika Sanitarna, 3 (1987), 65–69.
[20] DOMKA F., GĄSIOREK J., *Sposób wykorzystania odpadowego siarczanu amonowego*, Pat. PRL, Nr 88019, 1977.

OCENA AKTYWNOŚCI BAKTERII *DESULFOTOMACULUM RUMINIS* W PROCESIE DEGRADACJI PROSTYCH SUBSTRATÓW ORGANICZNYCH

Zbadano stopień przyswajalności prostych substratów organicznych stosowanych jako jedyne źródło węgla w hodowli wyizolowanych bakterii *Desulfotomaculum ruminis*. Badania kinetyczne pozwoliły stwierdzić, że takie substancje organiczne, jak pirogronian sodowy, mleczan sodowy, glukoza, skrobia i *L*-glutaminian sodowy są dobrze przyswajalne przez wyizolowane bakterie. Wykazano również, że kwas *L*-askorbinowy, mrówczan sodowy, gliceryna, bursztynian sodowy i cytrynian sodowy należą do substancji, w obecności których wzrost bakterii jest nieznaczny, natomiast *L*-cystyna, octan sodowy, jabłczan sodowy, etanol i benzoesan sodowy powodują zahamowanie procesu desulfurykacji.

ОЦЕНКА АКТИВНОСТИ БАКТЕРИЙ *DESULFOTOMACULUM RUMINIS* В ПРОЦЕССЕ ДЕГРАДАЦИИ ПРОСТЫХ ОРГАНИЧЕСКИХ СУБСТРАТОВ

Исследована степень ассимиляции простых органических субстратов, применяемых как одиночный источник углерода в культурах выделенных бактерий *Desulfotomaculum ruminis*. Кинетические исследования позволили установить, что такие органические вещества, как пирогронат натрия, лактат натрия, глюкоза, крахмал и *L*-глутаминат натрия хорошо ассимилированы выделенными бактериями. Было также обнаружено, что аскорбиновая кислота, формиат натрия, глицерин, сукцинат натрия и цитрат натрия принадлежат к веществам, в присутствии которых умножение бактерий незначительно, зато *L*-цистин, ацетат натрия, малеат натрия, этанол и бензоат натрия вызывают заторможение процесса обессерения.