Environment Protection Engineering

Vol. 23 1997 No. 1–2

KRYSTYNA KOSIŃSKA*, TADEUSZ MIŚKIEWICZ**

EFFECT OF THE COD/SO_4 RATIO ON THE DESULPHURICATION OF SULPHATE-ENRICHED LIQUID MANURE FROM INDUSTRIAL PIG FARM BY *Desulfovibrio desulfuricans*

Desulfovibrio desulfurificans bacteria were grown on wastewater samples from industrial-scale swine fattening. The efficiency and rate of sulphate reduction were investigated in terms of the COD/SO_4 ratio which ranged from 0.4 to 3.1. Reduction efficiency was found to exceed 90% at COD/SO_4 values higher than 0.8. When the COD/SO_4 value increased, so did the efficiency of sulphate reduction, amounting to 97.8% at 3.1. It was found, furthermore, that the rate of sulphate reduction also increased with the increasing COD/SO_4 ratio.

1. INTRODUCTION

Sulphate bacteria *Desulfovibrio desulfuricans* are well known for their participation in the sulphur cycle and have been referred to in specialised literature a great number of times [1], [2]. They have raised the interest of many investigators, particularly those concentrating on the contribution of sulphates to the methanogenesis process [3], [4], on gypsum bioconversion to sulphur, as well as on the recovery of elemental sulphur [5]–[8]. The role of *D. desufluricans* consists in the reduction of oxidised sulphur forms, e.g., sulphates, which is known as sulphate respiration or biological desulphurication. To achieve the efficiency desired it is necessary that both carbon and sulphates be present in the substrate, as shown by equ. (1):

$$\text{lactate}^- + SO_4^{2-} \rightarrow \text{acetate}^- + 2\,CO_2 + S^{2-}. \tag{1}$$

* Institute of Environmental Protection, ul. Wystawowa 1, 51-618 Wrocław, Poland.

** Food Biotechnology Department, Wrocław University of Economics, ul. Komandorska 118/120, 53-345 Wrocław, Poland.

The contribution of various, chemically defined carbon sources to sulphate bacteria growth and biological desulphurication efficiency is still regarded as a problem of prime importance. Thus, MACPHERSON and MILLER [9] investigated sulphate bacteria growth on substrates with carbohydrates, alcohols, organic acid salts and amino acids as carbon sources. Of these, lactates (the component of a standard nutrient medium prepared by Starkey) were found to be best suited to the growth of *D. desulfuricans*.

Another problem concomitant with the removal of sulphates by biological desulphurication is the optimisation of the C/S ratio in the substrate. DOMKA and GĄSIOREK [10] obtained a 50% reduction of sulphates at a C/S ratio of 1.84. The increase of the C/S ratio slightly improved the efficiency of the process, but, at the same time, noticeably deteriorated its dynamics. In their more recent study, DOMAGAŁA and DOMKA [11], [12] achieved a 90% reduction of sulphates with a C/S ratio of 11.4 in the nutrient medium and in the presence of lactate as a carbon source. According to Domagała and Domka, the efficiency of biological desulphurication was affected not only by the the C/S ratio, but by the carbon source as well. In the presence of lactate, glucose and pyruvate, the optimum proportion of carbon to sulphur amounted to 9.0, 4.5 and 6.5–9.2, respectively.

The high efficiency of sulphate removal obtained with synthetic nutrient media under laboratory conditions encouraged us to upgrade the investigations by making use of *D. desulfuricans* bacteria to remove sulphates from industrial wastewater. That is why the present study involved a medium which consisted of non-sterile liquid manure from pig farms. The medium was enriched with organic substances, biogens and microelements. Sulphates were added, as their content in the manure was not high enough. The objective of the study was to find out whether or not sulphate respiration would occur in the non-sterile effluent from the pig farm, and to determine the effect of the COD/SO_4 ratio substituted for the C/S factor.

2. MATERIALS AND METHODS

Microorganisms. Sulphate-reducing bacteria, *D. desulfuricans*, were isolated from the hydrogen sulphide sources of the spa Busko Zdrój [13]. The microorganisms were adapted to the nutrient medium prior to inoculation [14].

Media. *D. desulfuricans* were grown on samples of industrial pig farm liquid manure which was brought from the pig farm and stored at 5 °C. The chemical parameters of the manure averaged as follows:

pH	7.9,
$COD_{(nf)}$, g of O_2/dm^3	5.3,
total nitrogen (Kjeldahl), g of N/dm^3	0.75,
ammonia nitrogen, g of N/dm^3	0.41
total phosphorus, g of P/dm^3	0.13,
phosphates, g of P/dm^3	0.09.

The nutrient media applied varied only in sulphate concentration which ranged from 1.04 to 6.46 g of SO_4^{2-}/dm^3. In this way, it was possible to differentiate the initial values of the COD/SO_4 ratio. The samples were treated predominantly with $FeSO_4 \cdot 7H_2O$ in amounts which enabled the COD/SO_4 ratio to be kept within 0.4 and 3.1 (table 1).

Table 1

Major parameters of the medium

No. of reactor	Initial values of parameters examined			
	COD/SO_4 (mg of O_2 dm^{-3}/ mg of SO_4 dm^{-3})	COD/SO_4 loads (mg of O_2/ mg of SO_4)	C/S (mg of C dm^{-3}/ mg of S dm^{-3})	COD/S (mg of O_2 dm^{-3}/ mg of S dm^{-3})
1	3.1	1.85	3.2	9.3
2	2.3	1.45	2.3	6.8
3	1.8	1.1	1.85	5.4
4	1.6	1	1.74	4.9
5	1.5	0.9	1.5	4.5
6	1.3	0.8	1.3	3.9
7	1.2	0.7	1.2	3.5
8	1	0.6	1	3.1
9	0.9	0.55	0.9	2.6
10	0.8	0.5	0.8	2.4
11	0.6	0.4	0.6	2.1
12	0.5	0.35	0.5	1.5
13	0.4	0.3	0.45	1.4
14*	–	–	1.8	–

* Starkey's medium

Bacterial culture. Sulphate respiration processes were run in a system of 13 hermetic glass reactors of a two-liter effective volume. The reactors were fed only once with 1.5 dm^3 of nutrient medium and 0.5 dm^3 of inoculum (which contained 12.8 g of organic substances) and then incubated at 38 °C. Incubation was discontinued when no changes were observed in the concentration of sulphates.

Analytical methods. Sulphates were determined in averaged samples gravimetrically, whereas the coefficients of the reduction rate were established graphically according to the following equation:

$$S_0/S_e = e^{-Kt} \tag{2}$$

where S_0 and S_e denote initial sulphate concentration and sulphate concentration after time t (g of SO_4/dm^3), respectively, K is directional coefficient in equ. (1) (h^{-1} or d^{-1}); and t indicates duration of the incubation process (h or d). COD was determined by the dichromate method. TOC was calculated in terms of the equation:

$$COD = 2.2595\ TOC + 0.725 \tag{3}$$

which describes the relationship between COD and TOC (figure 1) in the pig farm effluent [15]. The equation incorporates the results from analysis of filtered and non-filtered samples.

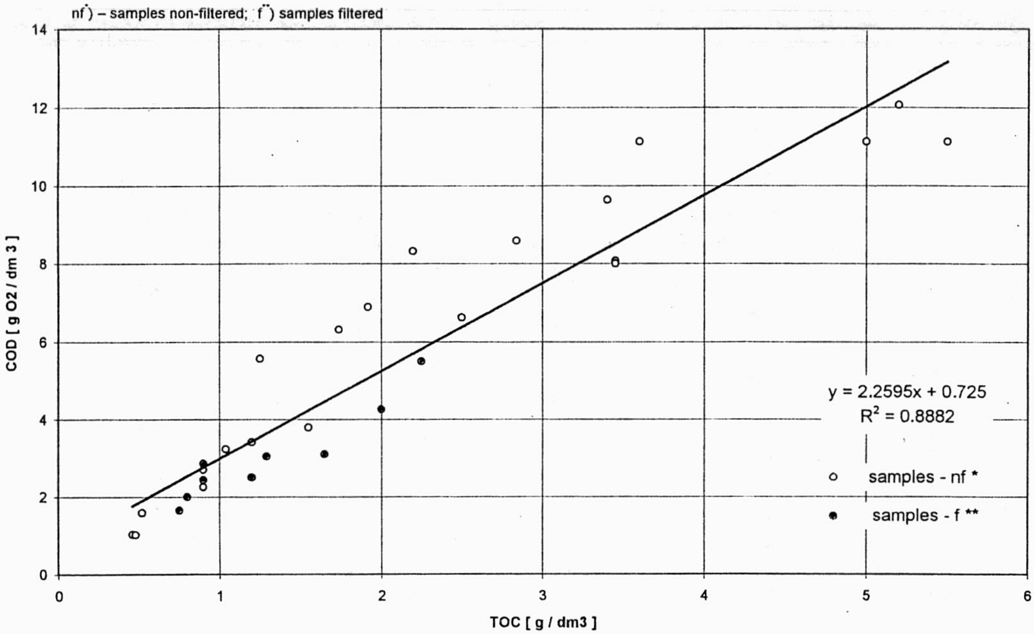

Fig. 1. COD versus TOC for COD(nf) and COD(f) ranging from 1.0 to 12.1 g of O_2/dm^3 and from 1.65 to 5.5 g of O_2/dm^3, respectively (based on the data reported by OLESZKIEWICZ [15])

3. RESULTS AND DISCUSSION

Table 2 relates the efficiency of sulphate reduction to the value of the COD/SO_4 ratio.

From these data it can be seen that the efficiency of sulphate removal increased with the increasing value of the COD/SO_4 ratio. Removal efficiencies higher than 90% were achieved at COD/SO_4 values exceeding 0.8. Below 0.8 the reduction of sulphates decreased noticeably. Thus, in terms of removal efficiency, the most advantageous values of the COD/SO_4 ratio ranged between 0.8 and 3.1. When COD/SO_4 was expressed in terms of C/S (table 1), the C/S values reported by DOMKA and GĄSIOREK [10] as the optimal ones were found to fall within the same range. In our study, the optimal value of C/S yielded a higher removal efficiency. And this is an indication that the wastewater from swine fattening proves to be a good substrate for *D. desulfuricans* bacteria.

Table 2

Efficiency of removal versus COD/SO_4

No. of reactor	COD/SO_4 (mg of O_2 dm^{-3}/ mg of SO_4 dm^{-3})	Incubation time (h)	Removal efficiency (%)
1	3.1	64	97.8
2	2.3	64	97.4
3	1.8	88	97.4
4	1.6	88	96.9
5	1.5	88	96.7
6	1.3	88	95.4
7	1.2	88	92.2
8	1	136	95.2
9	0.9	140	92.1
10	0.8	160	90.6
11	0.6	184	68.8
12	0.5	184	55.6
13	0.4	184	48.2

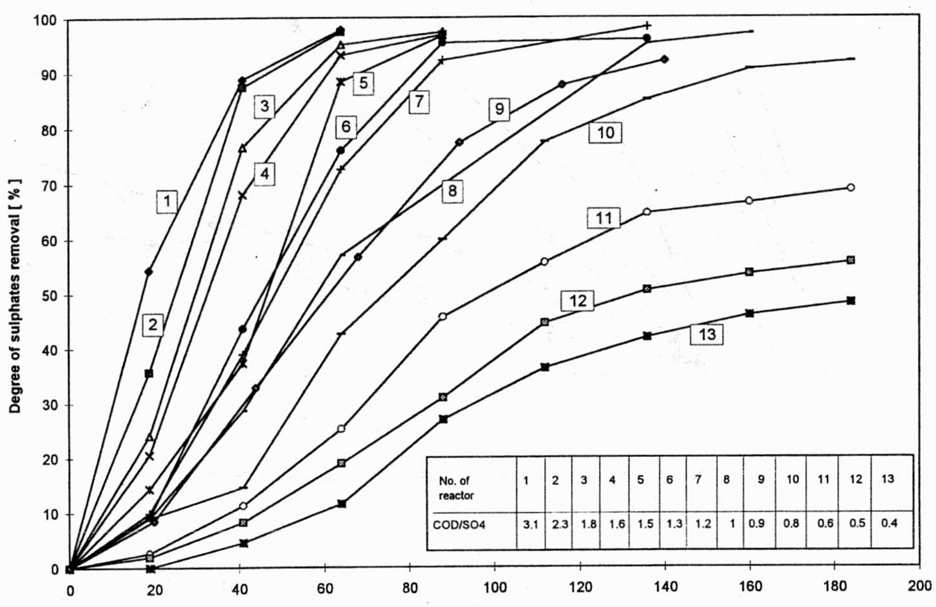

Fig. 2. Sulphate reduction at varying initial COD/SO_4 values

Figure 2 shows removal efficiency variations as a function of time. As shown by these plots, the initial value of the COD/SO_4 ratio contributed markedly to the biological desulphurication of sulphates. The time required to achieve a 90% removal efficiency approached 40 h in reactor No. 1 at COD/$SO_4 = 3.1$, and increased to

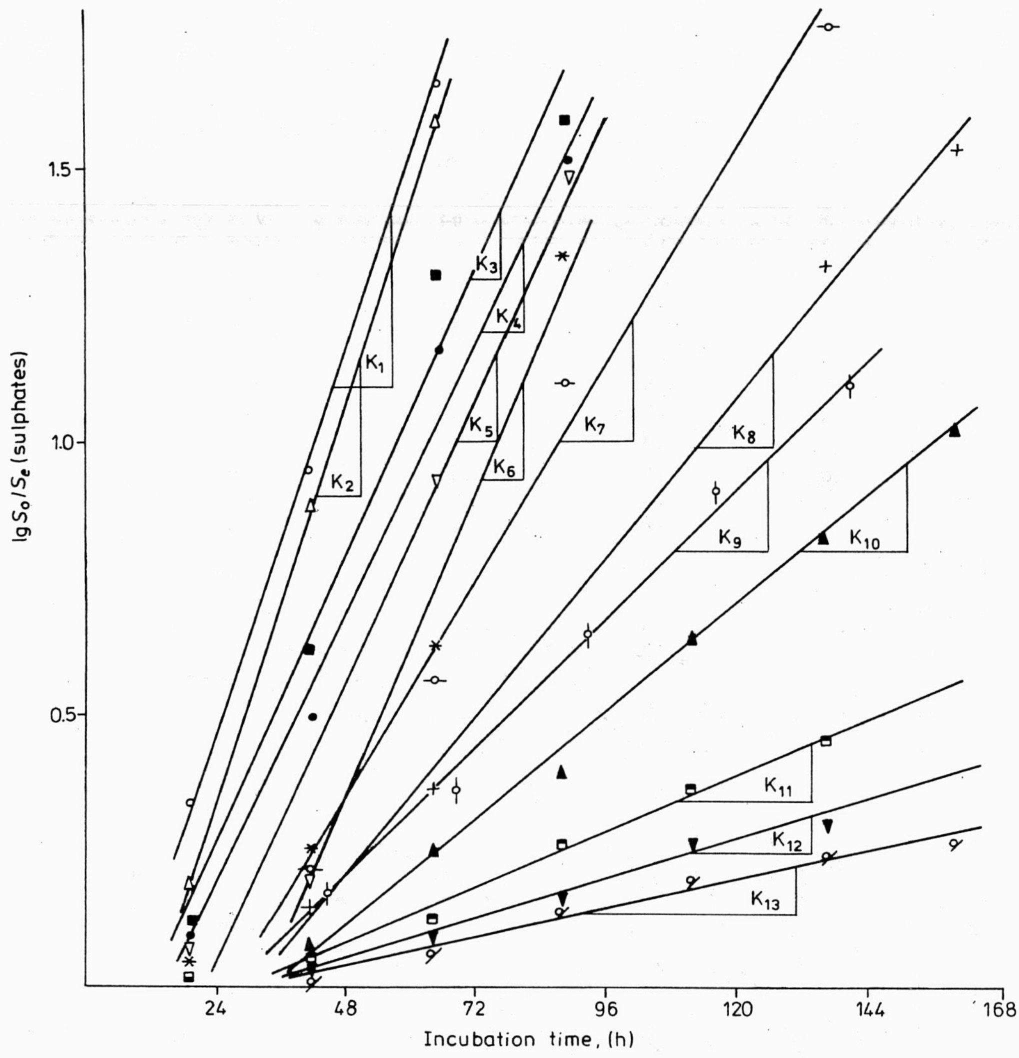

Fig. 3. Coefficients of specific rate for sulphate removal at varying COD/SO_4 ratio (calculated in terms of $S_0/S_e = e^{-Kt}$)

No. of reactor	1	2	3	4	5	6	7	8	9	10	11	12	13
COD/SO_4^{2-}	3.1	2.3	1.8	1.6	1.5	1.3	1.2	1	0.9	0.8	0.6	0.5	0.4

about 140 h in reactor No. 9 at $COD/SO_4 = 0.9$. When the COD/SO_4 values fell below 0.8, the reduction of sulphates was noticeably inhibited. This phenomenon (also reported by DOMKA and GĄSIOREK [10]) was likely to be associated with an insufficient content of organic carbon compounds in the medium; their oxidation provides electron supply to sulphur which acts as a final acceptor in the sulphate respiration process. Unlike Domka and Gąsiorek, we observed no inhibition of sulphate reduction at increased COD/SO$_4$ values. On the contrary, when the COD/SO$_4$ ratio increased, so did the reduction of sulphates. At COD/SO$_4$ = 3.1 (maximum value), the removal efficiency was the highest, amounting to 97.8%.

What seems to play an important role in the removal of sulphates is the fixation of hydrogen sulphide in the form of FeS. HILTON and OLESZKIEWICZ [16] showed that hydrogen sulphide concentration contributed considerably to the microbiological conversion of sulphur. The rise in H_2S concentration inhibited methanogenesis and favoured sulphate respiration at the same time. H_2S is also believed to affect biological desulphurication; yet, the problem is still far from being well understood.

Our experimental results were also interpreted in terms of the sulphate reduction rate, and the coefficient of specific rate k ($k = K \cdot 2.303$) appeared to be a useful tool. Table 3 relates the rate of sulphate removal to the COD/SO$_4$ ratio. Relevant plots are shown in figures 3 and 4.

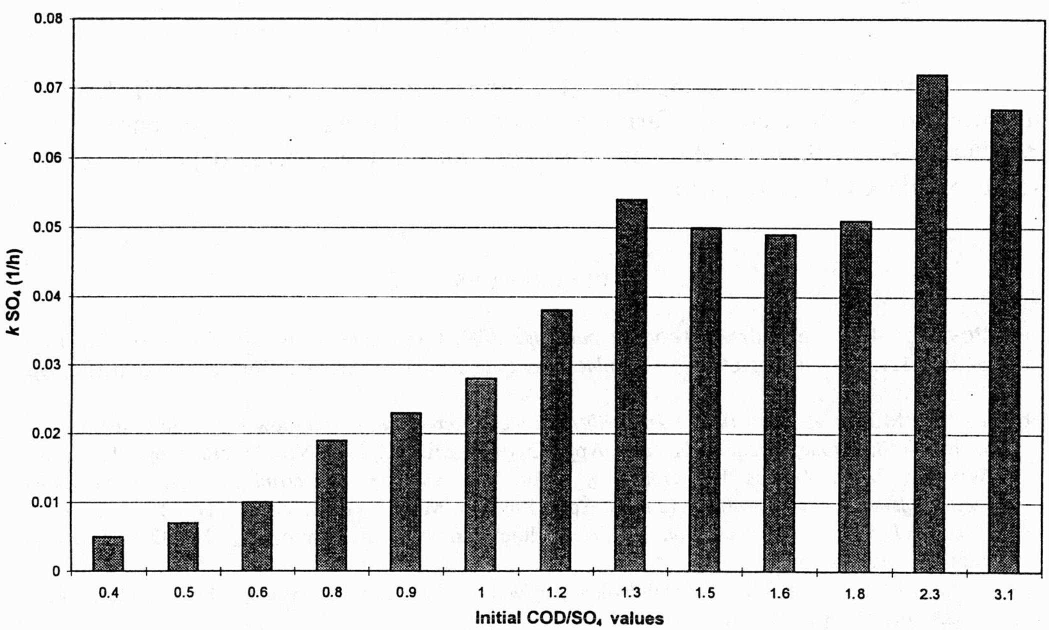

Fig. 4. Coefficients of specific rate for sulphate removal versus initial COD/SO$_4$ values

As shown by the data in table 3, sulphates were removed at the fastest rate when the COD/SO$_4$ ratio ranged between 1.3 and 3.1 (relevant values of the coefficient k varying from 0.049 to 0.072 h^{-1}). When the initial COD/SO$_4$ values fell below 1.3, the rate of removal was inhibited, and the half-period of reduction increased noticeably.

Table 3

Rates of sulphate removal by biological desulphurication

No. of reactor	COD/SO_4 (mg of O_2 dm^{-3}/ mg of SO_4 dm^{-3})	Time interval (h)	K (h^{-1})	k (h^{-1})	r^2	$t_{1/1}$ (days)
1	3.1	0–64	0.0293	0.067	0.999	0.43
2	2.3	19–64	0.0311	0.072	0.999	0.4
3	1.8	19–88	0.0221	0.051	0.975	0.57
4	1.6	19–88	0.0214	0.049	0.984	0.59
5	1.5	19–88	0.0218	0.05	0.951	0.57
6	1.3	41–88	0.0233	0.054	0.971	0.54
7	1.2	41–136	0.0165	0.038	0.99	0.76
8	1	41–160	0.0121	0.028	0.997	1.03
9	0.9	44–140	0.01	0.023	0.995	1.26
10	0.8	41–160	0.0081	0.019	0.997	1.54
11	0.6	41–136	0.0043	0.01	0.993	2.89
12	0.5	41–136	0.003	0.007	0.991	4.12
13	0.4	41–136	0.0024	0.005	0.984	5.25

The investigations revealed that desulphurication of sulphate-enriched liquid manure from industrial pig farming could be achieved in the presence of *D. desulfuricans* and that the efficiency and dynamics of the process depended on the value of the COD/SO_4 ratio.

REFERENCES

[1] POSTGATE J.R., *The sulphate-reducing bacteria*, 1984, Cambridge University Press, pp. 9–50.

[2] SCHIFF J.A., FRANKHAUSER H., *Assimilatory sulfate reduction*, Springer-Verlag, 1981, Berlin, pp. 153–198.

[3] BRYANT M.P. et al., *Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H_2-utilising methanogenic bacteria*, Appl. Environ. Microb., 1977, Vol. 33, No. 5, pp. 1162–1169.

[4] WINFREY M.R., ZEIKUS J.G., *Effect of sulfate on carbon and electron flow during microbial methanogenesis in fresh water sediments*, Appl. Environ. Microb., 1977, Vol. 33, No. 2, pp. 275–281.

[5] CORK D.J., *Gypsum bioconversion to sulphur*, Illinois Institute of Technology, 1982, Department of Biology, manuscript.

[6] SADANA J.C., MOREY A.V., *Microbiological production of sulfide from gypsum*, J. Scien. Industr. Res., 1962, Vol. 21c, pp. 124–127.

[7] BURGESS S.G., WOOD L.B., *Pilot-plant studies in production of sulphur from sulphate enriched sewage sludge*, J. Scien. Food Agric., 1961, No. 12, pp. 326–335.

[8] BROWN R.S., *Regeneration of scrubber effluent containing sulfate radicals*, 1980, US Patent No. 4,242,448.

[9] MACPHERSON R., MILLER J.D.A., *Nutritional studies on Desulfovibrio desulfuricans using chemically defined media*, J. Gen. Microbiol., 1962, Vol. 31, pp. 365–373.

[10] DOMKA F., GĄSIOREK J., *Effect of the concentration of available-carbon compounds on the microbial reduction of sulphates*, Acta Microbiol. Polonica, 1975, series B, Vol. 7(24), No. 2, pp. 97–101.

[11] DOMAGAŁA Z., DOMKA F., *Kinetic model of dissimilatory sulfate reduction*, Env. Prot. Eng., 1992, Vol. 18, No. 1–2, pp. 99–108.

[12] DOMAGAŁA Z., DOMKA F., *Estimation of the effect of Desulfotomaculum ruminis bacteria on the process of degradation of simple organic substrates*, Env. Prot. Eng., 1991, Vol. 17, No. 3–4, pp. 83–92.

[13] DOMKA F., GĄSIOREK J., *Activity of Desulfovibrio desulfuricans bacteria in hydrogen sulphide containing waters of the Busko Region*, Balneologia Polska, 1975, Vol. XX, No. 1–2, pp. 217–224.

[14] KOSIŃSKA K., MIŚKIEWICZ T., ILNICKA-OLEJNICZAK O., *Influence of temperature as well as the amount and type of inoculum on the sulphate respiration process for industrial pig farm liquid manure*, Env. Prot. Eng., 1995, Vol. 21, No. 1–4, pp. 83–99.

[15] OLESZKIEWICZ J.A. et al., *Optimization of wastes treatment with reference to biogas and protein recovery*, Report JB-5-535-7, 1981, US EPA.

[16] HILTON B.L., OLESZKIEWICZ J.A., *A comparison of anaerobic reactors operating with and without the addition of sulfates*, Water Poll. Res. J. Canada, 1987, Vol. 22, No. 3, pp. 444–455.

WPŁYW WSKAŹNIKA COD/SO_4 NA DESULFURYKACJĘ WZBOGACANYCH W SIARCZANY ŚCIEKÓW Z PRZEMYSŁOWEGO TUCZU TRZODY CHLEWNEJ Z UŻYCIEM BAKTERII *Desulfovibrio desulfuricans*

Bakterie *Desufovibrio desulfuricans* hodowano na ściekach z przemysłowego tuczy trzody chlewnej. Badano wpływ wartości COD/SO_4 w przedziale od 0,4 do 3,1 na efektywność i szybkość usuwania siarczanów. Wykazano, że usunięto ich ponad 90%, gdy COD/SO_4 był wyższy od 0,8. Stwierdzono, że im wyższy był COD/SO_4, tym skuteczniejszy był rozkład siarczanów i wyniósł on 97,8%, gdy COD/SO_4 = 3,1. Wykazano również, że wraz ze wzrostem COD/SO_4 zwiększała się także szybkość rozkładu siarczanów.