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INFLUENCE OF TEMPERATURE AS WELL AS THE AMOUNT AND TYPE OF INOCULUM ON THE SULPHATE RESPIRATION PROCESS FOR INDUSTRIAL PIG FARM LIQUID MANURE

A batch sulphate respiration process as a possible means for mutual reduction of sulphates and organics from industrial wastes was investigated. Experiments were carried out with *Desulfovibrio desulfuricans* bacteria and the industrial pig farm liquid manure enriched with ferrous sulphate as a source of sulphates. Special attention was paid to the effect of temperature and inoculum on the removal of sulphates and organics. It turned out that the best reduction of sulphates (more than 99%) and organics (72–77%) was achieved at the temperature of 38 °C and with inoculum from a bottom deposit of the reactor. The adaptation of *D. desulfuricans* to the substrate used was necessary.

1. INTRODUCTION

Microbial reduction of sulphates is one of the three anoxic respiration processes being recognized in natural environment in which oxidized forms of non-organic compounds such as sulphates, nitrates and carbonates are the final hydrogen acceptors. Nitric respiration (denitrification) and carbonate respiration (methanogenesis) are generally applied in the wastewater treatment as well as in the solid waste disposal and the sewage sludge processing. Sulphate respiration process has not found so far wide application due to the formation of metabolites such as sulphides and hydrogen sulphide which are toxic and useless for energy production.

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Facing, however, the steadily growing environmental pollution with natural sulphur compounds, their fate and the influence on natural ecosystem as well as the possibilities of their decomposition and neutralization in microbial processes are under study. Numerous investigations of the sulphate respiration process were carried out within the complex study of methanogenesis occurring in sewage sludge or industrial effluents. When studying the influence of sulphates on the methanogenesis course in freshwater bottom deposits, WINFREY and ZEIKUS [17] have found that sulphates, depending on their amount, inhibit formation of methane in the bacteria growing substrate. Thus, they have confirmed the results obtained by CAPPENBERG [6], who found earlier that sulphates in 0.1% concentration totally inhibited methanogenesis. Similarly, BRYANT et al. [2] have proved the inhibitory effect of sulphates on methane formation from the organic substrates in form of lactate, pyrogronate and ethanol. In every case, the addition of sulphate has considerably reduced or totally stopped formation of methane.

In the 1950s and 1960s, BUTLIN et al. [4], [5] and WONG [18] suggested the application of the sulphur dissimilative transformation to the industrial production of elementary sulphur. Butlin presented a microbial/chemical method which included microbial generation of H_2S from sulphates being followed by its chemical conversion into elementary sulphur. On the other hand, Wong developed an exclusively microbial method consisting in the decomposition of sulphates into H_2S during the sulphate respiration process followed by its oxidation into elementary sulphur by purple or green bacteria. Similar research was conducted by CORK [7] and SADANA and MOREY [16] who made the effort to apply the microbial conversion of gypsum into elementary sulphur using of *Desulfovibrio desulfuricans* and *Chlorobium thiosulphatophilum*. BURGESS and WOOD [3] have tested the possibilities of obtaining sulphur from sulphate-rich wastewater sludges by means of *Desulfovibrio desulphuricans*. BROWN [1] has patented a method of eliminating SO_2 from exhaust gases formed during fossil fuel combustion. According to this method, sulphates are formed from SO_2 in chemical way and next they are decomposed by *Desulfovibrio* into sulphides and hydrogen sulphide which are finally oxidized to elementary sulphur by *Beggiatoa* bacteria.

The sulphate salinity of industrial effluents limits the applicability of methanogenesis for their treatment. The aerobic methods of wastewater treatment do not allow the removal of sulphates which may disturb the operation of wastewater treatment plant at concentrations exceeding 2 g/dm^3. In the case of high concentrations of sulphates, supplementary chemical processes (e.g., their precipitation) might be necessary. The sulphate solid wastes, e.g., gypsum and phosphogypsum, also may present certain hazard for environment.

The investigations were carried out with the aim of recognizing both the possibilities and conditions of the sulphate respiration utilization for simultaneous removal of organics and sulphates in highly concentrated wastewater coming from the industrial pig farm.

The purpose the research was to obtain an active bacterial population able to reduce sulphates (via adaptation of the pure bacterial culture of *Desulfovibrio desufuricans* to non-sterile industrial waste). We also determined the bacterial population growth rate and generation time and estimated the effect of temperature as well as the amount and type of inoculum on the course of respiration process.

2. MATERIALS AND METHODS

Bacteria. Sulphate reducing bacteria (SRB) isolated from hydrogen sulphide springs of Busko Zdrój by GĄSIOREK and DOMKA [11] were used. SRB were stored and reproduced using Starkey's fluid lactate nutrient medium according to RODINA [15].

Substrates. The liquid manure coming from the industrial pig farm has been applied as a substrate. This type of wastewater is a rich source of easily biodegradable organics, nutrients and microelements. Since it does not contain its own oxidized sulphur forms, it was enriched with ferrous sulphate.

Methods of bacterial culture. The sulphate respiration process was conducted in a batch system, using air-tight glass reactors (active capacity of 2 dm^3). The reactors were filled up once with a selected substrate and a given amount of inoculum, and then incubated in a thermostate-controlled room. The gas pressure in the reactors was adjusted via a safety valve.

Analytical methods. The following parameters have been determined in the mixed samples:

- concentration of organic substrates as COD (after filtration), g O_2/dm^3;
- concentration of sulphates, using gravimetric method, g SO_4^{2-}/dm^3;
- dry mass of volatile suspended solids (VSS), using gravimetric method, g/dm^3;
- specific growth rate of *D. desulfuricans* was calculated from the formula:

$$m = (\lg X_2 - \lg X_1)/\lg e(t_2 - t_1) \tag{1}$$

where:

m – specific growth rate, h^{-1},
X_1, X_2 – VSS concentrations, mg/dm^3,
t_1, t_2 – time between subsequent measurements, h;

- generation time of SRB was calculated from the equation:

$$t_d = \ln 2/\mu \tag{2}$$

where t_d is the time of doubling the mass of bacteria, h or d;

- rate constants of organics and sulphates removal were determined graphically according to the equation:

$$S_0/S_t = e^{-Kt} \tag{3}$$

where:

S_0 – initial concentration of substrates, g O_2/dm^3 or g SO_4^{2-}/dm^3,
S_t – concentration of substrates over time t, g O_2/dm^3 or g SO_4^{2-}/dm^3,
K – rate constant of the substrate removal, h^{-1} or d^{-1},
t – time, h of d;

- influence of temperature on the rate of substrate removal was established from the Arrhenius equation:

$$k = A\mathrm{e}^{-E/RT} \tag{4}$$

where:

k – specific rate constant of substrate removal, h^{-1} or d^{-1},
A – directional slope coefficient (tg a), cal·mole^{-1},
E – activation energy, cal·mole^{-1},
R – universal gas constant, 1.987 cal·mole^{-1}·K^{-1},
T – absolute temperature, K.

3. RESULTS

3.1. PHYSICAL-CHEMICAL CHARACTERISTICS OF LIQUID MANURE

The raw liquid manure was conveyed from a farm and stored at a temperature of +5 °C. Basic pollution indices of the raw substrate are presented in table 1. Due to filtration the concentration of organics in the raw manure has been reduced by ca. 40%, which means that their major part is present in the colloidal and dissolved forms. About 55% of total nitrogen were present in the form of ammonium nitrogen, whereas 65% of total phosphorus were constituted by phosphates. The liquid manure, like Starkey's nutrient medium, provided sufficient supply of nutrients.

Table 1

Chemical characteristics of the raw liquid manure

Pollution indices	Value		
	Minimum	Maximum	Average
COD-nf, g O_2/dm^3	3.3	6.6	5.3
COD-f, g O_2/dm^3	1.6	3.9	2.9
Total nitrogen, g/dm^3	0.3	1.2	0.74
Ammonium nitrogen, g $N\text{–}NH_4/dm^3$	0.2	0.7	0.41
Total phosphorus, g/dm^3	0.31	0.56	0.4
Phosphates, g PO_4^{3-}/dm^3	0.13	0.4	0.27
Sulphates, g SO_4^{2-}/dm^3	0.15	0.2	0.16

nf = non-filtered.
f = filterd.

3.2. BACTERIA ADAPTATION

The aim of the bacteria adaptation to liquid manure was to obtain the population of a high physiological activity under conditions of non-sterile waste used. The first adaptation phase covered a series of successive passages of the pure *D. desulphuricans* culture on a modified, sterile Starkey's substrate. The modification consisted in replacing the lactate carbon in the nutrient medium with an equivalent amount of organic carbon occurring in the liquid manure. At this phase no chemical analysis was carried out. After a preliminary adaptation, SRB were passaged during the second phase onto a non-sterile substrate of the raw liquid manure enriched with up to 3 g of ferrous sulphate /dm^3. The activity of bacterial population was estimated basing on the rate of sulphate consumption. The results of the adaptation at the second phase are presented in figure 1. At the beginning, SRB population required 80 days to remove ca. 50% of sulphates from the substrate which contained about 1 g of SO_4^{2-} /dm^3. After adaptation, however, about 95% of sulphates were removed by the bacteria as soon as within 14 days. The bacterial population, which was subject to adaptation, removed sulphates with the efficiency reaching 86–95% at the time being reduced to 5–8 days.

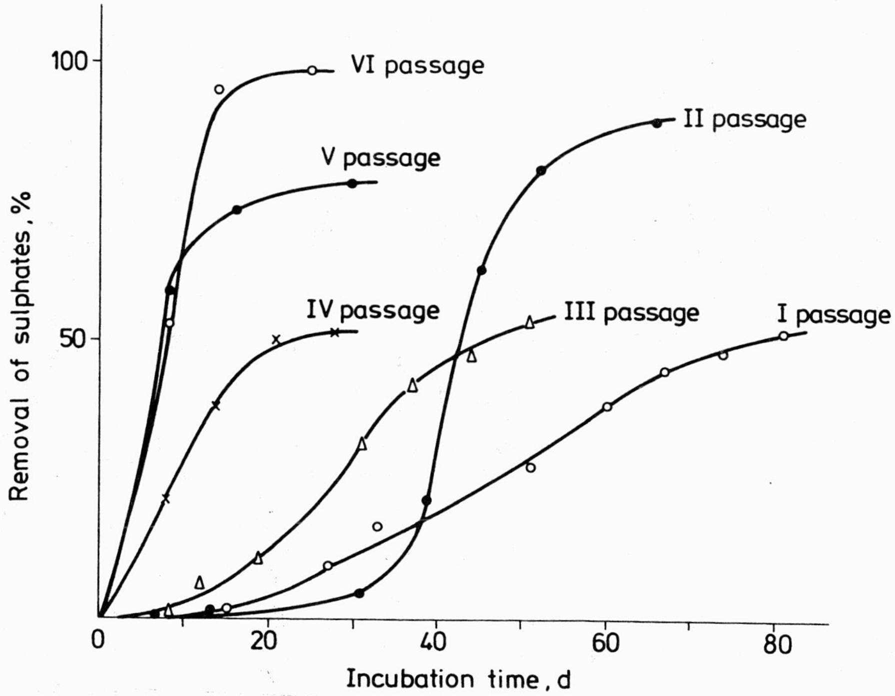

Fig. 1. Adaptation process of *D. desulfuricans* culture using a liquid manure from pig farm as a substrate

3.2. SPECIFIC GROWTH RATE OF POPULATION AND GENERATION TIME

Based on VSS analysis, the growth rate curve for SRB was plotted as presented in figure 2. It is possible to distinguish on the curve all the phases characteristic of microbe growth, i.e., lag (I) phase, phase of logarithmic growth (II), equilibrium phase (III) and decay phase (IV). The specific growth rate of the bacterial population under study in the logarithmic phase (calculated from the equation (1)) amounted to 0.00547 h^{-1} and proved that the growth of bacteria was very slow. The time necessary for doubling the mass of bacetria, as calculated from formula (2), totalled about 5.3 days.

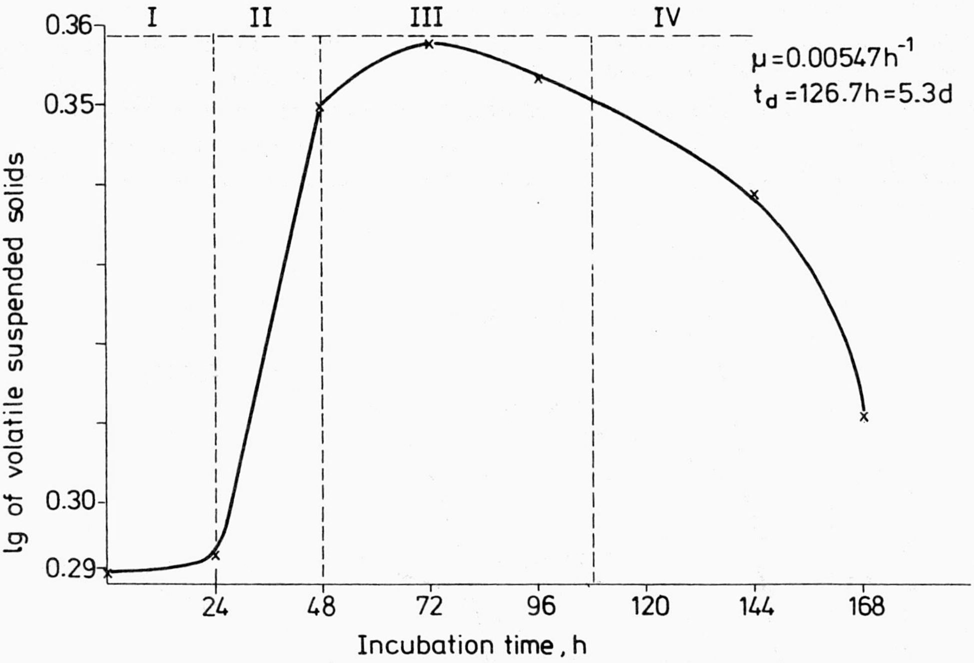

Fig. 2. Growth curve of bacteria population cultured on a liquid manure from pig farm during of the sulphate respiration process

3.4. EFFECT OF TEMPERATURE

The influence of temperature on the process and the rate of pollutant removal was studied over the temperature range from 20 °C to 45 °C. The variations of the amounts of both sulphates (*A*) and organics (*B*), depending on temperature, are presented in figure 3. At the temperature of 45 °C no changes in the amount of sulphates were noticed. Changes in the amount of organics have not, however,

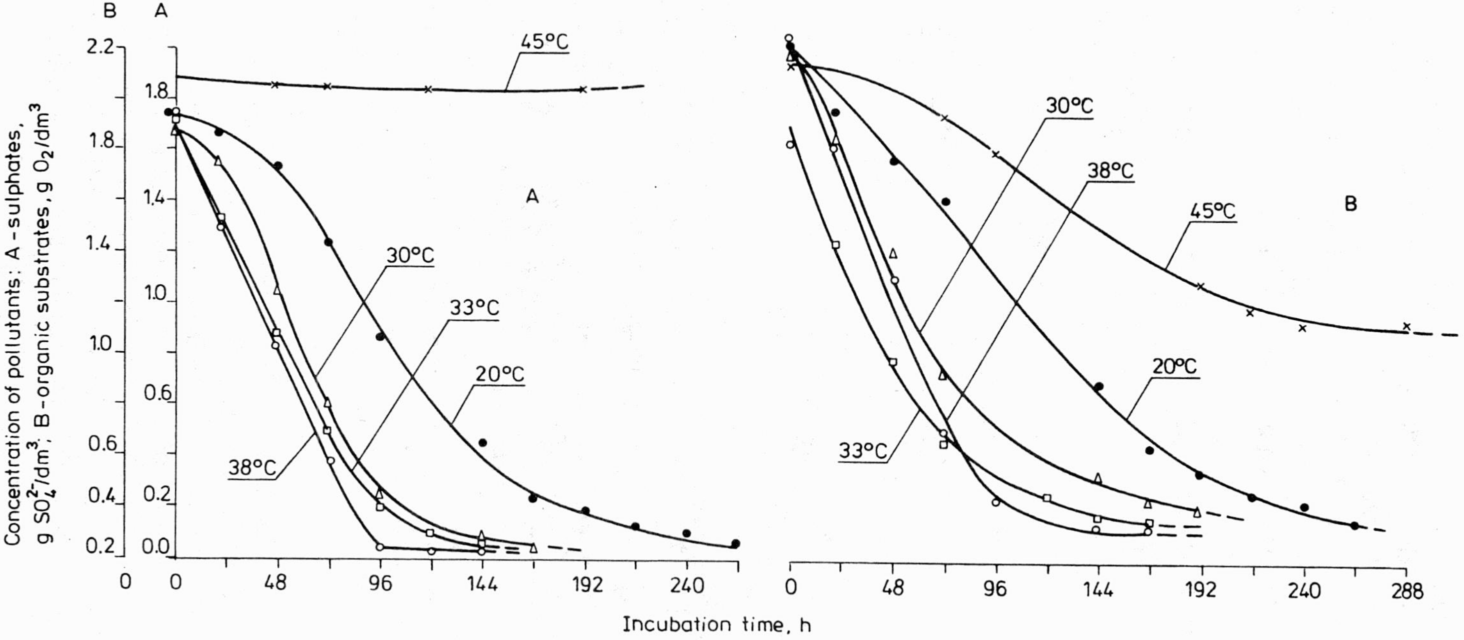

Fig. 3. Variations of the pollutants under study
during the batch process of sulphate respiration as a function of incubation temperature

resulted from sulphate respiration process, they were caused rather by the activity of another populations of thermophilic microbes in the liquid manure and inoculum, or by the denaturation of organics.

At the temperatures of 20–38 °C the concentrations of sulphates were reduced from 1.7 g of SO_4^{2-}/dm^3 to below 0.01 g of SO_4^{2-}/dm^3 (the removal exceeded 99%) over various periods of time. At the temperature of 38 °C the process was the slowest (96 h).

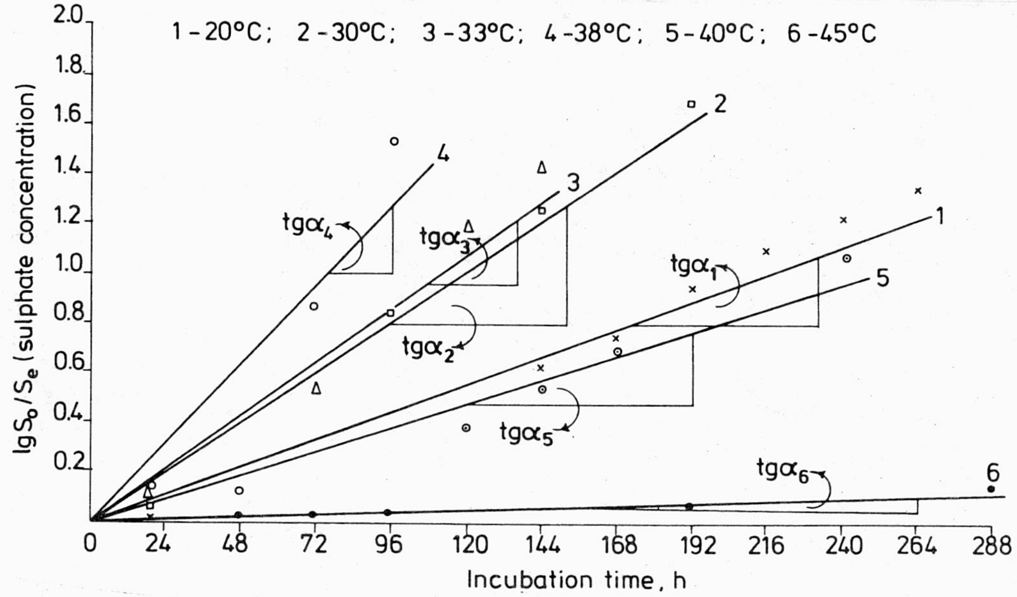

Fig. 4. Determination of the coefficients of specific decomposition rate (k) and half-life period of sulphates ($t_{1/2}$) for pig farm wastewater during the process of sulphate respiration at various temperatures (according to the model $S_0/S_e = e^{-Kt}$)

The similar influence of temperature was obserwed in the case of organic pollutants. The decrease in COD (from 1.8–2.2 g of O_2/dm^3 to below 0.5 g of O_2/dm^3, i.e., 72–77% removal) at the temperature of 38 °C was the fastest and lasted only 96 h.

The rates of the removal of sulphates and organics were also characterized by the constants of their removal rates and half-life periods (figures 4 and 5). The values of the parameters are given in table 2.

Temperature rose in the range of 20–38 °C which was connected with an about threefold increase in the rate of sulphate removal (from $k = 0.0127$ h^{-1} at the temperature of 20 °C to $k = 0.0366$ h^{-1} at 38 °C) and an almost twofold increase in the rate of COD removal (from $k = 0.0074$ h^{-1} to $k = 0.0161$ h^{-1} at the temperatures of 20 °C and 38 °C, respectively). The half-life period of substrates was reduced similarly: for sulphates from 2.27 d to 0.79 d and for organics from 3.9 d to 1.79 d at the temperatures of 20 °C and 38 °C, respectively. A temperature higher than 38 °C

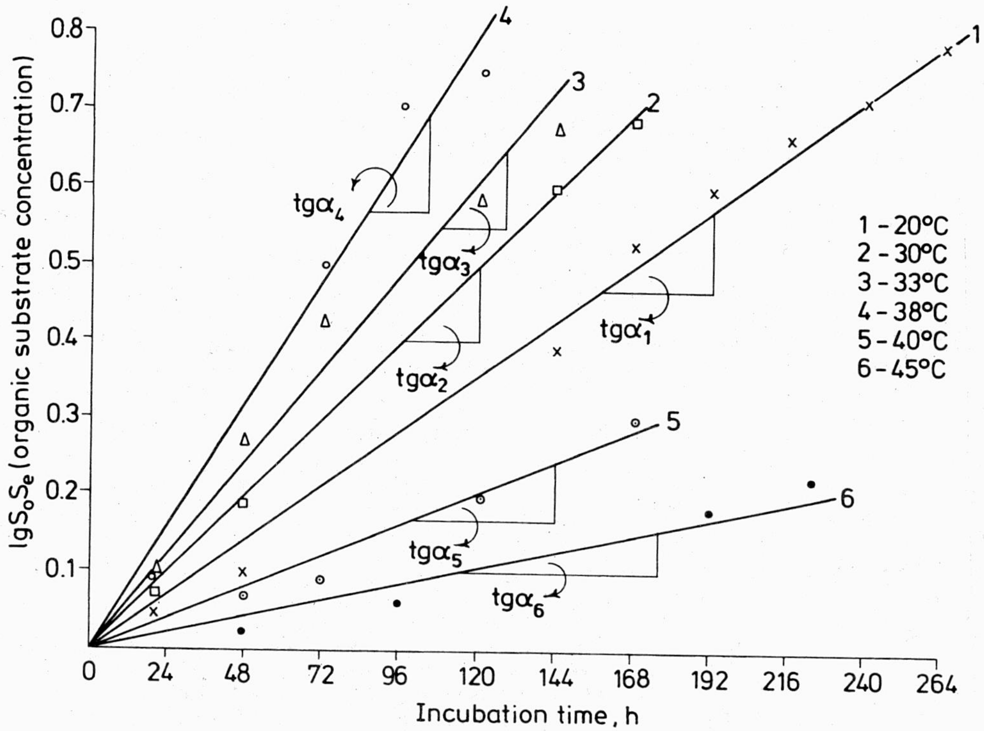

Fig. 5. Determination of the coefficients of specific decomposition rate (k) and half-life period of organic substrates ($t_{1/2}$) for pig farm wastewater during the process of sulphate respiration as a function of temperature (according to the model $S_0/S_e = e^{-Kt}$)

Table 2

Characteristic parameters of the substrate removal rate of sulphate respiration batch process at different temperatures according to the model $S_0/S_e = e^{-Kt}$

Process temperature	Pollutants	Process rate constant			
		K (h^{-1})	k (h^{-1})	$t_{1/2}$ (d)	r^2
20 °C	SO_4^{2-}	0.0055	0.0127	2.27	0.0975
	COD	0.0032	0.0074	3.9	0.99
30 °C	SO_4^{2-}	0.01	0.023	1.26	0.967
	COD	0.0041	0.0094	3.07	0.983
33 °C	SO_4^{2-}	0.0103	0.0237	1.22	0.977
	COD	0.0047	0.0108	2.63	0.981
38 °C	SO_4^{2-}	0.0159	0.0366	0.79	0.956
	COD	0.007	0.0161	1.79	0.968
40 °C	SO_4^{2-}	0.0047	0.0108	2.67	0.905
	COD	0.0018	0.0041	7.04	0.903
45 °C	SO_4^{2-}	0.0006	0.0014	20.63	0.97
	COD	0.001	0.0023	12.55	0.955

brought about an abrupt reduction of the rate of substrate removal, whereas the half-life period increased many times.

The influence of temperature on the rate of substrate removal according to Arrhenius' equation is shown in figure 6. It follows from it explicitly that at the temperatures exceeding 38 °C, the process of sulphate respiration was inhibited. For

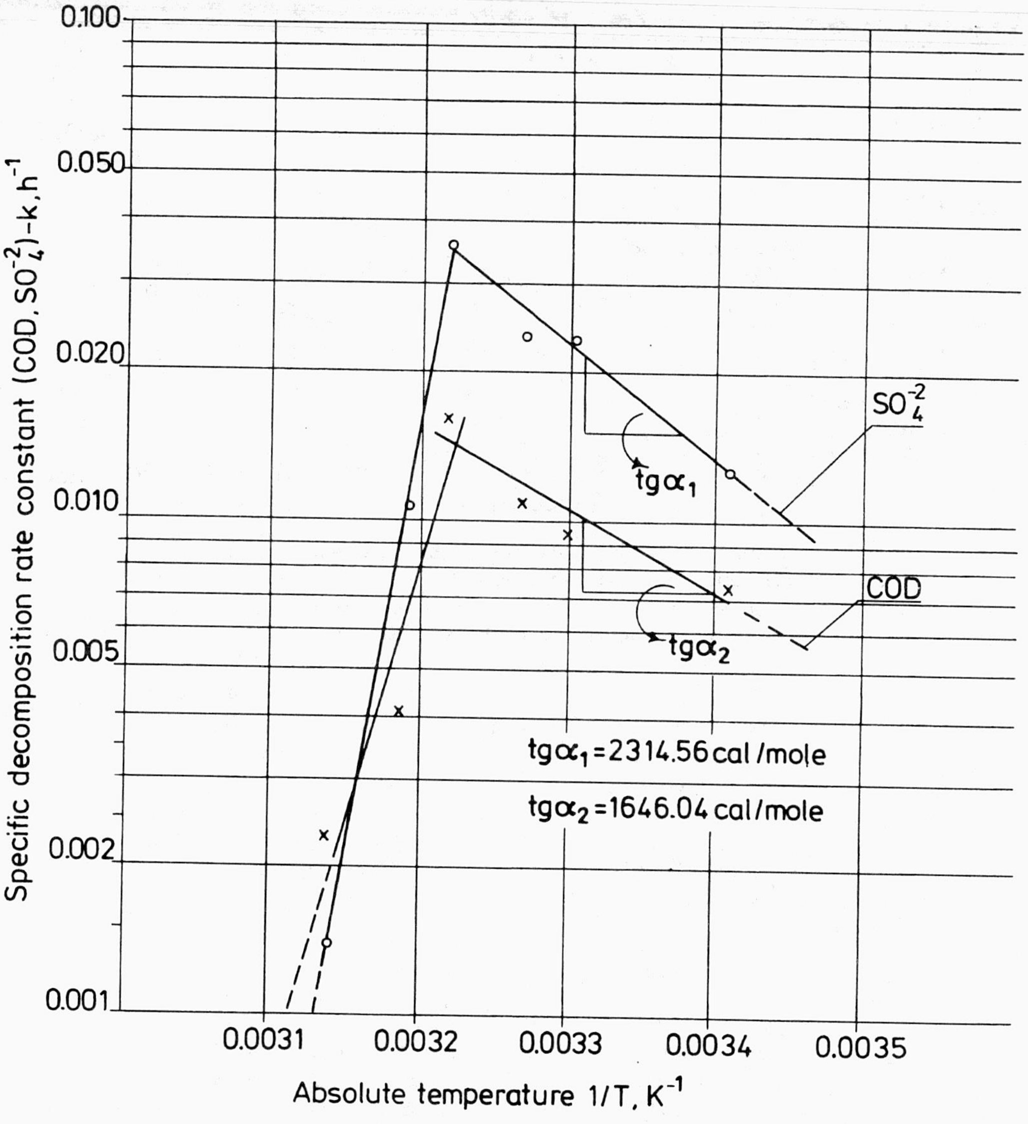

Fig. 6. Influence of temperature on removal dynamics of the pollutants under study from pig farm liquid manure during the batch process of sulphate respiration (according to Arrhenius' equation)

the temperatures within 20–38 °C, activation energy for sulphates amounted to 2314.6 cal·mole^{-1}, whereas that for organics (COD) being equal to 1646 cal·mole^{-1}. Further research was carried out at the temperature of 38 °C.

3.5. EFFECT OF THE AMOUNT AND TYPE OF INOCULUM

Variations in sulphate (*A*) concentrations and COD (*B*), depending on the initial amount of inoculum, are presented in figure 7. The curves 1–5 corresponded successively to the following amounts of biomass in the inoculum: 0 g of VSS/dm^3, 0.405 g of VSS/dm^3, 1.62 g of VSS/dm^3, 3.23 g of VSS/dm^3 and 5.39 g of VSS/dm^3. In the reactors 2–5, the sulphate concentrations were decreased from 1.7–1.8 g of SO_4^{2-}/dm^3 to the values lower than 0.01 g of SO_4^{2-}/dm^3 (over 99% reduction) in different time; however, they depended on the initial amount of inoculum. The shortest time for sulphate removal (96 h) was noticed in the reactor with the highest biomass concentration (VSS equal to 5.39 g/dm^3). The reduction of organics (COD) from 1.7–1.9 g of O_2/dm^3 to the values lower than 0.5 g of O_2/dm^3 (reduction by about 70%) was noticed simultaneously in the same reactors at different time periods, depending on the initial amount of inoculum. In the control reactor (without

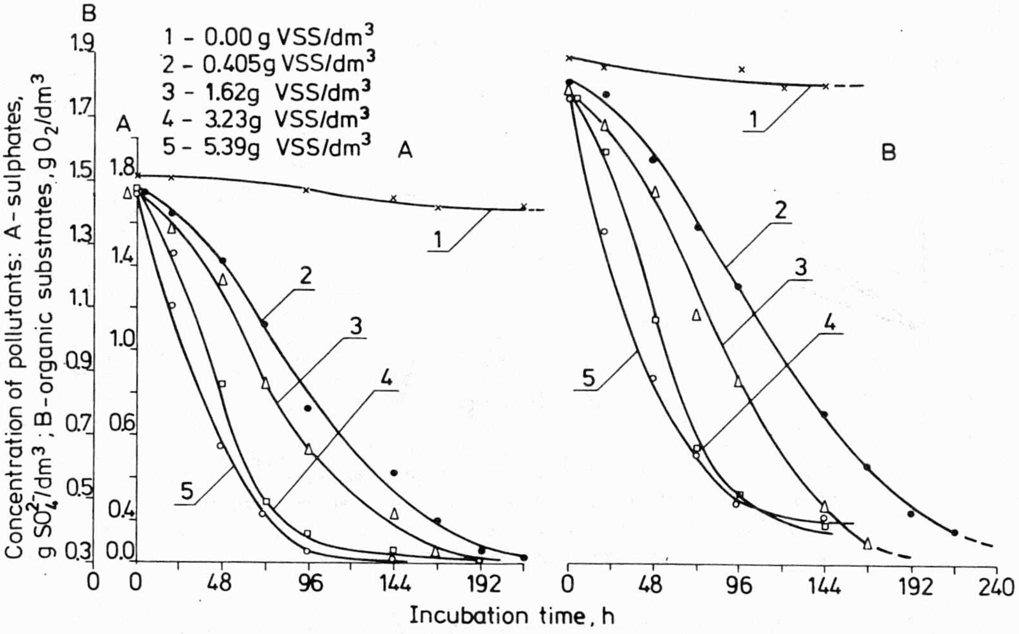

Fig. 7. Variations of pollutants under study during the batch process of sulphate respiration as a function of the inoculum amount (at 38 °C)

inoculum), no visible changes in the concentration of both pollutants under study were noticed.

The dynamics of sulphate removal, depending on the inoculum amount, was determined by calculating the coefficients of specific removal rate and half-life periods. These relationships are presented in figure 8 (sulphates) and figure 9 (organic substrates). The values of coefficients are included in table 3.

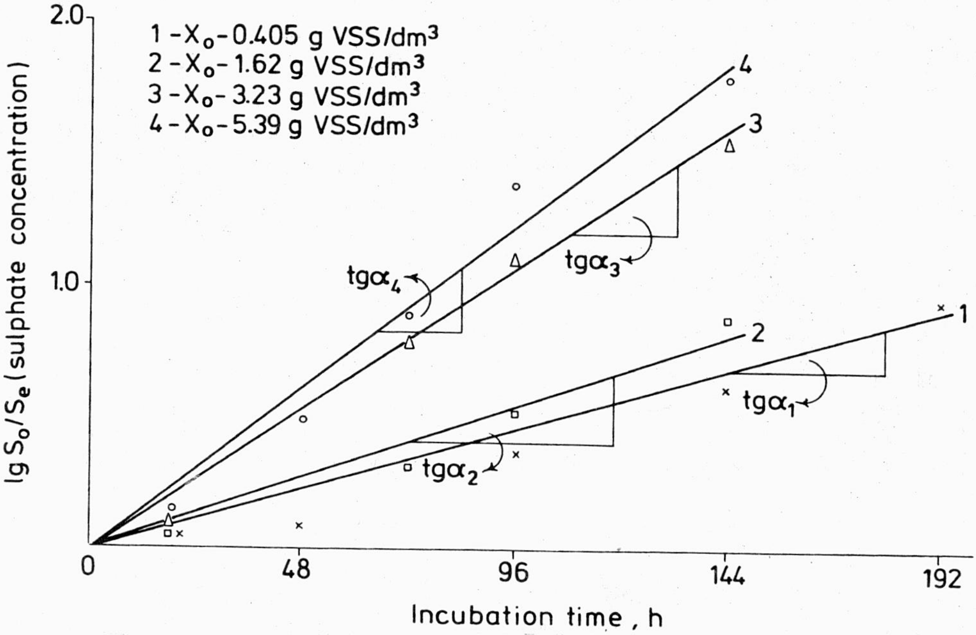

Fig. 8. Determination of the coefficients of specific decomposition rate (k) and half-life period of sulphates ($t_{1/2}$) during the batch process of sulphate respiration as a function of the inoculum amount (at 38 °C, according to the model $S_0/S_e = e^{-Kt}$)

The increase in the inoculum amount took place together with an almost threefold increase in the rate of sulphate removal (from $k = 0.0127$ to $k = 0.0341$ h^{-1}) and with an almost twofold increase in the rate of COD removal (from $k = 0.0069$ to $k = 0.0138$ h^{-1}). It was accompanied by the respective decrease in the half-life periods (from 2.27 to 0.85 d for sulphates, and from 4.18 to 2.1 d for COD). These results proved that the amount of inoculum applied and the rate of sulphate respiration measured are interrelated.

Sulphate respiration depended also on the inoculum type. Two types of inoculum were tested. The first one was a bottom deposit from the inoculum container, whereas the other one was supernatant from the container. The results obtained

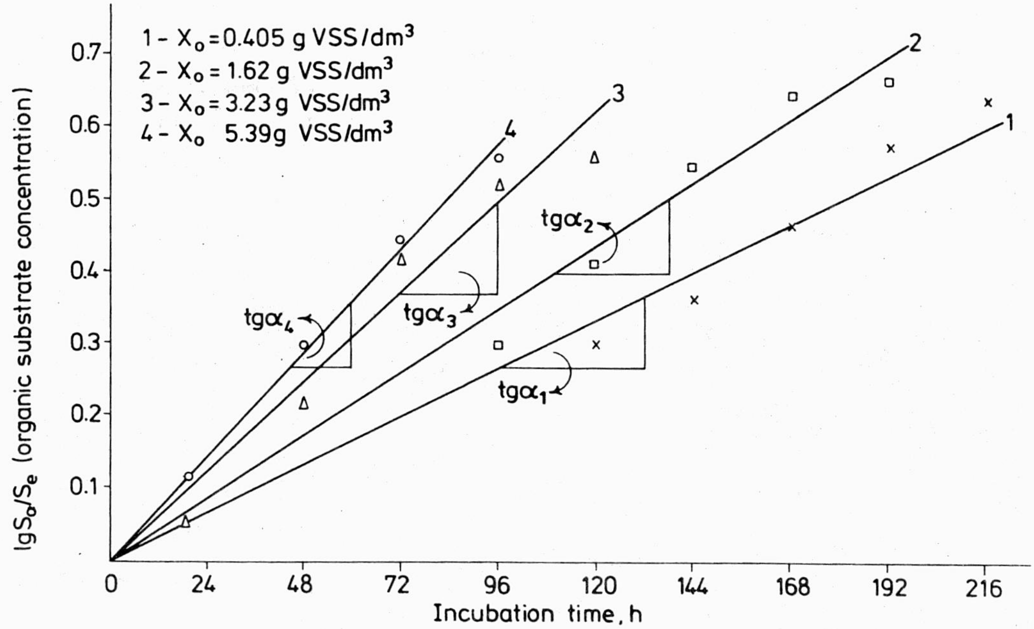

Fig. 9. Determination of the coefficients of specific decomposition rate (k) and half-life period of organic substrates ($t_{1/2}$) for pig farm wastewater during the batch process of sulphate respiration as a function of the inoculum amount (at 38 °C, according to the model $S_0/S_e = e^{-Kt}$)

Table 3

Characteristic parameters of the substrate removal rate from the liquid manure applying batch process of sulphate respiration at different amounts of inoculum (temperature of 38 °C, model $S_0/S_e = e^{-Kt}$)

Parameters	Initial amount of inoculum (g VSS/dm³)							
	5.39		3.24		1.62		0.4	
	SO_4^{2-}	COD	SO_4^{2-}	COD	SO_4^{2-}	COD	SO_4^{2-}	COD
K (h^{-1})	0.0148	0.00597	0.0118	0.0051	0.0064	0.0038	0.0055	0.0030
k (h^{-1})	0.0341	0.0136	0.0272	0.0117	0.0147	0.0088	0.0127	0.069
$t_{1/2}$ (d)	0.85	2.1	1.06	2.47	1.96	3.28	2.27	4.18
r^2	0.965	0.996	0.972	0.922	0.957	0.977	0.935	0.977

using these inocula are presented in figure 10 and in table 4. It appeared that inoculum from the bottom deposit was much more active because of higher number of SRB present in this type of inoculum than in the supernatant.

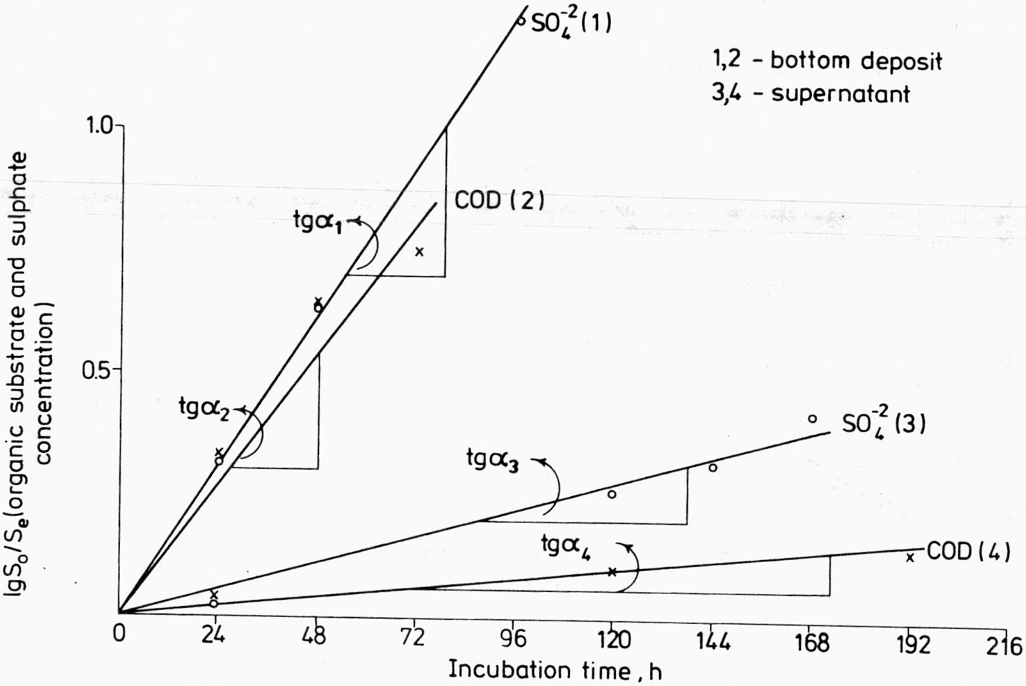

Fig. 10. Determination of the coefficients of specific decomposition rate of the pollutants under study (k) during the batch process of sulphate respiration using different types of inoculum (at 38 °C, according to the model $S_0/S_e = e^{-Kt}$)

Table 4

Characteristic parameters of the sulphate respiration process for liquid manure applying different inoculum types (batch process at temperature of 38 °C, model $S_0/S_e = e^{-Kt}$)

Parameters	Inoculum type			
	Bottom deposit		Supernatant	
	COD	SO_4^{2-}	COD	SO_4^{2-}
K (h^{-1})	0.0107	0.0128	0.00074	0.0025
k (h^{-1})	0.0246	0.0295	0.0017	0.0058
$t_{1/2}$ (d)	1.17	0.98	16.99	4.98

4. DISCUSSION

The research conducted has proved that bacteria adaptation period was unavoidable due to the application of a substrate whose chemical composition was not defined. The adaptation has increased considerably the activity of *D. desul-*

furicans population. It was very important in the case of applying these bacteria to industrial wastewater treatment. This problem was not often discussed in the literature concerning sulphate respiration process because pure substrates readily degradable by SRB (e.g., lactate, pyrogronate, acetate, ethanol) were applied, for instance, by BRYANT et al. [2], DOMKA and GĄSIOREK [8], FORSBERG [9], GĄSIOREK and DOMKA [11], MACPHERSON and MILLER [12] and MILLER and HUGHES [14].

Growth of the population adapted to the liquid manure substrate was slow and its generation time – long ($m = 0.0055$ h^{-1}, $t_d = 5.3$ d). According to GAJKOWSKA-STEFAŃSKA [10] it is characteristic of many anoxic and anaerobic microbes and specially of those involved in sugar and spirit industry wastewater treatment. The parameters of the biomass growth dynamics (growth rate coefficient, population generation time) are very essential as their values affect the operation of flow-through wastewater treatment systems. Inappropriate values of these parameters can be the cause of washing out the biomass.

Temperature is a key parameter of the process. Its increase up to 38 °C accelerates organics removal and sulphate decomposition, afterwards the processes slow down abruptly. Hence the temperature of 38 °C may be suggested as the optimal for the sulphate respiration process. This conclusion is not contradictory to the statement of MIDDLETON and LAWRENCE [13] who have suggested that the process goes on at its best at the temperature of 31 °C. But they did not investigate the process at higher temperatures. GĄSIOREK and DOMKA [11] also dealt with the problem of the temperature influence on the microbial reduction of sulphates. In that case, the temperature of 35 °C proved to be the best. The presented results suggest that the sulphate respiration belongs to mesophyll processes, which may have an influence on its application cost.

Inoculum was another essential factor influencing the rate of the process. Its amount applied to initiate the process evidently influenced the rate of removal of sulphates and organic substrates. It is particulary important in the case of the population being used which was characterized by a slow growth and long generation time. In such a case, it is necessary to use a large amount of highly active inoculum, i.e., inoculum from the bottom deposit rather than from the supernatant. In the light of the results obtained, the application of inoculum in the form of supernatant, which is reported by MIDDLETON and LAWRENCE [13], is controversial.

In this paper, an attempt was made to study the sulphate respiration process. Such a process allowed removal of organics and sulphates. It turned out that removal of both those pollutants was convergent which may have an advantageous practical meaning. *D. desulfuricans* bacteria may be used not only to the reduce the sulphates but, at the same time, to eliminate organic pollutants as well. The possibility of their simultaneous removal and a high efficiency of the process are so attractive that the research is worth continuing.

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WPŁYW TEMEPRATURY, WIELKOŚCI I RODZAJU INOKULUM NA PRZEBIEG PROCESU ODDYCHANIA SIARCZANOWEGO W ŚCIEKACH Z PRZEMYSŁOWEGO TUCZU TRZODY CHLEWNEJ

Badano możliwość wykorzystania oddychania siarczanowego jako sposobu jednoczesnego usuwania siarczanów i związków organicznych ze ścieków przemysłowych w warunkach statycznych. Do badań stosowano bakterie *Desulfovibrio desulfuricans* i ścieki z przemysłowego tuczu trzody chlewnej wzbogacone w siarczan żelazawy jako źródło siarczanów. Szczególną uwagę zwrócono na wpływ temperatury oraz inokulum na stopień usuwania siarczanów i związków organicznych. Okazało się, że najwyższą redukcję siarczanów (ponad 99%) i związków organicznych (72–77%) uzyskano, gdy temperatura wynosiła 38 °C, a inokulum pochodziło z osadu w reaktorze. Wykazano, że w badanym procesie konieczna była adaptacja *Desulfovibrio desulfuricans* do stosowanych substratów.

ВЛИЯНИЕ ТЕМПЕРАТУРЫ, ВЕЛИЧИНЫ И ВИДА ИНОКУЛУМ НА ПРОТЕКАНИЕ ПРОЦЕССА СУЛЬФАТНОГО ДЫХАНИЯ В СТОЧНЫХ ВОДАХ ИЗ ПРОМЫШЛЕННОГО ОТКАРМЛИВАНИЯ СВИНЕЙ

Исследована возможность использования сульфатного дыхания как способа одновременного удаления сульфатов и органических соединений из промышленных сточных вод в статических условиях. Для исследований были использованы бактерии *Desulfovibrio desulfuricans* и сточные воды из промышленного откармливания свиней, обогащенные сульфатом железа как источником сульфатов. Особенное внимание было обращено на влияние температуры, а также инокулум на степень удаления сульфатов и органических соединений. Оказалось, что самой большой редукции сульфатов (свыше 99%) и органических соединений (72–77%) достигнули тогда, когда температура составляла 38 °Ц а инокулум происходило из осаждений в реакторе. Было обнаружено, что в исследуемом процессе необходимой была адаптация *Desulfovibrio desulfuricans* для применяемых исходных веществ.