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CONTENTS

LILIANNA BARTOSZEK*, JANUSZ A. TOMASZEK*

ANALYSIS OF THE SPATIAL DISTRIBUTION OF PHOSPHORUS FRACTIONS IN THE BOTTOM SEDIMENTS OF THE SOLINA–MYCZKOWCE DAM RESERVOIR COMPLEX

The statistical analysis was performed to compare average contents of phosphorus fractions in the bottom sediments between the lacustrine zone and the riverine zone in the Solina Reservoir, between the main object and the retarding one as well as between all the research stations of the analysed reservoirs. It was found that the deposits from the stations localized in the lacustrine zones of both reservoirs did not differ significantly in regards to total phosphorus content, inorganic phosphorus, and non-apatite, inorganic phosphorus fractions, however, they were characterized by significantly different contents of organic phosphorus.

1. INTRODUCTION

The concentrations of biogenic substances determine trophic conditions of a reservoir [1–3]. High content of phosphorus in the bottom sediments testifies to intensive primary production that is connected with high trophy of waters.

Phosphorus compounds deposit in the bottom sediments of reservoirs due to sedimentation processes. Along the axis of dam reservoirs, the following zones can be distinguished: the riverine, transitional and lacustrine zones differing in phosphorus circulation. In the riverine zone, mainly sedimentation of phosphorus compounds, associated with thick mineral and organic suspension, as well as dragging of deposit deep inside the reservoir over the bottom take place. Sedimentation of little loamy and organic particles, containing phosphorus, occurs together with a decrease of the water flow speed in the transitional zone. The conditions in this zone, decreasing water flow, and a high content of mineral salts in water are favourable to primary production. Periodic internal supply of phosphates can occur in this zone because of fine-grained deposit easily undergoing resuspension. Biological circulation and sedimentation with

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decayed organic remains as well as periodic release of the dissolved phosphates from the sediments, in favourable conditions, are considered significant elements of the phosphorus circulation within the dam in the lacustrine zone [4, 5].

Decomposition processes are intensive in the bottom sediments. Emission and movement of the released phosphates into other layers and zones of the reservoir can be stimulated by currents in the active bottom zone, by weak water movements in the metalimnion and hypolimnion, by fish activity, bentos, and bentoplancton as well as by gases released from the deposits [6]. Variability of phosphorus respective fractions in different places of the sediments can reflect differences in the sources of phosphorus. Relatively more iron-phosphorus compounds are met in the deposits of these reservoirs which are more subject to greater inflow of sewage [7, 8].

The aim of the work was to analyse the spatial distribution of the phosphorus fraction content in the bottom sediments of the upper San dam reservoirs complex (the main reservoir – the retarding one).

2. EXPERIMENTAL

The Solina Reservoir is the most voluminous and the deepest body of water behind a dam in Poland. Together with the Myczkowce Reservoir it forms a cascade (Fig. 1) that serves the complex of Hydroelectric Power Stations of Solina –Myczkowce S.A. The Solina–Myczkowce complex of dam reservoirs is constituted of two bodies of water that are very different in terms of their morphometric parameters (Table 1). The waters of the San River (of which 90% are derived from the hypolimnion of the Solina Reservoir) form the main tributary feeding the Myczkowce Reservoir [9].

The samples of bottom sediment were collected at four stations around the Solina Reservoir, i.e. : Centralny (1), Zapora (2), Brama (3), Skałki (4), of average depths of ca. 45, 55, 14 and 15 m, respectively, as well as at two stations in the Myczkowce Reservoir, i.e. Myczkowce Zapora (5), Myczkowce Zabrodzie (6). of approximate depths of 11 and 3 m, respectively. Sampling was carried out 1–2 times a month from May to November 2005 (9 samples) and once a month (except in May) between April and November 2006 (7 samples). The 0–5 cm superficial layer was taken for analysis, averages being calculated for three sediment cores sampled with a gravity corer. The interstitial water was separated by centrifugation (at 4000 r.p.m.). The obtained residue was air dried at room temperature and at 60 °C, and then ground and sieved. The fraction of <0.9 mm grain size was stored for examination in hermetically closed PE bags at a temperature of 4 °C in the dark. The harmonized SMT protocol was applied in analysing the fractionation of phosphorus in the sediments [10–12]. The fractions obtained were as follows: inorganic phosphorus (IP), organic phosphorus (OP), apatite phosphorus (AP, calcium-associated forms) and non-apatite inorganic phosphorus

(NAIP, the forms associated with oxides and hydroxides of Al, Fe and Mn). The bottom sediments were mineralized in concentrated HNO_3 (microwave digestion method – UniClever II Plazmatronika). Phosphorus forms in the solutions of extracts and mineralized bottom sediments were analysed colorimetrically in accordance with the PN-EN 1189:2000 standard. An Aquamate spectrophotometer (Thermo Spectronic, UK) was used for colorimetric determinations.
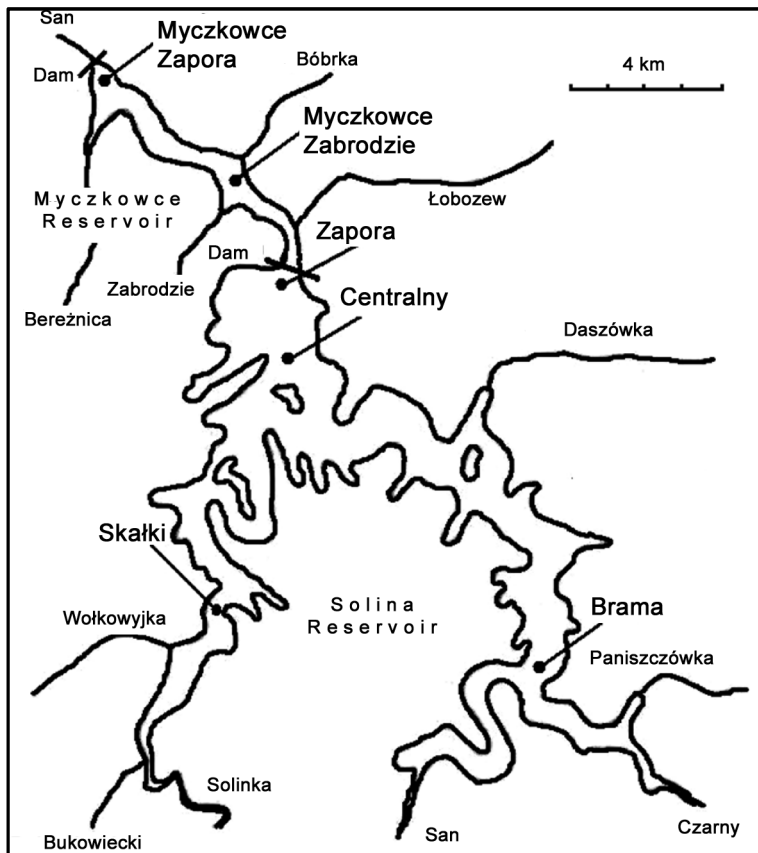
Fig. 1. Sampling locations at the Solina and Myczkowce Reservoirs

The t-Student test, the test of Cochran–Cox (the so called t test with a separate variance analysis) and the nonparametric test of Kolmogorov–Smirnov were applied to compare the average values of the two groups. The variance analysis (ANOVA – the normality test of Shapiro–Wilk, the test of Levene of variance uniformity, Fisher–Snedecor's test, the parametric test of Scheffe and the nonparametric test of Kruskal–Wallis) was used to evaluate the differences between the average values in a few groups, at the assumed significance level $\alpha = 0.05$ [13].

Morphometric parameters of the cascade of the Solina–Myczkowce Reservoirs

Parameter	Solina Reservoir	Myczkowce Reservoir
Area [ha]	2200	200
Maximum volume [Mm^3]	502	10
Average depth (max) [m]	22 (60)	5 (15)
Catchment area [km^2]	1174.5	1248
Hydraulic retention time [d]	155–273	2–6

3. RESULTS AND DISCUSSION

The analysis of phosphorus fractionation in the bottom sediments with the SMT method allowed one to separate four fractions: NAIP, AP, OP and IP. The content of the NAIP fraction containing the forms associated to oxides and hydroxides of Al, Fe and Mn ranged from 0.096 to 0.316 mg P·g^{-1} of d.w. in the sediments of the Solina Reservoir and from 0.123 to 0.331 mg P·g^{-1} of d.w. in the sediments of the Myczkowce Reservoir (Table 2).

The content of the AP fraction (the forms associated with calcium) varied in a rather wide range from 0.199 to 0.414 mg P·g^{-1} of d.w. in deposits of the Solina Reservoir in comparison to the range of this fraction from 0.255 to 0.332 mg P·g^{-1} of d.w. in deposits of the Myczkowce Reservoir. Organic phosphorus – OP, the fraction covering all organic compounds in which phosphorus can occur in the bottom sediments, varied in a similar range from 0.189 to 0.385 mg P·g^{-1} of d.w. and from 0.188 to 0.327 mg P·g^{-1} of d.w. (Solina, Myczkowce respectively). The IP fraction (inorganic phosphorus) informs on a general content of inorganic compounds with phosphorus. The IP fraction content ranged from 0.407 to 0.685 mg P·g^{-1} of d.w. in the Solina Reservoir and from 0.446 to 0.644 mg P·g^{-1} of d.w. in the Myczkowce Reservoir.

The average content of the NAIP, OP and IP fractions showed the trend of increase similarly as the average of total phosphorus with the depth of the reservoirs from which the sediments were collected. The statistical analysis (based on the tests: Cochran–Cox and Kolmogorov–Smirnov) confirmed the significance of differences (p <0.05) of the average contents of the above mentioned phosphorus fractions between the deposits of zones: the lacustrine zone and the zone being under the influence of tributaries in the shallower parts of the Solina Reservoir.

A detailed test of Kruskal–Wallis, on account of similar values of the average contents of NAIP fractions, distinguished two groups of sediments: (1) at the Centralny, Zapora and Myczkowce Zapora stations as well as (2) at the Skałki and Myczkowce Zabrodzie stations. The deposits collected at the Brama station placed themselves, from the point of view of similarities, between these groups, not showing statistically

significant differences with respect to the sediments from the stations: Centralny and Skałki as well as Myczkowce Zabrodzie (Fig. 2).

T a b l e   2

Content of phosphorus and its fractions [mg P·g⁻¹ d.w.] and fractions contribution [%]
in P_{tot} in the bottom sediments of the Solina – Myczkowce Reservoirs

Station		$P_{tot.}$	NAIP		AP		OP		IP	
		[mg P·g⁻¹ d.w.]	[mg P·g⁻¹ d.w.]	[%]	[mg P·g⁻¹ d.w.]	[%]	[mg P·g⁻¹ d.w.]	[%]	[mg P·g⁻¹ d.w.]	[%]
Centralny $n = 16$	average	0.912	0.237	26.0	0.320	35.2	0.320	35.2	0.561	61.7
	median	0.894	0.235	25.6	0.333	36.6	0.311	34.5	0.570	62.4
	minimum	0.826	0.207	20.5	0.206	21.9	0.286	29.6	0.457	55.3
	maximum	1.009	0.316	33.5	0.370	40.3	0.385	44.5	0.603	66.1
	S.D.	0.05	0.03	3.0	0.05	5.3	0.03	3.6	0.04	3.5
Zapora $n = 15$	average	0.931	0.263	28.3	0.320	34.2	0.323	34.8	0.588	63.0
	median	0.928	0.262	27.8	0.350	36.2	0.326	33.4	0.594	64.5
	minimum	0.849	0.224	23.1	0.199	22.6	0.297	30.4	0.506	56.7
	maximum	1.014	0.306	34.8	0.414	40.8	0.365	39.7	0.685	69.9
	S.D.	0.05	0.03	3.1	0.06	5.5	0.02	3.0	0.06	4.0
Brama $n = 16$	average	0.857	0.196	22.9	0.339	39.7	0.291	34.0	0.537	62.7
	median	0.861	0.194	22.9	0.338	40.2	0.290	34.1	0.541	63.4
	minimum	0.766	0.153	17.6	0.288	31.1	0.245	30.8	0.481	55.6
	maximum	0.929	0.236	27.7	0.387	47.9	0.347	37.4	0.567	67.6
	S.D.	0.05	0.02	2.4	0.03	3.8	0.03	1.9	0.02	3.1
Skałki $n = 16$	average	0.689	0.126	18.3	0.306	44.5	0.218	31.7	0.438	63.5
	median	0.686	0.125	17.7	0.308	45.0	0.218	31.6	0.438	63.8
	minimum	0.650	0.096	14.1	0.278	39.5	0.189	27.7	0.407	58.4
	maximum	0.726	0.180	24.8	0.336	49.0	0.249	36.3	0.467	68.4
	S.D.	0.02	0.02	3.2	0.02	3.05	0.02	2.5	0.02	2.4
Myczkowce Zapora $n = 16$	average	0.869	0.259	29.6	0.294	34.0	0.288	33.1	0.556	64.0
	median	0.872	0.251	29.8	0.293	33.6	0.293	32.6	0.551	64.2
	minimum	0.724	0.179	24.4	0.255	29.5	0.198	27.4	0.454	58.9
	maximum	0.996	0.331	34.0	0.326	38.5	0.327	37.7	0.644	67.7
	S.D.	0.08	0.05	3.3	0.02	2.4	0.03	2.4	0.05	2.9
Myczkowce Zabrodzie $n = 15$	average	0.754	0.184	24.3	0.302	40.2	0.244	32.3	0.490	65.1
	median	0.756	0.181	24.4	0.297	39.6	0.258	32.7	0.486	65.0
	minimum	0.665	0.123	18.5	0.278	36.7	0.188	28.2	0.446	62.6
	maximum	0.826	0.224	27.6	0.332	49.9	0.289	36.1	0.556	67.5
	S.D.	0.04	0.03	2.6	0.02	3.5	0.3	2.5	0.03	1.5
Solina $n = 63$	average	0.846	0.205	23.8	0.321	38.5	0.288	33.9	0.530	62.7
	median	0.873	0.209	24.0	0.326	39.2	0.299	33.6	0.538	63.7
	S.D.	0.11	0.06	4.7	0.04	6.0	0.05	3.1	0.07	3.3
Myczkowce $n = 31$	average	0.813	0.222	27.0	0.298	37.0	0.267	32.7	0.524	64.5
	median	0.797	0.209	26.4	0.297	37.1	0.268	32.7	0.508	65.0
	S.D.	0.09	0.05	4.0	0.02	4.3	0.04	2.5	0.05	2.4

Fig. 2. Statistical distribution of NAIP fraction contents [mg P·g^{-1}of d.w.]
in the bottom sediments of the Solina–Myczkowce Reservoirs

Fig. 3. Statistical distribution of IP fraction contents [mg P·g^{-1}of d.w.]
in the bottom sediments of the Solina–Myczkowce Reservoirs

Statistically similar differentiation of the average contents was observed in the IP
fraction, however, the sediments from the Brama station showed similarity to all de-
posits of the first group with statistically significant difference with the sediments
from the Skałki station (Fig. 3). The evaluation of statistical significance of OP frac-
tion average values (by means of Scheffe's parametric test) showed rather consider-
able differentiation of organic phosphorus among the deposits from particular stations.

The deposits from the stations: Centralny, Zapora and Brama did not show statistically significant differences, however, the deposits from the Myczkowce Zapora station showed similarity to those of the Brama station in the content of OP fraction. From the statistical point of view, the deposits from the Skałki and Myczkowce Zabrodzie stations, characterized by the lowest content of organic compounds with phosphorus, clearly differed from other deposits (Fig. 4).

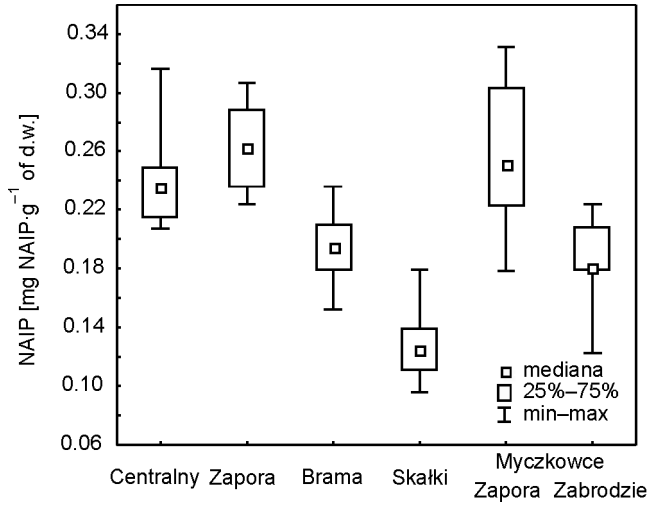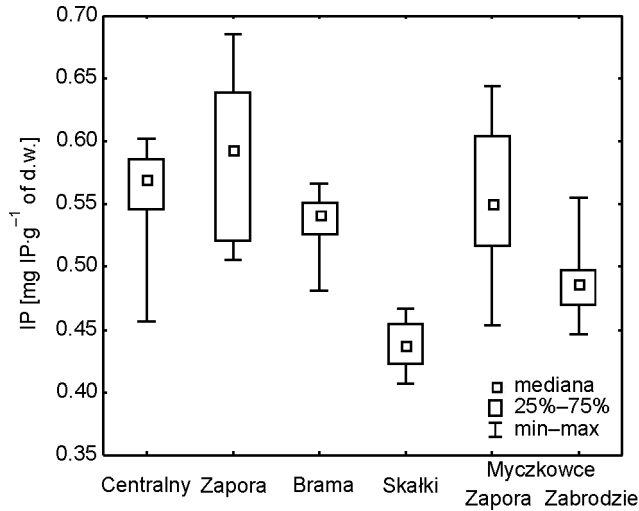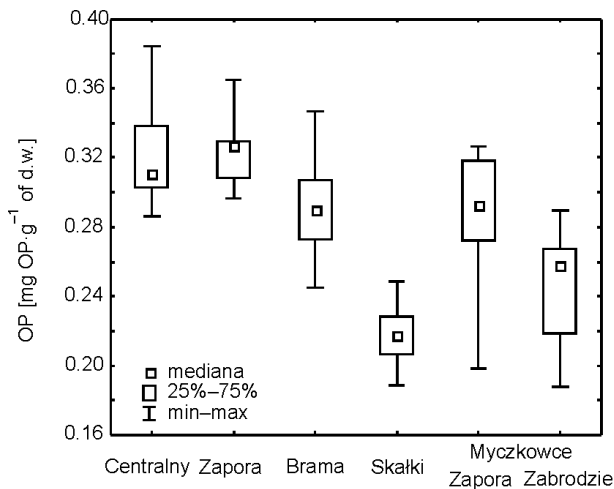Fig. 4. Statistical distribution of OP fraction contents [mg P·g^{-1}of d.w.]
in the bottom sediments of the Solina–Myczkowce Reservoirs

The average content of the AP fraction seemed to be similar in the deposits from almost all the stations. No statistically significant differences were found in the content of the apatite fraction in the sediments within reservoirs. The variance analysis (ANOVA) showed only statistically significant differences in the content of apatite compounds among the deposits from the Myczkowce Zapora (the lowest AP) and Centralny as well as Brama stations and also among the deposits from the Myczkowce Zabrodzie and Brama stations (Fig. 5). However, the comparison between the averages in the two examined groups (by means of the Kolmogorov–Smirnov's test) did not show statistically significant differences *(p* <0.05) considering AP fraction content between the sediments of the zones: the lacustrine zone and the one being under the influence of tributaries in the shallower parts of the Solina Reservoir.

The deposits of the Myczkowce Reservoir were characterized by the higher average content of the NAIP fraction and by the lower one of AP and OP in comparison to the deposits of the Solina Reservoir. The content of IP fraction was comparable in the deposits of both reservoirs (Table 2). Statistical analysis (based on the tests: t-Student, Cochran–Cox and Kolmogorov–Smirnov) confirmed the occurrence of significant differences between the averages in the two examined groups ($p < 0.05$), be-

tween the content of AP and OP fractions in the deposits of the main reservoirs and the retarding one and it did not show statistically significant differences considering the contents of NAIP and IP fractions in the deposits of both reservoirs.
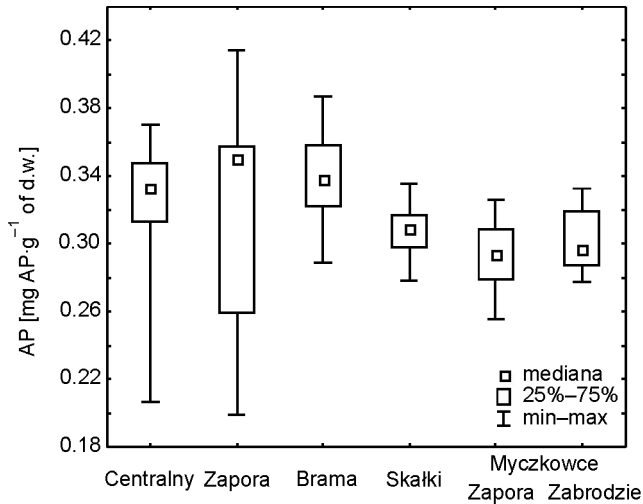

Fig. 5. Statistical distribution of AP fraction contents
[mg P·g^{-1} of d.w.] in the bottom sediments of the Solina–Myczkowce Reservoirs

Considering spatial differentiation of phosphorus content in the sediments of the discussed reservoirs more information about the distribution of phosphorus is provided by the proportion of particular fractions in total phosphorus. The proportion of the NAIP fraction in total phosphorus was the smallest (on the average from 18.3% to 29.6%) in the deposits of both reservoirs (Table 2). The proportion of AP fraction in total phosphorus was highest in the deposits of the Solina Reservoir riverine zone (on average 39.7% – Brama, 44.5% – Skałki) and in the deposits of the Myczkowce Reservoir (34.0% – Myczkowce Zapora, 40.2% – Myczkowce Zabrodzie). The percentage content of the AP fraction was comparable to the OP fraction (35.2% – Centralny, 34.2% – Zapora) in the deposits of the Solina Reservoir lacustrine zone. The average percentage contents of IP and NAIP fractions was higher in the deposits of the retarding reservoir and those of OP and AP fractions lower than in the deposits of the main reservoir.

In the bottom sediments of the Bort-Les-Orgues Reservoir (France) the percentage obtained by SMT method was: 59% NAIP fraction, 25% OP and 16% AP (of total phosphorus) [8, 14]. The average proportion of NAIP fraction in the bottom sediments from four stations of the Chinese lake was 72%, 41%, 24% and 12% (in total phosphorus). The proportion of OP and AP fractions varied from 12% to 24% and from 11% to 70% respectively, in total phosphorus. Generally, IP fraction was higher than the OP one [15]. Alkalization of lakes admittedly progresses together with the increase

of lakes trophy, therefore the increase of the proportion of the apatite fraction in total phosphorus could be theoretically treated as an indicator of the eutrophication of reservoirs [16]. However, as per Kaiserli et al. [17], the AP fraction was the predominant phosphorus fraction in the deposits of mesotrophic lakes. Jin et al. [15] also found, that the NAIP fraction content was higher than the AP fraction content in the deposits (from the two research stations) of the Chinese Taihu Lake, more contaminated with sewage and the opposite situation: AP > NAIP was observed in the deposits (from other two stations) less subject to anthropogenic contaminations. Perrone et al. [18] observed higher percentage of the NAIP fraction in the deposits collected from the deeper lake part and the higher content of AP and OP fractions in the deposits of the shallower parts.

In the Bort-Les-Orgues Reservoir, phosphorus mainly came from anthropogenic contamination (NAIP and partially OP) whereas the apatite fraction (AP) coming from the detritus was lower than 20%. Thus the highest amount of phosphorus, in the reservoir deposits, was of allochthonous origin [8, 14]. Brigault and Ruban [19] found that from the allochthonous sources of phosphorus, ca. 30% comes from domestic and industrial effluents (mainly NAIP), 60% from agriculture (NAIP + OP).

Significant proportions of the apatite and organic compounds in the total phosphorus content were found in the deposits of the Solina and Myczkowce Reservoirs, however, taking into consideration generally low content of this element in the deposits, they testify to low trophy level of the analysed reservoirs.

4. CONCLUSIONS

By means of the statistical analysis, the comparison of the phosphorus fraction average contents in the bottom sediments was made between the lacustrine and riverine zones in the Solina Reservoir, between the main reservoir and the retarding one as well as among all the research stations of the Solina–Myczkowce dam reservoirs complex. It has been found that the deposits of the lacustrine zone and the one being under the influence of tributaries are significantly different in relation to the content of NAIP, IP and OP fractions, whereas the deposits from the stations localized in the lacustrine zones of both reservoirs (Centralny, Zapora, Myczkowce Zapora) did not differ significantly in regards to the total phosphorus content of IP and NAIP fractions, however, they were characterized by significantly different contents of organic phosphorus (OP). The deposits collected at the Brama station showed great similarity to deposits of the lacustrine zone and the deposits from the Skałki and Myczkowce Zabrodzie stations clearly differed from other deposits. Statistically significant differences in the apatite fraction content were found neither in the deposits within reservoirs nor between the sediments of the lacustrine zone and the one being under the influence of tributaries in the shallower parts of the Solina Reservoir. However, it has been ob-

served that the deposits of the main reservoir and the retarding one differ significantly between themselves in the content of apatite (AP) and organic (OP) compounds with phosphorus. The retention of phosphorus in the deposits of dam reservoirs, especially spatially extensive ones, can take place by means of various mechanisms. As it results from the carried out analysis, the different distribution of phosphorus fraction in the bottom sediments does not necessarily have to be caused by the different character of deposits, connected with the depth changes along the longitudinal axis of the reservoirs.

The differentiation between the content of total phosphorus and the content of particular fractions in the deposits collected in the zone being under the influence of tributaries in the shallower parts of the Solina Reservoirs (Brama and Skałki), resulted presumably from different management and use of the catchment of the Solinka River, the San River and the Czarny Stream [20]. Bottom sediments of the central parts of both reservoirs as well as the sediments affected by the San River and the Czarny Stream could have been subject to greater sewage inflow resulting in the higher content of NAIP fraction. The proportion of the NAIP fraction in total phosphorus was the smallest in the deposits of both reservoirs that can suggest that the catchment of the Solina–Myczkowce dam reservoirs complex is not so much subject to anthropogenic contaminants inflow as the catchments of other reservoirs, especially lowland ones, usually are.

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EWA KUKUŁA***, ADAM MASŁOŃ*, RENATA GRUCA-ROKOSZ**

NOXIOUSNESS OF ODOURS
AND PROPERTIES OF WASTEWATER SLUDGE
PROCESSING WITH BIOPREPARATION

The aim of the study was to verify the effectiveness of decrease of odour production during sludge stabilization with selected groups of microorganisms in conditions similar to those occurring on sludge storage pads. The experiments were carried out on a laboratory scale; the biopreparation EM-bio was used. It was found that in anaerobic conditions, biopreparation modified biochemical processes in a small degree and decreases the redox potential. Clear decrease of odour noxiousness of the wastewater sludge for samples with biopreparation was not observed. In the continuation of these studies sludge processing at higher redox potentials should be investigated.

1. INTRODUCTION

In wastewater treatment plants, odour problems occur as a result of odour emission from the utilization and storage of screenings, greet and sludge. The odours form during biochemical processes occurring in technological facilities, drying beds, storage pads and during sludge transport to the place of the final utilization. In the case of sludge treatment, considerable amounts of odour are produced during the stabilization processes and dewatering [1, 2]. In 1993, complaints pertaining to the noxiousness of odours represented 43% of all complaints [3].

The modification of the biochemical processes in wastewater sludge could lead to a significant limitation of noxious odour. During the decomposition of organic matter

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by microorganisms both in the anaerobic and the aerobic cycles, intermediate products are generated which are extremely odour noxious to the environment. These are such by-products as sulfur compounds – hydrogen sulfide, mercaptans, sulfides, nitrogen compounds such as ammonia, and pyridine. Microorganisms added to wastewater sludge can change biochemical processes and significantly limit production of odorous gases [4, 5]. This leads to a new method with a possibility of sludge stabilization without odour production. The achievement of noxious odour limitation by this method is particularly rational when odour emission takes place over a large surface area, for example in sewage treatment plants where storage pads exist for the temporary disposal of sewage sludge.

The aim of the study was to verify the effectiveness of decrease of odour production during sludge stabilization with selected groups of microorganisms in conditions resembling those on sludge storage pads.

2. EXPERIMENTAL

The experiments were carried out on a laboratory scale. The biopreparation EM was used [6], activated in the molasses solution. This preparation, being a mixture of microorganisms such as acid milk bacteria, photosynthetic bacteria, yeast and actinomycetes, has been used in agriculture for soil improvement and in animal rising as a probiotic up to now. In order to check influence of actinomycetes, additional cultures obtained from composted sewage sludge were added. Isolates and cultures were run on the Pochon nutrient medium.
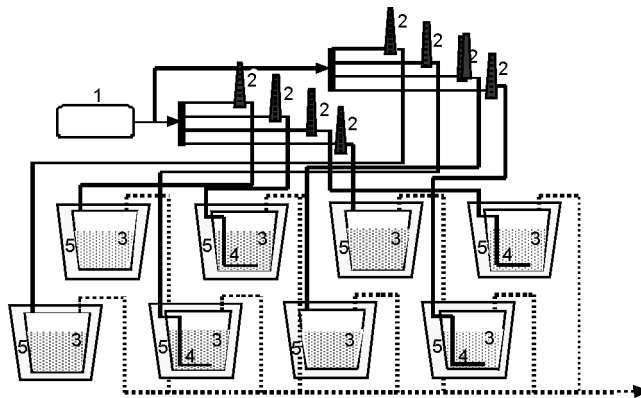

Fig. 1. Scheme of the research station: 1 – blower, 2 – rotameter, 3 – 30 dm^3 container, 4 – aeration system, 5 – thermal isolation of mineral wool

The investigated sludge originated from a municipal sewage treatment plant. This was return sludge, thickened for 3 days in the gravity thickener, conditioned by polye-

lectrolyte and dewatered. Eight tests containing 30 kg of sludge were prepared. Sludge was thoroughly mixed by mechanical stirrer and placed in a closed, thermally insulated container in a heated room (Figs. 1, 2).

Fig. 2. Research station; volume of the container 30 dm^3

The temperature of the room was maintained between 18 and 21 °C. Sludge processing consisted in the following tests:

- control test 1without aeration,
- control test 2 with aeration,
- test 3 with biopreparation EM,
- test 4 with biopreparation EM and aeration,
- test 5 with biopreparation EM and additional actinomycetes,
- test 6 with biopreparation EM, additional actinomycetes and aeration,
- test 7 with biopreparation EM, exposed to light,
- test 8 with biopreparation, aeration and exposed to light.

During processing, air was flowing only above the surfaces of the sludge in tests 1, 3, 5 and 7 with the flow rate of 120 dm^3/h. The sludge in tests 2, 4, 6 and 8 was aerated from the bottom through the volume of the sludge at constant flow rates.

The experiment was carried out for 41 days, at 14 day intervals sludge and gas samples were taken. The samples of wastewater sludge were taken by means of a special sampler for soil sampling (Egner stick) disinfected previously. The samples were taken according to Polish Norm PN-R-04031:1997. The range of analyzed parameters included: temperature, reaction (PN-Z-15011-3:2001), redox potential (PN-ISO 11271:2007), mass and dry mass (PN-EN 12880:2004, using lyophilisation process), volatiles in dry weight (PN-Z-15011-3:2001), total nitrogen and organic carbon on the elemental analyzer Flash 1112 (ThermoQuest). Total organic carbon was determined following the removal of inorganic carbon by contact with the vapour of HCl in desiccators [10]. Analysis of nitrogen mineralization by soil microflora was based on the extraction of the sludge sample of 5 g in 50 ml of water. The probe was shaken for 30 min and then centrifuged, finally the following parameters were determined: am-

monia nitrogen according to PN-ISO 5664, using UDK 132 Semiautomatic Distilla-
tion Unit, VELP, nitrate nitrogen (PN-C-04576-08:1982), nitrite nitrogen (PN-EN
26777:1999) and phosphate phosphorus (PN-EN ISO 6878:2005).

Odorimetric analysis of exhausted air was performed using the dynamic dilution
method, by changing the flow rate of air. Estimation of the odour intensity was per-
formed using *n*-butanol solutions [3].

Table 1

Results of wastewater sludge processing

Parameter	Day	1	2	3	4	5	6	7	8
pH	1	6.9	6.9	6.9	7.0	7.0	7.0	7.0	6.9
	13	7.0	7.1	7.2	7.7	7.4	7.1	7.1	7.2
	27	8.0	8.0	8.0	8.0	8.0	8.0	7.9	7.8
	41	8.0	8.0	8.0	8.0	7.9	8.0	7.9	7.8
Dry weight [%]	1	15.9	15.9	15.9	15.9	15.9	16.1	16.1	16.2
	13	16.2	16.6	16.0	15.6	15.7	15.7	15.7	16.0
	27	15.9	15.7	16.1	15.7	15.9	15.6	15.9	15.8
	41	15.5	15.2	15.9	15.3	15.1	15.3	15.3	15.3
Organic dry weight [%]	1	64.5	64.5	64.5	66.4	64.3	65	64.9	65.2
	13	65.4	65.9	65.4	63.9	64.8	64.4	64.0	65.0
	27	64.0	63.9	64.0	63.0	63.3	63.5	63.6	63.2
	41	63.1	62.9	64.1	62.2	62.4	62.6	62.8	62.4
Total nitrogen [% of d.w.]	1	5.7	5.5	5.6	5.5	5.3	5.5	5.6	5.5
	13	4.9	5.1	4.9	5.3	4.8	5.1	5.2	4.9
	27	4.5	4.9	4.8	5.0	4.6	5.0	4.8	4.8
	41	4.5	4.7	4.4	4.5	4.5	5.0	4.6	4.2
Organic carbon [% d.w.]	1	36.0	35.3	36.3	34.9	34.7	35.5	36.1	35.4
	13	30.7	31.8	32.5	32.9	31.2	30.2	33.6	31.6
	27	30.4	30.3	30.2	31.6	30.4	30.8	30.3	30.1
	41	29.1	30.2	29.9	30.0	29.8	30.6	29.6	29.6

Microbiological determination, based on standard testing procedures, included:
the total number of mesophilic bacteria (PN-EN ISO 6222:2004), total number of bac-
teria spores, MPN of I and II phases nitrifying bacteria, the number of *Clostridium
perfringens* bacteria (PN-EN ISO 7937:2005), the number of coliforms (PN-75/
C-04615/05, PN-77/C-04615/07), the presence of *Salmonella* (PN-Z-19000-1:2001).

3. RESULTS AND DISCUSSION

In the wastewater sludge before tests (test 1), the reaction medium was inert, the
dry weight was low and equalled 15.9% but organic compounds – only 64.5% of the
dry mass (Table 1). The sludge contained 5.7% of total nitrogen, medium amount of

organic carbon – 36%; the ratio of organic carbon to total nitrogen was low however, and equalled approximately 6.32.
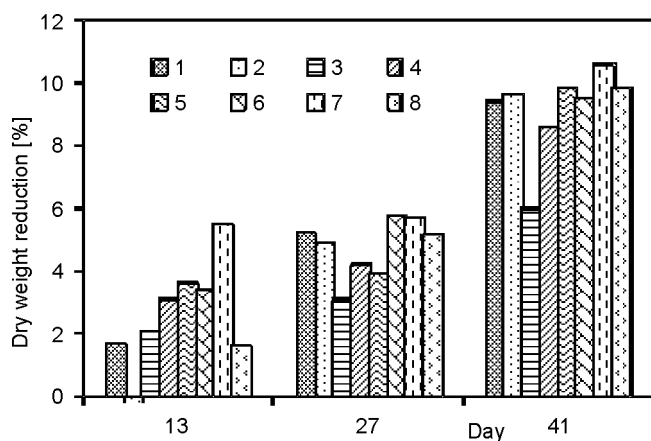

Fig. 3. Range of dry weight reduction during the experiment

During the experiments, the reaction medium was neutral but pH was slowly increasing, reaching after a month ca. 8 in all tests. The sludge mass, dry weight and organic dry weight decreased (Fig. 3). From the 13th to 41st day of the experiment a similar mass losses occurred in all aerated tests (2, 4, 6 and 8) and amounted to 2.5% of initial mass. The mass in other tests decreased marginally less but the most in the test 3 with biopreparation – 2.1%, and the least in the control test 1 – 1.9%. The largest degree of organic dry weight removal occurred in tests with biopreparation and ranged between 12.5% and 14.4%. In both control tests the value of this parameter was lower, in the test 1 it was equal 11.3% and in the test 2 – 11.8%.

After 41 days of the experiment, the content of organic carbon in all containers was very similar and ranged between 29.1% and 30.6% and the total nitrogen ranged between 4.2% and 5.0% (Table 1). The highest degree of organic carbon removal occurred in control test 1 and amounted to 26.8%, whereas in the test 3 with biopreparation it reached 22.6%. The maximum total nitrogen depletion equalled 28.6%, and occurred in control test 1, whereas in the test 3 with biopreparation a lower value equal 26.1% was obtained (Table 1). In the other aerated tests nitrogen and carbon losses were similar and a bit lower than in test 3.

The investigation of nitrogen mineralization by microflora showed that after two weeks of processing a large depletion of nitrates extracted from the sludge occurred (Fig. 4). Initial nitrate nitrogen values ranged between 119.3 mg N/kg d.w. and 192.7 mg N/kg d.w. and they decreased to the range of 7.6–18.8 mg N/kg d.w. The greatest decrement occurred in tests 3–5. In the control tests, the nitrate content decreased by up to 11%. After 13th day of experiment, the nitrate nitrogen contents extracted from

all aerated tests were in the range 31–38 mg/kg d.w. and were higher than in the not aerated samples (23–25 mg/kg of d.w.). Simultaneously, the ammonia nitrogen amount increased considerably in the extracts. At the beginning of the experiment, the content of ammonia nitrogen extracted from the sludge amounted from 0.55 to 1.55 mg N/kg d.w. By the 41st research day it increased to the values of 7.4–9.3 mg N/kg d.w. The highest concentration occurred in the control test 1. In the other tests, the amount of ammonia was lower. The nitrite nitrogen content extracted from the sludge changed between 9.4 and 38.7 mg N/kg d.w. and did not depend on the method of sludge processing.

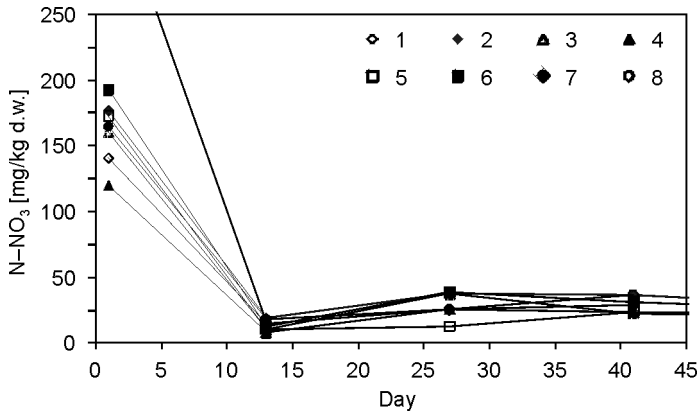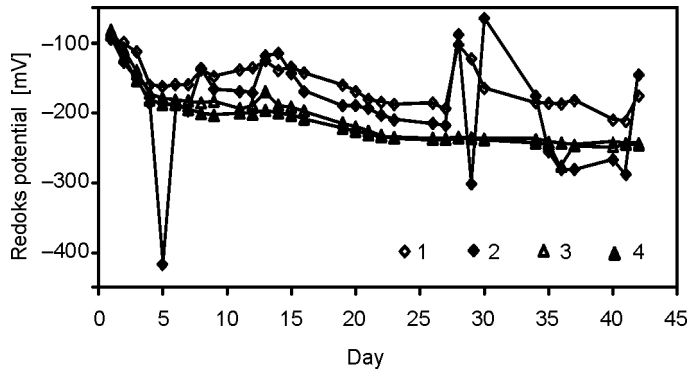Fig. 4. Time dependence of nitrate nitrogen content extracted from the sludge

Fig. 5. The *in situ* redox potentials during the experiment at the depth of 14 cm

Concentration of phosphate phosphorus at the beginning of the tests was about 0.6 mg P/g d.w. After two weeks it increased to about 0.9–1.1 mg P/g d.w. At the end of tests its values were around 1.8 mg P/g d.w. There were no significant differences between tests.

The *in situ* redox potential in tests 1–4 at the depth of 14 cm gradually decreased (Fig. 5). After 40 days of the experiment it changed in the range from –210 to –270 mV. It was lower in the tests with biopreparation than in control test during large part of experiment.
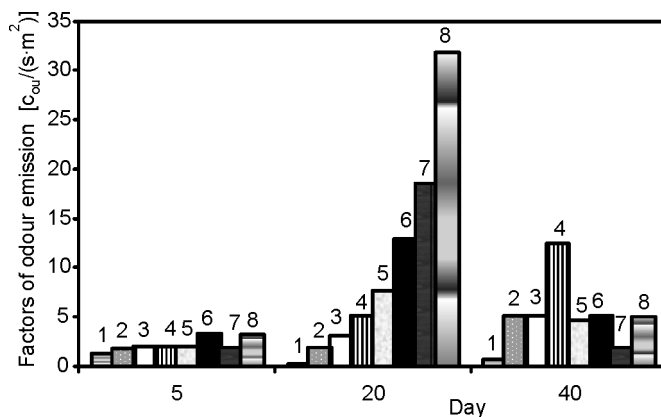

Fig. 6. Factors of odour emission during the experiment

Odour measurements (Fig. 6) showed that the number of odour units coming from aerated samples was greater than from the non-aerated samples. A progressive increase of the odour noxiousness occurred in all samples with biopreparation.

The results of bacteriological analyses demonstrated a lack of significant differences in the microflora of tests (Table 2). The wastewater sludge was characterized by a high number of vegetative bacteria. In every test after the period of transformation the number of these bacteria decreased. However, reduction in their number was smaller than during the mesophillic or thermophillic fermentation process [7]. In all samples of sewage sludge, the quantity of bacteria spores increased. In samples without aeration the proportional participation of bacteria spores was higher.

The investigated sludge contained a large number of *coli* and of *Salmonella* bacteria [6]. The sanitary effect was not observed taking into account the presence of *Salmonella*. After 41 days of the experiment these bacteria were present in all samples. Especially large number of *coli* including *E. coli* O157 indicated a potential health risk. According to obligatory rules in countries of the European Union, the majority of organisms from sewage sludge are listed in the second group of risk with exception of *Salmonella* and *E. coli* O157 and of several mycobacteria which are listed in the third group of risk [8, 9]. The titre of *E. coli* in investigated sewage sludge increased almost imperceptibly, which proved about a minimal reduction in the number of these bacteria.

The number of *Clostridium perfringens* increased in all investigated samples when the number of nitrifying bacteria decreased. It indicated poor oxygen conditions in

samples. The lower number of nitrifying bacteria might also be a cause of the lack in the odour noxiousness limitation of the studied wastewater sludge.

<div align="right">T a b l e 2</div>

<div align="center">Results of bacteriological analyses</div>

Parameter	Day	1	2	3	4	5	6	7	8
Total number of bacteria [cfu/g d.w.]	13	$3.2 \cdot 10^8$	$3.4 \cdot 10^8$	$4.1 \cdot 10^8$	$4.9 \cdot 10^8$	$3.7 \cdot 10^8$	$3.9 \cdot 10^8$	$3.5 \cdot 10^8$	$3.0 \cdot 10^8$
	27	$5.4 \cdot 10^8$	$4.8 \cdot 10^8$	$3.5 \cdot 10^8$	$9.5 \cdot 10^8$	$3.0 \cdot 10^8$	$3.5 \cdot 10^8$	$6.1 \cdot 10^7$	$3.6 \cdot 10^8$
	41	$1.8 \cdot 10^8$	$1.2 \cdot 10^8$	$6.1 \cdot 10^7$	$2.4 \cdot 10^8$	$6.9 \cdot 10^7$	$1.60 \cdot 10^8$	$1.4 \cdot 10^8$	$1.5 \cdot 10^8$
Ratio of total number of bacteria to total number of bacteria spores [%]	13	0.55	0.58	0.29	0.31	0.72	0.47	0.46	0.59
	27	1.07	0.52	2.39	0.23	2.02	0.93	7.41	1.60
	41	1.07	0.74	2.01	0.57	1.60	0.52	1.27	0.71
Number of *Clostridium perfringens* [cfu/g d.w.]	13	$1.0 \cdot 10^5$	$9.2 \cdot 10^4$	$8.7 \cdot 10^4$	$1.2 \cdot 10^5$	$1.4 \cdot 10^5$	$1.2 \cdot 10^5$	$1.1 \cdot 10^5$	$1.1 \cdot 10^5$
	27	$1.2 \cdot 10^5$	$1.6 \cdot 10^5$	$1.0 \cdot 10^5$	$1.1 \cdot 10^5$	$1.7 \cdot 10^5$	$1.5 \cdot 10^5$	$2.0 \cdot 10^5$	$2.0 \cdot 10^5$
	41	$1.1 \cdot 10^5$	$1.6 \cdot 10^5$	$2.2 \cdot 10^5$	$1.7 \cdot 10^5$	$1.8 \cdot 10^5$	$2.3 \cdot 10^5$	$2.1 \cdot 10^5$	$1.3 \cdot 10^5$
Titre of *Coli*	13	$2 \cdot 10^{-7}$	$2 \cdot 10^{-7}$	$2 \cdot 10^{-7}$	$2 \cdot 10^{-7}$	$2 \cdot 10^{-7}$	$2 \cdot 10^{-7}$	$2 \cdot 10^{-7}$	$2 \cdot 10^{-7}$
	27	$7 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$5 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$2 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$2 \cdot 10^{-6}$	$8 \cdot 10^{-6}$
	41	$7 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$2 \cdot 10^{-6}$
Titre of II phase nitrifying bacteria [1 g d.w.]	13	$4 \cdot 10^7$	$4 \cdot 10^7$	$5 \cdot 10^7$	$5 \cdot 10^7$	$4 \cdot 10^7$	$4 \cdot 10^7$	$5 \cdot 10^7$	$4 \cdot 10^7$
	27	$4 \cdot 10^6$	$4 \cdot 10^6$	$4 \cdot 10^6$	$5 \cdot 10^5$	$10 \cdot 10^5$	$4 \cdot 10^6$	$5 \cdot 10^6$	$4 \cdot 10^6$
Presence of Salmonella sp. [100 g of sludge]	13	pres.	pres.	pres.	pres.	pres.	pres.	pres.	pres.
	41	pres.	pres.	pres.	pres.	pres.	pres.	pres.	pres.

4. CONCLUSIONS

• The analysis of initial results indicated that the biopreparation in a small degree modified biochemical processes and caused redox potential to decrease.

• The clear decrease of odour noxiousness of the wastewater sludge for samples with biopreparation was not observed in applied conditions. The aeration process caused an increase of the number of odour units released to the environment during sludge processing.

• A sanitary effect was not achieved in the experimental conditions.

• Very small air space in the containers with wastewater sludge caused almost an-aerobic conditions. The intensity of aeration, applied in the experiment improved aerobic conditions to a small degree. Also the composition of bacterial microflora indicated that the availability of oxygen was limited.

• The faintly alkaline reaction of the sludge proved that the growth of lactic acid bacteria was limited.

• It is supposed that low redox potential was a reason of a weak growth the micro-organisms from biopreparation.

• In the continuation of these studies conditions for the tests should be changed. The course of processes of wastewater sludge processing at higher redox potential should be investigated.

ACKNOWLEDGEMENT

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EFFICIENCY OF POLLUTANT REMOVAL BY FIVE MULTISTAGE CONSTRUCTED WETLANDS IN A TEMPERATE CLIMATE

In recent years, an increase in interest in hybrid constructed wetland systems (HCWs) has been observed. These systems are composed of two or more filters with different modes of sewage flow. Based on over eight years of monitoring, carried out at five local HCWs located in the Pomerania Region of Northern Poland, the effective removal of organic matter (from 74.9 to 95.5% COD) in the loading range 1.5–17.0 g COD·m^{-2}·d^{-1} was confirmed. Efficiency of total nitrogen (TN) removal varied from 23.4 to 79.2%. The most effective removal of TN (2.0 g·m^{-2}·d^{-1}) occurred in the system with vertical flow compartments with intermittent loadings.

1. INTRODUCTION

Agricultural regions of Poland are inhabited by approximately 30% of the country's population. Almost all villages use water from local supply systems. Due to the scattered households in the countryside, installation of central sewage systems is technically difficult and economically expensive. Therefore, individual household treatment systems are often used instead. Most Polish villages (ca. 41.5%) use local sewage systems or septic tanks. It is estimated that about 1 km^3 per year of untreated wastewater in these regions is discharged to surface water or to the soil.

Protection of the natural environment in Poland has led to regulations demanding thorough wastewater treatment in rural areas [1, 2]. For this reason, the idea of treating wastewater in constructed wetlands (CW) has gained in popularity [3, 4]. During the last twenty years, some three hundred facilities of this type have been constructed in Poland.

In the temperate climate, constructed wetlands are built mainly as soil filters with vertical or horizontal flow. In the vertical flow constructed wetland (VF CW) the dominating processes are organic matter mineralisation and ammonia nitrification due to

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better oxygen conditions in comparison with the horizontal flow constructed wetland (HF CW) [5–7]. Until recently in Poland and Germany, mainly one stage systems with horizontal flow of sewage have been used. Problems with clogging and unstable efficiency of nitrogen removal caused interest in VF CWs owing to better oxygen transfer characteristics within the beds, enhanced by intermittent loading of sewage [6, 8, 9]. Single stage VF CWs, with the unit area of 4 $m^2 \cdot pe^{-1}$, provide effective organic matter removal for loadings up to 40 g $COD \cdot m^{-2} \cdot d^{-1}$ and the effluent meets very strict Austrian requirements irrespective of season and air temperature (final effluent concentrations below 90 $mg \cdot dm^{-3}$ COD, 25 $mg \cdot dm^{-3}$ BOD_5) [9]. According to Sardon [10], removal efficiency of BOD_5 as high as 86% is achievable by HF CWs at the BOD loading rate of 21.0 $g \cdot m^{-2} \cdot d^{-1}$ while 95% removal is achievable by VF CWs at 22.1 g $BOD \cdot m^{-2} \cdot d^{-1}$. In Canada VF CWs used for landfill leachate treatment provided up to 99% removal of BOD_5 and up to 97% decrease of ammonium nitrogen with the hydraulic loading rate (HLR) of 40 $mm \cdot d^{-1}$ and the BOD load rate of 42 $kg \cdot ha^{-1} \cdot d^{-1}$ [11]. It has also been indicated that the maximum allowable loading of organic matter applied to a hydrophyte bed is 25 g $COD \cdot m^{-2} \cdot d^{-1}$ and that effective nitrification and nitrogen removal in VF CWs take place when HLR does not exceed 300 $mm \cdot d^{-1}$ [12, 13]. However, excessive loading of organic matter may cause bed clogging with a resulting decrease in pollutant removal efficiency [14, 15]. Thus, according to Alvarez [16] effective pretreatment of raw wastewater (e.g. anaerobic pretreatment) could minimize the danger of clogging and improve the efficiency of pollutant removal in CWs as well as resulting in a reduction of 30–50% of the necessary wetland area.

In recent years, an increase in interest in hybrid constructed wetland systems has been observed [6–8]. These systems are composed of two or more filters with both VF and HF CWs. HCWs combine the benefits of both types of beds, with claims of better final effluent quality (lower organic matter concentration, complete nitrification and partial denitrification) [6, 11, 16]. In addition, HCWs require a smaller unit area and achieve higher efficiency of pollutant removal in comparison with that of one stage systems.

However, the influence of the sewage application and discharge regimes on removal efficiency of pollutants in HCWs remains unrecognized. Also there is lack of knowledge regarding the efficacy of systems in the post-vegetative period (November to March), especially when the temperature falls below 4 °C.

In order to explore these issues, an evaluation was carried out of hydrophyte bed performance during the period of 1998–2006. The investigations were conducted at facilities with various bed configurations and sewage flow modes.

The aim of this paper is the evaluation of seasonal and long-term performance of five HCWs, as well as assessment of the relationship between pollutant removal efficiency and bed configuration. The effects of influent loading rates of and VF bed configuration on the removal efficiency were also analyzed.

2. METHODS

The studies were carried out at five local Hybrid Constructed Wetlands, treating primary settled domestic wastewater in Darżlubie, Wieszyno, Wiklino, Sarbsk and Schodno in the Pommerania Region of Poland. Final discharge of treated effluent was to the Baltic Sea. All facilities were planted with reed. The characteristics of the HCW systems studied are shown in Table 1.

Table 1

Characteristics of the hybrid constructed wetland systems

Plant	Q [$m^3 \cdot d^{-1}$]	Configuration	Area [m^2]	Depth [m]	Hydraulic load [$mm \cdot d^{-1}$]	Unit surface area [$m^2 \cdot pe^{-1}$]
Sarbsk	29.7	HF VF	1610 520 ∑2130	0.6 0.5	18.5 38.6 13.9	8.5 2.6 ∑9.1
Wiklino	18.6	HF I VF HF II	1050 624 540 ∑2214	0.6 0.4 0.6	17.7 46.9 34.4 8.4	7.0 2.0 3.4∑12.4
Wieszyno	24.5	HF I VF HF II	600 300 600 ∑1500	0.6 0.6 0.6	40.8 81.7 40.8 16.3	3.0 1.5 3.0 ∑7.5
Schodno	2.2 (winter) 8.9 (summer)	HF I VF I HF II VF II Willow plantat.	416 307 432 180 ∑1300	0.6 0.45–0.6 0.6 0.45–0.6	5.3–21.4 7.2–28.9 5.1–20.6 12.2–9.4 1.7–6.8	27.8–6.4 20.5–4.7 28.8–6.6 12.0–2.8 ∑20–6.7*
Darżlubie	56.7	HF I Cascade bed HF II VF HF III	1200 400 500 250 1000 ∑3350	0.6 0.6 1.0 0.6	47.3 141.2 113.4 226.8 56.7 16.9	2.0 0.67 0.8 0.4 1.7 ∑5.6
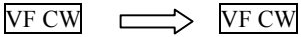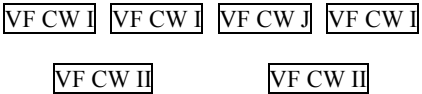
*In the summertime the wastewater treated is used for irrigation of a willow plantation with the surface area of approximately 400 m^2.

In all the HCWs studied, the first biological stage of treatment was horizontal flow (HF) CW. The analysed systems differ from one another in the order and number of subsequent stages (Table 2). In Sarbsk, sewage is introduced without resting to two VF beds working in parallel. At Wiklino two VF CWs work alternately with fortnightly resting.

In Wieszyno, two VF beds work in series and are loaded without resting. In Schodno, ca. 65% of the sewage is pumped intermittently to one of four VF beds. The remaining sewage is pumped directly to the HF II bed.

Operating conditions of vertical flow constructed wetlands (VF CWs)

Plant	Configuration		Operation condition of VF beds
Wiklino	VF CW	VF CW	alternately, intermittent
Wieszyno	VF CW $\Longrightarrow$	VF CW	in series, continuous
Sarbsk	VF CW	VF CW	parallel, continuous recirculation into HF
Darżlubie	VF CW	VF CW	parallel, continuously
Schodno	VF CW I VF CW I VF CW J VF CW I VF CW II	VF CW II	VF I – 4 compartments alternately, intermittent VF II – 2 compartments alternately, intermittent

Wastewater treated in HF II bed is pumped into two beds of VF II that alternate with an intermittent loading regime. In summer, treated wastewater is directed for irrigation to the willow plantation (*Salix viminalis*) and in winter it is finally discharged directly from the HCW to soil by the means of a drainage system.

Combined spot samples were taken monthly at each stage of the HCWs from 1998 to 2006 (Table 3) and analysed for pH, total suspended solids (TSS), organic matter (BOD$_5$, COD), total nitrogen, ammonium nitrogen, nitrate, nitrite, total phosphorus. Analyses were carried out according to the methods of Polish Standards and recommendations given in the Environment Ministry Decree [1]. Hydraulic load rates to each first stage were also recorded, based on the pump hour run meters.

Removal efficiency η was calculated as a quotient of pollutant load difference between the influent (L_{inf}) and effluent (L_{out}) after each treatment stage:

$$\eta = \frac{L_{inf} - L_{out}}{L_{inf}}$$

The mass removal rate (MRR) was calculated based of the following equation:

$$MRR = \frac{C_{inf}Q_{inf} - C_{out}Q_{out}}{A}$$

where: A is the surface area of CW [m^2], Q_{inf} and Q_{out} − average values of wastewater discharged in inflow and outflow [m^3·d^{-1}], C_{inf} and C_{out} − average concentrations of contaminant in inflow and outflow [mg·dm^{-3}].

3. RESULTS AND DISCUSSION

3.1. WASTEWATER QUALITY AND POLLUTANT REMOVAL EFFICIENCY

Average pollutant concentrations in the influent and effluent of the HCWs studied are shown in Tables 3 and 4.

Table 3

Average concentrations for each parameter in the raw influent

Parameter	Schodno $n^x = 18$	Darżlubie $n^x = 21$	Wiklino $n^x = 88$	Wieszyno $n^x = 18$	Sarbsk $n^x = 38$
TSS, mg·dm^{-3}	156.6 ± 51.2	359.5 ± 87.9	539.3±127.2	1269.5 ±167.6	819.9 ±208
COD, mg O$_2$·dm^{-3}	880.0 ±189.2	837.5 ±156.3	466.3± 92.7	1021.9 ±251.2	687.6 ±162.9
BOD$_5$, mg O$_2$·dm^{-3}	448.5 ±123.2	401.5 ± 51.3	265.2± 51.7	657.3 ±118.5	420.0 ± 87.2
TN, mg·dm^{-3}	96.1 ± 36.7	176.3 ± 35.6	104.1± 10.2	114.0 ± 22.1	73.8 ± 21.9
N-NH$_4^-$, mg·dm^{-3}	78.0 ± 28.5	82.6 ± 23.4	87.3± 9.0	84.8 ± 15.3	47.1 ± 13.7
N-NO$_3^-$, mg·dm^{-3}	0.1	1.3 ± 0.3	0.8± 0.2	1.0 ± 0.4	0.9 ± 0.1
N$_{org}$, mg·dm^{-3}	16.6 ± 3.9	90.8 ± 26.8	16.2 ± 5.3	27.9 ± 8.9	25.9 ± 7.3
TP, mg·dm^{-3}	14.6 ± 3.9	15.3 ± 0.8	15.2± 0.7	20.1 ± 1.2	11.9± 0.9

n^x – number of samples.

Table 4

Average concentrations for each parameter in the effluent from HCWs

Parameter	Schodno $n^x = 18$	Darżlubie $n^x = 21$	Wiklino $n^x = 88$	Wieszyno $n^x = 18$	Sarbsk $n^x = 38$
TSS, mg·dm^{-3}	48.6 ±20.1	92.0±27.3	36.3 ±17.2	106.4 ±31.7	45.6 ±49.9
COD, mg O$_2$·dm^{-3}	178.1±38.1	210.5 ± 67.8	31.5 ±8.9	175.9 ±99.3	44.2 ±15.9
BOD$_5$, mg O$_2$·dm^{-3}	96.6 ±20.1	72.0 ± 21.4	10.9 ±4.1	85.9 ±53.6	19.0 ±1.7
TN, mg·dm^{-3}	37.2±9.9	56.5 ± 16.9	21.7±5.5	87.3 ±14.8	27.6 ±8.5
N-NH$_4^-$, mg·dm^{-3}	30.6 ± 8.7	30.3± 11.5	6.0±4.3	67.1 ±14.2	16.8 ±11.2
N-NO$_3^-$, mg·dm^{-3}	0.3	5.9± 2.8	9.6 ±6.7	0.6 ±0.3	5.03 ±9.38
N$_{org}$, mg·dm^{-3}	7.2 ±1.3	22.5± 5.6	4.3 ±1.7	19.53 ±12.91	5.8 ±2.3
TP, mg·dm^{-3}	3.5 ±0.9	6.9± 2.1	7.2 ±1.6	14.6±3.9	8.9±3.1

n^x – number of samples.

Influent wastewater quality varied considerably between the HCWs, that at Wieszyno receiving the highest concentrations, high concentrations of TSS, BOD_5 and COD at the beginning of the biological stage of treatment indicating improper septic tank operation. On the other hand, considerable variation in influent quality was observed at Schodno, with high values of standard deviation observed (reaching 30%). Very high concentrations of organic matter in the influent to Darżlubie and Schodno HCWs suggested that, together with domestic wastewater, manure liquid from a farm and sewage from a food company were entering the system.

According to current Polish Standards [1], sewage discharged from less than 2000 pe and above 50 pe has to fulfil the following criteria: $BOD_5 \leq 40$ mg·dm^{-3}, COD ≤ 150 mg·dm^{-3}, and SS ≤ 50 mg·dm^{-3}. Discharges to nutrient sensitive areas must meet additional criteria: TN ≤ 30 mg·dm^{-3}, TP ≤ 5 mg·dm^{-3}. The effluent from the systems at Wiklino and Sarbsk fulfilled Polish Standards [1]. All other facilities, in spite of considerable pollutant removal efficacy, did not provide proper effluent quality.

The relative ability of the HCWs to remove organic matter (expressed as COD) was:

Wiklino > Sarbsk > Wieszyno > Schodno > Darżlubie
95.5% > 93.6% > 84.7% > 79.8% > 74.9%

The relative average TN removal of the systems was:

Wiklino > Darżlubie > Sarbsk > Schodno > Wieszyno
79.2 % > 67.9% > 62.6 % > 61.3% > 23.4 %

The highest efficiency of nitrogen removal was observed in the Wiklino HCW in which the VF beds were operated alternately and wastewater was introduced intermittently. Likewise at the Schodno facility, high efficiency of TN removal was observed despite very high concentrations in the inflow. These observations confirm that the HCWs are successful at nitrogen removal.

Table 5

Mean values of loads and mass removal rates (MRR)
of pollutants in HCWs [g·m^{-2}d^{-1}]

Plant	COD		BOD_5		TN	
	Load	MRR	Load	MRR	Load	MRR
Schodno	6.1	4.8	3.1	2.4	0.7	0.4
Darżlubie	14.2	10.6	6.8	5.6	3.0	2.0
Wieszyno	16.7	13.8	10.7	9.3	1.9	0.4
Wiklino	3.9	3.7	2.2	2.1	0.9	0.7
Sarbsk	9.6	9.0	5.9	5.6	1.0	0.65

As well as nitrogen removal, intermittent loading of VF beds operating alternately with resting periods (Wiklino) was especially effective at enhanced organic matter removal. In Table 5, the mean organic matter (COD and BOD_5) and nitrogen inlet loads are compared with the mass removal rate (MRR).

A wide range of loadings was applied in Schodno HCW, receiving almost one tenth the load rate of Wieszyno. However, the maximum allowable loadings given in the literature (COD = 40 $g·m^{-2}·d^{-1}$ and TN = 20 $g·m^{-2}·d^{-1}$) [9, 10] were not exceeded. Studies in Spain [17] on HF beds with BOD load ranging from 0.8 to 23.0 $g·m^{-2}·d^{-1}$ and on VF beds from 12.8 to 29.8 $g·m^{-2}·d^{-1}$ showed 80.0% and 95.0% BOD_5 removal, respectively. In the Polish HCWs organic matter loading ranged from 0.8 (Schodno) to 10.7 $g·m^{-2}·d^{-1}$ (Wieszyno), while the removal efficiency ranged from 78.5% (Schodno) to 95.9% (Wiklino).

Based on analysis of the obtained results, it could be concluded that the Schodno HCW, in spite of receiving the lowest loading rates, did not provide the highest pollutant removal efficiency. In contrast, high organic matter loading in Wieszyno resulted in poor nitrogen removal efficiency despite satisfactory organic matter removal. The highest MRR of TN was obtained in the Darżlubie HCW in spite of quite high values of organic matter loading (Table 5). The MRR was almost three times higher than the value of 0.7 $g·N·m^{-2}·d$ given for systems in Denmark [7]. The obtained results suggest that the MRR of pollutants from 1 m^2 changed in proportion to the loading value.

At the same time, organic matter in the HCWs was removed highly effectively over a wide loading range, irrespective of the HCW configuration. However, the MRR of TN related more closely to the HCW configuration than to the applied nitrogen loading.

3.2. SEASONAL CHANGES OF POLLUTANT REMOVAL

Seasonal changes in the quality of wastewater influent and effluent are presented in Fig. 1. The analysed systems were categorised into two groups, based on influent TSS, BOD_5, COD concentrations: 1) Wieszyno, Sarbsk and Wiklino – treatment of wastewater from multistage family houses – low concentrations of pollutants applied during the growing season (Figs. 1a, 2a); 2) Darżlubie and Schodno – higher concentrations of applied pollutants during the growing season (Figs. 1b, 2b). Regardless of season, performance of group 1 HCW was better than that of group 2, the effluent BOD and TN of which exceeded Polish permissible values in the vegetative season [1].

Comparison of seasonal average removal efficiencies for Wiklino and Darżlubie is presented in Fig 3.

Whilst BOD removal efficiency was not seasonally dependent, TN removal was ca. 10% higher in the growing season at both facilities. Other differences were rela-

tively small and may have been caused either by flow irregularity and/or fluctuations of inlet pollutant concentrations.
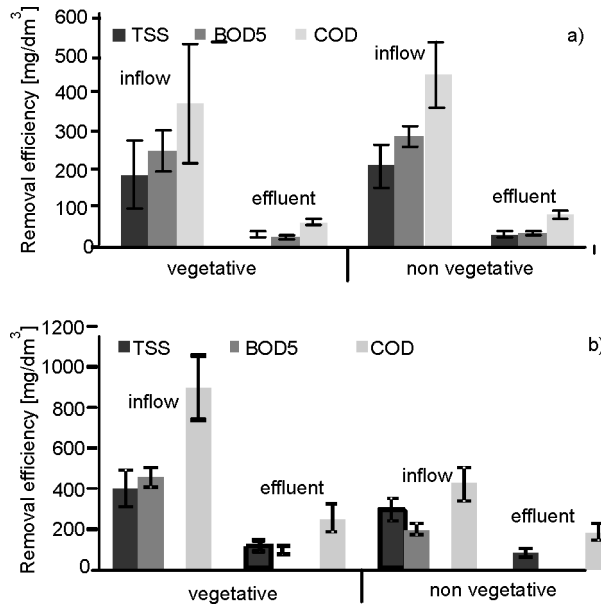
Fig. 1. Organic matter and total suspended solid concentrations
in vegetative and post vegetative season in a) Wiklino, b) Darżlubie
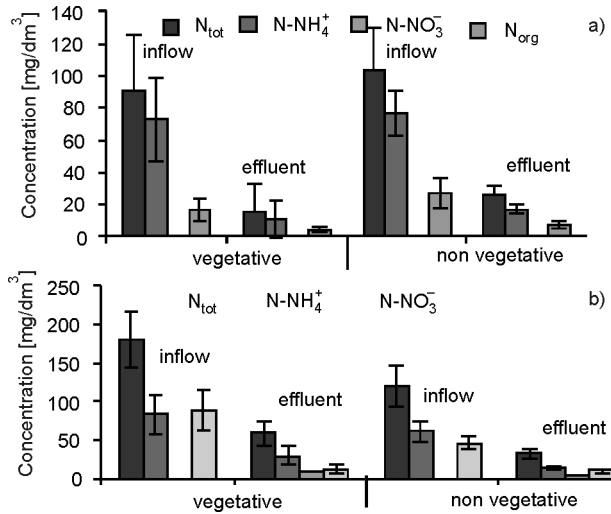
Fig. 2. Concentrations of nitrogen compounds
in vegetative and post vegetative season in a) Wiklino, b) Darżlubie
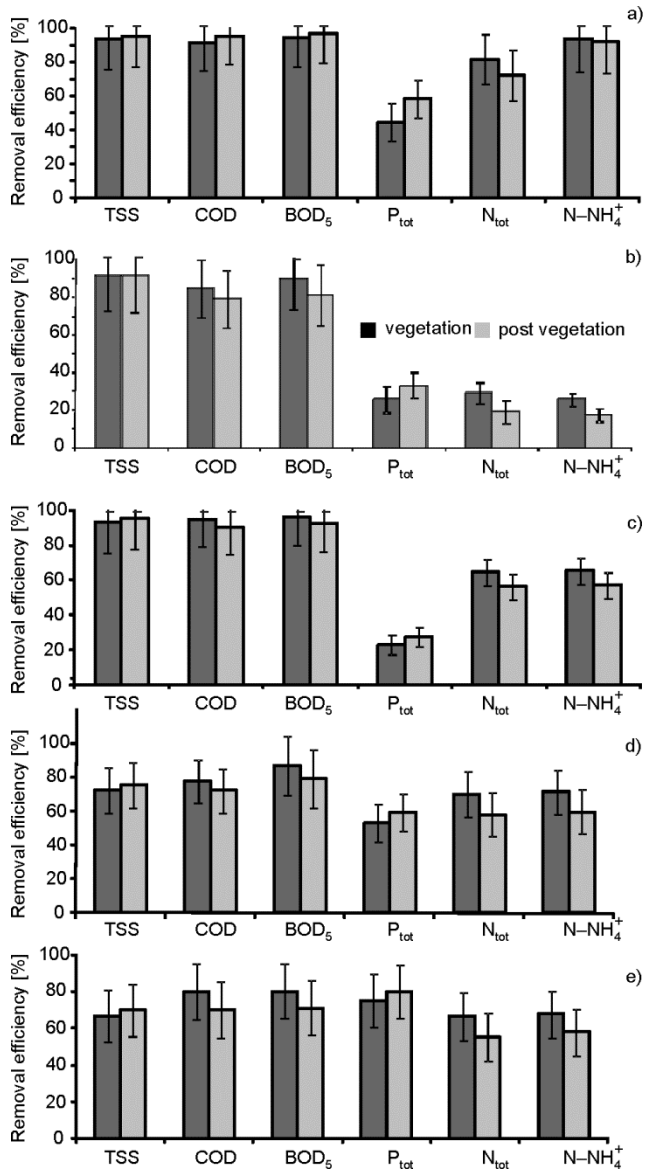
Fig. 3. Removal efficiency of pollutants in vegetative and post vegetative
seasons: a) Wiklino, b) Wieszyno, c) Sarbsk, d) Darżlubie, e) Schodno

4. CONCLUSIONS

Hybrid constructed wetlands ensure stable and effective removal of organic matter
in the load range 1.5–17.0 g $COD \cdot m^{-2} \cdot d$, irrespective of the bed configuration used.

Mass removal rates of total nitrogen range from 0.4 to 2.0 g TN·m^{-2}·d^{-1} depending on the configuration of VF CWs used and the sewage input regime.

Organic matter and total phosphorus removal efficiency was not season dependent, in contrast to the removal efficiency of nitrogen compounds.

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INFLUENCE OF FERTILIZATION WITH COMPOST
FROM MUNICIPAL SLUDGE ON THE CONTENT
OF DRY MATTER AND THE YIELD OF
ENERGY WILLOW PLANTED ON LIGHT SOIL

An assessment has been done of the yielding of nine clones of energy willow planted in Kościernica near Koszalin on light soil of IVb–V class, fertilized with the compost from municipal sludge in the dose of 10 t·ha^{-1} of dry matter and with Hydrofoska 16 fertilizer in two doses (562.5 kg·ha^{-1} and 1.125 kg·ha^{-1}). The highest content of dry matter in shoots was obtained on the objects without any fertilization. Fertilization with the compost decreased the content of dry matter in the shoots and increased the yield of fresh matter in comparison with the testing object without fertilization.

1. INTRODUCTION

Sludges constitute troublesome waste in sewage treatment plants and are still difficult to recycle [1, 2]. In 2008, a total of 978 900 tons of dry matter of sludges were produced in Poland in industrial and municipal sewage treatment plants [3]. In industrial sewage treatment plants, the quantity of the sludges produced decreased annually from 700 300 tons in 2000 to 411 600 tons in 2008, while in municipal waste treatment plants it increased from 359 800 tons in 2000 to 567 300 tons in 2008. In accordance with the state ecological policy and the guidelines of the National Plan of Waste Management 2010 [4], in 2018 the following recycling from the foreseen quantity of 706.6 thousand tons of sludges from municipal sewage treatment plants 9.5% was predicted for the purposes of reclamation, for use in agriculture and in nature 9.5%, 20.6% for composting and 60.7% thermal neutralization. The guidelines included in

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Directive 99/31/EC concerning waste disposal [5] which are transferred to the Polish law in the Ordinance by the Minister of Economy as of 12 June 2007, prohibit starting from the day of 1 January 2013 any disposal of such sludge which contains over 5% of general organic carbon, characterised by the heat of combustion of over 6 MJ/kg of dry matter [6]. The use of sludges for the production of compost, which will then be used among others for the fertilization of the crop of energy plants, will also allows an active inclusion in the realization of the EU objectives related to the power industry. The Ordinance by the Minister of Economy as of 14 August 2008 imposes an obligation on power stations to use agricultural raw materials for energy purposes [7]. In those areas where light soils dominate, fertilization with compost from municipal sludges or also sludges may increase their productivity, the retention of rain water and may step up the soil forming process [2, 8–12].

The purpose of the present research was an assessment of the influence of the fertilization of energy willow with compost from municipal sludges and being enriched with mineral fertilizers on the content of dry matter and the yield of the shoots of nine clones in the second, third and fourth year of their crop in a place with a deep level of underground waters, weak soils yet with a relatively favourable distribution of precipitation in the vegetation period.

2. MATERIALS AND METHODS

Field experiments with nine clones of shrubby willow were conducted in Kościernica near Koszalin on a field which had been fallowed for 10 years, on light soil rated among IVb–V class, with the mechanical composition of light clayish sand with an acid reaction, and an average content of assimilable phosphorus in the soil, yet with a low content of potassium and magnesium. In the first decade of April 2005, willow cuttings were planted at the abundance of 33.2 thousand of ferns per one hectare. One year old shoot outgrowths were cut after the first vegetation of willow in the winter of 2005/2006. In spring 2006, a strict experiment was set up with the method of random split blocks in a dependent layout in three repetitions, where the first class split blocks were four fertilizer combinations, and the second class split blocks were nine clones of willow. The experimental plot had the area of 34.5 m^2 (2.3×15.0 m^2). Within the framework of fertilizer combinations, the following were randomized:

a) objects without any fertilization,

b) objects fertilized with compost (10 t·ha^{-1} of dry matter),

c) objects fertilized with compost (10 t·ha^{-1} of dry matter) and Hydrofoska 16 in the dose of 562.5 kg·ha^{-1} containing 90 kg·ha^{-1} of N, 90 kg·ha^{-1} of P$_2$O$_5$ and 90 kg·ha^{-1} of K$_2$O in a pure component,

d) objects fertilized with compost (10 t·ha^{-1} of dry matter) and Hydrofoska 16 in the dose of 1125.0 kg·ha^{-1} containing 180 kg·ha^{-1} of N, 180 kg·ha^{-1} of P_2O_5 and 180 kg·ha^{-1} of K_2O in a pure component.

Compost from municipal sludges with an attestation issued by the University of Technological and Natural Sciences in Bydgoszcz was purchased from the Waste Recycling Company in Sianów. The compost contained 66.7% of dry matter as well as significant quantities of nutrients and alkali metals, while the quantities of heavy metals were in accordance with the Polish standards. In the dose of 10 Mg·ha^{-1} of compost, 3906 kg of organic matter, 174.6 kg of nitrogen in total and 160.1 kg of phosphorus and also considerable quantities of alkali metals: 20 kg of sodium, 32.5 kg of potassium, 110 kg of calcium and 17.2 kg of magnesium were all carried to the soil. In the year 2006, the compost was applied on 7 days before spreading of Hydrofoska 16, which was then mixed with the soil. The dose of Hydrofoska 16 was divided into two parts, which were applied in the interval of one month. In the years 2007, 2008 and 2009 re-fertilization with Hydrofoska 16 was applied on c and d objects with the omission of compost before the start of the vegetation of willow. Nine clones of willow: 1047, 1054, 1023, 1013, 1052, 1047D, 1056, 1018 and 1033 were investigated. The shoots were every time mowed from 1/3 of the area of the experimental plot after the second vegetation in February 2008, after the third vegetation in February 2009 and after the fourth vegetation in November 2009. On the day of mowing, the yield of fresh matter of the shoots and the content of dry matter were evaluated. The data of the yield of biomass was statistically processed with the use of Statistica programme. Analyses of variances were performed, the gravity of the effects was evaluated with the F test and the significance of the factors examined was calculated with the method of variance components.

3. RESULTS

Precipitation in all years of the experiment was above 753 mm in the period from January to December, and from 459 mm in the period from April to October in 2008 to 654 mm in 2007. The highest precipitations (1062 mm) occurred in 2007 which was considered very wet. The year 2008 with the precipitation of 855 mm was well wet and the years 2006 and 2009 with the precipitation of 753 mm and 787 mm, respectively, were wet [21].

Yields of fresh and dry matter depending on fertilization and the content of dry matter in willow shoots in the years of 2006–2009 are presented in Table 1. In the table and subsequent ones, NIR$_{0.05}$ is the smallest significant difference on the confidence level of $\alpha = 0.05$. One asterisk (*) denotes significance on the confidence level $\alpha = 0.05$, two asterisks (**) $\alpha = 0.01$, (***) – $\alpha = 0.001$. Fertilization with the com-

post (case b) and with Hydrofoska 16 (cases c and d) decreased the content of dry matter in the shoots in comparison with that in the testing object without any fertilization (case a). The composition of fertilizer significantly influenced the yields of the fresh and dry matter of the shoots. Fertilization with the compost (case b) increased the yield of fresh matter by 2.2 t·ha^{-1} in average, i.e. by 6.9% and the yield of dry matter by 1.0 t·ha^{-1}, i.e. by 6.9% in comparison with the testing object (case a). On the objects with the compost, fertilization with the Hydrofoska 16 in the dose of 562.5 kg·ha^{-1} (case c) contributed to an increase of the yield of fresh matter by 13.8 t·ha^{-1}, i.e. by 43.3% and the yield of dry matter by 5.8 t·ha^{-1}, i.e. by 40.3% in comparison with the testing combination a.

Table 1

Yield of fresh and dry matter depending on fertilization
and content of dry matter in willow shoots in the years of 2006–2009
in Kościernica. The average from 9 clones and 3 harvests

Composition of mixture of fertilizers			Contents of dry matter in shoots [%]	Yield [t·ha^{-1}]	
Case	Fresh matter of compost [t·ha^{-1}]	Hydrofoska 16 [kg·ha^{-1}]		Fresh matter	Dry matter
a	0	0	44.5	31,9	14,4
b	15	0	44.1	34,1	15,4
c	15	562.5	43.4	45,7	20,2
d	15	1125.0	43.5	50,4	22,4
NIR$_{0.05}$			0.8*	2.0***	1.1***

Fertilization with the compost and Hydrofoska 16 in the dose of 1125.0 kg·ha^{-1} (case d) increased the yield of fresh matter by 18.5 t·ha^{-1}, i.e. by 58.0% and the yield of dry matter by 8.0 t·ha^{-1}, i.e. by 55.6% in comparison with those of the testing object (case a).

Table 2

Effect of synergism of the crop years and the fertilizer combinations on the yield
of fresh matter of willow shoots. An average from 9 clones of willow

Harvest of shoots	Crop years	Yield of the fresh matter of willow for the fertilizer combinations [t·ha^{-1}]			
		a	b	c	d
February 2008	II	21.4	21.4	31.3	36.1
February 2009	III	31.7	32.9	46.7	48.2
November 2009	IV	42.5	47.9	59.2	67.0
NIR$_{0.05}$		3.4**			

In the second and third crop years, the differences in the yield of fresh as well as of dry matter between the object fertilized with the compost (b) and the testing object without any fertilization (a) were insignificant, in contrast to the fourth year, when the differences were considerable (cf. Tables 2, 3). In each crop year, an additional fertilization with Hydrofoska 16 (cases c and d) was favourable to obtain higher yields of dry matter of shoots (compare with cases a and b).

T a b l e 3

Effect of synergism of the crop years and the fertilizer combinations on the yield of dry matter of willow shoots. An average from 9 clones of willow

Harvest of shoots	Crop years	Yield of the dry matter of willow for the fertilizer combinations [t·ha^{-1}]			
		a	b	c	d
February 2008	II	9.04	8.81	12.59	14.46
February 2009	III	13.35	13.66	19.53	20.66
November 2009	IV	20.84	23.64	28.44	32.22
NIR$_{0.05}$		1.85**			

T a b l e 4

Effect of synergism of clones and the fertilizer combinations on the yield of dry matter of willow shoots. An average from 3 harvests

Willow clone	Yield of the dry matter of willow for the fertilizer combinations [t·ha^{-1}]			
	a	b	c	d
1047	16.3	17.1	21.1	29.3
1054	12.7	15.0	19.6	24.5
1023	15.5	16.1	27.0	22.0
1013	16.2	16.5	20.7	18.6
1052	12.1	12.2	16.3	25.3
1047D	14.3	13.8	21.8	23.2
1056	16.0	17.2	18.2	14.9
1018	13.0	14.7	19.8	23.3
1033	13.5	15.9	17.2	20.9
NIR$_{0.05}$	3.2***			

No significant increase of the yield of dry matter was observed for all the clones apart from that No. 1033 on objects fertilized with the compost (case b) in comparison with those unfertilized (Table 4). The increase of the yield of dry matter within the range from 2.2 to 11.5 t·ha^{-1}, i.e. from 13.8% to 74.2%, were obtained with all the clones on the objects fertilized with the compost and with Hydrofoska 16 in the dose of 562.5 kg·ha^{-1} (case c) in comparison with the object without any fertilization. Di-

versified reaction of the clones in the yield of dry matter was observed on the objects fertilized with the compost in the dose of 15 t·ha^{-1} and with Hydrofoska 16 in the dose of 1125 kg·ha^{-1} (case d). Yield of dry matter increased for eight clones examined (from 2.4 t·ha^{-1} to 13.2 t·ha^{-1}, i.e. from 14.8% to 109.1%) in comparison with the testing object.

4. DISCUSSION

An increase of the productivity of energy willow based on agricultural science methods has a large significance; however, there are still very few experimental studies in this area in Poland [8, 13–15]. In many countries, willow crops are situated in the vicinity of sewage treatment plants where municipal liquid wastes or sludges are used for the purpose of fertilization [11, 16, 17]. The willow is counted among plants with intense water requirements [15, 18]. On the grounds of research it was recognized that those areas where the annual precipitation exceeds 575 mm and there are soils counted included in the complexes of agricultural usefulness such as rye very good (4), rye good (5), cereal and fodder strong (8), cereal and fodder weak (9) and weak and very weak grasslands (3z), are fit for the crop of willow in Poland [19]. The experiment was located in Kościernica on the soil of a weak rye complex (IVb–V class) and the rain water was its only source for the willow.

The content of dry matter increased annually from the level of 41.0% after the second year of the crop to 48.6% after the fourth year of the crop, which was similar to what was observed in the research by Stolarski and Szczukowski [13, 15]. In the author's research, with the passage of the crop years, the yield of the fresh and dry matter of willow shoots grew from 27.5 t·ha^{-1} and 11.2 t·ha^{-1} after the second year to 54.1 t·ha^{-1} and 26.3 t·ha^{-1} after the fourth year, respectively. In the research by Stolarski et al. [13], the yield of dry matter of willow wood, which was acquired very year, was higher than in the author's research and was 16.89 t·ha^{-1}·year^{-1} and in the four year cycle: 24.99 t·ha^{-1}·year^{-1}. The difference in the yield of dry matter of willow between the research by Stolarski and that presented here resulted from the quality of soil and its abundance in water. In Obory near Kwidzyń, the experiment with willow was conducted on heavy fen soil produced from heavy flour clay, in a strong cereal and fodder complex of IIIb class.

In the present research, a favourable influence of fertilization with the compost on the yields of willow shoots increased with the passage of crop years; it achieved significant rises in the fourth year only by 5.4 t·ha^{-1}, i.e. by 12.7% in the yield of fresh matter and by 2.8 t·ha^{-1}, i.e. by 14.4% in the yield of dry matter in comparison with those of the objects without any fertilization. It is seems that the yield generating effect of the compost could emerge as a consequence of the improvement of the soil

fertility as a result of the fertilizing activity of the compost [9] and its stimulating influence on the abundance and activeness of oligotrophic and macrotrophic bacteria as well as total fungi and cellulotic fungi [20].

No data concerning the reaction of the clones of energy willow to fertilization with the compost from municipal sludges and with Hydrofoska 16 compound fertilizer is to be found in the literature. In the present research, the differences between those clones which on the average obtained the largest and lowest contents of dry matter in shoots were 3.9%, i.e. 8.9% of the average content in the experiment, and with the yield of dry matter: 4.4 t·ha^{-1}, i.e. 24.3% of the average yield of dry matter in the experiment. On the objects fertilized with the compost from municipal sludges, only clone No. 1033 reacted with a significant increase of the yield of fresh matter by 6.2 t·ha^{-1}, i.e. by 20.6% in comparison with that of the object without any fertilization. In the previous research, it was similarly confirmed that the recommendations concerning fertilization with compost with reference to species cannot be generalized in relation to the willow, yet its effects should be examined on the level of a clone [14]. A beneficial effect of the combined fertilization with the compost and Hydrofoska 16 similarly as in the case of fertilization with the compost only on the yields of fresh and dry matter increased with the passage of the crop years; it annually achieved higher average increases of the yield, but the differences in these yields between high and low doses of Hydrofoska 16 were significant only in two per three harvests. According to the literature data, the yields of willow depend on the course of weather, the quality of soil, fertilization applied and the length of the harvest rotation [13–15]. In foreign experiments, the productivity of willow was also different, depending on the location of the field, fertilization and watering. For example, in Sweden in three year rotations, the yield fluctuated from 7 to 20 t·ha^{-1}·year^{-1} of dry matter, and in Germany it ranged from 6 to 14 t·ha^{-1}·year^{-1} [21], in the United States in four year rotations in the range from 15 to 20 t·ha^{-1}·year^{-1}, and in Wales in the range from 6 to 12 t·ha^{-1}·year^{-1} [22].

5. CONCLUSIONS

No access of willow roots to the underground water caused a periodic reaction of the plants to the stress of drought even though hydrothermal conditions for the crop of willow in the years of 2006–2009 in Kościernica were favourable: from January to December the precipitation was from 753 mm to 1062 mm and in the periods from April to October, it was from 459 mm in the year 2008 to 654 mm in the year 2007.

Fertilization with the compost in the dose of 10 t·ha^{-1} of dry matter reduced the content of dry matter in shoots by 0.4% and increased the yield of fresh matter by 2.2 t·ha^{-1}, i.e. by 6.9% and dry matter by 1.0 t·ha^{-1}, i.e. by 6.9% in comparison with the testing object without any fertilization.

The favourable influence of fertilization with the compost on the yields of fresh and dry matter increased with the passage of the crop years achieving significant rises only in the fourth year with the yield of fresh matter by 5.4 t·ha^{-1}, i.e. by 12.7% and with the yield of dry matter by 2.8 t·ha^{-1}, i.e. by 14.4% in comparison with the objects without any fertilization.

The application of the combined fertilization with the compost and with Hydrofoska 16 in both doses examined (562.5 kg·ha^{-1} and 1,125.0 kg·ha^{-1}) on the average decreased the content of dry matter in the shoots by 1.1% and 1.0%, respectively and increased the yield of fresh matter on the average by 13.8 t·ha^{-1}, i.e. by 43.3% and by 18.5 t·ha^{-1}, i.e. by 58.0%, respectively and the yield of dry matter on the average 5.8 t·ha^{-1}, i.e. by 40.3% and by 8.0 t·ha^{-1}, i.e. by 55.6% in comparison with the testing object without any fertilization.

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JEREMI NAUMCZYK*, MAŁGORZATA KUCHARSKA*

TANNERY WASTEWATER TREATMENT
BY ANODIC ELECTROOXIDATION
COUPLED WITH ELECTRO-FENTON PROCESS

Investigations were performed on tannery wastewater, previously coagulated with $FeCl_3$. An electrolyzer was equipped with two graphite cathodes and $Ti/SnO_2/PdO_2/RuO_2$ anode, divided by the diaphragm made of asbestos fibre. The current density was: cathodic 2.0 A/dm^2 and anodic 2.1 A/dm^2. After 55 min of the process the catholyte was transferred into the anodic space and the process was continued. After 55 min of electro-Fenton process, the COD was reduced by 52.0% (current efficiency of COD removal was 10.6%). Electrooxidation continued by the anodic process resulted in elimination of ammonia in 55 min and a total reduction of COD by 72.9%. The coupled process d could be considered a simulation of the combined process under flow conditions. The effect of this process was compared to that of a two-steps process: Fenton process followed by indirect anodic oxidation.

1. INTRODUCTION

Tannery wastewater treatment represents a difficult technological problem. After conventional treatment, including sedimentation and biological process, effluents usually do not meet the requirements for COD, total solids and ammonia. Recently, a more effective and non-selective processes – advanced oxidation processes (AOPs) and electrooxidation have been extensively investigated.

Tannery wastewater treatment by anodic electrooxidation has been reported in few papers [1–5]. Results of present studies show that chlorine generated on the anode removes ammonia and reduces the COD value by 77.6% [1]. $Ti/SnO_2/PdO_2/RuO_2$ (SPR) anode is reported the most effective one [1]. Fenton process resulted in a reduction of COD by 63.7% [6].

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Fenton oxidation is particularly attractive due to its simplicity and high efficiency in removal of organics [7–10]. In the Fenton process, hydroxyl radical, a very strong oxidant, is generated according to the equation:

$$Fe^{2+} + H_2O_2 + H^+ \longrightarrow Fe^{3+} + HO^\bullet + H_2O \qquad (1)$$

Additionally, a modified Fenton reaction has been reported as effective for treatment of wastewater polluted with pharmaceuticals [11].

In the last decade, application of the electrochemical method for Fenton process, named electro-Fenton (EF), has been reported. EF process can be conducted in several ways. In the former one, ferrous ion and oxygen are externally applied, but H_2O_2 is generated on the cathode, according to the reaction:

$$O_2 + 2H^+ + 2e^- \longleftrightarrow H_2O_2 \qquad (2)$$
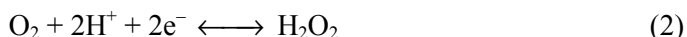
Several cathodes such as mercury pool [12], graphite [13, 14], vitreous carbon [12], carbon-poly(tetrafluoroethylene) O_2 fed [14–17] can be applied. Those cathode materials allow one to conduct reaction (2) due to a high overvoltage of the hydrogen reduction. At the cathode, reduction of Fe^{3+} to Fe^{2+} is also possible.

In an another way, H_2O_2 is externally applied, while a sacrificial iron anode is used to generate ferrous ions [18–20], or H_2O_2 is externally applied and Fe^{2+} ions are electrogenerated via reduction of Fe^{3+} ions or $Fe(OH)_3$ sludge [16]. Both Fe^{2+} and H_2O_2 may also be electrogenerated at a sacrificial anode and cathode via two electroreduction of sparged oxygen [21].

In the case when H_2O_2 is generated, the cathode electrolyzer can be divided with a diaphragm into the catholyte and anolyte spaces. The advantage of an electrolyzer without a diaphragm is its lower current resistance, which results in lower energy consumption. However, in such a case, possibility of undesired decomposition of H_2O_2 exists according to the reaction (2) [17]. Various materials are used as diaphragms – sintered glass discs, synthetic fibres and cation exchange membranes [14].

As anodes, titanium plates were used mostly covered with precious metal oxides – DSA®. The way of preparing of the cathode material is very important. Results obtained by Da Pozzo et al. [14] show that a gas diffusion (carbon–PTFE) cathode is much more suitable than a graphite cathode. Additionally, Fort et al. [22] proved that the addition of azobenzene in gas diffusion cathode improved its efficiency, due to the reduction of the voltage of reaction (2).

EF process was applied to many types of wastewaters – landfill leachate [18, 20, 23], urban wastewater [13], wastewater after regeneration of ion exchangers [24]. This process was also widely used for oxidation of same organics – formaldehyde [25], aniline [21], dyes [16], atrazine [12], non-ionic surfactants [26], phenols [27], chlorophenols [28] and many others.

However, the EF process was applied to tannery wastewater only in one study [19]. Iron cathode and anode sized $6 \times 7.5 \times 2$ mm^3 were used, placed at the distance of

6 cm, in a reactor with 45 cm^3 wastewater sample. At pH 3.0 and 7.2, after addition of 1670 mg/dm^3 of H_2O_2, the drop in COD after 10 min was 72% and 58%, respectively (initial value 2810 mg/dm^3). When EF process was conducted with the use of the carbon cathode and DSA type anode and after external addition of H_2O_2, the results seem to be more promising.

A high content of chlorides and ammonia is characteristic of tannery wastewater, thus the generation of chlorine at the anode should result in a significant removal of ammonia and COD. The aim of the studies presented in this paper was the evaluation of the effectiveness of the combined EF process with anodic electrooxidation by means of the anodic generated chlorine.

2. MATERIALS AND METHODS

Experiments were performed in an electrolyzer divided into anodic and cathodic parts by the asbestos fibre diaphragm. Dimensions of both parts were the same – 138×120×30 mm^3 and the volumes of catholyte and anolyte were 320 cm^3 each. As a cathode, two graphite plates were used, previously anodically activated with the current density of 3.0–3.3 A/dm^2 for 4 min, followed by the cathodic reduction to the potential of 1.5 V against a silver chloride electrode. The activation was conducted according to the Do and Yeh procedure [2]. Ti/SnO$_2$/PdO$_2$/RuO$_2$ (SPR) anode was used, with dimensions 10×10 cm^2. At the beginning of the process, both catholyte and anolyte spaces were filled with tannery wastewater (previously coagulated). Characteristics of those wastewaters was as follows: pH – 3, conductivity – 14566 μS/cm, COD 708 mg/dm^3, N–NH$_4$ – 404 mg/dm^3, Cl$^-$ – 4930 mg/dm^3. Wastewaters were initially saturated with oxygen and then constantly aerated.

During the experiments, the catholyte was transferred into the anodic space at specific time intervals (50, 55 and 60 min), the catholyte space was filled with a new sample of wastewater and the process was continued by indirect anodic oxidation. This treatment was a simulation of the continuous (flow) process.

During the experiment, the cathodic current density was 2.0 A/dm^2, anodic current density 2.1 A/dm^2. The concentration of ferrous ions (added as FeSO$_4$) was 1500 mg/dm^3, the latter value turned to be the best in a classic Fenton process [6].

The effectiveness of the EF process was monitored by the determination, after specific intervals, the following parameters: temperature, N-NH$_4$ content and COD. pH was monitored constantly and (if necessary) adjusted with 1 M H$_2$SO$_4$ to 3.0–4.5. In the anolyte, temperature, chlorides, free and total chlorine, N-NH$_4$ and COD were determined. pH was monitored constantly and adjusted to the value of 4.0–4.5 with 1 M NaOH. Additionally, the voltage was measured. The catholyte current efficiency of H_2O_2 synthesis was determined in the following way: 10% solution of KJ was placed in the catholyte space of the electrolyzer and the process was conducted for

15 min. Iodine volatilized from the catholyte space was absorbed in 0.5 M $Na_2S_2O_3$. The current efficiency was calculated based on the total produced iodine.

Results of other investigations of those wastewaters (Fenton process, Fenton process followed by anodic electrooxidation and anodic electrooxidation) [6] show that the SPR anode is the most suitable and the optimum current density is 2.0 A/dm^2.

3. RESULTS AND CONCLUSIONS

Figure 1 shows time dependences of the COD values during all investigated processes. For the Fenton process, the H_2O_2/Fe^{2+} doses were 4000/1500 mg/dm^3 and pH was 3.5. These parameters were assumed as the optimum ones [1].
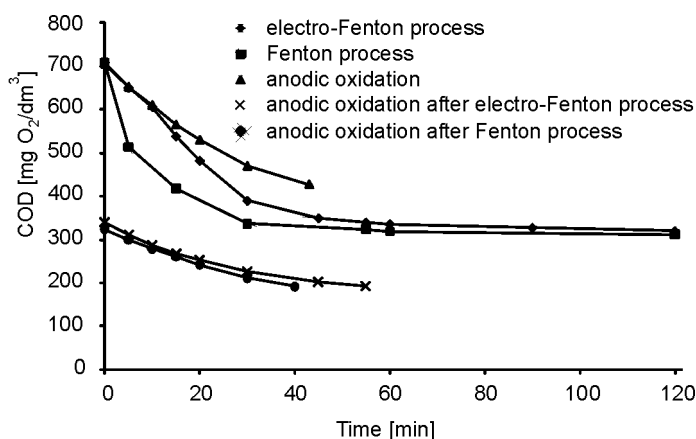
Fig. 1. COD during all investigated processes

During first 30 min of the EF process, an efficient reduction of COD was observed (44.9%). From 55 to 120 min the COD decrease was insignificant – from 52.0% to 54.8%, respectively. During the first 5 min the rate of the process was increasing.

In the case of a classic Fenton process, at the beginning COD decreased rapidly and then the process slowed down. Starting from 45 min, the both processes were similarly effective, the Fenton process being a little more efficient. After 55 min of the EF process, the COD decreased to 340 mg/dm^3 O_2 (52.0%). The rate of both, EF and classic Fenton processes, depended on the concentration of hydrogen peroxide. That is why only in case of the EF process, the rate of COD removal increased in the first 5 min – at the beginning the concentration of H_2O_2 was very low (in the classic Fenton process, the total dose of H_2O_2 was added at the beginning). Due to this, at the beginning the rate of the Fenton process was much higher than the rate of EF process. Insignificantly higher final effectiveness of the classic Fenton process could be explained by the higher pH in the EF process.

The increase in concentration of H_2O_2 in the EF process (Fig. 2) was rapid at the first 30 min (to 240 mg/dm^3), then it slowed down (327 mg/dm^3 after 120 min), despite the H_2O_2 consumption in EF process continuously decreasing. This could be explained by polarization of the electrode (evidenced by the increase in voltage in spite of increasing temperature), reaction between H_2O_2 and hydroxyl radicals

$$H_2O_2 + 2OH^\bullet \longrightarrow 2H_2O + O_2 \tag{3}$$
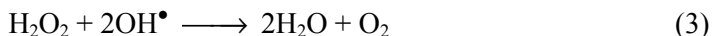
and degradation of H_2O_2 caused by temperature increase and air flow. Also another cathodic reactions can take place.
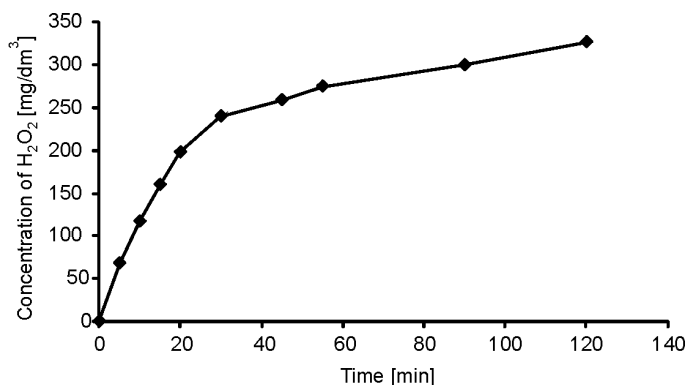
Fig. 2. H_2O_2 concentration during the electro-Fenton process

During the process, no permanent changes in the characteristics (activity) of the cathode were observed – the results were reproducible. A cathodic reaction of H_2O_2 synthesis describes Eq. (2). A summary of indirect cathodic oxidation of pollutants could be described as follows:

$$2H_2O + O_2 + 4e^- \longrightarrow 4OH^- \tag{4}$$

An apparent current efficiency of the indirect cathodic oxidation of organic pollutants (expressed as the current efficiency of the reduction in COD, assumes that 96 500 C corresponds to 8 g of O_2) for 30, 60 and 120 min of the process was relatively low and equalled: 34.6, 20.3 and 10.6%, respectively. The effectiveness of the process referred to the content of H_2O_2 remaining in the solution after a specified time could be calculated based on reaction (2).

For 30, 60 and 120 min of the process, the effectiveness was: 18.8, 11.0 and 6.3%, respectively. The total efficiency of H_2O_2 synthesis and of oxidation of organic pollutants was also not high and for 30 min of the process equalled 53.4%. The current efficiency of H_2O_2 synthesis determined with the use of KJ solution was much higher – 87.7%. This difference in the current efficiency described above was probably caused by decomposition of H_2O_2 due to the phenomena discussed above.

Due to cathodic reactions described with Eqs. (2) and (4), some increase in pH was observed, thus strong acid had to be added to maintain pH between 3.0–4.5. Theoretical amount of strong acid required to neutralize OH^- ions is 229 mmol/h but in practice it was ca. 3% lower. Increase in temperature was slightly higher than in the case of anodic oxidation, probably due to a need of higher voltage (9.2–14.0 V) to obtain the current density of 2 A/dm^2, caused by a diaphragm increasing electrical resistance. During the cathodic process, no decrease in ammonia concentration was observed. However, in anodic oxidation ammonia was removed after 55 min. Under flow conditions, the retention time for catholyte and anolyte must be the same (the same volume). It can be therefore assumed that optimum conditions for the coupled process should include the EF process lasting 55 min at the current density of 2.0 A/dm^2, followed by anodic indirect oxidation, also in 55 min at 2.1 A/dm^2.

The time dependences of COD during anodic electrooxidation in the coupled process, as well as during electrooxidation of wastewater after classical Fenton process [21] are also shown in Fig. 1. Both curves are very close to each other. The final COD removal in the coupled process was 72.9%. For the Fenton process accompanied with electrooxidation, corresponding to the time of ammonia removal, the final effect is a little higher – 73.8% COD removal [1]. Anodic electrooxidation (Fig. 1) of coagulated wastewater resulted in 43 min for complete ammonia removal and 39.3% for COD removal (to 428 mg/dm^3) [1]. The effect was much less outstanding than in the coupled process.

Ammonia concentrations during the anodic oxidation of wastewaters after EF process are shown in Fig. 3, being similar to those observed for anodic oxidation of wastewaters only after coagulation, presented elsewhere [1]. The current efficiency of electrooxidation of ammonia was by 1.3% lower (41.1%) and reduction of COD was by 0.5% higher (43.5%) than those in wastewater only after coagulation. The current efficiency of COD removal was 8.8% being also higher than that in previous studies (8.5%) [1].
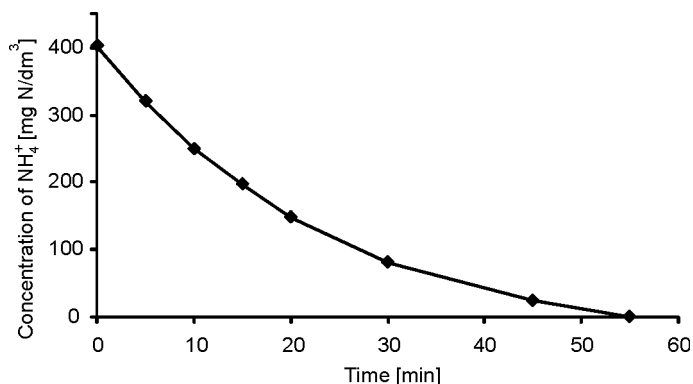
Fig. 3. Ammonia concentration during electro-Fenton process

The main disadvantage of the coupled electrooxidation process is a significant energy consumption, due to high electrical resistance caused by the presence of a diaphragm. Energy consumption per 1 kg of $N-NH_4$ was 130.5 kW/h, being almost twice higher than that for the electrooxidation. Another disadvantage of the coupled process is a need of adding acid or base solutions. The principal advantage of the coupled process is much higher COD removal and possibility to conduct the process in one reactor.

Due to scarce literature data, the results presented above cannot be compared with those of other authors. Do and Yeh [29] used a sintered glass disc as a diaphragm, graphite cathode and SPR anode. They found that the cathodic efficiency of phenol oxidation is higher than the anodic one and it decreased with an increase in the amount of electrical charge. The increase from 300 to 2500 C resulted in the decrease in the cathodic current efficiency from 83.5 to 56.1% and in the anodic one – from 80.4 to 57.4%. Those efficiencies were relatively high but mostly due to a high initial concentration of phenol (3222 mg/dm^3). Degradation of phenol was below 40%. This significant decrease in the current efficiency has been explained by degradation of hydroxyl radicals on the cathodic surface. The authors investigated also the influence of temperature on the effectiveness of cathodic and anodic process and they found that the optimum is about 35 °C. Similar results were obtained by Sudoh at al. [27] with the use of Pt anode and graphite cathode.

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CONTENT OF SELECTED HEAVY METALS IN WATER AND RIVERBED SEDIMENTS OF THE UTRATA RIVER

The results of study of heavy metals content in water, suspended solids and bottom sediments of the Utrata River have been presented. The highest concentrations were observed for the dissolved form of Pb (0.35 mg/dm^3). All heavy metals under investigation were present in suspended solids (over 90%). Remarkable amount have also been found in sediments [mg/kg] Zn – 174, Cu – 76, Pb – 49, and Cd – 2.5. Water extracts showed lower concentrations, while EDTA extraction was several times more efficient (over 26%).

1. INTRODUCTION

Development of civilisation has led to introducing heavy metals into the natural environment in amounts remarkably excessing the natural load. Bottom sediments accumulate many elements, some of which are heavy metals, hazardous for natural environment, as well as animals and people. Their amount might be several orders of magnitude higher than in the water phase [1–5]. Heavy metals are considered to be stimulating, or – on the other hand – limiting factors for life activities. Metals as free cations and those bound to suspended solids and bed sediments of water bodies harm biocenosis. Depending on their concentration, oxidation number and tendency to create complexes, they might become toxic substances for all living organisms. Toxicity of metals is dependent on their form. The total contents of metals in environmental samples, eg. bottom sediments, wastewater sediments or soil, does not allow one to determine real danger imposed by their forms which can be absorbed by life forms like plants, or health hazard for animals and people [6–8].

Mobility of heavy metals and their availability for organisms can be estimated based on chemical indicators (fractionation analysis of sediments) and biological ones (estimation of heavy metals contents in plants and life forms inhabiting the area of

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sediments being analysed). Speciation and its analysis are most often used to carry out this kind of study, since they allow one to determine forms of heavy metals in environmental samples [9]. According to the IUPAC definition, speciation study leads to identification and/ or determination of one or more chemical forms of a given metal in a sample [10], and speciation is defined as determining specific forms and configurations which can be taken by a given element [11].

In studies of heavy metals in environmental samples, single (simple) extraction is applied. It simulates natural conditions for transfer of elements from a sample directly to solution [9, 12]. Procedures applied here simulate natural environmental phenomena (eg. acid rain), which enables estimation of bioavailability and mobility of heavy metals. Apart from single extraction, sequential extraction is used, enabling one to separate heavy metals into chemical forms that can be released to the solution in different environmental conditions. Application of sequential analysis allows estimation of percentages of mobile metal forms, available for plants [13–15]). When combined with modern statistical methods, the following factors can be assessed as well: bioavailability, migration, accumulation, biomagnification, toxicity, solubility or sorption [16].

2. MATERIALS AND METHODS

Sediment samples were taken along midstream of the Utrata River, within the borders of the town of Pruszków, from 11 sampling stands (Fig. 1) and additionally from collector sewer from heat and power plant, located nearby some of the sampling stands.
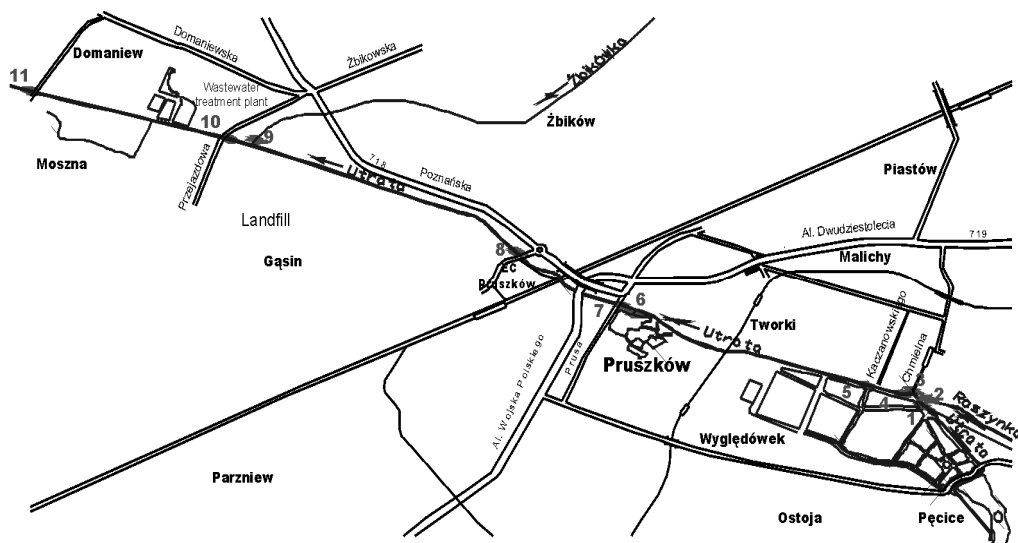

Fig. 1. Map of the Utrata River at the section of Pruszków town with location of sampling points (1–11)

Sediment samples were taken with Kajak probe by KC Denmark Research Equipment, from top layer of bottom sediments, on the depth of 10–15 cm below the sediment surface. The total of 28 water samples and 24 sediment samples were collected.

Water samples were filtered on filter drains. Suspension left on drains was mineralised with acid mixture of HNO_3 and $HClO_4$ (3:1). Sediment samples were dried at room temperature. Air dry deposit was thoroughly stirred and sieved, getting the fraction < 1 mm. One gram of sediments was weighed out from dried and sieved samples, to be mineralized with the acid mixture. After mineralization of the suspension and sediments, the following heavy metals were determined in the filtrate (water) and in solutions: Fe, Mn, Zn, Cu, Pb, Cd. To determine the total content of heavy metals in water, graphite furnace atomic absorption spectrometry (GFAAS) was used, while in mineralized solutions – flame atomic absorption spectrometry (FAAS). Effects of metal extraction from bottom sediments were compared for distilled water and 0.1 M EDTA solution. The extraction was carried out for 1 h after adding reagents in the ratio of 10 g of sediments/100 cm^3 solution. Extracts after filtration were analysed to determine the content of heavy metals.

3. RESULTS AND DISCUSSION

Water and bottom sediment quality were analysed in the period of 2003–2006 in the Utrata River [17]. The results of the study showed that water quality varies and depends on the place of collecting samples. Characteristics of water was slighlty basic (pH 7.1–8.0). Higher values of pH were observed downriver. River waters were well aerated. Closer to collector sewers of the heat and power generation plant, water was oversaturated with air and showed high manganese index (62 mg/dm^3). Manganese index values increased (over 10 mg/dm^3) downriver, from sampling stand 6. The analysed waters were highly mineralized, which is proved by high value of electrical conductivity (average 947 µS/cm). Analyses of the depths of the river show various contents of suspended solids in sampling stands as well as depending on period of research. Higher values were observed in winter period, lower ones in spring. Riverbed sediments were slightly basic (pH 7–7.6). Main ingredients of sediments were mineral substances, accounting for 86.2%. According to Stangenberg classification, these sediments can be classified as mineral sediments. The highest concentration of organic matter was observed in sediments taken from sampling stands 1–5 (19.4%), while the lowest in the area around sites 8 and 9 (5.4%). Reasearch sites upriver were situated within agricultural area, while sites from 6 to 11 in urban, highly industrialized area. Study of metals dissolved in water and bound in the suspension showed significant differences between sampling stands. Lead occurred in the highest concentrations in a dissolved form (0.35 mg/dm^3). The lowest concentration in water was

determined for Cd (0.01mg/dm^3). Ranges, medians and average contents for heavy metals are given in Table 1.

Table 1

Concentration of metals in the depths of the river [mg/dm^3]

Concentration	Fe	Mn	Zn	Cu	Pb	Cd
Average	0.09	0.02	0.04	0.07	0.35	0.01
Median	0.06	0.01	0.02	0.07	0.32	0.01
Minimum	0.03	0.01	0.01	0.06	0.27	0.01
Maximum	0.21	0.14	0.10	0.08	0.49	0.03

Contents of metals in suspension accounted for ca. 99% for iron, manganese and zinc, while for copper, lead and cadmium ca. 91% (Table 2). Lead was the least bounded with suspension and it was present mainly in a dissolved form. The process of bounding metals in water depends, among others, on temperature, amount of suspension, biological activity of organisms [17–19] as well as geological base. In many cases, reaction of solution is important as well. In slightly basic reaction, metal ions (especially zinc) change into slighlty soluble forms, which at the same time tend to adsorb on suspensions [20].

Table 2

Concentrations of metals in suspension [mg/dm^3]

Concentration	Fe	Mn	Zn	Cu	Pb	Cd
Average	47.46	3.71	6.93	0.73	3.42	0.13
Median	35.86	2.02	6.87	0.59	3.59	0.13
Minimum	15.76	0.79	3.83	0.15	1.62	0.09
Maximum	95.49	21.36	11.13	1.79	4.27	0.17

Bottom sediments of the Utrata River analyzed in this study were characterised by high content of heavy metals. Content range (in mg/kg) was: for zinc 71–338, copper 8.2–281, lead 49.6–111.6 and cadmium 1.25–4.39, respectively. The values of average contents of metals allowed one to arrange them in order of occurence, depending on their concentrations, in the following way: Zn > Cu > Pb > Cd (Fig. 2. Table 3).

For the analyzed sediments, the most distinctive area was the one located in the neighbourhood of municipal waste and heat and power generation plant. Sediments sampled there were characterised by the lowest reactivity, while their conductivity was many times higher than the values measured in other stands. For these sediments, the highest concentrations of metals were determined (Fig. 2).

Total content of heavy metals in bottom sediments is used only to estimate the level of pollution. It cannot be the basis to determine concentrations of elements that are potentially bioavailable. This kind of estimate can be based on the results of single

or sequential extraction. Extraction allows one to identify metal forms and assess their mobility in aquatic environment. Bioavailability of metals usually depends on particular factors: reaction (pH), electrical conductivity of organic matter, oxidizing-reducing properties, as well as heavy metals characteristics and their initial form.
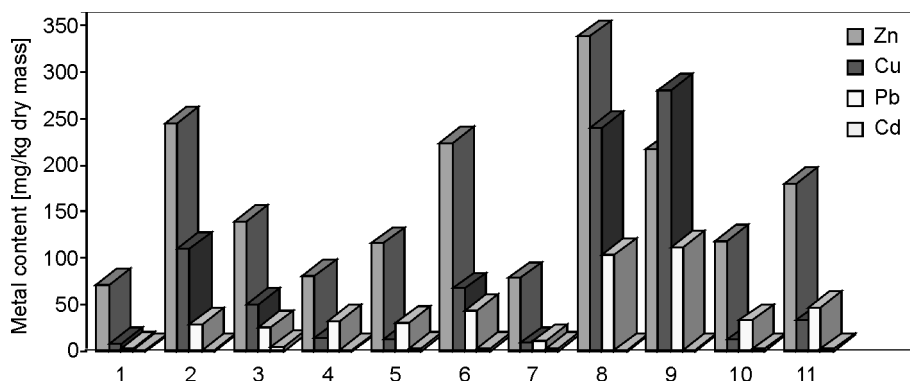
Fig. 2. Concentration of heavy metals in riverbed sediments of the Utrata River

T a b l e 3

Concentrations of heavy metals in riverbed sediments [mg/kg d.w.]

Concentration	Fe	Mn	Zn	Cu	Pb	Cd
Average	9651.65	392.67	164.52	76.44	49.70	2.52
Median	3819.74	110.38	151.87	33.36	31.92	2.45
Minimum	987.98	26.92	71.13	8.21	23.60	1.25
Maximum	36382.21	1536.21	338.36	281.25	111.63	4.39

In laboratory-scale tests, effects of Zn, Cu, Pb and Cd extraction from sediments were compared for those with the use of EDTA and H_2O. The results prove that adding 0.1 M EDTA to sediments leads to the transfer of metals deposited in sediments into the solution. Results for metals extracted with EDTA were much higher than those for solutions with distilled water (Fig. 3).

Applying 0.1 M EDTA led to the release of 45–189 mg/kg Zn, 7–68 mg/kg Cu, 6–100 mg/kg Pb and 0.5–2.0 mg/kg Cd from the sediments, which accounted for 34, 40, 37 and 26% of the total contents of Zn, Cu, Pb and Cd, respectively. The highest increase of solubility was observed for sediments taken in the last part of the analysed river area. Much lower, but still noticeable values were measured for sediment extraction with distilled water. Lead and cadmium were released to the environment the most efficiently (18% and 7.7% respectively), the lowest efficiency was that for zinc (1.2%) and copper (3.5%). After using water and EDTA, it was observed that metals occurring in sediments are intensively washed out, which makes them more bioavailable. Taking into account slight concentrations of metals in depths of the river and

their high content in riverbed sediments, the effectiveness of metal transfer from sediments into water might be high. Presence of complexing substances (eg., EDTA), mobilizing metals into water solution, is a significant threat to aqueous biocenosis. Plants absorb chelated metals in motion intensely, which results in their concentration in biomass exceeding the demand [21].
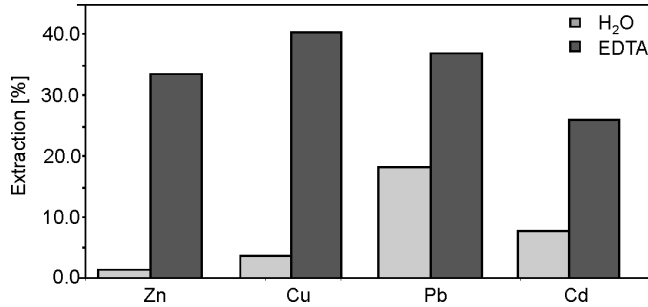
Fig. 3. Percentage of extraction of metals from sediments into water and EDTA

In Poland, there are not legally biding regulations referring to classification of bottom sediments which would comply with commonly used categorization of polluting elements. The only estimate is the one carried out while monitoring quality of inland surface waters. It is based on geochemical criteria and estimates sediment quality in terms of its pollution with detrimental organic elements and heavy metals. The first Polish geochemical classification of riverbed sediments and lake bed sediments was made by the Polish Geological Institute and is used in State Environmental Monitoring. Sediments were divided into 3 classes according to their metal content, based on geochemical criteria [22]) (Table 4).

Table 4

Classification of water sediments based on geochemical criteria

Metal [mg/kg]	Geochemical background	Class I slightly polluted sediments	Class II moderately polluted sediments	Class III polluted sediments
Cadmium (Cd)	<0.5	<1	<5	<20
Copper (Cu)	6	<20	<100	<200
Lead (Pb)	10	<50	<200	<500
Zink (Zn)	48	<200	<1000	<2000

According to this classification, sediments from the Utrata River, referring to the metal contents, can be assessed mainly as slightly or moderately polluted. The sediments are polluted mainly with copper and cadmium. The content of Cu, at majority of sampling stands, exceeded the value set for class II. The analysis of the results of the

research led to the conclusion that a high proportion of the sediments is contaminated with cadmium, in some cases the concentrations exceeded the values for class II. The content of zinc and lead were slightly lower. Their content was usually classified as belonging to class II, sometimes class I.

It is impossible to determine the contents of metals in sediments being safe for organisms, using geochemical criteria. The USA and Canada have introduced criteria enabling assessment of ecotoxicity of waste. They are based on two critical (limiting) values of the treshold effect level (TEL) – the content of a given element, above which its toxic effect can be observed and probable effect level (PEL) – the content of a given element, above which its toxic effect on organisms is often observed. Having analysed the sediments in terms of their ecotoxicity, it can be claimed that generally they can impose danger to aquatic life. Cadmium content in all the samples exceeded TEL (0.7 mg/kg), and usually it exceeded also PEL (3.5 mg/kg). The concentrations of other analysed metals were higher than TEL (Cu – 36 and Pb – 35mg/kg) only in a few sampling stands in the industrialized area. They tended to slightly exceed PEL (Cu 197, Pb 91, Zn 315 mg/kg).

4. CONCLUSIONS

The high content of heavy metals in the surface layer of sediments showed continuous inflow of pollution to the waters of the Utrata River. Their source might be surface run-offs from the river basin, from nearby roads and from the collector sewers.

Concentrations of analysed metals were low in the depths of the river. The most dissolved forms were determined for lead. Over 90% of contamination was connected to suspension. The riverbed sediments of the Utrata River are most polluted with cadmium and zinc, while copper and lead were in lower concentrations.

The total content of metals in sediments can be applied only to assess their level of contamination. It is not useful to assess the mobility or bioavailability of metals in the environment. This estimate can be done based on concentration values determined in speciation analysis. Extraction of metals into water and EDTA solution carried out for the purpose of this study proved that Zn, Cu, Pb and Cd get into dissolved forms easily and they become more bioavailable for organisms inhabiting the Utrata River. Metals in sediments are easily washed out into water phase. It is especially noticeable in the case of lead and cadmium.

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AGROINDUSTRIAL WASTES AS UNCONVENTIONAL SUBSTRATES FOR GROWING OF *BACILLUS* STRAINS AND PRODUCTION OF BIOSURFACTANT

The study was aimed at the development of economical methods for biosurfactant production by the use of unconventional substrates. The research investigated the potential of utilising agroindustrial wastes to replace synthetic media for cultivation of *Bacillus* strains and biosurfactant production. In total, 21 of the waste products from dairy, sugar, fatty, and fruit and vegetable processing industries, breweries, distillery were examined. Three bacterial strains were identified by 16S rRNA gene sequencing: *Bacillus subtilis* (I′-1a), *Bacillus* sp. (T-1), *Bacillus* sp. (T′-1). Biosurfactant production was examined in an indirect way by measuring of surface tension (ST), blood agar lysis, oil spreading and drop collapsing tests. The best unconventional substrates for bacteria growing and biosurfactant production at 30 °C under aerobic conditions were molasses, brewery effluents, and fruit and vegetable decoction from the processing factory.

1. INTRODUCTION

Currently, the main drawback for widespread use of biosurfactants is disadvantageous economics of their production. Although biosurfactants have numerous advantages (being non-toxic, nonhazardous, biodegradable, environmentally friendly, selective, effective under extreme conditions, with wide potential in industrial applications, and unique surface-active properties), their production costs higher than those of synthetic surfactants is a major drawback. Biosurfactants could potentially replace synthetic surfactants if costs of their production were lowered substantially. Achieving

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this goal requires finding alternative inexpensive substrates and highly efficient microorganisms for biosurfactant production. The choice of inexpensive raw materials is important to the overall economics of the process because they account for 50% of the final product cost. The best way to reduce substrate cost for biotechnology at present is to use wastes with the right balance of carbohydrates and lipids to support optimum bacterial growth and biosurfactants production, and which are either free or carry a cost credit for environmental benefit [1]. As is known, millions tons of hazardous and non-hazardous wastes are generated each year throughout the world. There is a great need for better management of these wastes via the concept: reduce, reuse, and recycle [1]. So far, several renewable substrates include various agricultural and industrial by-products and waste materials have been intensively studied for microorganism cultivation and biosurfactant production at a laboratory scale, for example: olive oil mill effluent [2], waste frying oil [3], oil refinery wastes [4], soapstock [5, 6], molasses [7, 8], whey [7, 9, 10], starch wastes [10–12], cassava flour processing effluent [13] and distillery waste [9].

In the present research, the potential of agroindustrial wastes has been investigated to replace synthetic media for supporting the growth of the *Bacillus* strains and biosurfactant synthesis.

2. MATERIALS AND METHODS

Isolation, identification and characterization of bacterial isolates. Bacterial strains (T-1, T′-1 and I′-1a) used in this study were isolated from sludge of a 100 year old oil refinery in Czechowice-Dziedzice (Poland) as described by Berry et al. [14] and Płaza et al. [15, 16]. The aged sludge was acidic (pH 2), highly contaminated with polycyclic aromatic hydrocarbons. Bacterial isolates were identified based on the 16S rRNA gene sequence analysis. A direct-colony, polymerase chain reaction (PCR) was exploited to amplify the 16S rRNA gene in a 30-cycle PCR using universal primers 27F and 1492R. The PCR conditions used were: initial denaturation at 95 °C for 8 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and elongation at 72 °C for 1 min, followed by elongation at 72 °C for 10 min. The amplified PCR products were purified using the Qiagen-PCR purification kit as per the manufacturer's instructions. The purified PCR products were sequenced from both ends at the DNA Sequencing Core facility of the University of Michigan at Ann Arbor. The 16S rRNA gene sequences were analysed at the Ribosomal Database Project (RDP) II (http//:rdp.cme.msu.edu). The top 10 most homologous sequences were aligned using the CLUSTALW program v1.83 at the European Bioinformatics site (www.ebi.ac.uk/clustalw). The similarity matrix was prepared using the DNAdist program in the PHYLIP package with Jukes–Cantor corrections. Isolates were identified

as the genus/species to which they showed highest 16S rRNA gene sequence similarity in the RDP database.

Isolates were maintained on agar slants (SMA–Standard Methods Agar, bioMerieux) containing 8 g of peptone, 2.5 g of yeast extract, 1 g of glucose and 15 g of agar per 1 dm^3 of distilled water at 4 °C.

Isolates growth on solid media and in liquid cultures. In the experiment, the following solid and liquid wastes were used: whey, diary wastewaters, brewery effluents, brewery spent grain, sugar wastewaters, beet pulp, molasses, soapstock, oil slime, acidic wastewaters from fatty factories, fusel, slop, potato decoction, apple and citrus pomaces, fruit and vegetable decoction from the processing factory. The wastes were collected and stored at −18 °C until needed.

Whey was boiled for 10 min, cooled to 4 °C, and solid particles were removed by filtration through the cotton. The operation was repeated. Spent grain, apple and citrus pomaces (100 g) were mixed with 1 dm^3 of distilled water and incubated at 4 °C by 24 h. After sedimentation, solid particles were removed by filtration through the cotton. Beet pulp (100 g) was mixed with 1 dm^3 of distilled water, boiled by 30 min, cooled and then filtered through cotton. Molasses (100 g) was diluted in 1 dm^3 of distilled water. Potato peels were collected and washed with tap water followed by distilled water. Then blenching operation was carried out by immersing the peels in boiling water for 20 min. After sedimentation, the potato substrate was filtered through cotton. All the wastewaters were filtered through cotton to remove solid particles. Concentrations of the waste products prepared in this way and other liquid wastes were established as 100%.

20 g/dm^3 of agar were then added to solid and liquid samples of wastes, the mixtures were sterilized in an autoclave under 1 atm at 121 °C for 15 min and poured into plates. The concentrations (in vol. %) of the media used were: 100, 50, 25 and 10. The minimum medium (MM) of the following composition (g/dm^3): KNO_3 – 3.0, Na_2HPO_4 – 2.2, KH_2PO_4 – 1.4, $NaCl$ – 0.1, $MgSO_4 \cdot 7H_2O$ – 0.6, $CaCl_2 \cdot 6H_2O$ – 0.04, $FeSO_4 \cdot 7H_2O$ – 0.02 was used to dilute the wastes. 24 hour slant cultures of isolates to be tested were spread on the medium with a microbiological loop. The plates were incubated for 72 h at 30 °C. The growth was examined after 24, 48 and 72 h. As the control, the bacterial growth on minimum medium (MM) was evaluated. All growth studies were done in triplicate.

The bacterial suspensions, obtained from a nutrient agar slant incubated for 24 h at 30 °C, in the liquid Standard Methods medium of the following composition (g/dm^3): peptone – 8, yeast extract – 2.5, glucose – 1 was adjusted to $OD_{600 \text{ nm}}$ 0.65 (ca. 10^7–10^8 CFU/cm^3). Then 3 cm^3 of the bacterial suspensions were inoculated in 300 cm^3 Erlenmeyer flasks containing 150 cm^3 of sterilized organic wastes as nutrient sources. The cultures were grown aerobically at 30 °C for 96 h with constant shaking (110 rpm). Growth curves were obtained by monitoring the optical density at 600 nm on an

UV/VIS spectrophotometer (Varian). Three independent experiments were conducted for each bacterial strain and for each chosen organic waste product.

Determination of biosurfactant production. Hemolytic activity was carried out as described by Carrilo et al. [17] with the minor modifications. Isolated strains were screened on blood agar plates containing 5 vol. % of blood and incubated at 30 °C for 96 h. Hemolytic activity was detected as the presence of a clear zone around a colony.

The drop collapse technique was carried out in polystyrene lid of a 96 microwell plate (Biolog, Harward, CA, USA) as described by Jain et al. [18] and Bodour and Maier [19]. 100 µl bacterial cultures were added to wells of a 96 well microtiter plate lid, than 2 µl of crude oil were added to the surface of the cultures.

Oil spread technique was carried out according to Morikova et al. [20] and Youssef et al. [21]. 50 cm^3 of distilled water were added to Petri dishes followed by addition of 100 µl of crude oil to the surface of the water. Then, 10 µl of the bacterial cultures were put on a crude oil surface. The diameter d of the clear zone on the oil surface was observed.

Surface tension (ST) was determined with a Kruss processor tensiometer (model K12 Kruss, Germany) by the plate method. Before measuring, the bacterial cultures were centrifuged at 10 000g for 20 min and the supernatant was used for the surface activity measurements. To increase the accuracy, an average of three independent experiments was used for the study. Surface tension of water was taken 71.79 ± 0.3 mN/m.

All the assays were performed in triplicate with distilled water as a control.

3. RESULTS AND DISCUSSION

The isolates were screened and selected for further studies as described by Płaza et al. [15]. Three bacteria (T-1, T′-1 and I′-1a), halothermotolerant Gram-positive spore forming species were chosen for identification. The 16S rRNA gene sequences showed that the isolates were *Bacillus* spp.; I′-1a was identified as *B. subtilis*, but T-1 and T′-1 were identified as *Bacillus* sp. The 16S rRNA gene sequencing could not clearly assign isolates T-1 and T′-1 to any species of *Bacillus* as both isolates showed higher than 99% similarity to two distinct species (*B. subtilis* and *B. licheniformis* for T-1 and *B. subtilis* and *B. amyloliquefaciens* for T′-1). The morphological and biochemical characteristics of the three isolates were presented by Płaza et al. [22].

The results of growing *Bacillus* strains on solid media are presented in Table 1 (concentrations of all wastes was 100%). *Bacillus* strains grew very well on the following solid media: two brewery effluents, beet pulp, molasses, slop and fruit and vegetable decoction. Two from three brewery wastewaters tested appeared to be good candidates to replace conventional media, probably due to right balance of carbohydrates and lipids to support optimum growth of the strains.

Table 1

Growth of *Bacillus* strains on solid media
composed of various waste products[a]

Waste products	*Bacillus* strains		
	T-1	T′-1	I′-1a
1. Whey 1	–	–	–
2. Dairy wastewater	–	++	+
3. Whey 2	–	–	–
4. Brewery effluent 1	++	+++	+++
5. Brewery effluent 2	+	+	+
6. Brewery effluent 3	+++	+++	+++
7. Brewery spent grain	++	+	+
8. Sugar wastewaters	++	+	–
9. Beet pulp	+++	++++	+++
10. Molasses	++++	++++	++++
11. Soapstock 1	–	–	–
12. Soapstock 2	–	–	–
13. Oil slime	–	–	–
14. Acidic fatty wastewater 1	–	–	–
15. Acidic fatty wastewater 2	–	–	–
16. Fusel	–	–	–
17. Slop[b]	+++	+++	+++
18. Potato decoction	++	++	++
19. Apple pomace	+	+	+
20. Citrus pomace	+	+	+
21. Fruit- and-vegetable decoction	+++	+++	+++

[a]++++ very good growth, +++ good growth,
++ medium growth, + poor growth, – no growth.
[b]Concentration of slop was 50 vol. %.

Results presented in Fig. 1 show that only 4 wastes (brewery effluents, molasses and fruit and vegetable decoction) from all tested ones were good substrates for growth of the isolates at 30 °C in liquid media under aerobic conditions. Molasses and fruit and vegetable decoction were found to be the best substrates for growing of *Bacillus* strains. Both waste products had high values of COD and BOD, and nutritional components of both wastes were efficiently utilized for biomass built-up.

In this work, the protocol proposed by Youssef et al. [21] was applied to screen production of biosurfactants by *Bacillus* strains growing at 30 °C in brewery effluents, molasses and fruit and vegetable decoction. In the first method used to screen biosurfactant producing isolates blood agar lysis was used. From all of *Bacillus* strains growing in brewery effluents, molasses and fruit and vegetable decoction, hemolytic activity was observed (Table 2). Youssef et al. [21] reported 13.5% of the hemolytic strains to lower the surface tension to the values below 40 mN/m. Various compounds are

produced by microorganisms which can cause lysis of red blood cells however they do not necessarily have to be surface active molecules. For this reason, many authors suggested that this method should be supported by other techniques based on surface activity measurements [8, 19].
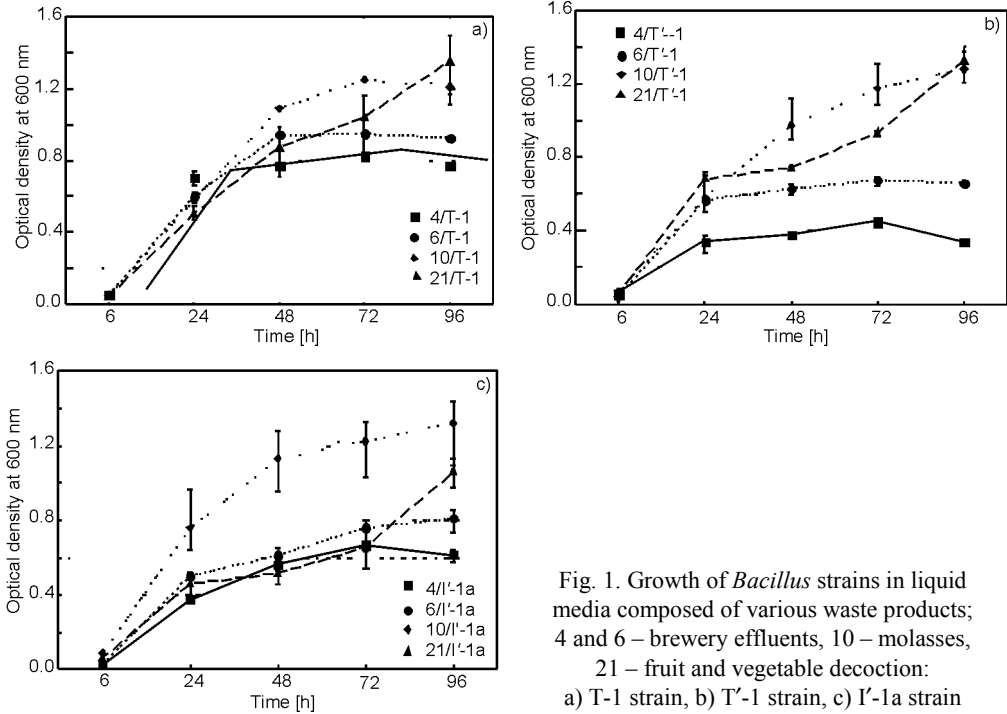

Fig. 1. Growth of *Bacillus* strains in liquid media composed of various waste products; 4 and 6 – brewery effluents, 10 – molasses, 21 – fruit and vegetable decoction: a) T-1 strain, b) T′-1 strain, c) I′-1a strain

The surface active properties of the isolates therefore were tested by the following methods: drop collapse method, oil spreading technique, and finally surface tension measurements. Both, the drop collapse and oil spreading techniques can be used as qualitative and quantitative assays. They have several advantages in requiring small volumes of samples, are rapid and easy to carry out, and do not require specialized equipment. The drop collapse method is not as sensitive as the oil spreading technique in detecting low levels of biosurfactant production [21]. In our investigation for *Bacillus* strains growing in molasses medium both methods gave positive results. In the case of the fruit and vegetable decoction, negative drop collapse technique results and low values of the oil spread technique were observed. For brewery effluents, oil spread method showed positive reaction in the case of three strains (T-1, T′-1, I′-1a) and drop collapse technique only for two strains (T-1 and I′-1a) (Table 2).

Although the use of both drop collapse and oil spreading techniques constitute an easy and quick assay to screen biosurfactant production. However, the surface tension measurements were carried out to confirm the results obtained. In Table 2, the values

of the surface tension are presented. In most of the *Bacillus* strains the surface tension was lower than 40 mN/m. Only one strain (T′-1) growing in brewery effluent showed surface tension over 40 mN/m. *Bacillus* strains decreased the surface tension to 26.7 –28.8 mN/m and to 30.8–36.5 mN/m for molasses and fruit and vegetable decoction, respectively. Results showed that those waste products had similar potential for growing of *Bacillus* strains and biosurfactant production, and can replace synthetic media. The use of economic substrates meets one of the requirements for a competitive process for biosurfactant production. Our early investigation confirmed that three *Bacillus* strains were also able to grow and produce biosurfactant in brewery effluent medium under aerobic and thermophilic conditions during the phase of stationary growth [22].

T a b l e 2

Surface active properties of isolates growing in liquid media
composed of various waste products

Waste products	Strains	Surface tension σ [mN/m]	Drop collapse method	Oil spread method d [mm]	Hemolytic activity
4. Brewery effluent	T-1	37.73±1.05	+	4.67	+
	T′-1	42.73±0.58	–	17.67	+
	I′-1a	33.05±0.17	+	6	+
6. Brewery effluent	T-1	34.43±0.30	+	4.33	+
	T′-1	39.21±0.98	–	4	+
	I′-1a	31.54±0.57	+	10	+
10. Molasses	T-1	26.64±0.43	+	28.33	+
	T′-1	29.25±0.93	+	40	+
	I′-1a	28.45±0.54	+	40	+
21. Fruit and vegetable decoction	T-1	31.55±0.91	–	2.33	+
	T′-1	36.55±0.28	–	2.67	+
	I′-1a	30.82±0.64	–	6.67	+

Among the potential biosurfactant producing microbes, *Bacillus* species are known to produce cyclic lipopeptides including surfactins, iturins, fengycins and lichenysins as the major classes of biosurfactants [11, 23]. Surfactin, one of the most effective surfactants produced by *Bacillus* strains, showed a ST of 25 mN/m, IT < 1.0 mN/m and CMC of 0.025 g/dm^3 [23].

In the present investigation, it was shown that three *Bacillus* strains were capable of growing and producing biosurfactants in media composed of brewery effluents, molasses and fruit and vegetable decoction from processing factory at 30 °C.

Medium composition is critical in determining properties of biosurfactants. Makkar and Cameotra [8] cultivated *B. subtilis* MTCC 2423 and 1427 under thermophilic conditions using molasses as a carbon source; the biosurfactant production lowered

the ST of the medium to 29 and 31 mN/m for MTCC 2423 and 1427, respectively. Potato substrates were evaluated as a carbon source for surfactant production by *B. subtilis* ATCC 21332 [12, 24]; ST dropped from 71.3 to 28.3 mN/m, and CMC of 0.10 g/dm^3 was obtained. In addition, Nitschke and Pastore [13] used a cassava-flour processing effluent as a substrate for surfactant production by *B. subtilis* LB5a and *B. subtilis* ATCC 21332, which reduced ST of the medium to 25.9 and 26.6 mN/m, respectively. Joshi et al. [7] studied biosurfactant production using molasses and cheese whey under thermophilic conditions by four *Bacillus* strains. ST was reduced to 34–37 mN/m for isolates grown under both static and shaken modes.

4. CONCLUSION

Bioconversion of industrial wastes into useful products (biosynthesis) has the potential of being a source of new materials and can convert industrial wastes into commercial products, and still reduce pollution.

Our preliminary investigation confirms that the three *Bacillus* strains can grow on various waste products (brewery effluents, molasses and fruit and vegetable decoction) as organic media (microbiological nutrients) and can produce biosurfactants. Replacing traditional microbiological media with agroindustrial wastes as substrates for biosurfactant production holds great potential. Moreover, this will reduce many management problems of processing industrial waste. The isolation and characterisation of biosurfactants produced by *Bacillus* strains growing on various wastes will be taken into consideration in the future research. However, the use of wastes as feedstock for bioprocesses generates new analytical and methodological difficulties concerning the measurement of the product accumulated. To overcome the problem new methods should be developed based on the waste used [18].

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DETERMINATION OF KINETIC AND STOICHIOMETRIC
PARAMETERS OF ACTIVATED SLUDGE MODELS

An overview of methods aiming at the determination of selected parameters of activated sludge models has been presented. The attention was paid to the model parameters being the most sensitive and dependent on the composition of biomass and/or substrate. These parameters should be determined experimentally. Methods based on the measurements of oxygen uptake rate are widespread use in this area. For the parameters associated with ordinary heterotrophic organisms the method is well-known and regarded as standard. At the same time for many parameters associated with polyphosphate accumulating organisms the procedures have not been elaborated so far.

1. INTRODUCTION

Activated sludge models (ASMs) elaborated by the IWA task group [1] and other mathematical descriptions of biological wastewater treatment processes, i.e. the model presented by Barker and Dold [2], are widespread used for simulation and optimisation of activated sludge systems. The application of the model to a certain activated sludge system should be preceded by its calibration. Model calibration is understood as the estimation of the model parameters to fit a certain set of data obtained from the full-scale wastewater treatment plant (WWTP) [3]. The starting point for the model calibration is usually the set of default parameters which is implemented in the software used or provided with the model description. In general, two model calibration approaches exist: the mathematical optimisation approach and the process engineering approach. The former is based on mathematical calculations, whereas the latter relies on the experience and the process understanding of the modeller. In many works, the process engineering approach is combined with the mathematical one, by applying the sensitivity analysis to check, if the model is indeed sensitive to changes in the parame-

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ters that were calibrated. It was proved many times that sensitivity analysis allows identification of the most sensitive parameters in the activated sludge model. The reduced parameter set of the most sensitive parameters found by Weijers and Vanrolleghem [4] for ASM1 included: the yield coefficient for heterotrophic (Y_H) and autotrophic (Y_A) biomass, maximum specific growth rate for heterotrophic (μ_H) and autotrophic (μ_A) biomass, specific decay rate coefficient for heterotrophs (b_H), substrate half-saturation constant for heterotrophic organisms (K_S), oxygen half-saturation constant for autotrophic organisms (K_{OA}) and correction factor to adjust for either the change in μ_H associated with anoxic conditions or for the fact that only a portion of biomass can denitrify ($\eta_{anoxic,H}$). In the models comprising biological phosphorus removal, the kinetic and stoichiometric coefficients related to polyphosphate accumulating organisms (PAOs) occurred to be the most sensitive apart from several parameters mentioned above for ASM1. According to the parameter importance ranking elaborated by Brun et al. [5] for ASM2d, the following parameters belong to the most sensitive: specific decay rate coefficient for PAOs (b_{PAO}), maximum specific growth rate for PAOs (μ_{PAO}), poly-P storage rate constant (q_{PP}), and hydrolysis rate constant (K_H). Makinia et al. [6] found that the effluent concentrations of $P-PO_4^{3-}$ was influenced by the greatest number of parameters of ASM2d and ASM3P, and most of them were associated with PAOs. Other authors stated that the most sensitive parameters of the BioWin AS model are yield coefficients related to PAOs [7].

Various methods have been proposed for the purpose of determination of kinetic and stoichiometric parameters as well as for wastewater and sludge characterisation [8–10]. In this work, the authors focus on the kinetic and stoichiometric parameters of the activated sludge models. Due to the fact that ASMs and ASM-based model describe generally biological wastewater treatment processes, it is obvious that the methods for determination of the parameters are mainly biological tests. However, also other methods, for example titration techniques have been proposed. Due to the fact that the majority biological wastewater treatment processes involve oxygen consumption, the methods based on the measurement of oxygen uptake rate (OUR) occurred to be dominating. Nitrate utilisation rate (NUR), ammonium uptake rate (AUR) and phosphorus uptake rate (PUR) are also useful to assess some of the kinetic and stoichiometric parameters.

The application of OUR tests for the determination of the most sensitive parameters of ASMs and ASM-based models has been discussed in the paper. The aim of the study was to estimate the reliability of these tests, indicating their advantages and disadvantages and to show the gaps in the elaboration of the procedures.

2. STOICHIOMETRIC COEFFICIENTS

Stoichiometric coefficients occurred to be more sensitive and influential parameters than kinetic parameters in ASMs and ASM-based models [7, 9]. Thus, their de-

termination is a very important task. To the key stoichiometric coefficients in the models describing activated sludge systems belong Y_H, Y_A, and yield coefficients for biological phosphorus removal. There are several crucial yield coefficients associated with PAOs dependent on the model. In ASM2 and ASM2d these are yield coefficient for PAO biomass formed from polyhydroxyalkanoates (PHA) (Y_{PAO}), yield expressing amount of phosphate released per 1 mg of substrate (Y_{PO4}), yield expressing PHA requirement for polyphosphate storage (Y_{PHA}). The EAWAG Bio-P module to ASM3 comprises the same yields as ASM2 and ASM2d and, additionally, yield coefficient for PAO biomass grown on PHA under anoxic conditions ($Y_{PAO,NO}$). In ASM2 and ASM2d, instead of $Y_{PAO,NO}$, Y_{PAO} and a constant reduction factor for anoxic conditions were used. The experimental estimation of Y_{PAO} or $Y_{PAO,NO}$ is complicated due to the problems with the differentiation between ordinary heterotrophs and PAOs. Thus, the values of these parameters were estimated theoretically based on heterotrophic yield (Y_H) [11]. The decrease of Y_{PAO} compared to Y_H depends on a substrate. For acetate, the 15% reduction was calculated, whereas for propionate the reduction factor was equal to 7% [11]. Mathematical description of biological phosphorus removal in the BioWin AS model is extended in comparison to ASMs. The model comprises seven yield coefficients relating to PAOs [2], the most sensitive being: the yield expressing amount of phosphate released per 1 mg of acetate ($Y_{P/Acetate}$), yield coefficient expressing the fraction of phosphate stored in the releasable polyphosphate form (Y_{lowPP}), yield coefficient expressing the amount of PHA stored when 1 mg of acetate or propionate is sequestered ($Y_{P/PHA,seq}$) [7]. In spite of the fact that all these parameters are sensitive, Y_{lowpp} is relatively stable for PAO and its determination seems to be not necessary. For the other two parameters, the methods of their determination have not been published so far. $Y_{P/Acetate}$ corresponds with the yield of phosphorus release to substrate uptake (Y_{PO4}), which is used in ASM2, ASM2d and ASM3P models [1, 11–12]. The default value of Y_{PO4} in ASM2 and ASM2d models is equal to 0.4 mg P·mg COD^{-1}, whereas in ASM3P model, it is slightly lower and equal to 0.35 mg P·mg COD^{-1} [11].

At least two methods are known for the determination of Y_H. Sollfrank and Gujer [13] and Brands et al. [14] suggested the addition of a certain amount of raw wastewater during OUR test and measurement of the substrate oxidation rate ($r_{o,ex}$). The contribution of degradable COD is calculated by the subtraction of the inert fraction from the filtered COD. Y_H was calculated according to the following equation:

$$Y_H = \frac{COD_{degradable} - \int_{0}^{t} r_{o,ex}(t)dt}{COD_{degradable}} \tag{1}$$

Kappeler and Gujer [15] advised to estimate Y_H as the ratio of biomass COD to the filtered substrate COD. The determination of biomass and substrate concentrations

should be made periodically during the OUR test. The ratio of $S(0)/X(0)$ in this test should be high, i.e. based on the COD it should equal 20/1.

$$Y_H = \frac{\Delta COD_{biomass}}{\Delta COD_{soluble}} \qquad (2)$$

Y_H can be also estimated as the slope of the dependence between substrate and biomass concentration. It can be made during the OUR test performed under the same conditions as those described in [15]. The samples should be taken at equal intervals during the exponential growth phase. The last two methods are based on the same assumption that there is a linear relation between substrate (S) and biomass (X) concentration, which is actually derived from the definition of yield coefficient [16].

$$\Delta X = Y_{xs} \Delta S \qquad (3)$$

All mentioned above procedures for determination of Y_H are relatively simple and reliable. It was found that Y_H did not differ significantly for municipal wastewater, whereas in the activated sludge systems designed for industrial wastewater it can vary in a wide range [17]. Thus, for activated sludge systems, which treat industrial waste-water or municipal wastewater with the high contribution of industrial wastewater, the determination of Y_H is recommended. It is usually assumed that in the WWTP treating municipal wastewater Y_H is equal to 0.67 mg COD·mg COD^{-1}.

The yield coefficient for autotrophs (Y_A) can be determined from the OUR test, in which a known pulse of ammonium ($S_{NH}(0)$) is added to activated sludge [9]. What is important, ammonium should be added to the sludge already aerated for a longer period of time, which is in the endogenous phase. In such conditions, the increase of OUR will be connected with the growth of autotrophs. Y_A can be calculated from the equation:

$$Y_A = \frac{4.57 S_{NH}(0) - \int_0^t r_{o,ex}(t)dt}{S_{NH}(0)} \qquad (4)$$

In the activated sludge models, the value of Y_A at the level of 0.24 g COD·g N-NO$_3$$^{-1}$ is usually assumed.

3. KINETIC PARAMETERS CONNECTED WITH THE MONOD EQUATION

The Monod equation is used to express limitation of the variety of substrates (carbon compounds, ammonium, oxygen) in all contemporary applied models describing activated sludge systems. What is more, the values of two parameters of the model equation, i.e. the maximum biomass specific growth rate and the saturation constant,

may change in a wide range for ordinary heterotrophs even with regard to municipal wastewater. For example, the literature values of maximum specific growth rate of heterotrophic biomass varied from 0.6 to 13.2 d^{-1} [18]. Thus, it is suggested determining the maximum specific biomass growth rate and the saturation constant within the model calibration process at least for ordinary heterotrophs.

Both kinetic parameters of the Monod equation can be estimated within a single respirometric test, however estimation of μ_H is simple and reliable, whereas the precise estimation of K_S is more difficult. The value of μ_H is determined as the slope of the OUR changes in time, if these changes are presented in a semi-logarithmic coordinate system (Fig. 1). This determination is in agreement with the principles of bioprocess engineering. In order to determine K_S, in agreement with these principles too, several OUR tests with various initial substrate concentration should be performed. Such procedure was recommended by Cech et al. [19]. However, it is a time-consuming and laborious task.

Fig. 1. Determination of μ_H based on the results obtained within the OUR test performed according to [15]; $S(0)/X(0)=20/1$, synthetic municipal wastewater

At least two simpler procedures for estimation of μ_H and K_S were described [15, 20]. In both methods, the same condition must be fulfilled, i.e. the excess of substrate should be used in order to prevent substrate limitation. The procedure presented in [15] was applied successfully by the authors of this work and it can be recommended. According to this procedure, the batch OUR test should be carried out with a very small amount of activated sludge biomass ($S(0)/X(0) – 20/1$ based on COD). In

order to prevent the growth of autotrophs, allylthiourea (ATU) should be added at the beginning of the test. During the first period of the test, OUR increases due to the unlimited heterotrophic growth, then it suddenly decreases because the system reaches the limiting concentration of readily biodegradable substrate as it was shown in Fig. 1. Kappeler and Gujer [15] described briefly how to estimate μ_H and K_S based on the same data by plotting the measured OUR and simulated data, using the Monod equation and trial-and-error method. The users should be conscious that the application of Kappeler and Gujer method for determination of μ_H might give its elevated values [15]. It is due to the fact that the experimental conditions favour fast growing organisms [21].

The estimation of Monod equation parameters for autotrophs is more complicated than for ordinary heterotrophs as literature data and our own experience indicate. In order to estimate the ammonium half-saturation coefficient for autotrophic biomass (K_{NH}), an analogous procedure elaborated for ordinary heterotrophic organisms (OHOs) by Cech et al. [19] can be applied. By this method, Novák et al. [21] estimated the value of K_{NH}. They carried out the series of OUR tests with different initial concentrations of ammonium ions. Spanjers and Vanrolleghem [22] described a simpler procedure for the simultaneous estimation of K_{NH} and μ_A. This method is based on the addition of a known amount of ammonium to an activated sludge in the endogenous state and record the respiration rate, until the endogenous respiration rate is reached again. Spanjers and Vanrolleghem [22] suggested using of a very low initial values of $S(0)/X(0)$ equal to 1/200 based on COD. The results of the OUR test should be then simulated using the following equation of the model presented in [22], what finally allows the determination of the values of K_{NH} and $\mu_{max,A}$.

$$r_0 = (4.57 - Y_A)\, \frac{1}{Y_A}\, \mu_{max,A}\, \frac{S_{NH}}{K_{NH} + S_{NH}}\, X_{BA} \tag{5}$$

This model is in certain aspects simplified, in other aspects, it is an extended version of ASM1. The drawback in the application of the methodology proposed in [22] is the fact that the parameters were not estimated individually but as a combination. It may lead to the errors in their estimation. Moreover, the use of Eq. (5) requires the knowledge of Y_A and autotrophic biomass concentration (X_{BA}).

Even more complicated is the determination of kinetic parameters of Monod equation for PAOs. The literature data are very limited in this area. Rieger et al. [11] assumed the values of μ_{PAO}, half saturation constant for phosphorus in poly-phosphate storage (K_{PS}), half saturation constant for PHA (K_{PHA}) based on the so-called P-release and P-uptake tests carried out in anaerobic and aerobic conditions, respectively. They were made within the calibration of the EAWAG Bio-P module for ASM3. This calibration revealed that the values of maximum biomass growth rate and saturation constants were higher than the default values of ASM2d. Although the values of the above-mentioned kinetic parameters were estimated in [11] within biological batch tests, there was hardly any information, how it was actually done.

At the same time several attempts have been made in order to estimate the kinetic parameters of biological phosphorus removal from wastewater [18, 23]. However, they have not included saturation constants or specific growth rate of PAOs. To the most often estimated kinetic parameters of biological phosphorus removal from wastewater belong the ones expressing phosphorus release rate and different substrate uptake rates [18, 23]

4. DECAY COEFFICIENTS

Heterotrophic decay coefficient (b_H) in the aerobic conditions should be estimated in the respirometric test with the activated sludge only. It is a standard, simple and widespread used method [9, 15]. In order to prevent growth of autotrophs, ATU should be added at the beginning of the experiment. Literature data suggested that the endogenous respiration might last up to several days. At the same time the own experience indicated that the duration of this type of test is not longer than one day, usually in the range of 6–12 h (Fig. 2). Presenting the changes of endogenous respiration rate of sludge versus time in a semi-logarithmic coordinates system, b_H should be estimated as the slope of the decreasing line. Similar test can be also carried out under anoxic conditions. The value of b_H under anoxic conditions is usually by 40–50% lower than that under aerobic conditions [24].
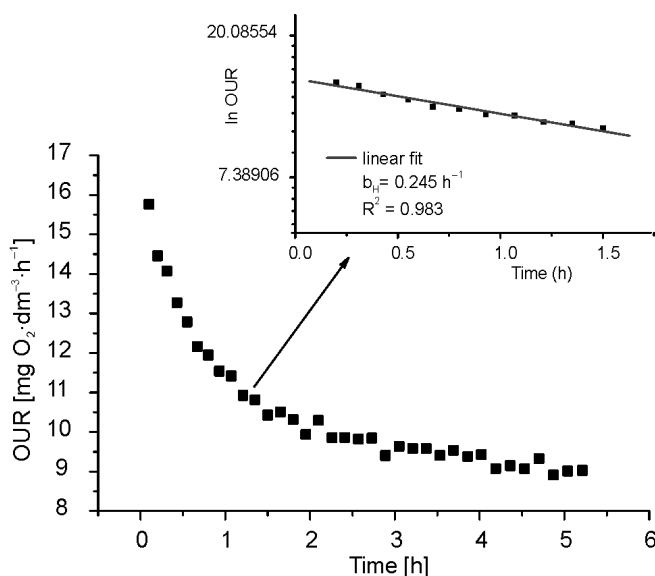

Fig. 2. Determination of b_H based on the results obtained within the OUR test.
The results of the experiments performed according to [15];
activated sludge taken from Combined Wastewater Treatment Plant in Łódź

Spanjers and Vanrolleghem [22] described the procedure for the simultaneous determination of b_H and b_A under aerobic conditions. It can be done by the addition of an optimum mixture of acetate and ammonium to the sludge in the endogenous state. ATU is not added. More details are given in [22]. It should be pointed out that according to the authors the procedure needed development and more replications. Thus, it is difficult to recommend it unequivocally.

There is a lack of detailed, standard procedures for the determination of decay coefficients for PAOs. At the same time it should be noticed that several attempts have been made in this area. It was necessary in order to use the activated sludge models. Siegrist et al. [24] suggested that aerobic decay rate of PAO (b_{PAO}) might be close to the estimated aerobic decay rate of polyphosphate (b_{PP}). The value of b_{PP} can be estimated within the laboratory tests or plant measurements of poly-P content in sludge under aerobic conditions. Another method for the estimation of b_{PAO} based upon the wash-out test was suggested by Rieger et al. [11]. However, its verification is difficult due to the shortage of information about executive and calculation procedures.

5. HYDROLYSIS CONSTANTS

The procedure to determine hydrolysis constants depends on the assumptions, whether hydrolysis is dependent on the heterotrophic biomass concentration. If it is assumed that hydrolysis can be expressed by first order kinetics with respect to the concentration of slowly biodegradable substrate (X_S), the determination of hydrolysis constant k_H is simple and reliable:

$$r_H = k_H X_S \tag{6}$$

A respirometric test with the initial ratio $S(0)/X(0)$ equal to 1/2 based on COD should be carried out. At the beginning of this experiment, ATU should be added. Once the readily biodegradable substrate (S_S) is removed, the further decrease of the respiration rate is governed by the hydrolysis of X_S. The value of k_H can be determined either by simulation of the changes of OUR curve using ASM1 or by the linear regression made in the appropriate range. More details are presented in [15].

Assuming that hydrolysis is limited by heterotrophic biomass concentration (X_{BH}), to be more precise by X_S/X_{BH}, and there is a maximum rate of hydrolysis, another procedure for the estimation of parameters in the following equation should be applied.

$$r_{HX} = K_{HX} \frac{\dfrac{X_S}{X_{BH}}}{K_X + \dfrac{X_S}{X_{BH}}} X_{BH} \tag{7}$$

This procedure was elaborated by Ekama et al. [20] and involved the monitoring of respiration rate in a test operated under a daily cyclic square-wave feeding pattern. During the first 12 h substrate (wastewater) is supplied to the reactor, and then for about next 12 h it is not. The second stage of this test allows the determination of K_X and K_H. Details are given in [20, 25].

6. CONCLUSIONS

Activated sludge models and ASM-based models contain a high number of parameters. Not all of them require experimental determination. It should be made with regard to the most sensitive and the composition of biomass and/or substrate dependent parameters, in particular. Respirometric methods are very useful for the purpose of the determination of stoichiometric and kinetic parameters of the activated sludge models. However, the knowledge about methods which should be applied for the determination of these parameters is miscellaneous. For several parameters, determination procedures have been elaborated and can be called standard methods. First of all, it concerns the parameters associated with ordinary heterotrophic organisms (OHOs). To this group of parameters belong Y_H, μ_H, K_S and b_H. Also the methods for the estimation of hydrolysis rates and hydrolysis saturation constants (k_H, K_H, K_X) are well known and verified by various scientists. The methods for the determination of parameters associated with autotrophs have been also proposed, however, in several cases they need to be developed. For example, it concerns the method for the determination of b_A. Many parameters associated with PAOs occur to be very sensitive. However, the methods for their determination have many gaps or even have not been elaborated yet. In Table 1, an overview of the methods for the determination of the activated sludge model parameters discussed in this work has been presented.

T a b l e 1

Overview of the methods of determination of selected kinetic
and stoichiometric parameters of the activated sludge models

Group of parameters	Microorganisms	Parameter	Method(s) of determination	Ref.
1	2	3	4	5
Stoichiometric	OHO	Y_H [g COD·g COD^{-1}]	Batch test at $S(0)/X(0)$=20/1 Measurements of biomass and substrate COD	[15]
	Autotrophs	Y_A [g COD g·N-NO$_3^{-1}$]	Batch test with addition of NH$_4$Cl to the endogenous sludge	[9]

Table 1 continued

1	2	3	4	5
Stoichiometric	PAO	Y_{PAO} [g COD·g COD_{PHA}^{-1}]	Theoretical or by calibration \with batch experiments	[11]
		Y_{PO4} [g P·g COD_{PHA}^{-1}]		
		Y_{PHA} [g COD_{PH}·g P^{-1}]	Theoretical	[11]
		$Y_{P/Acetate}$ [g P·g COD^{-1}]		[2]
		$Y_{P/PHAseq}$ [g COD_{PHA}·g $COD_{Acetate}^{-1}$]		
Kinetic growth rate constants	OHO	μ_H [d^{-1}]	Batch test at $S(0)/X(0)=20/1$	[15]
	Autotrophs	μ_A [d^{-1}]	Batch test with addition of NH_4Cl to the endogenous sludge	[22]
	PAO	μ_{PAO} [d^{-1}]	Batch test – lack of details	[11]
Kinetic saturation constants	OHO	K_S [g COD·m^{-3}]	(1) Batch test with various initial concentrations of COD	[19]
			(2) Batch test at $S(0)/X(0)=20/1$	[15]
	Autotrophs	K_{NH} [g N·m^{-3}]	(1) Batch test with various initial concentrations of $N–NH_4^+$ (2) Batch test with addition of NH_4Cl to the endogenous sludge	[9]
	PAO	K_{PS} [g P m^{-3}]	Batch test – lack of details	[11]
		K_{PHA} [g PHA g X_{PAO}^{-1}]		
Kinetic decay rate constants	OHO	b_H [d^{-1}]	Batch test with activated sludge. Measurements of endogenous respiration rate	[15]
	Autotrophs	b_A [d^{-1}]	Simultaneous estimation of b_H and b_A in a batch test. Addition of acetate and ammonium to endogenous sludge	[22]
	PAO	b_{PAO} [d^{-1}]	Estimation on the basis of b_{PP}	[24]
Hydrolysis rate and saturation constants	OHO	k_H [d^{-1}]	Batch test at $S(0)/X(0)=20/1$ assumption of 1st order kinetic for hydrolysis	[15]
		K_H [d^{-1}] K_X [g COD·g COD^{-1}]	Measurement of respiration rate in a test operated under a daily cyclic square-wave feeding pattern	[20]

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DYNAMICS OF METHANOGENIC ARCHAEAL COMMUNITIES BASED ON THE RRNA ANALYSIS AND THEIR RELATION TO METHANOGENIC ACTIVITY

Changes in phylogenetic groups of methanogenic microorganisms during methane fermentation have been studied. Phylogenetic groups of methanogens were quantified and visualized by hybridization of oligonucleotide probes complementary to rRNA of the major phylogenetic groups. At the beginning of fermentation, *Eubacteria* are the main group and the count of Archaea constitutes only an insignificant percentage of the population of microorganisms. During the process of fermentation, a very significant increase in methanogenic microorganisms was recorded after 70 days of the process in progress. It is reflected in the quantity and the composition of released biogas.

1. INTRODUCTION

Biogas plants may play important roles in providing society with a sustainable mix of energy. Such plants convert biomass and biological waste into the so-called biogas, i.e. a mix of CH_4, CO_2 as well as H_2O containing in addition traces of N_2, H_2S. Methane is later converted into energy [1–3].

The understanding of the microbiology of such continuously operated industrial plants is still very rudimentary. The microbial community of an anaerobic digester is composed of large number of different organisms belonging to the bacteria and Archaea domains. These microorganisms represent the major phylogenetic groups within the anaerobic digester community, including such physiological groups as hydrolytic bacteria, fermentative acidogenic bacteria, acetogenic syntrophic bacteria, hydrogenotrophic and acetotrophic methanogens, and sulphate reducing bacteria. For better understanding and control of this diverse community, the groups must be analysed quantitatively. Because of difficulties with cultivation of the methanogenic microorganisms, for their quantification fluorescence *in situ* hybridization (FISH) was used with rRNA-

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targeted fluorescent oligonucleotide probes [4, 5]. This technique makes it possible to identify cells through detecting fluorescence originating from marked oligonucleotide probes which underwent hybridization with a complementary nucleic acid occurring in a given cell. Oligonucleotides used in phylogenetic investigations enable one not only to detect bacteria from specific groups, but also to determine their abundances in samples [6]. Additionally, this technique provides a reliable measure of activity or, at least, potential activity of cells, because ribosomes undergo hybridization, and their amount depends on metabolic activity of a cell [7].

The purpose of this study was to determine the dynamics of methanogenic archaeal communities and their relation to methanogenic activity in a reactor.

2. MATERIALS AND METHODS

Object of study. Methane fermentation was conducted in five laboratory fermenters of the capacity of 10 dm^3. Maize silage from an experimental cultivation intended as biomass for methane fermentation constituted the raw material for fermentation. The chemical composition of silage maize biomass is presented in Table 1.

T a b l e 1

Chemical composition of silage maize biomass

Factor	Value
FAO coefficient	550
Dry mass	37.05%
Mineral compounds	2.3%
Organic compounds	82%
Lignin	1.43%
Cellulose	18.39%
Hemicellulose	19.59%
Carbon	42.03%
Nitrogen	1.77%
Hydrogen	5.98%

For T0 sample, the composition of fermentation feedstock was as follows: maize silage biomass – 1800 g; water 6 dm^3; inoculum (post-fermentation liquid from previous processes of methane fermentation) – 0.5 dm^3. The fermentation was conducted in thermally stable conditions ($T = 36$ °C) for the period of 90 days. The process was conducted at the Department of Chemical Proecological Processes, the Faculty of Chemistry, NCU in Toruń.

Collection of samples and microbiological analyses. Collection of samples depended on parameters of biogas released during fermentation. The composition of

biogas was determined by means of multigas monitor (Gas Data). Sampling of fermentation liquid was done with a sterile syringe. A single collection amounted to ca. 30 ml of the liquid. Then, a relevant volume of the collected liquid was filtered through a polycarbonate filter with pores of 0.22 µm in diameter. This volume was dependent on the bacteria count and was determined individually for each sample. Filters with the biomass retained on their surface were subject to the following microbiological analyses:

Determination of the total number of microorganisms. The total number of microorganisms was determined by the method of direct counting under an epifluorescence microscope (Nicon Eclipce T300). For the determination purpose, microorganisms retained on the filter surface were being stained with the pigment DAPI.

Determination of the number of living and dead microorganisms. In order to perform this determination, microorganisms retained on the filter surface were subject to LIVE/DEAD staining. In the procedure of staining and identification, the diagnostic set LIVE/DEAD (Invitrogen) was applied [8].

Determination of the number of methanogenic microorganisms. Determination was performed with the FISH method [9, 10]. In the process of hybridization, oligonucleotide probes were applied, complementary to rRNA of the major phylogenetic groups of methanogenic microorganisms (Table 2). Also Eubacteria and Archaea were included in the phylogenetic analysis. Termini 5′ of oligonucleotides were marked with the fluorescent pigment Cy3. Conditions of the hybridization process for particular phylogenetic groups are presented in Table 3.

T a b l e 2

Sequences of oligonucleotides applied in the FISH process

Group of microorganisms	Sequens (5′ $\longrightarrow$ 3′)	References
Archaea	GTGCTCCCCCGCCAATTCCT	[10]
Methanosarcinales	GGCTCGCTTCACGGCTTCCCT	[10]
Methanomicrobiales	CGGATAATTCGGGGCATGCTG	[11]
Methanobacteriales	ACCTTGTCTCAGGTTCCATCTCC	[10]
Methanococcales	GCAACATAGGGCACGGGTCT	[10]
Eubacteria	GCTGCCTCCCGTAGGAGT	[4]

Microscopic analysis. In all microscopic analyses, the epifluorescence microscope (Nicon Eclipse T300) with digital registration of images was used. During the analyses, each time 40 randomly selected fields of view were analysed. On the obtained images, labelled cells were counted; an average value was calculated and then expressed per number of cells in the unit volume of the fermentation liquid. All results were statistically analysed with the analysis of variance applying the software Statistica 6.0.

3. RESULTS AND DISCUSSION

Processes leading to methane production in the anaerobic environment require co-operation between many species of microorganisms and result from interactions be-tween organisms of various metabolisms [12]. During the fermentation, an increase in the total number of microorganisms was observed. However, between sample T2 and T3, i.e. between the 42nd and the 70th day of fermentation, reduction in the total number of microorganisms was observed from 44.5×10^8 to 39.2×10^8. At the same time, the increased contribution of dead cells was recorded with respect to living ones (Table 3). However, in the subsequent measurements, both the total number of cells, as well as the contribution of living cells increased significantly.

T a b l e   3

Total number (*TN*) of microorganisms in the fermentation liquid
and the contribution of living and dead cells

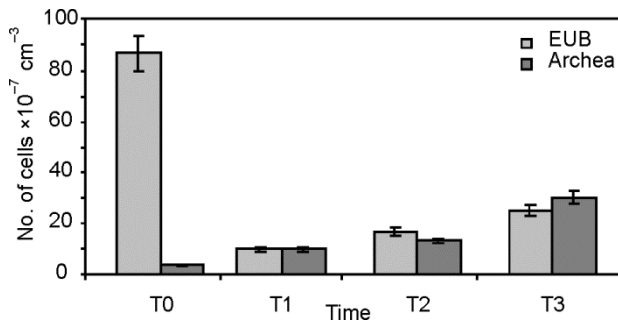| Sample	$TN \pm SD$ [No. of cells$\cdot 10^{-8} \cdot$cm^{-3}]	Living cells [%]	Dead cells [%]
T0	38.7±5.4	86	14
T1	44.5±3.2	89	11
T2	39.2±4.9	79	21
T3	68.0±5.9	95	5

Fig. 1. The count of Eubacteria and Archaea during methane fermentation

Changes observed in Fig. 1 are characteristic of transformations during methane fermentation and are similar to those described by Montero et al. [13]. At the begin-ning of fermentation, Eubacteria are the main group and the count, Archaea constitut-ing only an insignificant percentage of the population of microorganisms. The count of Archaea increases during the experiment, however the count of Eubacteria basically always remains higher [13].

During the conducted methane fermentation, the count of individual groups of methanogens differed considerably in particular phases of the process (Table 4,

Fig. 2). During the process of fermentation, a very significant increase in methanogenic microorganisms was recorded after 70 days of the process in progress. It is reflected in the quantity and the composition of released biogas (Table 5).

Table 4

Conditions of the hybridization process FISH

Parameter	*Methano-microbiales*	*Methano-bacteriales*	*Methano-sarcinales*	*Methano-coccales*	*Eubacteria* and Archaea
Hybridization buffer					
Formamide concentration	20	30	45	45	35
NaCl 5 M [µl]	360	360	360	360	360
Tris HCl 1 M pH 7.4 [µl]	40	40	40	40	40
Formamide [µl]	400	600	900	900	700
H_2O [ml]	1198	998	698	698	700
SDS 10% [µl]	2	2	2	2	2
Temperature [°C]	46	46	46	46	46
Time [min]	120	120	120	120	120
Washing buffer					
Formamide concentration	20	30	45	45	35
NaCl 5 M [µl]	2150	1020	300	300	800
Tris HCl 1 M pH 7.4 [µl]	1000	1000	1000	1000	1000
EDTA 0.5M pH 8.0 [µl]	500	500	500	500	500
H_2O [ml]	46.3	47.43	48.15	48.15	47.65
SDS 10% [µl]	50	50	50	50	50
Temperature [°C]	48	48	48	48	48
Time [min]	15	15	15	15	15

The volume of the released biogas was significantly increasing till the 15th day and then once again from about the 60th day of the process, and the percentage contribution of methane in the gas increased only after 50 days of the process. The obtained results related to the dynamics of methanogenic microorganisms, as well as the volume and parameters of biogas, indicate that the intensive methanogenic processes started relatively late. Also the absence of such compounds as H_2S or NH_3 in the nascent biogas indicate quite untypical course of the process.

Among the analysed phylogenetic groups, *Methanosarcinales* are the only ones who use up acetate in the production of methane [14]. Their count increases if the concentration of acetate increases in a reactor; the latter one being a product of de-

composition of complex chemical compounds [15, 16]. Therefore, the count of *Methanosarcinales* at the beginning of the process is low and it increases only after a certain time, when bacteria of an acetogenic phase deliver acetate. During further fermentation phases, the count of this group of methanogens is generally different and depends on the availability of substrate.

T a b l e   5

Characteristics of released biogas and composition of
populations of methanogenic microorganisms

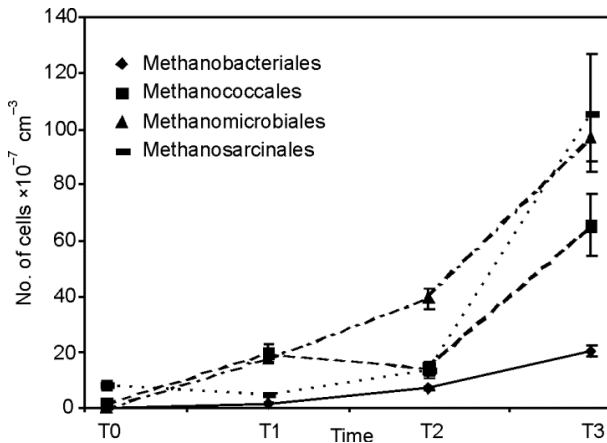| Sample/ day of fermentation	Volume of biogas [cm^3/d]	Contents				Number of methanogenic microorganisms $\times 10^{-7}$ cm^{-3}			
		CH$_4$ [%]	H$_2$S [ppm]	H$_2$ [ppm]	NH$_3$ [ppm]	*Methano- bacteriales*	*Methano- coccales*	*Methano- microbiales*	*Methano- sarcinales*
T$_0$ /0	116	0	0	0	1	0	1.8	0	8.2
T$_1$/42	2050	10	3	0	0	1.8	19.9	17.6	4.7
T$_2$/70	4300	49	0	22	0	7.1	14.1	39.3	13.5
T$_3$/88	1750	50	0	20	0	20.5	65.6	97.3	105.5

Fig. 2. Dynamics of populations of methanogenic
microorganisms during the methane fermentation

From the data presented in this paper, it appears that the count of *Methanosarcinales* in the initial phase of fermentation decreased (Table 5, Fig. 2). Therefore one can assume that the inoculum contained significant quantities of microorganisms from this group, and then their number was decreasing due to the absence of acetate in the fermentation tank. After the increase in the concentration of substrate (acetate) which resulted from the activity of acetogenic bacteria, the count of *Methanosarcinales* increased, and this increase proceeded till the end of the process, what might result from the constant presence of acetate in the fermentation tank.

Some isolated strains of *Methanosarcina* may also use hydrogen, however, its significance as a substrate for this phylogenetic group in complex populations of microorganisms was not yet thoroughly examined. According to Ahring [17] in most of the conditions prevailing in anaerobic reactors, *Methanosarcinales* are not able to compete for hydrogen with autotrophic methanogens. This assumption can constitute an explanation for observations by Padmasiri et al. [14] and Lee et al. [16], who state that during the periods when the count of *Methanosarcinales* decreases, the count of autotrophic methanogens increases and the other way around. In the presented studies, relationships similar to the aforementioned observations proceeded only during the first 40 days of the process, when the count of *Methanosarcinales* decreased insignificantly, whereas the count of other groups increased. However, at the final stage of this process, the increase in the count was related to all analysed methanogens (Fig. 2). These discrepancies could be caused by differences in the chemical composition of the fermentative mass or slightly different fermentation conditions, which determined the development of specific groups of microorganisms.

The significance of autotrophic methanogens, as well as their competition in the population living in the bioreactor has not been studied yet as thoroughly as the bacteria using acetate [18]. In the experiment performed by Padmasiri et al. [14] concerning the fermentation of pig faeces, it was observed that *Methanobacteriales* were the most abundant group at the beginning of the process, the number of which dropped in subsequent stages of the process, and *Methanomicrobiales* dominated among autotrophic methanogens. The domination of this group was also observed during the fermentation of sewage sludge [16, 19, 20]. Similar results were obtained in this paper, where *Methanomicrobiales* after the 40th day of the process were the most abundant group of autotrophic methanogens (Table 5, Fig. 2).

Montero et al. [18] and Lee et al. [16] in their studies on fermentation of miscellaneous resources (sewage sludge, synthetic glucose, whey) proved that the count of autotrophic groups of methanogens is higher at the beginning of the process, and then they are partially replaced by heterotrophic methanogenic microorganisms. During the processes studied in this paper, the count of *Methanosarcinales* was changing exactly in accordance with the described schema. At T0 time, these microorganisms dominated among methanogens, and their significant contribution could result from the introduced inoculum. In the subsequent sample (T1) their count and contribution in populations of methanogens significantly decreased, and after that (T2), their count increased, whereas the contribution in the whole population was still low. However, in the last sample (T3), the count of *Methanosarcinales* was the highest among the analysed methanogens and consequently their contribution in the population was also the highest. From the research by Hori et al. [15] it appears that the concentration of dissolved hydrogen is the main factor influencing the domination of autotrophic methanogens in the environment. The predominance of methanogenic bacteria that use up hydrogen could be caused by the availability of this substrate in high concentra-

tions at the end of fermentation, which resulted in the development of these microorganisms. Furthermore in each anaerobic environment, hydrogen is also used by other microorganisms, e.g. homoacetogenic bacteria or bacteria reducing sulphates, therefore microorganisms compete for this substrate. One should assume that conditions conducive to the development of methanogenic bacteria prevailed in fermenters, therefore they were winning the competition for hydrogen, and their count reached much higher values than in the experiments where a different substrate was subject to fermentation. The number of autotrophic methanogens can also be connected with the presence of syntrophic bacteria decomposing propionate and fatty acids. as well as interactions of these microorganisms [18].

It appears from the above presented discussions that changes in the population size of microorganisms occur during the fermentation. Consequently, these changes may significantly affect the number of nascent biogas, its composition and the quality. On the one hand, the applied raw material is the cause of these changes, and on the other hand, the observed changes in the population of methanogens result from interactions of microorganisms, which unfortunately are seldom taken into consideration.

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APPLICATION OF CONSTANT ELECTRIC FIELD
IN SIMULTANEOUS INTENSIFICATION OF DEWATERING OF
WASTEWATER SLUDGE AND FILTRATE PURIFICATION

Because the application of pressure in electro-dewatering of wastewater sludge (and consequent compression) affects processes of electroosmosis and electrophoresis, the study aimed at determining the effect of vacuum and constant electric field on the specific resistance of sludge and quality of filtrate. It was found that both the current intensity and direction affects the biogen content in sludge dewatering filtrate. An increase in the intensity of the current applied to the filtering barrier was accompanied by an increase in the effectiveness of the removal of ammonium nitrogen (filtering barrier as an anode) and orthophosphates (filtering barrier as a cathode). A constant electric field was shown to considerably affect the specific resistance of sludge filtration.

1. INTRODUCTION

Sludge dewatering in a wastewater treatment plant is one of the most important stages in its processing. However, sludge dewaterability is restricted mainly due to its high organic matter content and sludge particle compressibility [1–3]. Due to the latter quality, a compact layer of sludge, called cake sludge, is formed on the surface of the filtering barrier during the dewatering process, obstructing the water flow from above. It is a layer of low porosity and it results in high specific resistance of filtration, thereby hampering water flow [4–7].

Effectiveness of dewatering is usually improved by applying very high pressure, although this does not improve the process to a satisfactory extent. High pressure usually causes further increase in the thickness of the layer which adheres to the filtering barrier and negatively affects the filtration effectiveness. Electro-dewatering and associated electroosmosis and electrophoresis [8–10] is a new technique applied in sludge processing. Electroosmosis involves movement of the liquid phase of a solution rela-

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tive to the solid phase, caused by the difference of electrical potential in the system. Electroosmotic movement of water has been described both on a microscopic scale and in model systems [11–13]. Electrophoresis, on the other hand, involves the movement of charged colloidal particles relative to the surrounding liquid, caused by an electric field. These two processes may largely improve the effectiveness of dewatering, improving its structure on the way.

Wastewater sludge is a heterogenic system containing large amounts of colloidal matter with a surface charge, which enables the application of an electrical current to make the particles move in a specific direction. When a constant electric field is applied to sludge, the matter particles contained in it may be attracted or repelled by electrodes, depending on the charge. Such actions ultimately result in an increase in the dry matter of sludge, i.e. its better dewatering. Considering the operating costs of such devices, the effects are greater than the outlays and are economically justified [10, 14, 15].

Formation of considerable amounts of filtrate which may contain large amounts of impurities is a problem inseparable from sludge dewatering. Since filtrate is returned to the main process line of wastewater treatment, it may reduce the effectiveness of removal of impurities in the plant. However, the intensification of wastewater treatment processes by removal of nitrogen and phosphorus compounds in recent years has not been accompanied by similar progress in the purification of filtrate.

The experiments presented in this paper were conducted on a device which enables the application of a constant electric field to wastewater sludge, while dewatering it under a vacuum. A constant electric field was used to determine its effect on the value of the specific resistance of sludge filtration and the characteristics of filtrate.

2. MATERIALS AND METHODS

The experiment was conducted in wastewater sludge following anaerobic fermentation, obtained from the "Łyna" Wastewater Treatment Plant in Olsztyn, where community wastewater is treated in a high-performance system which involves removal of carbon, nitrogen and phosphorus compounds. Consequently, the sludge being processed contained large amounts of these compounds. Anaerobic fermentation of wastewater sludge is conducted under mesophilic conditions at 32–36 °C. Table 1 shows the fundamental parameters of the wastewater sludge used in the study.

Sludge filtrate which ran off during the sludge filtration process contained 89.62–138.64 mg $N-NH_4/dm^3$ and 140.22–504.03 mg PO_4/dm^3. Their specific resistance of filtration was high and ranged from 4.67×10^{12} to 3.21×10^{13} m/kg.

The experiment was carried out with a model of a filtering funnel (Fig. 1) in which electrodes were made of steel, enabling the connection of constant electric field. Filter paper (0.2 mm thick, 80 g/m^2) was used as the filtering barrier. Wastewater sludge was

treated with a vacuum at 4.9 N/cm² according to the method developed by Coackley and Jones, employing a theoretical model of filtration developed by Carman.

Physicochemical characteristics
of the sludge used in the experiment

Parameter	Range of values
pH	6.85–7.59
Moisture [%]	96.04–97.31
Dry matter [% d.m.]	2.69–3.96
Ash [% d.m.]	38.55–41.89
Volatile substances [% d.m.]	61.45–58.11
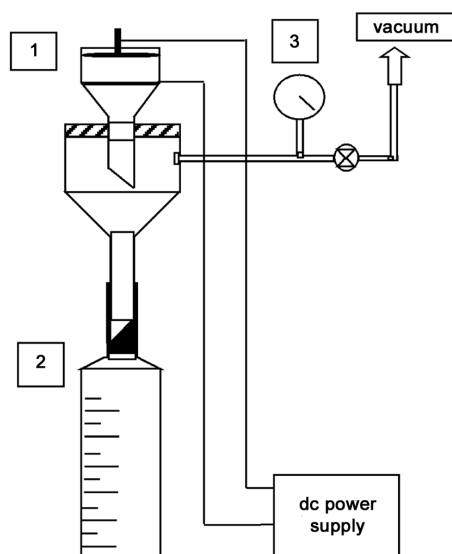
Fig. 1. Apparatus for vacuum electro-dewatering
of wastewater sludge: 1 – modified filtering funnel,
2 – measuring cylinder, 3 – vacuum gauge

A current rectifier with adjustable voltage and current intensity was used as a source of direct current. The series of experiments differed in direct voltage (6–48 V), current values (0.05–0.55 A) and its direction. The maximum duration of the sludge dewatering process was 30 min. The pH value of the filtrate was determined with an electrode; other parameters determined included temperature, ammonium nitrogen and orthophosphates. Ammonium nitrogen was determined by distillation according to Kjeldahl, whereas phosphates were determined colorimetrically with ammonium molybdenate and ascorbic acid. Dry matter content, hydration, ash and volatile substances were determined in the sludge according to the methodology given in [16].

3. RESULTS AND DISCUSSION

3.1. EFFECT OF CONSTANT ELECTRIC FIELD
ON THE SPECIFIC RESISTANCE OF SLUDGE FILTRATION

Dewatering of wastewater sludge accompanied by electroosmosis and electrophoresis considerably affects fluctuations of the specific resistance of sludge filtration depending on the current direction and the electric field intensity. The effect of current intensity was determined at a constant voltage of 38 V.
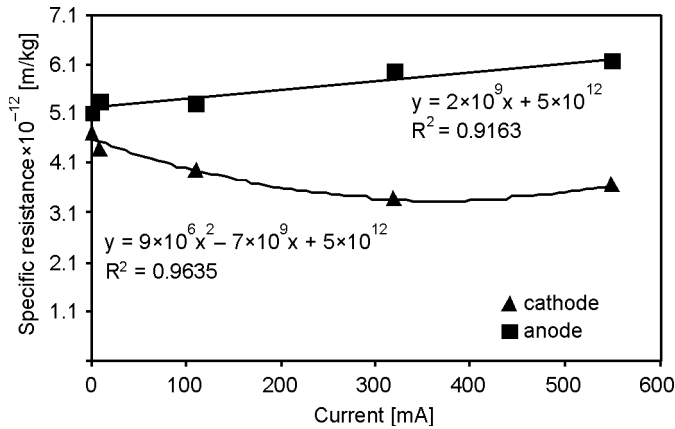

Fig. 2. The effect of current direction on the specific
resistance of wastewater sludge (V = 38 V)
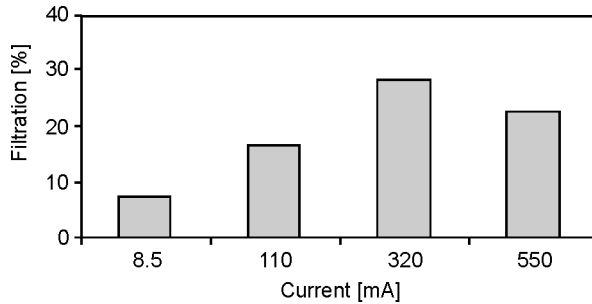
Fig. 3. Effect of electro-dewatering on the specific resistance
of sludge filtration. The filtering barrier as a cathode (V = 38 V)

The specific resistance of the sludge which was not treated with a constant electric field was equal to 4.73×10^{12} m/kg and 5.13×10^{12} m/kg (Fig. 2). When the filtering barrier was employed as an anode, the specific resistance slightly increased. However, when it was used as a cathode, the specific resistance decreased considerably (Fig. 2)

at low values of electric field intensity (8.5 mA). The lowest value of 3.38×10^{12} m/kg was reached at the current intensity of 320 mA. Compared to the sludge dewatering without constant electric field treatment, the value was lower by 28.54% (Fig. 3). The resistance of sludge filtration increased to 3.65×10^{12} m/kg at the current intensity of 550 mA, but it was still lower by 22.83 %. Similar relationships were observed in dry matter content in dewatered sludge. According to reports of other studies, better results can be achieved when high pressure and sludge conditioning is employed in sludge electro-dewatering.

In contrast to conventional methods of dewatering, which yield sludge containing 20–25% of dry matter, electro-dewatering enables an additional increase of dry matter content to over 50% [10, 15]. Much poorer effects of dewatering, such as those presented in this paper, result from the application of low vacuum and from omitting the conditioning process. The effect of high pressure methods is much stronger, leading to increased effectiveness of the process.

3.2. QUALITY OF FILTRATE FROM SLUDGE DEWATERING

Filtrate obtained from sludge dewatering contained variable amounts of ammonium nitrogen and orthophosphates, depending on the direction of the current applied and its intensity.
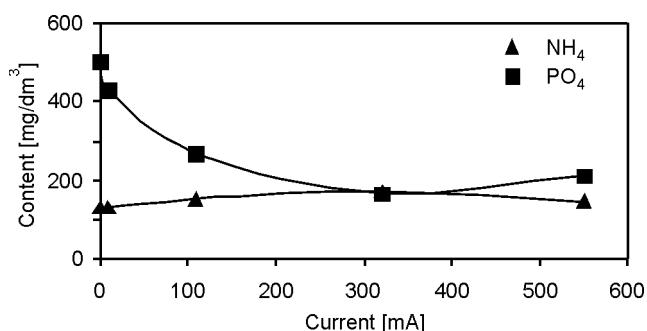

Fig. 4. Ammonium nitrogen and orthophosphate content
in the filtrate. Filtering barrier as a cathode

When the filtering barrier was used as the negatively charged electrode (cathode), the ammonium nitrogen content changed slightly (Fig. 4). The concentration of the compound within the current intensity range used in the experiment changed from 133.02 to 172.79 mg N-NH_4/dm^3. The orthophosphate content decreased considerably and its lowest concentration (170.23 mg PO_4/dm^3) was obtained at the direct current intensity of 320 mA. Compared to the initial value in the filtrate, this value was lower by 66.23 % (Fig. 5).
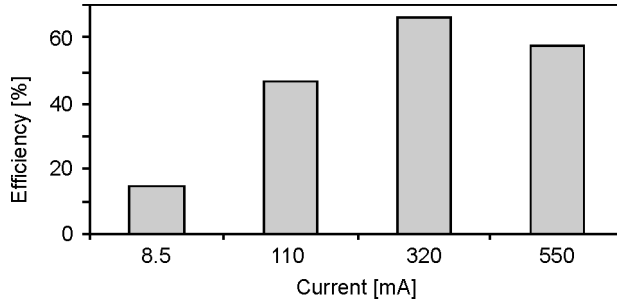
Fig. 5. Effectiveness of orthophosphate removal. Filtering barrier as a cathode

The current of 550 mA produced a slight increase in orthophosphate content, although the effectiveness was still high – 57.82%.
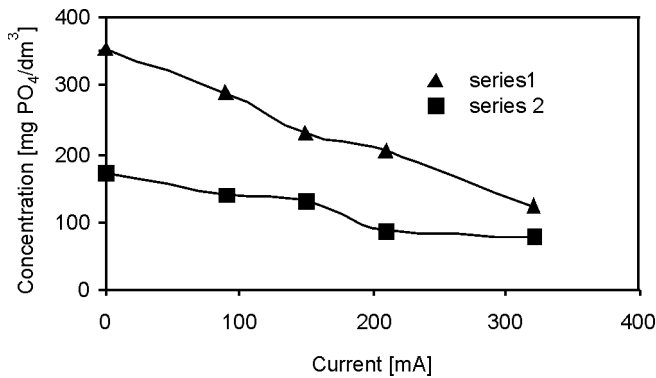

Fig. 6. The effect of electro-dewatering on the orthophosphate content in the filtrate. Filtering barrier as a cathode. Initial concentration: series 1 – 351.86 mg PO_4/dm^3, series 2 – 172.30 mg PO_4/dm^3
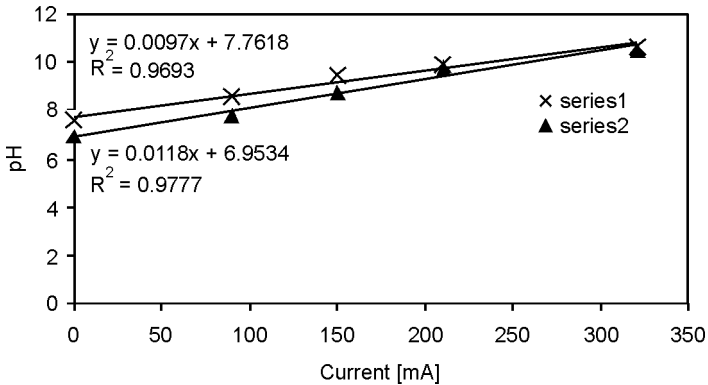

Fig. 7. The effect of electro-dewatering on the filtrate pH. Filtering barrier as a cathode. Initial concentration: series 1 – pH = 7.59, series 2 – pH = 6.96

The initial orthophosphate content affected the effectiveness of their removal in the process of electro-dewatering (Fig. 6). With a high initial concentration of 351.82 mg PO_4/dm^3, the effectiveness of orthophosphates removal was much better as compared to that at the initial value of 127.30 mg PO_4/dm^3.

The process of electro-dewatering was accompanied by changes of pH of the filtrate. The changes depended on the current intensity applied; its increase resulted in the maximum pH of 10.64 (Fig. 7). An increase in the filtrate pH results from the water electrolysis which takes place on the electrodes. Hydrogen ions (H^+) usually form on the anode, whereas on the cathode, hydroxyl ions (OH^-) are formed which are present in the filtrate after the dewatering process. Similar changes of pH in sedimentary waters confirmed Saveyn et al. [17] using the model sludge.
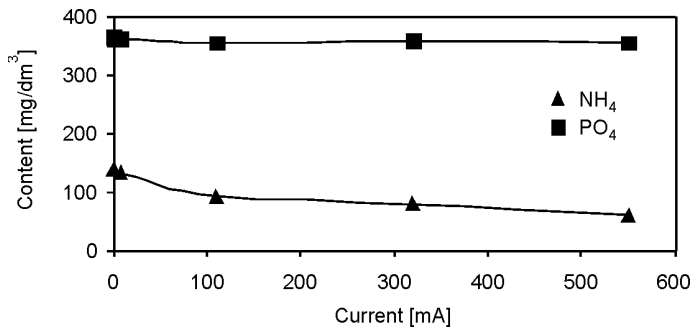

Fig. 8. Ammonium nitrogen and orthophosphate content
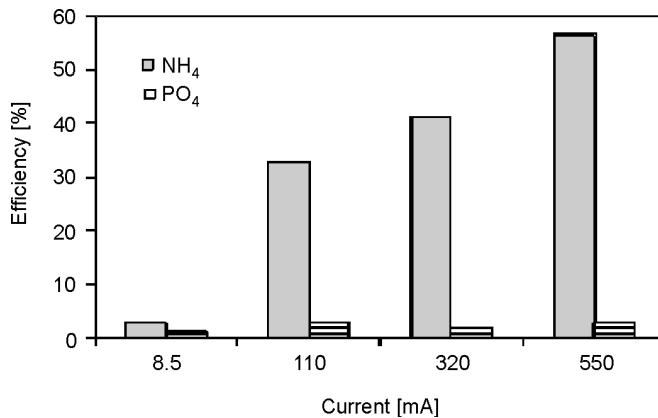in the filtrate. Filtering barrier as an anode

Fig. 9. Effectiveness of ammonium nitrogen and orthophosphate
retention. Filtering barrier as an anode

When the filtering barrier was used as an anode, the orthophosphate content in the filtrate changed slightly, decreasing from the initial value of 367.43 mg PO_4/dm^3 to

357.25 mg PO_4/dm^3 with increasing current intensity (Fig. 8). The effectiveness of their removal was low and reached only 2.9 % (Fig. 9). On the other hand, the ammonium nitrogen content in the sample decreased with increasing current intensity from the initial value of 138.63 mg NH_4/dm^3 to 60.21 mg NH_4/dm^3. The maximum effectiveness achieved at the highest current intensity was equal to 56.57 % (Fig. 9).

4. CONCLUSIONS

The results of the investigation confirm the possibility of using constant electric field to simultaneous increase the dewaterability of sludge and removing nitrogen and phosphorus from water sedimentary. It was demonstrated that retention of nutrients in the sediment depends on the intensity and direction of current flow. The resulting efficiency of retention of ammonium nitrogen in the model vacuum device was 56.57% (filtering barrier as an anode), while that of orthophosphate – 66.23% (filtering barrier as a cathode).

Electro-dewatering method can successfully be used in the processing of sludge in the sewage treatment plant "Łyna" in Olsztyn. Sludges used for studies after anaerobic digestion are dewatered in centrifuges and outflow of sludge filtrate goes to the main technological treatment including inflow sewage. Implementation of the proposed technology will increase the efficiency of wastewater treatment, as well as increase the value of sewage sludge fertilizer.

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METHANE EMISSION FROM THE NIELISZ RESERVOIR

The results of measurements conducted in 2010 of methane emission from the surface of the Nielisz Reservoir on the River Wieprz have been presented. This is a lowland reservoir located in the forested areas of the South-Eastern Poland (The Central Roztocze). The content and isotopic composition of methane in the upper (1 cm) layer of the bottom sediment have also been examined. The methane flux ranged from 15.98 to 383.85 $mmol \cdot m^{-2} \cdot d^{-1}$, while concentrations of the gas in pore water ranged from 133.33 to 1265.45 $\mu mol \cdot dm^{-3}$, and the value of $\delta^{13}C\text{-}CH_4$ was within the range –8.05 and –56.22‰. The fractionation coefficients ($\alpha_C CH_4\text{-}CO_2$) between methane and $\sum CO_2$ equalled approximately 1.05. The results of investigations suggest that methane in the sediment of the Nielisz Reservoir is produced by acetate fermentation. Levels of emission of this gas to the atmosphere can be compared with the values reported for large tropical reservoirs.

1. INTRODUCTION

The global warming of recent years has stimulated research on greenhouse gases emission to the atmosphere from both aquatic and terrestrial environments [1–6]. The main causal agents underpinning enhancement of the greenhouse effect are considered to be CO_2 and CH_4 as emitted excessively by human civilisation. Although methane remains in the air much more briefly than CO_2, its potential for causing global warming while it remains is 23 times higher [7]. Over the last 300 years, the atmospheric concentration of methane has more than doubled [8]. It has been estimated that about 7% of the carbon gases emitted from anthropogenic sources originate in dam reservoirs [9], while over 40% of methane emissions to the atmosphere derive from natural wetlands or croplands kept periodically underwater [10].

Emission of greenhouse gases from dam reservoirs reflects decomposition of flooded terrestrial biomass, biomass produced in the reservoir and organic matter in various states of decomposition introduced into the body of water via tributaries. Bio-

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genic CH_4 is formed by the two main processes of acetate fermentation and CO_2 reduction. CO_2 and CH_4 are produced by hydrolytic decomposition:

$$CH_3COOH \rightarrow CO_2 + CH_4$$
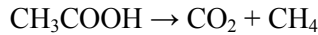
Acetate can also be oxidized to CO_2 and H_2O, the former then being reduced metabolically to CH_4, wherein hydrogen serves as the source of electrons [9]:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$
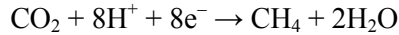
The application of stable carbon isotope analysis to CH_4 ($\delta^{13}C_{CH_4}$) and also to co-existing CO_2 ($\delta^{13}C_{CO_2}$) may yield a quite precise determination of the mechanism underpinning methane formation in sediments [11–14]. Thus the main purpose of this study was to determine the flow of methane into the atmosphere from the Nielisz Reservoir, as well as and the mechanism underpinning its formation in sediments.

2. METHODS AND MATERIALS

Study site description. The Nielisz Reservoir was put into operation in 2008. It is located in Eastern Poland (Lubelskie Voivodship, Zamość District, Nielisz Community), along the middle reaches of the Wieprz River at its confluence with the Por River.
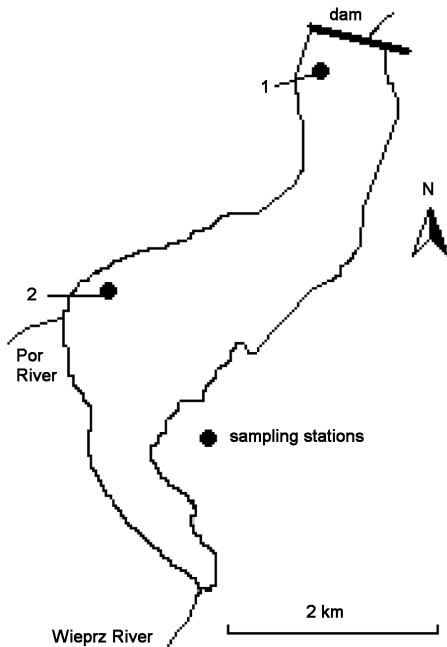

Fig. 1. Locations of the sampling stations
in the Nielisz Reservoir; morphometric properties
of the reservoir: volume – 19.5 mln m^3,
area – 10.6 km^2, mean depth – 2 m,
maximum depth – 4 m

Surrounded by coniferous forests, the reservoir has at its dam end a hydroelectric power plant of the capacity of 362 kW. Basic tasks of this body of water include: protection against flooding, reduced fluctuations in water level during the breeding season for birds, energy supply, recreation and amateur fishing. The locations of the sampling stations for the reservoir studied are shown in Fig. 1. Station 1 was located near the dam, while station 2 was situated near the place where the Por River flows into the Nielisz Reservoir.

Sampling and methods. Gas samples were taken from the studied reservoir in June, July and September 2010 (Table 1). The CO_2 and CH_4 fluxes were measured using a static chamber method, specifically a stainless steel chamber (of 0.3 m × 0.3 m × 0.16 m) equipped with a dry battery-driven fan and a small vent stopped by a silicon septum for sampling. Five gas samples from the chamber air headspace were withdrawn manually into gastight syringes 0, 10, 20, 30 and 40 min after deployment. All samples were transported to the laboratory and analysed for gas concentrations within 4 h. Gas fluxes were calculated from a linear regression of time dependences of gas concentration within the chamber, and expressed in $\mu mol \cdot m^{-2} \cdot d^{-1}$. Positive changes indicated emissions of gas from the reservoir.

Simultaneously with the methane emission study, sediment cores were taken from the stations using a gravity sediment corer (by KC Kajak, Denmark). Sampled cores together with overlying water were transported to the laboratory immediately.

Although sediment cores are normally processed for sediment gases in helium filled glove bags, the failure to measure sediment nitrogen ensured that cores were processed in the open within a few hours of collection. Pore water from the top 1 cm layer of sediment was recovered to gastight glass vials, using a modified pore water squeezer [15]. Immediately after collection, the samples of water in vials were acidified using 6 M HCl (final concentration ca. 50 mM) to quantitatively convert all carbonate anions into CO_2 [16]. The gas concentrations in the overlying and pore water were analyzed using the headspace equilibration technique. Gases were extracted from the water in glass gastight vials, a known volume of water being replaced with helium. Water was equilibrated in the vials with added helium by means of 5 min of vigorous shaking. The gas phase was immediately analyzed for the concentrations and isotopic composition of CH_4 and CO_2.

The CH_4 and CO_2 concentrations in gas samples were analyzed using a Pye Unicam gas chromatograph (model PU-4410/19) equipped with a flame ionization detector (FID) and a stainless steel column packed with a Haye Sep Q, 80/100 Mesh, 6 ft long and of 2 mm ID. The GC was also equipped with a methanizer to detect low levels of carbon dioxide. The methanizer is packed with a nickel catalyst powder and heated to 380 °C. When the column effluent mixes with the FID hydrogen supply and passes through the methanizer, CO_2 is converted to CH_4. The carrier gas was helium at

the flow rate of 30 cm^3/min. The carbon isotopic compositions of CH_4 and CO_2 were determined with an IRMS DELTA^{Plus} Finnigan on line with GC/CIII.

Sediment subsamples were dried and further measured for loss on ignition (LOI) at 550 °C for 4 h. The contents of organic total carbon and total nitrogen were measured using an elemental analyser (Flask 1112, ThermoQuest). The nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic compositions were detected using an IRMS DELTA^{Plus} Finnigan coupled with the elemental analyser. Total organic carbon and $\delta^{13}C$ was measured following the removal of inorganic carbon by contact with the vapour of HCl in a desiccator [17].

Stable carbon and nitrogen isotopic compositions were reported in standard δ notation ($\delta^{13}C$, $\delta^{15}N$) expressed as "per mil": δR (‰) = $(R_a/R_{b(sample)}/ R_a/R_{b(standard)} − 1] \cdot 10^3$, where R_a/R_b are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios relative to the PDB and air standards, respectively.

The carbon isotope separation between CH_4 and ΣCO_2 was expressed in δ notation as the fractionation factor ($\alpha_C CH_4$-CO_2): $\alpha = \delta^{13}C_{CO_2} + 1000/\delta^{13}C_{CH4} + 1000$ [14].

3. RESULTS AND DISCUSSION

Selected parameters characteristic of physical and chemical properties of water and atmospheric air are as shown in Table 1.

Table 1

Main features of air and water

Station	Sampling date	Temperature of air [°C]	Temperature of water [°C]	Oxygen concentration [mg·dm^{-3}]	pH
1	17.06.2010	23	22.5	5.80	8.51
	13.07.2010	29	25.2	9.17	8.93
	21.09.2010	13	15.1	8.87	8.09
2	17.06.2010	22	20.2	7.92	8.46
	13.07.2010	29	26.7	8.21	8.55
	21.09.2010	15	13.5	9.80	8.36

Figure 2 shows time dependences of methane concentration in the static chamber during the research period at the two sampling stations. The largest difference between the initial methane concentration and that recorded in the 40th minute at station 1 was only 7.2 µmol dm^{-3}, while the highest CH_4 concentration was 8.2 µmol dm^{-3}.

A significantly larger amount of methane in air samples collected from the static chamber was observed at sampling station 2 (except in July 2010). In June and September, the differences between the methane concentrations at the beginning and end

of the experiment were of ca. 72 and 49 µmol·dm^{-3}, respectively. The highest concentration was 91 µmol·dm^{-3}.
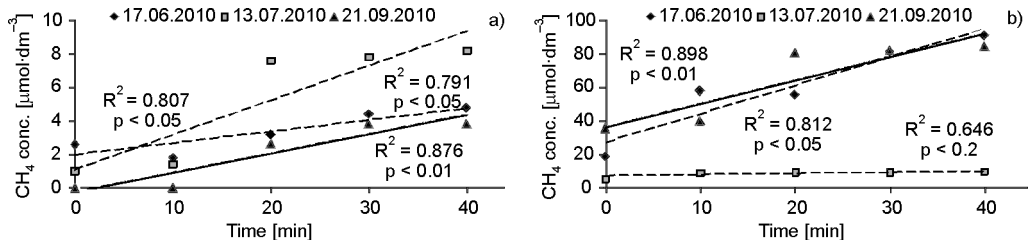

Fig. 2. Time dependences of concentrations of methane in the chamber: a) station 1, b) station 2

In line with regression equations, the change in methane concentration at the time determined the rate of emission of this gas to the atmosphere (Fig. 3). The lowest flow of methane at the water–atmosphere interface was of 15.98 mmol·m^{-2}·d^{-1}, while the highest was of almost 384 mmol·m^{-2}·d^{-1}. In June and September, the methane emissions at station 2 were respectively about 24 and about 12 times higher than at station 1. The exception was in July when the methane flow into the atmosphere was slightly more than twice as high at station 1, but did not exceed the value of 47 mmol·m^{-2}·d^{-1}.
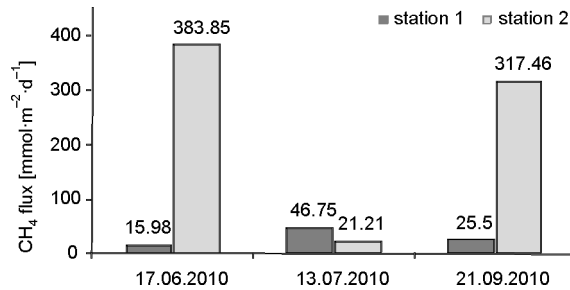

Fig. 3. Fluxes of CH$_4$ at the water–air interface in the studied reservoir

The average emission of methane from reservoirs of the temperate zone has been estimated to range from 0.6 to 5 mmol·m^{-2}·d^{-1} (1.25 mmol·m^{-2}·d^{-1} on average), while the corresponding figures for tropical reservoirs range from 1.25 to 93.75 mmol·m^{-2}·d^{-1} (18.75 mmol·m^{-2}·d^{-1} on average) [9]. It was, however, found that the methane emission from a tropical reservoir was slightly above 237 mmol·m^{-2}·d^{-1} [18]. As can be seen, the determined flow rates of methane for the Nielisz Reservoir are very high, and comparable with (or even in excess of) emissions from tropical reservoirs. However, it should be noted that the reservoir is relatively "young", with flooded areas of land capable of providing large amounts of labile organic matter and stimulating methanogenesis. Immediately after the flooding of a reservoir area there is a large emission of greenhouse gases, which eventually decreases slightly and then remains relatively

constant throughout the life of the waterbody. For example, in the case of Arctic reservoirs, the maximum emission of greenhouse gases occurs from 3 to 5 years after water retention in the reservoir [19]. A positive correlation between temperature and methanogenesis is well documented (cf. e.g. [20, 21]), thus it is surprising that in September (when water and air temperatures were lowest) so high level of methane emissions at station 2 was recorded. Certainly, further research will verify whether the phenomenon in question was a short-lived one or not.

Table 2

Results of analysis of CH_4 and CO_2 in pore water,
as well as of C and N analysis in sediment of The Nielisz Reservoir

Station	Sampling date	Gas concentration [μmol·dm⁻³]		Isotope ratio			Data of bulk sediment		
		CH_4	$\sum CO_2$	$\delta^{13}C_{CH_4}$ [‰]	$\delta^{13}C_{CO_2}$ [‰]	$\alpha_C CH_4\text{-}CO_2$	$\delta^{13}C$ [‰]	$\delta^{15}N$ [‰]	LOI [%]
1	17.06.2010	1172.73	3066.67	−56.62	−9.90	1.049	−18.60	3.39	8.04
	13.07.2010	1047.27	1233.33	−57.35	−10.29	1.050	−23.54	3.48	1.37
	21.09.2010	1265.45	1973.33	−56.22	−9.41	1.050	−19.98	4.28	6.32
2	17.06.2010	133.33	2133.33	−58.05	−12.27	1.049	−26.53	2.27	13.02
	13.07.2010	300.00	2822.33	−57.03	−11.11	1.049	−25.94	0.39	5.40
	21.09.2010	273.33	2493.33	−56.95	−9.90	1.050	−26.12	1.98	4.29

Methane concentrations in sediment pore water ranged from 133.33 to 1265.45 μmol·dm⁻³ (Table 2), and values about threefold higher were recorded at station 1. Methane concentration in the sediment pore water only correlated very poorly with the content of organic matter and total organic carbon in the surface (0–1 cm) layer of sediment (Figs. 4a, b). A strong dependence of $\delta^{15}N$ on $\delta^{13}C$ in bottom sediment was shown (Figs. 4c, d).

Studies of the isotopic composition of carbon and nitrogen in sediments enable determination of the origin of organic matter (allochtonous and autochthonous matter) deposited in bottom sediments [17, 22]. Depletion of isotope ^{12}C in organic carbon and higher values of $\delta^{15}N$ are characterized by an autochthonous origin of matter [23–25]. Autochthonous organic matter is more easily degradable than the allochtonous one, and it contributes to the formation of anoxic conditions and processes entailing methanogenesis. It is also known that algae decompose to methane and carbon dioxide tenfold more rapidly than lignocellulose does [26]. Figures 4c, d show clearly that the concentration of methane in pore water increases with $\delta^{15}N$ and $\delta^{13}C$ values. Based on these results it may be suggested that the process of methanogenesis is not dependent on the quantity of organic matter but rather on the origin of organic matter in sediments. Autochthonous matter is a better substrate for the methanogenesis than the matter of terrigenous origin. It remains in contrast with the results of Murase and Su-

gimoto [12] who argue that the amount of deposited organic matter is the dominant factor of methanogenesis in sediments.
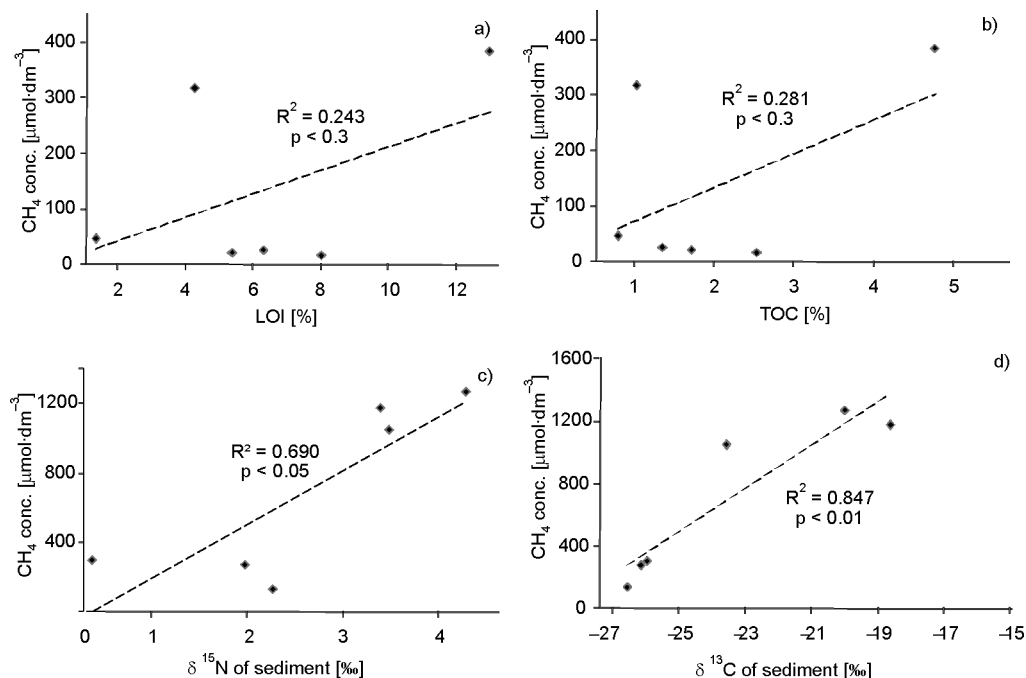

Fig. 4. Dependences of methane concentration on a) loss on ignition, b) total organic carbon content in sediment, c) δ^{15}N value for sediment samples, d) δ^{13}C value for sediment samples

The dependence shown in Fig. 4d can also be explained based on preference for the lighter isotope of carbon among methanotrophic bacteria in the process [14]. This is how increased production of methane will tend to cause depletion of the substrate, with ^{12}C and thus the δ^{13}C value being increased in the sediments.

The values of $\delta^{13}C_{CH_4}$ varied within the range from −58.05‰ to −56.22‰, while the values of $\delta^{13}C_{CO_2}$ changed from −12.27‰ to −9.41‰ (Table 2). The resulting values of $\delta^{13}C_{CH_4}$ are characteristic of freshwater reservoirs [27–29]. Biogenic CH_4 is mainly formed by the acetate fermentation and CO_2 reduction processes. The first mechanism is more common in (sulphate-poor) freshwater environments in which organic matter is readily available [14]. Methane produced by acetate fermentation has a δ^{13}C value within the range from −65‰ to −30‰, while methane formed by CO_2 reduction has δ^{13} values between −110‰ and −60‰ [30].

Figure 5 shows a positive correlation between δ^{13}C-CH$_4$ values and methane concentration, as well as between δ^{13}C-CH$_4$ and $\delta^{13}C_{CO_2}$ values. Knowledge of the δ^{13}C carbon dioxide which coexists with methane is helpful in identifying sources of CH_4.

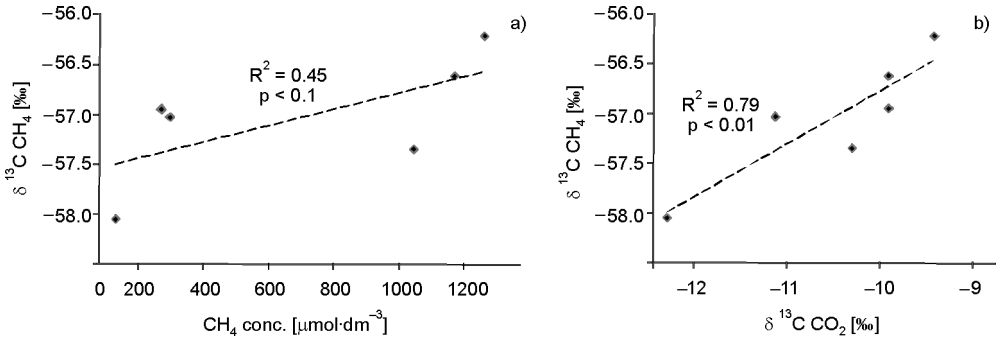Fig. 5. Dependences of $\delta^{13}C_{CH_4}$ on methane content (a)
and $\delta^{13}C_{CO_2}$ (b) in porewater of the Nielisz Reservoir

Knowing the coefficient of isotopic fractionation ($\alpha_C CH_4\text{-}CO_2$), a methanogenesis mechanism can be specified. In marine environments, where methane is usually formed through the reduction of CO_2, $\alpha_C CH_4\text{-}CO_2$ values vary in the range 0.05 to 1.1. However, in freshwater ecosystems, where fermentation is dominated by methyl fermentation, the values of $\alpha_C CH_4\text{-}CO_2$ range from 1.04 to 1.055 [14]. The $\alpha_C CH_4\text{-}CO_2$ values were either 1049 or 1050 for the Nielisz Reservoir. Based on both the $\delta^{13}C\text{-}CH_4$ value and that of the coefficient $\alpha_C CH_4\text{-}CO_2$, it can be seen that methane in the reservoir in question is generated from acetate fermentation.

4. SUMMARY

• Methane flows at the water–atmosphere interface were within the range of 15.98–384 $mmol \cdot m^{-2} \cdot d^{-1}$, and are thus comparable with those found to be typical of tropical reservoirs.

• Concentrations of methane in the sediment pore water ranged from 133.33 to 1265.45 $\mu mol \cdot dm^{-3}$ and correlated positively with the $\delta^{15}N$ and $\delta^{13}C$ values. It was concluded that the autochthonous organic matter is a better substrate for the methanogenesis than the matter of terrigenous origin.

• $\delta^{13}C_{CH_4}$ values reached levels typical of freshwater reservoirs and varied from −58.05‰ to −56.22. Both $\delta^{13}C_{CH_4}$ values and $\alpha_C CH_4\text{-}CO_2$ coefficient values (~1.05) confirm that methane in the Nielisz Reservoir is formed by acetate fermentation.

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BARBARA TCHÓRZEWSKA-CIEŚLAK*

MATRIX METHOD FOR ESTIMATING THE RISK OF FAILURE IN THE COLLECTIVE WATER SUPPLY SYSTEM USING FUZZY LOGIC

Collective water supply system (CWSS) belongs to the so called critical infrastructure with a priority importance for urban agglomerations. The draft of European standard *Security of drinking water supply. Guidelines for risk and crisis management* defines the concept of risk in CWSS and principles of management and protection in crisis situations. In the paper, a method for estimating the risk of failure in CWSS, based on the assumptions of classical matrix methods while information from the system operation is inaccurate, was proposed. The proposed method is based on fuzzy logic, which allows one to incorporate incomplete data and develop a risk evaluation system.

1. INTRODUCTION

Collective water supply systems (CWSS) are the foundation of existence of urban agglomerations. As one of the elements of an expanded urban infrastructure the system is exposed to the risk of an external nature (floods, droughts, blackout, incidental pollution of water sources, earthworks, ground temperature changes as well as acts of vandalism and even terrorism) and internal nature (technical failure resulting from processes of aging, fatigue, or hydraulic conditions, technical defects of materials and failures being a result of poor workmanship or wrong operation) [1, 2]. As a result of mentioned above incidents CWSS or one of its subsystems (intake, pumping and storage, distribution) may fail, the consequences are born by water consumers and water supply companies [3, 4]. A measure of the risk of failure in CWSS is a function of the probability of the occurrence of undesirable events and possible losses resulting from such events. In the analyses of risk in CWSS, we often find the problem of the ambiguity of the available operation data or data from the experts. A theory that can be

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used in such a case is the theory of fuzzy sets [5–7]. The concept of fuzzy sets was introduced in 1965 by Zadeh [7] from the University of Berkeley (California), who based on the work of Polish mathematician (creator of multi-valued logic) Jan Łukasiewicz. Fuzzy logic can be viewed as a multi-valued logic. Unlike in the classical set theory, the limit of the fuzzy set is not precisely determined but a gradual transition occurs from non-membership of elements in a set, through their partial membership, to full membership. This gradual transition is described by the so called membership function μ_A. Fuzzy sets can be used to describe various linguistic concepts related to risk analysis (small, medium, large, very large). Membership function μ_A assigns a value from the interval [0, 1], $\mu_A: X \rightarrow [0, 1]$ to each element x of the universe X. Most commonly used shapes for membership functions are Gaussian, triangular or trapezoidal [5–7].

2. MATERIALS AND METHODS

2.1. FUZZY MODEL OF THE RISK OF FAILURE OF WATER NETWORK

Risk is a measure of the probability and severity of the adverse effect [8, 9]. For the CWSS, the measure of risk (r) is defined as:

$$r = f(P,C) = \sum_S PC \tag{1}$$

where: S – a series of the successive undesirable events (failures), P – the probability (likelihood) of S or a single failure (a point value, depending on the frequency of failure), C – a point value of losses caused by S or a single failure. Depending on the frequency of a given failure, the point weights for the parameter P are presented in Table 1 [9, 11].

Table 1

Criteria for a descriptive point scale for the parameter P_i ($i = 1, 2, 3$)

P_i	Probability of failure
1	low probability, once in (2–10) years and less often
2	medium probability, once in (0.5–2) years
3	high probability, once in (6–12) months and more often

The criteria and the point weights for the assumed descriptive point scale for the parameter of losses C_j are presented in Table 2 [11, 12].

Criteria for a descriptive point scale for the parameter C_j, ($j = 1, 2, 3$)

Point weight C_j	Description
1	small losses: perceptible organoleptic changes in water, isolated consumer complaints, financial losses up to $5 \times 10^3 \, €$
2	medium losses: considerable organoleptic problems (odour, changed colour and turbidity), consumer health problems, numerous complaints, information in local public media, financial losses up to $10^5 \, €$
3	large losses: endangered people require hospitalisation, professional rescue teams involved, serious toxic effects in test organisms, information in nationwide media, financial losses > $10^5 \, €$

Based on Eqs. (2) and Tables 1, 2 two-parametric risk matrix was formulated:

$$r_{ij} = \begin{vmatrix} r_{11} & r_{12} & r_{13} \\ r_{21} & r_{22} & r_{23} \\ r_{31} & r_{32} & r_{33} \end{vmatrix} = \begin{vmatrix} 1 & 2 & 3 \\ 2 & 4 & 6 \\ 3 & 6 & 9 \end{vmatrix}$$

According to the basic matrix for risk assessment given above we can analyse various undesirable events, assuming the following scale of risk: tolerable risk (r_T), controlled risk (r_C), and unacceptable risk (r_U).

For risk analysis of water mains failure the membership function class type t (a triangular function, Eq. (2)), the membership function class type γ (Eq. (3)) and the membership function class type L (Eq. (4)), were proposed [13, 14].

$$\mu_A(x,a,b,c) = \begin{cases} 0 & \text{for } x \le a \\ \dfrac{x-a}{b-a} & \text{for } a < x \le b \\ \dfrac{c-x}{c-b} & \text{for } b < x \le c \\ 0 & \text{for } \quad x > c \end{cases} \qquad (2)$$

$$\mu_A(x,a,b) = \begin{cases} 0 & \text{for } x \le a \\ \dfrac{x-a}{b-a} & \text{for } a < x \le b \\ 1 & \text{for } x > b \end{cases} \qquad (3)$$

$$\mu_A(x,a,b) = \begin{cases} 1 & \text{for } x \le a \\ \dfrac{b-x}{b-a} & \text{for } a < x \le b \\ 0 & \text{for } x > b \end{cases} \qquad (4)$$

Table 3 shows the linguistic characterization, type and parameters of the membership function.

Linguistic characterization, type and parameters of the membership
function for P parameter, $\overline{P} = \{A_1, A_2, A_3)$

A_i	Linguistic characterization	Type of the membership function	Parameters of the membership function		
			a	b	c
A_1	low probability	type L (Eq. (4))	–	0.125	0.5
A_2	medium probability	triangular t (Eq. (2))	0.125	0.50	0.75
A_3	high probability	type γ (Eq. (3))	0.5	0.75	–

Table 4 shows the linguistic characterization, type and parameters of the membership function for the C parameter.

Linguistic characterization, type and parameters of the
membership function for the C parameter, $\overline{C} = \{B_1, B_2, B_3\}$

B_j	Linguistic characterization	Type of the membership function	Parameters of the membership function		
			a	b	c
B_1	small		0.0	0.0	1.5
B_2	medium	triangular t (Eq. (2))	0.5	1.5	2.5
B_3	large		1.5	3.0	3.0

Figure 1 shows the forms of the membership function for the parameters P and C.
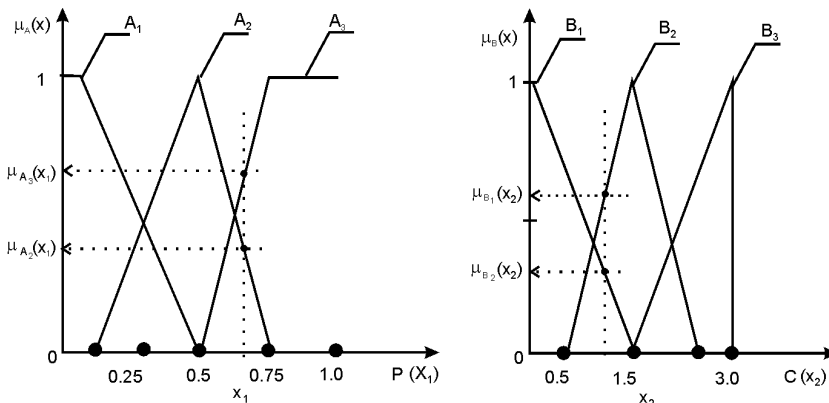

Fig. 1. Form of the membership function for the parameters $P(x_1)$ (left) and $C(x_2)$ (right)

2.2. EXPERT SYSTEM USING FUZZY RULES OF INFERENCE

A fuzzy decision model calculates the output value based on the multiple input values. The model does not analyse the exact values of the arguments, only their grade of membership in fuzzy sets, and thus the output value, being a base for the decision making process, is determined. The model consists of four main blocks [13]:

• The fuzzification block, which converts a vector of numbers (the crisp input values) into a vector of the grades of membership (e.g. a singleton method). For each input several fuzzy sets can be attributed. For each crisp value the membership to all sets connected with given input is calculated. For risk analysis, the input values for the model are risk parameters: variable x_1 (probability) and variable x_2 (losses). For the value of the probability, variable x_1 may belong to three defined sets: A_1 – low probability, A_2 – medium probability, A_3 – high probability, for the parameter of losses C variable x_2 may belong to the sets: B_1 – small, B_2 – medium, B_3 – large.

• The block of rules – determining the relationship between the inputs and outputs of the expert system (determination of the rules of inference). The base of rules represents the expert knowledge of possible values of variables characterizing the analysed system. The base is a set of fuzzy rules: $\{R_1, R_2, …, R_M\}$ in a *if-then* form.

• The inference block – the determination of a fuzzy conclusion of the expert system. There are several models of inference; the most popular models are Mamdani –Zadeh or Takagi–Sugeno models [5].

• The defuzzification block – the crisp output value is calculated for the inferred fuzzy set. We obtain a specific result, which completes the decision making process. The transformation of fuzzy set into the determined value can be made by various methods [5, 13].

3. RESULTS

3.1. MODEL OF EXPERT SYSTEM FOR RISK ANALYSIS IN CWSS OF THE TAKAGI–SUGENO TYPE

The basic feature of the Takagi–Sugeno–Kang (TS) inference model is the lack of defuzzification block because the result of the model is not in a fuzzy form. A typical rule in a TS fuzzy model has the form [5–7]:

If x_1 is A_i and x_2 is B_j, then output is $y = r_{ij}$.

The most frequently used function is the first order polynomial:

$$y = p_0 + \sum_{i=1}^{N} p_i x_i \tag{5}$$

where $p_0, p_1, …, p_N$ are numerical weights. For a zero order TS model $p_i = 0$ for $I > 0$.

The output level y_i of each rule is weighted by the firing strength w_i of the rule. The output from the model takes the form:

$$y = \frac{\sum\limits_{i=1}^{3}\sum\limits_{j=1}^{3} w_{ij} r_{ij}}{\sum\limits_{i=1}^{3}\sum\limits_{j=1}^{3} w_{ij}} \tag{6}$$

$$w_{ij} = \mu_{Ai}(x_1) T \mu_{Bj}(x_1) \tag{7}$$

where T is the algebraic product.

For the proposed model of assessing the risk of failure in CWSS a multi-input single-output (MISO) TS model was proposed, Matlab program (fuzzy toolbox) was used and the following assumptions were made: model consists of two inputs (value of the probability x_1 and value of the consequences x_2). Each variable is defined by fuzzy sets: $\{A_1, A_2, A_3\}$ for variable x_1 (Table 3, Fig. 1) and $\{B_1, B_2, B_3\}$ for variable x_2 (Table 4, Fig.1). The output from the model is a positive real number r_{ij} describing the risk of failure in CWSS. It is defined by three possible values: $r = \{r_T, r_C, r_U\}$.

Table 5 presents a description of the base of rules for the proposed model, developed based on literature data [10, 13, 15]. This base of rules was introduced into the base of Matlab Fuzzy Toolbox.

Table 5

Base of rules of the expert system: risk analysis in CWSS

Number of the rule	Description
R1	If x_1 is A_1 and x_2 is B_1 then $y = r_{11}$
R2	If x_1 is A_1 and x_2 is B_2 then $y = r_{12}$
R3	If x_1 is A_1 and x_2 is B_3 then $y = r_{13}$
R4	If x_1 is A_2 and x_2 is B_1 then $y = r_{21}$
R5	If x_1 is A_2 and x_2 is B_2 then $y = r_{22}$
R6	If x_1 is A_2 and x_2 is B_3 then $y = r_{23}$
R7	If x_1 is A_3 and x_2 is B_1 then $y = r_{31}$
R8	If x_1 is A_3 and x_2 is B_2 then $y = r_{32}$
R9	If x_1 is A_3 and x_2 is B_3 then $y = r_{33}$

The particular risk sets are:
- the tolerable risk set: $r_T = \{r_{11}, r_{12}, r_{21}\}$,
- the controlled risk $r_C = \{r_{13}, r_{22}, r_{31}, r_{23}\}$,
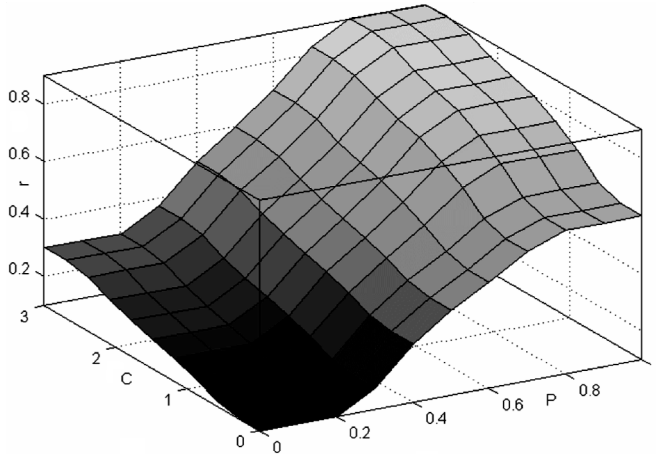- the unacceptable risk $r_U = \{r_{32}, r_{33}\}$.

Fig. 2. The risk surface

The risk graph generated from the Matlab Fuzzy Toolbox program is shown in Fig. 2.

4. CONCLUSIONS

In the proposed MISO TS model for assessing the risk of failure in CWSS, each of the input variables ($x_1 = P$ and $x_2 = C$) is defined by three linguistic values, respectively:

• for x_1 (Table 1): low probability (A_1), medium probability (A_2), and high probability (A_3);

• for x_2 (Table 2): small (B_1), medium (B_2), large (B_3).

Inference in the proposed expert model is based on the Takagi–Sugeno–Kang procedure (the risk of failure in CWSS). The concept of fuzzy inference presented in the paper may be considered as a tool to support decisions made in the process of managing risk of failures in CWSS. Based on the determined risk, the system operator makes decisions concerning the operation or modernization (repair) of the system or its individual components, in particular: if as a result of risk analysis the model generates a value of tolerable risk then the system operator decides to allow the system to operate. If as a result of risk analysis the model generates a value of controlled risk then the system operator decides to allow the system to operate but under the condition that modernization or repair will be undertaken. If as a result of risk analysis the model generates a value of unacceptable risk then the system operator decides not to allow the system to operate and initiates an immediate action to reduce risk. The proposed model can be used both for a comprehensive risk analysis in CWSS as well as for the

partial analysis of its individual elements. For example, in the process of modernization, the components with the highest risk of failure will be modernized first.

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MAGDALENA GAJEWSKA*

FLUCTUATION OF NITROGEN FRACTION
DURING WASTEWATER TREATMENT
IN A MULTISTAGE TREATMENT WETLAND

Factors influencing COD and removal and transformation of N fractions have been investigated in the conditions of horizontal (SSHF) and vertical (SSVF) flow reed beds. Multistage treatment wetland in Wiklino ensured a stable and very effective removal of all the analysed pollutants. Based on the resultsof investigation, it could be assumed that the working conditions of SSHF beds play an important role in the transformation of particulate fractions to colloidal ones and colloidal to dissolved fractions of COD and Org-N while the SSVF bed was very effective in the removal of dissolved fractions.

1. INTRODUCTION

At present, evaluation of the effectiveness of a wastewater treatment plant (WWTP) is based upon determination of organic matter content expressed by BOD and COD as well as the removal of nutrients (N and P). Although the size of pollutants discharged to a WWTP plays an important and often underestimated role in characterizing treat ability and hence the degree of contaminant removal of wastewater. According to Tiehm [1], the size of the suspended solids has a considerable impact on separation processes such as sedimentation, flocculation and filtration. Many authors confirm that the biological degradation rate in terms of COD reduction is influenced by particulate size distribution [1–3].

In order to obtain efficient nitrogen removal in the denitrification process, sufficient amounts of bioavailable carbon source should be ensured. In the case of treatment wetlands (TWs), the preferred approach is to use an internal carbon source already present in wastewater. According to Tanner [4], nitrogen removal in many TWs occurred concurrently with organic matter removal due to limited carbon sources. In

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biofilter systems such as TWs, particulates from the wastewater are naturally trapped by filtration, causing headloss due to the clogging of the filter layer. Although accumulation of organic matter is a typical feature of both natural and treatment wetlands, the over net inputs of external organic matter present in wastewater and outputs from decomposition potentially contribute to clogging in the pore space in sub-surface flow TWs [5–9].

Most of the initial total nitrogen (TN) can be successfully removed by nitrification and denitrification in a wastewater treatment plant. In contrast, the portion of dissolved organic nitrogen (DON) which has not been converted into inorganic N forms is very difficult to remove from wastewater [10]. Recently it has been found out that wastewater treatment leads to the formation of dissolved and colloidal organic compounds resistant to biochemical degradation, similar to humic acids [11].

So far very few studies have been conducted on organic matter and N speciation and the available data focus mainly on investigation in a conventional WWTP. Since data on nitrogen speciation during wastewater treatment in TWs is rather limited, the goal of the study was to investigate both organic matter (COD) and N speciation during wastewater treatment in a multistage treatment wetland (MTW). The investigations carried out bring information about factors and conditions influencing (limiting) COD and N fractions removal and transformation in the changing condition of SSVF and SSHF beds.

2. STUDY FACILITY AND METHODS

2.1. CHARACTERISTICS OF THE TW STUDIED

The studies were carried out in a full scale multistage treatment wetland (MTW) situated in Wiklino, a village in northern Poland near Słupsk. Wastewater after mechanical treatment in a three chamber settlement tank (with two day retention time) was pumped into the wetland responsible for biological treatment. This system consisted of a subsurface horizontal flow bed (SSHF I) followed by two subsurface vertical flow beds (SSVF) working with intermittent lodgings and the second SSHF II bed.

Table 1

Characteristics of Wiklino MTW

WWTP	Flow [m^3·day^{-1}] (pe)	Configuration	Effective grain size [mm]	HRT [day]	Area [m^2]	Depth [m]	Hydraulic load [mm·day^{-1}]	Unit area [m^2·pe^{-1}]
Wiklino	20.5 (220)	SSHF I	2–6	12.3	1050	0.6	19.5	4.7
		SSVF		–	312	0.4	65.7	1.4
		SSHF II		6.3	540	0.6	38.0	2.4
					Total 1902			Total 8.5

The basic characteristic of the applied solution is presented in Table 1. The MTW is covered in 95% by *Phragmites australis* with the plant density of 200–300 m^{-2}, and by a few other species (*Typha latifolia*) and grasses, e.g. *Carex.* sp. The matrix medium was coarse sand with the porosity of 0.4 and with the permeability coefficient $k = 110$ m·day^{-1}. The coefficient of graining non-uniformity $U = d_{60}/d_{10}$ equalled 0.35.

2.2. SAMPLING PROGRAM AND LABORATORY ANALYSES

Samples were collected every two or three weeks during the period from June 2008 to October 2009 (29 sampling events). The composite samples of influent and effluent as well as those after subsequent stages of treatment were collected for nitrogen and organic matter characterization.

At this stage of the investigation, standard analyses of chemical properties (COD, BOD, TN, NH_4^+-N, NO_3^--N, NO_2^--N, TKN, TSS and VSS) were carried out in accordance with the Polish Standard Methods and the procedures adopted by Hach Chemical Company which are in accordance with APHA 2005 [12, 13]. Additionally, COD$_f$ was also analysed after filtration in aqueous phase through a millipore nitrocellulose filter with the pore size of 0.45 µm. Furthermore, the content of volatile suspended solids in the total suspended solids was determined as loss on ignition.

The quantification (dissolved, colloidal, and particulate) of organic matter and N was based on the filtration of the influent and subsequent stage effluents through a series of filters (0.1, and 1.2 µm pore size millipore nitrocellulose filters). The dissolved fraction was obtained by filtration of a wastewater sample through a 0.1 µm pore size filter. The filtration of the wastewater sample through a 1.2 µm pore size filter enabled obtaining a non-dissolved fraction (particulate) and the sum of the colloidal and dissolved ones. Having the value of the dissolved fraction, it was possible to calculate the colloidal one. Then the filtrates were analysed for COD, NH_4^+-N, and TKN. Organic nitrogen concentration was estimated based on the difference between TN and the summation of NH_4^+-N, NO_3^--N and NO_2^--N concentrations. The temperatures of wastewater and air as well as dissolved oxygen and redox potential were measured directly in sampling points by Multi 340i/SET by WTW. Organic nitrogen concentration was estimated based on the difference between TN and the summation of NH_4^+-N, NO_3^--N, NO_2^--N concentrations.

3. RESULTS AND DISCUSSION

3.1. POLLUTANT CONCENTRATIONS AND EFFICIENCY REMOVAL

The characteristics of pollutants, in the influent and effluent and after the subsequent stages of treatment in MTW, with the range as well as mean values with stan-

dard deviation, are presented in Table 2. As is seen, the mean and median values did not vary significantly. Additionally, the standard deviation was lower than 30% of mean values, which suggests that the data were normally distributed. Thus, the mean values were taken under further consideration.

Table 2

Characteristics of wastewater in the Wiklino MTW

Parameter	Influent				After SSHF I			
	Mean	SD	Median	Min–Max	Mean	SD	Median	Min–Max
Flow, $m^3 \cdot day^{-1}$	20.5	0.7	20.3	19.9–22.1	19.3	0.8	19.5	18.7–20.8
pH	6.9	0.06	7.0	6.9–7.1	7.05	0.2	7.1	6.9–7.4
TSS, $mg \cdot dm^{-3}$	392.9	259.8	348.4	162.1–922.4	80.0	48.6	84.6	24.6–132.6
VSS, $mg \cdot dm^{-3}$	269.5	188.7	195.5	125.6–644.3	43.3	33.8	28.5	10.2–96.1
TN, $mg \cdot dm^{-3}$	130.5	8.1	129.1	119.9–146.7	72.1	18.1	68.3	44.2–92.5
NH_4^+-N, $mg \cdot dm^{-3}$	86.2	17.5	95.1	57.1–105.4	50.8	16.9	54.7	26.1–70.3
Org-N, $mg \cdot dm^{-3}$	43.5	15.87	49.9	23.3–66.2	19.1	10.3	18.9	6.6–33.7
NO_3^--N, $mg \cdot dm^{-3}$	0.9	0.5	0.7	0.1–1.8	1.1	0.6	1.3	0.2–2.2
COD, $mg \cdot dm^{-3}$	660.3	212.6	604.8	382.1–965.3	234.1	126.6	167.2	110.4–425.7
COD_f, $mg \cdot dm^{-3}$	283.6	69.1	280.4	184.4–00.6	56.4	19.7	62.2	22.3–78.6
BOD, $mg \cdot dm^{-3}$	323.8	126.2	280.6	194.8–500.7	33.5	24.6	30.3	10.5–73.5
T_s, °C	12.7	5.7	12.5	8.1–17.5	11,7	5,6	12.0	8.0–16.2
O_2, $mg \cdot dm^{-3}$	0.5	0.2	0.4	0.0–0.9	1,8	1,0	1.5	0.6–2.1
Redox, mV	–242.7	21.8	–240	–301.5—140.5	–87,3	14,2	–85	–146.5—25.5
	After SSVF				Effluent (after SSHF II)			
Flow, $m^3 \cdot day^{-1}$	18.1	1.0	18.3	17.1–19.4	16.9	1.1	17.3	15.9–18.1
pH, $mg \cdot dm^{-3}$	7.1	0.23	7.1	6.7–7.4	6.9	0.3	6.9	6.6–7.2
TSS, $mg \cdot dm^{-3}$	64.5	24.9	67.8	22.4–96.4	25.1	11.1	28.3	6.5–40.1
VSS, $mg \cdot dm^{-3}$	23.6	7.6	25.0	14.2–36.9	9.7	6.7	6.6	1.2–29.7
TN, $mg \cdot dm^{-3}$	49.0	18.3	47.8	14.7–73.5	22.8	6.3	20.9	15.9–34.5
NH_4^+-N, $mg \cdot dm^{-3}$	33.7	16.6	33.5	7.6–53.5	10.5	4.9	10.1	0.7–15.3
Org-N, $mg \cdot dm^{-3}$	7.9	3.4	8.9	4.2–14.1	4.2	3.6	4.0	2.4–9.1
NO_3^--N, $mg \cdot dm^{-3}$	9.1	5.4	9.1	4.2–19.8	8.2	5.3	6.8	1.9–15.6
COD, $mg \cdot dm^{-3}$	75.4	31.3	76.8	35.6–115.5	59.6	29.8	45.7	30.4–104.3
COD_f, $mg \cdot dm^{-3}$	47.0	23.3	46.4	10.0–78.6	26.6	14.8	24.1	3.8–54.5
BOD, $mg \cdot dm^{-3}$	14.7	6.6	15.6	5.7–23.5	6.7	3.8	4.7	3.6–12.4
T_s, °C	9.9	5.6	10.0	8.5–11.7	11.1	5.6	10.5	8.2–11.1
O_2, $mg \cdot dm^{-3}$	3.9	1.1	3.8	2.8–4.9	2.1	1.1	2.0	1.1–3.2
Redox, mV	210.5	24.5	212.0	105–320	32.6	5.2	31.5	–10–60.5

The concentrations of pollutants were much higher than those for domestic wastewater discharged to TWs [14–16]. Vymazal [15] reported BOD_5 concentration in the influent almost fourfold lower and that of TSS over sixfold lower. Only in France, where raw wastewater is treated in two stage vertical flow TWs, the concentrations of

BOD and TSS in the influent were similar to the concentrations observed in this investigation [17]. The concentration of TN in the influent was two- to even three-fold higher than that reported in the literature [14–17]. The reasons for higher pollutant concentrations could be lower water consumption by the person equivalent (ca. 100 $dm^3 \cdot day^{-1}$), lack of rain water infiltration to the sewer system, and what is undoubted, incorrect operation of the septic tank (too high concentration of TSS discharged to the MTW). Nitrogen in the influent was present mainly in the form of NH_4^+-N (ca. 65%) and Org-N (over 34%), which confirms that in the septic tank the conditions were favourable for the ammonification process. The hydraulic and pollutant loads discharged to the first stage of treatment (SSHF) in the MTW investigated are consistent with the range values reported elsewhere [4, 14–17] under conditions protecting the facility against clogging.

The COD/BOD and BOD/N ratios bring information about biodegradability for microbiological transformations. Additionally in this study, COD_f/BOD is presented as an indicator of easy degradable dissolved organic matter [18]. The wastewater studied was characterized by a typical COD/BOD ratio – ca. 2.0, and BOD/N – ca. 2.5. Furthermore, the COD_f/BOD ratio was less than 0.9, which suggests that organic matter is mostly present in an easy biodegradable dissolved fraction, and comparing with the high content of VSS in TSS, the rest was probably present in easy decomposable suspended organic matter [18].

The MTW investigated ensured a stable and very effective removal of all the analysed pollutants. Consequently, the concentration of the pollutants in the effluent did not exceed Polish permissible values [12]. The total removal efficiency of BOD_5 and COD was equal to 97.9% and 90.6% respectively. Among the N species, Org-N was removed with the highest effectiveness – 90.3%, the removal effectiveness of NH_4^+-N was 87.8%, and that of TN was only 82.5%. An unexpectedly high efficiency of N species was observed in the first stage of treatment (SSHF). The removal efficiency of TKN was over 46% and was accomplished with very high BOD removal, ca. 80.5%, which suggests potential heterotrophic competition for oxygen, whose supply in this stage of treatment is strongly limited. The SSVF bed, which is usually used for nitrification, showed the lowest efficiency of NH_4^+-N removal, equal to 31.1%, although the Org-N removal was the highest – 58.6%. Both BOD and COD_f were present in each stage of the treatment, which indicates the availability of organic carbon. Thus, denitrification was not limited by this factor. Neither the fluctuation of alkalinity nor pH confirmed the sequential of conventional nitrification and denitrification.

3.2. FLUCTUATION OF COD AND N FRACTIONS

Table 3 presents a detailed fractionation of organic matter (COD) and total organic nitrogen (TON) including the dissolved (< 0.1μm), colloidal (0.1 –1.2 μm) and par-

ticulate (> 1.2 µm) ones. The analysis of the organic matter (COD) and Org-N fractions showed the highest contribution of particulate forms of both COD (65.2%) and TON (67.2%) in the influent. The contributions of the colloidal fractions were almost similar for COD (25.4%) and Org-N (26.4%), while the contributions of the dissolved fractions were the lowest and equalled 9.4% for DCOD and 6.0% for DON.

Table 3

COD and Org-N fractions in the analysed MCW Wiklino [%]

Stage of treatment	Organic matter (COD)			Organic nitrogen (ON)		
	Dissolved (DCOD)	Colloidal (CCOD)	Particulate (PCOD)	Dissolved (DON)	Colloidal (CON)	Particulate (PON)
Influent	9.4	25.4	65.2	6.0	26.8	67.2
After SSHF I	16.3	35.4	48.3	12.5	26.4	61.1
After SSVF	32.7	27.2	40.1	18.8	28.1	53.1
Effluent (after SSHF II)	41.5	37.1	21.4	31.2	20.6	48.2

The particulate fractions of COD and Org-N were removed in all the stages of treatment with the highest efficiency removal, 98.5 and 94.2% respectively (Table 4, and Fig. 1). Both the COD and Org-N fractions were effectively removed and/or transformed into other fractions in the first stage of treatment. Long retention time in SSHF I with limited oxygen conditions (0.5 $mgO_2 \cdot dm^{-3}$) and redox potential equal to – 240 mV create favourable conditions for a very effective transformation of the colloidal fraction into the dissolved one (Tables 2 and 3, Fig. 1).

Table 4

Removal efficiency of COD and Org-N fraction in subsequent stages of treatment in the MTW Wiklino [%]

Stage of treatment	Removal efficiency of COD fractions			Removal efficiency of Org-N fractions		
	Dissolved (DCOD)	Colloidal (CCOD)	Particulate (PCOD)	Dissolved (DON)	Colloidal (CON)	Particulate (PON)
After SSHF I	38.5	50.6	73.7	7.6	56.4	59.9
After SSVF	35.3	75.1	73.3	37.5	56.9	64.1
After SSHF II	49.8	46.1	78.8	26.7	65.9	59.5
Total	80	93.4	98.5	57.7	93.6	94.2

The removal of the dissolved Org-N was 7.6%, being the lowest one in SSHF I, which could be explained by very high concentrations of 95.1 $mg \cdot dm^{-3}$ NH_4^+-N in the wastewater influent (Table 2), which is the most preferable form of nitrogen for plants and microorganisms [19]. The second stage of treatment in SSVF with oxygen condi-

tions (3.8 mg $O_2 \cdot dm^{-3}$) and redox potential equal to 212 mV enhanced the removal of the dissolved Org-N and colloidal COD (Tables 2 and 4).
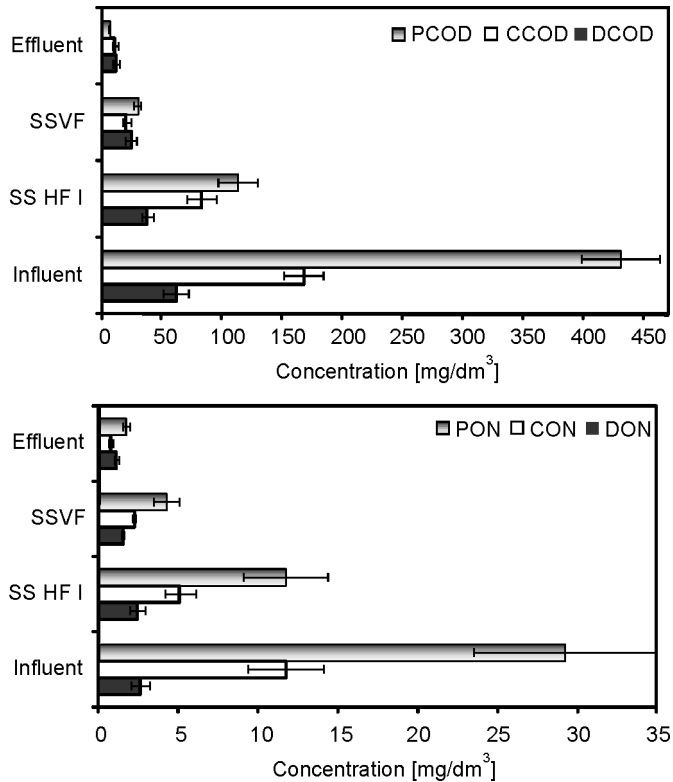

Fig. 1. The fluctuation of organic matter (upper)
and org-N fraction (lower) in the MTW Wiklino

The particulate and colloidal fractions of both COD and Org-N transformed in the SSHF I stage into adequate dissolved Org-N and colloidal COD, were rapidly removed from the wastewater during treatment in SSVF. Thus, the working condition of the SSVF bed and its role can be compared with those of an oxygen biological reactor in a conventional WWTP (but with much shorter retention time).

In the case of the MTW Wiklino, well aerated wastewater was entered into the last stage of treatment (SSHF II), where favourable conditions existed for further transformation of the particulate form into colloidal one, and the colloidal form into the dissolved one. This stage of treatment was designated for the polishing of the treated wastewater and denitrification process, which can confirm the results obtained in this stage of treatment. The most effective removal of dissolved organic compounds (49.8%), and both the colloidal and dissolved Org-N fractions accomplished with

a very high efficiency of TN (56.2%) and organic matter (49.0%) removal proved the importance of this stage of treatment (Tables 2 and 4, Fig. 1).

During the subsequent treatment stages at MTW, the forms changed significantly, and finally COD and Org-N were present in the effluent, mainly in the dissolved and colloidal fractions, except particulate Org-N (PON), which still consisted of 48.2% of total Org-N (TON) (Table 3). Both the dissolved and colloidal fractions could be products of the treatment processes as in the case of organic matter produced during cell decay, previously recognized as a soluble microbial product (including cell debris), and could be potentially hardly degradable [20, 21]. Such refractory compounds are similar to humic acids (fluvic and humin acids), and according to Nguyen [6] are the predominant stable organic matter fraction accounting for 63–96% of deposit in subsurface TWs. According to Pagilla [2], TON in the effluent from a conventional WWTP in the USA consists of 10% CON and 85% of DON. Such results may suggest that DON and CON as well as CCOD and DCOD can originate from the microbiological decomposition of raw wastewater, and can be both humic acids and microorganisms. The same authors assumed that the concentrations of PON ranged from 20% to 42% of TON in the treated wastewater, which is in accordance with the value achieved in this investigation. Similar results for wastewater treated with extensive methods and natural methods (TWs) could suggest that part of particulate Org-N and COD is recalcitrant, thus resistant to any treatment processes applied. Such compounds resistant to biochemical degradation, similar to humic acids can modified the properties of the recipient and could be responsible for trace metals transport and increase of their bioavailability in the ecosystem [11, 22].

4. CONCLUSIONS

The investigated MTW in Wiklino ensured a stable and very effective removal of all the analysed pollutants. Consequently, the concentration of pollutants in the effluent did not exceed Polish permissible values (Dz.U.nr 137 item 984). The efficiency removal of BOD5 and COD were equal to 97.9% and 90.6%, respectively. Among N species, Org-N was removed with the highest effectiveness – 90.3%, whereas the effectiveness of NH_4^+-N removal was 87.8% and that of TN was only 82.5%. The analysis of the organic matter (COD) and Org-N fractions showed the highest contribution of particulate forms of both COD (65.2%) and TON (67.2%) in the influent. The share of colloidal fractions were almost similar for COD (25.4%) and Org-N (26.4%), while the contribution of the dissolved fractions was the lowest and equalled 9.4% for DCOD and 6.0% for DON in the influent. During the subsequent treatment stages at MTW, the forms changed significantly, and finally COD and Org-N were present in the effluent, mainly in the dissolved and colloidal fractions. Based on the achieved

results it could be assumed that the working condition of SSHF beds play an important role in the transformation of particulate to colloidal and colloidal do dissolved fractions of organic matter and Org-N. The SSVF bed was very effective in the removal of the dissolved fraction, which can suggest that very effective microbiological processes take place in it.

The investigation proved that a part of particulate organic matter and Org-N present in raw wastewater is resistant to treatment.

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MARCIN DĘBOWSKI*, MARCIN ZIELIŃSKI*, ANGELIKA KLUK*, ŁUKASZ CHOMAŃSKI*

EFFECT OF AQUA KEM GREEN CHEMICALS
ON TECHNOLOGICAL EFFECTIVENESS
OF GASIFICATION OF PLANT BIOMASS

Development of sailing tourism is contributes to degradation of the natural environment, waters of lakes and rivers in particular. Wastewaters produced on sailboats and yachts and deposited in their chemical toilets are usually discharged directly to aquifers, with a minor part being discharged to collectors for these are located in a few ports only. Thus, it seems indispensable to develop a complex system of collection and neutralization of such wastewaters. It is, however, a difficult process for it requires solving both logistic and technological problems. One of the technological problems may involve adopting an optimum method for neutralization of the wastewaters that usually contain chemical agents for the removal of unpleasant odours, e.g. Aqua Kem Green type. A solution to this problem may be co-gasification of wastewaters from sailboats with plant biomass in the fermentation process in a biogas works.

1. INTRODUCTION

Poland is a country with great natural wealth of lakes, accumulated mainly in the Great Lakeland of the Warmia and Mazury Region. These beautifully located areas are highly attractive from the recreational point of view and serve the constantly developing tourism, e.g. sailing. Unfortunately, this development is becoming the cause of advancing degradation of the natural environment. Cases of discharging wastewaters from chemical toilets of sailboats directly to lake waters recently increased. A lack of system solutions for the collection of produced wastewaters or insufficiently stipulated requirements for chemical toilets exploitation additionally contribute to long-lasting degradation of this outstanding landscape and deterioration of water purity.

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Worthy of notice is the fact that each year the Great Masurian Lakeland is visited by over 50 000 sailors, which means that 10 000–12 500 sailboats and yachts are sailing in this area. Ca. 40% of the latter are equipped in chemical toilets [1]. The use of chemical toilets involves application of chemicals that, among others, assure neutralization of unpleasant odours. Contemporarily, the chemical agents used in chemical toilets are produced based on bronopol.

An exceptionally high activity of bronopol (2-bromo-2-nitropropane-1,3-diol) has been observed especially against strains of Gram-negative bacteria. It has become a highly popular bactericide (as a substitute of formaldehyde) not only in toilet preparations but also in the cosmetic and pharmaceutical industry.

One of such chemicals is Aqua Kem Green. Unfortunately, the mixture of fecal matter and chemical substances produced upon the use of this type of agents requires appropriate treatment. Nowadays, treatment of wastewaters from yachts proceeds in wastewater treatment plants located on the area of the Great Masurian Lakeland. However, managers of these plants often refuse to collect sewages from chemical toilets both from yachts, coaches or camping sites. These plants are operating based on the method of activated sludge, therefore an additional load of discharged impurities increases their demand for energy that is necessary for the exploitation of aeration tanks. They are also highly overloaded with additional discharges of wastewaters in the summer season. What is more, microflora of the activated sludge is susceptible to toxic substances occurring in wastewaters. Hence, a good solution to this problem appears to be making use of agricultural biogas works or selected fermentation tanks of municipal wastewaters treatment plants. Anaerobic biodegradation of sewages has a number of advantages [2]. It results in the production of sewage gas containing a high-energy component – methane, whilst microorganisms of anaerobic sludge are capable of decomposing many toxic chemical compounds [3, 4].

Biogas works are usually located on rural areas with easy access to fermentation substrate. The Great Masurian Lakeland is, undoubtedly, the area used for farm production; hence a vast increase is expected here in biogas production investments. Costs of purchase of fermentation substrate may be reduced by means of mixing plant biomass with wastewaters. In this case, the mixing of, e.g. maize silage with wastewaters from sail boats seems advisable. This will enable diluting chemical substances contained in wastewaters from chemical toilets, reducing sewage load in the neighbouring wastewater treatment plants, and most of all will make the wastewaters a source of energy. Finally, this means development of economic mechanisms that will allow one to establish a network of effective collectors for chemical toilets.

The objective of this study was to find the optimum dose of bronopol containing chemicals that would neither contribute to inhibition of fermentation processes nor disturb significantly the activity of a biogas works.

2. EXPERIMENTAL

The experiment was conducted at the laboratory of the Department of Environment Protection Engineering at the University of Warmia and Mazury in Olsztyn. It was conducted in two variants by the same method. Difference was the use of various loading of the active tank volume with feedstock of organic compounds. In the former variant assays were carried out with the level of 2.5 kg o.d.m./m^3 and in the latter – 5.0 kg o.d.m./m^3

Analyses were made for the effect of the dose of Aqua Kem Green chemicals at various loadings of tanks with a load of carbon compounds on the final technological effects of the methane fermentation process. The objective of this experiment was to determine the dose of the bronopol containing chemical agents that would not disturb the fermentation process which has a direct impact on biogas production.

Prior to exact fermentation with the use of a system of respirometers, a substrate (maize silage) was appropriately conditioned and prepared (it was subject to 10 min mixing and chopping with a type 3 blixer (Robot Coupe) for proper disintegration).

The tested doses of Aqua Kem Green chemicals containing bronopol were administered into measuring devices that enable determining the degree of degradability of organic matter as well as monitoring the quantity and composition of biogas produced. In the experiment, use was made of Oxi-Top Control respirometric kits by the WTW Company which consisted of reaction tanks tightly coupled with measuring and recording devices. The research method used in the study enabled determining the activity of anaerobic sludge, susceptibility of the applied organic substrates to biodegradation, and quantity and composition of gaseous metabolites. The devices recorded and analyzed changes in the partial pressure inside the measuring tank evoked by biogas production in anaerobic processes run by microorganisms. In each of the experimental variants, 50 cm^3 of anaerobic sludge obtained from a municipal waste water treatment plant were administered to the measuring tank, followed by various doses of the substrate and various doses of chemical agent applied for the proper functioning of chemical toilets (e.g. on sailboats): Aqua Kem Green. The 100% dose was assumed to be the dose of bronopol containing chemical whose quantity recommended by the producer mixes at the ratio of 50/50 with feedstock for fermentation.

The complete measuring kit, made of a reaction tank and a measuring-recording device, was fixed in a thermostatic cabinet with hysteresis not exceeding ± 0.5 °C. Measurements were performed at 35 °C for 20 days, with the pressure in the reaction tank being measured every 15 min. Before the end of the measurement, 30% sodium base (NaOH) was introduced to a special container mounted inside the reaction tank, which enabled carbon dioxide to precipitate from the gaseous phase. A decrease of pressure in the reaction tank corresponded to the content of carbon dioxide, whereas otherwise the value of pressure depended on the content of methane. In respirometric analyses, calculations are based on the equation of ideal gas:

$$n = \frac{pV}{RT} \tag{1}$$

where: n – number of moles of gas, p – gas pressure [Pa], V – gas volume [m^3], R – universal gas constant (8.314 J/(mol·K)), T – temperature [K].

The content of carbon in the gaseous phase was calculated from the following equation:

$$n_{CO_2} + n_{CH_4} = \frac{p_1 V_g}{RT} \times 10^{-4} \tag{2}$$

where: $n_{CO_2} + n_{CH_4}$ – number of produced moles of carbon dioxide and methane, p_1 – difference in gas pressure in a measuring tank at the beginning and at the end of the experiment caused by oxygen consumption and absorption of the generated CO_2 [hPa], V_g – volume of gaseous phase in a measuring tank [cm^3], R – gas constant, T – temperature of incubation [K], 10^{-4} – conversion factor.

The content of carbon dioxide in the gaseous phase was calculated from the following equation:

$$n_{CO_2} = \left(\frac{p_1 V_g - p_2 (V_g - V_{KOH})}{RT} \right) \times 10^{-4} \tag{3}$$

where: n_{CO_2} – number of produced moles of carbon dioxide [mol], p_2 – difference in gas pressure in an appropriate measuring tank at the end of the experiment minus pressure recorded at the beginning of the experiment minus pressure recorded in a blank sample after addition of KOH solution [hPa], V_{KOH} – volume of KOH solution [cm^3].

The content of methane in the gaseous phase was calculated from the following equation:

$$n_{CH_4} = n_{CO_2 + CH_4} - n_{CO_2} \tag{4}$$

Results of respirometric analyses allowed one to determine the rate of biogas production as affected by doses of the chemical agents Aqua Kem Green. Measurements of pressure inside the tank, made in 15 min intervals, enabled determining the rate of the process. Reaction rate constants were determined based on the experimental data by the method of non-linear regression using the Statistica 8.0 PL software. The iterative method was applied, in which in each iterative step a function is replaced with a linear differential relative to the determined parameters. The coefficient of concordance φ_2 was adopted as a measure of curve fitting (with the parameters determined in the study) to experimental data. This coefficient is a ratio of the sum of squared deviations of values calculated based on the determined function from experimental data, to the sum of squared deviations of experimental values from the mean value. Thus, the lower the value of the φ_2 coefficient, the better consistency is obtained. The fitting of

the model to experimental points was adopted at the level at which the coefficient of concordance did not exceed 0.2.

3. RESULTS AND DISCUSSION

The applied 0.187 cm^3 dose of Aqua Kem Green chemical enabled producing 118 $m_N^3/T_{o.d.m.}$ of biogas at the constant for all samples loading of the measuring tank with a feedstock of organic compounds, i.e. 2.5 $kg_{o.d.m.}/m^3$. The total volume of biogas produced in this sample reached 295 cm^3, whereas methane content accounted for 59%. The rate of biogas production resulting from anaerobic metabolism reached 0.25 $m_N^3/(T_{o.d.m.}\cdot h)$. Increasing the dose of the bronopol containing chemical to 150% caused a negligible decrease in biogas production to 117 $m_N^3/T_{o.d.m.}$. The total quantity of biogas produced accounted for 294 cm^3 and methane content oscillated at the level of 58%. The rate of the reaction was observed to decrease slightly and reached 0.24 $m_N^3/(T_{o.d.m.}\cdot h)$. In turn, decreasing the dose of the chemical agent to 75% and 50% enhanced the production of biogas to the level of 124 $m_N^3/T_{o.d.m.}$. The total volume of sewage gas was 310 and 311 cm^3, with the reaction rate of ca. 0.26 $m_N^3/(T_{o.d.m.}\cdot h)$ and methane content at the level of 64% and 60%. Once Aqua Kem Green was applied at the dose of 25%, the efficiency of the process reached 129 $m_N^3/T_{o.d.m.}$, its rate accounted for 0.27 $m_N^3/(T_{o.d.m.}\cdot h)$, the total quantity of biogas produced for 322 cm^3 and methane content for 59%. In the blank sample with no bronopol containing chemical added, the production of biogas reached 127 $m_N^3/T_{o.d.m.}$, the total volume of biogas obtained reached 318 cm^3, and methane content remained at the level of 57%.

Application of the same dose of Aqua Kem Green chemical in another variant, at a constant loading of the measuring tank for all samples with the feedstock of organic compounds, i.e. 5.0 $kg_{o.d.m.}/m^3$ led to slightly higher efficiency of the process. The applied 0.187 cm^3 dose enabled producing 130 $m_N^3/T_{o.d.m.}$ of biogas. The total volume of biogas produced in this sample reached 325 cm^3, whereas methane content accounted for 60%. The rate of biogas production resulting from anaerobic metabolism reached 0.27 $m_N^3/(T_{o.d.m.}\cdot h)$. Increasing the dose of the bronopol containing chemical to 150% caused a negligible decrease in biogas production to 129 $m_N^3/T_{o.d.m.}$. The total quantity of biogas produced accounted for 323 cm^3 and methane content oscillated at the level of 60%. The rate of the reaction was 0.27 $m_N^3/(T_{o.d.m.}\cdot h)$. In turn, decreasing the dose of the chemical agent to 75% decreased the production of biogas to the level of 127 $m_N^3/T_{o.d.m.}$. The total volume of sewage gas was 317 cm^3, with the reaction rate of ca. 0.26 $m_N^3/(T_{o.d.m.}\cdot h)$ and methane content at the level of 63%. The dose of 50%

caused increase in biogas production to 130 $m_N^3/T_{o.d.m.}$. The total volume of sewage gas was 326 cm^3, with the reaction rate of ca. 0.27 $m_N^3/(T_{o.d.m.}\cdot h)$ and methane content at the level of 60%. Once Aqua Kem Green was applied at the dose of 25%, the efficiency of the process reached 131 $m_N^3/T_{o.d.m.}$, its rate accounted for 0.27 $m_N^3/(T_{o.d.m.}\cdot h)$, the total quantity of biogas produced– for 329 cm^3 and methane content – for 62%. In the blank sample with no bronopol containing chemical added, the production of biogas reached 136 $m_N^3/T_{o.d.m.}$, the total volume of biogas obtained reached 340 cm^3, and methane content remained at the level of 59%.

The volume of biogas produced, the content of methane and the rate of sewage gas production expressed per normal conditions (blank sample) obtained the experiment are presented in Tables 1, 2.

Table 1

Results of respirometric measurements with the use of various doses of Aqua Kem Green at loading of the measuring tank with a feedstock of organic compounds 2.5 $kg_{o.d.m.}/m^3$

No.	Dose of Aqua Kem Green	Biogas production		Methane content [%]	Rate $[m_N^3/(T\ o.d.m.\cdot h)]$
		$[m_N^3/T\ o.d.m.]$	$[cm_N^3]$		
1	150% (0.281cm^3)	117.60	294.0	58.0	0.24
2	100% (0.187cm^3)	118.12	295.3	59.0	0.25
3	75% (0.141cm^3)	123.90	309.7	59.4	0.26
4	50% (0.094cm^3)	124.60	311.5	61.2	0.26
5	25% (0.047cm^3)	128.62	321.6	59.3	0.27
6	–	127.05	317.6	57.3	0.26

Table 2

Results of respirometric measurements with the use of various doses of Aqua Kem Green at loading of the measuring tank with a feedstock of organic compounds 5 $kg_{o.d.m.}/m^3$

No.	Dose of Aqua Kem Green	Biogas production		Methane content [%]	Rate $[m_N^3/(T\ o.d.m.\cdot h)]$
		$[m_N^3/T\ o.d.m.]$	$[cm_N^3]$		
1	150% (0.281 cm^3)	129.32	323.3	60.0	0.27
2	100% (0.187 cm^3)	129.85	324.6	59.7	0.27
3	75% (0.141 cm^3)	126.87	317.2	63.7	0.26
4	50% (0.094 cm^3)	130.37	325.9	60.1	0.27
5	25% (0.047 cm^3)	131.42	328.6	62.3	0.27
6	–	135.97	339.9	59.2	0.28

Bronopol is a catalyst of oxidation of compounds containing thiol groups (SH) to disulfides [6]. This reaction is additionally characterized by a rapid oxygen consump-

tion. In turn, one electron reduction of an oxygen molecule leads to generation of a strongly reactive superoxide anion. It oxidizes intracellular thiols, including glutathione. The latter is one of the most common low-molecular antioxidants. Oxidation results in the generation of glutathione disulfide. The reaction itself proceeds owing to a specific structure of a glutathione molecule, being a tripeptide containing cysteine, which, in turn, contains thiol groups (SH) susceptible to the action of superoxide anions. Oxidative stress results in disruption of the physical continuity of cellular structures and in disorders of biochemical transformations. Cell metabolism is thereby disturbed, which is manifested by inhibition of its growth and development [5, 6]. The results of examination show that such inhibition proceeds already at a 50% dose of Aqua Kem Green chemical at the first stage of experiment with anaerobic reactor load of feedstock reaching 2.5 $kg_{o.d.m.}/m^3$ as well as at the lowest dose of the chemical applied at the latter stage (tank load – 5 $kg_{o.d.m.}/m^3$). This is indicated by a decrease in the process rate and in the intensity of biogas production.

Free radicals (superoxide anions) might be neutralized owing to the action of catalase and superoxide dismutase (intracellular bacterial enzymes), according to the following reaction:

$$2H^+ + 2O_2^- \xrightarrow[\text{dismutase}]{\text{superoxide}} 2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Yet, the rate of oxidation of thiols determined by bronopol concentration is also of significance in terms of the neutralization of bronopol. Once bronopol concentration is so high that the concentration of enzymes in a cell appears to be insufficient, the oxidative stress occurs.

The bactericidal effect of bronopol may be neutralized under anaerobic conditions, which has been proved by applying relatively low concentration of the bronopol containing agent at the first stage of the experiment. With its concentration reaching 25%, the volume of biogas produced was not reduced but even increased, which points to the enhanced activity of fermentation bacteria. Shepherd et al. [7] prove that another reaction may occur in which bronopol is consumed and neutralized. It does not require oxygen and is slower than oxidation of thiols where bronopol is only a catalyst. This reaction is determined by the concentration of thiols [7] and affords the possibility of neutralizing wastewaters containing bronopol in the course of methane fermentation.

4. CONCLUSIONS

Increasing concentration of Aqua Kem Green chemical leads only to minor inhibition of fermentation processes in anaerobic reactors, which results from effective neutralization of bronopol occurring in that chemical agent.

Introduction of a low dose of Aqua Kem Green chemical (25%) to anaerobic reactors (with the load of 2.5 $kg_{o.d.m.}/m^3$) does not affect the fermentation processes.

Increasing the load of the reactor with a feedstock of organic compounds from 2.5 to 5 $kg_{o.d.m.}/m^3$ has no significant effect on the effectiveness of biogas production.

Wastewaters produced on yachts and accumulated in chemical toilets with the described chemical agent may be dosed together with plant biomass to agricultural biogas works, without any significant disturbances in the biological processes run therein.

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