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USING THE ATP TEST IN WASTEWATER TREATMENT IN THE SILESIA PROVINCE

The research conducted in recent years enabled one to develop a test for qualitative and quantitative analyses of ATP concentration in wastewater and sewage sludge performed in short time directly under field conditions. Both the test itself and the comparison of the obtained results complement the main classic procedures. The test allows one to characterize the impact of raw wastewater on the treatment process and on the intoxication of organisms inhabiting wastewater treatment units. They also provide information on the microbiological pollution in the treated wastewater. Moreover, they can be used to monitor the treatment process itself.

1. INTRODUCTION

For many years, the researchers sought methods enabling quick identification of the microbiological threats in water. The adenosine triphosphate (ATP) test has been more and more often used in practice [1, 2]. At present, the test is mainly used to assess the hygiene conditions in various industries [3–5]. These include the food industry (production lines, water, technological devices), pharmaceutical industry (biological contamination of medicines and cosmetics), chemical industry and the utilities sector (microbiological pollution of drinking water and usable hot water) [6–8]. The latter application seems particularly important for public facilities such as hospitals [9] or seasonally inhabited hotels. The main advantage the ATP tests offer is identifying the existing threats in real time. Importantly, the test is simple and easy to conduct. At the same time, it is highly precise and can be performed at the sampling point [10, 11]. The producers of the latest ATP tests also indicate that they can be used to assess the operation of biological wastewater treatment plants [12].

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ATP is determined by the bioluminescence method based on the oxidative decarboxylation reaction of luciferin. The reaction is described by:

$$\text{ATP} + \text{O}_2 + \text{luciferin} \xrightarrow{\text{luciferase, Mg}^{2+}} \text{oxyluciferin} + \text{AMP} + \text{products} + \text{light} \quad (1)$$

The luciferase enzyme catalyses the transformation of luciferin to oxyluciferin where transition of the molecule to its ground state results in the emission of light measured with a luminometer. The measured bioluminescence intensity is proportional to the light quanta emitted during the ATP hydrolysis reaction. The determined light impulse intensity, given in relative light units (RLU), is correlated with the ATP concentration in the sample [1, 10, 13].

The total ATP (tATP) content in water or wastewater is the sum of the cellular ATP (cATP, present in the living cells) and dissolved ATP (dATP, coming from the decomposed cells). The ATP content per bacterial cell differs and depends on the species, physiological conditions and the metabolic activity of microorganisms. It ranges between 0.1 and 0.5 fg[3] per bacterial cell (Table 1) [1, 12].

Table 1

ATP content in the microorganism cells [1]

Microorganism	ATP [fg/cell]
Pseudomonas fluorescens	0.6
Leuconostoc mesenteroides	0.7
Escherichia coli	1.0
Lactobacillus sp.	2.0–2.2
Bacteria mixture	1.0

The tATP and dATP measurements in influent/effluent of wastewater treatment plant and in the bioreactor are recommended to control the current plant operation. The data enables the calculation of the cATP content (the difference between tATP and dATP) in raw and treated wastewater, the biomass stress index (BSI), and the active biomass ratio (ABR) [12]. The description of the bacteria physiological condition is very important for the operation and efficiency of wastewater treatment plants. The determination of the described parameters provides information on the potential intoxication of wastewater, sludge condition and its activity as well as the process effectiveness. Consequently facilitates optimization of the process that are related both to the catchment area of the treatment plant and the treatment process performance [14, 15].

Both dATP and cATP occur in raw wastewater, which enables one to establish the BSI. The analysis is based on the assumption that a part of the ATP contained in the wastewater supplied to the plant should occur in the bacterial form. Exceeding a certain dATP level may

[3]1 fg is 10^{-15} g.

indicate the presence of toxic substances in the wastewater. The importance of this indicator increases when there are industrial plants in the catchment area of the treatment plant. The industrial plant effluents can negatively affect the biological treatment processes [15–17].

When the operation of bioreactors with activated sludge is analysed, two parameters should be taken into consideration such as BSI and ABR. In the treated wastewater, the possibility to determine cATP content is indicated. The value provides information on the occurrence of microorganisms in the effluent, which can point to the improper operation of a bioreactor or secondary settling tank. The test producer gives the limit values for particular indicators, which enables the researchers to characterize directly the conditions such as: proper, or requiring preventive actions, or alarming ones that require immediate corrective actions (Table 2) [12].

Table 2

Assessment of the wastewater treatment plant operation related to the selected indicators recommended by LuminUltra Technologies, Ltd. (Canada) [12]

Sampling point	Parameter	Proper conditions	Required preventive actions	Required corrective actions
Influent	BSI, %	<50	50–70	>75
Bioreactor	BSI, %	<30	30–50	>50
	ABR, %	>25	10–25	<10
Effluent	cATP, ng/cm^3	<50	50–250	>250

The test results can be useful in the current control of the plant operation as they complement the conventional methods.

2. EXPERIMENTAL

Research objectives. The aim of the research was to determine the wastewater toxicity and the operation of a bioreactor with the ATP test. At the same time, the physicochemical analyses of raw and treated wastewater were conducted and the sludge content in the bioreactor was examined. The research was conducted in three wastewater treatment plants in which five various treatment systems were used. The investigation was conducted once for each selected plant in the same season. Additionally, the archive data was used to apply the results obtained in the following research to the assessment of the raw and treated wastewater quality and bioreactor operation.

2.1. WASTEWATER TREATMENT PLANTS

The research was conducted in three municipal wastewater treatment plants situated in the Upper Silesia urban area. Five different process lines for wastewater treatment were used (Figs. 1–3). Each process line was analysed separately.

Wastewater treatment plant 1. The plant serves approximately 50 000 inhabitants and the 24 h wastewater flow is 6600–7690 m^3. The separate sewage system makes about 80% of the whole system. It was built in the years 2000–2005. The combined sewage system constitutes the remaining 20%. It was constructed throughout the entire 20th century. There are seven sewage pumping stations in the plant catchment area. In the area, there are also large meat processing plants.

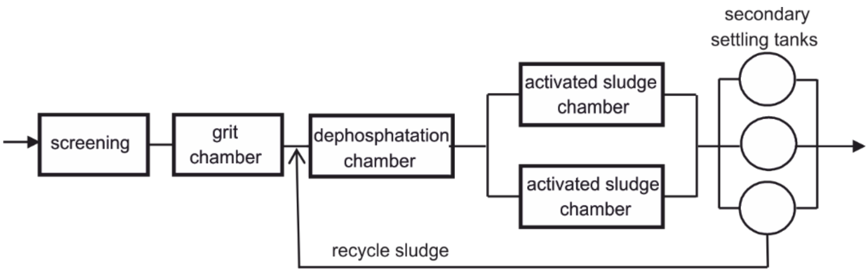

Fig. 1. Block diagram of the wastewater treatment plant 1

The plant technological system (Fig. 1) consists of screening, aerated grit chamber, dephosphatation chamber and two bioreactors whose total volume is 16 700 m^3. The reactors act as oxidation ditches in the biological treatment process. The wastewater is aerated with mechanical rotors with transverses. The rotor work is controlled with the oxygen content which enables simultaneous denitrification. The sludge age is 25 days whereas its content is 3500 mg TSS/dm^3. When increased contents of phosphates are found in the outlet, the phosphorus is precipitated with iron compounds. Then, the wastewater is transported into three secondary settling tanks, of the volume of 895 m^3 each. The excessive sludge is dewatered on the filtration presses and dried.

Wastewater treatment plant 2. The plant has two process lines supplied from independent collectors (Fig. 2). Approximately 12 000 inhabitants are connected to the process line I. The 24 h wastewater flow is 1370–1750 m^3. The separate sewage system makes approximately 50% of the collector catchment area, in which one sewage pumping station operates. There are no significant industrial plants in the collector catchment area. The supplied wastewater flows through a rotating sieve. Then, it is transported into the bioreactor that works in the Biolak system (process line I, Fig. 3a), whose total volume is 4340 m^3. The anaerobic part is separated in the bioreactor. It enables biological dephosphatation. The wastewater is aerated with a system of diffusers mounted on floats. It provides proper wastewater mixing and creation of the alternate nitrification and denitrification zones. The aeration system is operated with the ongoing control of the oxygen content. The sludge is 25 days old whereas its content in the chamber is approximately 1700 mg TSS/dm^3. Then the wastewater flows through a secondary settling tank combined with an aeration chamber. When the process is finished, the wastewater undergoes final aeration and is transported into the receiver.

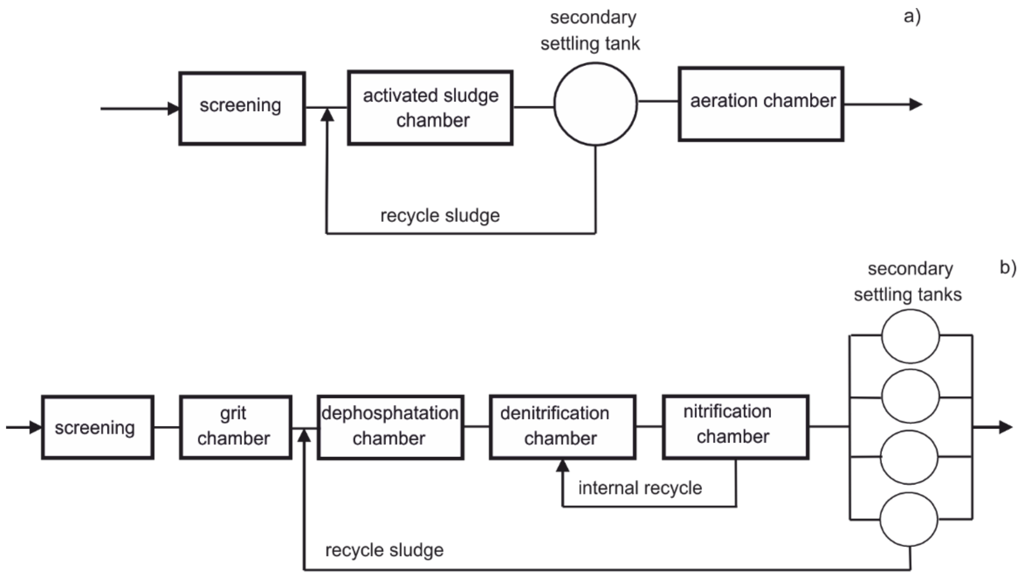

Fig. 2. Block diagrams of the wastewater treatment plant II:
a) process line I – Biolak, b) process line II – Phoredox

Approximately 28 000 inhabitants are connected to the process line II (Fig. 3b). The 24 h wastewater flow is 7506–7809 m^3. The separate sewage system, built in the 1970s and 1980s, dominates in the collector catchment area and serves approximately 65% of the inhabitants. The remaining residents use the combined sewage system in which two sewage pumping stations operate. Confectionery production plants also use the sewage system. The wastewater is treated with the Phoredox method. The process line II consists of the screening, grit chamber, dephosphatation chamber (730 m^3), denitrification chamber (4400 m^3) and nitrification chamber (4950 m^3). Mechanical mixers are used to keep the sludge suspended in the dephosphatation and denitrification chambers. The nitrification chamber makes use of the diffusers supplied with compressed air. The sludge age is 20–25 days and its content in the nitrification chamber is approximately 3800 mg TSS/dm^3. The wastewater is transported into secondary settling tanks (four tanks, 544 m^3 in volume) from which they flow into the receiver. It is possible to precipitate phosphorus with iron compounds (fed directly into the bioreactor) in both process lines.

The sludge management is performed together for both process lines. The excessive sludge is stabilized with oxygen and dewatered with filtration presses. Every year, approximately 2100 t of sludge are obtained. Its hydration is 20%. The sludge is removed and dried.

Wastewater treatment plant 3. The plant (Fig. 3) was designed to serve 125 000 inhabitants. Additionally, important food production plants are located in the area. The

24 h wastewater flow is approximately 32 000 m^3. The combined sewage system dominates in the catchment area.

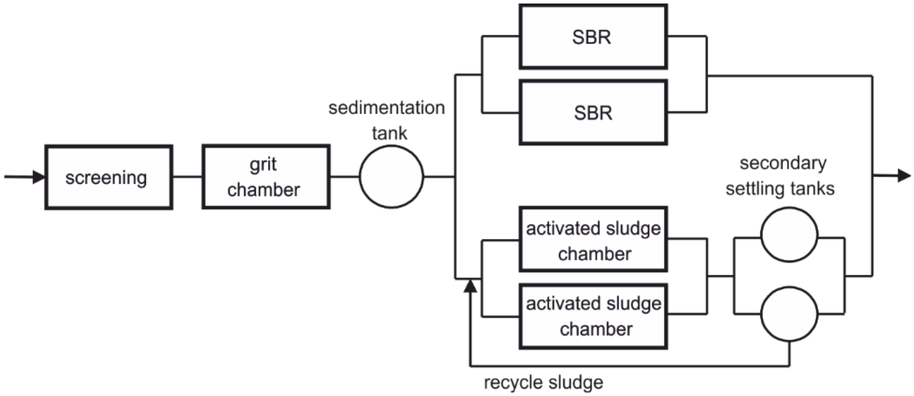

Fig. 3. Block diagrams of studied wastewater treatment plants

The wastewater supplied to the plant undergoes primary treatment (screening, grit chambers, and tanks) and then is separated into two streams. One of them (approximately 16 000 m^3/d) is directed into units that operate according to the Sequencing Batch Reactor (SBR) principle. The remaining part is transported into the process line consisting of the dephosphatation, predenitrification, denitrification and nitrification chambers, hereinafter referred to as activated sludge chambers (ASC). The wastewater is aerated with membrane diffusers. The sludge is suspended with mechanical mixers in each chamber. Additionally, the chemical precipitation of phosphorus is possible. The sewage sludge from both process lines undergoes fermentation in fermentation chambers.

2.2. ANALYTICAL METHODS

Sampling and analyses. The effectiveness of the discussed wastewater treatment plants was assessed. The wastewater samples were collected at the plant inlet area (after the screening process was completed) and at the plant outlet area (after going through the secondary settling tank). The following indicators were determined:

- pH according to internal research procedure; determination of pH of water and wastewater using a CX-401 Elmetron pH-meter, with pH electrode of a complex type ERH-111's Hydromet and a CT2B-121 Elmetron temperature sensor.
- Chemical oxygen demand (COD) according to PN-ISO 15705:2005 *Water quality. Determination of the chemical oxygen demand index (ST-COD)*. By the small-scale sealed tube method using a photometer Nanocolor 500 D and a Vario thermostat 3.
- Total suspended solids (TSS) according to PN-EN 872:2007 *Water quality. Determination of suspended solids*. By the method of filtration through glass fiber filters using the Sartorius filtration.

• Dissolved organic carbon (DOC) according to the internal research procedure. Determination of organic and inorganic carbon in water and wastewater using a Shimadzu total organic carbon analyzer TOC-5000A (the IR method).

• Total nitrogen according to PN-73/C-04576/12 *Water and wastewater. Tests on the contents of nitrogen compounds.* Determination of Kjeldahl nitrogen using a Buchi distillation apparatus unit K-314.

• Total phosphorus determined by the colorimetric method (ascorbic acid) using a UV-VIS Varian Cary 50 Scan UV-Vis spectrophotometer.

• For the sewage sludge, the total suspended solids (TSS) and all ATP forms (tATP, dATP and cATP) were determined.

ATP determination. The analyses were performed in accordance with the test producer's instructions. The Quench Gone Wastewater (QG21W) test kit and the luminometer (Berthold Technologies) were used for the analyses [2, 18].

First the luminase (luciferin–luciferase complex) activity was examined to check its bioluminescence in the samples. The luminescence intensity of the luminase-control reagent mixture (Ultra Check) should not be higher than 500 RLU. The tATP content was determined by the QG21W test kits. The subsequent procedures were as follows: extraction, dilution and the luminescence measurement. The dATP content was determined in the same way excluding extraction. 1 cm^3 and 0.1 cm^3 samples were used to determine tATP and dATP, respectively.

Taking into consideration the measured luminase luminescence signal and the conversion factor (depending on the sample amount and dilution level), the bioluminescence intensity in RLU was finally converted to the ATP content in the following way:

$$[\mathrm{tATP}] = \frac{\mathrm{RLU_{tATP}}}{\mathrm{RLU_{UC1}}} \times 11 \qquad [\mathrm{ngATP/cm^3}] \tag{2}$$

$$[\mathrm{dATP}] = \frac{\mathrm{RLU_{dATP}}}{\mathrm{RLU_{UC1}}} \times 101 \qquad [\mathrm{ng\ ATP/cm^3}] \tag{3}$$

$$[\mathrm{cATP}] = [\mathrm{tATP}] - [\mathrm{dATP}] \tag{4}$$

where: RLU_{UC1} – measured luminescence intensity for the luminase–control reagent mixture, RLU_{tATP} – measured luminescence intensity proportional to the tATP content, RLU_{dATP} – measured luminescence intensity proportional to the dATP content.

The parameters describing the treatment process were defined with the following formula:

$$\mathrm{BSI} = \frac{[\mathrm{dATP}]}{[\mathrm{tATP}]} \times 100\% \tag{5}$$

where: BSI – the biomass stress index, %, dATP – dissolved ATP concentration, ng ATP/cm^3], tATP – total ATP content, ng ATP/cm^3.

$$\mathrm{ABR} = \frac{[\mathrm{cATP}]}{\mathrm{TSS}} \times 100\% \tag{6}$$

where: ABR – the active biomass ratio, %, cATP – cellular ATP concentration, ng ATP/cm^3, TSS – total suspended solids in the bioreactor, mg/dm^3, 0.5 – a conversion factor from ATP concentration to dry biomass concentration (a biomass population contains 250 parts of biomass carbon per 1 part of intracellular ATP and that biomass is approximately 50% carbon on a dry basis) [12].

3. RESULTS AND DISCUSSION

The analysed wastewater treatment plants have been either built or modernised over the last two decades. They make use of modern solutions that give good results of the treatment processes, even though they differ significantly in the applied technologies. In the three places, the treatment is conducted with the under-loaded activated sludge, which should provide a proper reduction of suspension and carbon and biogenic compounds.

Each plant had specific process line solutions. No sludge fermentation was conducted in the plants 1 and 2. The excessive sludge was stabilized with oxygen either in the bioreactors with extended retention time or in separated stabilization chambers. In both cases, the wastewater retention time in the bioreactor exceeded 48 h. As a result, it was possible to abandon the use of sedimentation tanks. The wastewater treatment and sewage sludge processing were simplified and the range of the wastewater treatment plant service decreased. Additionally, for the Biolak system (wastewater treatment plant 2, process line I), final water aeration–water conditioning the secondary settling tank scheme was applied. This helped to avoid oxygen deficiencies in the receiver water below the wastewater discharge.

The raw wastewater supplied into particular plants had different values of the pollution indicators. The obtained concentrations indicate that the wastewater in the sewage systems was diluted with the inflow and infiltration water to various extents. Nonetheless, the values were close to those observed in the raw wastewater supplied into the treatment plants in the Silesia Province.

In the analysed treatment plants, the raw wastewater and treated wastewater transported into receivers were analysed. Additionally, the wastewater in the primary treatment was analysed in the treatment plant 3 (Tables 3–5). High removal levels of all analysed polluters was observed. In the treatment process, TSS, COD and total phosphorus decreased to 2–20 mg/dm^3, 19–45 mg O_2/dm^3 and approximately 0.5 mg P/dm^3 (reduction in indicators by 93.2–99.2%), respectively. The decrease in the total nitrogen and DOC was slighter reaching 83.3–93.8% (concentration in the treated wastewater ranged from 5 to 17 mg N/dm^3) and 84.2–90.7% (9–23 mg C/dm^3), respectively.

Table 3

Results of the physicochemical analyses and ATP test for wastewater in the wastewater treatment plant 1

Indicator	Raw wastewater	Treated wastewater
pH	7.02	6.49
TSS, mg/dm^3	424	20
COD, mg O_2/dm^3	840	19
DOC, mg C/dm^3	105.6	16.7
Total nitrogen, mg N/dm^3	101.0	16.9
Nitrate nitrogen, mg N/dm^3	–	12.2
Total phosphorus, mg P/dm^3	19.5	0.95
tATP, ng ATP/cm^3	316.0	18.3
cATP, ng ATP/cm^3	297.0	0.3
dATP, ng ATP/cm^3	19.0	18.0

Table 4

Results of the physicochemical analyses and ATP test for the wastewater treatment plant 2

Indicator	Process line I		Process line II	
	Raw wastewater	Treated wastewater	Raw wastewater	Treated wastewater
pH	7.88	7.57	7.85	7.57
TSS, mg/dm^3	402	10	360	4
COD, mg O_2 /dm^3	1,380	37	1,220	45
DOC, mg C/dm^3	84	9.2	93.9	10.6
Total nitrogen, mg N/dm^3	92.88	5.73	88.8	5.46
Total phosphorus, mg P/dm^3	9.1	0.62	9.1	0.5
tATP, ng ATP/cm^3	78.7	1.33	138	8.03
cATP, ng ATP/cm^3	69.3	0.0	125.3	6.37
dATP, ng ATP/cm^3	9.42	1.33	12.7	1.66

The results obtained after the primary treatment in the plant 3 are as follows. The removal level for TSS, COD, DOC and total phosphorus ranged between 42.1 and 51.0%. The total nitrogen demonstrated a significantly lower value (27.4%).

Table 5

Results of the physicochemical analyses and ATP test for the wastewater treatment plant 3

Indicator	Raw wastewater	Primary treatment	SBR outlet	ASC outlet
pH	7.68	7.45	7.31	7.28
TSS, mg/dm^3	236	116	2	2
COD, mg O_2 /dm^3	588	340	34	34
DOC, mg C/dm^3	223	115	20.8	23
Total nitrogen, mg N/dm^3	103.4	75.04	16.2	12.6
Total phosphorus, mg P/dm^3	20.85	10.2	0.57	0.55
tATP, ng ATP/cm^3	162.0	81.5	11.3	7.44
cATP, ng ATP/cm^3	138.5	70.3	6.92	5.81
dATP, ng ATP/cm^3	23.5	11.2	4.38	1.63

The level of tATP removal in the treatment process exceeded 90% in all the examined samples and correlated with the decrease in the COD and TSS values. For cATP, a lower concentration was also observed in effluent of the wastewater treatment plants. The values were 0.3 ng cATP/cm^3 (plant 1), 6.4 ng cATP/cm^3 (plant 2, process line I) and no ATP (plant 2, process line II), and approximately 6 ng cATP/cm^3 (plant 3). This indicator is essential when it comes to the microbiological characteristics of wastewater. It should be minimized as much as possible. For the raw wastewater, the dATP contents is particularly important. In the treatment plants 2 and 3, its concentration was approximately 8 times lower than that of the tATP, whereas in the treatment plant 1 it was 15 times lower. It is possible that the raw wastewater supplied into the treatment plants contained no toxic substances that could negatively influence the microorganisms present in the activated sludge. Thus, it did not have an adverse impact on the treatment plant operation.

Table 6

ATP test results for the sewage sludge

Indicator	Plant 1	Plant 2		Plant 3	
		Process line		SBR	ASC
		I	II		
TSS in the bioreactor, mg/dm^3	3480	1700	2964	nd	4000
tATP concentration in the bioreactor, ng ATP/cm^3	3900	913	2360	4720	3350
cATP concentration in the bioreactor, ng ATP/cm^3	3119	753	1989	3610	3304
dATP concentration in the bioreactor, ng ATP/cm^3	781	160	371	1110	46

The sludge age was relatively high in all the cases (20–24 days, Table 6), and the retention times for wastewater in bioreactors were different. The TSS values were 1700–4000 mg/dm^3. The ATP contents differed even more. The tATP content was 913–4720 ng/cm^3, whereas the dATP was 46–1110 ng/cm^3.

It seems possible to use the ATP test to determine the effectiveness of the treatment process (Fig. 4) as it can be used in the field conditions and the results are obtained quickly. It is particularly important for the ongoing monitoring of the treatment plant operation and possible remedial actions.

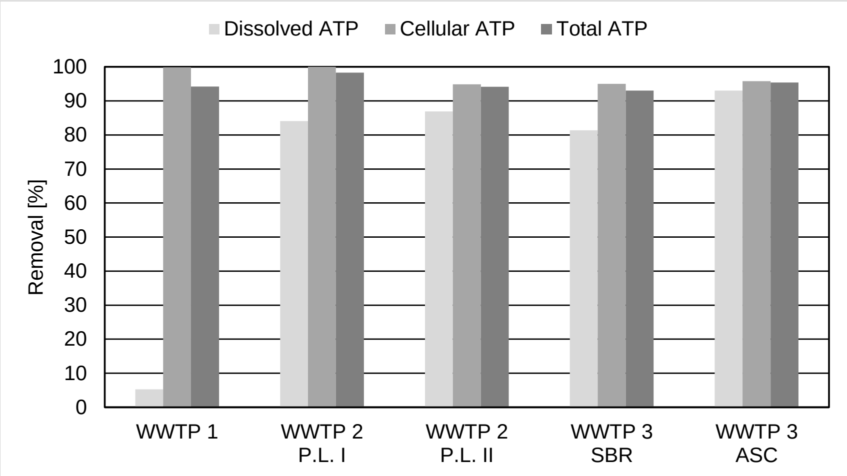

Fig. 4. Effectiveness of the operation of the wastewater treatment plant determined using the ATP test

All the analysed wastewater treatment plants serve agglomerations whose number of inhabitants is similar (over 100 000). The wastewater quality must meet the requirements of the strictest standards enlisted in the EU Directive and the Regulation of the Polish Ministry of the Environment of 24 July 2006 on the conditions that must be fulfilled when wastewater is introduced into waters or soils and on the substances acutely harmful for the aquatic environment [19]. The analysis of the obtained results indicates positive treatment effects. The values of most standardized indicators in the wastewater were lower than the maximum concentrations permitted by the above-mentioned Directive. The only exception was the total nitrogen, whose concentration slightly exceeded the permissible values in the treatment plants 1 and 3. Nonetheless, the obtained values did not differ from the results presented in the Silesia Province Marshal's reports on the performance of the National Programme for Municipal Wastewater Treatment (NPMWWT) [20, 21]. They confirm high treating effectiveness of the treatment plants put into use in recent years.

The total nitrogen concentrations in the treated wastewater that exceed permissible values are often observed in biological treatment plants. It is largely related to too low intensity of the denitrification process. Such a situation is observed when treatment is performed under good oxygen conditions or when there is no sufficient amount of carbon that serves as an indispensable substrate for the denitrification process. Consequently, the content of nitrates in the treated wastewater directed into receivers is high.

This can cause the denitrification in the receiver and moving the sludge onto its surface. It can also result in the increased TSS concentrations in the treated wastewater [22]. The problem is well-illustrated with the results obtained for the treatment plant 3, in which the removal of nitrogen compounds in the primary treatment was relatively low when compared with the removal of carbon compounds. Such a situation contributed to the C/N ratio that was too low in the biological treatment. For this reason, high contents of nitrogen in the form of the nitrate nitrogen could occur at the bioreactor outlet.

The ATP values obtained in the raw and treated wastewater confirm that the operation conditions in the three analysed facilities were good. It seems that the ATP analyses can quickly help to assess the plant effectiveness, particularly when it comes to small treatment plants with no laboratories. The test complements other indicators (BOD_5, COD, TSS, organic carbon) and enables analyses in the field conditions. Consequently, it helps to make rational decisions about the changes in the treatment process parameters.

Performing the qualitative ATP analysis of raw and treated wastewater and sludge provided additional information on the raw wastewater quality and their possible toxic impact, bioreactor operation, treated wastewater quality, and sewage system condition (Table 7) [14–17, 23]. It was possible to determine indicators typical of the treatment process, such as BSI for the raw wastewater characterising the dATP/tATP correlation. The BSI analysis is based on the assumption that a large tATP amount in the raw wastewater should occur in the cellular form. On the other hand, the increased dATP content may indicate the presence of toxic substances which leads to the cell decomposition. The wastewater quality is good when the BSI is lower than 50%. At 75%, it should be checked why such a situation occurred. Usually, it is related to the likely toxic influence of the industrial wastewater. The obtained BSI values (6–15%) indicate no toxic factors in the raw wastewater.

Table 7

Plant operation assessment with the ATP values

Sampling point	Parameter	Plant 1	Plant 2		Plant 3	
			Process line		SBR	ASC
			I	II		
Influent	BSI, %	6	12	9	15	15
Bioreactor	BSI, %	23	18	16	24	1
	ABR, %	44.8	22	33.6	nd	41.3
Effluent	cATP, ng/cm^3	0.3	0.0	6.37	6.92	5.81

The BSI is also used to evaluate the bioreactor operation. However, its permissible values are different and result from the development of microorganisms that is characteristic of bioreactors. The BSI lower than 30% demonstrates that the bioreactor works properly. When it is higher than 50%, correction actions are required. In the analysed treatment plants, the BSI value for bioreactors corresponded to the guidelines. Nevertheless, it ranged between 1 and 24%. The parameter was slightly increased (16–24%) in the four analysed bioreactors. The increased BSI value could be influenced by the changes in the SBR operation conditions and the advanced sludge age that affects the growth of flocs often covered with filamentous bacteria. As a result, the sedimentation abilities of the sludge deteriorated and the endogenous respiration occurred.

The other parameter, i.e. ABR, also provides interesting data. Its values for the analysed facilities ranged between 22.0 and 44.8%. The LuminUltra Technologies Ltd. guidelines suggest that the treatment plant operation conditions are good when ABR is higher than 25%. Due to that fact, corrective actions should be considered for the process line I in the treatment plant 2. However, a more careful analysis of the bioreactor operation conditions shows that such actions were unnecessary. The cATP value at the outlet and the BSI were low. The discussed problem probably resulted also from the sludge age and long retention time for wastewater in the bioreactor. A significant amount of the sludge underwent mineralization. Due to that, the ratio of the biomass of living microorganisms to the suspension could be relatively low.

The cATP content in the treated wastewater is the final indicator for the effectiveness of the treatment plant operation. The obtained value was relatively low and did not exceed 7 ng cATP/cm^3 in the investigated treatment plants. This parameter, essential to characterize the wastewater transported into the receiver, describes the operation of the technological system based on the running off sludge. It should be as low as possible.

Table 8

tATP/COD and tATP/TSS ratios for the examined wastewater treatment plants

Wastewater treatment plant	Sampling point	tATP/COD	tATP/TSS
1	raw wastewater	3.16×10^{-4}	7.45×10^{-4}
2	process line I – Biolak	0.56×10^{-4}	1.95×10^{-4}
	process line II – Phoredox	1.13×10^{-4}	3.83×10^{-4}
3	raw wastewater	2.76×10^{-4}	6.86×10^{-4}
	primarily treated wastewater	2.40×10^{-4}	7.02×10^{-4}

The values of tATP/COD and tATP/TSS ratios in the raw wastewater are given in Table 8. The tATP and dATP contents and the quantities determined from these values were determined. They helped to analyse the quality of the wastewater supplied into the treatment plants, the conditions of the treatment process performance and its effects. Significant differences were observed both for the tATP/COD and tATP/TSS ratios in the raw wastewater (0.56×10^{-4}–3.16×10^{-4} and 1.95×10^{-4}–7.45×10^{-4}, respectively). The differences probably resulted from the technical condition of the sewage system. Collector oversizing, slight hydraulic gradients and damages caused by mining enabled the precipitation of suspended solids in the sewage tubes. When the flows are low, the wastewater can be filtrated through the sludge layers. In the rain period, the collectors can be flushed and the pollution of the supplied wastewater can periodically increase. Thus the quality of the wastewater supplied into the treatment units changes seasonally and depends on the precipitation level. Processes such as sludge sedimentation in the sewage system, its filtration through deposited grit and the sludge leaching can cause the decrease in the tATP content. A similar phenomenon can be provoked by the sedimentation process and keeping wastewater in the retention tanks before its pumping. It seems that the low tATP/COD and tATP/TSS ratios could indicate difficulties in the proper wastewater transportation. Importantly, these are only preliminary observations that should be confirmed with further research.

4. CONCLUSIONS

The need to quickly identify possible threats in wastewater treatment plants requires the introduction and development of tests used for qualitative and quantitative determinations of microorganisms. The performed ATP analyses confirm previous observations on the possibility of the ATP test application in practice. The results provided a lot of data on the quality of the raw and treated wastewater and the treatment process itself. The ATP/COD or ATP/TSS ratios offer information on wastewater transport and the sewage system condition.

The ATP tests can be complementary to typical analyses used in treatment plants. Standard methods such as microscopic analyses or colony plate method, are time-consuming and do not enable immediate reaction when a threat occurs. When analysing the application of this test to assess the adaptation level of microorganisms to the changing substrate, particularly in the industrial wastewater treatment, it seems the ATP test could replace the TTC (tetrazolium chloride) test to indicate cellular respiration. The ATP determination is useful when defining the threats related to the development of filamentous bacteria in the bioreactor. It is also valuable when researchers try to determine the decomposition of the microorganism cell membrane occurring at the anaerobic decomposition of the sewage sludge (fermentation). Using the running-off sludge, the test can also be applied to decide whether the technological system works properly.

It is important to remember that the ATP determination is a non-specific test. In other words, the biological material type cannot be distinguished. The test only enables the differentiation between cATP (living cells) and dATP (decomposed cells). Nonetheless, it is a very useful tool applied in the ongoing operations due to its simplicity, extremely short analysis time and the possibility of performing measurements in situ. Therefore it can be used for quick threat assessment and application of the necessary remedial actions.

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REFERENCES

[1] KOWAL K., LIBUDZISZ Z., *Technical Microbiology,* Wydawnictwo Politechniki Łódzkiej, Łódź 2000 (in Polish).

[2] PISTELOK F., WIERA B., STUCZYŃSKI T., *Threats identification for water health safety during exceptional events,* Instal., 2012, 3, 44 (in Polish).

[3] VENKATESWARAN K., HATTORI N., DUC M., KERN R., *ATP as a biomarker of viable microorganisms in clean-room facilities*, J. Microbiol. Methods, 2003, 52, 367.

[4] HAWRONSKYJ J., HOLAH J., *ATP. A universal hygiene monitor*, Trends Food Sci. Technol., 1997, 8, 79.

[5] DAVIDSON C.A., GRIFFITH C.J., PETERS A.C., FIELDING L.M., *Evaluation of two methods for monitoring surface cleanliness. ATP bioluminescence and traditional hygiene swabbing*, Luminescence, 1999, 14, 33.

[6] DELAHAYE E., WELTE B., LEVI Y., LEBLON G., MONTIEL A., *An ATP-based method for monitoring the microbiological drinking water quality in a distribution network*, Water Res., 2003, 37, 3689.

[7] DEININGER R., LEE J., *Rapid determination of bacteria in drinking water using an ATP assay*, Field Anal. Chem. Technol., 2001, 5 (4), 185.

[8] DEININGER R., LEE J., CLARK R., *Rapid detection of bacteria in drinking water and water contamination case studies,* Front. Earth Sci., 2011, 5 (4), 378.

[9] COOPER R., GRIFFITH C., MALIK R., OBEE P., LOOKER N., *Monitoring the effectiveness of cleaning in four British hospitals,* Am. J. Infect. Control, 2007, 35, 338.

[10] HAMMES F., GOLDSCHMIDT F., VITAL M., WANG Y., EGLI T., *Measurement and interpretation of microbial adenosine triphosphate (ATP) in aquatic environments*, Water Res., 2010, 44 (13), 3915.

[11] FARRIS L., HABTESELASSIE M.Y., PERRY L., CHEN S.Y., TURCO R., REUHS B., APPLEGATE B., *Luminescence techniques for the detection of bacterial pathogens*, [in:] M. Zourob, S. Elwary, A. Turner (Eds.), *Principles of Bacterial Detection. Biosensors, Recognition Receptors and Microsystems*, Springer, New York 2008, 213–230.

[12] LuminUltra Technologies, Ltd., *Test Kit Instructions. Test Kit Product: QuenchGone21^{TM} Wastewater Test Kit Product: QG21W-50/QG21Wb-25,* Canada.

[13] SHAMA G., MALIK D., *The uses and abuses of rapid bioluminescence-based ATP assays*, Int. J. Hyg. Environ. Health, 2013, 216, 115.

[14] EATON A., CLESCERI L., RICE E., GREENBERG A., *Standard methods for the examination of water and wastewater*, American Public Health Association, American Water Works Association, Water Environment Federation, Washington 2005.

[15] TAM N., *Effects of wastewater discharge on microbial populations and enzyme activities in mangrove soils*, Environ. Pollut., 1998, 102, 233.

[16] DALZELL D., CHRISTOFI N., *An ATP luminescence method for direct toxicity assessment of pollutants impacting on the activated sewage sludge process*, Water Res., 2002, 36, 6, 1493.

[17] DALZELL D., ALTE S., ASPICHUETA E., DE LA SOTA A., ETXEBARRIA J., GUTIERREZ M., HOFFMANN C.C., SALES D., OBST U., CHRISTOFI N., *A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge*, Chemosphere, 2002, 47, 535.

[18] PISTELOK F., WIERA B., STUCZYŃSKI T., KOPYTKO M., The study on the use of the ATP assay to optimize the operation of the water treatment plant and water supply, [In:] Water Environment Man, X Jubilee Scientific-Technical Conference, 29–30 March, 2012 (in Polish).
[19] *The Regulation of the Polish Ministry of the Environment of 24 July 2006 on the conditions that must be fulfilled when wastewater is introduced into waters or soils and on the substances acutely harmful for the aquatic environment*, Dz.U. 2006.137.984 (in Polish).
[20] Ministry of the Environment (Republic of Poland). National Programme for Municipal Wastewater Treatment. Report on implementation of the national program for municipal wastewater treatment in 2008–2009. Warsaw, September 2010 (in Polish).
[21] Ministry of the Environment (Republic of Poland, *National Programme for Municipal Wastewater Treatment. Report on implementation of the national program for municipal wastewater treatment in 2010–2011*, Warsaw, November 2012 (in Polish).
[22] PARK J., YOO Y., *Biological nitrate removal in industrial wastewater treatment: which electron donor we can choose*, Appl. Microbiol. Biotechnol., 2009, 82, 415.
[23] CHU L., WANG J., WANG B., XING X.-H., YAN S., SUN X., JURCIK B., *Changes in biomass activity and characteristics of activated sludge exposed to low ozone dose*, Chemosphere, 2009, 77, 269.