Environment Protection Engineering

Vol. 40 2014 No. 4

DOI: 10.5277/epe140405

KATARZYNA BUDZIŃSKA[1], BOŻENA SZEJNIUK[1], ANITA JUREK[1], ADAM TRACZYKOWSKI[1], MAGDALENA MICHALSKA[1], KRZYSZTOF BERLEĆ[1]

EFFECTIVENESS OF REMOVING MICROBIAL POLLUTANTS FROM WASTEWATER BY THE ACTIVATED SLUDGE METHOD

Wastewater contains numerous saprophytic microorganisms, pathogenic and potentially pathogenic. Detection of bacteria indicating the sanitary state of polluted waters shows the possibility of occurring of pathogenic microorganisms in that environment. In the presented paper, the effectiveness of removing microbiological pollutions from wastewater treated by the activated sludge method has been evaluated, by estimating the elimination of *E. coli*, faecal streptococci, rods of the genus *Salmonella* and bacteria of the family *Enterobacteriaceae*. Experimental research showed that often in spite of a high degree of elimination of bacteria at individual stages of treatment, they are still identified in wastewater discharged to the water environment. The fact of occurring bacteria of the family *Enterobacteriaceae* and rods of *Salmonella* in treated wastewater discharged to surface waters is particularly worrying.

1. INTRODUCTION

Municipal wastewater treatment plants can be classified as serious sources of emission of microbiological pollutions to the environment, including viruses, bacteria and protozoans pathogenic for people. They pose a potential threat to human health and contribute to a progressing degradation of waters making wastewater receiving bodies [1]. Microorganisms most frequently isolated from wastewater belong to the families *Enterobacteriaceae, Pseudomonadaceae, Lactobacillaceae* and *Micrococcaceae*. Moreover viruses, protozoans and numerous filamentous fungi are identified [2]. It is commonly known that raw municipal wastewater contains a great number of pathogenic and opportunistic microorganisms, as well as those antibiotic resistant

[1]Department of Animal Hygiene and Microbiology of the Environment, University of Technology and Life Sciences, Mazowiecka 28, 85-084 Bydgoszcz, Poland, corresponding author K. Budzińska, e-mail: katarzyna.budzinska@utp.edu.pl

including multidrug resistant, mainly of intestinal origin [3]. In processes of wastewater treatment, a considerable reduction in pathogenic microorganisms occurs; however successive stages of processing (dehydratation) may lead to an increase in their concentration [4]. The presence of pathogenic microorganisms depends mainly on two factors, that is on the occurrence of pathogens among people inhabiting the given area connected to the sewerage system and on the ability of pathogenic microorganisms to survive in wastewater and during processes of sewage sludge processing [5]. In 1 g of excrements of an ill person there are found, on average: 10^8 cells of *Escherichia coli*, 10^6 rods of *Salmonella*, 10^6 *Shigella*, 10^6–10^7 units of viruses, 10^5 oocysts of protozoans of the genus *Giardia* and many other pathogenic microorganisms. Kinde and Atwill [6] report that people with acute salmonellosis can excrete from 10^8 to 10^{11} bacterial cells of *Salmonella* in 1 g of excrements. According to Olańczuk-Neyman et al. [7], the following species of the genus *Salmonella* are most frequently identified in wastewater: *S. thompson, S. virchow*, *S. dublin* and *S. infantis*. Along with household wastewater, bacteria occurring permanently in the intestinal tract of people and animals such as: enteric rods of the family *Enterobacteriaceae*, faecal streptococci are introduced to water receiving bodies, there can also be bacteria causing many diseases of gastro-intestinal character. Therefore insufficiently treated wastewater may pose threat to people using surface waters, where run-offs from wastewater treatment plants are discharged, for recreational purposes. Detection of bacteria indicating the sanitary state in polluted waters suggests the possibility of the occurrence of pathogenic bacteria [8].

Disinfection of wastewater should be definitely regarded the most appropriate way of protection of water environment against microbiological pollution. In technological practice, two groups of methods for disinfection of treated wastewater are used: chemical methods, consisting in chlorination, ozonization, using peracetic acid or performic acid, and physical ones, involving the use of UV radiation or membrane methods. All those methods are characterized by a number of advantages and disadvantages, connected with particular conditions of realization, mainly with their costliness [9, 10].

Under specific conditions, even small or very small amounts of discharged wastewater can pose a threat to areas under particular protection. In spite of the fact that municipal wastewater contains large amounts of microorganisms including pathogenic agents, reducing their population has never been the priority of the wastewater treatment plant [3, 10]. According to George et al. [11], it is necessary to conduct research determining the effectiveness of eliminating pathogenic microorganisms from wastewater using indicator bacteria for this purpose.

The aim of this study was to evaluate the effectiveness of wastewater treatment by determining the elimination of *E. coli*, faecal streptococci, rods of the genus *Salmonella* and bacteria of the family *Enterobacteriaceae* from the studied environment.

2. MATERIALS AND METHODS

Collecting wastewater samples for the study. The subject of this study was household wastewater deriving from a mechanical and biological wastewater treatment plant in Kuyavian-Pomeranian voivodeship. The following samples were collected to the analysis: raw wastewater (A), wastewater after treatment on sieve-grit chamber (B), wastewater from the denitrification chamber (C), wastewater from the nitrification chamber (D) and treated wastewater discharged to the river (E). Wastewater samples were collected according to the instructions of collecting, handling and storage given in EN 25667-2 and PN-EN ISO 5667-3. Analyses of the collected wastewater were made 7 times in the autumn–winter period and the spring–summer period. The number of *Escherichia coli* bacteria, faecal streptococci, rods of the genus *Salmonella* and the number of bacteria of the family *Enterobacteriaceae* was determined in the wastewater (cells$\cdot$cm^{-3}).

Determination of the number of Escherichia coli. Quantitative determination of *Escherichia coli* in wastewater was carried out based on calculation of the most probable number (MPN) with a 3-tube set, using McCrady's tables. For initial analyses, the fermentation tube technique was applied, with the use of a liquid medium with lactose and bromocresol purple (Mac Conkey Bulion, Merck). At the first stage, decimal dilutions of wastewater from 10^{-1} to 10^{-8} were made in three replications. Then the samples were incubated at 37 °C for 24–48 h. A change in the liquid medium colour from violet into yellow as a result of decomposition of lactose and the presence of gas in the Durham tube was regarded as a positive result. Positive and doubtful samples were confirmed using the solid selective medium – Tergitol-7-agar with addition of 1% TTC (2,3,5-triphenyltetrazolium chloride). Incubation for 24 h at 37 °C. TTC and tergitol 7 cause the inhibition of growth in the most of Gram-positive bacteria. Bacteria fermenting lactoses produce acids which results in a decrease in pH of the medium. This is observed through change in coloration of the medium from green into yellow, thanks to the presence in the medium of the pH indicator – bromothymol blue. Apart from selective properties, TTC contained in the medium performs the role of a diversifying factor. Microorganisms having ability to reduce TTC to red formazan at the lack of ability to decompose lactose will grow in the form of colonies with dark red colour. In contrast, bacteria lactoso(+) reducing TTC will grow in the form of colonies with yellow and orange to brick-red. *Escherichia coli* and bacteria of the coli group grow in the form of yellow or yellow and orange colonies, sometimes with a rust-coloured centre, the medium around colonies under the filter changes colour into yellow [PN-77/C-04615].

Determination of the number of faecal streptococci. To determine the number of faecal streptococci in wastewater, azide dextrose broth (Merck) was applied at the first stage of the study. Azide and sulfate(IV) present in the medium inhibit the development of Gram-negative accompanying bacteria, and Gram-positive bacteria are slightly inhibited by low concentrations of crystal violet, whereas streptococci do not respond to the action of this compound at such concentration. Cultures made in this way were incubated at 37 °C for 24–48 h. Turbidity of the medium (PN-82/C04615.25) was regarded the positive result of the initial analysis. In the next stage, from each positive culture in tubes with the multiplying-selective medium scratch inoculations were made on kanamycin esculin azide agar. Incubations were performed at 37 °C for 24–48 h. Positive result of the analysis confirming the presence of faecal streptococci was determined based on milky-white, small colonies along with black colour of the medium. Based on positive results of the confirmed analysi,s the final result was determined in the form of the most probably number of cells bacteria per 1 cm^3 of wastewater (PN-77/C-04615).

Determination of the number of bacteria of the genus Salmonella. The method for detecting *Salmonella* rods in all materials analysed involved determination of the presence of those bacteria with the use of culture on multiplying and differentiating-selective media, according to the standard PN-Z-19000-1. At the first stage of the study, 1% buffered peptonic water was used, and then selective multiplication on a liquid differentiating-multiplying medium according to Rappaport with an addition of tetrationate and malachite green. Following this, cultures were transferred on the BPLA agar medium with brilliant green, phenol red and lactose and on the XLD medium with xylose, lysine and sodium deoxycholate. Typical colonies of *Salmonella* on the BPLA medium grew in the form of pale pink colonies, around which characteristic pink coloration of agar occurred. On the XLD agar, typical bacterial colonies grew in the form of small colonies with the black centre surrounded with a light red zone. The final identification involved the use of serological tests – the polyvalent serum HM and the microtest API 20 E.

Determination of the total number of bacteria of the family Enterobacteriaceae. The number of bacteria of the family *Enterobacteriaceae* was determined on Mac Conkey agar by the plate method using the deep inoculation technique. Determination included distributing 0.1 cm^3 of an appropriate dilution on Petri plates with a solid medium. The prepared cultures were incubated at 37 °C for 24 h. After the incubation staining with Gram's method and the cytochrome oxidase test were performed. The total number of bacteria of the family *Enterobacteriaceae* was calculated from:

$$L = \frac{10C}{\left(N_1 + 0.1N_2\right)d}$$

where: L is the total number of *Enterobacteriaceae* cells per 1 cm^3 of wastewater, C – the sum of all colonies grown on plates taken for calculation, N_1 – the number of plates from the first calculated dilution, N_2 – the number of plates from the next calculated dilution, d – the dilution index corresponding to the first calculating dilution.

3. DESCRIPTION OF THE TREATMENT PLANT

Both municipal and industrial wastewater is delivered to the wastewater treatment plant. Wastewater is collected by means of a system of gravitational sewerage and delivered to the treatment plant through pumping stations PI, PII, PIII. The amount of wastewater delivered to the wastewater treatment plant is given in Table 1.

Table 1

Amount of wastewater flowing to the treatment plant

Type of wastewater	Amount of sewage [$m^3 \cdot d^{-1}$]	
	Outside sugar beet harvest	During sugar beet harvest
Municipal wastewater	998	998
Fat processing plant	523	523
Sugar factory	40	310
Wastewater disposal station	10	10
Total	1571	1841

At the beginning of the technological system of wastewater treatment, devices for the initial mechanical treatment of wastewater are used, with a spiral sieve with a press, an aerated oblong grit chamber and grease removal tank, cooperating with screw conveyors for transporting screenings and sand. Intake hoppers of screw conveyors are ended with chutes directed immediately to containers of the volume of 2.2 m^3 each. Raw wastewater flows to the treatment plant with two pressing pipelines DN 350 mm. Before the entry of pipelines to sieve-grit chambers, there is a chamber of valves with a distributor and four knife gate valves installed. The sieve-grit chamber VSR 400 Kombi as a combined device for mechanical wastewater treatment consists of a spiral sieve for separation of solid pollutions together with a conveyor taking out and dehydrating screenings, an aeration oblong grit chamber for separating sand and removing fats from wastewater. The overflow chamber collects wastewater from the sieve-grit chamber, then it flows gravitationally to the dephosphatation chamber, where release of phosphorus takes place under anaerobic conditions. Apart from raw wastewater treated mechanically, sludge recirculated from the secondary sedimentation tank flows there (outside recirculation). Another facility is the denitrification chamber. Along the outside wall of the chamber, three mixers are installed, spaced about 10.5 m apart. Wastewater is discharged by two pipes to the nitrification chamber. It is made up of two combined containers. Two air collectors 200–250 mm in the nominal diameter are installed along the wall dividing the chamber into two containers. Four distributive pipelines go out of each, distributing air to the net of diffusers placed at the bottom of the container. Secondary sedimentation tanks are the terminal devices of wastewater treatment. One of the tanks works, while the other makes the reserve in the case of failure. Pollution concentrations in raw wastewater are presented in Table 2.

Table 2

Characteristics of the raw wastewater

Index	Outside sugar beet harvest	During sugar beet harvest
BOD_5 [mg O_2·dm^{-3}]	609.80	952
COD [mg O_2·dm^{-3}]	1275	2172
Total suspension [mg·dm^{-3}]	412	816
Total nitrogen [mg·dm^{-3}]	88.70	90.10
Total phosphorus [mg·dm^{-3}]	24.30	25.79

Pollution concentrations in wastewater after treatment by the activated sludge method at the assumed degree of reduction (98.5% BOD_5, 97% COD, 95% of total suspension, 86% of total nitrogen, 96% of total phosphorus) is shown in Table 3.

Table 3

Characteristics of the treated wastewater

Index	Outside sugar beet harvest	During sugar beet harvest
BOD_5 [mg O_2[mg·dm^{-3}]	9.15	9.52
COD [mg O_2·dm^{-3}]	43.44	43.44
Total suspension [mg·dm^{-3}]	20.6	20
Total nitrogen [mg·dm^{-3}]	12.42	12.61
Total phosphorus [mg·dm^{-3}]	0.97	1.03

4. RESULTS AND DISCUSSION

The performed analyses showed the occurrence of bacteria *E. coli* in raw wastewater in the autumn–winter period within the range from 4.5×10^4 to 9.5×10^5 cells·cm^{-3} and from 4.5×10^5 to 4.0×10^6 cells per 1 cm^3 of wastewater in spring –summer months (Table 4). The mean number of those bacteria for both research periods amounted to 5.42 and 6.08 logcells·cm^{-3}, respectively (Fig. 1). According to Tyagi et al. [12], bacteria *Escherichia coli* belong to the most sensitive indicators of microbiological pollution of water and wastewater. In untreated wastewater, the concentration of *E. coli* most often remains on the level from 10^6 to 10^7 cells·cm^{-3} [4, 7, 13]. Tsai et al. [14], in turn, detected from 8.6×10^3 to 6.0×10^3 cells·cm^{-3} in raw wastewater.

The study conducted by Szumialas et al. [15] indicated an exceptionally high level of bacteriological pollution of raw wastewater, ranging, on average, from 9.3×10^{18} to 1.8×10^{20} of faecal coliform bacteria per 100 cm^3 of wastewater. The process of mechanical wastewater treatment resulted in a slight decrease in the number of *E. coli*. The mean number of those microorganisms for the whole research period in mechanically treated wastewater decreased to a level of 3.5×10^5 cells·cm^{-3}, i.e. by 0.58 logarithmic unit (Table 4, Fig. 1).

Table 4

Number of *E. coli* [cells·cm^{-3}] in wastewater samples from individual stages of treatment

Period	No. of analysis	Wastewater				
		Raw	After mechanical treatment	From the denitrification chamber	From the nitrification chamber	Treated
Autumn –winter	1	4.5×10^4	2.5×10^4	1.5×10^4	9.5×10^3	4.5×10^3
	2	2.5×10^5	2.5×10^3	9.5×102	1.5×10^1	0.9×10^1
	3	4.5×10^5	9.5×10^4	4.5×10^4	2.5×10^4	9.5×10^2
	4	9.5×10^5	9.5×10^5	2.5×10^4	1.5×10^3	4.5×10^2
Mean		4.2×10^5	2.9×10^5	2.9×10^4	9.0×10^3	2.5×10^3
Spring –summer	5	4.5×10^5	9.5×10^4	2.5×10^3	9.5×10^3	2.5×10^3
	6	4.0×10^6	4.5×10^5	9.5×10^3	2.5×10^3	7.5×10^2
	7	9.5×10^5	7.5×10^5	9.5×10^3	4.5×10^3	9.5×10^2
Mean		1.8×10^6	4.3×10^5	7.2×10^3	5.5×10^3	1.4×10^3

In the study conducted after mechanical treatment, Kawamura and Kaneko [2] prove a decrease in the number of *E. coli* by 60–70%. Similarly, the percentage of reduction after this stage of the process of removing pollutions from wastewater accounting for 63.59% was observed by Budzińska et al. [4]. Stages of biological wastewater treatment resulted in a decrease in the number of indicator bacteria. In wastewater collected from the denitrification chamber, the mean number of *E. coli* amounted to 2.9×10^4 cells·cm^{-3} (the autumn –winter period) and 7.2×10^3 cells·cm^{-3} (the spring–summer period). In wastewater collected from the nitrification chamber, the number of those bacteria was 9.0×10^3 cells·cm^{-3} and 5.5×10^3 cells·cm^{-3}, respectively. The average number of those bacteria in treated wastewater discharged from the treatment plant amounted to 2.0×10^3 cells·cm^{-3}, whereas the largest number was determined in the first replication and it was 4.5×10^3 cells·cm^{-3}. Consecutive stages of wastewater biological treatment resulted in a decrease of the number of bacteria from 5.12 to 3.68 logarithmic units. In treated wastewater discharged to the water body, the average number of *E. coli* for the whole research period amounted to 2.78 log cells·cm^{-3} (Table 4, Fig. 1). Walczak and Donderski [3] reported the presence of 0.93×10^3 cells·cm^{-3} faecal coliform bacteria in treated wastewater. The authors recorded 97.28% eliminations of those bacteria.

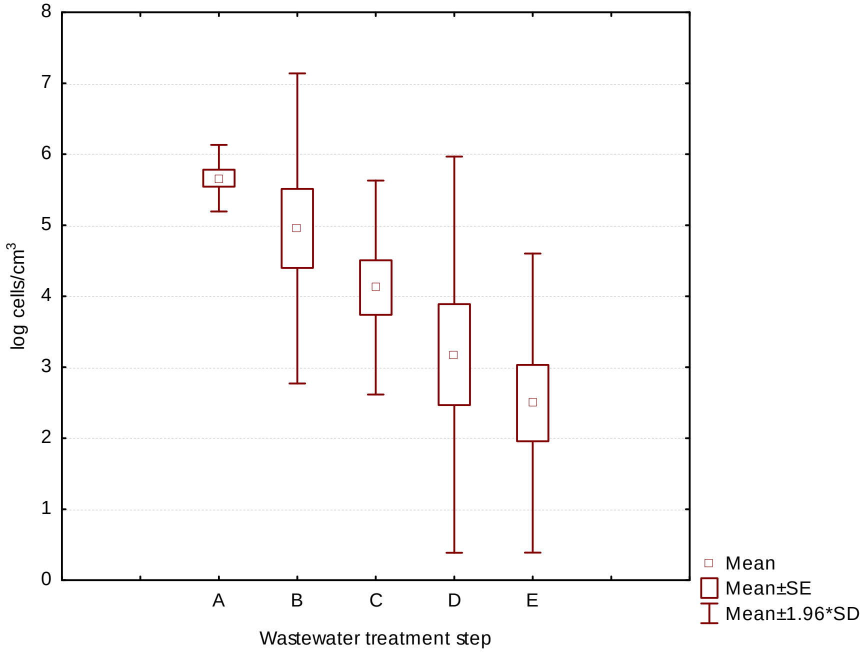

Fig. 1. Number of bacteria *E. coli* in wastewater on various stages of treatment: A – raw wastewater, B – after mechanical treatment, C – wastewater from the denitrification chamber, D – wastewater from the nitrification chamber, E – treated wastewater

Analysing the effectiveness of biological sewage treatment, Bonde [16] observed that the number of *E. coli* decreased by 95%, whereas Szumilas et al. [15] report that modern sewage treatment plants are able to reduce more than 99.99% of coliform bacteria by means of biological treatment. In the present study, elimination of *E. coli* in treated wastewater within the range from 99.40 to 99.92% was recorded (Table 8). Most strains of *E. coli* do not induce diseases in people, but some of them have virulence factors which can cause threat to life. Enterohaemorrhagic strains of *E. coli* (EHEC) have an impact on a growing incidence of haemorrhagic colitis (diarrhoea) and haemolytic uraemic syndrome (HUS) [13]. The study conducted by Martin et al. [17] proves that municipal sewage including household sewage, constitutes the reservoir of EHEC in the environment. This wastewater contain a high concentration of *E. coli* even after conventional treatment.

Faecal streptococci are the subject of a particular interest of hygienists and microbiologists in the aspect of indicators of sewage pollution. Sanitary assessment conducted for detecting the presence of faecal streptococci is regarded by many microbiologists a more appropriate procedure than detecting *E. coli* [9]. In raw wastewater, the average number of faecal streptococci oscillated within the range from 1.1×10^4 cells·cm^{-3} (the spring–summer period) to 2.1×10^4 cells·cm^{-3} (the autumn

–winter period), whereas the mean number of those bacteria for 7 replications was 1.7×10^4 cells·cm^{-3} (Table 5). The mean number of faecal streptococci in the whole period of the study remained on the level of 4 logarithmic units and was by 1.7log MPN·cm^{-3} lower than the concentration of *E. coli* in raw wastewater (Fig. 2). A high number of those bacteria was observed in the nitrification chamber, it was on average 3.1×10^2 cells·cm^{-3}(autumn–winter) and 3.2×10^2 cells·cm^{-3} (spring–summer). As a result of successive stages of wastewater treatment, the number of streptococci decreased. In treated wastewater, their average number amounted to 10 cells·cm^{-3} (Table 5).

Table 5

Number of faecal streptococci (cells·cm^{-3}) in wastewater samples from individual stages of treatment

Period	No. of analysis	Wastewater				
		Raw	After mechanical treatment	From the denitrification chamber	From the nitrification chamber	Treated
Autumn –winter	1	4.5×10^4	2.0×10^2	2.5×10^2	4.5×10^2	9.5×10^1
	2	1.5×10^4	2.5×10^2	0.9×10^1	9.5×10^1	n.o.
	3	2.0×10^4	2.5×10^3	9.5×10^1	4.5×10^2	2.5×10^1
	4	2.0×10^3	4.5×10^3	1.5×10^2	2.5×10^2	9.5×10^1
Mean		2.1×10^4	7.6×10^3	1.3×10^2	3.1×10^2	5.4×101
Spring –summer	5	2.5×10^4	2.0×10^4	4.5×10^2	7.5×10^2	9.5×10^1
	6	2.2×10^3	3.4×10^2	1.8×10^1	1.5×10^2	n.o.
	7	6.3×10^3	5.7×10^2	2.7×10^1	6.4×10^1	n.o.
Mean		1.1×10^4	7.0×10^3	1.7×10^2	3.2×10^2	3.2×10^1

n.o. – not observed.

The study by Szumilas et al. [15] indicates that the number of faecal streptococci in raw wastewater ranged from 5.6×10^5 to 1.8×10^6 bacteria in 100 cm^3 of wastewater. Walczak and Donderski [3] reported on average 7.3×10^2 cells·cm^{-3} of faecal streptococci in raw wastewater. Lalke-Porczyk et al. [18] conducted a study that aimed at evaluating the effectiveness of household wastewater treatment in a mechanical and biological treatment plant with a sand-cane filter. It was found that among the indicator bacteria of the sanitary state, the number of faecal streptococci decreased to the smallest degree. Their number in wastewater inflowing to the willow filter ranged from $1.12\cdot10^3$ to $1.54\cdot10^4$ cell in 100 cm^3, and in wastewater inflowing to the cane deposit – from $3.82\cdot10^3$ to $1.74\cdot10^4$ cells in 100 cm^3. Biological stage of wastewater treatment resulted in the elimination of those bacteria on average to the level of 1.83 (denitrification chamber) and 2.36 log MPN·cm^{-3} (nitrification chamber). In treated wastewater, streptococci were detected in 57% of collected samples. The mean number of those bacteria in wastewater discharged to the Noteć River was 1.05 log MPN·cm^{-3} (Table 5, Fig. 2).

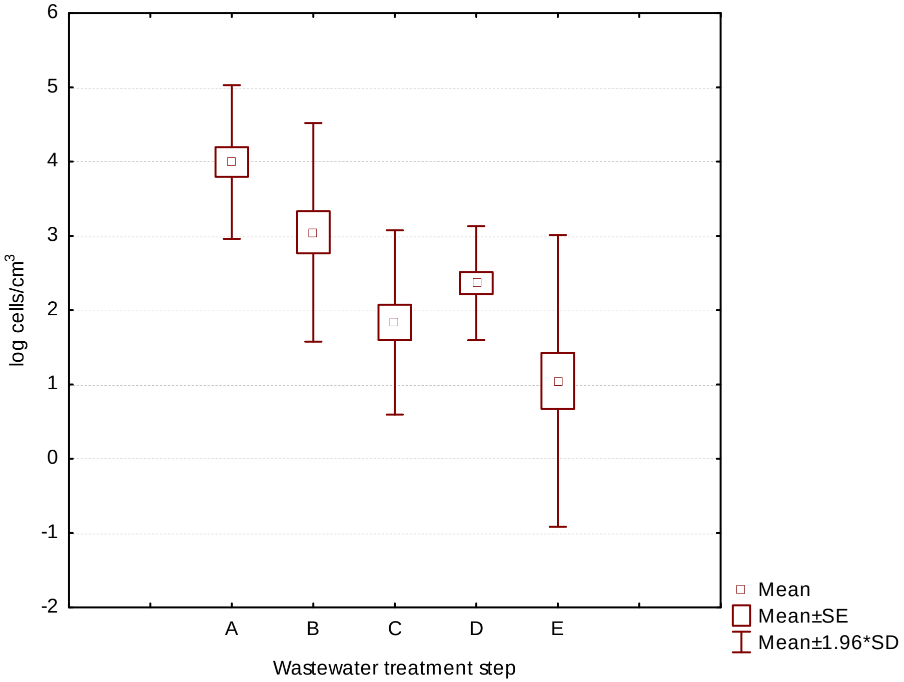

Fig. 2. Number of streptococci in sewage on individual stages of treatment: A – raw wastewater, B – wastewater after mechanical treatment, C – wastewater from the denitrification chamber, D – wastewater from the nitrification chamber, E – treated wastewater

In the present study, the elimination of faecal streptococci after the final stages of wastewater treatment amounted, on average, to 99.73% (3.19log cells·cm^{-3}) (Table 8, Fig. 2). In the willow treatment plant, the number of bacteria of this group decreased on average by 84.65%, and in the cane treatment plant by 87.26% [18]. According to Walczak and Donderski [3], subjecting sewage to mechanical and biological treatment with activated sludge eliminates faecal streptococci on average by 86%.

Bacteria of the genus *Salmonella* were determined in the case of 71% samples of raw wastewater. The number of the bacteria in untreated wastewater on average for the autumn–winter period was 2.8×10^2 cells·cm^{-3} and 1.2×10^1 cells·cm^{-3} in spring–summer. Subjecting wastewater to mechanical treatment resulted in a slight decrease in the number of *Salmonella* rods by 0.32log MPN·cm^{-3}. Next processes of removing pollutions in chambers with activated sludge contributed to elimination of those bacteria. It should be emphasized, however, that in treated wastewater the presence of rods of the genus *Salmonella* was determined in 43% of collected samples. The average number for wastewater discharged from the treatment plant was 1.9×10^0 cells·cm^{-3} (Table 6, Fig. 3).

Table 6

Number of *Salmonella* [cells·cm^{-3}] in wastewater samples from individual stages of treatment

Period	No. of analysis	Wastewater				
		Raw	After mechanical treatment	From the denitrification chamber	From the nitrification chamber	Treated
Autumn –winter	1	9.5×10^2	2.0×10^1	n.o.	n.o.	n.o.
	2	0.4×10^1	0.4×10^1	0.3×10^1	0.4×10^1	n.o.
	3	4.5×10^1	3.5×10^1	0.6×10^1	n.o.	0.3×10^1
	4	1.5×10^1	0.6×10^1	2.0×10^1	2.0×10^1	0.3×10^1
Mean		2.8×10^2	6.1×10^1	2.0×10^1	2.0×10^1	2.0×10^1
Spring –summer	5	3.5×10^1	3.0×10^1	1.1×10^1	0.7×10^1	0.7×10^1
	6	n.o.	n.o.	n.o.	n.o.	n.o.
	7	n.o.	n.o.	n.o.	n.o.	n. o
Mean		1.2×10^1	1.0×10^1	3.7×10^0	2.3×10^0	2.3×10^0

n.o. – not observed.

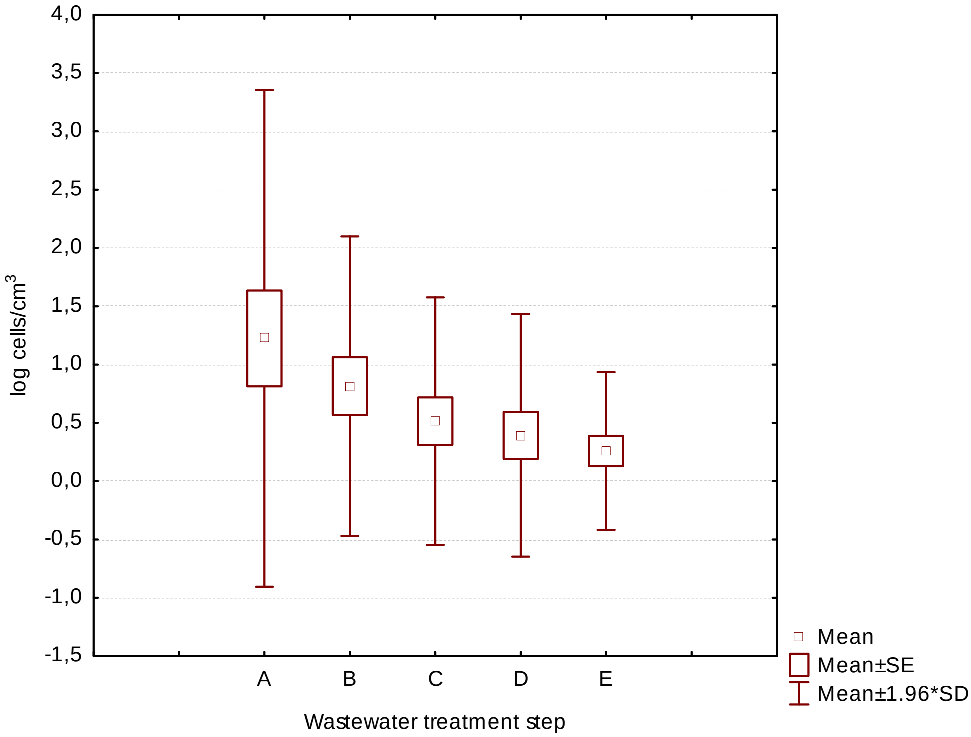

Fig. 3. Number of *Salmonella* rods in wastewater on individual stages of treatment:
A – raw wastewater, B – wastewater after mechanical treatment, C – wastewater from the denitrification chamber, D – wastewater from the nitrification chamber, E – treated wastewater

The elimination of *Salmonella* amounted to 99.77% on average (Table 8). Song et al. [19] observed a very low efficiency of removing those bacteria amounting to 1.81 logarithmic units. An important aspect in spreading rods of *Salmonella* is their resistance to unfavourable factors of the natural environment and ability to survive in various ecological niches for a longer period of time. It follows from the experiments, that *Salmonella* can survive in municipal wastewater for a relatively long time before they are completely eliminated and this is the period which constitutes an important part of the infection process not only in relation to *Salmonella* but alto to other enterobacteria [20]. Some pathogenic bacteria, including *Salmonella,* have such a low infectious dose that the oral administration of even small number of those bacteria may result in infection of the organism [5].

Table 7

Total number for bacteria of the family *Enterobacteriaceae* [cells·cm^{-3}] in wastewater

Period	No. of analysis	Wastewater				
		Raw	After mechanical treatment	From the denitrification chamber	From the nitrification chamber	Treated
Autumn –winter	1	4.4×10^5	8.5×10^4	1.2×10^5	1.4×10^4	5.0×10^2
	2	7.0×10^5	2.8×10^4	3.9×10^3	9.9×10^3	1.8×10^1
	3	3.3×10^5	6.1×10^4	1.5×10^3	2.3×10^4	4.2×10^2
	4	1.0×10^6	2.8×10^5	1.6×10^4	2.5×10^5	1.6×10^3
Mean		6.2×10^5	1.1×10^5	3.5×10^4	7.1×10^4	6.4×10^2
Spring –summer	5	1.9×10^6	1.3×10^5	1.3×10^4	6.8×10^3	1.5×10^3
	6	5.9×10^5	1.7×10^5	1.9×10^4	1.8×10^4	1.7×10^3
	7	6.3×10^4	2.7×10^3	2.0×10^3	2.0×10^3	1.2×10^2
Mean		8.5×10^5	1.0×10^5	1.1×10^4	8.9×10^3	1.1×10^3

Another group of microorganisms determined in the studied wastewater was bacteria of the family *Enterobacteriaceae*. In raw wastewater, the total number of those bacteria ranged from 3.3×10^5 to 1.0×10^6 cells·cm^{-3} in the autumn–winter. In the spring–summer number of bacteria of the family *Enterobacteriaceae* ranged from 6.3×10^4 to 1.9×10^6 cells·cm^{-3}. In both periods, the number of the bacteria after mechanical treatment slightly decreased to 10^5 cells·cm^{-3}. Treated wastewater still contained considerable amounts of bacteria of the family *Enterobacteriaceae,* in the sixth replication even 1.3×10^3 cells·cm^{-3}. The mean number for this type of bacteria in treated wastewater amounted to 6.4×10^2 cells·cm^{-3} (autumn–winter) and 1.1×10^3 cells (spring–summer) (Table 7). The study by Walczak and Donderski [3] indicated that the number of enterobacteria remained on a similar level (1.2×10^5 bacteria in 1 cm^3 of wastewater). In the study by Koivunen et al. [10] numerous species of bacteria from the family of *Enterobacteriaceae* was observed in raw wastewater.

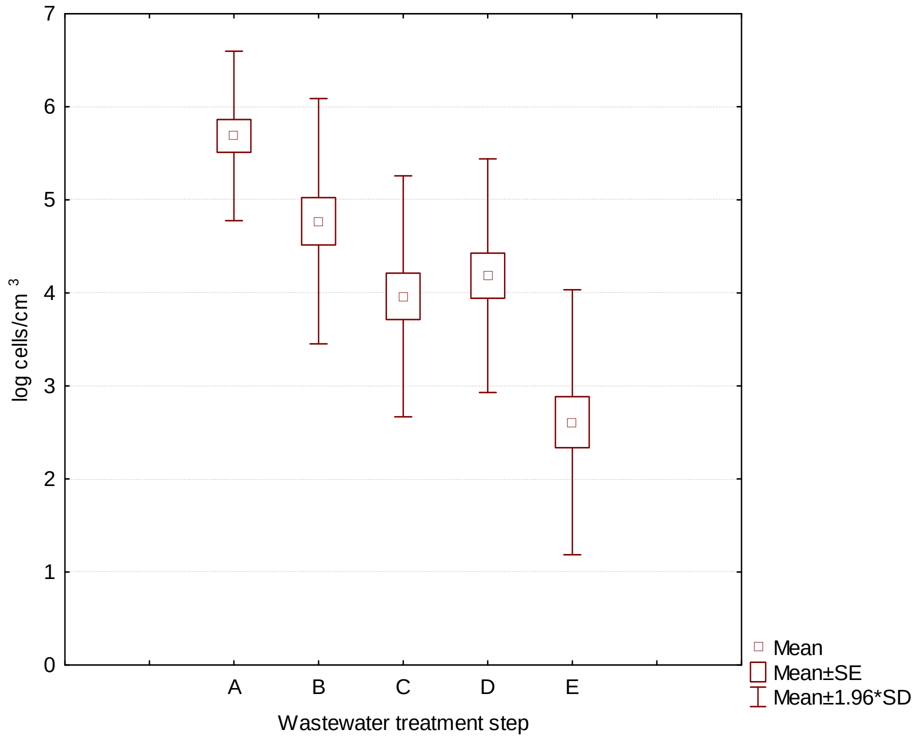

Fig. 4. Changes in number of bacteria of the family *Enterobacteriaceae* in wastewater: A – raw wastewater, B – wastewater after mechanical treatment, C – wastewater from the denitrification chamber, D – wastewater from the nitrification chamber, E – treated wastewater

Table 8

Level of elimination of indicator bacteria in wastewater after various processes [%]

Indicator bacteria	Mechanical treatment		Biological treatment		Secondary sedimentation	
	Autumn –winter	Spring –summer	Autumn –winter	Spring –summer	Autumn –winter	Spring –summer
Enterobacteriaceae family bacteria	82.26	88.23	88.35	98.95	99.85	99.87
Escherichia coli	30.95	76.11	97.86	99.69	99.40	99.92
Salmonella spp.	78.21	91.67	97.86	99.33	99.46	99.99
Faecal streptococci	63.81	36.36	98.52	97.09	99.74	99.71

In raw wastewater, the number of bacteria of the family *Enterobacteriaceae* remained at the level of 5.69 logarithmic units (Fig. 4). After the stage of mechanical treatment, a decrease in the number of enterobacteria by 0.92 log cells·cm^{-3} was observed. Subjecting wastewater to treatment in chambers with activated sludge resulted in the elimination of the microorganisms by 3.08 log cells·cm^{-3} in comparison with the number of those bacteria in raw wastewater (Fig. 4). The most effective elimination of indicator microorganisms was achieved in the case of *Salmonella* spp. The elimination rate ranged from 99.46% (in autumn–winter) to 99.99% (in spring–summer) (Table 8).

Walczak and Donderski [3] found that in the process of mechanical and biological wastewater treatment it is possible to achieve 96.18% elimination of bacteria of the family *Enterobacteriaceae*. Similarly high percentage of reduction is reported by other authors [10, 11]. It is necessary to increase the effectiveness of removing microbiological pollutions from wastewater, whereas introducing sewage disinfection would be the last barrier preventing from the spread of pathogenic microorganisms by water [21].

5. CONCLUSIONS

Microbiological analysis indicated a considerable bacteriological pollution of raw and treated wastewater with *E. coli,* faecal streptococci and bacteria of the family *Enterobacteriaceae*. The results of the study concerning the occurrence of indicator bacteria in treated wastewater showed that in spite of the high rate of their elimination, the number of microorganisms left in the wastewater is still large. The fact of occurring bacteria of the family *Enterobacteriaceae* and rods of the genus *Salmonella* in treated wastewater discharged to surface waters is particularly alarming.

REFERENCES

[1] PALESE A.M., PASQUALE V., CELANO G., FIGLIUOLO G., MASI S., XILOYANNIS C., *Irrigation of olive groves in Southern Italy with treated municipal wastewater: effects on microbiological quality of soil and fruits*, Agr. Ecosyst. Environ., 2009, 129, 43.

[2] KAWAMURA K., KANEKO M., *Microbial quality of human wastes and treatment plant effluent*, Water Sci. Techn., 1986, 18, 257.

[3] WALCZAK M., DONDERSKI W., *Elimination of indicators (TC, FC, FS) and Enterobacteriaceae family bacteria during the sewage treatment process*, Pol. J. Natur. Sci., 2007, 22 (2), 294.

[4] BUDZIŃSKA K., JUREK A., SZEJNIUK B., WROŃSKI G., *Efficiency of bacteriological pollution removal in sewage treatment using biological ponds*, Rocz. Ochr. Środ., 2011, 13, 1519 (in Polish).

[5] LANGELAND G., *Salmonella spp. in the working environment of sewage treatment plants in Oslo, Norway*, Appl. Environ. Microbiol., 1982, 43, 1111.

[6] KINDE H., ATWILL E. R., *Salmonella in sewage effluent raises ecological and food-safety concerns*, California Agriculture, 2000, 54 (5), 62.

[7] OLAŃCZUK-NEYMAN K., GENEJA M., QUANT B., DEMBIŃSKA M. , KRUCZALAK K., KULBAT E., KULIK-KUZIEMSKA I., MIKOŁAJSKI S., GIELERT M., *Microbiological and biological aspects of the wastewater treatment plant "Wschód" in Gdańsk*, PJOES, 2003, 12 (6), 747.

[8] BAUDISOWA D., *Evaluation of Escherichia coli as the main indicator of faecal pollution*, Wat. Sci. Tech., 1997, 35 (11–12), 333.

[9] KRZEMIŃSKA D., NECZAJ E., PARKITNA K., *Application of Fenton reaction for supporting biological wastewater treatment from the dairy industry*, Rocz. Ochr. Środ., 2013, 15, 2381 (in Polish).
[10] KOIVUNEN J., SIITONEN A., AEINONEN-TANSKI H., *Elimination of enteric bacteria in biological--chemical wastewater treatment and tertiary filtration units*, Water Res., 2003, 37, 690.
[11] GEORGE I., CROP P., SERVAIS P., *Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods*, Water Res., 2002, 36, 2607.
[12] TYAGI V.K., CHOPRA A.K., KAZMI A.A., KUMAR A., *Alternative microbial indicators of faecal pollution: Current perspective*, Iran. J. Environ. Health. Sci. Eng., 2006, 3 (3), 205.
[13] GRANT S.B., PENDROY C.P., MAYER C.L., BELLIN J.K., PALMER C.J., *Prevalence of Enterohemorrhagic Escherichia coli in raw and treated municipal sewage*, Appl. Environ. Microbiol., 1996, 62, 3466.
[14] TSAI Y., PALMER C.J., SANGERMANO L.R., *Detection of Escherichia coli in sewage and sludge by polymerase chain reaction*, Appl. Environ. Microbiol., 1993, 59 (2), 353.
[15] SZUMILAS T., MICHALSKA M., BARTOSZEWICZ M., *Characteristics of bacterial contamination of municipal wastewater from a large urban agglomeration and evaluation of the reduction of pollution in wastewater treatment*, Roczn. PZH, 2001, 52 (2), 155 (in Polish).
[16] BONDE G.J., *Pollution of a marine environment*, Water Poll. Contr. Fed. Washington, 1990, 2, 45.
[17] MARTIN M.T., RIVERA I.G., CLARK D.L., OLSON B.H., *Detection of virulence factors in culturable Escherichia coli isolates from water samples by DNA probes and recovery of toxin-bearing strains in minimal o-nitrophenolb-D-galactopyranoside-4-methylumbelliferyl-b-D-glucoronide media*, Appl. Environ. Microbiol., 1992, 58, 3095.
[18] LALKE-PORCZYK E., SWIONTEK BRZEZINSKA M., DONDERSKI W., *The role of hydrophyte systems in sewage treatment from rural areas*, Woda – Środowisko – Obszary Wiejskie, 2010, 10 (3), 119 (in Polish).
[19] SONG Z., YU M., YANG G., SUN Q., *Removal of microbiological contaminants in a sewage treatment system with constructed wetlands as tertiary treatment*, 3rd International Congress on Bioinformatics and Biomedical Engineering, DOI: 10.1109/ICBBE.2009.5163309.
[20] VENGLOWSKY J., PLACHA I., VARGOVA M., SASAKOVA N., *Viability of Salmonella typhimurium in the solid fraction of slurry from agricultural wastewater treatment plant stored at two different temperatures*, H. Saloniemi (Ed.), Proc. 9th International Congress in Animal Hygiene, Helsinki, 17–22 August, 1997, 2, 805.
[21] DAIMS H., TAYLOR M.W., WAGNER M., *Wastewater treatment: a model system for microbial ecology*, Trends Biotechnol., 2006, 24 (11), 483.