Polish Journal of Environmental Studies Vol. 10, No. 6 (2001), 457-461

Evaluation of Indicator Bacteria Removal in Wastewater Treatment Processes

K. Olańczuk-Neyman, H. Stosik-Fleszar, S. Mikołajski*

Hydro and Environmental Engineering Faculty, Technical University of Gdansk,
Narutowicza 11/12, 80-952 Gdansk, Poland
* Saur-Neptun Gdansk S.A. Poland

Received: 2 May, 2001
Accepted: 27 July, 2001

Abstract

The results of elimination of total coliforms, *E.coli* and enterococci from wastewater during mechanical-chemical treatment as well as biological treatment operated in the MUCT system in a full-scale wastewater treatment plant are presented. It was proved that the change of treatment technology resulted in improving bacteria removal efficiency - reduction of the number of total coliforms increased from 0.9 $\log_{10}$ to 2.5 $\log_{10}$ and *E. coli* from 1.0 $\log_{10}$ to 2.3 $\log_{10}$. The UV disinfection of effluent from the MUCT system allowed for further reduction of the number of bacteria to 3.4 logio when the dose of UV radiation equal to 40 mWs/cm^2 was applied and to 3.8 logio at the dose of 52 mWs/cm^2. The geometric mean number of bacteria in the effluent after UV disinfection with the dose 40 mWs/cm^2 was below 50/100 ml of total coliforms, 15/100 ml of *E.coli* and approximately 30/100 ml of enterococci.

Keywords: sewage treatment, microbial indicators, total coliforms, *E.coli,* enterococci, disinfection, UV

Introduction

In Poland, due to lack of adequate requlation, microbial quality of treated sewage is not examined. At the same time contaminated effluents from wastewater treatment plants contribute to the bacteriological pollution of marine coastal waters, resulting in limited possibilities of their use for recreation.

The effluent from the full scale wastewater treatment plant "Wschod" in Gdansk, amounting to 100,000 m^3/d on average, is discharged to the Vistula River inflowing to the Bay of Gdansk. The former mechanical-chemical treatment (M-Ch) involving mechanical screening and primary sedimentation, including precipitation with PIX coagulant in the presence of flocculant (acryloamid polymer) was modernized in 1998. The last chemical step (chemical treatment) was omitted and biological treatment system (MUCT) using activated sludge technology with biological denitrification and defosfatation followed by secondary sedimentation was introduced.

However, in this new treatment system raw, highly microbiologically polluted primary sewage sludge is recirculated to the process line as a substrate for production of volatile fatty acids, (that are indispensible in the process of biological defosfatation of wastewater). Liquefaction of the sludge during acid fermentation creates appropriate conditions for releasing the microorganisms formerly captured in the primary sludge of the treated wastewater. Also strongly microbiologically polluted effluent originating in the process of centrifuging of the sewage sludge is recirculated to the process line and mixed with the raw influent.

Evaluation of indicator bacteria removal efficiency of the plant before (M-Ch treatment) and after modernization (MUCT system) and pilot-scale investigation of UV disinfection of treated wastewater were performed.

Correspondence to: Prof. K. Olanczuk-Neyman, e-mail: kola@pg.gda.pl

Materials and Methods

Before modernization of the plant, in the time period from May to June 1998, grab samples of the inflowing raw wastewater and of the treated wastewater outflowing from the system after 3 hours (corresponding to the retention time) were collected. Altogether 40 samples were collected.

Collection and analytical procedures of physico--chemical analysis were conducted according to Polish Methods. The following physico-chemical parameters were examined: total suspended solids (TSS) (PN-72 C-04559/02), chemical oxygen demand (COD) (dichromate method) (PN-74/C-04578/03), biochemical oxygen demand (BOD_5) (PN-84/C-04578/04), N_{tot} (PN-73/C--04576/12), $N\text{-}NH_4^+$ (PN-73/C-04576/04), $N\text{-}NO_2^-$ (PN-73/C-04576/06), $N\text{-}NO_3^-$ (PN-82/C-04576/08), P_{tot} (PN-91/C-04537/09), together with bacteriological indicators: total coliforms and *E. coli.*

After modernization of the plant (MUCT system), in the period from July 1999 till April 2000, the samples of raw wastewater (mixed with the effluent from centrifuges) and of the effluent outflowing after 26 hours corresponding to retention time were collected. Altogether 26 samples were examined. Apart from the parameters measured before, this time turbidity (PN-79/C-04583/03), transmittance at 254 nm and enterococci were examined as well. Transmittance was measured with potable transmittance measuring device UV-TUV 5 Wedeco.

Coliforms and *E. coli* were enumerated using selective Fluorocult LMX Broth modified ace. to Manafi and Ossmer (Merck) [1]. The high nutritional quality of the broth enables high growth rate of coliforms and the presence of lauryl-sulphate to a large extent inhibits the growth of gram-positive bacteria. Two specific substrata are chromogenic X-GAL which is cleaved by coliforms to blue-green product, and the highly specific for *E. coli* (producing β-glucoronidase)- fluorogenic MUG are present. Bacteria were cultured from 24 to 48 hours at 37°C [2]. In case of a positive reaction for coliforms the broth turned a blue-green color (X-GAL reaction). *E. coli* was distinguished by the presence of β -D-glucoronidase (more specific than lactose fermentation) [3], which decomposes fluorogenic substrate - MUG. In the case of presence of *E. coli* the light blue fluorescence of the methylumbelliferyl in the broth (MUG-reaction) was visible under UV light (366 nm). The MUG positive tubes were confirmed for the presence of *E. coli* by indol production into tryptone water (Merck) after incubation at 44.5° for 24 h.

Table 1. Main characteristics of mechanical-chemical (M-Ch) and MUCT effluent.

Parameter	M-Ch	MUCT	M-Ch	MUCT	M-Ch	MUCT	MPC *
	Mean value		Minimum value		Maximum value		
Transmittance at 254 nm (%)	–	49.3	–	35.0	–	76	
Turbidity ($mgSiO_2/l$)	–	19.8	–	12.7	–	31.7	
TSS (mg/l)	47.0	12.5	28.0	8.0	78.0	19.0	50.0
COD (mgO_2/l)	279.5	38.0	222.0	32.1	396	45.9	150.0
BOD_5(mgO_2/l)	144.5	4.5	117.0	2.6	170.0	7.1	30.0
N_{tot} (mgN/l)	52.3	11.54	38.6	8.5	91.8	13.6	30.0
$N\text{-}NH_4^+$(mg/l)	38.89	0.87	30.2	0.03	43.1	7.10	6.0
$N\text{-}NO_2^-$(mg/l)	0.012	7.90	0.007	7.50	0.017	8.20	–
$N\text{-}NO_3^-$(mg/l)	0.1	9.51	0.07	6.60	0.13	19.10	30.0
P_{tot} (mgP/l)	1.65	0.88	0.90	0.33	2.61	2.60	5.0

* Maximum Permissible Concentrations for effluents discharging into surface waters or soil by Polish Regulations [5].

Table 2. Concentration of *E. coli* and total coliforms in influent and in effluent for two treatment systems.

Wastewater treatment system	Concentration of *E. coli* (×10,000) /Total coliforms (×10,000) (MPN/100 ml)		
	Geometric mean	Median	Range
M-Ch: – influent – ffluent	 2800/9920 240/1170	 2600/7000 230/620	 500 – 19,000/600 – 240,000 60 – 7,000/60 – 240,000
MUCT: – influent – effluent	 1400/5700 7.4/18.5	 1300/7000 6.2/24	 130 – 24,000/230 – 24,000 2.3 – 70/2.3 – 240

Enterococci were enumerated using Chromocult Enterococci Broth after 24 ± 4h incubation at 37°C (Merck). The sodium-azide present in this medium largely inhibits the growth of the accompanying, and especially the Gram-negative microbial flora while sparing the enterococci. The medium contains specific substrate 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (X-GLU) which is cleaved by (characteristic for enterococci) the enzyme β-D-glucosidase (stimulated in medium by selected peptones). This results in an intensive blue-green colour of the broth (a false positive results by most other β-D-glucosidase positive bacteria are inhibited by azide). Therefore, the colour-change of the broth confirms the presence of enterococci and D-streptococci [4]. All indicator bacteria were determined using multiple tube MPN test.

Disinfection of effluent was performed with Wedeco UV irradiation device E-2 with low-pressure mercury lamp Spectrotherm (kindly provided by Wedeco, Poznan) which was placed in the outflowing channel. A valve placed at the outflow from the device allowed for regulation of the flow in the range from 12 to 16 1/h. Other valves allowed for collecting samples of wastewater and as well as cleaning of lamps. Under the experimental conditions UV dose amounted from 40 to 52 mWs/cm^2. The dose of UV radiation was evaluated on the basis of data obtained from Wedeco and the flow of sewage, transmittance and the total operation time of the lamp. At the transmittance Tlcm=45% and flow rate of 12, 14 and 16 1/h, the dose of UV radiation was equal to 52, 45 and 40 mWs/cm^2, respectively. For each dose of UV radiation 4-6 series of investigations were performed. In each series total coliforms, *E. coli,* and enterococci were enumerated before and after exposure to UV radiation.

Results

Effectiveness of Wastewater Treatment

At the time when only mechanical-chemical treatment was performed in the plant, the values of COD and BOD_5 exceeded Polish Maximum Permissible Concentrations (MPC) (Polish Regulations, 1991). The same situation periodically occured in the case of TTS (Table 1). After the MUCT system was put to operation both physical and chemical quality of effluent satisfied the demands of the Polish MPC; with periodically occurred increased values of $N\text{-}NH_4^+$.

In the Table 2 comparison of concentrations off. *coli* and total coliforms in the untreated wastewater and in the effluent from the mechanical-chemical (M-Ch) and MUCT treatment are given. The geometric mean value of MPN total coliforms in raw wastewater was equal to 9.92 x 10^7/100ml and 5.7 x 10^7/100ml before and after modernization of the plant, respectively, while the corresponding *E. coli* concentrations were equal to 2.8 x 10^7/100ml and 1.4 x 10^7/100ml. In the effluent from the M-Ch plant the geometric mean value of total coliforms was 1.17 x 10^7/100ml and *E. coli* concentration equaled to 2.4 x 10^6/100ml, while the corresponding values for the MUCT plant were equal to 1.85 x 10^5/100ml and 7.4 x 10^4/100ml, respectively.

The logio reductions for mechanical-chemical and MUCT systems are summarised in Table 3. Total coliforms elimination as well as *E. coli* in M-Ch treatment was almost the same magnitude and amounted 0.9 logio and 1.0 logio respectively. In MUCT system the reductions of bacterial indicators amounted 2.5 $\log_{10}$ for total coliforms and 2.3 $\log_{10}$ for *E. coli.*

Table 3. Logio reductions in geometric mean of total coliforms and *E. coli* for two treatment systems.

Wastewater treatment system	Reduction of indicator bacteria number [$\log_{10}$]	
	Total coliforms	*E. coli*
M-Ch	0.9	1.0
MUCT	2.5	2.3

Although after modernisation of the plant the microbial quality of the outflow substantially improved, the number of bacteria still exceeded Polish Permissible Concentration for the water used for recreational purposes (1000 FC/100 ml), as well as the concentration advised by WHO for effluent from a wastewater treatment plant (1000 FC/100 ml).

Effectiveness of UV Disinfection

The results of investigations of the effectiveness of UV disinfection of sewage including total coliforms, *E. coli* and enterococci are presented in Table 4.

The results indicate that the doses of UV radiation in the range from 40 to 52 mWs/cm^2 caused a reduction of *E. coli* by 3.4 to 3.8 $\log_{10}$ and slightly smaller reduction of enterococci by 3.1 to 3.3 $\log_{10}$ The geometric mean number of bacteria in the effluent after UV disinfection with the dose 40 m W s/cm^2 was below 50/100 ml of total coliforms, up to 15/100 ml of *E. coli* and about 30/100 ml enterococci.

Discussion

The results of investigations of operation of full scale wastewater treatment plant proved that, depending on the technological process applied, substantial differences in efectiveness of removal of indicator bacteria take place.

At the time when non fully effective mechanical-chemical (M-Ch) plant was operated COD and BOD_5 exceeded MPC values, and reduction of the number of total coliforms and *E. coli* bacteria was only about 1.0 $\log_{10}$. After introduction of the biological MUCT system, effluent fully satisfied Polish MPC, and reduction of bacteria number increased to 2.5 $\log_{10}$ and 2.3 $\log_{10}$, respectively. Nevertheless, the number of indicator bacteria in the effluent from the MUCT system (geometric mean 7.4 x 10^4 /100 ml, range 2.3 x 10^4 - 70 x 10^4 E. coli/100 ml)

Table 4. UV disinfection effectiveness of MUCT wastewater treatment plant effluent.

Indicator bacteria	Dose nWs/cm²	n cycles	$N_0 \times 1000$		N		log N_0/N
			Geom. mean	Range	Geom. mean	Range	
E. coli	40	6	41	23 – 130	15	6 – 23	3.4
[MPN/100 ml]	45	5	77	23 – 240	13	5 – 23	3.7
	52	4	68	23 – 240	10	6 – 23	3.8
Total coliforms	40	6	85	19 – 700	41	23 – 240	2.9
[MPN/100 ml]	45	5	173	62 – 700	45	6 – 700	3.1
	52	4	122	62 – 240	50	6 – 240	3.3
Enterococci	40	6	49	24 – 70	32	23 – 60	3.1
[MPN/100 ml]	45	5	45	24 – 70	25	5 – 62	3.2
	52	4	41	24 – 70	17	5 – 62	3.3

No - MPN of bacteria in 100 ml, before disinfection
N - MPN of bacteria in 100 ml after disinfection

still exceeded the Polish permissible value for the waters used for recreational purposes (1000 FC/100 ml).

The UV disinfection of the effluent from the MUCT plant resulted in further reduction of the number of *E. coli* bacteria, ranging from 3.4 $\log_{10}$ at the dose of UV radiation equal to 40 mWs/cm^2 to 3.8 $\log_{10}$ at the dose of 52 mWs/cm^2. Maximal reduction of *E. coli* from wastewater treated in the full MUCT (mechanical and highly effective biological treatment) system and disinfected with UV rays amounted to 6.1 $\log_{10}$. This value was by about 1.0 $\log_{10}$ smaller than described by Rose et al. [6] in a full-scale water reclamation facility with biological treatment and chlorination.

Reduction of the number of enterococci in the effluent was in the range from 3.1 $\log_{10}$ at the dose of UV radiation equal to 40 mWs/cm^2 to 3.3 at 52 mWs/cm^2, and was lower than *E. coli* (in the range from 3.4 to 3.8 $\log_{10}$). The maximal investigated reduction of enterococci was similar to reduction of total coliforms.

The obtained investigation results regarding the dose of UV radiation are similar to the ones acquired by Lazarova et al. [7], who found out that the dose of UV radiation needed for secondary effluent disinfection to reach the concentration of bacteria below 2 x 10^3 FC/100 ml is approximately 35 mWs/cm^2.

In our case the number of *E. coli* in effluent after MUCT treatment, disinfected with the UV dose 40 mWs/cm^2, was in the range 6-23/100 ml (geometric mean 15/100 ml). That means irradiated effluent fully reached the Polish Permissible Concentration for water used for recreational purposes, and concentration advised by WHO for bacteriological quality of effluent from wastewater treatment plant. Also, the number of enterococci in the effluent disinfected with UV (40 mWs/cm^2), which was about 30/100 ml met the standards for bathing water of good quality due to Wyer et al. [8].

It is worth stressing, that contrary to limited usefulness of thermotolerant coliform group and *E. coli* (detection in environments without fecal contamination, low survival capability when compared with fecal pathogens), as ideal fecal indicators or pathogen index organisms in natural ecosystems, fecal streptococci (enterococci) have received widespread acceptance. They are not as ubiquitous as coliforms, are always present in faeces of warm-blooded animals, they do not multiply in sewage-contaminated waters [9] and are more environmentally - resistant [10]. Additionally, fecal streptococci show a close relationship with health hazards, mainly for gastrointestinal symptoms associated with bathing in waters [11].

The results of this study indicate that effluent from municipal wastewater treatment plant, even using highly effective biological processes, is heavily microbiologically polluted. Because of this, is necessary disinfect it, control its bacteriological quality, especially when discharged into surface waters used for recreational purposes. Bacteriological monitoring should involve, besides such indicators as coliforms and *E. coli,* more environmentally resistant - enterococci which are accepted as a more reliable indicators of fecal pollution.

References

1. GEISSIER K., MANAFI M., AMOROS I. AND ALONSO I.L., Quantitative determination of total coliforms and Es cherichia coli in marine waters with chromogenic and fluorogenic media. J.Appl. Microbiol., **88,** 280, **2000.**
2. FLEISCHER J., SCHLAFMANN K., OTCHWEMAH R., BOTZENHART K., Elimination of enteroviruses, other en teric viruses, F-specific coliphages, somatic coliphages and E. coli in four sewage treatment plants of southern Ger many. Journal of Water Supply: Research and Technology-AQUA **49,** (3), 127, **2000.**
3. SWIATECKI A, DYNER E., Wykorzystanie bakterii z grup coli w badaniach sanitarnych wod powierzchniowych. Analizy srodowiskowe. PIOS. Warszawa 55-64, 1993.
4. MANAFI M., SOMMER R., Rapid identification of en terococci with a new fluorogenic-chromogenic assay. Wat. Sci.Tech. **27,** 271, **1993.**

5. Polish Regulations. Dz. U. Nr 116 poz. 503. ZaJ.2 **1991.**
6. ROSE J.B, DICKSON L.J., FARRAH S.R, CARNAHAN R.P., Removal of pathogenic and indicator microorganisms by full-scale water reclamation facility. Wat. Res. 30, (11), 2785, **1996.**
7. LAZAROVA V, SAVOYE P., JANEX ML, BLATCH-LEY III E.R. POMMEPUY M, Advanced wastewater dis infection technologies: state of the art and perspectives. Wat. Sci. Tech. **40,** (4-5), 203, **1999.**
8. WYER M.D., FLEISHER J.M, GOUGH J, KAY D., MERRETT H, An experimental health-related classification for marine waters. Wat. Res. **33,** 715, **1999.** 9. Monitoring Bathing Waters. Ed. J. Bartram and G. Rees. E&FN Spon London **2000.**
10. **HILL** V.R., SOBSEY M.D., Microbial indicator reductions in alternative treatment systems for swine wastewater. Wat. Sci. Tech. **38,** (12), 119, **1998.**
11. WHO, Guidelines for Safe Recreational Water Environ ments. Draft for consultation. World Health Organization, Geneva **1998.**