Microbiological and Biological Aspects of the Wastewater Treatment Plant "Wschód" in Gdańsk

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Abstract

This study indicates that in the wastewater treatment plant "Wschód" in Gdańsk, working in the modified UCT system, the effectiveness of bacteria pollutant removal varies from 92 to 99% and almost 100% of parasites are removed. Despite this, the number of indicator bacteria and periodical presence of *Salmonella* in the effluent indicates that it is strongly bacteriologically polluted.

It was discovered that the number of indicator bacteria in primary sludge was by 1 to 3 orders of magnitude higher than in the excess activated sludge. Also, *Salmonella* was twice more frequently detected in the primary sludge than in excess activated sludge (70% and 30%, respectively). In contrast, the average number of invading helminths' ova (ATT) was over two times higher in excess activated sludge than in primary sludge. An efficient method for controlling activated sludge bulking resulting from intensive growth of *Microthrix parvicella* was dosing of PAX-16 (the doses from 2.5 to 4.8 g Al³⁺/kg d.m.·d).

Keywords: activated sludge, modified UCT system, removal of bacteria and parasites, filamentous bacteria

Introduction

Since the operation of Polish wastewater treatment plants (WWTPs) with multiphase activated sludge systems for integrated removal of organic carbon, nitrogen and phosphorus started only a few years ago, a number of operational problems have still not been fully elucidated. For instance, the data concerning the effectiveness of elimination of bacteria and parasites as well as biological characteristics of activated sludge and the reasons for periodical intensive growth of filamentous bacteria, are scarce. Also, sludge bulking due to periodical filamentous growth, resulting in foams and scum formation on the surface of bioreactors and deterioration of effluent quality, is not explained well, although it seems to be one of the most serious operational problems of multiphase, low-rate sludge systems [1, 2, 3, 4].

This paper presents the results of investigations concerning the biological aspects of operation of the modern WWTP "Wschód" in Gdańsk, where a multiphase activated sludge system co-operating with a unit for VFA generation from primary sludge is working. The investigations were carried out during the first two years of operation of the WWTP (2000-2001), in close co-operation with the operator of the facility, company Saur Neptun Gdańsk.

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The Study Area

The wastewater treatment plant "Wschód" in Gdańsk underwent upgrading and modernization in the years 1997-1998. The plant receives about 90,000 m³ of sewage per day.

Mechanical treatment units consist of mechanical screens, aerated sand traps with grease removal traps and radial-flow primary sedimentation tanks. Biological treatment units consist of 6 multiphase MUCT (modified UCT system) reactors and 12 radial-flow secondary sedimentation tanks. A typical MUCT system was additionally equipped with a transitional chamber which can optionally serve as a nitrification or denitrification chamber and with deaeration chamber, where the mixture of treated wastewater and activated sludge, recirculated from nitrification chamber to denitrification chamber, is de-oxidized. The plant is equipped with a three-chamber fermenter with complete mixing, co-operating with primary sedimentation tanks. Pre-fermented sludge, containing volatile fatty acids (VFAs), is discharged to sewage before primary sedimentation tanks. Thanks to this, sewage before biological treatment becomes enriched with organic substances, necessary for effective biological dephosphatation.

Since 2002 the treated sewage is transported via a pipeline into the Bay of Gdańsk and discharged 2.3 km away from the coastline.

In the period from 2000 to 2001 a two-year investigation of sewage and one-year investigation of sewage sludge were carried out. The samples were collected 1-2 times a month. Altogether 114 samples of sewage and 19 samples of sludge were collected. The samples of sewage were collected at the following sampling points (Fig. 1): after screens (sampling point no. 1), after primary sedimentation tanks (sampling point no. 2) and from the effluent (sampling point no. 3). The grab samples of raw sewage were collected at 9:00 a.m., the samples of mechanically treated sewage - at 11:00 a.m. and the samples of biologically treated sewage - at 10:30 a.m. next day. The mean samples were acquired by mixing the three grab samples collected at 5-minute intervals. The samples of sludge were collected from primary sedimentation tanks (sampling point no. 4) and raw excess sludge from secondary sedimentation tanks (sampling point no. 5). The samples of activated sludge for microscope analysis were collected once a month from the beginning and the final section of the nitrification chamber, from the aerobic, anoxic and deaeration chambers and from external activated sludge recirculation pipeline.

The samples were transported to the laboratory in a portable refrigerator and analyzed immediately.

Methods

Bacteria

The basic microbiological analyses included determinations of the following types of bacteria: the *coliform* bacteria, *E.coli*, faecal enterococci and *Clostridium perfringens*. The extended analyses also included determinations of *Salmonella*. The standard dilution method was used for determination of indicator bacteria (using from seven to ten dilutions) and standard membrane filters method. Bacteria were plated onto the following culture media: the *coliform*





1 - raw sewage (after screens, 2 – mechanically treated sewage, 3 – biologically treated sewage (after MUCT reactors and secondary sedimentation tanks), 4 – primary sludge, 5 – waste activated sludge (WAS), 6 – activated sludge from bioreactor.

bacteria and *E.coli* on the Fluorocult LMX broth medium with MUG substrate (according to Elmund *et. al.* [5]), presence of suspected *E. coli* was indicated by fluorescence in the UV rays and positive indol reaction, enterococci on Chromocult medium Enterococci broth and *Cl. perfringens* on TSC medium. All nutrient media were produced by Merck. The results of investigations were presented as MPN in 100 cm³ of sewage or sludge.

The prevalence of Salmonella was detected according to the following schedule. The 1 dm3 of sewage was centrifugated (4000 r.p.m) and obtained supernatant was filtered on a membrane filter with pores of 0.45 µm diameter. The membrane filters and the sediment left after centrifugation were used in further analyses. In the case of sewage sludge the 10 ml of sewage sludge samples were used. The samples were inoculated into the liquid selective-multiplication medium with acid sodium selenite (SF). The cultures were incubated at 37°C for 24 hours and then plated onto the following agars: weaklyselective MacConkey (MC) and two strongly-selective: Salmonella-Shigella (SS) and Wilson-Blair (WB) produced by Becton-Dickinson. Identification analyses were carried out using API 20 E tests, followed by serotyping. Identification analyses were performed in microbiological laboratory of Isolation Hospital in Gdańsk. The number per 1 dm³ of sewage and per 1 kg of d.m. of sludge were obtained after recalculation.

Helmints

The analyses of prevalence of *Ascaris sp.* and *Trichuris sp.* ova were carried out according to the method described by Wasilkowa (The guidelines of Health Department [6]). For analysis of prevalence of *Toxocara sp.* ova, flotation method described by Quin *et al.* (Gundłach *et al.* [7]) was used. Results were given as the number of parasites ova per dm³ of sewage and per 1 kg of d.m. of sludge. Altogether 59, samples of sewage and 12 samples of sludge were analyzed.

Activated Sludge

The basic biological investigations of activated sludge consisted of evaluation of composition of activated sludge in the multiphase bioreactor. Biocenosis fluctuations in the nitrification zone was especially analysed. The qualitative evaluation of bacteria in the samples of activated sludge was performed on the basis of microscope observations. Bacteria were divided into three basic groups:

1. cylindrical (rod-shaped), dispersed free swimming,

- 2. filamentous,
- 3. zoogleal and cocci.

The following features are characteristic of filamentous bacteria: shape and length of filaments (straight, curved, long, short), presence of sheath, formation of whirls and bands, filaments stuck in flocs, filaments with real or false branches. Identification of filamentous bacteria was performed by means of microscope observations of prepared slides stained with Gram method and Nessier method [8] at magnification x400 and x600. The bacteria of genus *Spirillum* and *Spirochaeta* were identified on the basis of motion. Three-stages scale was used in evaluation of activated sludge bacteria: x - rare (single cylindrical, filamentous or zoogleal bacteria in some microscopic fields), xx - common (several tens of rod-shaped bacteria, a dozen of filaments or a few zooglea in a microscopic field), xxx - numerous (several hundred rods, several tens of filaments or a dozen zooglea in a microscopic field). The qualitative analysis of activated sludge microfauna using microscopic magnification from x170 to x680 was performed.

The bulking phenomenon was evaluated on the basis of sludge volumetric index (SVI) and diluted sludge volumetric index (DSVI). Intensity of activated sludge foaming in bioreactors was evaluated visually - by estimating the percentage of a bioreactor's surface covered with foam and scum.

Physical and Chemical Analysis

In March 2002 investigations concerning the effectiveness of control of intensive filamentous bacteria growth by means of polialuminium chloride (PAX-16 produced by Kemipol) and ferric sulfate (PIX-113, produced by Kemipol) were performed. An existing installation for chemical precipitation of phosphorus was used for dosing the reagents to the outflow from nitrification chambers of the bioreactors. PAX-16 was introduced to bioreactor no. 4, where the most intensive foaming occurred and the layer of scum was the thickest. For 8 days the dose from 2.5 g Al³⁺/ kg d.m.·d to 4.8 g Al³⁺/kg d.m.·d and for the next 9 days the dose from 1.70 to 2.11 g Al³⁺/kg d.m.·d were introduced to the reactor. PIX-113 was dosed to bioreactor no. 5, in the amount from 6.5 to 9.3 g Fe/kg d.m.·d.

In grab samples of sewage outflowing from the secondary sedimentation tanks the values of COD and BOD₅ and concentrations of TSS, TN, NH_4 -N, NO_3 -N and TP were determined. The measurements were carried out according to the Polish Standards for water and wastewater: TSS (PN-72 C-04559/02), nitrates (PN-82 C-04576/08). Measurements of COD, total nitrogen, ammonia nitrogen and total phosphorus were carried out using the microanalitical methods of Merck company, spectrophotometer S12 and mineralizer CR3000 produced by WTW. BOD₅ was measured manometrically using OxiTop apparatus produced by WTW.

Results

Bacteriological Analyses

Sewage

The results of determinations of the most probable number (MPN) of indicator bacteria: coliforms, faecal coli (suspected *E.coli*), faecal enterococci and *Clostridium perfringens* in raw sewage, mechanically treated sewage and in the effluent are presented in Figs. 2, 3, 4 and 5. In raw sewage the average geometrical values were as follows: *coliforms* - 3×10^7 (7,48 log₁₀), *E.coli* - 1.1×10^7 (7.04 log₁₀), faecal enterococci - 2.3×10^6 (6.36 log₁₀), *Clostridium perfringens* - 1.1×10^7 (7.04 log₁₀).

The number of indicator bacteria (min-max) varied in range from about 2 \log_{10} (*coliform* bacteria 2 \log_{10} , *E.coli* 2.24 \log_{10} , faecal enterococci 2.36 \log_{10}) to about 3.00 \log_{10} (*Clostridium perfringens*).

In mechanically treated sewage the number of indicator bacteria (MPN) was similar to the MPN in raw sewage; however, the range of fluctuations was higher, from about 2.1 \log_{10} for *coliform* bacteria and *E.coli*, 2.25 \log_{10} for faecal enterococci to 4.7 \log_{10} for *Clostridium perfringens*. Temperature of sewage was found to affect the number of *E.coli*. The highest numbers of *E.coli* were detected in the periods when temperature of sewage was above 18°C (from May to October 2000 and 2001).

In the effluent from the WWTP, the fluctuations of number of indicator bacteria varied from approximately 1.5 \log_{10} for *Clostridium perfringens* to about 2.5 \log_{10} for other bacteria. Neither seasonal changes resulting from sewage temperature, nor effect of variable loading of activated sludge (from 0.055 to 0.092 kg BOD₅/kg·d) on the MPN of analyzed indicator bacteria was discovered.

Usually the geometrical average number of indicator bacteria in treated sewage was by about 2 orders of magnitude lower than in raw sewage.

In sewage inflowing to the WWTP, the Salmonella bacteria (in 1 l) were present in 22.7% of analyzed samples. In 2001 Salmonella was more frequently detected (33% of samples) than in 2000 (10% of samples). Salmonella was detected in 5% of analyzed samples of treated sewage. The following species occurred most frequently: S. thompson, S. virchow, S. dublin, S. infantis and S. from serological group C1 (Table 1). All serotypes listed above are capable of causing food poisoning in humans.



Fig. 2. The MPN of coliform bacteria in raw sewage, mechanically treated sewage and effluent from the WWTP.



Fig. 3. The MPN of probable *E.coli* bacteria in raw sewage, mechanically treated sewage and effluent from the WWTP.



Fig. 4. The MPN of faecal enterococci in raw sewage, mechanically treated sewage and effluent from the WWTP.



Fig. 5. The MPN of *Clostridium perfringens* in raw sewage, mechanically treated sewage and effluent from the WWTP.

| No. | Bacteria | Sewage | | Sludge | |
|-----|--------------------|--------|---------|---------|--------|
| | | Raw | Treated | Primary | Excess |
| 1. | S. virchow | х | Х | х | Х |
| 2. | S. thompson | х | | | х |
| 3. | S. gr. serolog. B | | | х | х |
| 4. | S. gr. serolog. C1 | х | | | х |
| 5. | S. infantis | х | | | |
| 6. | S. dublin | х | | | |
| 7. | S. livingstone | | | | х |
| 8. | S. gr serolog. D | | | х | |
| 9. | S. typhimurium | | | х | |
| 10. | S. hadar | | | х | |
| 11. | S. enteritidis | | | Х | |

Table 1. The species of Salmonella isolated from sewage and sewage sludge of the WWTP "Wschód" in Gdańsk.

Sludge

The number of indicator bacteria in primary sludge was substantially higher than in waste activated sludge. The geometrical mean (GA) of *coliform* bacteria and suspected *E. coli* in primary sludge reached $6.0x10^7$ and $4.6x10^7/100$ ml, respectively, and was by about an order of magnitude higher than in excess sludge. The GA number of faecal enterococci ($2.1x10^7/100$ ml) was by almost 2 orders of magnitude higher and the number of *Clostridium perfringens* ($4.3x10^5/100$ ml) was by more than 3 orders of magnitude higher in primary sludge [9].

Salmonella was isolated from 70% of primary sludge samples (S. virchow, S. from serological group B, S. typhimurium, S. hadar, S. from serological group D. and S. enteritidis) and 33% samples of excess activated sludge samples (S. livingstone, S. thompson, S. virchow, S. from serological groups B and C1) (Table 1).

Parasitological Analyses

Sewage

Raw sewage contained the highest number of *Ascaris sp.* parasites - between 0 and 8 active (invading) ova in 1 L and from 0 to 5 passive (non-invading) ova in 1 l. The content of parasitic ova in mechanically-treated sewage was significantly lower - from 0 to 2 per 1 L of active ova and from 0 to 1 per 1 l of passive ova. No *Trichuris sp.* ova were detected during the entire investigation period.

Sludge

The mean number of viable *Ascaris sp.*, *Trichuris sp.* and *Toxocara sp.* ova in primary sludge was 533, 267 and 233/kg d.m. and 2167, 500 and 0/kg d.m. in waste-activated sludge.

Only on rare occasions were the helminths ova not detected in sludge samples (i.e. in October 2001).

Biological Investigations of Activated Sludge

In the aerobic zone of bioreactor (nitrification chamber) zoogleal bacteria dominated. Additionally, mostly in the beginning part of this chamber, small filamentous bacteria growing out of flocs were found. The number of filamentous bacteria substantially increased in the periods of low temperature (January, February, November). Only occasionally single Spirillae and Spirochetae as well as free-swimming rod-shaped bacteria were present. 19 species of sedentary ciliates were detected. The dominant ciliate species was Epistylis plicatilis (from 360 to 2380 per ml), the subdominant was Vorticella sp. (from 200 to 680 per ml) and occasionally found genera were Opercularia and Zoothamnium. Among crawling ciliates, the most numerous species was Aspidisca costata (from 40 to 1880 per ml). The greatest number of this ciliate were found in the beginning part of the chamber. Two genera, of free swimming ciliata, Euplotes and Linotus were detected outside the periods of low temperature (from 40 to 120 per ml). Relatively low numbers of sporozoa and colourless flagellates were observed: Cochliopodium granulatum from 120 to 640 per ml, Arcella vulgaris from 80 to 560 per ml and the Amoeba sp. - from 40 to 240 per ml. In low temperature periods neither quantitative nor qualitative changes in this group were noted. The Rotatoria numbers were low (from 20 to 60 per ml).

In the anaerobic zone (the phosphates releasing), opposite as in the aerobic zone, zoogleal bacteria were scarce. The free swimming rod-shaped bacteria and various types/ genera of filamentous bacteria dominated. From several



Fig. 6. The doses of PAX-16 (g Al³⁺ per 1 kg MLSS per day) applied in the subsequent days of experiment.

tens to several hundreds per ml of sporozoa and colourless flagellates as well as single crawling ciliate from the *Aspidisca costata* were also observed.

In the anoxic zones (denitrification), the number of filamentous bacteria was smaller than in the anaerobic chambers and also fewer types of these bacteria were detected. The sporozoans were more numerous than colourless flagellates. The number of ciliates, which was very low in the I denitrification zone (1 free swimming, 2 crawling and 3 sessile species from the *Vorticella* genus), increased in the II denitrification zone (5 species of free swimming ciliates, 2 crawling and 7 sessile species).

A high number of ciliates was detected in the deaeration chamber: sessile *Epistilis plicatilis* (to 3000 per ml) and crawling *Aspidisca costata* (about 900 per ml).

Control of Activated Sludge Bulking and Foaming Using Chemical Methods

Problems with activated sludge bulking and foam and scum formation in bioreactors have occurred during periods of low temperature since 1998, but in winter season 2001/2002 especially intensive filamentous growth took place. The foams and scum of floating sludge gathered on the surface of bioreactor's chambers - especially of the nitrification chamber. The floating layer of activated sludge outflowed with wastewater from secondary sedimentation tanks, causing substantial deterioration of the quality of effluent from the WWTP. The concentration of total nitrogen increased by 30% and values of COD, BOD₅ and concentrations of TSS and total phosphorus increased 2-3 times.

During winter (2002), investigations of samples of scum of floating sludge from aerobic and anaerobic chambers of bioreactor were performed. A great number of filamentous bacteria was detected in the collected samples. The prevalence of filamentous bacteria both in aerobic and anaerobic chambers of bioreactor indicated that they belong to the A group of microorganisms according to Eikelboom and van Buijsen [8]. The A group is able to grow in all zones of bioreactor (regardless oxygen conditions). The gram-positive Microthrix parvicella, forming tangled whirls inside and outside the flocs, was a dominant species. The filaments without the sheath did not form branches. Poliphosphates granules stored in the cells were found. The result of sulphur test was negative. Occassionally Gram-positive Nostocoida limicola, forming tangled whirls like Microthrix parvicella, was detected. After staining the cells turned out to be almost round. No sheaths, branches, excrescences or granules of stored substances were detected. In samples of floating sludge single filaments of Gram-positive Cyanophyceae and Gram-negative bacteria type 021N were identified. The filaments of type 021N were strong, formed by spherical-shaped cells with well-visible cell walls, without branches or excrescences. The filaments of Cyanophyceae not containing sulphur granules were slightly curved, without branches or excrescences.

In March 2002 the effect of coagulant application was investigated. Two coagulants were used: polialuminium chloride (PAX-16) and ferric sulphate (PIX-113). In this period of time, the daily inflow of wastewater to the bioreactors varied from 20,000 to 26,000 m³/d. The average concentration of activated sludge (MLSS) in the experimental bioreactor (no.4) was approximately 4 g/l. Applied doses of polialuminium chloride (PAX-16) are presented in Fig. 6.

Prior to dosing of PAX-16, almost 100% of the nitrification chamber of the experimental bioreactor was covered with foam (Fig. 7). The volumetric activated sludge index (SVI) was approximately 240 ml/g (Fig. 8), while the dissolved sludge volumetric index (DSVI) was equal to about 160 ml/g. Nitrification zones in the other working bioreactors (no. 1, 3 and 5) were covered with foam in 60-80% (Fig. 7). In these reactors the average SVI value was about 215 ml/g (Fig. 8) and the DSVI value was about 150 ml/g.

On the fifth day of polialuminium chloride dosing (11th of March) the foam started to disappear and on the 9th day of the experiment (15th of March), 80%



Fig. 7. The surface of nitrification chamber of the experimental bioreactor (no. 4) covered with foam and scum. Dosing of polialuminium chloride - from 7th to 23rd of March 2002.

of the nitrification zone surface was free from foam and scum. On the 13th day of the experiment floating sludge occupied only about 5% of the surface of nitrification zone and secondary sedimentation tanks and it completely disappeared from denitrification zone of the experimental bioreactor (Fig. 7). After 14 day of experiment (20th of March), the SVI value decreased to approximately 150 ml/g (Fig. 8). In the meantime, the SVI value in other bioreactors (1, 3 and 5) increased to 245 cm³/g, on average. Further decrease of the SVI value was observed for about 12 days after the dosing of polialuminium chloride was halted. Then the value of this parameter increased again. In mid-April the temperature in the bioreactors increased from about 13°C to about 16°C. In the consequence, the seasonal, probably spontaneous reduction of filamentous bacteria populations in the bioreactors took place.

Simultaneous dosing of ferric sulphate (PIX-113) to bioreactor no. 5 (the doses varied from 6.5 to 9.3 kg Fe^{3+}/kg MLSS·d) was performed in the period from 24th of March to 4th of April 2002, did not result in filamentous growth control. Concentration of activated sludge (MLSS) in the reactor during the experiment fluctuated from 2.9 to 3.5 g/l. Neither the SVI and DSVI values decreased nor foam and scum reduced due to PIX-113 dosing.

Discussion

Over 2-years of investigations carried out thanks to funding by the State Committee for Scientific Research allowed for analyses of the aspects of the effectiveness of bacteria and parasite removal from sewage, and evaluation of sanitary conditions of generated sludge in the multiphase activated sludge system of the WWTP "Wschód". Another task, important for the operation of the WWTPs, was the evaluation of filamentous bacteria in biological treatment system which proved the possibility of chemical control of intensive growth of these microorganisms.

Bacteriological Investigations

During bacteriological investigations the elimination of indicator bacteria and potentially pathogenic *Salmonella* bacteria after subsequent stages of wastewater treatment was evaluated. In raw sewage the *E.coli* to enterococci ratio was equal to 7.7 and the share of *E.coli* bacteria in the total number of coliforms was equal to 36.7%. These values are similar to the average values calculated for municipal sewage of 11 cities in the USA - 5.1 and 37.7%, respectively [10].

It is of interest that the number of two analyzed groups of bacteria, not capable of forming spores (coliform and enterococci), was almost constant in the course of sewage treatment (fluctuating from $2\log_{10}$ to $2.5\log_{10}$). In contrast, high fluctuations of the number of spore-forming bacteria *Clostridium perfringens* in raw and mechanically treated sewage ($3\log_{10}$ and $4.5\log_{10}$, respectively) were observed. In biologically treated sewage the number *of Clostridium perfringens* decreased to $1.5\log_{10}$.

Decrease of differences between the geometrical average (GA) and median (M) in the course of sewage treatment for each group of bacteria indicate stabilization of bacteria number in treated sewage.

Effectiveness of bacteria removal from sewage is presented in Figs. 9-11. The results presented indicate that removal of bacteriological pollutants during mechanical treatment is relatively small. The highest removal effectiveness was noted in the case of faecal enterococci (by 44.4%) and anaerobic spore-forming *Cl. perfringens* (by 27.8%).

These values are consistent with the reports of Imhoff and Fair (1940) [10], who stated that during primary sedimentation from 5 to 40% of bacteria is removed. Investigations performed in the WWTP "Wschód" indicated that the number of coliform bacteria in mechanically treated sewage (after 2-3 hours of primary sedimentation) may be slightly higher than in raw sewage. This increase of the bacteria number probably results



Fig. 8. The values of sludge volumetric index (SVI) in the experimental bioreactor in comparison to the average SVI value in other bioreactors (1, 3 and 5).



Fig. 9. The effectiveness of *coliform* bacteria and *E.coli* bacteria removal in the processes of wastewater treatment.

from releasing of bacteria due to biological decomposition of suspended solids [11] as well as multiplication bacteria, especially in periods when temperature of sewage is high. This is indicated by the results of mechanically treated sewage: during the operation of fermenter (2000), the percentage share of *E.coli* bacteria in the group of coliform bacteria was twice as high (43%) as after the fermenter operation was stopped (24%). Also, the fact that the highest numbers of *E.coli* were found in mechanically treated sewage when temperature was above 18°C confirms the above statement.

According to Geldreich [10], the coliform bacteria can multiply in the primary sedimentation tank after 1 hour of sedimentation. The author, referring to other sources, reported that the number of coliform bacteria in the effluent from primary sedimentation tank, at the temperature of 10°C, can double in 1 hour time. The results of investigations carried out by Brandes [12] and Stosik-Fleszar [13] confirm the reports of possible multiplication of coliform bacteria in sewage and water.

Substantial removal of bacteria from sewage takes place in bioreactors, due to the following processes: sorption on the activated sludge flocs, antagonism, predation and competition for food. Fluctuations of temperature in the bioreactor in the range of 12° C to 21° C, inconsiderably affected the bacteriological quality of sewage outflowing from the plant. No relationship was also found between the load of activated sludge with pollutants in the range from 0.055 to 0.092 kg O₂/kg·d and the number of bacteria in the effluent from the WWTP [9], or between the concentration of suspended solids in treated sewage and the number of indicator bacteria [13].

In treated sewage, the share of *E.coli* bacteria in the total number of *coliform* bacteria was 30% - lower than in mechanically treated sewage (33.3%) and raw sewage (36.7%). It was indicated, that in the WWTP "Wschód", equipped with multiphase MUCT reactors, the effectiveness of *coliform* bacteria and faecal enterococci removal is over 99% and effectiveness of removal of anaerobic spore-forming *Cl. perfringens* bacteria is equal to ap-

2,50E+06 2,00E+06 1,50E+06 1,50E+06 1,00E+06 5,00E+05 0,00E+00 Influent Mech. Effluent

Fig.10. The effectiveness of removal of faecal enterococci in the processes of wastewater treatment.

proximately 90%. These values are within the range characteristic of traditional treatment methods by means of activated sludge [5, 10, 14, 15].

Despite the fact that effectiveness of bacteria removal in mechanical and biological treatment processes was high, still it was insufficient, since the number of bacteria in raw sewage was high (the geometrical average of MPN of coliform bacteria - 3.0×107, suspected E.coli - 1.1×107, faecal enterococci - $2,3 \times 10^7$). The geometrical average of suspected *E.coli* in treated sewage varied from 6.0×10^3 to $7.0 \times 10^{5}/100$ cm³. This is more than the recommended value for bathing places in Poland [16] and in the UE [17], equal to100/100 cm³. Also the admissible values, equal to 1000/100 cm3 and 2000/100 cm3, respectively, are exceeded. Improvement of microbiological quality of sewage could be accomplished either by application of the third stage of treatment (rapid filters) followed by disinfection, for instance with UV radiation [18, 19, 20, 21, 22] or application of membrane processes [23].

The periodical presence of pathogenic *Salmonella* in 1 dm³ of treated sewage is alarming.

In the study it was indicated that the number of indicator bacteria in primary sludge was from a few times higher (in the case of bacteria not capable of forming spores) to a few hundred times higher (in the case of forming spores bacteria) than in raw sewage. Moreover, primary sludge contained higher numbers of indicator bacteria than excess activated sludge: by 1 order of magnitude of coliform bacteria and suspected *E.coli* bacteria and by over 3 orders of magnitude of *Cl. perfringens*.

It should be pointed out that *Salmonella* was isolated in 70% of primary sludge samples and in 33% of excess sludge samples.

The numbers of indicator bacteria in sludges from the WWTP "Wschód" did not differ from sludges from other WWTPs in Poland [24, 25, 26] and in the world [27]. The fact that *Salmonella* was frequently detected suggests that sludge processing techniques should be scrutinized in order to find a possible method of *Salmonella* inactivation.



Fig.11. The effectiveness of removal of *Clostridium perfringens* in the processes of wastewater treatment.

| Crown of filomentous hestoria | Growth conditions | | | |
|---|-------------------|--------|----------------------------------|--|
| Group of filamentous bacteria | Aerobic | Anoxic | Anaerobic | |
| S (e.g. Sphaerotilus natans, type 1701) | - | - | - | |
| C (e.g. type 021N, <i>Thiothrix</i>) | - | - | -/+ (+ for sulphur microorg.) | |
| A (e.g. Microthrix parvicella, type 0092, Nostocoida limicola) | 0 | 0 | 0/+ | |
| F (e.g <i>Nocardia</i> -like actinomycetes) | ? | ?/- | -/? | |

Table 2. Reaction of filamentous microorganisms to methods combining kinetic selection (high concentration gradient) and metabolic selection in different culturing conditions (Wanner 2000).

-- limitation, 0 - no effect, + - stimulation, ? - uncertain effect

Parasitological Investigations

Raw sewage inflowing to the WWTP "Wschód" contained *Ascaris sp.* ova, though *Trichuris sp.* ova were not detected. During mechanical treatment the number of invading ova of *Ascaris sp.* decreased by approximately 77%. During biological treatment almost 100% of *Ascaris sp.* ova were eliminated. Only in 4% of examined samples of treated sewage were non-invading *Ascaris sp.* ova detected.

The average number of invading ova of all analyzed helminths in waste activated sludge was approximately 2670 per kg of d.m. This was more than 2.5 times higher than the average number of all helminths for primary sludge (about 1030 ova per kg d.m.). These values are similar to other authors' reports [28, 29, 30]. These results indicate the fundamental role of biological treatment processes in the removal of helminths ova.

Investigations of Activated Sludge and Control of Bulking and Foaming by Means of Chemical Methods

Though activated sludge collected from the nitrification chamber contained a relatively high number of sessile ciliates (including *Epistilis plicatilis* - the species storing large amounts of nitrogen in its cells up to 8% of d.m.) excreting extracellular mucus facilitating flocculation, plus crawling ciliates, it also contained (especially at low temperature periods) filamentous bacteria which disturbed the proper work of activated sludge. In these periods filamentous bacteria and rod-shaped bacteria were used to become dominant in anaerobic zone and their number gradually decreased in subsequent zones of the bioreactor.

It was indicated that problems related to activated sludge bulking as well as foaming and scum formation in all chambers of the bioreactors and secondary sedimentation tanks resulted from intensive growth of filamentous bacteria. The dominating species was *Microthrix parvicella*, belonging to the A group of filamentous bacteria. This group of microorganisms is capable of growth in all zones of bioreactors [8, 31] since they can assimilate substrate as quickly as floc-forming bacteria [32]. *Nostocoida limicola*, also belonging to the A group of filamentous bacteria, was also detected, though in smaller quantities. Both *Microthrix parvicella* and *Nostocoida limicola* produce surface-active substances and thus they also belong to the F group - bacteria responsible for foaming in the reactors. Occasionally, type 021N, belonging to *Cyanophyceae* (the group C of filamentous bacteria) was detected. This species grows in aerobic zones and is capable of assimilating reduced compounds of sulphur. *Microthrix parvicella*, *Nostocoida limicola* and type 021N belong to 10 species of filamentous bacteria most frequently occurring in the activated sludge systems [33].

According to Wanner [32], in the case when *Microthrix parvicella* and *Nostocoida limicola* are dominating in the multiphase activated sludge systems, where high gradient of substrate concentration occurs and different substrates are available, the mechanisms of kinetic or metabolic selection do not affect their intensive growth (Table 2). Therefore, it is recommended to apply non-specific methods, such as simultaneous dosing of aluminium or ferric ions [2, 32, 34, 35, 36].

According to the literature reports concerning possibility of foams and scum control in activated sludge bioreactors by using aluminium and ferric ions [2, 32, 34, 35, 36], in the WWTP "Wschód" polialuminium chloride (PAX-16) and ferric sulphate (PIX-113) were applied.

Application of ferric sulphate (PIX-113) did not result in filamentous bacterial growth limitation. During 12 days of dosing of PIX-113 (the doses from 6.5 to 9.3 Fe⁺³/kg MLSS·d) neither the SVI value improved nor were foams and scum in the experimental bioreactor reduced.

However, dosing of PAX-16 (the doses from 1.7 to 4.8 g Al.³⁺/kg MLSS·d) had a positive effect - foams and scum were eliminated and the SVI and DSVI values decreased. Positive effects of PAX-16 were achieved after 18 days of dosing, which is faster than in other wastewater treatment plants, where it took 4-6 weeks to observe improvement of conditions in bioreactors [2, 35, 36]. This was probably due to differences in the initial values of SVI and DSVI in various plants. In the WWTP "Wschód" the SVI before

PAX dosing was quite low (max. SVI about 250 cm³/g; max. DSVI about 230 cm³/g), while in Hellevoetsluis (the Netherlands) it reached about 250-350 cm³/g, in Stekene (Belgium) about 300 cm³/g, in Stockholm (Sweden) about 450 cm³/g and in Szczecinek (Poland) even 570 cm³/g.

Conclusions

Despite of effective removal of bacteria in the WWTP "Wschód" in Gdańsk (varying from 92 to more than 99%), the effluents are bacteriologically polluted. The number of indicator bacteria in treated sewage is higher than the admissible value for bathing waters in Poland and in the UE; moreover, *Salmonella* is occasionally detected in the effluent from the WWTP.

Therefore, if the effluents are discharged to receivers in protected regions, further treatment (for instance filtration on rapid filters followed by disinfection or membrane techniques application) should be considered.

Though biological treatment plays an essential role in bacteria and parasite removal, the contamination of primary sludge with bacteria is by 1-3 orders of magnitude higher than contamination of raw sewage. However, the excess activated sludge contains about 2.5 times more helminths ova (ATT) than primary sludge. This is probably due to different survival mechanisms of microbiological pollutants removed in various treatment processes.

It was proved that intensive growth of *Microthrix parvicella* and *Nocardia*-like bacteria in the bioreactors, causing activated sludge bulking as well as foaming and scum formation, especially during low temperature periods, can be effectively limited by the application of PAX-16 (the doses from 2.5 to 4.8 g Al.³⁺/kg MLSS·d).

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