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BACTERIOLOGICAL EVALUATION OF THE TREATMENT EFFICIENCY OF SURFACE WATER USED FOR WATER SUPPLY SYSTEM**

PART II

The efficiency of water disinfection process conduced by the Water and Sewerage Municipal Enterprise has been evaluated. It has been stated that from the sanitary viewpoint the state of tap water is unsatisfactory. Of numerous species of allochtonic bacteria, found both in the raw and treated water, there were many sporulating bacteria resistant to disinfection agents. Pathogenic bacteria such as *Corynebacterium pseudotuberculosis*, *Moraxella bovis* and relative pathogenic (*Bacillus cereus*) present in clean water give the evidence to the inadequacy of water disinfection.

1. INTRODUCTION

Pollution of surface water increases systematically with the industry and town developments as well as with the intensification of agriculture. This increasing pollution is of a special importance in the case of water supply system, since the efficiency of treatment process depends first of all on physicochemical and bacteriological parameters [1], [4], [7]–[9], [12], [15], [18], [19], [21].

The process of water treatment consists of many operations the most important of which — from epidemiological viewpoint — is disinfection process. At present, chlorine is most frequently used although ozone is more radical virio-and-bacteriocidal agent [13], [14]. It should be underlined that disinfecting properties of chlorine

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are, to a higher or smaller degree, decreasing due to its consumption for the oxidation of organic pollutants present in water. Thus only non-bounded part of chlorine is active as disinfectant [5].

The final effect of disinfection depends also on the water microflora sensitivity which is variable and varies depending on the species or strain. Such a reaction to the disinfectant is also stated in pathogenic microorganisms including viruses and bacteria. Resistance to chlorination was among others stated in *Salmonella typhosa*, *Shigella shigae* and *Sh. sonnei* [3], [17].

Another important fact is that quantitative and qualitative compositions of microorganisms in clean water and polluted water are different. Since in the latter sporogeneous forms are dominant, the efficiency of disinfection process is low. The purpose of the present paper was a bacteriological evaluation of the efficiencies of chlorine and ozone as disinfectants of the river Oława water used in water supply system.

2. MATERIAL AND METHODS

Water for analysis was sampled four times (May, August, November, 1979 and February, 1980) at the stand points situated on a slow-filter (raw water) and from the reservoir serving for water supply (clean water). Each time population sizes of psychro- and mesophylic bacteria growing on agar-agar and the coli titer were determined.

Pure cultures derived from all the morphologically differing colonies growing on agar-agar were subject to preliminary analyses, in which their staining ability by Gram method, behaviour towards free oxygen, sporulation ability, etc, were determined, and then to detailed analyses which made possible their identification and systematic classification according to BERGEY [2].

3. RESULTS AND DISCUSSION

Results of rutine bacteriological analyses of raw water samples are presented in tab. 1.

Preliminary identification analyses based on the selected morphological and biochemical features of those bacteria have shown that of 56 isolated strains 11 were able to form spores, 19 were Gram-positive, 25 Gram-negative and 11 Gram-unstable (tab. 2). Detailed diagnostics led to identification of the bacterial strains representing 20 kinds of bacteria (tab. 3).

It may be seen that the prevailing species are those belonging to *Bacillus* and *Pseudomonas* which being saprophytes live in soil and water, thus their presence is

Table 1

Bacteriological indices of tap water pollution

Sampling date	Total number of bacteria on agar-agar at 20°C after 72 h of incubation [cells/cm^3]	Total number of bacteria on agar-agar at 37°C after 72 h of incubation [cells/cm^3]	Coli titer
30.05.79	77×10^3	27×10^3	—
24.08.79	6600	7000	—
19.11.79	4000	166	3.0
5.02.80	5300	830	0.1

justified. There is, however, a number of species which usually do not occur in aquatic environment [12] and, as it follows from the tab. 3, they include also the conditionally pathogenic species. Their presence indicates that water is polluted with domestic sewage [6] and signalises the improper sanitary state of the water. Results obtained from the samples of treated water are given in tab. 4.

Table 2

Number of bacterial strains characterized by the selected morphological and biochemical features isolated from 4 samplings of raw water (slow filters)

Strains investigated	Number of strains	Percentage of strains
Total number of strains	55	100
Number of bacterial strains		
Gram-positive	19	34.5
Gram-negative	25	45.5
Gram-unstable	11	20.0
psychrophylic	32	58.2
mesophylic	24	43.6
sporogeneous	11	20.0
relative anaerobes	45	81.8
proteolytic	18	32.7
nitrate-reducing	33	60.0
ammonifying	23	41.8
decomposing urea	20	36.4
hydrolysing starch	14	25.5

Table 3

Bacterial species isolated from raw water (slow filters)

Genus and species	Number of strains	Sampling date	Characteristics of species
1	2	3	4
Acetobacter pasteurianus	1	III	saprophyte, isolated originally from bear, wine and wine graps
Acinetobacter lwofii	3	III, IV, V	pathogene, isolated from soil and water
Arthrobacter terregens	2	III	saprophyte, occurs in soil
Bacillus brevis	3	I, III, V	saprophyte, occurs in soil and as food pollutant
Bacillus firmus	3	I, V	saprophyte, occurs in soil
Bacillus megaterium	2	V	saprophyte, occurs in soil
Bacillus stearothermophilus	1	V	saprophyte, occurs in soil
Corynebacterium bovis	1	V	saprophyte, occurs in cow's udders, isolated from milk
Corynebacterium pseudodiphtheriticum	1	IV	saprophyte, occurs in nasopharynx mucosa in humans
Corynebacterium pseudotuberculosis	6	I, III, IV, V	pathogene, causes infections in homeothermal animals and sometimes in humans
Corynebacterium xerosis	1	V	saprophyte, component of the normal bacterial flora in humans
Eikenella corrodens	2	I	conditional pathogene, observed in infections of urinary tract and intestines
Flavobacterium, Pickett's group III	1	III	saprophyte, occurs in soil, water and plants
Klebsiella aerogenes	1	V	conditional pathogene, widespread in soil and water, component of bacterial flora in intestines of animals and humans; isolated in humans during infections of urinary and respiratory tracts
Kurthia sp.	2	V	—
Leptothrix ochracea	1	V	saprophyte, isolated from free-flowing waters containing iron
Micrococcus luteus	3	I, V	saprophyte, present in soil, air and water, commensal observed on human and animal skin
Micrococcus varians	1	I	saprophyte, occurs in soil, air and milk and its products
Neisseria elongata	1	V	—
Neisseria sicca	1	I	saprophyte, present in saliva, sputum mucous membranes and respiratory tracts
Pasteurella ureae	1	III	conditional pathogene, occurs in nasal fossa

1	2	3	4
Peptostreptococcus micros	2	III	conditional pathogene, accompanying flora isolated from lungs (gangrene) and uterus (postnatal septicemia); observed in some cases of appendicitis
Proteus inconstans	1	III	saprophyte, originally isolated from hospital wastewater, occurs in urine and stool
Pseudomonas aureofaciens	2	IV, V	saprophyte, occurs in soil and water
Pseudomonas chlororaphis	1	III	saprophyte, occurs in soil
Pseudomonas cichorii	1	V	plant pathogene, isolated from *Cichorium intybus* and *C. endivia*
Pseudomonas diminuta	1	III	saprophyte, occurs in water, isolated from hospital wastewater
Pseudomonas maltophilia	1	III	saprophyte, occurs in water, hospital wastewater, milk and frozen food
Pseudomonas mendocina	2	III	saprophyte, occurs in water and soil
Pseudomonas saccharophilia	1	I	saprophyte, isolated from swampy ground
Sporolactobacillus sp.	1	III	saprophyte, isolated from fodder
Staphylococcus epidermidis	2	IV, V	conditional pathogene, occurs in local abscesses, wound infections and catarrhs of mucous membranes
Streptobacillus moniliformis	1	III	pathogene, occurs in respiratory tract
Veillonella sp.	1	III	pathogene, occurs with pathogenic microorganisms, parasitizes in oral cavity of humans and respiratory tracts and animal intestines

Preliminary identification analyses allowed the isolation of 79 bacterial strains, of which 64 were sporogeneous. The majority (59) were Gram-positive, 8 Gram-negative and 12 Gram-unstable (tab. 5).

Detailed diagnostic analyses have allowed us to state that of 79 strains isolated from clean water 60 belonged to genus *Bacillus*, being thus resistant to disinfectants because of sporulation (tab. 6).

Summing up it may be stated that the total number of strains isolated from the treated water is higher than in raw water. From the above comparison it follows moreover that in treated water there appear species which are absent in raw water.

The above differences seem to prove that water for drinking purposes might have been also taken from other sources without being sent to slow filter from which the raw water was sampled. This hypothesis is additionally confirmed by the results of bacteriological analyses performed on 5 February 1980, showing that the number of bacteria in clean (treated) water was higher than that of psychrophylic bacteria in raw water.

Table 4

Bacteriological indices of the treated water pollution

Sampling date	Total number of bacteria on agar-agar at 20°C after 72 h [cells/cm^3]	Total number of bacteria on agar-agar at 37°C after 24 h [cells/cm^3]	Coli titer
30.05.79	7	40	100
24.08.79	2	2	100
19.11.79	3	7	100
5.02.80	160	6000	12.5

The interpretation of the investigations performed suggests another conclusions, namely that the majority of strains being conditional anaerobes must had been adapted in natural conditions to sudden oxygen deficites characteristic of water strongly polluted with wastewaters.

It has been stated, moreover, that most bacteria were able to reduce nitrates and that there were also numerous proteolytic, ammonifying and urea-reducing

Table 5

Number of bacteria possessing the selected morphological and biochemical features and isolated from drinking water during the investigations

Strains examined	Number of strains	Percentage of strains
Total number of strains	79	100
Number of bacterial strains		
Gram-positive	59	74.1
Gram-negative		
Gram-unstable	12	15.2
psychrophylic	38	48.1
mesophylic	42	53.2
sporogeneous	64	81.0
relative anaerobes	0.0	0.0
proteolytic	40	50.6
nitrate-reducing	72	91.1
ammonifying	36	45.6
decomposing urea	41	51.9
hydrolysing starch	51	64.6

Table 6

Bacterial species isolated from clean water

Genus and species	Number of strains	Sampling date	Characteristics of the species
1	2	3	4
Bacillus acidocaldarius	2	IV, V	saprophyte, occurs in soil and water
Bacillus amyloliquefaciens	1	IV	saprophyte, variety of *Bacillus subtilis*
Bacillus badius	2	II, III	saprophyte, present in excrements, air and food
Bacillus brevis	5	III, V	saprophyte, present in soil and as food pollutant
Bacillus cereus	6	I, IV, V	conditional pathogene
Bacillus cereus var. *mycoides*	3	V	saprophyte, occurs in soil
Bacillus cirroflagellosus	4	II, IV, V	saprophyte, characteristic of sea deposits
Bacillus coagulans	8	I, III, V	saprophyte, widespread, occurs in soil and food
Bacillus firmus	5	V	saprophyte occurs in soil
Bacillus globisporus	3	I, V	saprophyte, present in river water and soil
Bacillus licheniformis	1	II	saprophyte, occurs in soil and as food pollutant
Bacillus megaterium	11	II, IV, V	saprophyte, occurs in soil
Bacillus pumilus	1	I	saprophyte, occurs in soil
Bacillus popilliae	1	I	pathogene, occurs in soil
Bacillus psychrosaccharolyticus	1	II	saprophyte, occurs in soil and swamps
Bacillus sphaericus	1	V	saprophyte, occurs in soil
Bacillus subtilis	2	V	saprophyte, resistant to high temperatures, present in organic matter (plants, animals)
Bacillus species	1	I	saprophyte
Bacillus, Wolf and Barker group I	1	V	saprophyte
Corynebacterium pseudodiphtheriticum	1	I	saprophyte, present in nasopharynx mucosa in humans
Corynebacterium pseudotuberculosis	2	I, II	pathogene for homeothermal animals and sometimes for humans
Lactobacillus casei	1	III	saprophyte, isolated from pasteurozed milk
Lactobacillus leichmanni	1	II	saprophyte, isolated from pressed yeasts and cereal mash
Micrococcus luteus	1	I	saprophyte, occurs in soil, air, water, also observed on animal and human skin
Micrococcus varians	1	VI	saprophyte, occurs in soil and milk and its products
Moraxella bovis	1	I	pathogenic for cattle
Oscillospira guilliermondi	1	V	saprophyte, occurs in alimentary tracts of herbivorous animals

1	2	3	4
Pseudomonas aureofaciens	1	I	saprophyte, occurs in soil and water
Pseudomonas maltophilia	1	I	saprophyte, occurs in water, wastewater from hospitals, milk and frozen food
Pseudomonas fluorescens	1	V	conditional pathogene, occurs in soil and wastewater from hospitals; some biotypes pathogenic for plants
Sphaerotilus natans	3	V	saprophyte, characteristic of polluted waters
Sporolactobacillus sp.	3	III, V	saprophyte, isolated from the food for chicken
Staphylococcus epidermidis	1	VI	conditional pathogene, occurs in local abscesses and wound infections and catahrrs of mucous membranes

bacteria. This suggests, in turn, that the surface water was polluted with domestic wastes or liquid manure [10], [11], [16], [20]. As a general conclusion it may be stated that the water taken for water supply does not meet the bacteriological and sanitary standards.

The prevailing number of bacterial strains belongs to sporulating genera, hence their resistances to disinfectants and a low efficiency of the disinfection process. Numerous bacterial strains are not characteristic of aqueous environment, thus proving their allochtonic origin. Among these strains there are also some conditional pathogenes. Incidentally one observed an imbalance of the numbers of psychro- and mesophylic bacteria (tab. 1, and 4).

Under the above conditions it is necessary to increase the requirements concerning the protection of the Oława river water because of the final effects in its treatment and the efficiency of its disinfection in particular.

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BAKTERIOLOGICZNA OCENA SKUTECZNOŚCI PROCESU DEZYNFEKCJI WODY POWIERZCHNIOWEJ UJMOWANEJ DO CELÓW WODOCIĄGOWYCH

CZĘŚĆ II

Oceniono skuteczność procesu dezynfekcji wody w MPWiK we Wrocławiu. Stwierdzono niewłaściwy stan sanitarny wody ujmowanej do celów wodociągowych. Zarówno w wodzie surowej jak i uzdatnionej występowały liczne gatunki bakterii pochodzenia allochtonicznego, które nie są charakterystyczne dla środowiska wodnego. Na uwagę zasługuje także duża liczba bakterii przetrwalnikujących, szczególnie opornych na środki dezynfekcyjne. O niewłaściwej skuteczności dezynfekcji wody świadczy ponadto obecność bakterii patogennych, takich jak *Corynebacterium pseudotuberculosis*, *Moraxella bovis* i względnie patogennych *Bacillus cereus* w wodzie czystej.

БАКТЕРИОЛОГИЧЕСКАЯ ОЦЕНКА ЭФФЕКТИВНОСТИ ДЕЗИНФЕКЦИОННОГО ПРОЦЕССА ПОВЕРХНОСТНОЙ ВОДЫ СООРУЖЕННОЙ ДЛЯ ВОДОПРОВОДНЫХ ЦЕЛЕЙ

ЧАСТЬ II

Оценена эффективность дезинфекционного процесса в [MPWiK] во Вроцлаве. Обнаружено неправильное санитарное состояние воды сооруженной для водопроводных целей. Так в сырой как и в очищенной воде присутствовали многие породы бактерий аллохтонического происхождения, не будучи характерными для водной среды. Особого внимания заслуживает также большое количество склероцирующих бактерий, особенно строптивных на дезинфекционные средства. О неправильной эффективности дезинфекции воды свидетельствует также присутствие болезнетворных бактерий, таких как *Corynebacterium pseudotuberculosis*, *Moraxella bovis* и относительно болезнетворных *Bacillus cereus* в чистой воде.