

## MICROBIOLOGICAL ANALYSIS OF ACTIVATED SLUDGE IN MUNICIPAL WASTEWATER TREATMENT PLANT AT "KREMIKOV TZI" HOLDING

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### Summary

*The quantity of microorganisms biodegradants in samples activated sludge from municipal wastewater treatment plant at "Kremikovtzi" Holding, Sofia region, during different seasons was investigated. The total number of microorganisms was greatest during the summer. The representatives of genus Pseudomonas predominated in quantitative aspect among the bacteria. Molds were found during all seasons, regardless of their small quantity in the total number of microorganisms. Actinomycetes were isolated only during the summer months and yeasts were not found. The performed investigations give a possibility pure cultures to be used for recovering of the disrupted ratio of microorganisms in the activated sludge at extreme situations.*

### Introduction

The microbial community developing in the aerotanks of wastewater treatment plants is an example of a naturally formed ecosystem. This microbial community named "activated sludge" is a mix population of microorganisms which are considerably influenced by changes in the chemical, physical and biological environmental factors. The biocenosis of activated sludge is formed by the most resistant cultures, because of the lack of sterile conditions [2, 4, 8].

Studying the activated sludge of municipal and industrial wastewater, a great number of microorganisms has been isolated and the genera found most frequently are as follow: *Pseudomonas, Bacillus, Achromobacter, Enterococcus, Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Escherichia, Salmonella, Proteus, Streptococcus, Staphylococ-*

*cus, Micrococcus, Corynebacterium, Clostridium, Penicillium* [1, 12, 13, 15, 16, 19, 20].

A matter of interest is also the ratio of different groups of microorganisms in the wastewater. The determination of the dominating role of the microorganisms in the biocenosis of the activated sludge allows the treatment process to be controlled from outside. Many authors use a combination of pure cultures of microorganisms for treatment and detoxication of municipal and industrial wastewater [9, 14, 17, 18].

The aim of the present work was to analyse the most frequently found taxonomical groups of microorganisms and to determine their quantity in the activated sludge during the different seasons of the year.

### Materials and Methods

The studied samples of activated sludge have been taken for 2 years, each season - 2

samples from the municipal wastewater treatment plant at "Kremikovtzi" Holding

(Botunetz region near Sofia, Bulgaria).

Deflocculation and homogenization of the activated sludge was made in 0.01% sodium pyrophosphate for 8 min at constant agitation [5].

The following media were used for quantitative and taxonomical determination of the main physiological groups of microorganisms: meat peptone broth, meat peptone agar, medium for isolation of pseudomonade forms, medium of Endo, Levine, Hugh and Leifson, Czapek-Dox, Gause, Sabouraud, brewer must agar [11].

The quantitative determination of the microorganisms was done by solidphase cultivation in Petri dishes after logarithmic dilution and according to the method of the utmost dilutions.

The taxonomical determination of the bacteria was done by using morphological, cultural, physiological, biochemical characteristics, according to Bergey's manual [3].

The samples were cultivated at 20 - 25°C in order to create conditions close to the industrial ones.

The isolated precultures were stored by lyophilization. The lyophilizations were performed in laboratory lyophilizer type SMH15 "Usifroid" after freezing of the protected samples up to -34°C at working pressure 20 Pa to final temperature of the sample 22°C and further drying at 1 Pa pressure for 5 h. The protection media used were - sucrose 10%, gelatine 1.5%, skimmed milk 5.5%.

## Results and Discussion

The quantitative and taxonomical determination of the main physiological groups of microorganisms and their season distribution in the studied samples activated sludge has been shown in the performed investigations.

The results for the found out number of microorganism cells per 1 ml are presented in logarithmic scale in Fig. 1 and Fig. 2. The total number of microorganisms was the greatest during summer -  $7.6 \times 10^5$  cells/ml. This number was  $6.8 \times 10^5$  cells/ml and  $2.7 \times 10^5$  cells/ml during autumn and spring, respectively. The total number of microorganisms was smallest during winter -  $1.2 \times 10^5$  cells/ml. The number of the bacteria of genus *Pseudomonas*, genus *Alcaligenes* and family *Enterobacteriaceae* was greatest during summer -  $4.8 \times 10^5$ ,  $2.2 \times 10^4$  and  $2.6 \times 10^5$  cells/ml and this number was smallest during winter  $7.3 \times 10^4$  cells/ml,  $1.4 \times 10^3$  and  $1.2 \times 10^4$  cells/ml respectively. The quantity of the molds and bacteria of genus *Bacillus* was greatest during the winter -  $1.0 \times 10^3$  and  $1.5 \times 10^4$  respectively and smallest during the spring -  $2.4 \times 10^2$  and  $6.0 \times 10^3$  cells/ml. Actinomycetes were isolated only during the summer months -  $1.0 \times 10^3$  cells/ml and yeasts were not found during any of the seasons.

The quantity of the microorganisms during the different seasons did not vary significantly because no rapid, dangerous for the

biocenosis changes of the treated wastewater temperature can be registered.

The decrease in the number of the microorganisms was due to diminution in the concentration of the organic substances which led to progressive slow-down of the growth rate. At concentration of the organic substances less than 80 mg/l a transition of the microflora to a death phase was observed. The presence of infusoria and their active development in the system also decreased the quantity of the bacteria [6, 7].

The ratio of the different systematic groups, which was more than 1% of the total amount of microorganisms is presented in Fig. 3.

What predominated from the bacteria in a quantitative aspect were the representatives of genus *Pseudomonas*, whose percentage varied from 51.85 to 61.84 of the total amount of microorganisms depending on the season, followed by *Enterobacteriaceae* -10.00 to 28.95%. This fact determined the dominating role of these bacteria in the biodegradation process.

The bacteria of genus *Alcaligenes* represented 0.93 to 12.50% of the total amount of microorganisms depending on the season.

The representatives of genus *Bacillus* were 12.50 and 2.22% during the winter and the spring and less than 1% of all microorganisms found out during the summer and the autumn. The actinomycetes, isolated only

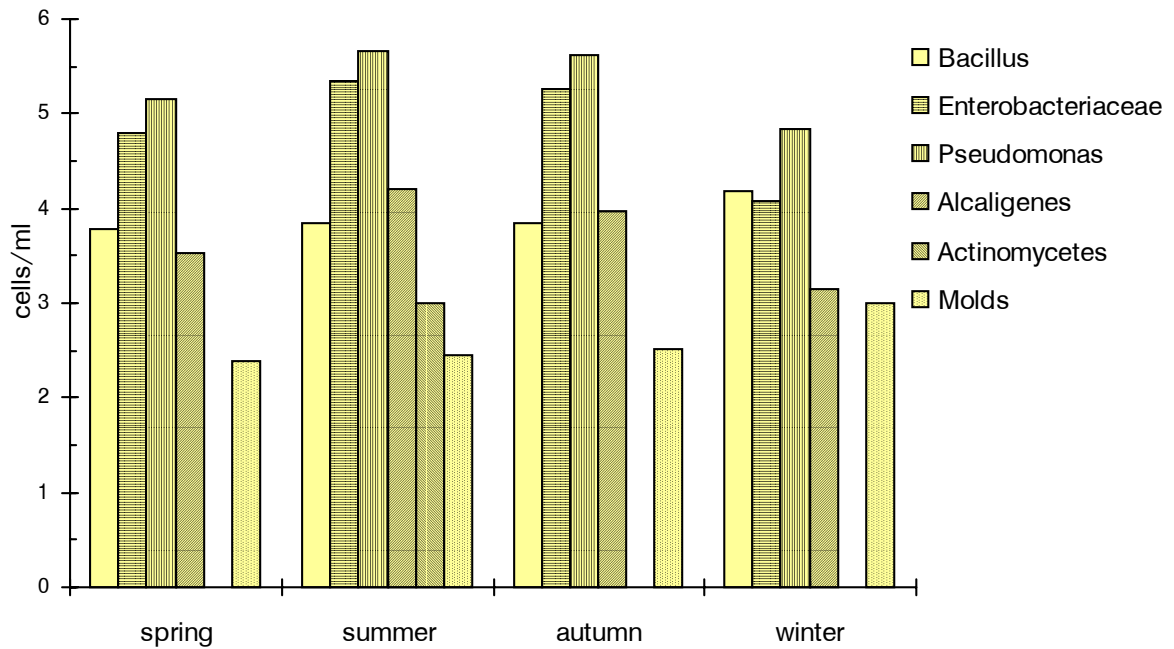


Fig. 1. Quantity of microorganisms in samples activated sludge depending on the season.

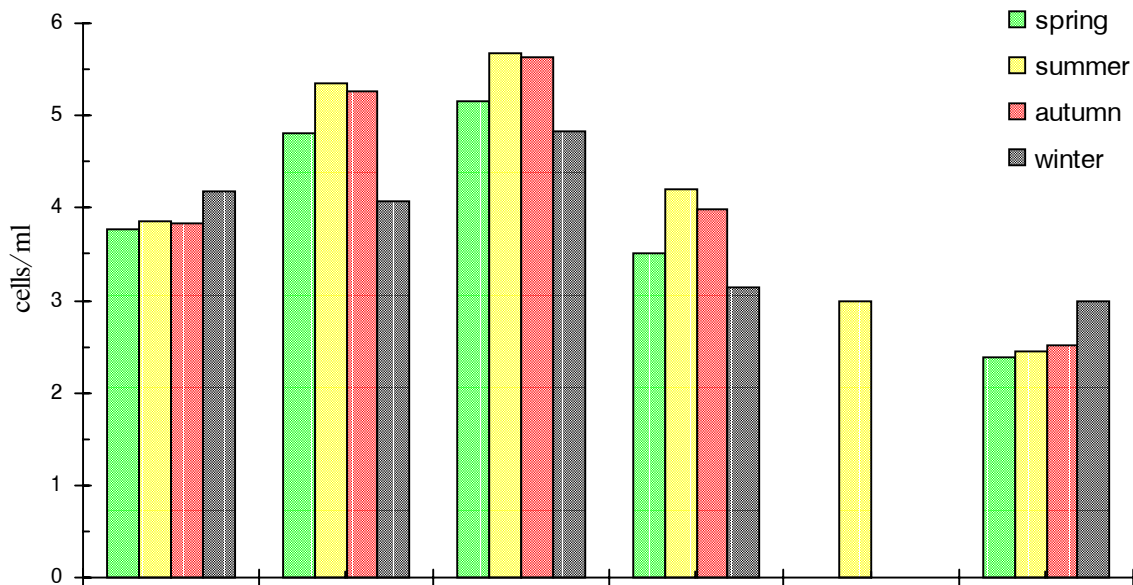


Fig. 2. Quantity of microorganisms in the established systematic groups depending on the season.

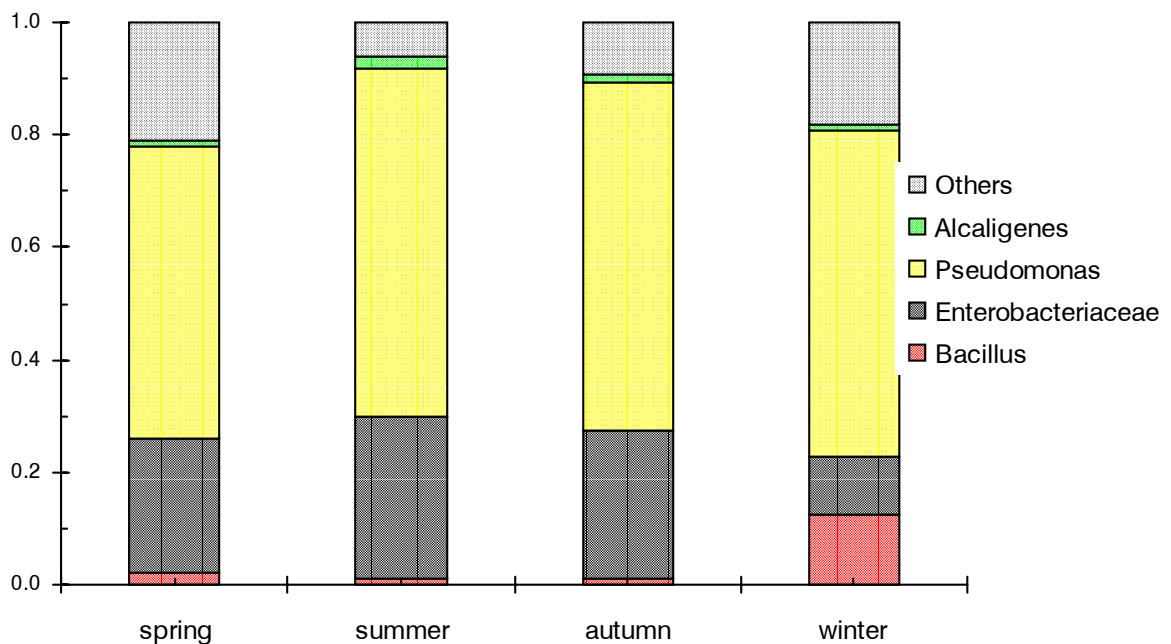


Fig. 3. Ratio of different systematic groups in regards to the total number of microorganisms.

during the summer months were also less than 1%. The molds regardless of their low percentage in the total amount of microorganisms - 0.04 to 0.83% - also participated in the processes of biological treatment of wastewater. Their presence was due to the low pH, excess of reducing compounds, nitrogen deficiency, etc. [4, 10]. The percentage of the not-identified bacteria was between 6.00 and 20.91 during the different seasons.

The presence of coli - bacteria required more profound investigation of the 128 samples of family *Enterobacteriaceae*, especially keeping in mind that varieties, pathogenic for the humans and animals could be found among them. It appeared that greatest was the percentage of *E. coli* - 49.22%, followed by *Citrobacter* - 26.56%, *Enterobacter* - 24.22% and *Proteus* - 1.56%.

The presented results showed that the most active role in the biodegradation process of municipal wastewater in the wastewater treatment plant at "Kremikovtzi" Holding was played by the representatives of genus *Pseudomonas*, followed by family *Enterobacteriaceae*, which predominated quantitatively. The percentage of *E. coli* was the biggest in *Enterobacteriaceae*. The presence of molds in the activated sludge during all seasons, regardless of their small amount showed that they played certain functions in the biodecomposing treatment processes.

A matter of interest is the ratio of the different groups of microorganisms in the activated sludge. At extreme situation, when this ratio is disrupted, the application of pure cultures can help to restore the equilibrium in the ecosystem.

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