

# **Determination of Microorganisms in Activated Sludge of Wastewater Treatment Unit**

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## **Summary**

The amount of microorganisms in samples activated sludge from municipal wastewater treatment unit at “Ristomya” south of Baghdad region, during different seasons was determined. The total number of microorganisms was greatest during the summer ( $7.6 \times 10^5$  cells/ml), whereas the total number of microorganisms was smallest during winter ( $1.2 \times 10^5$  cells/ml).

The representatives of genus *Pseudomonas* predominated in quantitative aspect ( $4.8 \times 10^5$  cells/ml) among the bacteria.

Actinomycetes were isolated only during the summer months, to reach its total number ( $1.0 \times 10^3$  cells/ml ). Molds were found during all seasons, regardless of their small amount in the total number of microorganisms that gave high number ( $1.0 \times 10^3$  cells/ml) in winter, whereas yeasts were not found.

The application of achieved experiments can help to store the equilibrium in the ratio of the different groups of microorganisms when is trouble in the activated sludge of wastewater treatment unit at extreme regions.

## **Introduction**

The microbial community developing in the aero pools of wastewater treatment units 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 [5]. 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, Staphylococcus, Micrococcus, Corynebacterium, Clostridium, Penicillium [1, 4, 9, 10].

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 [7]. The aim of the present work was to analyze 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 unit at (“Ristomya” south of Baghdad region,).

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

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 pseudomonad forms, medium of Endo, Levine, Hugh and Leifson, Czapek-Dox, Gause, Sabouraud, brewer must agar [3]. The quantitative determination of the microorganisms was done by solid phase 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 [2]. The samples were cultivated at 20 - 25°C in order to create conditions close to the industrial ones. The isolated precultures were stored by the refrigerator. The protection media used were - sucrose 10%, gelatin 1.5%, and 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 biogenesis 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 ratio of the different systematic groups, which as 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 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. [6].

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 64 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 unit at "Ristomya" station 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.

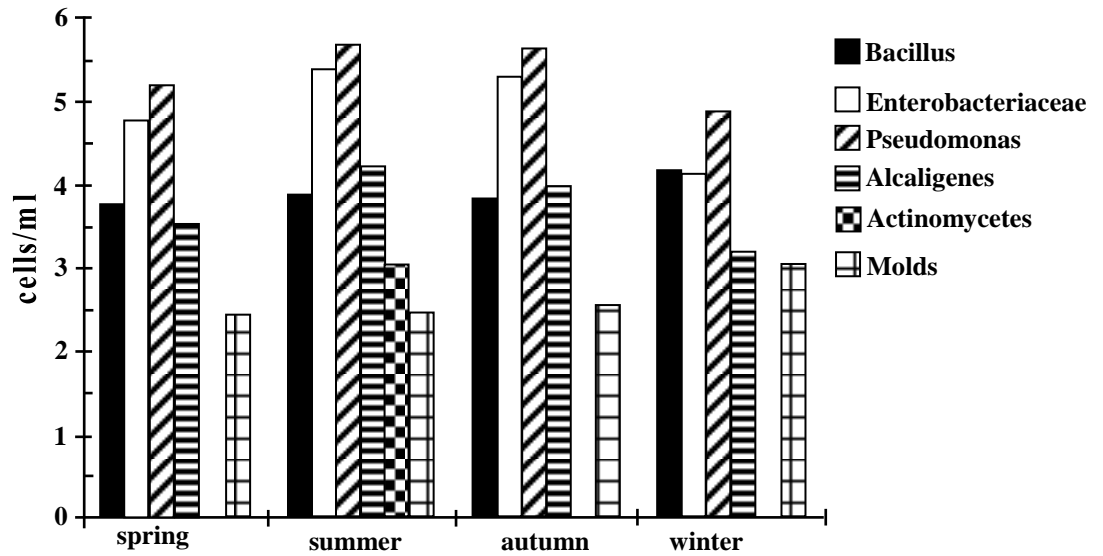


Fig.1. Effect of season on the total number of microorganisms in samples activated sludge.

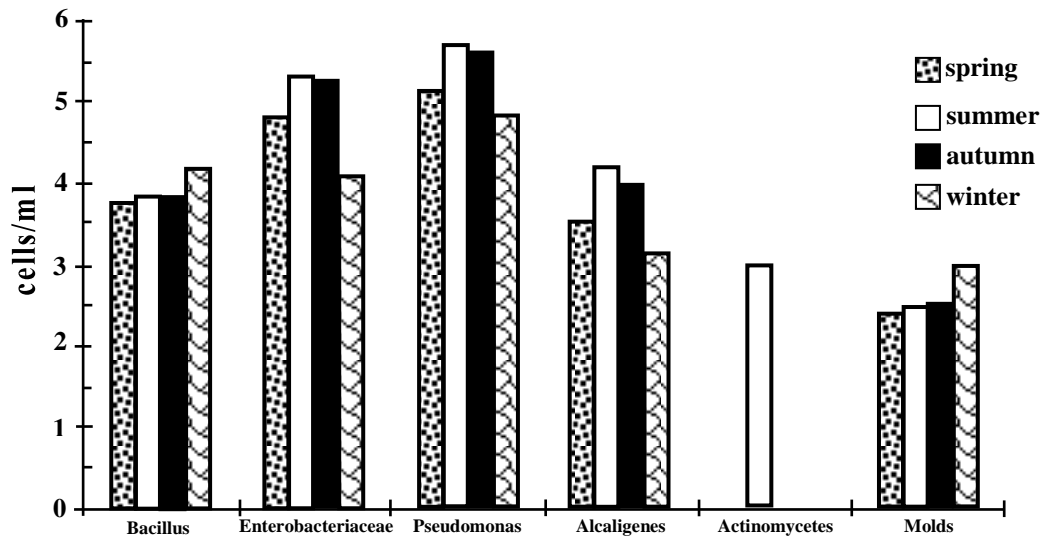


Fig. 2. Total number of each microorganism according to seasons.

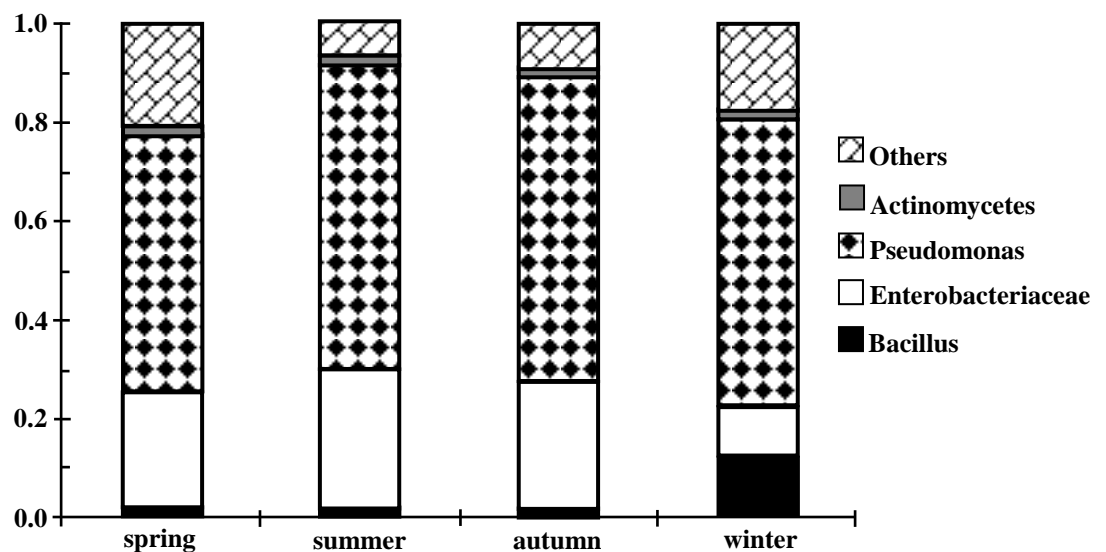


Fig. 3. The ratio of different microorganism in according to the total number.

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### الخلاصة

قدرت الاحياء المجهرية كميا ونوعيا في عينات من الوحل المأخوذ من وحدة معالجة المياه الثقيلة في منطقة الرستمية جنوب بغداد خلال فصول مختلفة من السنة. حصل أكبر عدد كلي للاحياء المجهرية في خلال فصل الصيف ( $10^5 \times 7.6$  خلية/مل) بينما اقل عدد كلي للاحياء المجهرية كان في فصل الشتاء اذ بلغ ( $10^5 \times 1,2$  خلية/مل). البكتريا الممثلة للجنس *Pseudomonas* تفوقت من حيث العدد على بقية الاجناس الاخرى حيث وصل عددها الى ( $10^5 \times 4.8$  خلية/مل). ال *Actinomycetes* عزلت خلال أشهر الصيف فقط، إذ بلغ عددها ( $10^3 \times 1,0$  خلية/مل).

الاعفان *Molds* وجدت خلال كل الفصول، بغض النظر عن كميتها القليلة في العدد الكلي للاحياء المجهرية، وقد ظهر أعلى عدد لها ( $10^3 \times 1,0$  خلية/مل) في الشتاء. الخمائر *Yeasts* لم تظهر في كل الفصول. التجارب التي أنجزت تعطي الامكانية للمزارع النقية لاستعمالها في استعادة ترتيب النسبة العددية المرتبكة للاحياء المجهرية في المياه الثقيلة عند المناطق التي تتجاوز فيها النسب المألوفة.