Microbiocenoses of Urban Anthropogenic Ecological Niches

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Abstract: *The aim of this research was to study the development of various associations of microorganisms in wastewaters and soil near wastewater treatment plant. It was found that the development of some groups of microorganisms is suppressed. Dozens of heterotrophic bacteria cells were found in the soils and wastewater. Evaluation of the seasonal dynamics of bacteria number during the year in the wastewater demonstrated the persistent microcenosis with denitrifiers, ammonifiers, both non-spore and spore-forming oligonitrophils, fungi and yeast present. The growth rate of bacteria was determined. It is shown that the microbial communities (bacteria, actinomycetes, fungi) present in the studied ecological niches gradually oxidize toxic compounds.*

Keywords:*bacterial associations, decomposer bacteria, microorganisms, wastewater*

1. Introduction

Anthropogenic changes caused by land use are quite common. Although the dramatic consequences of such changes for biodiversity at the landscape level are obvious and well known [1, 2], the consequences for soil microbial communities have not been sufficiently studied. In recent years, many researchers try to find different microbiological ways for pollution prevention and toxic wastewater treatment based on microbial biotransformation and degradation of foreign compounds [3]. The existing methods of neutralization of industrial wastes (mechanical, physicochemical) do not provide complete elimination of toxicants [4, 5].

The wastewaters that enter the wastewatertreatment plants (WWTP)are usually quite toxic. Decomposers play the main ecological and protective role in the processes of wastewater treatment from chemical pollutants and their analogues. The microbiological method can serve as an alternative to the existing bioremediation methods, as it is environmentally acceptable and sustainable in the case of the treatment of industrial multicomponent wastewaters [6, 7]. The use of microorganisms in the detoxification processes of contaminated substrates is one of the most promising areas of microbiology. The biochemical method based on the oxidative activity of activated sludge microorganisms is very efficient forthe treatment of domestic wastewaters with a stable composition [8, 9].

Nowadays, biological remediation methods are considered as one of the best for the treatment of contaminated wastewater and soils. Biodegradation is considered the most promising direction of the technologies for the restoration of soil systems contaminated with organic pollutants [3].

According to the literature[10], in the WWTP ecological system, the main role in oxidative processes belongs to heterotrophic bacteria, fungi and yeasts. However, not all organisms in the polluted system are environmentally significant, only those whose number exceeds 10^6cells/ml,gcan affect the system [11].

Previously, it was demonstrated that the large variety of generic and species composition of microorganisms, capable of destroying various pollutants, are present in WWTP ecological system, among which prevail non-spore rods of the Pseudomonas, Xanthomonas, Zoooglaea, Micrococcus genera[6, 12].

The study of the different industrial wastes cenoses composition using selective media showed that *Pseudomonas, Alcaligenes, Bacillus* genera prevail in these systems, moreover *Flavobacterium, Corynebacterium* genera are also quite common. It is possible that a significant part of the population of these microorganisms is able to destroy various pollutants, which is caused by a wide range of their decomposing activity and growth rate [13, 14].

*Pseudomonas fluorescens*strain is well knownfor its ability to decompose aromatic nitro compounds and is used for wastewater treatment [14]. A new decomposer, *Acetobactersp* OP_1, isolated from the anthropogenic ecological niche- acidic soil (pH-6),is capable to metabolize reduced (ammonium) and oxidized (nitrate) forms of nitrogen [15, 10].

The study of the microbocenoses composition of contaminated soils and wastewaters entering the WWTP, changes in the process of biological remediation under the conditions of interaction with native microflora and under the influence of natural and anthropogenic factors are of considerable scientific interest for both theoretical and applied microbiology.

2. Materials and methods

The **object** of the research was the local wastewaters from the Bozsu WWTP and the soil from the nearby territory. Soil samples were taken from 3 different sites near the WWTP - at a distance of 50 m – 1st site, 100 m – 2nd site and 200 m – 3rd site. Microorganisms from wastewaters and soil were isolated according to generally accepted microbiological protocols. Selective media were used for the growth of only selected microorganisms [16]: for ammonifying bacteriawere used meat-peptone agar (MPA), for spore-forming ammonifiers - MPA in combination with wort(1:1), for denitrifying bacteria-Giltai medium, for nitrifying bacteria – Vinogradsky medium, for oligonitrophils-Ashby's medium, for actinomycetes- starch-ammonia agar, for microscopic fungi -Czapek medium, for yeast -wort agar. The number of viable cells was determined by the method of serial dilutions with inoculation on a dense nutrient medium (with 0.95 confidence interval).

The growth rate was determined spectrophotometrically. The number of viable cells was determined by the method of serial dilutions with inoculation on a dense nutrient medium with a 0.95 confidence interval. The bacterial biomass was obtained by centrifugation at 6000 rpm and evaluated by optical density and weighting. The specific growth rate was calculatedaccording to the formula (1), developed byJerusalimsky N.D. [17]:

$$\mu hour^{-1} = \frac{2{,}3\ (\mathrm{Log}\ m1 - \mathrm{Log}\ m0)}{t1 - t0}, \quad (1)$$ where

m_0 –initial biomass at t_0 time
m_1 – final biomass at t_1 time
Identification was carried out by Bergey[18].

3. Results and discussion

The analysis of the microbiological study of wastewater and polluted soils revealed that its composition forms a rigorous selection processfor bacteria. Concentrated wastewaters are harmful for the studied groups of microorganisms. Dozens of cells were found in the wastewaters. After each stage of biological treatment, the number of bacteria increases.

Quantitative analysis of associations of microorganisms in wastewaters and nearby soils revealedits number and diversity (Table 1).

Table 1.The Number of Bacteria in Wastewater and Slightly Polluted Soils, thousands/ml

Bacterial Associations	Soil			Wastewater	
	1st site	2nd site	3rd site	Total	After biological treatment
Non-Spore Ammonifiers	100.0	120.0	167.0	380.0	510.0
Spore-FormingAmmonifiers	1.2	15.0	52.1	0.8	0.28
NitrifyingBacteria	0	0	0.1	0.5	0.2
Denitrifying Bacteria	35.2	70.6	10.1	60.0	42.0
Actinomycetes	0	0	1.0	2.8	1.7
Oligonitrophils	40.0	45.0	130.0	159.0	330.0
Microscopic Fungi	0	0.8	2.7	1.2	0.5
Yeasts	0	0	0.3	3.0	1.0

During the study, we have found the high number of non-spore and spore-formingammonifiers growing on MPA and MPA in combination with wort, which indicates an increased ability of bacteria to decompose nitrogen-containing compounds. The number of non-spore and spore-forming ammonifiers reached 100-510 thousands/ml, 0.8-52.1 thousands/ml, respectively, which is consistent with the data from the literature [6].These results indicate that domestic wastewaterscontain a substrate for ammonification, which consequently causes an increase in the number of microorganisms of this group.

Oligonitrophils were numerous, its number was almost at the ecologically significant level. The identification of a significant number of bacteria growing on synthetic media indicates their ability to grow, digest and degrade synthetic compounds, including the substances that are hard to decompose. The number of denitrifiers in the wastewaters was 60 thousands/ml, biological treatment reduced the numberto 42.0 thousands/ml.

In the next series of experiments, we evaluated the seasonal dynamics of bacteria number during the year in wastewaters. Our results demonstrated persistent microcenosis in the studied wastewaterswith denitrifiers, non-spore and spore-forming ammonifiers, oligonitrophils, fungi and yeast present(Table 2). Despite the fact thatthe formed microcenosis of bacteria persists during the year the number of bacteria changes significantly depending on the time of the year and the nature of incoming wastewaters. Possibly, it is due to the changing weather conditions and the nature of incoming wastewater with a heterogeneous composition.

Table2.Dynamics of Bacteria Number During the Year Depending on the Season and Wastewater Content

Bacterial Associations	The number of microorganisms in the wasterwaters			
	Spring	Summer	Autumn	Winter
Wasterwaters				
Non-Spore	690.0	124.0	132.0	76.0

Ammonifiers				
Spore-FormingAmmonifiers	4.0	-	1.0	0.1
Oligonitrophils	310.0	205.0	212.0	160.0
Actinomycetes	175.0	163.0	190.0	152.0
Microscopic Fungi	60.0	15.0	25.0	6.1
Yeasts	3.0	0.9	0.3	0
After Biological Treatment				
Non-Spore Ammonifiers	630.0	33.0	123.0	170.0
Spore-FormingAmmonifiers	5.0	3.0	1.0	0.80
Oligonitrophils	211.0	136.0	164.0	170.0
Actinomycetes	189.0	150.0	110.0	93.0
Microscopic Fungi	11.0	18.0	15.0	11.7
Yeasts	0.1	0.07	0.03	0.002

The growth rate of bacteria in the wastewaters, initiated by an easily accessible organic substrate (glucose), showed that the number of non-spore bacteria changes slightly, and doubles at a glucose concentration of 50 mg/l.The growth rate of spore-forming bacteria doubles at a glucose concentration of 100 and 500 mg/l. Interestingly, the maximal growth rate of non-spore bacteria in the wastewaterswas 8-10 times higher than that of spore-forming bacteria (Table 3). These results indicate that non-spore bacteria have higher adaptability to extreme environmental conditions.

Table 3.The Specific Bacterial Growth Rate in the Wastewaters (μ h^{-1}) in the Presence of Glucose

Glucose concentration, mg/l	Growth Rate, (μh^{-1})	
	Non-spore bacteria	Spore-formed bacteria
Control	0.026	0.0025
10.0	0.032	0.0031
20.0	0.033	0.0037
50.0	0.053	0.0052
100.0	0.040	0.0050
500.0	0.032	0.0051
1000.0	0.028	0.0028
10.0 g/l	0.030	0.0023
25.0 g/l	0.036	0.0018
50.0 g/l	0.053	–

Thus, the study of various associations of microorganisms in the urban ecological niche, with high anthropogenic pollution, allowed us to establish that microbiological processes slowdown in extreme conditions (high content of toxicants). The number of bacteria does not reach an environmentally significant level. However, in some niches, there are rich microbial population (bacteria, actinomycetes, fungi) that gradually oxidize toxic compounds.

4. Conclusion

The role of microorganisms in the biodegradation of various pollutants that are difficult to decompose is generally recognized. Depending on the nature of the pollutants, different types of microorganisms participate in the biodegradation; this is especially evident during the highly polluted wastewaters treatment. In this research, the change in the microbial composition of wastewater treatment plants and the surrounding area is traced. It was shown that a large number of pollutants and heterogeneity of wastewaters during the entire season reduces the composition of microbial species and has an ambiguous effect on itsquantity. The biological treatment of wastewaters causes the development of wide range of microorganisms, including spore-forming and non-spore bacteria, microscopic fungi, and yeast.

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