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Comparison of bioindicator eukaryotes of activated sludge biocenoses on two water-treatment plants: a case study

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Abstract

Activated sludge biocenoses were compared on waste-water treatment plants in the city of Kazan, Russian Federation and the city of Teplice, Czech Republic. Based on Palia-Kovnatski index, *Acanthamoeba* in Kazan, Epistylis in Teplice, and *Acanthamoeba* and *Centropyxis* were dominant genera in both plants. The major subdominant generas identified were *Arcella*, *Opercularia* and *Aspidisca*. This indicates high nitrification ability, high water purification potential and matured activated sludge. Chemical composition of the waste-water was identified as the main factor determining the sludge biocenoses diversity. Higher sludge biodiversity (Shannon, Margalef, and Sorensen indexes) was found in Kazan corresponding to more concentrated inflow water.

Introduction

Currently, an assessment of the quality of wastewater incoming to and outgoing from a waste-water treatment plant (WWTP) is carried out mainly on the basis of chemical analyses. This brings accurate data with low detection limits however in longer time period required for analyses, without evaluation of the biological effect and providing limited information in conditions of multicomponent industrial effluents. The solution of the problem is in the complex use of chemical and biological monitoring of the treated waters and biocenoses of active sludge, since biological control methods allow an integral assessment of the conditions for the functioning of the bioagent (activated sludge) at any point in the biological treatment of wastewater. Nevertheless since their establishment the methods of bioindication have not developed significantly (Zhmur *et al*. 1997; Wanner *et. al* 2000).

The influence of the biodiversity of a mixed population of microorganisms in water treatment processes on the composition of treated effluent is confirmed by earlier findings as well as by experience from operating wastewater treatment plants (Wanner *et. al* 2000; Ghanizadeh and Sarrafpour 2001; Seviour and Nielsen 2010; Zhmur 2003). However, the question remains open

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✉ *Corresponding author:* josef.trogl@ujep.cz whether the differences in the biological purification process can affect qualitative composition of the activated sludge biocenosis in wastewater. Recent works show the importance of knowledge of the composition and changes in the physiological state of microorganisms as indicator for assessing and predicting the effectiveness of biological oxidation process (Akhmadullina *et al.* 2016; Babko *et al.* 2014; Hreiz *et al.* 2015; Shchegolkova *et al.* 2016). Since this issue is not only of theoretical but also practical significance, we decided to carry out this case study and compare the activated sludge biocenoses of two wastewater-treatment plants (WWTP). The two localities chosen included the WWTP of the city of Kazan, Russian Federation, that is the industrial heart of autonomous Tatarstan Republic with ~1.2 mil. equivalent inhabitants. The second one, the city of Teplice, Czech Republic, is aSpa city with ~155 000 equivalent inhabitants. Both plant types show similarities in incoming water and sludge but differ in technology pattern. A comparison of the indicator organism profiles from two treatment plants by bio-indication (Miller *et al.* 2009) was carried out. The aim was the identification of dominant bioindicator microorganisms and evaluation of biodiversity (on the genus level) and similarities of sludge microbial communities based on diversity indices, especially Palia-Kovnatski.

Table 1. Composition of wastewater in studied waste-water treatment plants.

Components (mg/L)	**WWTP Kazan**			**WWTP Teplice**		
	Influent (mg/L)	**Efluent[1] (mg/L)**	**purification rate (%)**	**Influent (mg/L)**	**Efluent[1] (mg/L)**	**purification rate (%)**
BOD_5	148.9	9.6	93.6	157.4	12.1	92.4
COD	576	68	88.2	335.1	50.2	85.1
Suspended solids	231.4	10.03	95.7	169.2	15.1	91.1
Total nitrogen	31.94	18.6	41.8	35.3	14.3	60.3
Total phosphorus	3.1	1.9	38.7	4.8	1.5	68.6

[1] Purified water

Abbreviations: BOD biochemical oxygen demand; COD chemical oxygen demand; WWTP waste-water treatment plant

Experimental

Sludge samples formed from similar urban waste-waters (for composition see Table 1) originated from two waste-water treatment plants. The first was localized in Kazan, Russian Federation, with traditional configuration of activation process (mechanical and biological wastewater treatment in aeration tanks that foresee the regeneration zone and aeration) (Fig. 1a). The second plant was by the spa city with low industry Teplice, Czech Republic, with nitrification-denitrification configuration (Fig. 1b). Samples were collected from the regeneration phase of activation process.

Bioindication was carried out according to standards of Russian Federation (Bolotina 1987) using microscopes Micmed-5 (LOMO, Russia) and Lambda DN45 TH59F (Lambda Praha, Czech Republic). A small drop of liquid sludge on a microscopy glass slide was covered by cover glass and observed first at low magnification (eyepiece 10 or 15, lens 8), and then at large magnification (eyepiece 10 or 15 lens 40). Microscopic examination was carried out without removing the hand from the micrometer screw in order to be able to change focal lengths and consider the entire surface of the field. Identification of genera was carried out according to atlas of water and sludge microorganisms (Sládeček and Sládečková 1996, 1997). A genus-level of the sludge microbial community was investigated. Previous experiences of wastewater treatment aiming to control the activation process on-line showed that this level is sufficient for such purpose (Akhmadullina *et al.* 2016; Zakirov *et al.* 2013).

All analyses were carried out in triplicate and evaluated using Microsoft Excel.

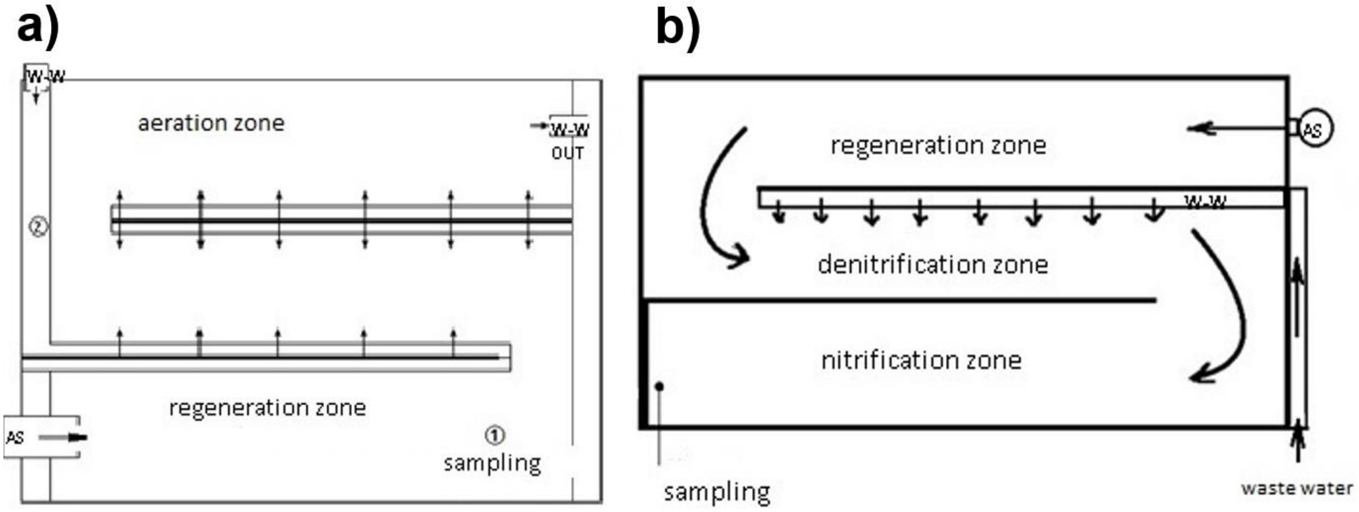

Fig. 1. Scheme of biological treatment processes and sludge sampling sites: a) Waste water treatment plant Kazan; b) Waste water treatment plant Teplice. AS – activated sludge, W-W – waste water.

Table 2. Classification of determined indicator organisms.

Type	**Subtype**	**Class**	**Genus at the locality**	
			Kazan	**Teplice**
Sarcomastigophora	*Mastigophora*	*Phymomastigophorea*	*Astasia*	–
		Zoomastigophorea	*Bodo*	*Bodo*
	Sarcodina	*Lobosea*	*Acanthamoeba* *Arcella* *Centropyxis*	*Acanthamoeba* *Arcella* *Centropyxis*
		Filosea	*Trinema* *Euglypha*	*Trinema* *Euglypha*
Ciliophora	*Ciliata*	*Oligohymenophora*	*Paramecium*	–
		Kinetophragminophora	*Holophrya* *Chilodonella* –	*Holophrya* *Chilodonella* *Colpidium*
		Peritricha	*Epistylis* *Opercularia* *Vorticella* *Thuricola*	*Epistylis* *Opercularia* – –
		Polyhymenophora	*Aspidisca*	*Aspidisca*
	Suctoria	*Suctorida*	*Podophrya* *Tokophrya*	– *Tokophrya*
Nemathelminthes	–	*Rotifera*	*Lecane Cephalodella* *Rotaria* *Gastrotricha Callidina* *Gastrotricha* – –	*Lecane Cephalodella* *Rotaria* – – – *Encentrum* *Philodina*
		Nematodes	*Tobrilus* *Nematoda*	*Tobrilus* *Nematoda*
Annelida	–	*Oligochaeta*	*Aelosoma*	*Aelosoma*

Table 3. Indexes of biodiversity and similarities (Shitikov *et al.* 2003).

WWTP	Indexes		
	Shannon	Margalef	Sorensen
Kazan	3.88	3.42	0.77
Teplice	2.94	2.84	

Results and Discussion

Results of hydrobiological analyses are summarized in Table 2. These visual observations and calculated indexes of biodiversity and similarity (Table 3) show a highly similar profile of indicator organisms of the two studied sludge samples. Further they confirmed that composition of the waste water is the main determinant of the qualitative composition of microbial communities.

The more rich genetic diversity of activated sludge treatment facilities in Kazan indicates to a more complex composition of treated wastewater. This was expected considering a half million inhabitants of the metropolis and industrial infrastructure development compared to Teplice with almost ten times less inhabitants. Systematization of data taking into account the specific conditions of existence of the identified biomarkers allowed us to classify active sludge of both WWTP as low-loaded, nitrifying, and performing the complete oxidation of pollutants. However, a comparison of the dominant aquatic biocenosis based on Palia-Kovnatsky index (*D*) (Table 4) confirmed the expected effect of arrangement of the biological treatment process on the composition of a mixed population of microorganisms (Table 4).

Table 4. Palia-Kovnatsky indexes (D) of dominance in the plants at the two localities studied.

Class	Genus	Palia-Kovnatsky index (*D*) at the locality	
		Kazan	Teplice
Phymomastigophorea	*Astasia*	1.6	–
Zoomastigophorea	*Bodo*	0.096	0.748
Lobosea	*Acanthamoeba*	14.56	4.988
	Arcella	3.2	4.239
	Centropyxis	14.88	18.703
Filosea	*Trinema*	3.072	0.935
	Euglypha	14.24	17.706
Oligohymenophora	*Paramecium*	1.632	–
Kinetofragminophora	*Holophrya*	1.152	0.623
	Colpidium	–	0.062
	Chilodonella	0.32	0.623
Peritricha	*Epistylis*	3.552	18.516
	Vorticella	5.12	–
	Opercularia	4.416	8.354
	Thuricola	0.096	–
Polyhymenophora	*Aspidisca*	7.168	2.431
Suctoria	*Podophrya*	0.032	–
Rotifera	*Gastrotricha*	1.408	–
	Rotaria	0.064	0.249
	Lecane	0.672	0.374
	Cephalodella	1.28	0.249
	Encentrum	–	0.249
Nematodes	*Tobrilus*	0.032	0.249
	Nematoda	0.064	0.249

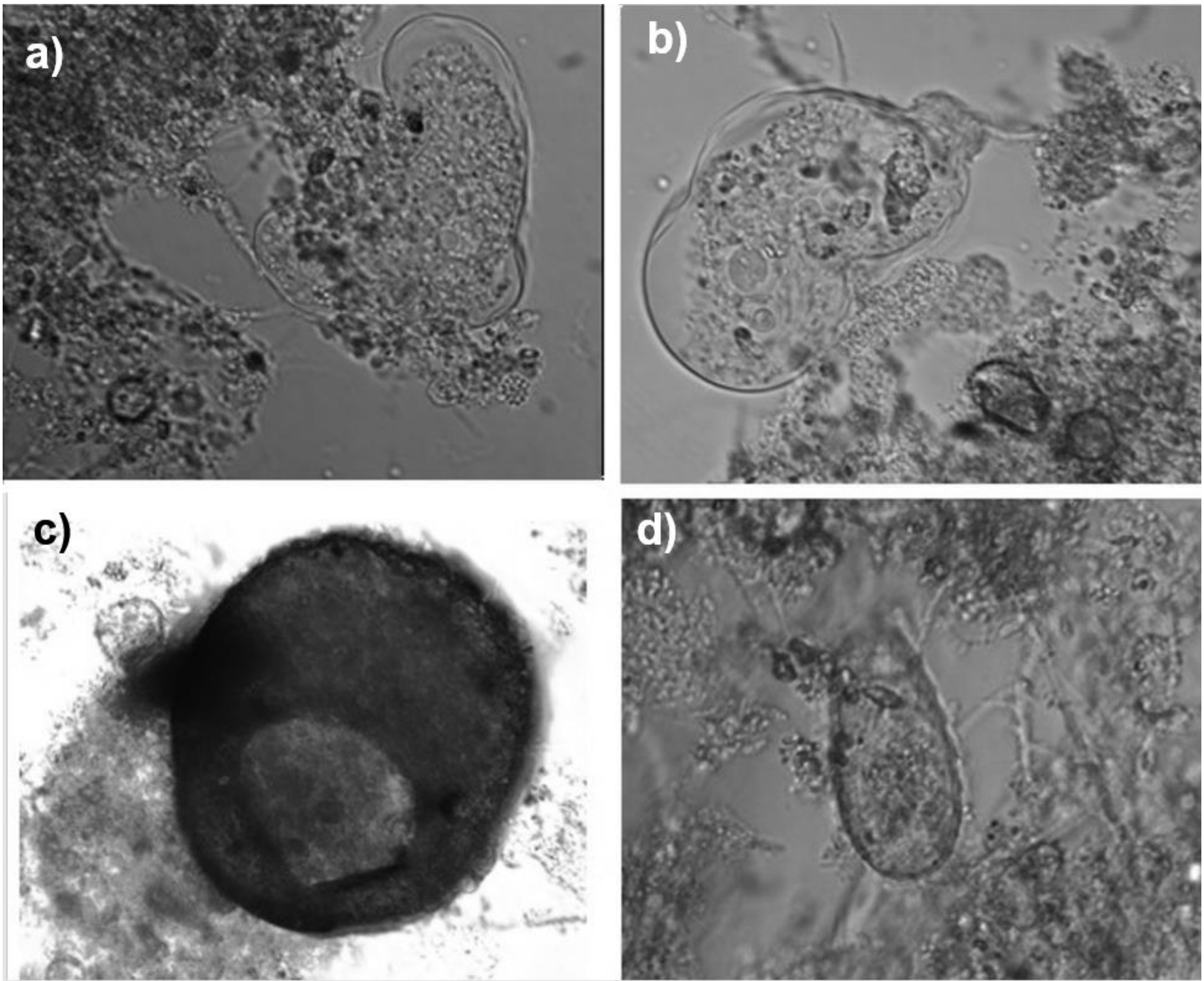

Fig. 2. Dominant genera of the Waste water treatment plant of the city of Kazan: a1, a2) *Acanthamoeba*; b) *Centropyxis*; c) *Euglypha*.

Amorphous genera *Acanthamoeba*, *Centropyxis*, and *Euglypha* were dominant for the WWTP of the city of Kazan ($D \geq 10$) (Fig. 2). Subdominant basic bio-indicators of water treatment process ($D<10$) were mainly ciliates – i.e. g. *Aspidisca* (Fig. 3).

These are characteristic for nitrifying sludge and exhibit a wide range of tolerance to a variety of environmental factors**.** The presence of large ciliates of the genera *Vorticella* and *Opercularia*, also subdominant bioindicators, and high turbidity of the sludge liquid provide the evidence of deep regeneration of the activated sludge accompanied by its deflocculation (Fig. 3). In such conditions amorphous amoebea become competitive since they are capable of both saprozoic and holozoic type of nutrition (Zhmur 1997).

Dominant bioindicator organisms found in WWTP Teplice, as expected, were the amorhous genera *Centropyxis* and *Euglypha*, typical for deep nitrifying sludge. As a confirmation of the latter fact is the dominance of peritrichous ciliates of the genus *Epistylis*, testifying to the high quality and good cleaning properties of nitrifying sludge. Among subdominants amorphous genera *Acanthamoeba*, *Arcella*, and ciliates *Opercularia* and *Aspidisca* prevail, characterizing ripe sludge.

In this experimental case study, the relationships between the main technological factors (modification of the process) determining the functioning of the biological unit system and the biocenosis of the activated sludge were first investigated. The main impact of the results aims on the praxis of WWTP control (especially in the Czech Republic and Russian Federation, however, applicable worldwide) and its possible changes. Currently, an assessment of the quality of incoming and treated wastewater on WWTP is carried out, predominantly on the basis of chemical analysis data. In this regard, the informative nature of the chemical control of the treatment facilities is drastically reduced, causing an unrealistic reflection of the conditions for the functioning of the biocenosis of the activated sludge. This can result in low quality of the biologically treated effluents (Wanner *et. al* 2000; Balymova *et al.* 2011). Also toxic and poorly oxidizable components of industrial effluents might act in synergy on a mixed population of microorganisms and thus negatively affect the biological oxidation process. The complex use of chemical and biological monitoring of the treated waters and biocenosis of active sludge is possible way to resolve this problem, since biological control methods allow an integral assessment of the conditions for the functioning of the bioagent (activated sludge) at any point in the biological treatment of wastewater. In addition they are fast and they do not require sophisticated pricy equipment (except for optical microscope).

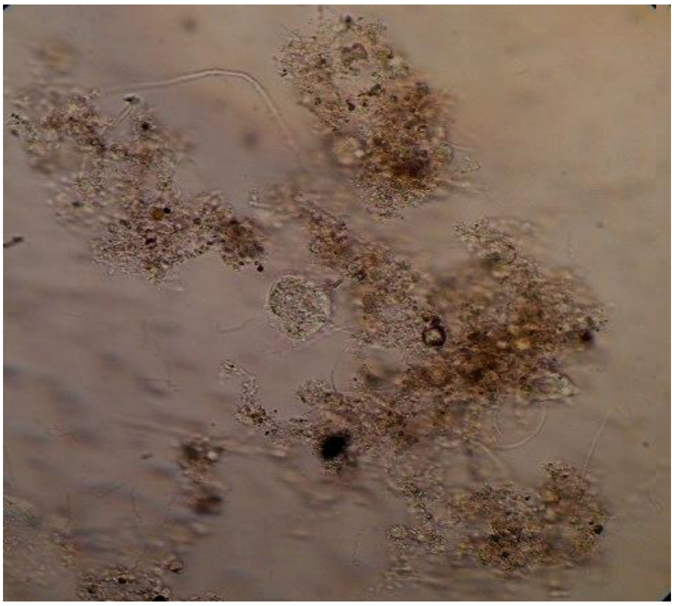

Fig. 3. Deflocculating fibers of activated sludge (WWTP Kazan).

Conclusions

This study compared the qualitative composition of microbiota of two waste-water treatment plants. Composition of the waste-water was determined as the main factor determining composition of the sludge biocenoses. Higher sludge biodiversity (based on Shannon, Margalef, and Sorensen indexes) was found in Kazan corresponding to more concentrated inflow water.

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