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SHORT COMMUNICATIONS

High Efficiency of Removal of Pathogenic Microorganisms at Wastewater Treatment Plants in the City of Moscow

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Abstract—While most pathogenic bacteria are efficiently removed from wastewater during biological treatment, some pathogens, notably *Arcobacter*, may be abundant in the purified water. Using 16S rRNA gene profiling, the composition of microbial communities of municipal wastewater in the city of Moscow was studied before and after biological purification at the Lyubertsy wastewater treatment plant. Fecal contaminants of the genera *Acinetobacter*, *Aeromonas*, *Arcobacter*, *Bacteroides*, *Streptococcus*, and *Veillonella*, which include human pathogens, predominated in the influent wastewater. After treatment, the relative abundance of these bacteria decreased by 50–100 times. Predominant organisms in the microbiome of the effluent water were bacteria characteristic of activated sludge, including the nitrifiers of the genera *Nitrospira* and *Nitrosomonas*, as well as phosphate- and glycogen-accumulating microorganisms. Thus, pathogenic bacteria, including *Arcobacter*, are effectively removed at the Moscow wastewater treatment plant.

Keywords: wastewater, pathogen, microbial community, molecular analysis

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The main function of wastewater treatment plants is to remove organic and inorganic pollutants from wastewater before it is discharged into the environment or sent for reuse. Wastewater treatment plants must also remove fecal contaminants and microbial pathogens from the wastewater (Kristensen et al., 2020). In particular, wastewater treatment can eliminate pathogenic members of the genera *Escherichia*, *Campylobacter*, *Salmonella*, and *Shigella* by over 99% (Mølgaard et al., 2002).

As a rule, wastewater treatment at large wastewater treatment plants is carried out in three stages. At the first stage, water is treated using physical methods (clarification); the second stage involves biological treatment in bioreactors with activated sludge (AS), and the third stage is the final post-treatment of water, including disinfection with ultraviolet irradiation. Consortia of microorganisms developing in AS bioreactors oxidize organic substances and remove nitrogen and phosphates. Microorganisms coming with wastewater, including pathogens, can be adsorbed on AS particles and removed together with excessive AS; as a result, their proportion in the purified water diminishes very significantly (Mølgaard et al., 2002).

Usually, the presence of fecal contaminants and bacterial pathogens is detected using the classical methods of cultivation on selective media or real-time PCR for marker genes. Culture-based screening previously was the gold standard for evaluation of microbiological quality of wastewater (Di Cesare et al., 2020). However, these methods ensure detection only of some indicator species, such as *Escherichia coli* and other enterobacteria (Koivunen et al., 2003; Frigon et al., 2013). Since many pathogens are difficult to culture under standard conditions or are not assessed at all, the real effectiveness of their elimination may remain unknown. As a result, the role of wastewater treatment facilities in protecting the environment from release of pathogenic microorganisms is difficult to assess (Kristensen et al., 2020).

Application of molecular techniques enriched our knowledge on the diversity and genetic potential of microbial communities of wastewaters and AS of treatment facilities, as well as on the presence of pathogenic microorganisms (Hultman et al., 2018; Karaolia et al., 2021). These works also showed that, even though biological wastewater treatment effectively eliminated most pathogens, some of them (e.g., *Arcobacter*) remained in the purified water and represented dominant components of the microbial communities (Kristensen et al., 2020).

Although wastewater treatment facilities of Moscow are among the largest in the world, most of the studies of the associated microbial communities have

Table 1. Principal characteristics of wastewater

Parameter, units	Clarified wastewater	Purified wastewater
Biochemical oxygen demand (in 5 days of incubation), mg/dm^3	183 ± 26	1.31 ± 0.18
Chemical oxygen demand, mg/dm^3	343 ± 51	58 ± 12
Ammonium ions, mg/dm^3	52.6 ± 4.2	<0.05
Nitrate ions, mg/dm^3	<0.1	<0.1
Nitrite ions, mg/dm^3	<0.1	0.17 ± 0.02
Sulfate ions, mg/dm^3	53.3 ± 6.9	72.6 ± 9.4
Phosphate ions, mg/dm^3	8.4 ± 1.1	0.74 ± 0.10

been carried out using the classical approaches. For instance, Zagainova et al. (2022) analyzed wastewaters at all treatment stages using culture-based techniques to identify potential pathogens: *Pseudomonas aeruginosa*, *E. coli*, *Raoultella ornithinolytica*, *Aeromonas hydrophila*, *A. caviae*, *A. molluscorum*, *Enterococcus hirae*, *E. faecium*, *E. faecalis*, *Clostridium perfringens*, *Streptococcus lutetiensis*, and *S. suis*. There have been only a few studies to investigate microbial communities of wastewaters and AS of treatment facilities using modern molecular genetic techniques. Shchegolkova et al. (2016) employed 16S rRNA gene profiling to determine the composition of the microbial communities of wastewater and AS at treatment facilities processing municipal wastewater and wastewaters of a slaughterhouse and an oil refinery plant. It was shown that the content of most known microbial pathogens (e.g., *Streptococcus* and *Trichococcus*) detected in influent wastewater decreased significantly in AS (Shchegolkova et al., 2016). In our previous work, we used molecular methods to characterize the composition of AS communities at nine large-scale municipal wastewater treatment facilities of Moscow (Begmatov et al., 2022). However, in this study we did not compare the composition of microbial communities of wastewater before and after purification, and therefore it was impossible to identify changes in their composition and assess the efficiency of pathogen removal.

The goal of the present work was to characterize the composition of the microbial communities of wastewater before and after biological purification at the Lyubertsy wastewater treatment plant of Moscow. With a capacity of 3×10^6 m^3/day, this facility is one of the largest in Europe and serves to collect and purify municipal and industrial wastewaters. The water samples analyzed in our study represented clarified wastewater after primary sedimentation and water after biological treatment in a bioreactor employing a three-stage anaerobic/anoxic/aerobic process known as the University of Cape Town (UCT) technology.

The chemical composition of water was determined in the MGULAB Laboratory Center by means of ion chromatography and spectrophotometry. To describe the composition of microbial communities, cells were collected by centrifugation from 5 L water samples. Total DNA was isolated using a DNeasy Power Soil Pro Kit (Qiagen). Fragments representing the V3–V4 variable region of microbial 16S rRNA genes were amplified by PCR using the universal primers 341F (5'-CCTAYG GGDBGCWSCAG) and 806R (5'-GGA CTA CNVGGG THTCTAAT) (Frey et al., 2016). The obtained PCR products were sequenced on the Illumina MiSeq platform (in the 2 × 300 bp format). Overlapping paired reads were merged using FLASH v.1.2.11 (Magoc et al., 2011). Reads from both samples were combined, and low-quality and chimeric sequences were discarded. After filtration, 16S rRNA gene fragment sequences were clustered into operative taxonomic units (OTUs) at the 97% identity level. To determine the relative abundances of OTUs in the samples, all reads (including low-quality and singleton reads) were mapped to OTU sequences at 97% global identity threshold . All these procedures were carried out using the USEARCH v.11 software package (Edgar, 2010). The taxonomic identification of OTUs was performed by searches against the SILVA v.138 database using the VSEARCH v.2.14.1 algorithm (Rognes et al., 2016). The obtained 16S rRNA gene fragment sequences were deposited in the NCBI Sequence Read Archive (SRA) database and are available via BioProject PRJNA945245.

Chemical analysis of wastewaters showed that application of the UCT technology resulted in efficient removal of organic compounds (as assessed by biological and chemical oxygen consumption), nitrogen, and phosphorus (Table 1).

The composition of the microbial communities of wastewater samples before and after treatment was analyzed based on 19750 and 14930 filtered 16S rRNA gene fragment sequences, respectively. Altogether, 1186 and 1467 species-level OTUs were detected in these samples. The values of the Shannon's index also indicated a higher diversity of the community of the purified water (5.45 vs. 4.81 in the influent unpurified water).

In agreement with our expectations, the two microbial communities differed significantly in their composition. After wastewater purification, the rela-

Table 2. Changes in the relative abundances of dominant microbial community members after wastewater treatment

Phylum	Genus	Share in the CWW community, %	Share in the PWW community, %
Relative abundance decreased in the purified water			
Actinobacteriota	*Collinsella*	1.52	0.04
Bacteroidota	*Bacteroides*	2.23	0.05
	Cloacibacterium	1.04	0.15
	Macellibacteroides	1.69	0.07
	Prevotella_9	1.11	0.03
Campylobacterota	*Arcobacter*	13.89	0.23
	Arcobacteraceae*	3.42	0.15
	Pseudarcobacter	1.66	0.05
	Sulfurospirillum	1.19	0.05
Firmicutes	*Blautia*	2.22	0.06
	Faecalibacterium	1.53	0.01
	Streptococcus	12.30	0.23
	Trichococcus	2.33	0.12
	Veillonella	1.68	0.02
Fusobacteriota	*Hypnocyclicus*	1.94	0.03
Proteobacteria	*Acinetobacter*	6.71	0.35
	Aeromonas	2.59	0.16
	Tolumonas	1.26	0.01
Total		**60.30**	**1.80**
Relative abundance increased in the purified water			
Bacteroidota	env.OPS_17	0.03	1.02
Chloroflexi	1-20	0.06	1.39
	A4b	0.02	1.45
Cyanobacteria	Ancylothrix_8PC	0.00	1.23
	Tychonema_CCAP_1459-11B	0.00	2.98
Nanoarchaeota	*Woesearchaeales*	0.11	7.01
Nitrospirota	*Nitrospira*	0.18	3.68
Patescibacteria	*Ca*. Nomurabacteria	0.09	2.71
Proteobacteria	*Ca*. Competibacter	0.51	11.30
	Chitinivorax	0.01	1.32
	Dechloromonas	0.77	1.90
	Nitrosomonas	0.03	2.57
Total		**1.80**	**38.55**

* A separate genus in the family *Arcobacteraceae* according to SILVA v.138 taxonomy.

tive abundances decreased more than twofold for 307 genera, increased more than twofold for 306 genera, and did not change significantly for 45 genera (changed less than twofold). Table 2 lists the data on the relative abundances of the genera constituting at least 1% of the microbiome in the samples of clarified wastewater before biological treatment (CWW) and of purified wastewater (PWW).

The predominant groups of bacteria detected primarily in CWW were members of fecal microbiota frequently found in wastewaters: *Collinsella* (Azcarate-Peril et al., 2021), *Bacteroides*, *Prevotella*, *Arcobacter* (Fisher et al., 2014) and related genera of the family *Arcobacteraceae*, *Blautia* (Koskey et al., 2014), *Faecalibacterium*, *Veillonella*, and other. The range of potential pathogens includes members of *Bacteroides*, *Arco-*

bacteraceae, *Streptococcus*, *Acinetobacter*, *Aeromonas*, and *Veillonella* (Altwegg et al., 1989; Wexler, 2007; Collado et al., 2011; Antunes et al., 2014); their total share in the CWW microbiome amounted to 44.5%. All these bacteria were efficiently eliminated during purification and constituted only 1.2% of the PWW community. In particular, the efficiency of *Arcobacter* elimination was over 98%, although a previous study by Kristensen et al. (2020) reported that the efficiency of its elimination from wastewaters was low. Their investigation of 14 municipal wastewater treatment plants in Denmark showed that the proportion of *Arcobacter*, including the human pathogens *A. cryaerophilus* and *A. butzleri*, ranged from 0.9 to 15% in influent water and from 1.6 to 13% in effluent water after final purification. Therefore, wastewater-mediated dissemination of pathogenic members of the genus *Arcobacter* may represent a serious problem (Kristensen et al., 2020). Apparently, at the Moscow wastewater treatment plant, *Arcobacter* cells are efficiently absorbed by AS, in contrast to the facilities assessed by Kristensen et al. (2020).

The microbiome of purified effluent water was dominated by bacteria that are typical for AS of wastewater treatment facilities and play a central role in removing of nitrogen (*Nitrospira* and *Nitrosomonas*) and phosphorus (*Dechloromonas*), as well as by glycogen-accumulating bacteria *Ca.* Competibacter. The high abundance of these functional groups is consistent with the high efficiency of removing of nitrogen and phosphorus. We also detected microorganisms of the phyla *Bacteroidota* (env.OPS_17), *Chloroflexi* (1-20, A4b of the class *Anaerolineae*), *Proteobacteria* (*Chitinivorax*), *Nanoarchaeota*, and *Patescibacteria*, which were among the dominant groups of the AS microbiomes at Moscow wastewater treatment plants characterized previously (Begmatov et al., 2022). Apparently, these bacteria proliferate in AS and are partially washed out to enter the effluent purified water.

Thus, analysis of the composition of wastewater microbial communities at the Lyubertsy wastewater treatment plant showed that all the most abundant potential pathogens in the influent water, including members of the genus *Arcobacter*, were removed very efficiently, and their relative abundance in effluent water decreased by 50–100 times. Considering that the total concentration of microorganisms in purified water is usually by two orders of magnitude lower than in wastewaters before treatment (Kristensen et al., 2020), the actual efficiency of pathogen removal was even higher.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interests.

Statement on the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

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AUTHOR CONTRIBUTIONS

Sh.A. Begmatov performed isolation of metagenomic DNA, analysis of 16S rRNA gene sequences and drafted the manuscript; A.V. Mardanov carried out 16S rRNA gene sequencing; A.G. Dorofeev and N.V. Pimenov performed sample collection, and N.V. Ravin was responsible for data analysis and preparation of the final version of the manuscript.

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