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Impact of maintenance on domestic wastewater treatment systems

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ABSTRACT

Wastewater treatment systems are important sources of contaminants of emerging substances, including pharmaceuticals, and personal care products. Onsite wastewater treatment systems provide alternative solutions to centralized systems; although they are becoming increasingly popular, little is known about the effect of maintenance on their performance. In the current study, chemical and microbiological parameters in the effluents from two identical on-site wastewater treatment systems were analyzed, one being properly maintained while the other not maintained at all. Taxonomic profiles vastly differed from each other, and organic micropollutants are present at higher concentrations in the effluent of the non-maintained unit. The results highlight the importance of proper maintenance.

KEYWORDS

onsite wastewater treatment systems, maintenance, activated sludge, organic micropollutants

1. INTRODUCTION

Despite today's urbanization, approximately half of the world's population still lives in rural areas [1] and might not have access to proper sanitation systems. In Central and Eastern Europe 30% of the population lives in settlements with less than 2,000 inhabitants, and only 9% of those settlements were connected to centralized treatment plants as of 2014. About 80% of wastewater from humans is discharged without proper treatment, contributing greatly to the pollution of waters. Organic pollutants include biodegradable substances as nutrients, Biological Oxygen Demand (BOD), Total Organic Carbon (TOC), as well as organic micropollutants, e.g., Polycyclic Aromatic Hydrocarbons (PAHs), pesticides, pharmaceutical and personal care products. Micropollutants in wastewater cause environmental problems, while some of them, including toxic metals, biocides or antibiotics are also capable of inducing antibiotic resistance in bacteria [2]. Inorganic pollutants include sulfides, chlorides, compounds of trace minerals, nitrogen and phosphorous. In many cases, centralized wastewater treatment is not feasible, due to economical or geographical reasons, thus many studies have discussed the advantages of decentralization (reviewed in [3]). Centralized treatment systems can be a huge financial burden, especially in lower-income areas [1], and wastewater collection systems must also adapt to geographical characteristics of the area [4]. In these cases, decentralization appears as the perfect alternative to centralization. The most important characteristics of On-site Wastewater Treatment Systems (OWTSs) are scalability and applicability. Building sewage collection systems is not required, lowering investment costs, and making the implementation relatively easy. The popularity of decentralized systems is rising, and adequate technology exists for these systems to meet standards. A properly functioning unit is capable of removing nutrients and micropollutants; however, performance mainly depends on the size and technology [3], also hydrodynamic conditions (dead zones and hydraulic shortcuts). These can also affect the mass transfer between substrate and biomass in the reactor [5]. Aeration plays a major role in the generation of flow field, which

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has a direct effect on water age and the average residence time in the reactor [6]. Several case studies showed that the abandonment of the systems could lead to under-performance [7, 8]. Only a small number of studies assessed the removal efficiencies of pollutants in detail, e.g., [9]. Improper technology, design, and most importantly the lack of maintenance can result in low performance, or even complete system failure [10, 11]. Due to lack of information or unwillingness, owners might not maintain their systems [12]. Simple sanitation systems use low-cost technologies, providing only basic treatment. Pit latrines retain fecal matter and discharge the liquid; however, the control of water pollution is not emphasized. In the case of more advanced technologies, environmental protection is the priority. The most promising technologies are Membrane BioReactors (MBR). These systems tend to comply with modern hygienic standards, and it is possible to reuse the treated wastewater and byproducts [13]. A basic domestic treatment is a septic tank, which settles suspended solids, and achieves anaerobic digestion to a degree. Pathogen removal is ineffective; therefore, a post-treatment step is needed. A constructed wetland is yet another low-cost treatment system, requiring less infrastructure, offering more diversity of design, leading to efficient treatment [13]. The most common treatment systems today are activated sludge systems. The type of on-site activated sludge system analyzed in the current study has been in use at more than 1,000 homes in Hungary. In water-scarce regions, irrigation by treated wastewater helps in lowering water needs, while decreasing environmental pollution [14]. Irrigation is widespread in these areas, and many studies have covered its advantages and disadvantages [14, 15]. Apart from cost-efficiency, the reuse could contribute to plant growth and higher yields but could decrease soil quality. Effects on plant health and soil quality depend on the properties of substances in wastewater, and the extent of irrigation [14, 15]. Through prolonged irrigation, heavy metals can accumulate, posing environmental and health risks [15]. Pathogenic bacteria are transferred to soil and plants, posing indirect health risks [16]. In case of anaerobic reactors, the treated effluent does not alter the number of total bacteria in soils and on crop significantly, nevertheless, community composition of bacterial population can change. Adequately operating modern systems are usually capable of removing a large spectrum of microorganisms [16, 17], and most of these systems possess a so-called “global core community”, i.e., the most abundant species, and their relative abundances in a given habitat, and is defined when assessing biological diversity [18, 19].

The main goal of this study is to assess the differences between two identical, but differently maintained OWTSs, by analyzing the microbial compositions and organic micropollutant levels. One unit was maintained properly, while the other not maintained at all. The hypotheses driving this study were the following:

1. Lack of maintenance has a negative effect on effluent quality, leading to high micropollutant levels;
2. Proper maintenance leads to the development of a microbial community similar to the global core community, while improper maintenance causes deviation.

2. MATERIALS AND METHODS

2.1. Sampling site and treatment units

Two activated sludge units of the same kind (Ökotech-Home Ltd. [20]) capable of serving 2–6 Population Equivalents (PE) were analyzed. Fermentation and denitrification take place in the anaerobic chamber. At the bottom of this chamber, the sludge and the sewage move to the anoxic chamber, and then to the aerobic chamber. Aerated wastewater moves to the post-settler, from where treated wastewater is drained into the recipient. According to the manufacturer, maintenance by the owner should include weekly check-ups of the system, strainer, aeration and the sludge container, while every three months, the air pump filter should be checked, and the system should be cleaned. Excess sludge should be removed regularly (1–180 days) and the basket filter emptied monthly. At the site of the maintained unit (M), the treated wastewater flows from the treatment unit to a 2 m^3 storage tank, and then into an infiltration system. At the site of the unit NM, the treated wastewater moves to an infiltration system directly from the post-settler. The produced sludge is circulated with the help of a pump. Schematic diagrams and images of an activated sludge unit are presented in [21].

2.2. Sample handling and storage

Sampling was performed in 2019 according to national standards (28/2004) [22] as described previously [21]. After the on-site measurements, post-settlers of both units, and the storage tank of the unit M were sampled for the analysis of DeoxyriboNucleic Acid (DNA), pesticides, Total Petroleum Hydrocarbons (TPH), PAH compounds, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nitrogen and phosphorous forms, salts, and detergents. The samples were stored in sterile plastic or glass bottles at either 4 °C, or frozen.

2.3. Analytical methods

Analysis of Oxidation Reduction Potential (ORP), pH, conductivity, and temperature were performed at each sampling site by using a Hach HQ40d portable multi-parameter meter, using the appropriate electrodes. Samples were taken for DNA analyses. Measurements of pesticides, TPH, PAH compounds, COD, BOD, Total Suspended Solids (TSS), nitrogen and phosphorous forms, salts, and detergents were performed by the accredited laboratory of Bálint Analitika Ltd. [23].

2.4. Microbial tests

Bacterial enumeration was done by laboratory of E.R.Ö.V. Ltd., as described in [21]. Detection of Salmonella spp., heterotrophic plate counts, and analysis of Enterococci and thermotolerant coliforms were performed according to Hungarian standards, respectively. The post-settler of NM and the storage tank of M were sampled for microbial tests, as these are the last steps before treated wastewater is discharged.

2.5. Questionnaire

A questionnaire was given out to the owners of the units to address maintenance behavior and chemical uses. Questions were related to the implementation and maintenance of the unit, treatment, and handling of the sludge and effluent, as well as the use of cleaning products. The owners gave their consent to participate in this study. The questionnaire was submitted to the Scientific and Research Ethics Committee of the Hungarian Health Science Council and did not consider the research using this questionnaire to be subject to authorization.

2.6. DNA extraction, sequencing and taxonomic classification of metagenomics samples

DNA extraction and sequencing was performed as described previously [21]. For the post-settler of unit M (M/A) and post-settler of unit NM (NM/A) samples, results of the single-end shotgun sequencing results were used for analysis. Adapter sequences were trimmed by the BBDuk tool (version 1.0.0). Kraken 2 (version 2.1.1) and then Bracken (version 2.7) with PlusPF database (May 17, 2021) were applied for taxonomical classification of the trimmed reads. Data analysis was performed using the R programing language, in RStudio version 4.2.0.

3. RESULTS AND DISCUSSION

3.1. Maintenance behavior of the owners

The owners of the first unit NM get their water from a municipal drinking water supply. They decided to use the system partly because there is no sewer network available, and partly because of environmental consciousness. Greywater is used for irrigation and toilet flushing, however the owners do not tend to use biodegradable detergents, and do not contribute to the proper operation of the unit. According to the owners, they were told the system will "simply work" without the need of maintenance. Parts of the unit are checked on less than once a year. The owners state that they have basic knowledge about the operation of the system and that the excess sludge must be removed from time to time, however it had never been removed by the time of sampling. According to the owners, the unit is not working properly, and it also emits an unpleasant smell. The owners of the second household unit M get their drinking water from a drilled well. There is a sewage system available, but according to the owners, they chose to use an OWTS because they consider themselves environmentally conscious. Greywater is not utilized, but the owners try to use natural or biodegradable products. They have adequate knowledge about the operation of the system, and at the time of installation, they were given a manual. Every crucial part of the unit is checked regularly. Excess sludge is checked regularly, emptied every three months, and according to the owners, the system works well. The sludge is used as compost for trees and bushes. In the case of this system, treated wastewater is moved from the post-settler to a 2 m^3 short-term storage tank after that the water is infiltrated.

3.2. Effluent quality

Removal efficiencies can only be evaluated if both raw and treated wastewater is analyzed. However, there was no opportunity to perform composite sampling. Nonetheless, the quality of effluents was evaluated. Results of on-site measurements (pH, temperature, conductivity, ORP), and chemical parameters are shown in Table 1. In case of the unit M/A, conductance of the effluent is significantly lower than in the post-settler of the one NM/A, which continued to decrease in the storage tank (M/B). pH and temperature readings are close to each other in the post-settlers but are

Table 1. On-site measurement results and chemical parameters

Parameter	Unit	Non-maintained post-settler (NM/A)	Maintained post-settler (M/A)	Maintained storage tank (M/B)
pH	—	7.34	7.58*	8.55*
Temperature	°C	24.1	23.6*	17.1*
Conductivity	μS cm^{-1}	1,605	703*	501*
Oxidation Reduction Potential (ORP)	mV	−358.0	166.0*	−178.3*
COD	mg L^{-1}	921	42*	<30*
BOD	mg L^{-1}	438	4*	3*
Inorganic nitrogen	mgN/L	155.0	12.1	10.7
Organic nitrogen	mgN/L	5.0	3.9	<0.5
Total nitrogen	mgN/L	160.0	16.0*	10.7*
Total phosphorous	mgP/L	26.50	84.00*	0.99*
Total suspended solids	mg L^{-1}	360	66*	18*
Total salts at 105 °C	mg L^{-1}	1712.0	<0.2	<0.2
Total salts at 600 °C	mg L^{-1}	1072.0	<0.3	<0.3

*data has previously been published in [24].

different when compared to the storage tank of the maintained unit. There is a significant difference between ORP values of the two post-settlers. In M/A the conditions are oxidative, while in NM/A highly reductive. In M/B the reductive conditions are most likely due to the lack of aeration.

COD and BOD were remarkably lower in M/A when compared to NM/A, while almost zero in M/B. Nitrogen forms, suspended solids, salts, oils and fats were similarly lower in M/A, even lower in M/B compared to NM/A. Total Phosphorous (TP) and nitrate values were higher in M/A than in NM/A, but TP decreased in the M/B. The reason behind the high TP in case of M/A can be due to an already high phosphorous level in the water source of the household, the excess usage of cleaning agents, or improper treatment of raw wastewater by the unit. To define this, further studies would be needed. The legal limits of effluents below Population Equivalent (PE) 600 in Hungary is 300 mg L^{-1} for COD, 80 mg L^{-1} for BOD, and 100 mg L^{-1} for TSS; thus unit NM did not meet these limits. The results of TPH, PAH, and pesticide measurements are it is shown in Table 2. Regarding TPH, NM/A contained high amounts of long-chain hydrocarbons, M/A had lower TPH concentrations, which continued to decrease in M/B. The results are similar in the case of PAH compounds and pesticides. M/A had more than ten times less PAHs compared to NM/A, and even less in M/B. All results, however, were under the limits. Nearly all pesticide content came from the mosquito-repellent, and Diethyltoluamide, as samples were taken in the summer.

The results of measurements confirm the differences in pollutant content between the two units. Not only the maintained unit had lower concentration of pollutants, but these further decreased in the storage tank, meaning it helps in reaching a lower pollutant level, as shown in [21], however a long-term storage of effluent is more beneficial than short-term storage [21].

3.3. Taxonomic classification

The core of wastewater treatment is the biodegradation, oxidation, and reduction of molecules by microbes. These have been thoroughly studied in wastewater treatment plants, but in small-scale systems little is known about microbial communities [25]. According to both Shannon and Chao1 diversity indexes, the non-maintained unit was more diverse than the maintained unit (Shannon: 5.524 and 4.887, Chao1: 1,189 and 328, respectively). At a kingdom level, both post-settlers harbor a high relative abundance of bacteria. Both post-settlers have the highest relative abundance for Proteobacteria. This phylum has a high diversity; it includes aerobic, anaerobic, and facultative anaerobic bacteria [26]. NM/A has higher abundance of Bacteroidetes and Actinobacteria, and a lower abundance of Firmicutes. Bacteroidetes and Firmicutes represent more than 90% of the total gut community; both are successful carbohydrate-digesting bacteria [27, 28]. Euryarchaeota and Synergistetes phyla are also abundant. Chloroflexi is not present in NM/A in a significant amount, as opposed to M/A (data not shown). Both post-settlers contain roughly the same orders of bacteria, with a few notable differences in abundance (data not shown). Burkholderiales and Pseudomonadales are present in a high ratio in M/A. The Thiotrichales order is not present from NM/A. These are aerobic bacteria capable of oxidizing sulfide forms. Desulfobacterales, Desulfovibrionales and Aeromonadales are present in NM/A, but not in the M/A. The species in the two latter orders are mostly anaerobic or obligately anaerobic, which coincides with the low oxygen-content of the post-settler. Regarding family composition, the units show a striking difference. The mostly aerobic Comamonadaceae, Thiotrichaceae, and Moraxellaceae families are present in M/A in a high ratio, but not in NM/A. Aeromonadaceae, and Desulfobulbaceae are only present in NM/A. The families found in unit NM are facultative anaerobic and obligate anaerobic species, capable of sulfate-reduction (data not shown). At the genus level, there is also a remarkable difference. Apart from the Bacteroides genus, member of the gastrointestinal microbiota, and the Pseudomonas genus harboring a diverse metabolism, and many unclassified reads, the composition of the post-settlers is almost completely different. The abundance is close to zero for Thiothrix and Acinetobacter in NM/A, compared to the high abundance in M/A. The opposite is the case for Desulfobulbus, Aeromonas and Tolumonas, with close to zero abundance in M/A, and relatively high abundance in NM/A (data not shown). The Moraxellaceae family includes the Acetinobacter genus, with human pathogens. The Pseudomonaceae family also

Table 2. TPH, PAH and pesticide measurement results of the units analyzed

Parameter	Unit	Non-maintained post-settler (NM/A)	Maintained post-settler (M/A)	Maintained storage tank (M/B)
C5-C12 (TPH)	μg L^{-1}	48.8	0.8	0.8
C13-C40 (TPH)	μg L^{-1}	18,500.0	14.7	6. 7
PAH without naphtalene	μg L^{-1}	0.291	0.007	0.005
Total naphtalene	μg L^{-1}	0.567	0.058	0.041
Total PAH	μg L^{-1}	0.858	0.065	0.046
Diethyltoluamide	μg L^{-1}	79.30	7.72	0.43
Total atrazine	μg L^{-1}	not detected	0.01	0.02
Bentazon	μg L^{-1}	not detected	0.30	0.05
Total pesticides	μg L^{-1}	79.30	8.03	0.50

Table 3. Microbial components of treated wastewater effluents. before discharging

Bacteria	Unit	Non-maintained post-settler (NM/A)	Maintained storage tank (M/B)
Enterococcus	CFU mL^{-1}	1,700	13
Thermotolerant coliform	CFU mL^{-1}	4,300	2
Heterotrophic plate count (22 °C)	CFU mL^{-1}	1,375,000	640
Heterotrophic plate count (37 °C)	CFU mL^{-1}	335,000	630
Salmonella spp.	–	present	present

includes several human pathogens. The microbial composition also reflects that NM/A has a highly reductive environment. The data show that the microbial composition of M/A better resembles the global core community, the Proteobacteria phylum is present in more than 80%, the diversity is lower than in unit M, and there is a clear dominance pattern [19].

3.4. Pathogenic bacteria

The presence or absence of pathogenic bacteria is an important factor in assessing effluent safety. Thus, the presence of multiple pathogen-containing genera was evaluated. The Mycobacterium, Clostridium, Legionella, and Klebsiella genera were only identified in the non-maintained post-settler, while Pseudomonas, Enterobacter, Bacteroides and Salmonella genera were present in both post-settlers. In the Mycobacterium genus, *M. intracellulare* and *M. avium* were the most abundant species. In the Clostridium genus, *C. botulinum* had the highest relative abundance among the 17 identified species in sample NM/A. In the Legionella genus only *L. pneumophila* was identified. *K.* pneumoniae was the most abundant of its genus, found only in the non-maintained unit. Regarding Pseudomonas, 10 species were found in the maintained unit, opposing to 100 in the non-maintained one. In the Enterobacter genus, *E. hormaechei* was the most abundant in NM/A, but this species was not present in M/A. In the Bacteroides genus, 10 species were found in M/A, and 22 in NM/A. *S. enterica* was the only species found in the Salmonella genus, with a slightly higher relative abundance in the maintained unit.

3.5. Microbial components of treated wastewater

As treated wastewater could be reused, it is important to know its microbial composition. Bacteria analyzed were present in a remarkably low concentration (Colony Forming Unit per milliliters, CFU mL^{-1}) in M/A; however Salmonella spp. was present in both cases, nicely corresponding with metagenomics results. Results are shown in Table 3.

4. CONCLUSION

This study presented the main differences between the chemical and microbial characteristics of two identical on-site wastewater treatment systems. As it was expected, there is a significant difference between the units; the lack of maintenance changed the taxonomic profile entirely, the analysis of micropollutants supports this conclusion. The non-maintained unit had higher concentration of the pollutants analyzed compared to the maintained one, as the different microbial composition results in different processes. It is important to note, that this study has a few limitations. Drinking water sources and raw wastewater were not analyzed, thus removal efficiency cannot be calculated, which may mask whether differences only stem from the different maintenance behavior. Based on this, far-reaching conclusions cannot be drawn. However, these results highlight the need for further detailed analyses on the treatment efficiency of OWTS and the effect of maintenance on organic micropollutant removal. It is highly recommended that OWTS manufacturers provide a manual for maintenance, which the owners strictly follow.

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REFERENCES

[1] A. G. Capodaglio, “Integrated, decentralized wastewater management for resource recovery in rural and peri-urban areas,” *Resources*, vol. 6, no. 2, 2017, Paper no. 22.

[2] I. Mantis, D. Voutsa, and C. Samara, “Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods,” *Ecotoxicol Environ. Saf.*, vol. 62, no. 3, pp. 397–407, 2005.

[3] G. Libralato, A. V. Ghirardini, and F. Avezzù, “To centralize or to decentralize: An overview of the most recent trends in wastewater treatment management,” *J. Environ. Manage.*, vol. 94, no. 1, pp. 61–68, 2012.

[4] F. Pasciucco, I. Pecorini, and R. Iannelli, “Planning the centralization level in wastewater collection and treatment: A review of assessment methods,” *J. Clean. Prod.*, vol. 375, 2022, Paper no. 134092.

[5] T. Karches, “Detection of dead-zones with analysis of flow pattern in open channel flows,” *Pollack Period.*, vol. 7, no. 2, pp. 139–146, 2012.

[6] T. Karches, “Effect of aeration on residence time in biological wastewater treatment,” *Pollack Period.*, vol. 13, no. 2, pp. 97–106, 2018.

[7] N. Moelants, G. Janssen, I. Smets, and J. Van Impe, "Field performance assessment of onsite individual wastewater treatment systems," *Water Sci. Technol.*, vol. 58, no. 1, pp. 1–6, 2008.

[8] P. Reymond, R. Chandragiri, and L. Ulrich, "Governance arrangements for the scaling up of small-scale wastewater treatment and reuse systems - Lessons from India," *Front Environ. Sci.*., vol. 8, 2020, Paper no. 72.

[9] X. Guo, Z. Liu, M. Chen, J. Liu, and M. Yang, "Decentralized wastewater treatment technologies and management in Chinese villages," *Front Environ. Sci. Eng.*, vol. 8, no. 6, pp. 929–936, 2014.

[10] I. Chirisa, E. Bandauko, A. Matamanda, and G. Mandisvika, "Decentralized domestic wastewater systems in developing countries: the case study of Harare (Zimbabwe)," *Appl. Water Sci.*, vol. 7, no. 3, pp. 1069–1078, 2017.

[11] B. Tang and Z. Zhang, "Essence of disposing the excess sludge and optimizing the operation of wastewater treatment: Rheological behavior and microbial ecosystem," *Chemosphere*, vol. 105, pp. 1–13, 2014.

[12] J. Lienert and T. A. Larsen, "Considering user attitude in early development of environmentally friendly technology: A case study of NoMix toilets," *Environ. Sci. Technol.*, vol. 40, no. 16, pp. 4838–4844, 2006.

[13] J. Boguniewicz-Zabłocka and A. G. Capodaglio, "Sustainable wastewater treatment solutions for rural communities': public (centralized) or individual (on-site) – Case study," *Econ. Environ. Stud.*, vol. 17, no. 44, pp. 1103–1119, 2017.

[14] A. Singh, "A review of wastewater irrigation: Environmental implications," *Resour. Conserv. Recycl.*, vol. 168, 2021, Paper no. 105454.

[15] S. Ofori, A. Puškáčová, I. Růžičková, and J. Wanner, "Treated wastewater reuse for irrigation: Pros and cons," *Sci. Total Environ.*, vol. 760, 2021, Paper no. 144026.

[16] B. Cui and S. Liang, "Monitoring opportunistic pathogens in domestic wastewater from a pilot-scale anaerobic biofilm reactor to reuse in agricultural irrigation," *Water*, vol. 11, no. 6, 2019, Paper no. 1283.

[17] M. Momba, J. Ebdon, I. Kamika, and M. Verbyla, "Using indicators to assess microbial treatment and disinfection efficacy," in *Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project)*, J. B. Rose and B. Jiménez-Cisneros, Eds, Michigan State University, 2019.

[18] A. M. Saunders, M. Albertsen, J. Vollertsen, and P. H. Nielsen, "The activated sludge ecosystem contains a core community of abundant organisms," *ISME J.*, vol. 10, no. 1, pp. 11–20, 2016.

[19] L. Wu, D. Ning, B. Zhang, Y. Li, P. Zhang, and X. Shan, "Global diversity and biogeography of bacterial communities in wastewater treatment plants," *Nat. Microbiol.*, vol. 4, no. 7, pp. 1183–1195, 2019.

[20] Biological Wastewater Treatment (in Hungarian), ÖkoTechHome, 2022. [Online]. Available: www.okotechhome.hu/. Accessed: Mar. 8, 2023.

[21] J. Knisz, P. Shetty, R. Wirth, G. Maróti, T. Karches, I. Dalkó, M. Bálint, E. Vadkerti, and T. Bíró, "Genome-level insights into the operation of an on-site biological wastewater treatment unit reveal the importance of storage time," *Sci. Total Environ.*, vol. 766, 2021, Paper no. 144425.

[22] *28/2004*, Ministry of Environmental Protection and Water Management. Decree No. 28 of 2004 (XII. 25.) KvVM of the Ministry of Environmental Protection and Water Management Concerning Emission Standards of Water-pollutant Substances and Laying Down Rules of Application, Hungary, 2004.

[23] Bálint Analitika Ltd (in Hungarian). [Online]. Available: www.balintanalitika.hu. Accessed: Mar. 8, 2023.

[24] E. Vadkerti and J. Knisz, "Irrigation usability of wastewater treated by an on-site small wastewater treatment unit," in *Proceedings on the Advanced Technologies in the Sewerage Community Systems*, Minsk, Vodokanal, February 12–13, 2020, pp. 36–40.

[25] I. N. Balcom, H. Driscoll, J. Vincent, and M. Leduc, "Metagenomic analysis of an ecological wastewater treatment plant's microbial communities and their potential to metabolize pharmaceuticals," *F1000Res*. vol. 5, 2016, Paper no. 1881.

[26] Y. Fukuyama, M. Inoue, K. Omae, T. Yoshida, and Y. Sako, "Anaerobic and hydrogenogenic carbon monoxide-oxidizing prokaryotes: Versatile microbial conversion of a toxic gas into an available energy," *Adv. Appl. Microbiol.*. vol. 110. pp. 99–148. 2020.

[27] H. J. Flint and S. H. Duncan, "Bacteroides and Prevotella," in *Encyclopedia of Food Microbiology*, C. A. Batt and M. L. Tortorello, Eds, 2nd ed., Elsevier, 2014, pp. 203–208.

[28] F. Magne, M. Gotteland, L. Gauthier, A. Zazueta, S. Pesoa, P. Navarrete, and R. Balamurugan, "The firmicutes/bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients?," *Nutrients*, vol. 12, no. 5, 2020, Paper no. 1417.