

REMOVAL OF PATHOGENS FROM DAIRY WASTEWATER BY UV TREATMENT

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Abstract: *Nutrient-rich wastewater has become a valuable resource for farmers, but if used untreated, the wastewater can pose serious risks for human health. There is potential for wastewater reuse in Romanian agriculture, but different wastewater treatment for agricultural reuse is only tested experimentally. Wastewater samples collected from the lagoon of a cow farm in Romania were tested to determine the microbiological load, expressed as efficiency removal of pathogens at UV treatment with germicidal lamps. Duration of treatment were of 1, 5, 10, 15, 20 and 30 minutes, and highest efficiency was of 87%, showing that UV treatment can be used at large scale for the disinfection of dairy wastewater before its reuse as irrigation water.*

Keywords: *Wastewater, pathogens, health risk, disinfection, UV*

1. Introduction

In recent years, wastewater recycling in agriculture has gained importance as component of agricultural water supply in several water-scarce countries, [1] were farmers in many arid and semiarid areas were forced to find solutions to irrigate their crops. The composition of wastewaters depends on their origin but generally, major contaminants found include organic compounds, xenobiotics, metals, suspended soils, nutrients (mainly nitrogen and phosphorus) and pathogenic microorganisms, [2].

Various studies have pointed out that the pathogens contained in wastewater pose a serious threat to human health and increase the risk for bacterial, parasitic and viral infections in consumers of wastewater irrigated crops, estimating the risk of infection based on the microbial concentration of reused wastewater, [3, 4]. Human and animal pathogens, phytopathogens and antibiotic resistant bacteria and their genes are important biological contaminants that can be transported by wastewater and/or be enriched in soil. Many pathogens are able of survival in the environment (e.g., water, soil, crops) long enough to allow transmission to humans, [5].

Thus, many countries have introduced standards and regulations for the physical-chemical and microbiological parameters of quality of wastewater used for irrigation of food and energy crops. Usually, limits are imposed for physical-chemical (turbidity, pH, salinity or electrical conductivity, suspended solids, heavy metals, BOD, COD, nutrients) and microbiological (*E. coli*, *Salmonella*, *Shigella*, fecal coliforms, fecal enterococci, nematode eggs) parameters, [6]. Regulatory frameworks on the quality of wastewater used in irrigation differ from country to country and compliance with these frameworks requires the analysis of the treated wastewater prior to its reuse.

Romania's accession to the European Union requires compliance with European requirements, and the reuse of wastewater effluents is in line with Objective 6 of the European Union's Sustainable Development Strategy. In Romania, reuse of irrigated water is not largely practiced (there is a low demand for the global use of treated wastewater), and different wastewater treatment methods for reuse are only addressed at the experimental level. In Romania, STAS 9450-88 imposes limits for the microbiological quality of wastewater used for irrigation, and refers to total coliform bacteria, faecal coliform bacteria, faecal *Streptococcus*, and *Salmonella* bacteria. Depending on the microbiological parameters, wastewater is classified into three categories of use:

category M1 – usable for all categories of soil and crops; category M2 – usable for all soils and crops, except very permeable soils and plants destined for food and fodder consumption in fresh state or conserved by freezing, without thermal processing; category M3 – usable for lands with ground level deeper than 4 meters and for crops whose products are processed thermally industrially, or for vegetable non-food crops.

Table 1 presents the limits of microbiological parameters allowed by the Romanian standards of wastewater reuse in irrigated agriculture.

Table 1: Romanian limits of microbiological parameters for wastewater reuse in agriculture

Parameter	Category		
	M1	M2	M3
Total coliform bacteria, probable number / dm ³	absent	> 100 - 100000	> 100000 - 1000000
Faecal coliform bacteria, probable number / dm ³	absent	Max. 10000	> 100000 - 1000000
Faecal <i>Streptococcus</i> , probable number / dm ³	absent	Max. 10000	> 100000 - 1000000
<i>Salmonella</i> bacteria	absent / 1000 cm ³	absent / 300 cm ³	absent / 100 cm ³

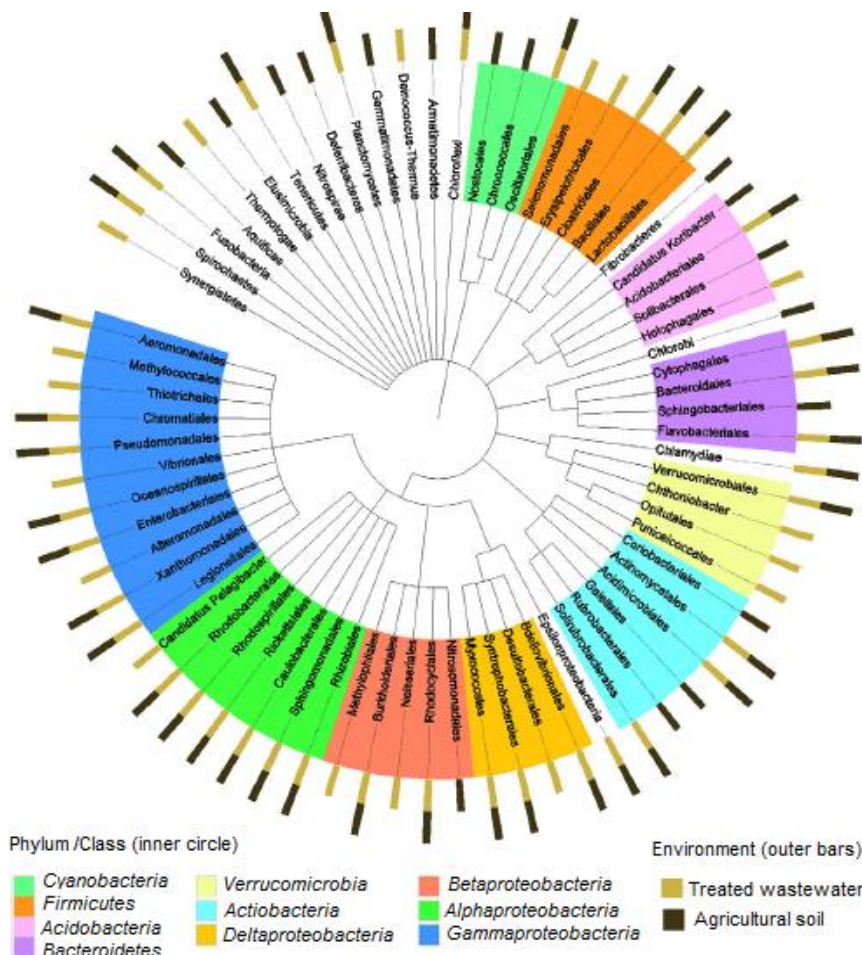


Fig. 1. Dendrogram representation of the bacterial diversity observed in treated wastewater and agricultural soil, [7]

Advanced treatment of wastewater can significantly reduce the concentrations of the specific pathogens in the wastewater and thus, the risk of disease transmission is reduced. However, treated wastewater still contains high loads of bacteria. Disinfection is a necessary step to destroy

or inactivate microorganisms and prevent the spread of dangerous diseases. Disinfection is the process of removing pathogenic micro-organisms from the wastewater. The application of the disinfection process is necessary in the case of industrial waters from slaughterhouses, breeding establishments, tanning, canning factories, food industry where fermentation processes take place. UV water disinfection has been successfully tested for many years, with no negative consequences, while the scope of application extends to many new areas. Of all the methods of wastewater disinfection, UV is one of the most efficient, economical and non-polluting methods available, being considered the fastest, most effective and safest.

UV disinfection has been increasingly developed for water and wastewater disinfection due to its broad-spectrum efficiency against various pathogens and non-formation of disinfection by products, [8]. UV disinfection has been recommended as a substitute for chemical additives for water treatment. Recently, UV disinfection systems have been installed in many water treatment plants in North America and Europe, and small household UV disinfection systems are also available, [9]. UV light disinfects water by permanently deactivating bacteria, spores, molds, viruses and other pathogens, thus destroying their ability to multiply and cause disease. For an effective UV disinfection, it is necessary that a sufficient amount of UV light penetrates through the water and is delivered to the target organisms, [10].

The most common UV sources for current UV disinfection systems are low-pressure (LP) and medium-pressure (MP) mercury lamps. LP lamps have monochromatic emission at a wavelength of 253.7 nm, while MP lamps have polychromatic emission light at a broad range of wavelengths, from 200 to 600 nm, [11]. The effectiveness of a UV disinfection system depends on the characteristics of the wastewater, the intensity of UV radiation, the amount of time the microorganisms are exposed to the radiation and the reactor configuration.

2. Description of laboratory testing method

Wastewater samples were collected from the lagoon of an industrial level cow farm with a total of 570 heads, of which 370 mature cows and 200 calves. The irradiation of UV wastewater was performed using a germicidal UV lamp LBA-e.2 x 15 W, with the power of UV tubes of 30 W, dimensions (Lxhxa) 470x178x70 mm, with wavelength 253.7 nm, and working temperature 10-35 ° C. One liter of wastewater was introduced into a plexiglass parallelepiped tank so that the height of the liquid layer was 2 cm. The germicidal lamp, having a surface roughly equal to that of the wastewater tank, was suspended by means of a metallic device at a distance of 5 cm from the surface of the wastewater (Fig. 2).

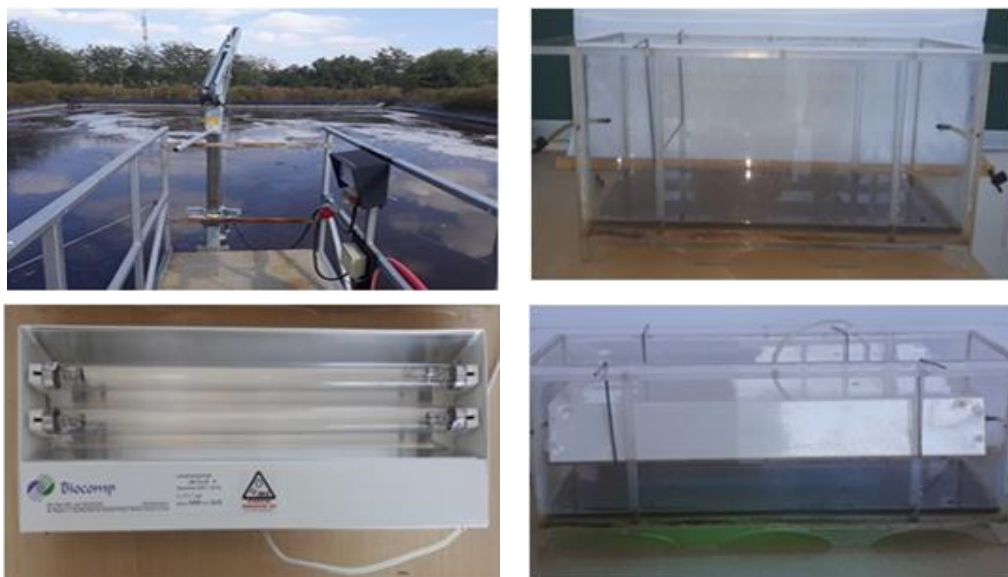


Fig. 2. Laboratory setup for UV treatment of dairy wastewater

In order to determine the total number of germs contained initially the wastewater, and the reduction of germs after UV treatment at different periods of time (1, 5, 10, 20 and 30 minutes), first it was prepared the Plate Count Agar culture medium, with the following composition (g / l): peptone 5.0; glucose 1.0; yeast extract 2.5; agar 15. Culture medium was weighed and prepared in 500 mL Erlenmeyer flasks and sterilized in the autoclave at 121 °C for 15 minutes (Fig. 3).



Fig. 3. Preparation of culture medium

During UV treatment, wastewater samples were taken on the side of the tank with a sterile tip pipette. In tubes containing 9 ml of sterile physiological saline serum, 1 ml volumes of UV-treated wastewater were pipetted at each established treatment time. The wastewater samples were homogenized on a Vortex device for a few seconds and then analyzed in order to determine the total number of germs, by the culture method in Petri dishes.

Into each sterile Petri dish was placed 1 ml of three dilutions of the UV-treated wastewater samples at different time intervals. The culture medium, sterilized and cooled to 45°C, was poured over the wastewater samples, then homogenized by circular movements. After solidification, the Petri dishes were incubated at 37 °C for 48 hours and the resulting colonies of pathogens (germs) were counted. All microbiological analyses were performed in a sterile microbiological hood.



Fig. 4. Inoculation of culture medium with UV treated wastewater and colonies of bacteria in Petri dishes

The result of the determination (expressed as colony forming units per ml - CFU / ml) was multiplied by the dilution factor.

3. Results obtained in wastewater treatment using UV germicidal lamp

The effectiveness of UV treatment, or the percentage inhibition of bacterial growth, was calculated with the equation:

$$UV \text{ rate of inhibition (\%)} = \frac{N_0 - N_p}{N_0} \cdot 100$$

where: N_0 – value of CFU/ml in untreated (initial) sample; N_p - value of CFU/ml in UV treated samples.

Table 2: Results of microbiological determinations on UV treatment

	Duration of UV treatment of wastewater					
	Initial sample	1 minute	5 minutes	10 minutes	20 minutes	30 minutes
Total number of germs, CFU/ml	$7 \cdot 10^7$	$7 \cdot 10^7$	$5 \cdot 10^7$	$4 \cdot 10^7$	$2 \cdot 10^7$	$9 \cdot 10^6$
Rate of inhibition, %	-	0	28%	42%	71%	87%

The results obtained show that the efficiency of UV treatment increases in proportion to the increase in treatment time. As expected for this high turbidity wastewater (529 FAU, determined with the Spectroquant Nova 60 Spectrophotometer before treatment), UV radiation was short-lived being absorbed only by the particles in suspension. Even though the height of the wastewater layer was relatively low (2 cm), the effectiveness of the treatment can be considered quite good under the tested conditions. For this type of wastewater, in treatment plants dedicated to dairy wastewaters, it is recommended to have a particulate removal treatment (decantation or filtration) in advance to UV or other disinfection treatment.

To assess the efficiency of UV treatment on the particulate matters in suspension from the wastewater, kinetic absorbance determinations at 600 nm for 2500 seconds were performed on a UV VIS spectrophotometer, with absorbance recording at 10 seconds, in 'kinetic' of the program.

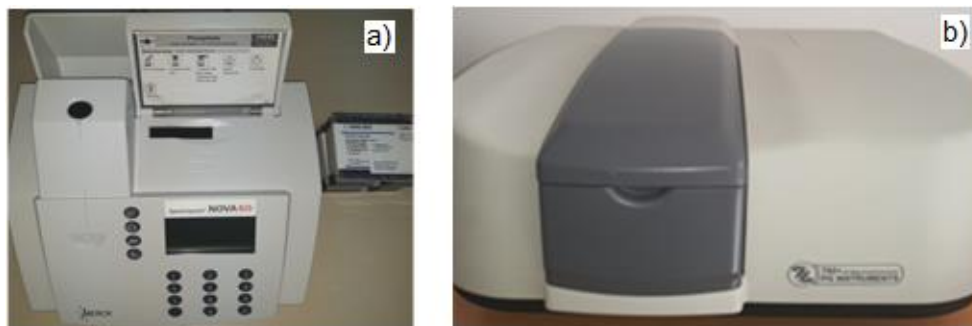


Fig. 5. Spectroquant Nova 60 Spectrophotometer (a) and T912+ PG Instruments UV VIS spectrophotometer (b)

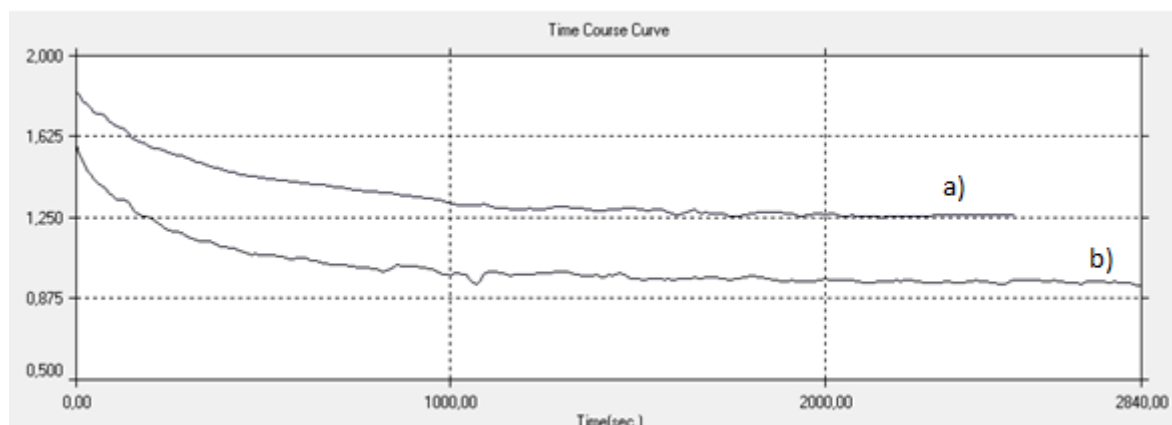


Fig. 6. Variation in time of absorption for wastewater samples:
a) sedimentation curve over time for the UV-treated sample for 30 minutes; b) untreated wastewater

Figure 6 shows that UV radiation caused an increase in wastewater turbidity, which was also easily observed visually at the end of the 30 minutes of UV treatment.

4. Conclusions

Inadequate microbial quality of treated wastewater is a challenge for developing countries, and limits agricultural reuse of wastewater. Human and animal pathogens, phytopathogens and antibiotic resistant bacteria and their genes are important biological contaminants that can be transported by wastewater and/or be enriched in soil, and further transmitted to humans and animals.

The microbial pathogens contained in the wastewater (bacteria, viruses, protozoa, and nematodes) can be removed by physical and biochemical treatment processes. Affordable technological solutions with minimal environmental impacts must be developed in order to ensure wastewater treatment processes compatible with sustainable uses.

For an effective UV disinfection, it is necessary that a sufficient amount of UV light penetrates through the water and be delivered to the targeted microorganisms. In the tested conditions, pathogen removal rate of 87% was achieved for a treatment period of 30 minutes, but at large scale, the removal rate can be of 100% and is influenced by wastewater initial characteristics, the length of wastewater tank and the characteristics of UV disinfection systems.

Acknowledgements

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References

- [1] Pedrero, F., I. Kalavrouziotis, J.J. Alarcón, P. Koukoulakis, and T. Asano. "Use of treated municipal wastewater in irrigated agriculture - review of some practices in Spain and Greece." *Agric. Water Manag.*, vol. 97, pp. 1233–1241, 2010.
- [2] Bitton, G. *Wastewater Microbiology*, Third Edition, Hoboken, New Jersey, John Wiley and Sons, Inc., 2005.
- [3] Elgallal, M., L. Fletcher, and B. Evans. "Assessment of potential risks associated with chemicals in wastewater used for irrigation in arid and semiarid zones: a review." *Agric. Water Manag.*, vol. 177, pp. 419-431, 2016.
- [4] Moazeni, M., M. Nikaeen, M. Hadi, S. Moghim, L. Mouhebat, M. Hatamzadeh, and A. Hassanzadeh. "Estimation of health risks caused by exposure to enteroviruses from agricultural application of wastewater effluents." *Water Res.*, vol. 125, pp.104-113, 2017.
- [5] Campos, C. "New perspectives on microbiological water control for wastewater reuse." *Desalination*, vol. 218, pp. 34–42, Elsevier Ltd., 2008.
- [6] Becerra - Castro, C., A.R. Lopes, I. Vaz – Moreira, E.F. Silva, C.M. Manaia, and O.C. Nunes. "Wastewater reuse in irrigation: a microbiological perspective on implications in soil fertility and human and environmental health." *Int. J. Environ.*, vol. 75, pp. 117–135, 2015.
- [7] Letunic, I., and P. Bork. "Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy." *Nucleic Acids Res.*, vol. 39, pp. 1–4, 2011.
- [8] Hijnen, W.A., E.F. Beerendonk, and G.J. Medema. "Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review." *Water Res.*, vol. 40 (1), 3-22, 2006.
- [9] Brownell, S.A., A.R. Chakrabarti, F.M. Kaser, L.G. Connolly, R.L. Peletz, F. Reygadas, M.J. Lang, D.M. Kammen, and K.L. Nelson. "Assessment of a low-cost, point-of-use, ultraviolet water disinfection technology." *J. Water Health* 6 (1), pp. 53-65, 2008.
- [10] Kollu, K., and B. Ormeçi. "Effect of particles and bioflocculation on ultraviolet disinfection of *Escherichia coli*." *Water Research*, vol. 46, pp. 750-760, 2011.
- [11] Guo-Qiang, L., Wang W-L., Huo Z-Y., Yun Lu, and Hu H-Y. "Comparison of UV-LED and low-pressure UV for water disinfection: Photoreactivation and dark repair of *Escherichia coli*." *Water Research*, vol. 126, pp. 134-143, 2017.