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Article

Study on the efficiency of conventional wastewater treatment processes in relation to variation of microorganisms' populations

DANIELA SIMINA STEFAN[1], CAMELIA UNGUREANU[1*], IOSIF LINGVAY[2], ATTILA TOKOS[2], GHEORGHE PAUNA[1], MIRCEA STEFAN[3], IONELA ROXANA MUSTACIOSU[4]

[1]National University of Science and Technology POLITEHNICA Bucharest, Faculty of Chemical Engineering and Biotechnologies, Gheorghe Polizu 1-7 Street, district 1, Bucharest, Romania, daniela.stefan@upb.ro
[2]ICPE BISTRITA S.A., Parcului 7 Street, Bistrita, Bistrita Nasaud, Romania, iosiflingvay@yahoo.com, attila.tokos@icpebn.ro
[3]University Titu Maiorescu, Pharmacy Faculty, Dambovnicului 20 Street, district 4, Bucharest, Romania, mircea.stefan@prof.utm.ro
[4]Gral Medical SRL, Biochemistry Department, Traian Popovici 79-91 Street, district 3, Bucharest, Romania, ionela_mustaciosu@yahoo.com
**Corresponding author: camelia.ungureanu@upb.ro*

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Abstract
Ensuring effective disinfection of wastewater remains a critical challenge for environmental protection and public health under the new European Directive for wastewater 2024/3019. This study evaluates how conventional treatment processes impact the removal efficiency of microorganisms. Standard treatment steps were assessed, including mechanical processes such as primary sedimentation and coarse screening, biological oxidation, and secondary clarification, to determine their role in reducing both microbial contamination and organic and inorganic loads. The research focused on measuring the removal efficiency of suspended solids, ammonium, chemical oxygen demand (COD-Cr), biochemical oxygen demand (BOD5), total nitrogen, total phosphorus, and microbial populations (fungi, mesophilic bacteria, total and fecal coliforms, fecal streptococci, and selected pathogenic bacteria) at three key points: influent, post-biological treatment, and final effluent. Results showed that microbial removal efficiencies at the final discharge stage ranged from 89% to 100%. Despite these high removal rates, a notable proportion of microorganisms still reached natural receiving waters, potentially impacting aquatic ecosystems and public health. This highlights the need for implementing more effective and environmentally sustainable disinfection methods in the final treatment stage. The findings provide a foundation for further research aimed at optimizing disinfection strategies in relation to influent characteristics, operational control, and compliance with environmental safety requirements.

Keywords: *conventional wastewater treatment, wastewater quality indicators, microorganisms populations, pathogens, efficiency of treatment*

INTRODUCTION

The processes applied in municipal wastewater treatment are essential for managing the chemical and microbiological parameters of effluents. The efficiency of removing contaminants that exceed the maximum permissible discharge limits into natural receptors, as regulated by NTPA 001 (Romanian technical standard for wastewater discharge limits), largely depends on the physicochemical and microbiological composition of the raw water [1÷3]. These waters typically contain elevated levels of ammonium, organic compounds of both natural and anthropogenic origin, total phosphorus, and microorganisms that indicate fecal and pathogenic contamination [4, 5].
Wastewater treatment generally involves primary mechanical processes —screening, grit and grease removal, and primary clarification—to take out settleable solids and floatable. This is followed by secondary stages dedicated to the biological degradation of organic carbon and nitrogen

transformation and removal by nitrification under aerobic conditions and denitrification in anoxic zones (often with an upstream anaerobic zone when phosphorus removal is required), and finally the treated effluent is separated from the biomass through secondary settling [4÷6]. Throughout these stages, the concentrations of coarse particles, suspended solids, sand, organic carbon compounds, ammonium, phosphate, and microorganisms are progressively reduced [7,8].

Key elements targeted by the treatment process include nutrients such as carbon, nitrogen, and phosphorus, as well as toxic substances and microbial contaminants. Microorganisms play a particularly important role in the degradation of organic matter and in nutrient transformations, including nitrification-denitrification and mineralization processes. At the same time, they can serve as indicators of potentially pathogenic domestic wastewater contamination [9,10].

The most relevant groups of microorganisms commonly present in wastewater include fungi and yeasts, which play essential roles in fermentation processes [11÷17], mesophilic bacteria and total coliform bacteria involved in lactose fermentation, and fecal indicator and pathogenic bacteria exemplified by *Escherichia coli, Salmonella spp., Listeria spp*., and *Staphylococcus aureus* [18,19].

This research aimed to assess the removal efficiency of several specific parameters during treatment. Wastewater samples were collected from the Calarasi Water Treatment Plant, operated by ECOAQUA Calarasi. The study focused on standard treatment steps such as mechanical processes (primary sedimentation and coarse screening), biological oxidation, and secondary clarification, evaluating their role in reducing microbial contamination as well as organic and inorganic loads. The assessment included measuring the removal efficiencies of suspended solids, ammonium, chemical oxygen demand (COD-Cr), chemical oxygen demand determined using potassium dichromate (COD-Cr), biochemical oxygen demand (BOD_5), total nitrogen (TN), total phosphorus (TP), and microbial populations (fungi, mesophilic bacteria, total and fecal coliform bacteria, fecal streptococci, and selected pathogenic bacteria).

MATERIALS AND METHODS

To characterize the efficiency of the ECOAQUA Calarasi wastewater treatment plant, samples were collected from three key points: Input, Post-Bio, and Output. All influent, intermediate, and effluent measurements presented in this study were obtained from grab samples collected at a single, representative moment of plant operation. Although every effort was made to sample under typical dry-weather conditions, grab sampling does not account for the diurnal flow and load fluctuations—morning peaks, industrial discharges, wet-weather dilution, or occasional process upsets—that a municipal plant routinely experiences. Consequently, the calculated removal efficiencies reflect the instantaneous performance at the time of sampling rather than a true flow-weighted daily average.

From a physicochemical perspective, the following parameters were determined using specialized equipment: pH (measured with a pH meter, Model 370 Jenway, England), suspended solids (2100Q IS Portable Turbidimeter, LED, 0–1000 FNU, Hach, Germany), chemical oxygen demand determined in the presence of potassium dichromate (COD-Cr), and biochemical oxygen demand over 5 days (BOD_5), measured using an Oxitop Control IS6 system (WTW, British-American company). In addition, total phosphorus and ammonium concentrations were analyzed with a DR3900 Laboratory Spectrophotometer (Hach, Germany).

For the microbiological analyses, samples were collected in sterile vessels, transported in refrigerated conditions (4°C) using sterile containers, and analyzed immediately upon arrival in the laboratory to ensure accuracy and safety. Prior to publication of the results, the operator's consent was obtained. Water samples intended for microbiological testing were collected in accordance with SR ISO 5667-2. All plating procedures were carried out in triplicate to enhance the reliability of the enumeration process. The detailed procedures for sample preparation and analysis are described in reference [19].

The removal efficiency of chemical and microbiological parameters was calculated using the following formula [20]:

The efficiency of chemical and microbiological parameters removal was calculated using the next relationship [20]:

$$\text{Removal efficiency, \%} = (C_{input} - C_{output})/C_{input} \times 100 \quad (1)$$

where:
C_{input} concentration of the parameter to the input of the treatment plant;
C_{output} concentration of the parameter to the output process or Post BIO.
In addition, the chemical and microbial removal efficiency (%) was calculated to express the proportion of concentration and CFU/ml eliminated during treatment stages [20].
To further quantify treatment performance, the log reduction value (LRV) was computed for each bacterial group using the equation [20]:

$$LRV = \log 10\ C_{input}/C_{output} \quad 2)$$

The relationship between log reduction values and removal efficiency (%) was illustrated to highlight the correspondence between logarithmic reduction units and percentage removal.

$$\text{Removal Efficiency (\%)} = (1-10^{-LRV})\times 100 \quad (3)$$

RESULT AND DISCUSSION

To better illustrate the effectiveness of the treatment stages, the removal efficiencies of the main chemical parameters were assessed and are summarized below.
As can be seen in Figure 1, the removal efficiency of ammonium, which had an initial concentration of 62.5 mg/L, was approximately 80% after the biological stage and exceeded 98% in the final effluent. The resulting concentration of 1.12 mg/L is safely below the maximum permissible limit of 2 mg/L. This performance can be attributed to the high efficiency of aeration and biological denitrification processes, which remove approximately 80% of ammonium, while sludge settling processes are responsible for the remaining reduction.
Organic load, expressed as COD-Cr, started at an initial concentration of 402.5 mg O_2/L. These were reduced by approximately 43% after the biological treatment and by up to 88.5% upon discharge, reaching concentrations below the maximum allowable limit of 125 mg O_2/L.
Total nitrogen entered the plant at a concentration of around 52 mg/L. The removal efficiency reached approximately 75% during the biological stage and about 92% overall.
Total phosphorus, with an initial concentration of 4.8 mg/L, showed a removal efficiency exceeding 33% in the biological stage and over 85% for the entire process, with the highest reduction observed during secondary settling.
Finally, the reduction efficiency of the 5-day biochemical oxygen demand (BOD_5) was about 43% at the biological reactor stage, increasing to over 95% in the final effluent.

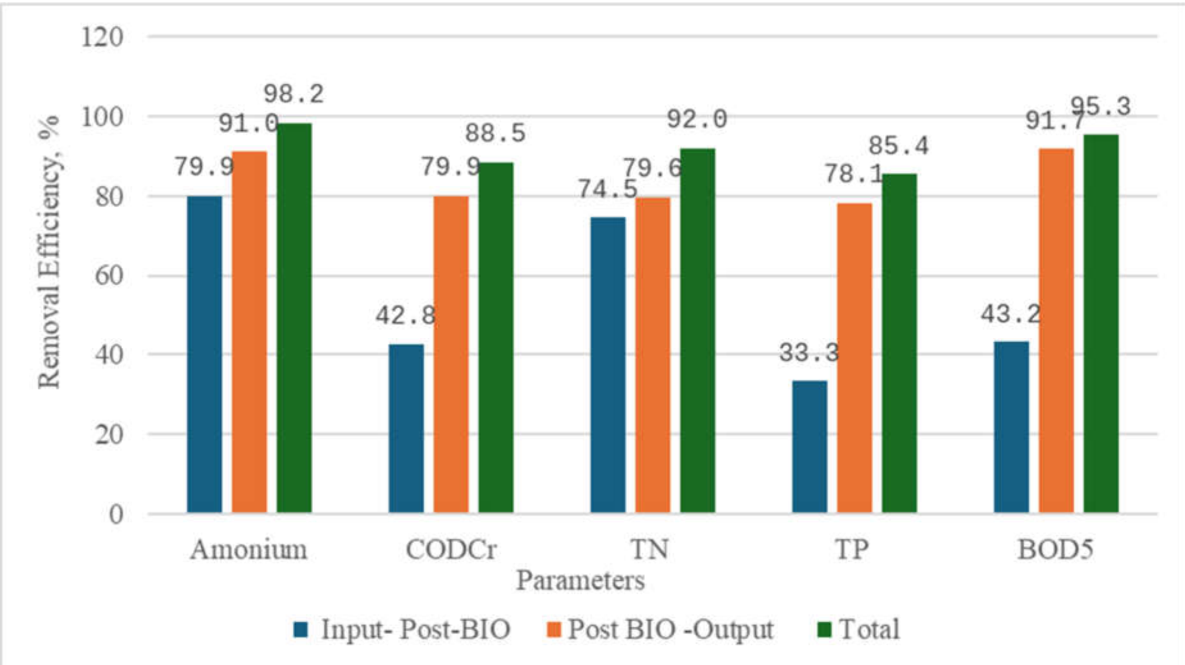

Fig. 1. Comparative removal efficiency (%) of specific chemical parameters across the wastewater treatment process

The removal efficiency for the different categories of microorganisms studied increases progressively throughout the treatment process. The efficiency of fungal removal rises to approximately 64% during the biological stage and reaches around 90% in the final effluent. The concentration of mesophilic bacteria decreases by 84% after biological treatment, with the removal efficiency further improving to 99% in the effluent. For total coliform bacteria, the removal efficiency exceeds 98% in the final discharge. Fecal coliform bacteria show a reduction of over 99%, while fecal streptococci are removed with an efficiency of more than 93%. The enormous microbial load that entered with raw sewage in the wastewater treatment plant was progressively reduced by several complementary pathways. In primary clarification, bacteria physically adsorb to or become embedded in settleable solids, so when these solids settle the associated cells are removed. In the biological stage, floc-forming activated-sludge organisms further scavenge free-floating microbes onto their surfaces, while protozoan grazers and bacteriophages prey on them and the prolonged aeration/starvation cycle imposes oxidative and nutrient stress. Secondary clarification then separates the biomass flocs—and the bacteria tied to them—from the treated effluent. Although the overall number of microorganisms decreases significantly, reaching removal rates above 90%, a notable fraction persists in the treated effluent. These residual microorganisms can affect aquatic food chains and pose health risks to fish and mollusk species in the receiving environment. Therefore, it is essential to assign particular importance to the final disinfection step. Introducing a dedicated disinfection stage into the technological flow is strongly recommended to further enhance microbiological safety.

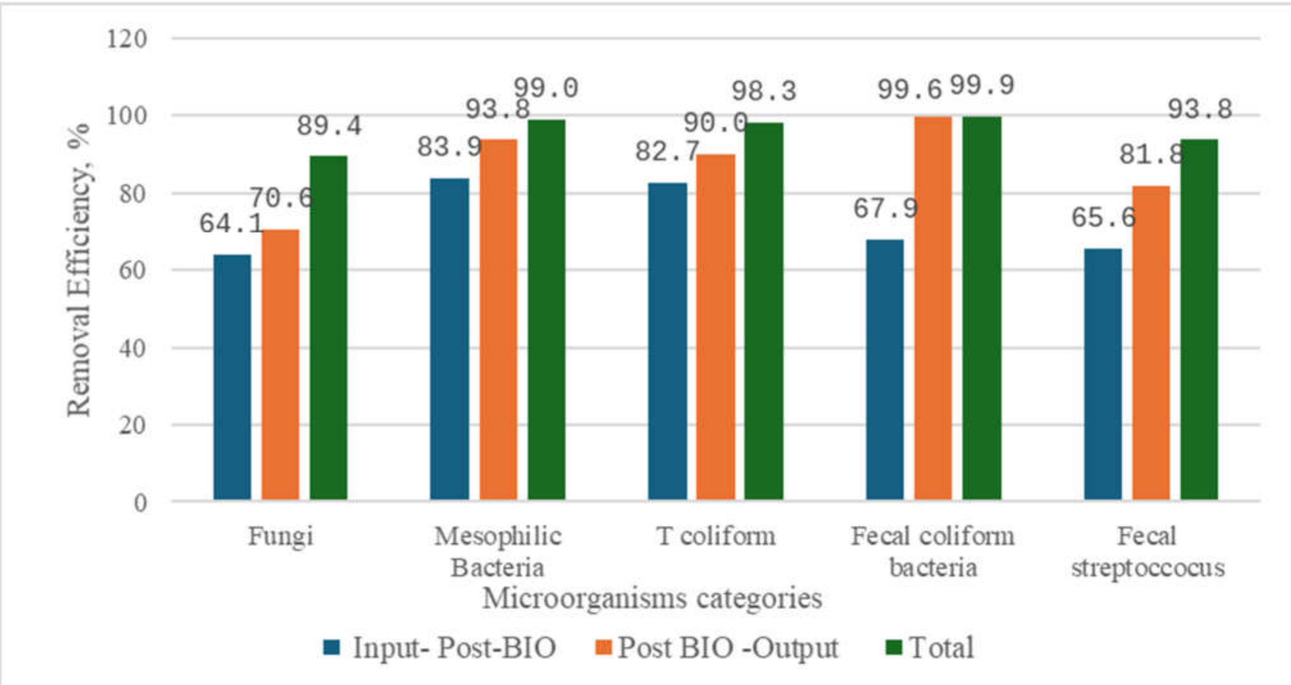

Fig. 2. Comparative removal efficiency (%) of microorganism categories across the wastewater treatment process

The removal efficiencies calculated for the assessed bacterial indicators revealed substantial reductions during the treatment process. *Listeria spp.* was completely removed (100% efficiency), while *E. coli* and *Salmonella spp.* demonstrated removal rates exceeding 99%. *Staphylococcus aureus* showed a slightly lower efficiency of approximately 97%, potentially reflecting its lower initial abundance or variable susceptibility to treatment. These results underscore the high overall performance of the biological treatment system in reducing microbial contamination. Percent removal was derived from mean CFU/mL counts obtained in triplicate, and no error bars were included in the efficiency plot for clarity. The y-axis scale was adjusted to enhance the visual differentiation between bacterial groups.

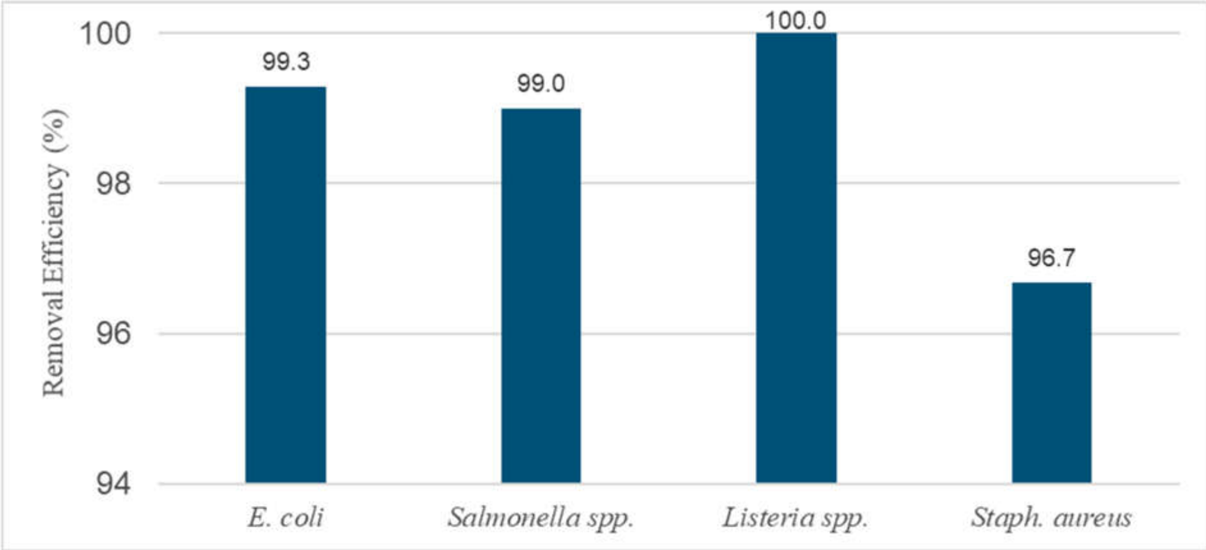

Fig. 3. Comparative removal efficiency (%) of target bacterial indicators across the wastewater treatment process

Table 1 presents the log reduction values (LRVs) achieved for each microorganism, illustrating the performance of the treatment stages in minimizing bacterial contamination.

Table 1. Log Reduction Values (LRVs) of selected microorganisms across the treatment process

Microorganism	Influent (CFU/mL)	Effluent (CFU/mL)	Log Reduction Value (LRV)
Fungi	2.8×10^{16}	3×10^{15}	0.98
Mesophilic Bacteria	3.9×10^{13}	3.9×10^{11}	2
Total coliforms	2.6×10^{6}	4.5×10^{4}	1.76
Faecal Coliforms	1.4×10^{5}	2×10^{2}	2.85
Faecal *Streptoccocus*	3.2×10^{9}	2×10^{8}	1.2
E. coli	2.7×10^{5}	1.9×10^{3}	2.15
Salmonella spp.	3×10^{5}	3×10^{3}	2
Listeria spp.	2.5×10^{3}	1 (assigned)	3.4
Staph. aureus	1.5×10^{2}	5	1.48

Note: Effluent counts < detection limit were set to 1 CFU/mL for calculation purposes

The log reduction values (LRVs) calculated for each bacterial group further illustrate the effectiveness of the treatment process. *Listeria spp.* exhibited the highest reduction, exceeding 3 log units, while *E. coli* and *Salmonella spp.* showed reductions of approximately 2 log units. *Staphylococcus aureus* demonstrated a lower LRV of 1.48, potentially due to its lower initial abundance and environmental persistence. Notably, the influent samples presented a pronounced dominance of fecal indicators, with *E. coli* counts exceeding *Staphylococcus aureus* by a factor of over 1,800. Overall, these results confirm the high efficacy of biological treatment while underscoring the need for continued monitoring of residual contamination. Based on percentage removal, all bacterial groups achieved high removal efficiency (>95%), reinforcing the robustness of the treatment system.

The relationship between log reduction values (LRV) and removal efficiency (%) illustrates the nonlinear nature of microbial inactivation. As shown in Figure 4, an LRV of 1 corresponds to approximately 90% removal, whereas an LRV of 2 achieves 99% efficiency. Increasing LRV further results in near-complete elimination of target microorganisms, with LRVs above 3 exceeding 99.9% removal. This exponential relationship highlights the importance of achieving higher log reductions to ensure microbiological safety, especially for pathogens with low infectious doses. The discrete reference points displayed on the curve serve as benchmarks commonly cited in wastewater disinfection performance standards.

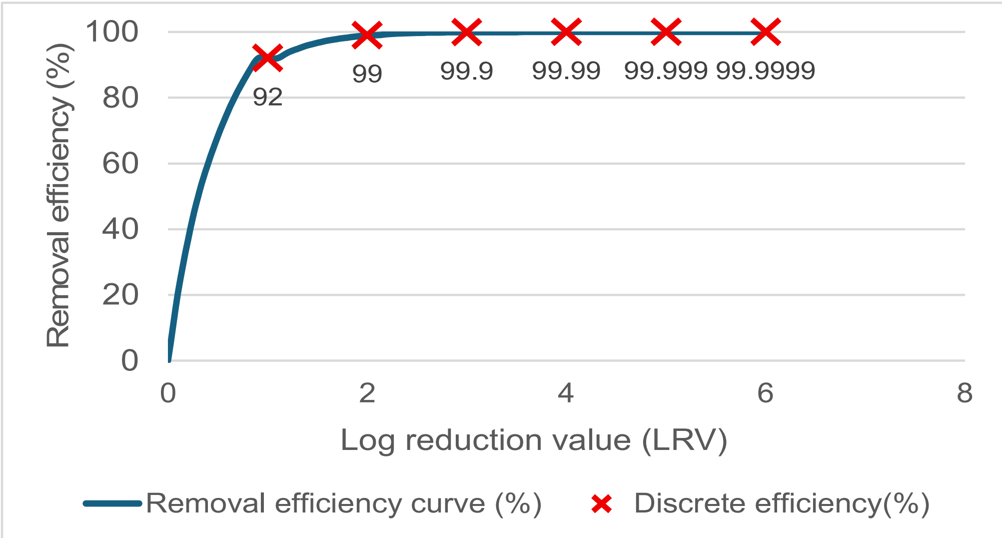

Fig. 4. Relationship between log reduction value (LRV) and microbial removal efficiency (%). *Note:* The curve shows the relationship between LRV, and microbial removal efficiency calculated as $(1-10^{-LRV}) \times 100$

CONCLUSIONS

The ECOAQUA Calarasi wastewater treatment plant demonstrates high efficiency in purifying water from both physical-chemical and biological perspectives, in accordance with the current legislation. Regarding microbial populations, a significant number of microorganisms were observed in the influent, with their concentrations progressively decreasing throughout the treatment stages.

This reduction is mainly attributed to the biological processes that transform complex macromolecular compounds containing carbon, nitrogen, and phosphorus (such as carbohydrates, lipids, proteins, uric acid) into simpler bio transformed compounds (acids, alcohols, amines, amino acids) or mineral substances (phosphates, nitrates, bicarbonates, acetic acid, pyruvic acid).

During these biotransformation processes, the overall content of macromolecular substances decreased, which led to a significant reduction in coliform bacteria populations. However, the population of fecal streptococci remained relatively high, as these microorganisms primarily degrade the intermediate compounds resulting from hydrolysis and redox processes, further converting them into mineral forms.

The removal efficiency for most microorganisms exceeded 99%. For fungi and fecal streptococci, the efficiency was approximately 90÷93%, as these groups are involved in the degradation of smaller molecules during mineralization.

The removal efficiency for pathogenic microorganisms, including *Escherichia coli*, *Salmonella spp.*, *Listeria spp.*, and *Staphylococcus aureus*, reached approximately 99.9%, demonstrating the plant's capacity to significantly reduce public health risks associated with treated effluents.

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