CET CHEMICAL ENGINEERING TRANSACTIONS

VOL. 127, 2026

A publication of AIDIC

The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Laura Capelli, Marzio Invernizzi, Selena Sironi

ISBN 979-12-81206-28-1; **ISSN** 2283-9216

DOI: 10.3303/CET26127056

Effect of *Eisenia Foetida* Population Density on the Efficiency of a Pilot-Scale Tohá Vermifiltration System for Non-Domestic Wastewater Treatment

Steeven Rivera Vasquez[a,*], Miguel Guerra-Saldaña[a], Robert Domínguez Reátegui[a], Jacson Rios Ojanama[b], Luis Cuzco Trigozo[a], Jhon J. López-Rojas[a]

[a]Universidad Nacional de San Martín, Facultad de Ecología, Perú
[b]Universidad César Vallejo, Programa Académico de Maestría en Gestión Ambiental y Desarrollo Sostenible, Peru
striverav15@gmail.com

Non-domestic wastewater generated by municipal markets often presents high pollutant loads that exceed regulatory standards, causing severe damage to sewage infrastructure and aquatic ecosystems. Decentralized and ecological treatment alternatives, such as vermifiltration, are required to address this issue. This study evaluated the effect of *Eisenia foetida* population density on the efficiency of a pilot-scale Tohá system for the treatment of commercial wastewater from a central municipal market. Two parallel reactors were operated with identical local substrates (coarse gravel, sand, coconut fiber, sawdust, and compost) but different earthworm biomasses: 350 g (System 1) and 750 g (System 2). A baseline characterization revealed that the influent's initial concentrations for Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Total Suspended Solids (TSS), and Oils and Greases (O&G) exceeded the Maximum Allowable Values. Wilcoxon and T-tests were employed to evaluate the systems' performance. Results demonstrated that the higher population density in System 2 yielded superior removal efficiencies of 96.4 ± 3.7 % for O&G, 74.6 ± 6.4 % for COD, 77.2 ± 6.7 % for BOD_5, and 78.5 ± 5.9 % for TSS, successfully bringing the effluent within regulatory limits. It is concluded that increasing *E. foetida* density in the Tohá system enhances the degradation of physicochemical pollutants, providing a sustainable solution for non-domestic wastewater management.

1. Introduction

Water is a vital resource, but its quality is continuously threatened by untreated anthropogenic discharges (Yang et al., 2021). Non-domestic wastewater from commercial activities generates large volumes of effluents with elevated physicochemical pollutant loads (Binti, 2017). The direct discharge of these effluents into the sewage system causes severe operational issues, including pipe corrosion, blockages, and reduced efficiency in centralized treatment plants (Anaya and Zegarra, 2015). Developing countries face significant challenges, as a vast majority of wastewater is discharged without prior treatment, endangering environmental and public health (Ferreira et al., 2021). Specifically, commercial activities discharge effluents that consistently exceed the Maximum Allowable Values for parameters such as Oils and Greases (O&G), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), and Total Suspended Solids (TSS) (Rodrigo, 2023). Therefore, the implementation of decentralized, cost-effective, and efficient treatment technologies is essential (Sosa et al., 2014). Vermifiltration has emerged as a sustainable biofiltration technology that combines the symbiotic action of earthworms and microorganisms to degrade organic matter and reduce physicochemical pollutants (Arora and Saraswat, 2021). The Tohá system, a specific configuration of vermifiltration, utilizes filtering layers and the earthworm *Eisenia foetida* to achieve high removal efficiencies while being economically viable and ecologically sustainable (Singh et al., 2017). Previous applications have demonstrated its effectiveness in treating domestic wastewater by significantly reducing organic matter and suspended solids (Castillo and Chimbo, 2021). Furthermore, the system has shown adaptability in treating industrial effluents with high toxic loads (Namaldi and Azgin, 2023). Despite the proven effectiveness of the Tohá system, the specific impact of

Paper Received: 29 May 2026; Revised: 17 July 2026; Accepted: 29 July 2026
Please cite this article as: Rivera Vasquez S., Guerra-Saldaña M., Domínguez Reátegui R., Rios Ojanama J., Cuzco Trigozo L., López-Rojas J.J., 2026, Effect of Eisenia Foetida Population Density on the Efficiency of a Pilot-Scale Tohá Vermifiltration System for Non-Domestic Wastewater Treatment, Chemical Engineering Transactions, 127, 331-336 DOI:10.3303/CET26127056

earthworm population density on the removal of high-strength commercial wastewater parameters requires further investigation to optimize reactor design. This study aims to assess the effect of *Eisenia foetida* population density on the removal efficiency of O&G, COD, BOD_5, and TSS in a pilot-scale Tohá system treating non-domestic wastewater from a municipal market. The specific objectives include characterizing the initial pollutant load, evaluating the removal efficiencies of two systems with different earthworm biomasses (350 g and 750 g), and statistically comparing their performance to establish optimal operational conditions.

2. Materials and methods

This section details the location of the study, the configuration of the experimental units, and the analytical procedures employed.

2.1 Study area

The collection of non-domestic wastewater was carried out at the central municipal market of Moyobamba, Peru, located at the approximate geographical coordinates of 6.03° S, 76.97° W, and an altitude of 874 m above sea level. The experimental treatment system was implemented at the "La Tipishca" Agricultural Research and Production Center of the Instituto de Educación Superior Tecnológico Público "Alto Mayo" (IESTP Alto Mayo) in Moyobamba, situated at 6.05° S, 76.98° W, and 871 m above sea level. Furthermore, the analyses for TSS, COD, and BOD_5 were conducted at the Sanitary Engineering laboratory of the Faculty of Ecology (FECOL) at the Universidad Nacional de San Martín (UNSM), while O&G were evaluated by an external environmental laboratory (NKAP Laboratorios Ambientales).

2.2 Experimental design

To evaluate the effect of *Eisenia foetida* population density on organic matter and physicochemical pollutant removal, the experimental setup was structured following a sequential logical order:
Description of the treatment system: Two pilot-scale Tohá systems (System 1 and System 2) were designed to evaluate the biological degradation of physicochemical pollutants. An elevated 150 L storage tank was installed at a height of 1.10 m to allow the raw wastewater to flow by gravity into the biofilters (Figure 1).
Operation and hydraulic description**:** To maintain an optimal operation, the volumetric flow rate (Q) was determined according to Eq(1) (Alvarado, 2017):

$$Q = \frac{V}{t} \tag{1}$$

where Q is the flow rate (L/s), V is the volume (L), and t is the time (s). Consequently, the hydraulic loading or irrigation rate (IR) was calculated using Eq(2) to ensure it remained below the maximum threshold of 1 $m^3/m^2/day$ (Salazar, 2005):

$$IR = \frac{Q}{A} \tag{2}$$

where IR is the irrigation rate ($m^3/m^2/day$) and A is the cross-sectional area of the filter (m^2). A continuous flow rate of 150 mL/min was maintained, establishing an optimal IR of 0.19 $m^3/m^2/day$.

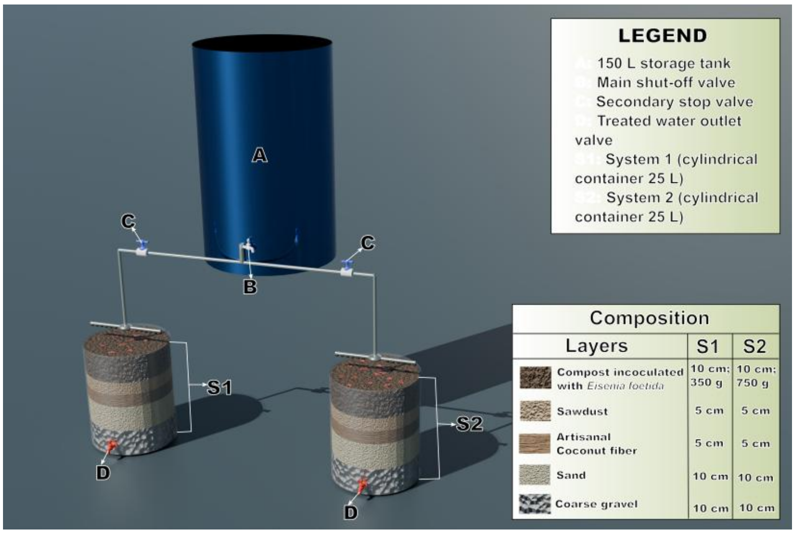

Figure 1: Schematic design of the pilot-scale Tohá systems

Evaluated factors: The primary independent variable was the earthworm population density. The reactors consisted of cylindrical containers with a volume of 25 L, a height of 41 cm, and a diameter of 32 cm. The biofiltration bed was assembled using five layers of locally available substrates to promote ecological sustainability. From bottom to top, the foundational strata for both systems included 10 cm of coarse gravel, 10 cm of sand, 5 cm of artisanal coconut fiber, and 5 cm of sawdust. To evaluate the effect of population density, the top active layer varied between the reactors. System 1 was equipped with a 10 cm layer of compost inoculated with 350 g of Eisenia foetida (yielding a spatial density of 4.35 kg/m²). System 2 contained a 10 cm layer of compost inoculated with 750 g of E. foetida (equivalent to 9.33 kg/m²). The nominal empty bed contact time (EBCT) for both reactors was approximately 2.8 hours.

2.3 Sampling and analytical methods

Prior to the treatment phase, the earthworms underwent a 7-day acclimatization period in the organic substrate. During this phase and the subsequent operation, the environmental conditions were monitored to ensure optimal earthworm development, maintaining an average pH of 6.9 and a temperature of 24.4 °C.
Wastewater samples were collected from the influent storage tank and from the discharge valves of both reactors on days 5, 10, 15, and 20 of continuous operation. The monitored parameters included O&G, COD, BOD_5, and TSS. The sampling protocols utilized amber glass bottles for O&G and plastic containers for the remaining parameters. In the laboratory, the analytical methods were conducted as follows: TSS concentrations were determined by direct measurement using a DIST1 sensor after homogenizing the samples. COD was evaluated through a colorimetric method using a DR 900 colorimeter, which involved sample dilution (1:20 ratio), a 2-hour digestion phase, and subsequent cooling. BOD_5 was measured by recording the initial dissolved oxygen with an H198193 meter, incubating the samples in 300 mL Winkler bottles at 20 °C for 5 days, and registering the final oxygen concentration. Lastly, O&G levels were analyzed according to the EPA Method 1664 Rev. B (2010). The removal efficiency (E) of the biofilters for each parameter was calculated using Eq(3):

$$E = \frac{(C_i - C_f)}{C_i} x100 \quad (3)$$

where E is the removal efficiency (%), C_i is the initial concentration of the parameter in the influent (mg/L), and C_f is the final concentration in the treated effluent (mg/L).

2.4 Data analysis

To evaluate the pollutant reduction and the effect of *Eisenia foetida* population density, descriptive and inferential analyses were performed. The Shapiro-Wilk test was applied to determine the normality of the data distribution. To statistically compare the initial influent and final effluent concentrations, the non-parametric Wilcoxon test was utilized for O&G and TSS, while the paired samples T-test was applied for COD and BOD_5. Furthermore, to evaluate regulatory compliance, both the initial influent concentrations and the final treated effluents were directly compared against the Maximum Allowable Values (MAV) established by Peruvian environmental regulations (D.S. N° 010-2019-VIVIENDA). Finally, an independent samples T-test was conducted to compare the removal efficiencies between the systems, evaluating how a higher *Eisenia foetida* population density favors the reduction of physicochemical pollutants. All statistical analyses were generated using the R version 4.5.1 (www.r-project.org).

3. Results and discussion

The findings of this study encompass the baseline properties of the raw effluent and the comparative pollutant removal performance of the biofilters.

3.1 Initial wastewater characterization

The raw non-domestic wastewater collected from the municipal market presented elevated pollutant concentrations that significantly exceeded the Maximum Allowable Values (MAV) for discharge into the sewage system (Table 1). These high initial loads reflect the diverse commercial activities of the market, which generate complex organic effluents that can cause severe blockages and corrosion in sanitation infrastructure, a common operational issue reported by Uría (2021), and specifically observed in markets handling hydrobiological products as noted by Carhuallanqui-Torres et al. (2025).
After treatment, a significant overall reduction in pollutant concentrations was achieved across all evaluated parameters ($p < 0.05$). Regarding regulatory compliance, System 2 consistently outperformed System 1, successfully bringing the effluent completely within the MAV limits for all physicochemical parameters (Table 1). In contrast, System 1 only achieved regulatory compliance for O&G and TSS.

3.2 Effect of earthworm density on removal efficiency

Following the acclimatization phase under optimal environmental conditions, both Tohá systems demonstrated a robust capacity to degrade pollutants. However, their removal efficiencies varied based on the *Eisenia foetida* population density.

Table 1: Initial concentration and final concentrations of the pilot-scale Tohá systems

Parameter	MAV (mg/L)	Initial Concentration	Final Concentration ST 1 (mg/L)	Final Concentration ST 2 (mg/L)
O&G	100.0	202.9 ± 167.6	5.16 ± 2.53*	3.18 ± 1.82*
COD	1,000.0	2,480.0 ± 1,457.4	1,145.0 ± 644.4*	562.5 ± 189.6*
BOD_5	500.0	1,595.8 ± 938.0	653.0 ± 315.1*	317.5 ± 81.1*
TSS	500.0	776.5 ± 394.2	240.0 ± 108.3*	151.5 ± 31.8*

** Significant differences between influent and effluent concentrations ($p < 0.05$) according to Wilcoxon (O&G, TSS) and paired T-tests (COD, BOD_5).*

Statistical evaluation confirmed differences in the removal efficiencies between the two systems for COD, BOD_5, and TSS, heavily favoring the reactor with the higher biomass (Figure 2). This superior performance can be scientifically attributed to the intensified biological and mechanical actions of a larger earthworm population. *Eisenia foetida* actively consumes suspended organic particles, transforming them into vermicast containing highly active microbial consortia (Khwairakpam, 2020). Furthermore, the higher density of earthworms significantly increased bioturbation, creating microscopic channels in the filter bed (Arora and Saraswat, 2021). This passive aeration mechanism enhances oxygen diffusion, fostering a highly oxidative environment that accelerates the aerobic degradation of dissolved organic matter (Singh et al., 2017). Furthermore, specific removal calculations reveal that while System 2 removed a higher total mass of COD per day, System 1 exhibited a higher specific removal rate per gram of earthworm biomass (0.82 g COD/g biomass/day vs 0.55 g COD/g biomass/day), indicating different metabolic dynamics at varying population densities."

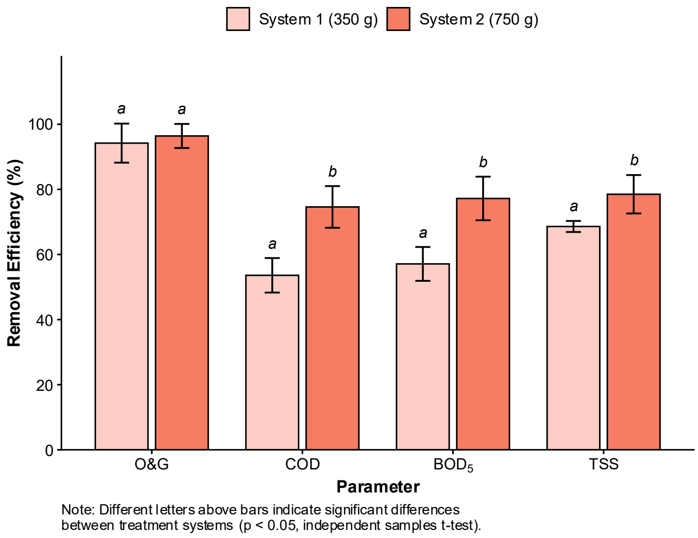

Figure 2: Comparative removal efficiencies between System 1 (350 g) and System 2 (750 g)

Conversely, the statistical analysis indicated no difference in the removal of O&G between the two systems ($p = 0.556$). Both reactors achieved remarkable O&G removal rates above 94 %. This phenomenon suggests that the degradation of fats and oils in the Tohá system is primarily governed by the physical filtration mechanisms of the substrate rather than the biological ingestion by the earthworms. The local materials incorporated, specifically coconut fiber and sawdust, provided a high specific surface area and structural porosity that effectively adsorbed the lipid molecules (Salazar, 2005) and can offer resilience against variable hydraulic shock loads (Sharma and Kazmi, 2014). The role of the earthworms in this specific process was primarily indirect, preventing physical clogging by consuming the accumulated bio-cake and maintaining the hydraulic conductivity of the filter bed over time, a mechanism described by Gwebu and Mpala (2022) and further supported by the anti-clogging intricacies detailed by Singh et al. (2018).

Despite the promising results, this study presents certain limitations that must be acknowledged. First, the evaluation lacked true parallel reactor replication, relying instead on longitudinal sampling to assess temporal performance. Second, the experimental phase was conducted at a pilot scale with a strictly controlled continuous flow rate. Consequently, the long-term operational stability, the physical clogging behavior of the filter media over extended periods, and the system's resilience to sudden hydraulic or organic shock loads, which are typical fluctuations in commercial market activities, require further investigation.

4. Conclusions

The implementation of the modified Tohá system proved to be a highly effective ecological alternative for degrading non-domestic wastewater generated by commercial markets. Increasing the population density of *Eisenia foetida* enhanced the biological degradation of key physicochemical pollutants. System 2 achieved superior removal efficiencies for COD, BOD_5, and TSS, successfully bringing all final concentrations completely within the regulatory Maximum Allowable Values. The higher earthworm density had a significant positive effect on the reduction of these parameters, although the removal of Oils and Greases remained statistically similar between both systems at highly efficient levels.

From an industrial and practical perspective, these outcomes demonstrate that decentralized vermifiltration is a viable strategy to manage complex commercial effluents. By effectively reducing pollutant loads below regulatory thresholds, municipal markets, restaurants, and similar non-domestic users can prevent the corrosion and blockage of centralized sewage infrastructure. Furthermore, the adoption of this technology offers a direct economic benefit by eliminating the supplementary operational costs and municipal fees associated with exceeding allowable discharge limits. The utilization of locally sourced substrates, such as artisanal coconut fiber and sawdust, also ensures that the system remains economically accessible for installation in developing regions.

Nomenclature

A – cross-sectional area of the filter, m²
BOD_5 – Biochemical Oxygen Demand, mg/L
C_f – final concentration in the treated effluent, mg/L
C_i – initial concentration in the influent, mg/L
COD – Chemical Oxygen Demand, mg/L
E – removal efficiency, %
IR – irrigation rate, m³/m²/day
O&G – Oils and Greases, mg/L
Q – volumetric flow rate, L/s
t – time, s
TSS – Total Suspended Solids, mg/L
V – volume, L

Acknowledgments

The authors gratefully acknowledge the "Alto Mayo" Public Technological Higher Education Institute (IESTP Alto Mayo) for providing the facilities at the "La Tipishca" Agricultural Research and Production Center for the implementation of the pilot systems. We also extend our gratitude to the Faculty of Ecology at the Universidad Nacional de San Martín (UNSM) for granting access to the Sanitary Engineering laboratory to conduct the necessary physicochemical analyses.

References

Alvarado E., 2017, Flow measurement manual (in Spanish), Technical Manual, Private Institute for Climate Change Research (ICC), Guatemala.

Anaya I., Zegarra C.R., 2015, Analysis and proposals to the regulatory framework of non-domestic wastewater discharges to sewerage networks (in Spanish), Master Thesis, Universidad del Pacífico, Lima, Peru.

Arora S., Saraswat S., 2021, Vermifiltration as a natural, sustainable and green technology for environmental remediation: A new paradigm for wastewater treatment process, Current Research in Green and Sustainable Chemistry, 4, 100061.

Binti S.N., 2017, Wet market wastewater regulatory framework in Malaysia, Master Thesis, Universiti Teknologi Malaysia, Johor Bahru, Malaysia.

Carhuallanqui-Torres M.N., Benites-Alfaro E., Lizarzaburu-Aguinaga D., Cabrera Carranza C., Jave Nakayo J., Suca-Apaza G.R., Atuncar Yrribari J.F., López Bulnes J., 2025, Worm-filter in the Recovery of Wastewater from the Commercial Activity of Hydrobiological Products, Chemical Engineering Transactions, 117, 433-438.

Castillo J.G., Chimbo J.E., 2021, Efficiency in organic matter removal using vermifilters (Eisenia foetida) in domestic wastewater for rural areas (in Spanish), Enfoque UTE, 12(2), 80–99.

Ferreira M.M., Fiore F.A., Saron A., Da Silva G.H.R., 2021, Systematic review of the last 20 years of research on decentralized domestic wastewater treatment in Brazil: state of the art and potentials, Water Science and Technology, 84(12), 3469–3488.

Gwebu L., Mpala C., 2022, Application of vermifiltration for domestic sewage treatment, In: Wastewater treatment, IntechOpen, London, UK.

Khwairakpam M., 2020, Vermifiltration for rural wastewater treatment, In: Earthworm Assisted Remediation of Effluents and Wastes, Springer Singapore, Singapore, 19–34.

Namaldi O., Azgin S.T., 2023, Evaluation of the treatment performance and reuse potential in agriculture of organized industrial zone (OIZ) wastewater through an innovative vermifiltration approach, Journal of Environmental Management, 327, 116865.

Rodrigo K. del R., 2023, Evaluation of non-domestic wastewater discharge and alternatives for the reduction of physicochemical concentrations, Moyobamba - San Martin (in Spanish), Bachelor Thesis, Universidad Nacional de San Martín, Tarapoto, Peru.

Salazar P.I., 2005, Tohá system: An ecological alternative for wastewater treatment in rural sectors (in Spanish), Bachelor Thesis, Universidad Austral de Chile, Valdivia, Chile.

Sharma M.K., Kazmi A.A., 2014, Effect of physical property of supporting media and variable hydraulic loading on hydraulic characteristics of advanced onsite wastewater treatment system, Environmental Technology, 36, 2634-2645.

Singh R., Bhunia P., Dash R.R., 2017, A mechanistic review on vermifiltration of wastewater: Design, operation and performance, Journal of Environmental Management, 197, 656–672.

Singh R., Bhunia P., Dash R.R., 2018, Understanding intricacies of clogging and its alleviation by introducing earthworms in soil biofilters, Science of the Total Environment, 633, 145-156.

Sosa D.B., Vigueras J.M., Holguín E.J., 2014, Biofiltration: A sustainable alternative for wastewater treatment (in Spanish), Vidsupra Visión Científica, 6(2), 56–60.

Uría M. del R., 2021, Quality infrastructure for compliance with non-domestic wastewater legislation in Peru (in Spanish), Master Thesis, Universidad Nacional Agraria La Molina, Lima, Peru.

Yang D., Yang Y., Xia J., 2021, Hydrological cycle and water resources in a changing world: A review, Geography and Sustainability, 2(2), 115–122.