Heterotrophic activity in the active layer of a vermifilter used for sewage treatment: effects of worm density and seasonality

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Abstract

The active layer of a vermifilter (VF) is composed of woodchips that contain heterotrophic bacteria and worms capable of removing organic matter. However, there is no information on heterotrophic microbial kinetics related to oxygen uptake rate (OUR_{end}), specific oxygen uptake rate ($SOUR_{end}$), and heterotrophic biomass yield (Y_H) as they relate to evaluating the removal of COD (chemical oxygen demand) and BOD_5 (biochemical oxygen demand) from wastewater. The goal of this study was to assess the heterotrophic activity of the active layer of a full-scale VF that treats rural wastewater. In addition, the effects of seasonality and earthworm density were determined. Heterotrophic activity was evaluated with a respirometer using active layer (wood chip) samples with earthworm densities of 1105 ± 982 earthworm/m^3 (Zone A) and 7221 ± 1699 earthworm/m^3 (Zone B), in fall-winter and spring-summer. The results show that the VF has a Y_H of 0.47 ± 0.1 and a growth rate (μ_{max}) of 0.05 ± 0.04 d^{-1}. The Y_H value was 0.39 in fall-winter and 0.54 in spring-summer, while the μ_{max} value was 0.07 d^{-1} in fall-winter and 0.02 d^{-1} in spring-summer. OUR_{end} and $SOUR_{end}$ increased 95% more in spring-summer than in fall-winter and a 64% more in Zone B than Zone A. This increase in heterotrophic activity corresponds to increases of 15.6% in COD removal and 12.2% in BOD_5 removal in spring-summer. These results indicate that seasonality and earthworm density affect kinetic parameters such as OUR_{end} and $SOUR_{end}$. Finally, the measurement of kinetic parameters allows the COD and BOD_5 removal efficiency of a VF to be estimated.

Keywords: Sewage treatment; Active layer; Heterotrophic activity; Respirometry; Kinetic parameters

1. Introduction

According to the World Health Organization monitoring program, 6 out of every 10 people do not have access to safely managed sanitation services. This lack of coverage in final wastewater treatment generates water quality deterioration, loss of biodiversity, and unhealthy environments that lead to the transmission of diarrheal diseases such as cholera, dysentery, and typhoid fever [1,2]. Therefore, wastewater must be treated prior to its discharge, a process carried out by conventional wastewater treatment plants (WWTP), which use technologies that include activated sludge. However, this technology is unsuitable for rural areas with populations of density below 300 inhabitants per km^2. In these areas, centralized technology has limitations such as the absence of sewerage system, energy costs for pumping systems, geographical and climatic limitations, low socioeconomic level, lack of technical assistance, skills to operate the systems. [3]. Therefore, it is necessary to find decentralized technologies that minimize the risks of wastewater discharges in these areas.

In such settings, vermifilters (VF) are an alternative to conventional WWTP. These nature-based systems operate with the synergistic activity of earthworms and microorganisms that change the physicochemical properties of wastewater [4]. The ingestion, burrowing, and excretion behaviors of earthworms reinforce this relationship, as they generate an aerated environment that promotes microorganism growth [5]. This facilitates the removal of organic matter, nutrients, and pathogens by aerobic microorganisms and reduces costs, as no injection pumps are used [6]. Some studies indicate that VF can reach removal efficiencies of 89.0% for organic matter measured as chemical oxygen demand (COD), 79.5% for total nitrogen, and 87.0% for total phosphorous [7, 8, 9]. However, as with any biological treatment systems, performance depends on operating conditions [10]. Earthworm density, temperature, filter medium, hydraulic loading rate, and organic loading rate are design and operating parameters and thus key factors that affect the removal organic matter, removal nutrient and greenhouse gases that reach 1975.5 mg/m^2h for CO_2, 36.5 mg/m^2h for CH_4 and 12.3 mg/m^2h for N_2O [5,7,11]. In a VF, biological processes occur in the active layer

, a filter medium that facilitates the adsorption of nutrients from sewage (e.g phosphorus and nitrogen), and that contains autotrophic and heterotrophic microorganisms [12,13]. Some studies have indicated that over 90% of organic matter removal in an aerobic medium could be related to heterotrophic aerobic bacteria [12,14]. However, few studies have addressed the behavior and growth of the heterotrophic biomass of the active layer of a full-scale VF [15]. To assess how this design and operating parameters affect organic matter removal efficiency, respirometry techniques can be used to model, understand, and monitor the biological processes that occur in a VF based on the estimation of kinetic parameters of the heterotrophic aerobic biomass [16]. Respirometry is defined as the measurement of the biological consumption rate of an inorganic electron acceptor [17]. This technique allows the aerobic biological processes achieved by the microorganism biomass to oxidize an organic substrate to be reproduced at laboratory scale [10]. Therefore, the characterization of kinetic parameters of the aerobic biological process provides activity values for the heterotrophic biomass responsible for removing organic matter from wastewater in a WWTP [17,18]. The main kinetic parameters in this analysis are OUR_{end}, $SOUR_{end}$, Y_H, and the maximum growth rate of the biomass (μ_{max}). For example, in an activated sludge system, it has been observed that a high OUR_{end} of 15.3 $mgO_2/L\cdot h$ or low μ_{max} of 0.047 reflects problems in the system operating conditions or even indicates toxicity levels in the influent [19,20]. This type of analysis therefore provides information for modeling and optimizing the main aerobic processes that degrade organic matter via heterotrophic biomass growth [21]. The respirometric method is one of the most widely used techniques for characterizing sewage and evaluating the microbial response in activated sludge systems [16]. However, it has proven to be highly effective in determining kinetic and stoichiometric parameters specific to particular types of biomasses, such as, moving bed biofilm reactor (MBBR) [17]. Therefore, although respirometry is a biological method used especially for activated sludge [18], technological advancements allow respirometry to be applied as a diagnostic tool in other types of unconventional plants [16], such as VF systems. Thus, the objective of this study is to use respirometry techniques to assess the heterotrophic activity of the active layer of a full-scale VF and determine the effects of seasonality and earthworm density on removal of the organic matter contained in wastewater.

2. Materials and Methods

2.1 Design and operating conditions

This study was carried out in a rural WWTP located in Copiulemu Commune, Concepción, in the Biobío Region, Chile. This region is a mediterranean climate. In dry months, rainfall reaches 35 mm and the temperature 25°C. In wettest month, rainfall reaches 350 mm and temperature from 0°C to 13°C [22,23]. This WWTP uses a sequential system that includes pretreatment for solids removal, followed by a VF and radiation ultraviolet for disinfection protected by a roof that covers the entire system. The VF is a vertical subsurface flow system, and its main characteristics are summarized in Table 1. Wastewater enters a rotary filter that has a helical screw to sweep solids from an external container to the VF. Once treated, this wastewater is stored in an accumulator tank and transferred via two pumps to the upper part of the VF, from where it is distributed over the surface via 24 sprinklers. The VF operates with a flow, hydraulic loading rate (HLR), and organic loading rate (OLR) of 160 m^3/d, 0.6

m^3/m^2d, and 0.5 $kgCOD/m^2d$, respectively. The filter material is stratified, with a lower layer of stones and an upper layer of wood chips measuring 0.92 ± 0.12 m. The latter layer is the active layer, where earthworms of the species *Eisenia foetida* and heterotrophic and autotrophic microorganisms are responsible for biological reactions [12,13]. The earthworm density was studied by taking samples between different seasons and different heights of the active layer. No differences were observed between seasons. Moreover, the Zone A of 104 cm and Zone B of 80 cm showed densities of 1105 ± 982 $earthworm/m^3$ and 7221 ± 1699 $earthworm/m^3$, respectively.

Table 1

2.2 Monitoring strategy

The monitoring strategy involved samples between May 2022 and November 2023, during the Southern Hemisphere fall-winter and spring-summer. Figure 1 shows a diagram with the VF and the active layer monitoring strategy. In Zones A and B of the VF, samples of the active layer were taken from different points by inserting a tube 12 cm in diameter and 100 cm in length. Wood chips were collected from the inside of the tube to calculate the earthworm density. Wastewater samples were taken from the pretreatment outlet (IN), the VF outlet (E1), and the UV outlet (E2). All samples were kept at a temperature of 4°C for less than 24 h until their analysis [24,25].

Figure 1

2.3 Analysis of liquid samples

The liquid samples – IN, E1, and E2 – were filtered with a Whatman membrane with a pore size of 0.7 µm and analyzed according to the protocol described in the standard method [26]. The organic matter present in the samples was determined by measuring COD (colorimetric method, 5220-D) and biological oxygen demand (BOD_5) (azide-modified Winkler method, 5210-B).

2.4 Respirometry analysis

The respirometry experiments were carried out to determine the kinetic response of the heterotrophic microorganisms in the active layer [16]. The respirometry test was applied to the solid samples of Zone A (1105 ± 982 worm/m^3) and Zone B (7221 ± 1699 worm/m^3) of the VF.

Figure 1

Wood chip biofilm was used to analyze the active layer. The solid sample was diluted in distilled water at a proportion of 1:20 to obtain concentrations in ranges of 1000-2000 mgSSV/L [10,25]. The experiment was performed using a BM-T respirometer. This is a batch method that includes a recirculation pump, with two experiments carried out: static OUR_{end} and $SOUR_{end}$ mode, which determines the endogenic oxygen uptake rate of the biomass, and dynamic R mode, which analyzes the evolution of the oxygen uptake rate to degrade an exogenous biodegradable substrate (sodium acetate). The samples were placed in a 1-liter tank in endogenous conditions to begin the experiment at a constant temperature of 20±1°C. The device allows agitation and aeration and includes an oximeter that measures and records data in BM-Advance software [10]. To assess the kinetic parameters of the heterotrophic activity, the autotrophic species were inhibited with 1-4 mg/ml of allylthiourea (ATU). Table 2 shows the main kinetic parameters related to heterotrophic activity that were calculated via BM-Advance, which is integrated into the device.

Table 2

2.5 Statistical analysis

To assess the effects of seasonality on COD, BOD_5, and the kinetic parameters OUR_{end} and $SOUR_{end}$, statistical analyses were performed using RStudio version 4.3.2, with a significance level of $p = 0.05$. Shapiro–Wilk and Levene tests were carried out to analyze the normality and homogeneity of variance, respectively. Subsequently, an ANOVA test was performed [27].

3. Results and discussion

3.1 Kinetic parameters and heterotrophic activity of the active layer

Table 3 shows the main kinetic parameters of heterotrophic biomasses from different wastewater treatment plants: OUR_{end} ($mgO_2/L\cdot h$), $SOUR_{end}$ ($mgO_2/gVSS\cdot h$), Y_H ($mgCOD_m/COD_s$); μ_H (d^{-1}), μ_{max} (d^{-1}), and K_d (d^{-1}). The kinetic parameters in the VF presented variations with respect to other types of technology.

Table 3

3.1.1 Oxygen consumption (OUR_{end} and $SOUR_{end}$)

In the VF OUR_{end} was 1.22±0.59 mgO_2/L·h and $SOUR_{end}$ was 1.24±0.63 mgO_2/gVSSh. These values are within the ranges observed in plants used for wastewater treatment such as CAS, UCT-MBR, and UASB-MBR which presented OUR values between 1.6 and 6 mgO_2/L·h. [10, 20, 28]. In the CAS studied by Arias et al. [19], OUR_{end} reached 15.3 mgO_2/L·h. This increase is caused by an overload of the heterotrophic biomass that affects wastewater treatment efficiency [19]. In the case of the CAS studied by Abdi et al. [15], OUR_{end} reached 0.05 mgO_2/L·h. Low values are explained by toxic substances present in the influent (i.e., sulfides) that affect biomass activity. Variations in OUR_{end} and $SOUR_{end}$ allow the metabolic function of heterotrophic microorganisms to be evaluated [20] and associated with different operating conditions to quantify and detect possible disturbances in a VF (i.e., influent retention times and organic loading rate, [19, 30]. Regarding organic loading rate (OLR), Llamas et al. [10] determined in a UASB-MBR that an OLR of 0.4 KgCOD/m^3d generates maximum OUR values ranging from 1.65 mgO_2/L·h to 2.61 mgO_2/L·h. At 0.7 Kg COD/m^3d, the OUR decreases due to system stabilization problems and at 0.2 Kg COD/m^3d the OUR decreases due to lack of substrate for microorganism growth [31,32]. In the case of the studied VF, the OLR was 0.54 Kg COD/m^3d; this design parameter is within the optimum range for this type of technology (0.2-0.6 Kg COD/m^3d) [5]. When the OLR is below 0.2 kg COD/m^3d, the growth of autotrophs outcompetes the growth of heterotrophs due to the limitation of organic carbon. This could reduce the COD removal efficiency below 77% [5]. Therefore, in VF the OUR_{end} and $SOUR_{end}$ can be used to determine the optimal design and operating parameters that affect the active layer [7,33,34) and organic matter degradation processes [5,35].

3.1.2 Yield coefficient (Y_H)

The Y_H in the VF was 0.47±0.11 mgCODm/CODs. This parameter is below the 0.6-0.8 mgCODm/CODs range of CAS, OSA, UCT-MBR, and UASB-MBR [20]. Abdi et al. [15], in a CAS, found low Y_H values, which reached 0.2 mgCODm/CODs due to the toxic effect of sulfides. Therefore, if the influent has no toxic effect, Y_H indicates the capacity of the system to generate biomass via heterotrophic activity [15]. In a VF, this process occurs in the active layer, which contains earthworms and microorganisms that form the biofilm [13]. Di Trapani et al. [29] compared the heterotrophic and autotrophic activity of the biofilm and suspended biomass in an IFAS-MBR. This study found that systems with suspended biomass promote heterotrophic activity, with a Y_H of 0.66, whereas systems with biofilm promote autotrophic activity, with a Y_A of 0.47. These differences can be explained by the trade-off that exists between microorganisms. For example, in MBBR stabilized promotes autotrophs of slow-growing specialized in nitrification achieving Y_A of 0.6 and Y_H of 0.45 [36]. Otherwise, in MBR (Membrane bioreactors), promotes heterotrophs of fast-growing specialized in removing organic matter achieving Y_H of 0.46 and Y_A of 0.18 [23,28,29].

3.1.3 Growth rate (μ_H) and endogenous decay constant (K_d)

The μ_{max} in the VF was 0.05±0.04 d^{-1}. This parameter is below the 0.4 – 9 d^{-1} range of CAS, OSA, UCT-MBR and UASB-MBR [10,20,29]. In CAS, Dehnavi et al. [20] and Abdi et al. [15] also found low values between 0.047-0.17 d^{-1}. This value is related to system operating conditions (i.e., influent toxicity, sludge state, hydraulic retention times). Regarding the VF, low μ_{max} values are explained by the low growth of the heterotrophic biomass due to the type of microbiome that develops in the active layer. Earthworms in a VF promote the development of heterotrophic microorganisms that participate in the degradation of organic matter and autotrophic microorganisms that participate in nitrification processes [11,37, 38]. The two microorganism types compete in the active layer and respond to different growth kinetics [39]. Leyva et al. [21] found that the immobilized biomass of the biofilm presents an autotrophic μ_{max} of 0.6-0.7 d^{-1} and heterotrophic μ_{max} of 0.02-0.11 d^{-1}, near the heterotrophic μ_{max} of the VF. Whereas heterotrophs grow quickly and depend on organic carbon sources, autotrophs grow slowly and depend on inorganic carbon sources [40,41]. Meanwhile, the earthworm gut acts as a reserve of denitrifying microorganisms. In the presence of nitrates, nitrites, and organic carbon of the medium, different facultative anaerobic denitrifiers survive in the external environment and compete with the microbiological community [5,7]. Oxygen is another factor that limit μmax in a VF, the burrowing behavior of earthworms introduces between 0.6 and 0.9 mg/L of oxygen in the active layer [42]. In contrast, moving bed biofilm reactors (MBBRs) can achieve oxygen concentrations between 1.5 and 5 mg/L through mechanical aeration, which enhances cellular synthesis within the biofilm [43]. With respect to kinetic parameter K_d, it is related to biomass loss and microorganism decay rate. The VF presented a loss of 3±1% of the biomass that developed in the active layer, which is among the characteristics of the microorganism and earthworm consortium [10]. In a VF there are various processes such as endogenous decay of microorganisms, destruction of extracellular polymeric substances, selection of slow-growing bacteria, and predation of bacteria by higher organisms such as earthworms [16,44,45]. This biomass loss is counteracted by humic substances and the retention capacity of the active layer [33,46], which promotes the development of the microbial community [47,48], generating an equilibrium between biomass growth and the loss rate.

3.2 Effects of seasonality on heterotrophic activity

Table 4 shows the kinetic parameters of the heterotrophic biomass in different seasons. The OUR_{end} (mg/L·h) and $SOUR_{end}$ (mg/gVSSh) parameters increased more than 95% in spring-summer relative to fall-winter. In a VF the main mechanisms that determine the removal of organic matter are related to the activity of earthworms that accelerate the development of the microbial community [5]. *Eisenia fétida* is a poikilothermic species that lives in an optimum range of 15-20°C [49]. Therefore, temperatures within this range improve the symbiotic relationship between the earthworms and heterotrophic microorganisms that determine organic matter removal [50]. This explains the increase in Y_H from 0.39 to 0.54 mgCODm/mgCODs. This increase in the heterotrophic biomass is consistent with other studies in which increases in COD removal of 6% to 15% in warm periods were observed [11,50]. However, in the early years of the VF, physical processes such as the adsorption mechanisms of the woodchip could maintain COD removal efficiency between 65% and 88% without season difference [8,31]. Meanwhile, kinetic parameter μ_{max}, decreased from 0.07 d^{-1} in fall-winter to 0.02 d^{-1} in spring-summer and K_d increased from 0.02 d^{-1} in fall-winter to 0.04 d^{-1} in spring-summer. These differences in K_d could be due to toxic compounds such as antibiotics in wastewater [11,51]. For example, Bermúdez et al. [52] and Dehnavi et al. [20] have found that there is a decrease in μ_{max} and variations in K_d in the presence of antiobiotics. This compound toxic can increase the energy required for generating specific inactivating enzymes to prevent the toxic effects, this could decrease μ_{max} from 0.04 to 0.005 [20,30], while maintaining OUR of 2.25 mg/Lh and Y_H of 0.54. Besides, variations observed in these kinetic parameters can also be produced by operating conditions in the VF [28]. En sprin-summer, a decrease of 90% in rainfall [22], combined with population fluctuation in tourist areas [53], can raise the organic loading rate (OLR), from 0.2 to 5.1 kg COD/m^{2}·d [54].The elevated OLR, driven by the increased influx of organic matter, clog the pores of the vermifilter and reduce the oxygen available in the system [55]. In extreme dissolved oxygen deficit, the aerobic bacterial population might undergo an endogenous phase [56] and restrict the growth of microorganisms, $\mu_{max.}$

Table 4

3.3 Effects of earthworm density on heterotrophic activity

Figure 2 shows significant differences between the seasons and monitored zones for OUR_{end} and $SOUR_{end.}$ In winter and spring, Zone B (7221 ± 1699 earthworm/m^{3}) presented greater oxygen uptake rates than Zone A (1105 ± 982 earthworm/m^{3}), with an increase in OUR of over 80% and an increase in SOUR of over 64%. Earthworm density is a VF design parameter [11, 57]. Earthworms can directly affect the formation of the biofilm, determining its morphology and final composition [7,33]. The body of the earthworm excretes stable aggregates and a mucus that increases the bacterial biodiversity involved in the degradation of organic matter from 0.8 to 1.1, according to the Shannon index [58]. Furthermore Li et al., 2013 [44], found that VF increases enzymatic activity between 9%-214% for protease, 11%-22% for dehydrogenase, 18%-32% for lipase, 12%-35% for amylase [59,60]. Therefore, a medium with greater earthworm density promotes the development of the active layer biofilm and can modify kinetic parameters [10,61]. Zones with a greater earthworm quantity generate environments with dissolved and colloidal organic matter that allow microorganisms to survive, reproduce, and form the biofilm [11,44,61].

Figure 3

3.4. Organic matter removal and heterotrophic activity in the VF

Figure 3 shows the COD and BOD_5 concentrations in IN, E1, and E2 of the VF in fall-winter and spring-summer, along with the oxygen uptake rate (OUR_{end}). The COD removal efficiency in the VF was 64.4% - 80.9% and the BOD_5 removal efficiency was 77.6% - 91.5%. A comparison of efficiency levels between seasons reveals no significant differences in E1: 284±77 mg/L (64.4%) for COD and 134±38 mg/L (77.6%) for BOD_5 in fall-winter and 332±55 mg/L (64.0%) for COD and 115±41 (82.6%) mg/L for BOD_5 in spring-summer. This is related to the decrease in the adsorption capacity of the active layer due to excessive VF operating times without cleaning or change of the filter medium [62]. In this case, solids increase in E1 settle quickly in the outlet chamber, which can occur when cleanings are more than eight months apart [63]. By contrast, significant differences were found in effluent E2: 266±47 mg/L (65.3%) for COD and 132±45 mg/L (79.3 %) for BOD_5 in fall-winter and 173±29 mg/L (80.9%) for COD and 55±27 mg/L for BOD_5 (91.5%) in spring-summer. As mentioned, *Eisenia fétida* lives in an optimal range of 15-20°C [49]; therefore, temperatures outside this range affect the synergy between the earthworm and the aerobic microorganism consortium responsible for organic matter degradation [50]. In this study, the temperature was 11±2°C in fall-winter and 17±4°C in spring-summer. This difference affects the metabolic activity of the earthworms and the symbiotic relationship with microorganisms that facilitate organic matter removal. However, the adaptability of earthworms to survive within a temperature range of 5–29°C and a pH range of 4.5–9 helps maintain system robustness, achieving removal efficiencies of 65.3–80.9% for COD and 79.3–91.5% for

BOD_5. [11]. The foregoing is consistent with the increase in OUR_{end} in the active layer, which went from 1.0 $mgO_2/gL·h$ in fall-winter to 1.6 $mgO_2/gL·h$ in spring-summer. Therefore, the increase in heterotrophic activity can facilitate COD and BOD_5 removal in the VF in spring-summer [5]. It can thus be concluded that the measurement of OUR allows the organic removal capacity of a VF to be estimated.

3.5 Sustainable design

The economic sustainability of the vermifiltration technology has also been enlightened by creating a bridge with the concept of circular bio-economy [39]. However, the lack of standardized systems significantly reduces the likelihood of valorizing solid waste or sewage without compromising public health. With a HLR of 0.6 $m^3/m^2·d$ and an OLR of 0.54 kg $COD/m^3·d$, the VF achieved a removal efficiency of 173 mg/L (65.3%) and 266 mg/L (80.9%) for COD. These values are below those reported in other studies, which have shown removal efficiencies ranging from 77.3% to 89.0% with HLR below 2 $m^3/m^2·d$ and OLR between 0.2–0.6 kg $COD/m^3·d$ [5]. On the other hand, Gutiérrez et al. [11] observed that VF systems do not remove Fecal Coliforms (FC) to safe levels in the effluents (i.e. 6.9 log_{10} MPN/100 mL). Therefore, the concentrations of COD and FC discard sewage reuse as a viable option for irrigation [64]. Regarding the active layer, although some authors suggest that the vermicompost generated by earthworms can be applied to agricultural soils to utilize adsorbed nutrients such as nitrogen and phosphorus [4,39], there is still insufficient evidence to demonstrate effective pathogen reduction in these solids without additional treatment [5]. Concerning emissions, few studies have assessed the impact of these systems on Greenhouse Gas generation [3]. Specifically in VF systems, Zhao et al. [7] and Huang et al. [65] demonstrated that these systems can generate emissions of up to 1975.5 $mg/m^2·h$ of CO_2, 36.5 mg/m^2·h of CH_4, and 12.3 mg/m^2·h of N_2O. In these systems, CO_2 is produced through organic matter oxidation under aerobic conditions; CH_4 through organic matter reduction in anaerobic conditions [55] and N_2O is generated via nitrification and denitrification of nitrogen compounds present in sewage [66].

4. Conclusions

Considering the results of this study, the following can be concluded:

- The microorganism growth rate in the system has a Y_H value of 0.39-0.54 mgCODm/CODs and a μ_{max} of 0.02-0.07 d^{-1}.
- Earthworm density affects kinetic parameters OUR_{end} and $SOUR_{end}$. Zone B (7221 ± 1699 earthworm/m^3) promotes heterotrophic species activity and generates increases of over 64% in OUR_{end} and $SOUR_{end}$ with respect to Zone A (1105 ± 982 earthworm/m^3).
- Seasonality affects heterotrophic species activity. Under temperature values of 17±4°C in spring-summer, the kinetic parameters OUR_{end} and $SOUR_{end}$ increased by 95% in the VF compared to fall-winter. Furthermore, the COD removal efficiency increased by 15.6% and BOD_5 removal efficiency increased by 12.2% in spring-summer.

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Author contributions

Gutiérrez, V.: Compilation and analysis of information, original draft, methodology, writing, review, and editing. **Gomez, G.**: Layout and graphic design and image editing. **Rodríguez, D.C.:** Review and editing. **Vidal, G**.: Conceptualization, validation, research, resources, original draft, writing, review and editing, visualization, supervision, and project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table Captions

Table 1. Summary of design and operating parameters of the vermifilter.

Table 2. Key kinetic parameters of the respirometry test.

Table 3. Kinetic parameters of heterotrophic biomasses in different WWTPs.

Table 4. Kinetic parameters of the heterotrophic biomass in a VF between seasons.

Parameter	Unit	Value
Volume	m^3	442
Area	m^2	276
Height	m	1.60
Influent flow	$m^{3/}d$	160
HLR	m^3/m^2d	0.60
Influent COD	kg COD	0.89
OLR	kg COD/m^2d	0.54
Carbon/nitrogen ratio	-	9
Active layer	-	Wood chips
Active layer height	m	0.92 ± 0.12
Active layer volume	m^3	254
Species	-	*Eisenia foetida*
Earthworm density Zone A	worm/m^3	1105 ± 982
Earthworm density Zone B	worm/m^3	7221 ± 1699

Note: HLR: hydraulic loading rate; OLR: organic loading rate; COD: chemical oxygen demand.

Table 1

kinetic parameters	Unit	Equation	Description
Oxygen uptake rate (OUR_{end})	$mgO_2/L\cdot h$	$\Delta OC/\Delta t$	Oxygen consumption rate
Specific oxygen uptake rate ($SOUR_{end}$)	$mgO_2/gVSSh$	OUR/VSS	Oxygen consumption rate of active biomass
Heterotrophic biomass yield (Y_H)	mgCODm/mgCODs	$1\text{-}\Delta OC/COD$	Biomass generated per unit of exogenous substrate
Endogenous decay constant (K_d)	d^{-1}	$SOURend/1.48$	Mortality constant of heterotrophic biomass
μ_H	d^{-1}	$Yhvss\cdot 24\cdot (U/X)\text{-}Kd$	Heterotrophic biomass growth rate
μ_{max}	d^{-1}	$\mu H\cdot (Ks+S)/S$	Maximum heterotrophic biomass growth rate

Note: Fcv: 1.48. Stoichiometric adjustment factor for reactions involved in the organic matter degradation process [10]; U: Substrate use rate (mgCODs/h); X_H: Active heterotrophic biomass; CODm: COD of microorganisms generated; CODs: COD of the substrate consumed; Ks: Semi – Saturation constant; S: COD removed.

Table 2

Type	**OUR_{end}** (mgO_2/L·h)	**$SOUR_{end}$** (mgO_2/gVSSh)	**Y_H** (mgCODm/mgCODs)	**μ_{max}** (d^{-1})	**K_d** (d^{-1})	**References**
UCT-MBR	2-6	-	0.57-0.74	0.40-2.40	0.26*	[28]
IFAS-MBR	-	-	0.64	8.99	0.19*	[29]
IFAS-MBR			0.66	3.66	0.24*	[29]
CAS	-	-	0.67	6.0	0.21*	[16]
OSA	-	-	0.67	6.0	0.32*	[16]
UASB-MBR	1.55	1.49	0.71*	-	0.03	[10]
UASB-MBRs	2.20	1.15	0.41*	-	0.02	[10]
CAS	3.60-15.30	0.9-4.0	0.86	2.28-3.00	0.06	[19]
CAS	0.053	-	0.23	0.17	0.01	[15]
CAS	1.76-2.28	-	0.73*	0.047	1.45*	[20]
VF	1.22	1.24	0.47	0.05	0.03	This study

Note: UCT: Anaerobic-anoxic-aerobic. MBR: Membrane bioreactors. MBRs: Membrane bioreactors with supported biomass. IFAS: Integrated fixed film activated sludge. CAS: Conventional activated sludge. OSA: Oxic settling anoxic. UASB: Upflow anaerobic sludge blanket. VF: Vermifilter. OUR: Oxygen uptake rate. SOUR: Specific oxygen uptake rate. VSS: Volatile suspended solids. CODm: COD of microorganisms generated; CODs: COD of the substrate consumed. * YH was calculated with fcv= 1.48. Stoichiometric adjustment factor for reactions involved in the organic matter degradation process [10]; *Kd was calculated with the decay rate bH.

Table 3

Parameter	Unity	Fall-Winter	Spring-Summer
OUR_{end}	mg/L·h	1.15	2.25
$SOUR_{end}$	mg/gVSSh	1.05	2.25
Y_H	mgCODm/ mgCODs	0.39	0.54
μ_{max}	d^{-1}	0.07	0.02
K_d	d^{-1}	0.02	0.04

Note. OUR: Oxygen uptake rate. SOUR: Specific oxygen uptake rate. VSS: Volatile suspended solids. CODm: COD of microorganisms generated; CODs: COD of the substrate consumed.

Table 4

Figure captions

Figure 1. Schematic representation of a VF and respirometry test.

Figure 2. Respirometry test in different seasons and with different earthworm densities. a) OUR_{end} and b) $SOUR_{end}$. Where ■ Zone A: 1105 ± 982 worms/m^3; ■ Zone B: 7221 ± 1699 worms/m^3.

Figure 3. Concentrations (mg/L) of a) COD and b) BOD_5 in IN (■), E1 (■), and E2 (■) and OUR_{end} in fall-winter and spring-summer

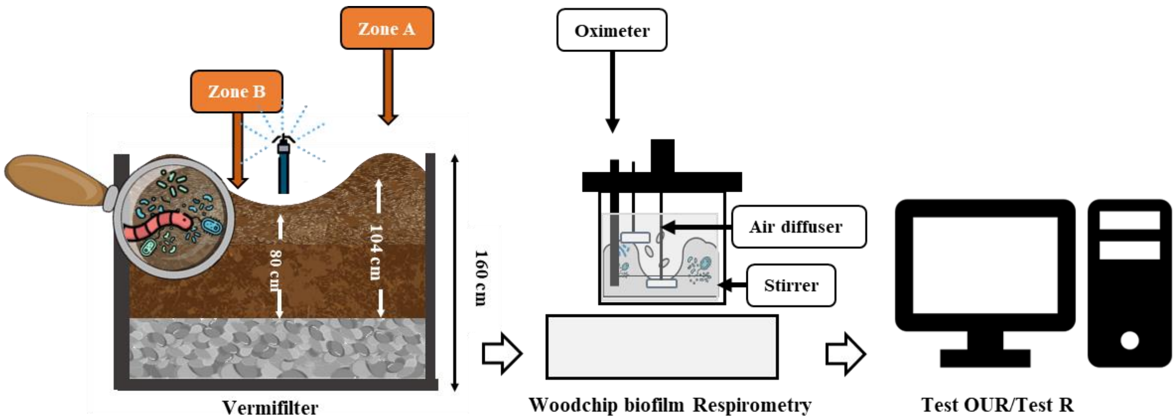
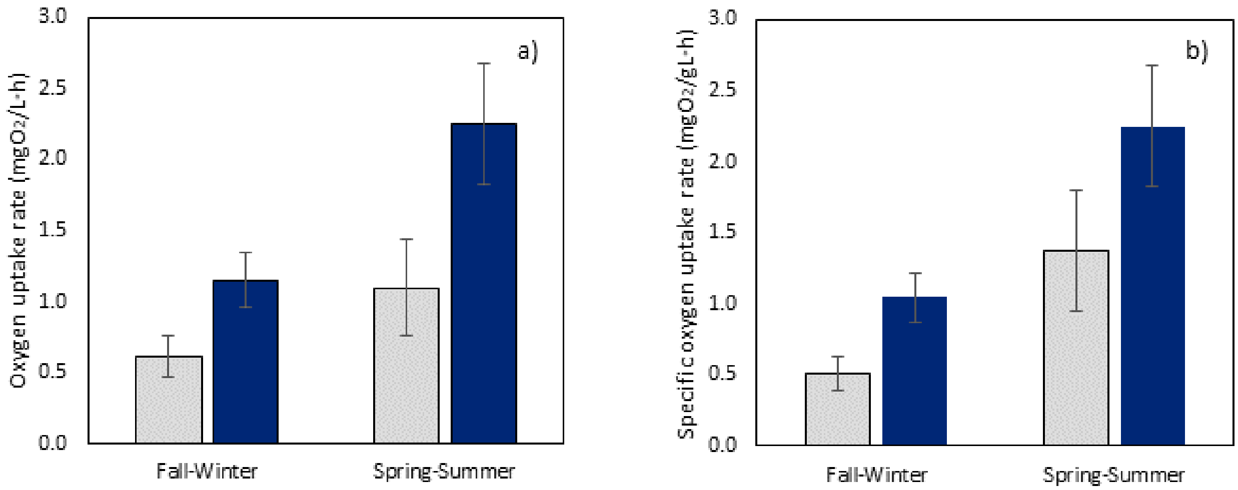

Zone A
Zone B
80 cm
104 cm
160 cm
Vermifilter
Oximeter
Air diffuser
Stirrer
Woodchip biofilm Respirometry
Test OUR/Test R

Figure 1



a)
Oxygen uptake rate (mgO2/L·h)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Fall-Winter
Spring-Summer
b)
Specific oxygen uptake rate (mgO2/gL·h)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Fall-Winter
Spring-Summer

Figure 2

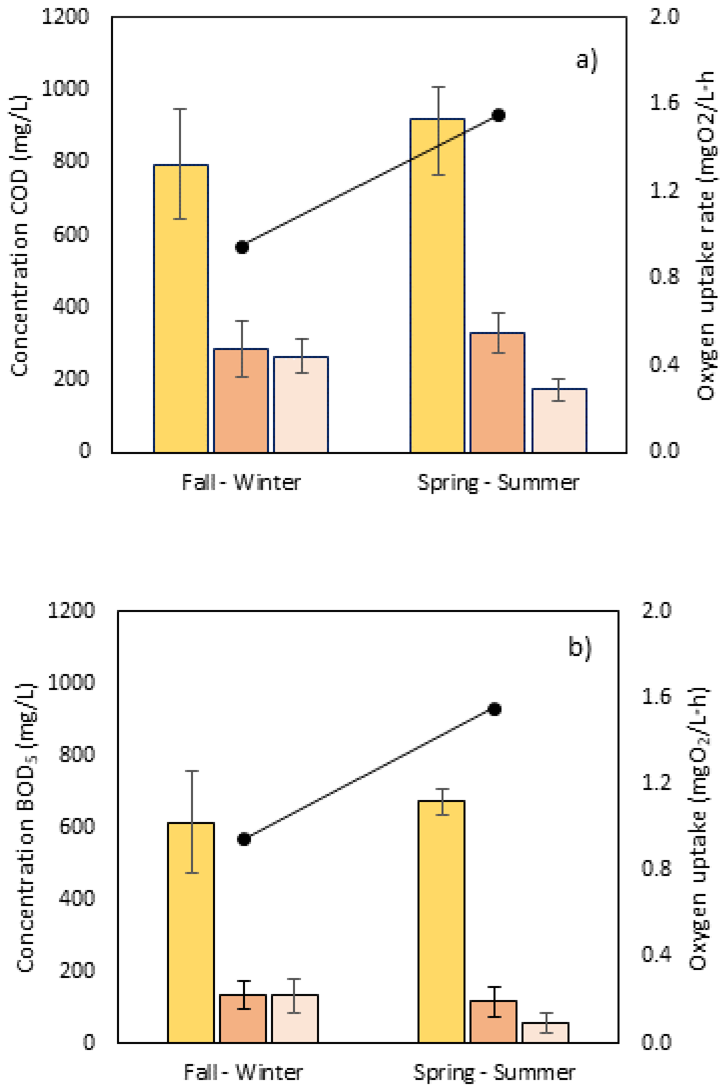

a)
Concentration COD (mg/L)
Oxygen uptake rate (mgO2/L·h
0
200
400
600
800
1000
1200
0.0
0.4
0.8
1.2
1.6
2.0
Fall - Winter
Spring - Summer
b)
Concentration BOD_5 (mg/L)
Oxygen uptake (mgO_2/L·h)
0
200
400
600
800
1000
1200
0.0
0.4
0.8
1.2
1.6
2.0
Fall - Winter
Spring - Summer

Figure 3