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INDICE GENERAL

INDICE DE TABLAS

INDICE DE FIGURAS

RESUMEN

Chile, es uno de los países más vulnerables a la escasez hídrica, y se estima que al menos el 25% de la sequía experimentada por el país desde el 2009, estaría relacionada con el cambio climático. Estos eventos de escasez hídrica afectan por, sobre todo, el suministro y el tratamiento de las aguas servidas que provienen de zonas rurales y que actualmente son administradas por Comités y Cooperativas. Aunque el 2018, la cobertura de agua potable llegó a un 99% en las zonas rurales concentradas, aún existen más de 300 mil viviendas que no cuentan con un sistema apropiado de abastecimiento y saneamiento de aguas residuales.

En regiones, las zonas rurales que presentan mayores problemas y que no cuentan con un abastecimiento formal de agua potable, se encuentran en el Biobío (68%), La Araucanía (71%), los ríos (62%) y Los lagos (64%). Por lo general, las viviendas que se encuentran en estas zonas no cuentan con la infraestructura suficiente para instalar un sistema de saneamiento adecuado y solo cuentan con pozos negros y fosas sépticas. Por lo tanto, los vermifiltros se han planteado como una solución viable para estas localidades.

La vermifiltración es un proceso bio-oxidativo en el que las lombrices detritívoras interactúan intensamente con microorganismos para eliminar los contaminantes que se encuentran presentes en el agua. Este sistema consta de un estanque relleno por diferentes capas filtrantes, con lombrices en la capa superficial que pueden bioacumular, biodegradar y/o biotransformar diferentes tipos de contaminantes. Aun cuando estos sistemas han sido presentados como una solución plausible para los sectores rurales, el 48% de los vermifiltros implementados presentan fallas por parámetros de operación que aún siguen siendo estudiados y que guardan relación con la tasa de carga de nutrientes, la tasa de carga hidráulica, el medio filtrante, la cantidad de lombrices y las emisiones de gases que pueden generar estos procesos de degradación. Sobre estas materias, aun no existe un consenso científico que indique cuales son los parámetros de funcionamiento óptimo de los vermifiltros y como las lombrices impactan el balance de los gases de efecto invernadero (GEI). Es por esta razón que el presente proyecto de investigación tiene como objetivo general “Evaluar los parámetros de diseño y operación de un vermifiltro a escala real, para optimizar el diseño y la operación de un vermifiltro para eliminar la materia orgánica, nutrientes y

controlar las emisiones de gases de efecto invernadero". En primer lugar, se evaluaron las condiciones de operación de un vermifiltro a escala real, para eliminar materia orgánica y nutrientes (Capitulo III, IV). Luego, se analizó el efecto de las lombrices y la estacionalidad sobre la capa activa de un vermifiltro a escala real (Capitulo V) y finalmente se determinaron las emisiones de gases de efecto invernadero de un vermifiltro a escala real (Capitulo VI). Para cumplir con este objetivo, se generó una revisión bibliográfica para evaluar la influencia de los parametros de diseño y operación en los vermifiltro, posteriormente se evaluó el desempeño de un vermifiltro en la zona rural para eliminar materia orgánica y nutrientes. Los estudios realizados en el VF consideraron la caracterización fisicoquímica y biológica del influente, efluente secundario y efluente final. Luego se caracterizó la capa activa del medio filtrante utilizando técnicas de respirometria. Para esto se consideró la estacionalidad (Otoño-invierno y Primavera-Verano) y la densidad de lombrices por zonas de la capa activa (Zona A: 1105 ± 982 lombrices/m^3; Zona B: 7221 ± 1699 lombrices/m^3). Para evaluar la cinética heterotrófica se determinó la tasa de consumo de oxígeno (OUR_{end}), la tasa especifica de consumo de oxígeno ($SOUR_{end}$), la biomasa generada (Y_H) y la tasa de crecimiento de microrganismos ($\mu_{max)}$. Finalmente se utilizó el método de la cámara estática para medir las emisiones de CO_2, CH_4 y N_2O en la capa activa considerando la estacionalidad.

Los principales resultados de esta tesis doctoral demostraron que los parametros óptimos de un VF están entre 3000-6000 lombrices/m^3, tasa de carga hidráulica (HLR) bajo 2.5 m^3/dm^2, tasas de carga orgánica (OLR) entre 0.2-0.4 kgDQO/m^{2}d, tiempos de operación de la capa activa (TO) que no superen los 24 meses y temperaturas entre 15°C y 30°C. En cuanto al desempeño del VF a escala real, este registró una eficiencia de eliminación de un 77% para la DQO, un 53% para el nitrógeno total (NT), un 36% para el fosforo total (PT) con incrementos en primavera verano de un 9% para la DQO y una disminución de NT en un 20%. Respecto a los coliformes fecales (CF), VF eliminó de 8,9 a 6,9 log10(MPN/100mL). Por lo tanto, la estacionalidad es un factor que afecta significativamente la eliminación de la DQO, NT y el VF no es capaz de eliminar coliformes en niveles seguros.

En cuanto a la capa activa (viruta de madera), los resultados mostraron Y_H de 0.39 en otoño-invierno y 0.54 en primavera-verano, mientras que μ_{max} fue de 0.07 d^{-1} en otoño invierno y 0.02 d^{-1} en primavera-verano. OUR_{end} y $SOUR_{end}$ incrementaron un 95% más en primavera Verano que en otoño-invierno y un 64% más en la zona B que en la zona A. Estos resultados indican que la estacionalidad y la densidad de lombrices, afectan los parametros cinéticos del VF que determinan la eliminación de la materia orgánica.

Finalmente, las emisiones generadas por una persona al año en un VF ($kgCO_2eq$/per·año) presentan los siguientes rangos, CO_2: 0.8 – 7.5 kg/per·año, CH_4: 0.1 – 0.5 $kgCO_2eq$/ per·año, y N_2O: 5.7 – 9.5 $kgCO_2eq$/per·año, respectivamente. Respecto al efecto de la estacionalidad, el CO_2 incrementa un 139% in primavera – verano comparado con el otoño-invierno. N_2O incrementa un 139% en otoño-invierno comparado con primavera-verano. Por otro lado, existe una correlación positiva entre la concentración de DQO del influente y las emisiones de CO_2 (r^2 = 0,7) y una correlación negativa entre la relación carbono/nitrógeno (C/N) y las emisiones de N_2O (r^2 = -0,6). Estos resultados muestran que la estacionalidad y las características del influente respecto a la carga orgánica y la relación C/N, tienen un efecto en las emisiones de gases de efecto invernadero en un Vermifiltro a gran escala.

ABSTRACT

Chile is one of the countries most vulnerable to water scarcity, and it is estimated that at least 25% of the droughts experienced in the country since 2009 are associated with climate change. These water scarcity events primarily affect the supply and treatment of wastewater originating from rural areas, which are currently managed by Committees and Cooperatives. Although by 2018 potable water coverage had reached 99% in concentrated rural areas, more than 300,000 households still lack an appropriate system for water supply and wastewater sanitation.

At the regional level, the rural areas facing the greatest difficulties and lacking a formal potable water supply are located in Biobío (68%), La Araucanía (71%), Los Ríos (62%), and Los Lagos (64%). Generally, households in these areas do not have sufficient infrastructure to install adequate sanitation systems and only have cesspools and septic tanks. Consequently, vermifilters have been proposed as a viable solution for these communities.

Vermifiltration is a bio-oxidative process in which detritivorous earthworms interact intensively with microorganisms to remove contaminants present in water. This system consists of a tank filled with different filtering layers, with earthworms in the surface layer that can bioaccumulate, biodegrade, and/or biotransform various types of contaminants. Although these systems have been presented as a plausible solution for rural areas, 48% of the vermifilters implemented show operational failures associated with parameters that are still under investigation, including nutrient loading rate, hydraulic loading rate, filter medium, earthworm density, and the greenhouse gas emissions generated during degradation processes. At present, there is no scientific consensus identifying the optimal operational parameters of vermifilters, nor is there clarity on how earthworms impact the balance of greenhouse gases (GHGs).

For this reason, the general objective of this research project was to "Evaluate the design and operational parameters of a full-scale vermifilter, in order to optimize its design and operation for the removal of organic matter, nutrients, and the control of greenhouse gas emissions." First, the operational conditions of a full-scale vermifilter were evaluated for the removal of organic matter and nutrients (Chapters III and IV). Subsequently, the effect of earthworms and seasonality on the

active layer of a full-scale vermifilter was analyzed (Chapter V), and finally, the greenhouse gas emissions of a full-scale vermifilter were quantified (Chapter VI).

To achieve this objective, a literature review was conducted to evaluate the influence of design and operational parameters on vermifilters. Thereafter, the performance of a vermifilter in a rural area was assessed in terms of organic matter and nutrient removal. The studies conducted on the vermifilter included the physicochemical and biological characterization of influent, secondary effluent, and final effluent. The active layer was then characterized using respirometric techniques, considering seasonality (autumn–winter and spring–summer) and earthworm density in different zones of the active layer (Zone A: 1105 ± 982 earthworms/m^3; Zone B: 7221 ± 1699 earthworms/m^3). To evaluate heterotrophic kinetics, the oxygen uptake rate (OURend), biomass yield (YH), and maximum microbial growth rate (μmax) were determined. Finally, the static chamber method was applied to measure CO_2, CH_4, and N_2O emissions from the active layer, also accounting for seasonal variation.

The main results of this doctoral thesis demonstrated that the optimal parameters for a vermifilter are a worm density of 3,000–6,000 earthworms/m^3, a hydraulic loading rate (HLR) below 2.5 m^3/d·m^2, organic loading rates (OLR) between 0.2–0.4 kg COD/m^2·d, active layer operating times (TO) not exceeding 24 months, and temperatures between 15 °C and 30 °C. Regarding its performance, the full-scale vermifilter achieved removal efficiencies of 77% for COD, 53% for total nitrogen (NT), and 36% for total phosphorus (PT), with seasonal increases of 9% in COD removal during spring–summer and a 20% reduction in NT removal. For fecal coliforms (CF), the vermifilter reduced concentrations from 8.9 to 6.9 $\log_{10}$ (MPN/100 mL). Therefore, seasonality significantly affects COD and TN removal, and vermifilters are not capable of reducing coliform concentrations to safe levels.

Regarding the active layer (wood shavings), the results showed Y_H values of 0.39 in autumn–winter and 0.54 in spring–summer, while μ_{max} was 0.07 d^{-1} in autumn–winter and 0.02 d^{-1} in spring–summer. OUR_{end} and $SOUR_{end}$ were 95% higher in spring–summer than in autumn–winter, and 64% higher in Zone B compared to Zone A. These findings indicate that seasonality and earthworm density affect the kinetic parameters of vermifilters, which in turn determine organic matter removal efficiency.

Finally, the annual emissions generated per person in a vermifilter (kg CO_2eq/person·year) fell within the following ranges: CO_2: 0.8–7.5, CH_4: 0.1–0.5, and N_2O: 5.7–9.5. With respect to seasonal effects, CO_2 emissions increased by 139% in spring–summer compared to autumn–winter, while N_2O emissions increased by 139% in autumn–winter compared to spring–summer. In addition, a positive correlation was identified between influent COD concentration and CO_2 emissions ($r^2 = 0.7$), and a negative correlation between the carbon-to-nitrogen ratio (C/N) and N_2O emissions ($r^2 = –0.6$). These results demonstrate that both seasonality and influent characteristics—particularly organic load and C/N ratio—significantly influence greenhouse gas emissions in large-scale vermifilters.

CAPÍTULO 1

INTRODUCCIÓN

SEGURIDAD HÍDRICA

El Cambio climático y el calentamiento global han generado fuertes repercusiones y problemas ambientales. Actualmente el panel intergubernamental del cambio climático afirma que el calentamiento ya alcanza 1,07°C y que, con la tasa actual de emisiones globales de gases de efecto invernadero, los 1,5°C se alcanzarían entre los años 2030 y 2052 (IPCC 2023). Para mitigar estos efectos, poner fin a la pobreza y promover una visión compartida de nuestro futuro que acelere las respuestas a los principales desafíos que enfrenta el mundo, el 2015 en la COP 21 de París, se creó la Agenda 2030 con 17 objetivos de Desarrollo Sostenible (United National, 2024).

Dentro de todos los ODS, el número 6 "Disponibilidad y la gestión sostenible del agua y el saneamiento para todos" (ODS N°6), es un uno de los más importantes, ya que plantea objetivos específicos sobre la cantidad y calidad del recurso hídrico. En otras palabras, sin seguridad hídrica, no es posible lograr otros ODS como la seguridad alimentaria ODS N°2, salud y bienestar ODS N°3, seguridad energética ODS N°7 y cambio climático ODS N°13 (PNUMA, 2019). Sin embargo, a pesar de su importancia, los esfuerzos mundiales han sido insuficientes para lograr el cambio que se requiere, poniendo en riesgo la seguridad hídrica del planeta que incluye el abastecimiento y la calidad de las aguas.

La degradación de los ecosistemas de agua dulce amenaza la biodiversidad, la seguridad alimentaria y los medios de subsistencia. Entre el 2017-2021, el 50% de los países ha reportado la degradación de uno o más ecosistemas relacionados con el agua dulce. Particularmente, existe una pérdida de agua superficial permanente en 364 cuencas hidrográficas por prácticas insostenibles y por el cambio climático, esto afecta a más de 93 millones de personas (United Nations, 2025). Esta situación resulta aún más grave al considerar los altos niveles de estrés hídrico existentes, que podrían generar el desplazamiento de unos 700 millones de personas al 2030. África septentrional y Asia central y meridional registran niveles de estrés hídrico superiores al 70%. Le siguen Asia occidental y Asia oriental, con niveles de estrés hídrico entre el 45% y el 55%. Por otro lado, si bien la población mundial que utiliza agua potable y servicios de saneamiento gestionados de manera segura aumentó a un 74,3% y un 54% durante el 2020, aún existen 2.000 millones de personas en todo el mundo que carecen de agua potable gestionada de manera segura y 3.600 millones de personas en todo el mundo que no cuentan con un sistema de saneamiento gestionado de manera segura (Naciones Unidas 2021).

REALIDAD NACIONAL

Chile presenta una posición privilegiada a nivel mundial en oferta del recurso hídrico, con una escorrentía media total de 51.281 m^3/persona/año (DGA 2016), que supera la media de América latina y el caribe. Sin embargo, factores antrópicos sobre los acuíferos, el uso intensivo de agua y su contaminación, han potenciado la escasez hídrica del recurso, ubicando a Chile dentro de los 25 estados del mundo con mayor estrés hídrico (WRI 2019). Este escenario afecta por, sobre todo, a las zonas rurales que se abastecen a través del programa de agua potable rural, APR (MMA 2020).

Desde 1964, la gestión del agua potable rural ha recaído en Comités y Cooperativas, responsables de operar y mantener la infraestructura de suministro hídrico (MOP 2015). Mientras que los comités se rigen por la Ley 19.418, las cooperativas lo hacen por la Ley de Cooperativas del Ministerio de Economía y Turismo (BID 2015). Hasta el año 2019, estas organizaciones han sido supervisadas por la Dirección de obras hidráulicas del Ministerio de obras públicas considerando solo estándares de calidad y cantidad para el agua potable. Sin embargo, la ley N°20.998 creó una nueva institucionalidad que cambio los APR por los servicios sanitarios rurales (SSR) para incorporar el saneamiento. Dentro de esta ley, se contempla la creación de la subdirección de servicios sanitarios rurales de la Dirección de Obras hidráulicas del MOP que debe asesorar a los operadores, formular y evaluar proyectos, contratar la inversión sectorial y actuar como unidad técnica. Con esto, se pone fin a la función que tenían las empresas de servicios sanitarios en cuanto a la prestación de atención técnica y administrativa a los APR de sus respectivas regiones. Aunque a fines del 2018, la cobertura alcanzada por los Comités y las Cooperativas llego a un 99% de agua potable en las zonas rurales concentradas (MOP), todavía existen más de 300 mil viviendas que no tienen un sistema formal de abastecimiento. Estas representan el 47,2% de la población rural total. De este universo, el 58,8% se abastece desde pozos, el 25,8% desde ríos, esteros, canales o vertientes y un 15,4% lo hace recurriendo a camiones aljibes (AMULÉN 2018). Al analizar estas cifras en términos regionales, los mayores problemas se concentran en la macrozona sur, siendo las regiones que poseen mayores proporciones de población rural con fuentes informales: La Araucanía (71%), Biobío (68%), Los lagos (64%) y los ríos (62%) (INE 2017). Con respecto al saneamiento rural que considera los sistemas de alcantarillado y el tratamiento de las aguas servidas, éste no ha formado parte de las tareas históricas correspondientes al programa de agua potable rural (Novoa, 2012) y se estima que un 80% de la población rural, no tiene servicios de tratamiento de aguas residuales (BID, 2015). Este déficit, se explica por diferentes causas entre las que destacan: 1) La inexistencia de sistemas de agua potable o alcantarillado de aguas servidas en las proximidades de la población objetivo. 2) El costo de la tecnología asociada al consumo energético (Novoa, 2012). 3) Las características particulares propias de la ruralidad (i.e. Dispersión de las viviendas, limitaciones geográficas y las complejidades que existen respecto al diseño y las técnicas disponibles, Lourenco and Nunes, 2020). Por lo tanto, resulta fundamental crear propuestas innovadoras que vayan más allá de las tradicionales soluciones aplicadas a poblaciones más concentradas para tratar los contaminantes de las aguas servidas (AMULÉN, 2018).

CONTAMINACIÓN DE LOS ECOSISTEMAS

El crecimiento industrial, agrícola y urbano de Las cuencas de la zona centro-sur, particularmente el que se puede observar en la cuenca del Bíobío, ha afectado la calidad de las aguas y ha alterado la ecología de los sistemas acuáticos (Habit et al., 2006; Figueroa et al., 2013; Alonso et al, 2017). Varios autores indican que los principales impactos, han sido causados por contaminantes de diferente naturaleza entre los que destacan los productos farmacéuticos, los productos de cuidado personal, Metales, Amonio y nitratos (Death and Winterbourn, 1995; Aedo et al., 2009; Orrego et al. 2009; Chiang et al. 2012; Alonso et al 2017) que se han incorporado tanto por las descargas domésticas, como también, por la irrigación y escorrentía desde la agricultura y la actividad forestal, infiltrándose hacia las aguas subterráneas con o sin autorización (Rozas et al. 2016). Aun cuando existen plantas de tratamiento, los sectores urbanos y rurales siguen siendo una de las fuentes principales de contaminación (Parra et al. 2013). Por ejemplo, algunas cuencas como el Río Maipo han sido declaradas zonas saturadas por 8 contaminantes. En particular, se han encontrado altas concentraciones de nitratos en aguas subterráneas asociadas a las aguas industriales y urbanas sin un tratamiento previo (MMA, 2022). Por otro lado, en la región de O Higgins, se encontró que en el 14% de los casos analizados, las concentraciones de nitratos en aguas superficiales superaron la norma (Arumi et al., 2020). Esta situación es un riesgo para el 76% de la población rural, que obtiene el suministro de aguas subterráneas (MINSEG, 2015; SISS, 2021). En las aguas servidas, se pueden encontrar los siguientes contaminantes: Material grueso, arenas, Grasas y aceites, sólidos en suspensión, sustancias con requerimientos de oxígeno y nutrientes, agentes patógenos y contaminantes emergentes (Alianza por el agua, 2008). Para cuantificar esta contaminación, se pueden medir los siguientes constituyentes de las aguas residuales: Constituyentes Físicos (e.i. Sólidos sedimentables, suspendidos y disueltos, Temperatura, Turbiedad, Color y Olor); Constituyentes Químicos (e.i. pH, Nitrógeno total "NT", Amonio "$N\text{-}NH_4$+", Nitrógeno total Kjeldah "NTK", Nitrito "NO_2^-", Nitrato "NO_3^-", Fósforo total, Fosfato "PO_4^{-3}", metales pesados, DBO_5, DQO, Carbono orgánico total "COT") y Constituyentes biológicos (i.e. Bacterias, Protozoos, virus, helmintos, rotíferos y algas) (Vidal 2014). Particularmente, dentro de los constituyentes químicos, también se encuentran los contaminantes emergentes que pueden ser agrupados en: Compuestos organoclorados, Fenoles, Hidrocarburos policíclicos aromáticos, Alquil bencenos lineales, bifenilos policlorados, esteroles y compuestos de cuidado personal y farmacéuticos (Alonso et al., 2017). Para eliminar estos contaminantes, los pozos negros, las letrinas y las casetas sanitarias con fosa séptica utilizadas por las viviendas rurales, no son suficientes (Novoa, 2012). Dentro de las tecnologías que se han evaluado, se encuentran sistemas convencionales como lodos activados (Vera et al., 2013; Mace and Mata-Alvarez, 2002), reactores anaeróbicos (Metcalf and Eddy, 2003; Kalyuzhni et al., 2006), Biodiscos (Metcalf & Eddy, 2003) y tecnologías no convencionales como los Humedales construidos (García and Corzo, 2008; Vymazal, 2008; Kadlec and Wallace, 2009) y los Vermifiltros (Arora et al., 2021).

SISTEMAS POR VERMIFILTROS

Según el estudio de la Unidad de Saneamiento Sanitario (SUBDERE, 2018), los VF destacan por su alta eficiencia de remoción y bajos costos de implementación en zonas rurales. Este sistema, patentado en 1986 por el Dr. José Tohá Castellá tras experiencias en Lufkin, Texas, fue implementado en 1994 en Melipilla para tratar aguas servidas de una comunidad de 1000 personas.

Los VF son plantas de tratamientos que utilizan lombrices y microorganismos para tratar las aguas servidas. Las lombrices confieren al sistema 2 propiedades principales, la ingesta y la degradación de los contaminantes del suelo (Singh et al., 2019). Cuando las lombrices ingieren la materia orgánica, esta pasa por la molleja y es triturada junto con los contaminantes que pueden contener (Dash, 1978; Sinha et al., 2008). En este proceso de ingesta y excreción, se modifican los atributos físicos, químicos y biológico del sustrato aplicado (Edwards, 2004; Liu et al., 2012). Por otro lado, el incremento de la porosidad y el área superficial generada por las cavidades que forman las lombrices, mejora la ingesta de los contaminantes (Jiang et al., 2016; Singh et al., 2018b), incrementa la capacidad de sorción del VF, aumenta el oxígeno disuelto de las capas, intensifica la degradación aeróbica, diversifica la población microbiana al generar condiciones favorables (Singh et al., 2018 a,b) y mejora los tiempos de retención de los contaminantes al aumentar la conductividad hidráulica y evitar que el sistema se atasque (Aira and Domínguez, 2009). Adicionalmente, el cuerpo de las lombrices genera una mucosa que incorpora microbios y enzimas que provienen de su intestino. Estos microbios y enzimas (i.e Amilasa, Celulasa, Fosfatasa, Proteasa, Lipasa, Quitinasa, Manasa) contribuyen con la mineralización de los contaminantes (Arora et al., 2014), con la eliminación de los patógenos al liberar compuestos antibacterianos y con la eliminación de las bacterias que no pueden desplazarse por las propiedades pegajosas de la mucosa. A su vez, esta misma mucosa, inhibe las actividades anaeróbicas y favorece la actividad de los microbios aeróbicos al mantener la relación carbono y nitrógeno para la degradación microbiana, con el aporte de proteínas, glucosas, aminoácidos y moléculas glicosiladas (Bajsa et al., 2003; Wang et al., 2011). Otros beneficios que se han identificado guardan relación con el incremento del trabajo realizado por los microbios en la formación de colonias y el comienzo de muchos mecanismos tales como la nitrificación, la desnitrificación, la oxidación de compuestos orgánicos (Arora et al., 2014) y la neutralización de contaminantes, por el calcio liberado desde el tubo digestivo de la lombriz (Singh and Kaur, 2014; Singh et al., 2018b).

En términos generales, si bien las lombrices pueden bioacumular, biodegradar o bio-transformar algunos tóxicos químicos (Jiang et al., 2016), los parámetros bajo los cuales puede operar un VF aún no están claros (Sartaj Ahmad et al., 2020). De hecho, en los sectores rurales, la implementación de este tipo de sistemas se reduce al 8% presentando fallas asociadas a su diseño y operación en el 48% de los casos (SUBDERE, 2012). A continuación, se describen los principales parámetros estudiados:

a) **Cantidad de lombrices**: Si bien las lombrices tienen una gran capacidad de aglomeración, pudiendo cohabitar entre 4.000 a 50.000 individuos por m^3, el funcionamiento de los VF depende de la capacidad que tiene la lombriz y los microorganismos presentes para degradar la materia orgánica del influente. Algunos experimentos han constatado que las actividades de excavación en la capa subsuperficial aumentarían las concentraciones de oxígeno y favorecerían los procesos de nitrificación por bacterias aeróbicas, cuando las cantidades fluctúa entre los 4.000 y 10.000 ejemplares por m^3 (Singh et al., 2019, Li et al., 2011, Vyamazal, 2005).

b) **Temperatura**: Las lombrices prefieren temperaturas entre 15-25°C (Singh et al., 2021). Bajas temperaturas pueden afectar la reproducción y su actividad metabólica al entrar en hibernación (Arora and Kazmi, 2015). En un estudio conducido por Wang et al. (2013), la eliminación de amonio durante el verano fue del 96% y en invierno fue del 55%. Por lo tanto, la aplicación de este tipo de tecnologías puede estar limitado para climas frío.

c) **Tasa de carga hidráulica (HLR)**: La tasa de carga hidráulica corresponde al volumen de agua servida por área del VF en el tiempo. Este valor representa un factor fundamental ya que determina el tiempo de contacto del agua servidas con el medio filtrante, afectando la adsorción, transformación y reducción de los contaminantes (Hughes et al., 2006). Se ha constatado que un factor idóneo para el HLR se encuentra entre 1 m^3/m^2d y 4 m^3/m^2d, rangos entre los cuales podrían sobrevivir las lombrices y los microorganismos, siempre y cuando, se mantenga una carga orgánica adecuada (Ghasemi et al., 2020; Zhao et al., 2012; Xia et al., 2008).

d) **Tasa de carga orgánica:** La carga orgánica medida como DQO/m^3d y la relación entre el C/N medida como la proporción entre la DQO y el nitrógeno total, tienen un rol importante en el tratamiento de aguas servidas. Cuando el influente tiene una carga orgánica baja, el oxígeno requerido para mineralizar los contaminantes es menor (Singh et al., 2019) y predominan ambientes aeróbicos en donde pueden crecer bacterias autótrofas (Elser et al., 2003; Makino et al., 2003). En algunas investigaciones, se ha constatado que a bajas cargas orgánicas (i.e 0,6 Kg DQO/m^{3}d), las bacterias autótrofas pueden generar la nitrificación del NH_4+ en el VF, y las bacterias heterótrofas pueden generar procesos de desnitrificación en el intestino de la lombriz. Este equilibrio puede invertirse, si las concentraciones de nitrógeno del medio aumentan con cargas orgánicas mayores o con influentes con una alta relación entre el carbono y el nitrógeno (Chowdhury and Bhunia, 2021).

e) **Medio filtrante:** El medio filtrante, es un elemento relevante en la eliminación de nutrientes. Algunos trabajos han constatado que los medios filtrantes con mejores resultados están conformados por material leñoso, compost, Dolochar y viruta de madera. En estos sistemas, la eliminación del fosforo ha estado entre un 20-60% (Samal et al., 2017; Singh et al., 2019), llegando incluso a un 70% (Zhao et al., 2014), particularmente el material leñoso, ofrece una mejor retención hidráulica por las partículas finas del suelo que mejoran las propiedades de adsorción (Arora et al., 2014). Por el contrario, al utilizar cuarzo y/o gránulos de cerámica, las concentraciones de fósforo aumentan en el efluente (Wang et al., 2014, Kumar et al., 2015; Arora et al., 2016) y la eliminación de nitrógeno disminuye por los daños que genera las astillas de cuarzo y las cenizas de madera en la lombriz (Arora et al., 2014).

f) Dimensiones: Respecto a las dimensiones ideales, se ha encontrado que la profundidad de los VF también tendría un impacto en la eficiencia del sistema. Tanto Nie et al. (2015), como Wang et al. (2014), han llevado a cabo experimentos en VF verticales, y han encontrado que existen una máxima eficiencia en la eliminación del Nitrógeno a 40 y 60 cm respectivamente.

Para mejorar la eficiencia de eliminación de la materia orgánica y nutrientes, se deben determinar valores para operar los VF con estos parametros. Sin embargo, en el contexto de cambio climático, la optimización debe incluir las emisiones que generan los procesos de degradación de contaminantes. Sobre estas materias, aun no existe un consenso científico que indique como las lombrices, podrían impactar el balance de los gases de efecto invernadero (GEI), de hecho, aun cuando algunos estudios realizados en procesos de vermicompostaje, han mostrado que las lombrices podrían incrementar entre un 33% y un 42% las concentraciones de CO_2 y N_2O, respectivamente (Lubbers et al., 2013). Particularmente en VF existen pocos estudios que han medido GEI. Mientras que Zhao et al. (2014) encontró un aumento en las concentraciones de CO_2 y CH_4 y una disminución del N_2O al incrementar la relación C/N. Huang et al. (2014), encontró que las concentraciones de CO_2 y CH_4 no tuvieron variaciones en función del C/N. Por otro lado, la cantidad de lombrices, puede ser un parámetro relevante. VF que utilizan aguas residuales industriales (i.e Productos lácteos y Purines de cerdos), registraron disminuciones de CO_2, CH_4 y N_2O cuando existen lombrices en el medio. Respecto al N_2O, es posible que exista un umbral de carga nitrogenada que determina si las lombrices incrementan o disminuyen este tipo de emisiones. Cuando la materia orgánica nitrogenada es baja, las lombrices estimulan la fijación del nitrógeno generando emisiones de N_2O, pero cuando el nitrógeno es alto y existe una alta densidad de lombrices, la estrategia de alimento de la lombriz induce a generar bajas emisiones respecto a un sistema sin lombrices (Borken et al., 2000). Por lo tanto, es necesario generan más investigaciones en esta línea (Singh et al., 2020), para dilucidar como la capacidad que tienen las lombrices para estabilizar el carbono y mineralizar los diferentes compuestos, puede causar un aumento en las emisiones netas de GEI del suelo (Janzen., 2006) o, por el contrario, una disminución.

CAPÍTULO 2

HIPOTESIS Y OBJETIVOS

2.1 HIPÓTESIS

Según él ultimo catastro, el 48% de los vermifiltros instalados en zonas rurales, pueden ser fuentes de contaminación por fallas de diseño y operación. Considerando que en zonas rurales no existen un control sobre parámetros como la temperatura, la densidad de lombrices, la carga hidráulica, la carga orgánica y la relación carbono/nitrógeno para eliminar la materia orgánica, los nutrientes y generar gases de efecto invernadero, se plantea la siguiente hipótesis:

Las bajas temperaturas estacionales en zonas rurales y bajas cargas de lombrices por deficiencias en el mantenimiento y en la operación del sistema, disminuyen la eliminación de la materia orgánica, los nutrientes y pueden incrementar la emisión de gases de efecto invernadero, respecto de estaciones con temperaturas más elevadas (CO_2, CH_4 N_2O).

2.2 OBJETIVOS

2.2.1 Objetivo general

Evaluar los parámetros de diseño y operación de un vermifiltro a escala real, para optimizar el diseño y la operación de un vermifiltro para eliminar la materia orgánica, nutrientes y controlar las emisiones de gases de efecto invernadero

2.2.2 Objetivos específicos

a) Evaluar las condiciones de operación de un vermifiltro a escala real, para eliminar materia orgánica y nutrientes.
b) Analizar el efecto de las lombrices y la estacionalidad sobre la capa activa de un vermifiltro a escala real.
c) Determinar las emisiones de gases de efecto invernadero de un vermifiltro a escala real y realizar un balance de masa.

CAPÍTULO 3

CRITICAL ANALYSIS OF WASTEWATER TREATMENT USING VERMIFILTERS: OPERATING PARAMETERS, WASTEWATER QUALITY, AND GREENHOUSE GAS EMISSIONS

Gutiérrez, V., Gómez, G., Rodríguez, D.C., Vidal, G. (2023). Critical analysis of wastewater treatment using vermifilters: Operating parameters, wastewater quality, and greenhouse gas emissions. *J. Environ. Chem*. Eng, 2, 11, 109683. https://doi.org/10.1016/j.jece.2023.109683.

Critical analysis of wastewater treatment using vermifilters: operating parameters, wastewater quality, and greenhouse gas emissions

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Abstract

Vermifiltration is a biooxidative process in which detritivorous worms interact intensely with microorganisms to eliminate contaminants present in the water. Although these systems have been presented as a plausible solution for the treatment of wastewater in rural areas, there are different parameters that can affect the removal efficiency and at a scientific level, there is no consensus on how to operate and design these systems, which It results in different alternatives and not very standardized forms of operation. This article presents a critical review of vermifiltration wastewater treatment, beginning with an analysis of related articles, followed by an evaluation of the influence of design and operational parameters, efficiency, and greenhouse gas generation. The main results of this review indicate that vermifiltration systems should use worm densities (DE) between 3000-6000 worms/m3, tributaries with hydraulic loading rates (HLR) below 2.5 m^3/dm^2, loading rates organic matter (OLR) not higher than 0.4 kgCOD/m^2.d, and filter material (FM) that includes wood chips to counteract the increase in NO3- and TP concentrations generated by nitrification and mineralization. Regarding the generation of greenhouse gases (GHG), more research is required in the area, since, although the HLR and OLR can affect the process, it has not been clearly defined whether the activity of the earthworms can generate carbon sinks or GHG sources. The results of this review article allowed us to define the most important operating parameters and the operating ranges to be considered in its application and operation in rural areas.

Keywords: Earthworm-microorganism consortium, operating conditions, rural areas, vermifilter, wastewater treatment.

1. INTRODUCTION

Worldwide wastewater production reaches 2,200 km^3/year. Around 40% of the world population lacks basic sanitation, and coverage is often much lower in rural areas than in urban areas. Twenty-five percent of urban inhabitants of developing countries lack access to sanitation services, while for rural populations in developing countries the figure reaches up to 82% [1]. The lack of adequate sanitation services leads to various diseases. The WHO estimates that 297,000 children under five die each year from diarrheal diseases caused by deficient sanitation, deficient hygiene, or unsafe drinking water [2]. Inadequate sanitation infrastructure and the elimination of phosphates, nitrates and organic matter is a challenging environmental issue [3] and a global problem that especially affects rural zones. These areas are characterized by low population densities and scattered homes; a decentralized wastewater management system is considered a feasible solution for rural wastewater treatment [3,4]. Nonconventional wastewater sanitation technologies are seen as alternatives to be considered for decentralized systems in small rural communities [4].

The vermifiltration is an example of nature-based solutions, to treat wastewater using vermiculture. The introduction of worms into the environment generated by the geo-microbial filter lends the system two main properties: a) ingestion of wastewater and b) degradation of soil pollutants [5]. The parameters under which a vermifilter can be operated have not yet been well studied and small modifications could alter the quality of the treated effluent and promote the generation of CH_4, CO_2, and N_2O emissions due to mineralization, nitrification, and denitrification processes that can occur in the system [6, 7]. The objective of this work is to carry out a critical review of wastewater treatment using vermifilters. It will provide a comprehensive analysis of the state of the art of this technology to assess the influence of operating and design parameters, filtration efficiency, and the generation of greenhouse gases from wastewater treatment using vermifilter systems. Considering the diversity of information and the lack of a consensus on the values and operating parameters, this bibliographical review will provide tools for the operation and design of these systems on a rural scale, defining key values for their operation and ranges of typical operations found in the literature.

2. VERMIFILTRATION

Vermifiltration is a bio-oxidative process in which earthworms interact with microorganisms, accelerating the stabilization of organic matter and significantly modifying its physical and biochemical properties. The microorganism-earthworm consortium is the main driver of the biochemical degradation of the organic matter contained in wastewater [8].

2.1 The role of earthworms

Earthworms are used in vermifilters to accelerate processes that occur in soil filtration during wastewater treatment [9,10]. Depending on the species, these animals can live for 3 to 7 years and double their population every 60-70 days [11]. It is important to note that the earthworm gut hosts millions of microbes that can fix nitrogen and decompose pathogens [10,12]. In soil, earthworms can modify physical-chemical properties via four processes: burrowing, ingestion, grinding, and excretion [13]. With their burrowing movements, earthworms increase hydraulic conductivity, improve soil filtration [14,15], and increase the oxygen concentration, favoring aerobic microorganism activity [5]. Via ingestion and grinding, earthworms generate microaggregates ranging from 50 μm to 250 μm [16,17], increase the soil surface area, improve adsorption of inorganic and organic matter, and facilitate the feeding of immobilized microbes in lower zones [9,18]. Finally, the body of the earthworm excretes stable aggregates and a mucus that incorporates microbes and enzymes such as amylase, cellulase, phosphatase, protease, lipase, chitinase, and mannanase that contribute to the mineralization of pollutants, favors aerobic microorganism growth, and contributes antimicrobial substances that inhibit pathogen growth [11,19-21].

2.2 Vermifiltration mechanisms

Vermifiltration is the process of treating wastewater through the joint action of earthworms and microorganisms that considerably minimizes water pollution [9]. Earthworms ingest and grind soil particles along with pollutants contributed by wastewater, modifying their physical, chemical, and biological properties. Subsequently, these modified properties aid in improving microbial activity in the vermifilter [21]. Figure 3.1 shows the main nutrient degradation and removal mechanisms in a vermifilter. The burrowing behavior of the earthworms contributes to increasing the oxygen in the effluent by 4-6 mg/L [22-24] and generating a redox potential of 52 and 70 mV in the active layer [1,6], facilitating aerobic microorganism growth in the surface layer [25,26]. Meanwhile, the presence of earthworm boosts up the denitrification because the gut acts as the storehouse of the denitrifiers [7]. The ingestion, grinding, and excretion behaviors of the earthworms hold the activity of anaerobic microorganisms that otherwise would be in a dormant state in the external environment [27]. The combination of both effects can change the microbiome of vermifilters [28]; the excretion different types of bacteria such as *Pseudomonas, Azoarcus, Burkholderia, Alcaligenes, Sphingobacteria*, and *Flavobacteria* results in degradation of organic compounds [29,30], while in the presence of *Myxcococcales, Bdellovibrionales Syntrophobacterales, Nitrospira*, and *Flavobacteria* nitrification and denitrification processes occur [28,30,31]. In terms of phosphorous concentrations, some studies have reported phosphorous increases in the effluent ranging from 100 to 300% [22,32,33]. This phenomenon can be explained by two factors: a) the microbial activity related to the earthworms, particularly bacteria of the genera *Myroides* and *Flavobacterium* that participate in the mineralization of phosphorous [8] from the filter material; and b) the vermicast generated by the earthworms that contains 1.16% nitrogen, 1.22% phosphorus, and 1.00% potassium [8]. As a solution to this increase in phosphorus, the use of a filtration material that promotes phosphorus adsorption during wastewater treatment is proposed, for example wood chip [34,35].

Regarding the generation of greenhouse gases (GHG), the processes that occur in a vermifilter [6,36] are similar to those that occur in the soil [37]. The generated CO_2 is produced by microorganism respiration [38,39], while the generated CH_4 is produced in areas with low aeration rates by methanogenic bacteria [40,41] and the generated N_2O is due to nitrification and denitrification processes [42] that occur during wastewater treatment [36]. Although few studies have assessed GHG generation in wastewater vermifilters [6,36], some research has indicated that earthworms can influence GHG generation mechanisms [39,43,44]. Whether their activity generates carbon sinks or GHG sources has not been clearly defined [37]

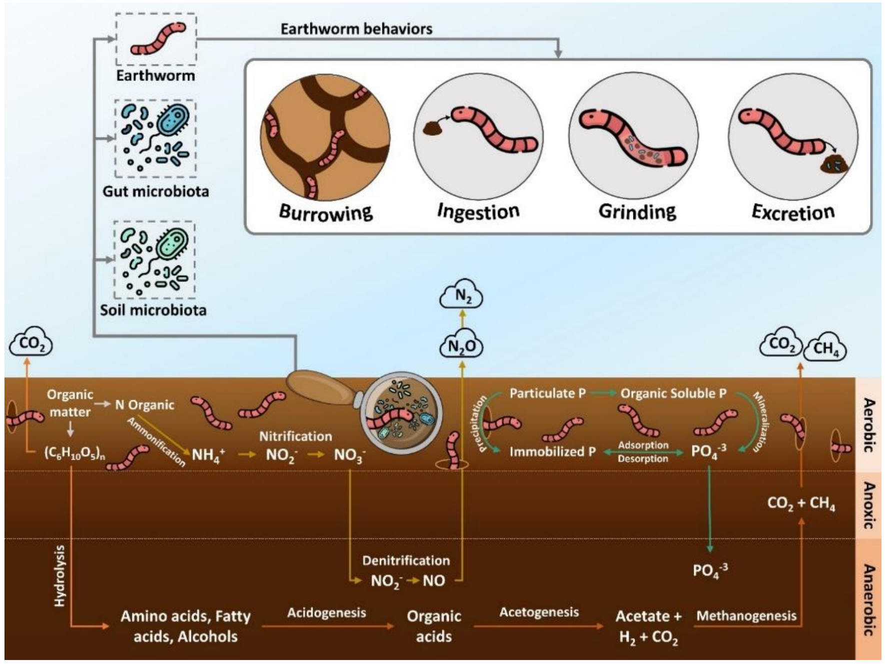

Figura 3.1 Schematic representation of organic matter and nutrient removal in vermifiltration and the role of earthworms and microorganisms

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3. BIBLIOMETRIC ANALYSIS

A bibliometric study of articles associated with this review was carried out. The words used for the search were "vermifiltration" or "vermifilter" and "sewage" or "domestic wastewater." To construct and visualize the bibliometric information, the VOSviewer 1.6.11 software tool was used, considering networks of co-occurrence of important terms extracted from the titles, abstracts, and keywords of the articles. The search was performed in the Web of Science database, where 101 publications were found based on the previously described criteria, published from 2009 to 2022. Figure 3.2 shows the networks of co-occurrence of relevant terms of this bibliometric analysis. Four clusters were identified that are differentiated with the colors red, purple, yellow, and light blue. The red cluster is related to terms that include nutrients, nitrogen, phosphorus, denitrification, nitrification, hydraulic loading rate, and constructed wetlands. The light blue cluster is related to terms that include organic matter, microbial communities, diversity, activated sludge, and wastewater. The yellow and purple clusters are smaller and are related to terms that include *Esenia fetida*, waste treatment, synchronous treatments, and pathogens.

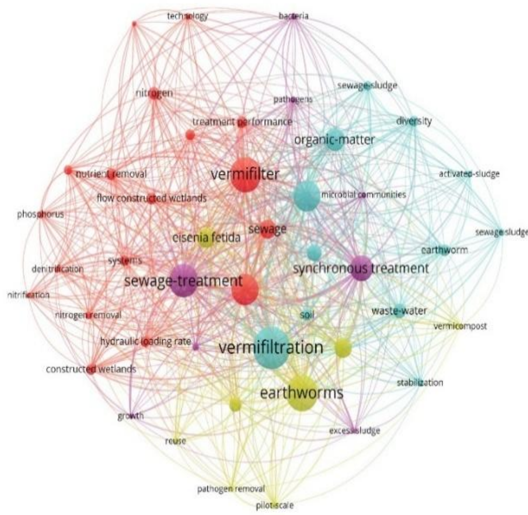

Figura 3.2 Representation of the bibliographic network of co-occurrence (VosViewer). The words used were “Vermifiltration” or “Vermifilter” and “Sewage” or “Domestic wastewater.”

In general terms, since vermifilters were applied for the first time more than 30 years ago by Dr. José Tohá Castellá [10], publications have had two main focuses: a) investigations that seek to determine the efficiency of a system in removing organic matter and nutrients from treated wastewater, achieving removal efficiencies between 75% and 90% for COD, 32% and 85% for TN, and 39% and 95% for TP [30,45]; b) investigations that seek to determine the diversity of microbial communities and the implementation of synchronous treatments with vermifilters and technologies such as biofilters with zooplankton, microbial fuel cells, and ultrafiltration systems [12,46-48]. This latter approach is especially important, as it could determine if the microorganisms in the effluent could put environmental safety at risk due to the presence of bacteria, protozoa, and metazoan [9,49] and, could limit the application of vermifilters to complementary technologies used to strengthen secondary and tertiary treatments at a low cost [50].

4. OPERATING CONDITIONS

In vermifilters earthworms can bioaccumulate, biodegrade, or biotransform some pollutants [51]; for them to do so, vermifilters must meet design and operating conditions that allow earthworm development. Variations in these parameters affect the efficiency of the vermifilter in removing organic matter, nutrients, and pathogens contained in wastewater [52,53]. In Table 3.1, the parameters that determine these operating and design conditions in a vermifilter are described.

Tabla 3.1. Design and operating parameters of vermifilters in sewage treatment

Influent	Design parameters				Operating parameters				Synchronous treatment	References
	Species	Earthworm density (earthworm/m³)	Active layer	Filter material height (m)	HLR (m³/m²d)	OLR (kg COD/m²d)	T (°C)	C/N		
Synthetic municipal	*Eeisenia fetida*	20000	Pindstrup / ceramsite	0.45	1	-	-	-	Ultrafiltration / recirculation	[48]
Real domestic	*Eeisenia fetida, Dendrobaena veneta*	5093-9458	Woodchips	1-1.60	0.48-0.96	0.34-0.68	9-13	4-7	-	[53]
Real domestic	*Eeisenia fetida*	4615	Woodchips / corn cobs	0.90	0.50-1.00	-	16.30-41.20	-	Microbial fuel cells / constructed wetlands	[47]
Real domestic	*Eeisenia fetida*	13263	Woodchips	1.40	1.30	1.20	-	23	zooplankton biofilter	[12]
Synthetic municipal	*Eeisenia fetida*	10000	Vermicompost / sand	0.25	3-7	0.6-50	-	4-14	serial system / constructed wetlands	[7]
Synthetic municipal	*Eeisenia fetida, Eleocharis palustris*	30000	Peanut shells / woodchips	0.90	0.50-1.50	-	21	-	-	[58]
Real domestic	*Eeisenia fetida*	5000-60000	Compost / soil	0.90	2-3-4	0.37	-	7.0	Gravel filter	[75]
Synthetic municipal	*Eeisenia fetida*	8481	Woodchips / compost	0.55	2.50	1.49		-	-	[1]
Real domestic	*Eeisenia fetida*	-	Woodchips	0.10-1.30	0.48	1.7-2.93	.	-	Zooplankton biofilter	[50]
Real domestic	*Eudrilus eugeniae*	-	Sawdust / cow dung	0.70	0.02	0.04	24-42	6.5	-	[65]
Real domestic	*Eeisenia fetida*	285	Sand	0.45	0.08	0.07	6.9- 28	14	Constructed wetlands	[61]
Real domestic	*Eeisenia fetida*	40000 – 80000	Vermicompost	0.27	0.89	0.22	-		Serial system	[56]
Real domestic	*Eeisenia fetida*	40000	Vermicompost / sawdust	0.27	0.89	0.40			-	[33]
Synthetic municipal	*Eeisenia fetida*	25000	Soil / sawdust	0.70	0.70	-	26	6	-	[45]
Real domestic	*Eeisenia fetida, Eudrilus eugeniae*	12000	Vermicompost / riverbed material	0.25	2.50	1.04	-	9	-	[32]
Real domestic	*Eeisenia fetida*	7143	Organic fraction / vermigratings	0.80	1	0.24	15-22.50	-	-	[62]
Synthetic municipal	*Eeisenia fetida*	15000	riverbed material / wood carbon / glass balls / mud balls	0.25	1.50	0.72	-		-	[22]
Synthetic municipal	*Eeisenia fetida*	30000	Vermigratings	0.25	1	0.45	2- 16		-	[52]
Synthetic municipal	*Eeisenia fetida*	8929	Soil / woodchips	0.53	1.05	0,11-0,43	10.40-37.90	2-5-10	Constructed wetlands	[36]
Synthetic municipal	*Eeisenia fetida*	3061	Soil / woodchips	0.55	0.06	0,01-0,02	6.50- 35.80	2-5-10	Constructed wetlands	[6]
Real domestic	*Eeisenia fetida*	16000	Ceramsite	-	4.20	0,39	5.20-33.90		-	[30]

Earthworm density: Earthworm density in the active layer, estimated from the size of the reactors. Generally, the earthworm density given in the article is in (g/L) and the weight of an individual earthworm is estimated at 0.5 g (Tahar et al. [53]); Active layer: Type of filter material; HLR: hydraulic loading rate; OLR: organic loading rate; T: temperature; C/N: Carbon and nitrogen rate.

4.1 Design parameters

Species (S) type and earthworm density (ED) are two important design parameters. As described in Table 1, although various species in the annelid phylum have been studied [32,53,54], the species *Eisenia fetida* acclimates more quickly and presents a greater reproduction capacity and survival rate relative to other species [21]. Regarding ED, Wang et al. [55] found that an increase from 9,000 earthworms/m^3 (i.e 4,500 g earthworm/m^3) to 33,000 earthworms/m^3 (i.e 16,500 g earthworm/m^3) increases the bacterial biodiversity involved in the degradation of organic matter from 0.82 to 1.11, according to the Shannon index. For ED greater than 40,000 earthworms/m^3 earthworms can reduce permeability and cause clogging due to vermicast production [56].

In terms of filter material (FM) and Filter material height (FMH), most designs involve a lower layer of gravel to support the vermifilter [7], while in the upper layers the use of pindstrup, woodchips and compost, among other materials, has been described. Most biological processes generated by earthworms and bacteria occur in this upper layer, which is known as the active layer [53,57]. In general terms, FM selection must depend on the organic sources it contains to facilitate earthworm survival [10,21] and the hydraulic conductivity it can achieve. Tejedor et al. [58], using a mix of woodchips and peanut shells, determined that optimal hydraulic conductivities vary from 250-700 mm/h, and that at conductivities < 100 mm/h, clogging can be observed in the system.

Figure 3.3 shows COD, NH_4^+, NO_3^-, TN, and TP removal efficiency as a function of FMH. In general terms, vermifilters with heights greater than 0.61 m are more efficient, as they improve the capacity for adsorption and distribution of pollutants in the system [21]. Certain restrictions must be considered, as although the evaluated designs reached 1.2 m, greater heights could favor anaerobic conditions in the lower layers since, in general, epigeic species such as *Eisenia fetida* prefer to be in the upper soil layers [59].

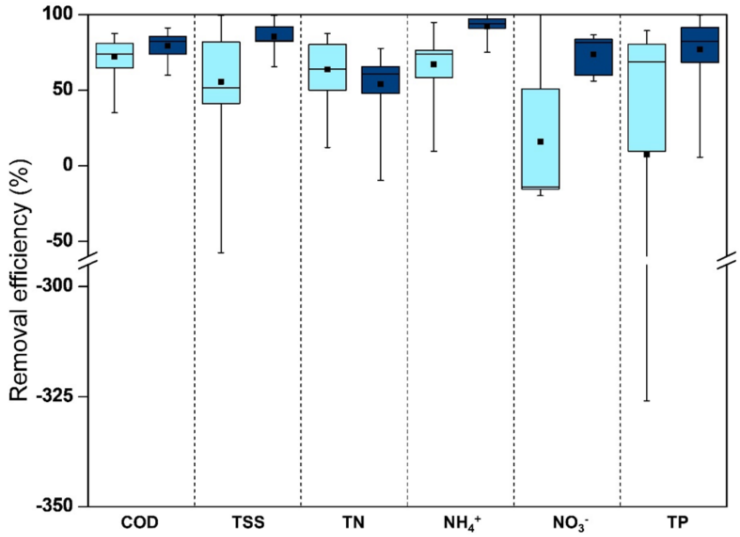

Figura 3.3. COD, TSS, TN, NH4+, NO3-, and TP removal efficiency in relation to vermifilter height. Where low: 0-0.60 m (■) and high: 0.61-1.6 m (■). Boxplots show the medians inside interquartile.

4.2 Operating parameters

The hydraulic loading rates (HLRs) and organic loading rates (OLRs) are important for vermifilter operation. As shown in Table 1, the HLRs and OLRs vary, with ranges of 0.02-7.00 m^3/m^2d and 0.01-5.00 kgCOD/m^2d, respectively.

In accordance with Arora and Saraswat [9], HLRs greater than 2.5 m^3/m^2d decrease the time required for microbial community growth, reducing pollutant retention in the FM, which has negative effects on COD, BOD_5, NH_4^+, and TN removal efficiency [7,21,22,60] Meanwhile OLRs below 0.2 kgCOD/m^3d could limit feeding and thus limit microbial growth and reduce removal of the organic matter contained in wastewater [7,25,26,61]. However, Zhao et al. [6] worked with an OLR of 0.01 kgCOD/m^3d, achieving a stable *Eisenia fetida* with 3571 earthworm/m^3, while Lavrnić et al. [61], with an OLR of 0.07 kgCOD/m^3d and 285 earthworm/m^3, had to 10 individual of *Eisenia fetida* for a period of 3 weeks to avoid the population removal. The main difference between the two studies lies in the active layer used by Zhao et al. [6], which, unlike that of Lavrnić et al. [61], contained a significant source of organic matter composed of a mix of peat soil and woodchips. It bears mentioning that OLR values of 5.00 kgCOD/m^3d can also have negative effects by reducing NH_4^+ removal. According to Chowdhury and Bhunia [7], this can be explained by the heterotrophs that, in making use of organic carbon, limit the growth of the autotrophs that generate nitrification of NH_4^+-N.

Figures 3.4 and 3.5 show TN, NH_4^+, NO_3^- and TP removal efficiency as a function of the OLR used by the vermifilters analyzed in this literature review. These results are consistent with the foregoing explanation, as it is possible to identify an optimal OLR range, which could span 0.2-0.4 kg COD/m^{2}d for wastewater [47,53,62]. If vermifilters operate below 0.2 kg COD/m^{2}d, earthworm survival could be at risk due to food shortage, and if they operate above 0.4 kg COD/m^{2}d, NH_4^+ removal could decrease and, incidentally, generate clogging in the system [21].

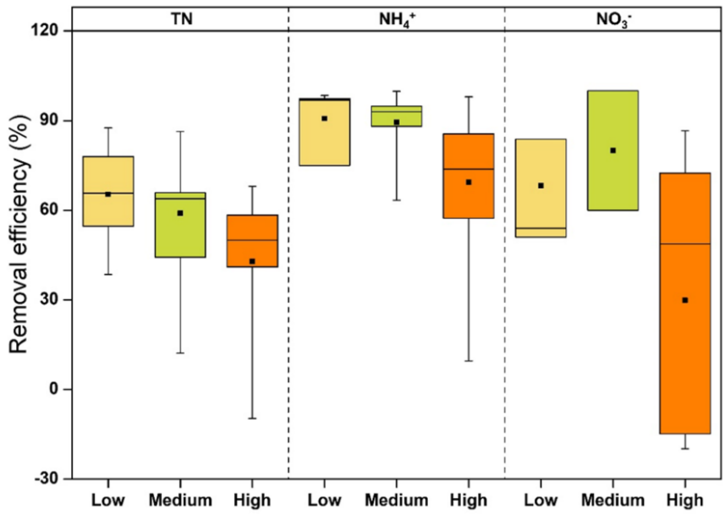

Figura 3.4. NH4+, NO3-, and TN removal efficiency in relation to organic loading rate. Where low: 0-0.2 kgCOD/m2d ( ), medium: 0.2-0.4 kgCOD/m2d ( ), and high: 0.4-1.2 kgCOD/m2d ( ). Boxplots show the medians inside interquartile.

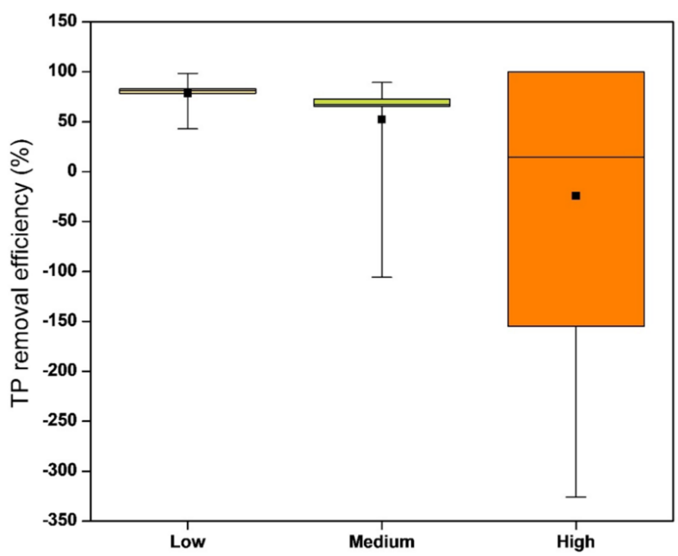

Figura 3.5. TP removal efficiency in relation to organic loading rate. Where low: 0-0.20 kgCOD/m2d ( ), medium: 0.21-0.40 kgCOD/m2d ( ), and high: 0.41-1.20 kgCOD/m2d ( ). Boxplots show the medians inside interquartile.

Regarding temperature, the optimum operating range for earthworms is between 15°C-30°C [63]; when the climate is cold, they tend to hibernate and migrate to the deepest layers, decreasing their reproductive and metabolic activity [10,52]. The carbon/nitrogen ratio (C/N) is an important parameter that can affect the earthworm/microorganism consortium and greenhouse gas generation [21]. If the ratio C/N increases, the CO_2 and CH_4 increases but, if the ratio C/N is low, increase N_2O. Zhao et al. [6] found that it is possible to maintain a balance between TN and GHG production (CO_2, CH_4, N_2O) when the ratio is 5:1.

4.3 Synchronous treatments

Some limitations of vermifiltration systems are related to the removal of microorganisms such as bacteria, protozoa, and metazoa [48], restrictions on working with greater HLRs, and system clogging due to high EDs and OLRs [1,5,48]. To overcome these limitations, some vertical vermifilters have incorporated synchronous treatments (ST), including anaerobic biofilters, ultrafiltration technologies, constructed wetlands, biofilters with zooplankton, and microbial fuel cells [7;12,47,48,64]. Vermifilters with zooplankton biofilters or microbial fuel cells have presented high organic matter, pathogen, and nutrient removal efficiencies, which in some cases can reach up to 90% for COD, TSS, NH_4^+, TP, fecal coliforms, and total coliforms [12,47,50].

5. QUALITY OF WASTEWATER TREATED BY VERMIFILTERS

The parameters that determine the wastewater treatment efficiency of a vermifilter will depend on the interaction among earthworms, microorganisms, and active layer composition [10]. Table 3.2 details the organic matter and nutrient removal efficiencies in wastewater treatment using vermifilters, as a function of active layer composition.

Tabla 3.2. Type of active layer and organic matter and nutrient removal efficiency.

Active layer		Operating parameters			Average removal efficiency ± SD (%)						
Material type	Earthworm density (earthworm/m^3)	Height (m)	HLR (m^3/m^{2}d)	OLR (Kg COD/ m^{2}d)	COD	TSS	TN	NH_4^+	NO_3^-	TP	Reference
Compost or Vermicompost	7000-7500	0.35	1.3	0.9-1.8	68.0 ± 2	-	-	-	-	-	[66]
	8000-8500	0.1	2.5	1.49	56.6 ± 20	-	-	42.7 ± 29	4.7 ± 38	16 ± 6	[1]
	9000-10000	0.2	3-7	0.6-5	-	34.3 ± 13	52.7 ± 8	-	-	-	[7]
	12000	0.15-0.25	1.5-2.5	1.04-0.72	64.6 ± 6	60.3 ± 9	-	65.5 ± 11	+15.1 ± 3	+197 ± 44	[22,32]
	30000-70000	0.1-0.2	1-1.5	0.45	76.1 ± 3	77.5 ± 14	-	-	-	-	[23,52]
Wood (woodchips, sawdust)	600-1300	0.4-0.8	0.2	0.05	77.3 ±11	-	50.7 ± 9	-	-	82 ± 9	[57]
	3000-4500	0.3-0.6	0.06-1	0.005-0.75	81.9 ± 3	-	79.5 ± 6	-	72.6 ± 13	87 ± 9	[6,47]
	5000-6500	1-1.3	0.47-0.95	0.3-0.59	83.4 ± 4	84.6 ± 8	54.1 ± 9	96.6 ± 3	-	65 ± 11	[53]
	8000-9500	0.35-0.7	0.47-1.05	0.1-0.4	70.4 ± 9	65.5 ± 40	62.5 ± 3	61.9	-	-	[36,53]
	12000-30000	0.3-1	0.7-1.3	0.2-0.94	89.0 ± 2	96.4 ± 2	23.8 ± 47	92.5 ± 2	-	+10 ± 7	[12,45]
Other material (soil, ceramsite)	200-300	0.33	0.08	0.07	66.5 ± 2	96.4 ± 1	47.5 ± 4	-	52.5 ± 2	44 ± 2	[61]
	7000-9000	0.35	0.2-1	0.05-0.24	77.2 ± 1	96.4 ± 6	63.8	90.0	-	-	[55,62]
	16000-17000	0.3-0.35	0.2-4.2	0.05-0.39	70.7 ± 4	90.13	65.7	92.1	-	81	[30,55]
	25000-33000	0.3-0.35	0.2-1.1	0.05-0.5	69.7 ± 3	-	64.2 ± 14	94.5 ± 5	+180.0	81 ± 1	[55,64]

Earthworm density: Earthworm density in the active layer, estimated from the size of the reactor. Generally, the earthworm density given in the article is in (g/L) and the weight of an individual earthworm is estimated at 0.5 g (Tahar et al. [53]); SD: standard deviation; HLR: Hydraulic loading rate; OLR: Organic loading rate; COD; TN; TP.

5.1 Removal of organic matter contained in wastewater via vermifilter

The main mechanisms related to organic matter removal have to do with the symbiotic action of earthworms and aerobic microorganisms [35]. To achieve efficient treatment, it is necessary to provide suitable conditions to increase earthworm activity, that is, promote the ingestion, burrowing, grinding, and mucus excretion behaviors of the earthworms, accelerating the development of a microbial community capable of assimilating organic matter. Table 3.2 shows COD high removal levels between 77.3±11.0% and 89.0 ±2.0% when vermifilters use the wood as an active layer, an HLR below 2 m^3/m^2d and an OLR between 0.2 and 0.6 $kgCOD/m^3d$. The operation in these ranges provides adequate contact time between the influent and the filter medium, carbon source in sufficient quantities to degrade organic matter and media with high porosity and surface areas that allow removal of organic pollutants by precipitation and adsorption [26]. Meanwhile, when compost or vermicompost is used as an active layer, COD removal efficiency is lower, and varies between 56.6 ± 20.0% and 76.1 ± 3.0 % [1,23,52].

5.2 Removal of nutrients contained in wastewater treated via vermifilter

The removal and transformation of nitrogen in its different chemical species depend on ammonification, nitrification, denitrification, biomass assimilation, filtration, and adsorption [51]. Meanwhile, phosphorous removal depends on the mineralization generated by earthworm-induced microbial activity and phosphorous retained by adsorption mechanisms [22,34]. Unlike organic matter, TN, NH_4^+, NO_3^-, and TP removal values present greater variations, fluctuating between +9.7% and 87.5% for TN [6,12], 9.6% and 99.8% for NH_4^+ [1,53], +593.0% and 86.7% for NO_3^-, and +326% and 99.7% for TP [32,47]. Table 3.2 presents studies on vermifilters that, using an active layer of wood fraction (operating parameters: ED between 600 and 6500 worm/m^3, HLR between 0.06 and 1.00 m^3/m^2d, and OLR between 0.05 and 0.59 $kgCOD/m^3d$), can reach maximum removal levels of 79.5±6.0%, 96.6±3.0%, 72.6±13.0%, and 87.0±9.0% for TN, NH_4^+, NO_3^-, and TP, respectively. It bears mentioning that the active layers that include wood fraction present lower initial concentrations of PO_4^{3-}, TP, and TN [33,65] and a better TKN and TP adsorption capacity in comparison to vermifilters that use materials such as cow dung or vermicompost [32,65,66]. Meanwhile, when ED is greater than 6500 worm/m^3, NO_3^- and TP concentrations increase by up to

180% and 197%, respectively. This could be caused by earthworms that favor the growth of ammonia-oxidizing bacteria that participate in nitrification and microbes that generate rapid mineralization of TP, thereby increasing phosphorous concentrations in the effluent [22,65,67]. Wang et al. [55,64] found a different result. Despite using a high ED of 25000 and 33000 worms/m^2, achieved phosphorous elimination efficiencies of 81.1±1.0%. An explanation could be that the active layer composed of padding soil/rice straws allowed improved adsorption and the combined treatment (anaerobic biofilter, trickling biofilter, and two vermifilters) contributed substantially to the general efficiency of the system.

5.3 Removal of pathogens contained in wastewater treated via vermifilter

Among the most important requirements in the current scenario is related to the removal of pathogens that can be transmitted in wastewater to meet standards that allow its reuse [5]. In this review, the vermifilters presented variations that ranged from 0.6 to 4 log reductions in Enterococus (ECC), total coliforms (TC), fecal coliforms (FC), *Escherichia coli* (EC), Salmonella (S), and fecal Streptococcus (FS) [32,33,56,62] b). There are various parameters that could explain pathogen removal. When Lavrnić et al. [61] worked with vermifilter that underwent significant decreases in earthworm population, pathogen removal reached 0.31 log for ECC, 1.26 log for TC, and 0.83 log for EC. Decreases in ED could generate a reduction in enzymes, microbes, and antibacterial substances provided by the earthworms [5,68]. Temperature, meanwhile, has been a little-studied variable. Arora and Kazmi [52] compared summer (35.4°C) and winter (15.6°C), finding drops greater than 1 log for TC, EC, and S. the highest pathogen removal values were found in studies that operated vermifilters with HLRs between 0.5 and 1 m^3/m^2d, reaching removal efficiencies greater than 3.5 log for TC and FC [33,47,52]. This relationship is not completely clear, as Pous et al. [12], upon generating shock with HLRs of 0.66 m^3/m^2d (750 l/d) and 2.65 m^3/m^2d (3000 l/d), decreased EC removal by 1 log, discarding wastewater reuse as a viable option. While there are various studies that support the capacity of vermifilters to remove pathogens via the action of the earthworm-microorganism consortium and the antibacterial activity of earthworm-related microflora [20,54,69,70], further research is needed to see the effects of operating parameters on these systems.

6. GREENHOUSE GAS (GHG) GENERATION DUE TO WASTEWATER TREATMENT VIA VERMIFILTER

The works of Zhao et al. [6] and Huang et al. [36] are among the few studies that have measured greenhouse gases in wastewater treated by vermifilters.

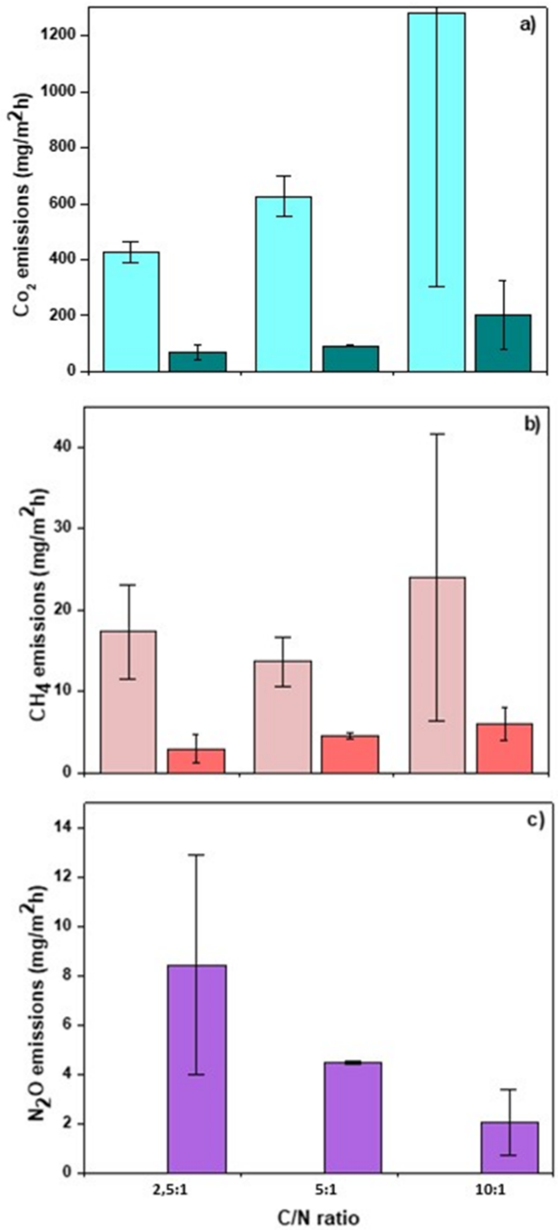

Figura 3.6. Average CO_2, CH_4, and N_2O emissions (kg/d) depending on the C/N ratio. Where a) CO_2 emissions, b) CH_4 emissions, and c) N_2O emissions. Where 8929 earthworms/m^3 and 3571 earthworms/m^3.

Figure 3.6 shows GHG emissions assessed in both experimental designs when they operated with different carbon-to-nitrogen ratios (C/N). The system designed by Zhao et al. [6] operated with the following parameters: ED: 3061 earthworms/m^3, HLR: 0.06 m^3/m^2d, OLR: 0.01-0.02 kgCOD/m^2d, and C/N ratio: 2.5-10, with fluctuations between 45.4 and 314 mg/m^2h for CO_2, 1.5 and 7.0 mg/m^2h for CH_4, and 2.4 and 12.3 mg/m^2h for N_2O. Meanwhile, the system designed by Huang et al. [36] operated with the following parameters: ED: 8929 earthworms/m^3, HLR: 1.05 m^3/m^2d, OLR: 0.11-0.43 kgCOD/m^2d, and C/N ratio: 2.5-10, with fluctuations between 399.9 and 1975.5 mg/m^2h for CO_2 and 11.53 and 36.53 mg/m^2h for CH_4. In both studies, CO_2 presented the highest concentrations.

A comparison of the results shows that the vermifilter designed by Huang et al. [36] emits, in some cases, 300% more GHG than that of Zhao et al. [6]. Various mechanisms can be involved in GHG generation. When organic matter and nutrient mineralization processes occur, CO_2 and CH_4 form part of the degradation process [71]. The increase from 0.01-0.02 kgCOD/m^2d to 0.11-0.43 kgCOD/m^2d could explain the more than 300% increase in these gases (see Figure 6). Specifically, CH_4 emissions reach no more than 3% of CO_2 emissions, suggesting that methanogenic bacteria are inhibited by the aerobic environmental conditions [72]. Meanwhile, N_2O increases from 2.0 mg/m^2d to 8.4 mg/m^2d when the C/N ratio decreases from 10 to 2.5, a result of denitrification processes that can convert up to 8% of the nitrogen in the influent to N_2O. This process is inhibited by the oxygen in aerobic environments, with the N_2O emission rate reaching only 12% of that of CO_2 in the case of Zhao et al. [6]. ED is another factor that can affect GHG generation. In the literature, there are some works that have compared ED with other influents (i.e., dairy products and pig slurry) and have observed differences in CO_2, CH_4, and N_2O emissions [71,72]. Luth et al. [71] found that, at low HLRs (i.e., 0.008 m^3/m^2d, flow rate: 2 l/d), the presence of earthworms can slightly increase CO_2 from 4.5 g/d to 5.1 g/d, and with greater HLRs (i.e., 0.12 m^3/m^2d, flow: 28 l/d), the presence of earthworms can decrease CO_2 from 14.3 to 9.4 g/d. For N_2O, the presence of earthworms generated a decrease in emissions with a low HLR (i.e., 0.008 m^3/m^2d, flow: 2 l/d), from 44 mg/d to 12 mg/d of N_2O, and with a high HLR (i.e., 0.12 m^3/m^2d, flow: 28 l/d), from 274 mg/d to 123 mg/d.

The author mentions that N_2O emissions depend on the relationship between the organic loading rate of the external environment and the earthworm gut, suggesting that high HLRs (i.e., 0.008 m^3/m^2d, flow: 2 l/d) would explain the decrease from 274 mg/d a 123 mg/d, due to the inhibition of denitrification activity in the gut, dilution of the added nitrogen inside the vermifilter, and the effect that earthworms have on the first layers of the medium [73]. The work of Zhao et al. [6] shows the opposite phenomenon, recording increases in N_2O from 2.0 to 12.3 mg/m^2h when the organic nitrogen of the medium increases (i.e., C/N of 10 to 2.5).

Considering all of the above, it is evident that there is space to produce more scientific evidence related to the generation of greenhouse gases and the operational conditions of a vermifilter. Preliminarily, Lubbers et al. [39] and Janzen [74] have found that the presence of earthworms in bioprocesses could increase CO_2 and CH_4 emissions by 33% and 42%.

7. SUMMARIZING

Vermifilters are considered low-cost and important systems in rural areas due to their implementation potential, especially in areas with limited access and scarce resources for the installation of conventional wastewater treatment systems. In these rural areas, septic tanks or upflow anaerobic filters are commonly used, however, these systems do not usually have high removal efficiencies and especially in the removal of nutrients and pathogens in wastewater, leading to contamination of wastewater. underground or surface water sources. In this way, vermifilters are considered a sustainable and effective option for wastewater treatment in rural areas, since in addition to their relatively low design cost, they can be built using materials available in the environment, such as compost, peat or sawdust. One last potential of these systems is the removal of pathogens, which in certain cases allows the treated water to be used for irrigation, which reduces the demand for freshwater resources and saves costs in the use of chemical agents such as chlorine to its disinfection. On the other hand, vermifilters do not require high energy costs for their operation, since they are based on the interaction of natural processes in a community of worms and microorganisms for the treatment of wastewater, which does not usually occur with conventional processes, where induced aeration systems or pumping systems are required, which increases the cost of treatment. Finally, the operational and design parameters of a vermifilter can vary according to the requirements of the wastewater treatment system and the expected efficiencies. Some common technical parameters in the design and operation of a vermifilter are; hydraulic load, organic surface load, hydraulic retention times, worm density, wastewater temperature and pH, although these are usually the most common operating parameters, other factors may also need to be considered such as the type of organic material used in the vermifilter, the size of the wastewater treatment system, and the flow rate of the wastewater in the influent.

8. CONCLUSION

According to the present review, the potential use of vermifilters in rural communities is high, since it is considered a low-cost system in terms of its implementation and allows adequate efficiencies to be achieved in terms of organic matter removal. There are multiple design and operation parameters that can affect the efficiency of the wastewater treatment of a vermifilter system, and it is for this reason that different design alternatives are presented in the environment, which gives rise to forms of operation. different. It is very important to know and consider the mechanisms that allow the elimination of organic matter, nutrients and pathogens present in wastewater and achieve a unification of the information that is presented at a scientific level. Given the different types of design and operating conditions, the main characteristics to consider in the operation of a vermifilter include, HLRs where, according to the bibliographic review based on the values of HLR and OLR, these can generate favorable conditions for the development of the Worm-microorganism consortium in the active layer without obstructions due to excess organic matter. Another important parameter is the number of worms/m3 in the active layer, which makes it possible to establish the optimal amounts to remove COD, TSS and NH4+, without increasing NO3- and TP in the effluent. The support medium is important to counteract the increase in NO3- and TP concentrations, it is concluded that it is possible to use filter materials that facilitate adsorption, such as sawdust and wood chips. Temperature is usually essential in these systems, since low temperatures could reduce the rate of elimination of pathogens.

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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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CAPÍTULO 4

PERFORMANCE OF A FULL-SCALE VERMIFILTER FOR SEWAGE TREATMENT IN REMOVING ORGANIC MATTER, NUTRIENTS, AND ANTIBIOTIC-RESISTANT BACTERIA

Gutiérrez, V., Monsalve, N., Gómez, G., Vidal, G. (2023). Performance of a Full-Scale Vermifilter for Sewage Treatment in Removing Organic Matter, Nutrients, and Antibiotic-Resistant Bacteria. *Sustainability*, 15, 6842. https://doi.org/10.3390/su15086842.

Performance of a full-scale vermifilter for sewage treatment in removing organic matter, nutrients, and antibiotic-resistant bacteria

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Abstract

The vermifilter (VF) is regarded as a sustainable solution for treating rural sewage. However, few studies have investigated the performance of a full-scale vermifilter. The objective of this study is to evaluate the performance of a full-scale vermifilter in reducing organic matter, nutrients, and antibiotic-resistant bacteria contained in sewage. Influent and effluents were obtained from a rural sewage treatment plant using a VF and UV disinfection system. The results show a significant removal ($p < 0.05$) of chemical organic demand (COD) (77%), biochemical oxygen demand (BOD5) (84%), total nitrogen (TN) (53%), and total phosphorus (36%). Seasonality is an influential variable for COD, BOD5, and TN removal. In addition, the molecular weight distribution shows that the VF does not generate a considerable change in distribution of organic matter (COD and total organic carbon (TOC)) and NH4+-N. The UV disinfection system eliminated 99% of coliform bacteria; however, they are not eliminated to safe concentrations. Therefore, it is possible to detect bacteria-resistant to the antibiotics ciprofloxacin, amoxicillin, and ceftriaxone of 63.5%, 87.3%, and 63.5%, respectively, were detected in the effluents, respectively. This study shows the potential of system for removal pollution and the need to optimize the VF to be a safe treatment.

Keywords: Sewage treatment; full-scale vermifilter; antibiotic-resistant bacteria; molecular weight distribution

5. Introduction

In 2010 the World Health Organization (WHO) recognized access to drinking water and sanitation as a human right. However, in 2020 45% of the sewage generated in the world was still discharged without safe treatment [1]. It has also been observed that sanitation coverage is often much lower in rural areas than in urban areas. The lack of adequate sanitation services can lead to environmental pollution, causing water quality deterioration, biodiversity loss, and changes in ecosystem structure and function [2]. In addition, because sewage contains a great variety of fecal microorganisms and pathogens, the creation of an unhealthy environment leads to the transmission of diarrheal diseases such as cholera, dysentery, and typhoid fever [1,3]. Meanwhile, lack of sewage treatment contributes to the propagation of antibiotic resistance.

The emergence of antibiotic-resistant bacteria (ARB) and their dissemination through the environment – recognized as one of the main problems of the 21st century [4] – limit the treatment of infectious diseases and increase the probabilities of morbidity and mortality in the population [5]. ARB can survive and multiply in the presence of antibiotics thanks to antibiotic resistance genes (ARG), which encode proteins that participate in various resistance mechanisms and can be transferred to other bacteria to make them resistant [6].

Therefore, the search for decentralized technologies that can be applied in rural areas with low populations and scattered homes to minimize the risks of inefficiently treated sewage discharge has become a priority [7-10]. Between the technologies that comply with this requirement include constructed wetlands (CW), upflow anaerobic sludge blankets (UASB) and moving-bed biofilm reactors (MBBR). However, they may have drawbacks that increase their operating costs, such as clogging of the substrate in CW [11]; sludge flotation and biomass washing at UASB [12] and the need for aireation in MBBR [13]. On the other hand, the vermifilters (VFs) function as aerobic biofilters by the burrowing behavior of the eartworms that increasing the oxygen. Therefore, they do not require induced aeration or pumping systems that increases the cost of treatment [14]. Also, the earthworms are able of eat all the suspended particles screened on the filter bed and avoid the generation of sludge of a conventional biofilter [15]. The VF are a bio-oxidative process based on the symbiotic relationship between earthworms and microorganisms used to biochemically degrade waste materials [16]. Because of this, VFs can be a treatment competitive with rural wastewater treatment plants (WWTP) due to their cost savings and ecological characteristics [15, 17]. Muga and Mihelcic [18] deem VFs sustainable technologies, as they maintain economic and environmental welfare and seek equitable social progress.

High VF contaminant removal performance has been observed, with chemical organic demand (COD), nitrogen, and phosphorous removal rates of 75-90%, 32-85%, and 39-95%, respectively [19-21]. They have also proved to be efficient in the removal of total coliform (CT), fecal coliform (CF), and ARB, with rates of 58-99%, 52-99%, and 100%, respectively [9, 22, 23]. However, there have been few studies on full-scale VFs, as most are conducted at laboratory scale, with VFs operated on a short-term basis under controlled experimental conditions [24, 25]. Furthermore, there are no studies associated with joint coliform and ARB removal efficiencies.

Therefore, the objective of this study is to assess the organic matter, nutrient, and ARB removal efficiency of a full-scale VF that treats sewage. In addition, this study will evaluate performance and the influence of seasonality through molecular weight distribution analysis with respect to organic matter and nutrient removal efficiency.

6. Materials and Methods

2.1 Design and operating conditions

This study was carried out in a rural WWTP in Copiulemu Commune, Concepción, Biobío Region, Chile. This WWTP uses a solid removal pretreatment system, a vermifilter (VF), and a UV radiation system as a tertiary treatment. The VF is a vertical subsurface flow system, the main characteristics of which are summarized in Table 4.1. Sewage enters a rotary filter with a helical plate to carry solids to a container outside the VF. The sewage is stored in a holding tank and transferred by two pumps to the upper part of the VF, distributing the sewage over the surface via 24 sprinklers.

Tabla 4.1. Summary of vermifilter design and operating parameters

Parameter	Unit	Value
Vermifilter volume	m^3	442
Vermifilter area	m^2	276
Vermifilter height	m	1.60
Influent flow	m^3/d	160
HLR	m^3/m^2d	0.60
OLR	kg COD/m^2d	0.50
Carbon/Nitrogen ratio	-	9
Temperature	°C	21
Active layer	-	woodchip
Active layer height	m	0.90 ± 0.12
Species	-	*Eisenia foetida*
Earthworm density zone A	$worm/m^3$	~ 1,100
Earthworm density zone B	$worm/m^3$	~ 7,000

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

The VF uses a flow rate, hydraulic loading rate (HLR), and organic loading rate (OLR) of 160 m3/d, 0.6 m3/m2d, and 0.5 kg COD/m2d, respectively. Figure 4.1 shows a schematic representation of the active layer, which included the filter material with a lower stone layer and upper woodchip layer (active layer) of 0.92 ± 0.12 m . The active layer contains *Eisenia foetida* species, which are distributed between different zones. The upper zone (zona A) has a height of 104 cm of woodchips and contains ~ 1,100 earthworms per cubic meter and the lower zone (zone B) has a height of 80 cm of woodchips and contains ~ 7,000 earthworms per cubic meter. Meanwhile, the disinfection system operates with a gravitational flow of 4.18 m^3/h. It is composed of 15 low-pressure, high-intensity mercury lamps, with an input power of 87 W and output power of 28 W.

Figura 4.1. Schematic representation of the active layer.

2.2 Monitoring strategy

The monitoring strategy involved taking samples between May 2022 and January 2023, covering the fall, winter, spring, and summer of the Southern Hemisphere. Figure 4.2 shows a schematic diagram of the monitoring strategy, which included collection of samples at the pretreatment outlet (IN), VF outlet (E1), and UV outlet (E2). The solid samples, meanwhile, were obtained by inserting a tube (12 cm in diameter and 100 cm in length) into the active layer. Woodchips were collected from inside the tube to calculate earthworm density. All the samples were stored for less than 24 h at 4°C prior to their analysis.

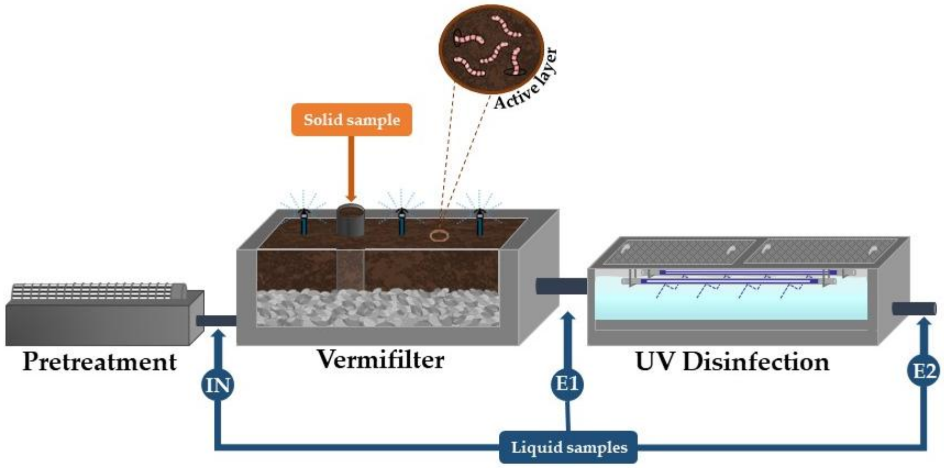

Figura 4.2. Schematic representation of the monitoring strategy. IN: pretreatment outlet; E1: vermifilter outlet; E2: UV (ultraviolet) outlet.

2.3 Physicochemical analysis

2.3.1 Liquid samples

To determine the quality parameters of IN, E1, and E2, the samples were filtered with a Whatman membrane with a pore size of 0.7 µm and analyzed based on the protocol described in the standard method [26]. The in situ parameters pH, temperature (T), dissolved oxygen (DO), electrical conductivity (EC), and oxidation-reduction potential (ORP) were measured using a multiparametric OAKTON-PC650 (Eutech Instruments; Singapore). DO was measured using an Oxi 330i handheld oximeter (WTW, Xylem Analytics Germany Sales, Oberbayern, Germany). The organic matter present in the samples was determined in the form of COD (colorimetric method, 5220-D), total organic carbon (TOC) (catalytic combustion oxidation and NDIR detection, TOC analyzer-LCPH, Shimadzu, Kyoto, Japan), and biological oxygen demand (BOD_5) (azide-modified Winkler method, 5210-B). Solids were measured based on total suspended solids (TSS) and volatile suspended solids (VSS). TSS were determined using gravimetric methods in which the sample was filtered (1.5 µm) and dried at 105°C. To determine VSS, the samples were dried for 30 min at 550°C and subtracted from the TSS value (gravimetric method, 2540-D). The analyzed nutrients were measured in the form of NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P (Shimadzu UV 1800 UV–Vis spectrophotometer, Kyoto Japan), total nitrogen (TN) (Spectroquant-Nova 60, Merck kits, Darmstadt, Germany), and total phosphorous (TP) (Spectroquant-Nova, Merck kits, Darmstadt, Germany).

2.3.2 Solid simples

pH, EC, and ORP were measured by diluting the active layer in distilled water at a ratio of 1:10 and stirring at 200 rpm for 1h [8]. Moisture content was determined by the weight loss of the sample at 105°C in 24 h [27]. The biofilm analyses were conducted by extracting 20 g of woodchips, which were suspended in 20 mL of distilled water and sonicated for 20 min to quantify them as volatile solids (VS) per area [26, 28].

2.4 Molecular weight distribution analysis of liquid samples

The IN and E2 samples underwent COD, TOC, and NH_4^+-N molecular weight distribution analysis. To this end, ultrafiltration (UF) was performed in a 450-mL shaken cell (Advantec UHP 76) at 20°C using nitrogen gas. The UF was carried out using three cellulose membranes with nominal molecular weight cut-offs of 10,000 Da, 5,000 Da, and 1,000 Da, allowing four different fractions to be obtained: compounds greater than 10,000 Da ("> 10,000 Da"), compounds between 5,000 and 10,000 Da ("5,000 – 10,000 Da"), compounds between 1,000 and 5,000 Da ("1,000 – 5,000 Da"), and compounds below 1,000 Da ("<1,000 Da") [29,30]. Each of the obtained fractions underwent COT, COD, and NH_4^+-N quantification, as mentioned in Section 2.3.1.

2.5 Microbiological analysis of liquid samples

The FC, TC, and ARB content of the IN, E1, and E2 samples was analyzed in accordance with the protocol described in the standard method [26]. FC and TC were determined by means of the multiple tube technique using the most probable number methodology (MPN/100mL). The presence of bacterial groups is determined by means of a presumptive and confirmatory test (Standard Method 9221-TC). ARB concentrations, meanwhile, were determined by antimicrobial susceptibily testing (AST) using the plate count technique [31]. MacCONKEY agar (Merck, Darmstadt, Germany) was used as culture medium and the plates were supplemented with the antibiotics amoxicillin (AMX), ceftriaxone (CTX), and ciprofloxacin (CIP) at concentrations of 32 µg/mL, 4 µg/mL, and 2 µg/mL, respectively [32]. Dilutions were prepared for IN, E1 and E2 samples and were incubated at 30°C for 24 hours. After incubation, the colonies capable of resisting the action of the antibiotic are counted, so this technique expresses the ARB concentration in colony-forming units (CFU/100mL).

2.6 Statistical analysis

Statistical analyses of the organic matter, nutrient, ARB, and coliform removals and the effect of seasonality were performed using RStudio version 1.2.1335, with a significance level of $p = 0.05$. Shapiro–Wilk and Fligner–Killen tests were conducted to analyze the normality and homogeneity of variance, respectively. Next, an ANOVA test was performed for the data with a normal distribution and a Kruskal–Wallis test for data without a normal distribution.

7. Results and discussion

3.1 Evaluation of vermifilter performance in organic matter and nutrient removal

Table 4.2 shows the results regarding the physicochemical and microbiological parameters in IN, E1, and E2. During the 6 months of monitoring, the full-scale VF presented statistically significant differences ($p < 0.05$) between IN and E2 in pH decrease (7.8 ± 0.4 to 6.8 ± 0.3), ORP increase (-25.4 ± 141.7 to 245.9 ± 127.5), and turbidity decrease (68.6%). Similarly, statistically significant removal efficiencies ($p < 0.05$) between IN and E2 were found for NH4+-N (81%), TN (53%), PO43--P (34%), TP (36%), COD (77%), BOD5 (84%), TSS (78%), and VSS (79%). The high standard deviation values of ORP, BOD5 and TSS, can be explained by variations in the influent, which affect the storage levels and the sedimentation of solids before being transferred by the two pumps to the VF [33].

Tabla 4.2. Physicochemical characterization of IN, E1, and E2 during the monitoring period

Parameter		Unit	IN (Mean ± SD)	Effluent (Mean±SD)	
				E1	E2
In situ	T	°C	17.1 ± 3.7	15.9 ± 3.6	15.8 ± 3.4
	pH	-	7.8 ± 0.4	6.9 ± 0.2	6.8* ± 0.3
	ORP	mV	-25.4 ± 141.7	181.8 ± 77.3	245.9* ± 127.5
	EC	µS/cm	1.6 ± 0.2	1.0 ± 0.4	1.2 ± 0.1
	DO	mg/L	1.2 ± 0.5	5.1 ± 0.6	5.0* ± 0.8
	Turbidity	NTU	210.8 ± 59.4	61.2 ± 22.6	66.2* ± 30.3
Nutrients	NH_4^+-N	mg/L	104.9 ± 35.3	23.0 ± 10.7	19.8* ± 8.7
	TN	mg/L	114.4 ± 32.5	57.5 ± 8.1	53.7* ± 6.0
	PO_4^{3-}-P	mg/L	10.3 ± 2.6	6.5 ± 2.4	6.8* ± 1.0
	TP	mg/L	12.5 ± 2.0	7.9 ± 2.9	8.0* ± 1.5
Organic matter	COD	mg/L	885.8 ± 89.9	286.0 ± 60.7	204.2* ± 38.4
	BOD_5	mg/L	486.3 ± 123.1	109.6 ± 27.5	79.4* ± 32.6
	TSS	mg/L	239.1 ± 101.4	67.3 ± 16.5	53.4* ± 17.9
	VSS	mg/L	186.6 ± 68.1	50.8 ± 18.9	39.5* ± 14.1
Microbiological	TC	log_{10}(MPN/100mL)	8.7 ± 0.7	6.5 ± 0.9	5.2* ± 1.0
	FC	log_{10}(MPN/100mL)	7.8 ± 1.0	5.9 ± 1.0	4.4* ± 1.3

Note: IN: Influent; E1: VF effluent; E2: UV effluent; SD: Standard deviation; NTU: Nephelometric Turbidity Unit; T: temperature; ORP: oxidation-reduction potential; EC: electrical conductivity; DO: dissolved oxygen; TN: total nitrogen; TP: total phosphorus; COD: chemical oxygen demand; BOD_5: biological oxygen demand, at five days; TSS: total suspended solids; VSS: volatile suspended solids; TC: total coliforms; FC: fecal coliforms; MPN: Most Probable Number. *: Statistically significant differences ($p > 0.05$) between IN and E2.

3.1.1 Organic matter

The VF achieved statistically significant removal efficiencies ($p < 0.05$) for COD and BOD_5 of 77% and 84%, respectively. These results are in line with those reported by authors such as Wang et al. [34], Zhao et al. [35], Tahar et al. [10], and Karla et al. [36], who reported COD and BOD_5 removal efficiencies above 75%. Organic matter removal in the VF is determined by the physicochemical modifications of the active layer, burrowing behaviors that promote the development of aerobic microorganisms, and earthworm excreta that modify the microbiome [24,37,38].

Table 4.3 presents different pilot- and full-scale VFs with their respective operating parameters and organic matter and nutrient removal efficiencies. With respect to active layer material, the woodchip allowed removal efficiencies of over 77% of COD and BOD_5. This coincides with results obtained from pilot-scale VFs, which report efficiencies over 75% [25,35,39]. These results can be explained as woodchip has been reported that have a hydraulic conductivity of 250-700 mm/h which prevents clogging [40] and adsorption properties that allow contaminant removal [10, 41].

Table S1 shows the physicochemical properties of the active layer at different earthworm densities. The high-density zone (Zone B) presents a pH of 4.8 ± 0.5 and an ORP of 367.5 ± 38.7 mV, while the zone with a low earthworm density (Zone A) presents a pH of 3.9 ± 0.2 and an ORP of 239.2 ± 29.6 mV. The increase in ORP suggests that a greater earthworm density increases the oxygen concentration and contributes to the degradation of organic matter. Regarding the pH in the layer that is active, the organic oxidation can lower alkalinity and generate organic acids that lower this pH [42]. However, this process can be neutralized by the calcium content in the earthworm gut [43]. To explain the increase in pH in Zone B, Singh et al. [42] and Hughes et al. [44] show that pH levels below 4.5 limit the performance of a VF.. On the other hand, Tahar et al. [10] indicate that high removal efficiencies are achieved at 5,000-10,000 worm/m3. Therefore, although densities of 1,100 worm/m3 are sufficient to maintain aerobic environments with high oxidation potentials (239.2 ± 29.6 mV). The worm may have problems neutralizing the active layer and may affect COD and BOD5 removal efficiency.

Regarding the operating parameters, HLR and OLR correspond to influential parameters in the organic matter removal [45, 46]. In general, Table 3 shows that pilot-scale VFs operate with HLRs less than 1 m^3/m^2d and OLRs less than 1 kg COD/m^2 [16, 27, 47]. Ghasemi et al. [45] and Jiang et al. [14] reported that operating with HLRs less than 2.5 m^3/m^2d and OLRs up to 0.6 kg COD/m^2d allows optimal growth of the microbial community without the system becoming clogged with organic matter. Meanwhile, Liu et al. [24], Ghasemi et al. [45], and Pous et al. [39] achieved COD and BOD_5 removal efficiencies greater than 70% in systems operating with HLRs over 1.3 m^3/m^2d and OLRs over 1 m^3/m^2, which is related to the use of systems complementary to the VF such as zooplankton biofilters and recirculation processes.

Tabla 4.3. Removal of organic matter and nutrients in pilot- and full-scale vermifilters with different operational configurations

Scale	Volume (m^3)	Active layer	Density (worm/m^3)	HLR (m^3/m^2d)	OLR (kg COD /m^2d)	Organic matter (% removal)		Nutrients (% removal)		Ref.
						COD	BOD_5	TN	TP	
Pilot	0.50	Woodchips	13,263	1.30	1.20	91	91	3	15	[39]
Pilot	1.50	Compost/ soil	5,000-6,000	2.00	0.37	60	-	-	-	[45]
Pilot	-	Woodchips	-	0.48	1.70 - 2.93	84	-	65	70	[25]
Pilot	0.10	Sand	285	0.08	0.07	68	-	60	45	[47]
Pilot	0.30	Organic fraction/ vermigratings	7,143	1.00	0.24	78	88	-	-	[27]
Pilot	0.02	Vermigratings	30,000	1.00	0.45	74	90	-	-	[11]
Pilot	0.10	Soil / woodchips	8,929	1.05	0.11 - 0.43		-	61	62	[48]
Pilot	0.45	Soil / woodchips	3,061	0.06	0.01 - 0.02	81	-	80	81	[35]
Full-scale	4.20	Ceramsite	16,000	4.20	0.39	68	78	-	-	[24]
Full-scale	**433**	**Woodchips**	**A:1,100 B: 7,000**	**0.50**	**0.60**	77	**84**	**53**	**36**	**Present study**

Note: T: temperature; HLR: hydraulic loading rate; OLR: organic loading rate; COD: chemical oxygen demand; BOD5: biological oxygen demand, at five days; TN: total nitrogen; TP: total phosphorus; A: zone A; B: zone B.

3.1.2 Nutrients

Table 4.2 shows that the VF achieved TN, NH_4^+-N, PO_4^{3-}-P, and TP removal efficiencies of 53%, 80%, 34%, and 36%, respectively, which were statistically significant ($p < 0.05$) between IN and E2. These results are in line with the findings of Huang et al. [48], Lavrnic et al. [47], and Tahar et al. [10], who found similar removal efficiencies. The main nitrogen transformation mechanisms occur through nitrification and denitrification [14]. Nitrification in the VF occurs by means of autotrophic microorganisms that develop in the presence of aerobic conditions generated by the burrowing behavior of the earthworms [46]. This explains the decrease in NH_4^+-N from 104.9 ± 35.3 mg/L to 19.8 ± 8.7 mg/L and the increase in NO_3^--N from 0.7 ± 0.6 mg/L to 29.6 ± 9.7 mg/L in E2. Meanwhile, the decrease in TN from 114.4 mg/L to 53.7 mg/L indicates the presence of denitrification processes, which occur in guts of the earthworms, as they store denitrifying bacteria [35, 46]. Regarding operating parameters (Table 3), TN removal has also been reported by authors such as Huang et al. [48], Arora and Kazmi et al. [16], and Lavrnic et al. [47] when operating with HLRs between 0.1 m^3/m^2d – 1.0 m^3/m^2d and OLRs between 0.01 – 0.6 Kg COD/m^3d. VFs that operate within these ranges optimize the formation of microorganism colonies in the active layer that participate in nitrification [15, 46]. Meanwhile, Thompkins et al. [25] and Ghasemi et al. [45] report TN removal efficiencies greater than 60% at HLRs greater than 1.0 m^3/m^2d and OLRs greater than 1 kg COD/m^{2}d, which is again attributed to the use of treatment systems complementary to the VF. Phosphorous removal depends mainly on the adsorption capacity of the active layer [43]. As in this VF study, other studies at pilot scale have achieved high removal efficiencies using woodchips [16, 25, 35, 48]; however, Pous et al. [39] reported removal efficiencies of only 15% in a pilot-scale VF. This result may be associated with earthworm density, as Kumar et al. [22] and [49] observed TP increases when using a density of 10,000 worm/m^3 in a laboratory-scale VF. Earthworms have microorganisms and enzymes capable of mineralizing TP and leaving it available as PO_4^{3-}-P. Therefore, earthworm density is considered a fundamental factor among operating parameters.

3.1.3 Molecular weight distribution analysis of organic matter and nutrients

Figure 4.3 shows the performance of the VF in terms of TOC, COD, and NH_4^+-N by means of a molecular weight distribution analysis. Organic matter in IN is distributed mainly in the fractions between 5,000 – 10,000 Da and >10,000 Da for COD and COT, respectively. Meanwhile, in E2 the COD distribution changes and is found mainly in the fraction >10,000 Da. NH_4^+-N is distributed mostly in fraction <1,000 Da in IN and E2, at proportions of 60.8% and 55.1%, respectively.

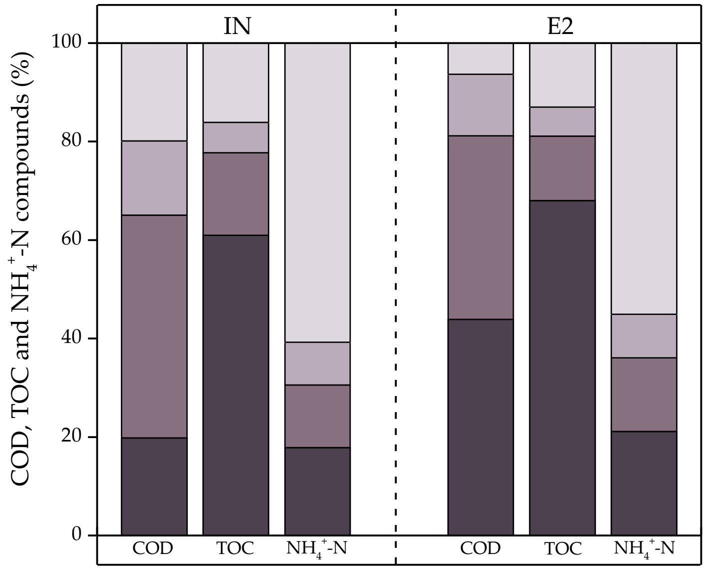

Figura 4.3. Molecular weight distribution of total organic carbon (TOC), chemical oxygen demand (COD), and NH_4^+-N in samples of the pretreatment output (IN) and UV output (E2). = <1,000 Da; = 1,000 – 5,000 Da; = 5,000 – 10,000 Da; = >10,000 Da.

In E2, there are decreases in COD and TOC in the <10,000 Da fractions of 8% and 4%, respectively. This can be explained by the symbiotic relationship between earthworms and microorganisms that allows the degradation of organic matter in fractions below 10,000 Da to be used as energy and a carbon source [50,51]. In E2 there are also increases in COD and TOC in the >10,000 Da fraction of 24% and 7%, respectively. These results were observed by Wang et al. [51] and Li et al. [52], who reported that organic matter in fractions below 100,000 Da can increase due to biodegradation and formation of aggregates with high molecular weights such as exopolysaccharides and humic substances [51]. However, organic matter with molecular weights above 100,000 Da can be retained in the filter medium. This can explain why the molecular distribution of COD and TOC does not undergo large variations. With respect to NH_4^+-N, the distributions in IN and E2 are similar. In both IN and E2 it is distributed mainly in the <1,000 Da fraction. Its distribution in this fraction may be associated with the fact that approximately 90% of the nitrogen comes from urine in the form of NH_4^+ [53]. In addition, the digestion process of earthworms leads to the excretion of ammonia and urea, which can be interconverted into NH_4^+ depending on the pH [16,54]. In E2, there is a decrease of 5.6% in the <1,000 Da fraction, which may be associated with nitrification mechanisms that occur in the system, through which NH_4^+-N is converted into NO_3^--N, decreasing its concentration. The fractions 1,000 – 5,000 Da and 5,000 – 10,000 Da remain unchanged. While the increase of 3.3% in the >10,000 Da fraction is associated with the release of proteins and DNA from the biofilm, cell debris, or ARG [51]. Therefore, it can be concluded that the VF does not generate a significant change in the complexity of the organic compounds and NH_4^+-N present in sewage. However, it is interesting to note and study the increase in COD and TOC in the >10,000 Da fraction, as it may also be associated with the high presence of TSS in E1 and the low microbiological compound removal efficiency of the UV disinfection system, which will be discussed below.

3.2 Effect of seasonality on the removal of organic matter and nutrients by a vermifilter

3.2.1 Organic matter

Figure 4.4 shows the COD and BOD_5 concentrations in IN and E2 in the fall-winter and spring-summer periods, with their respective removal efficiencies. A comparison of the removal efficiencies in each of the evaluated periods shows that the organic matter reduction was statistically significant ($p < 0.05$). In fall-winter, 72% and 78% of COD and BOD_5 were eliminated, respectively, while in spring-summer, the respective removals were 81% and 89%. Earthworms are poikilothermic species; therefore, they are affected by the season's temperatures of the medium [55]. Furlong et al. [56] determined that *Eisenia foetida* lives in an optimum range of 15 °C to 20 °C; thus, temperatures outside this range can affect organic matter, nutrient, and pathogen removal in a VF [16, 57]. In the studied full-scale VF, the temperatures were 10 ± 1°C in fall-winter and 15°C ± 5°C in spring-summer. These differences affect the growth and metabolic activity of earthworms and the microorganisms that are part of this symbiotic association [58]. Therefore, below 15°C it is possible that the decomposition and oxidation processes of the VF will be affected [59].

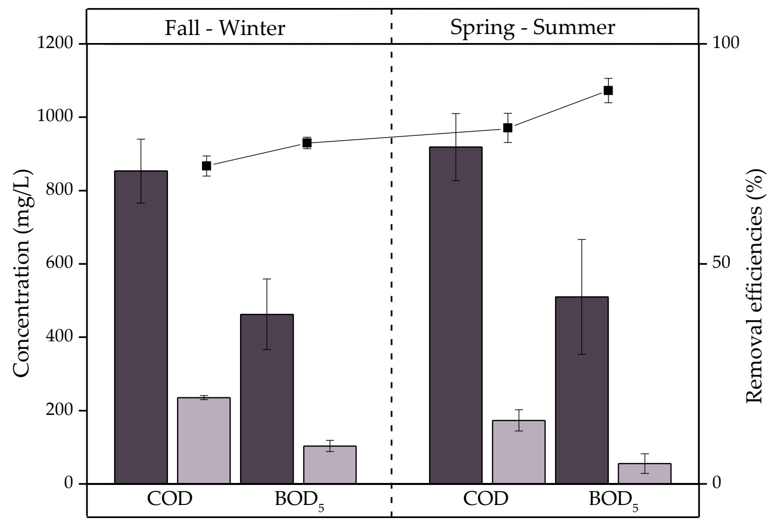

Figura 4.4. Concentrations (mg/L) of COD and BOD_5 in IN (), E2 () and removal efficiencies (%) in fall–winter and spring-summer.

3.2.2 Nutrients

Figure 4.5 shows the TN, NH_4^+-N, and NO_3^--N concentrations in IN and E2 in the fall-winter and spring-summer periods. The TN removal efficiencies were statistically significant ($p < 0.05$). In the fall-winter period the reduction was 61%, while in spring-summer it was 40%. This difference may be related to the greater growth and metabolic activity that can affect nitrogen transformation processes [42, 58]. Meanwhile, during the fall-winter period, there was a slightly significant increase in TN ($p = 0.06$) that could be explained by the seasonal rainfall that carries nitrogenous compounds and change the carbon-nitrogen ratio [60]. Zhao et al. [35] reported that the optimum carbon-nitrogen ratio is 5:1 to 10:1; changes to outside this range can lead to decreases in removal efficiency. During the fall-winter period, the carbon-nitrogen ratio was 6.0 ± 0.7, while during the spring-summer period it was 11 ± 0.8. These changes could explain the decrease in TN removal efficiency.

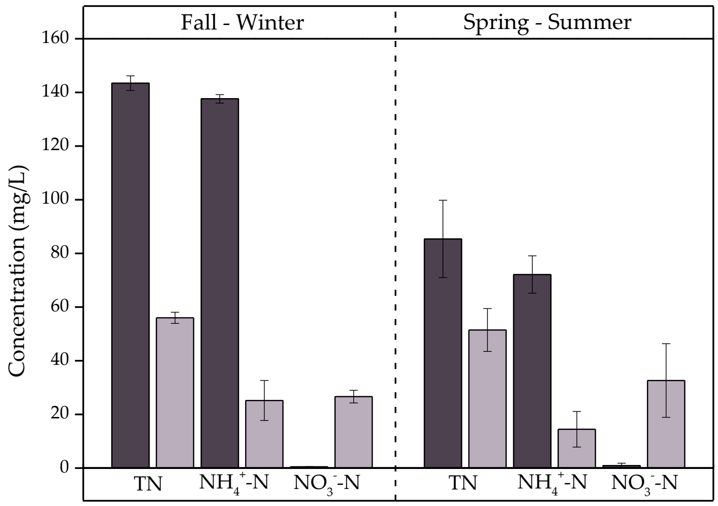

Figura 4.5. Concentrations (mg/L) of total nitrogen (TN), NH_4^+-N, and NO_3^--N in IN ( ) and E2 ( ) in fall-winter and spring-summer.

3.3 Removal of microbiological compounds by the vermifilter

3.3.1 Coliforms

Figure 4.6 shows the FC and TC concentrations and removal efficiencies obtained in IN, E1, and E2. There were average FC concentrations of 8.9 ± 8.9, 6.9 ± 7.1, and 6.2 ± 6.4 $\log_{10}$(MPN/100mL), respectively, and average TC concentrations of 8.5 ± 8.6, 6.9 ± 7.3, and 5.8 ± 5.8 $\log_{10}$(MPN/100mL), respectively.

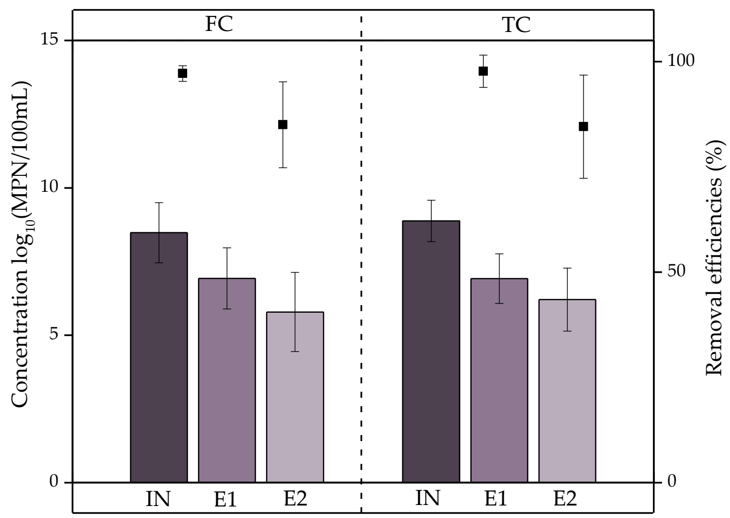

Figura 4.6. Fecal coliform (FC) and total coliform (TC) concentrations (log$_{10}$(MPN/100mL)) in IN (), E1 (), and E2 () and removal efficiencies (%).

The FC and TC removal efficiencies in E1 were on average 97.2% and 97.7%, respectively, which were statistically significant ($p < 0.05$). Arora et al. [23] reported FC and TC removal efficiencies of 99% and 90%, respectively, a result similar to that found by Kumar et al. [22], who reported removal efficiencies of 99.4% and 99.7%, respectively. Arora et al. [9], meanwhile, reported that pathogenic strains such as *Enterobacter, E. coli*, and *Pseudomonas* were not detected in the final effluent, showing a considerable removal of pathogens by the VF. These results were achieved with a laboratory-scale VF, that is, under controlled conditions; therefore, they suggest that a full-scale VF could reach these removal if its operating and design parameters are optimized.

Coliform removal efficiencies in a VF are associated with filtration, adsorption, and physicochemical conditions inadequate for their survival [16]. Due to the burrowing activity of the earthworms, oxygenation and microbial activity increase [9]; therefore, predation and competition can be influential factors in coliform reduction. Wand et al. [61], in studies on predation on *E. coli*, showed removal efficiencies close to 100% due to the activity of flagellated protozoa and *Bdellovibrio*. In addition, earthworms secrete mucus, the viscous and sticky nature of which restricts the movement of foreign microorganisms and aids in their destruction. Das and Paul [62] reported that earthworms ingest and destroy unfavorable microorganisms, decreasing the pathogen count. Finally, the antimicrobial properties of earthworms have already been documented, with 60% of pathogen removal attributed to them [9]. Authors such as Hussain et al. [63] and Chauhau et al. [64], studying earthworm tissue extracts, body paste, and coelomic fluid, reported that they can act as antimicrobial agents, although they did not exhibit activity against enteric bacteria; therefore, more studies on the behavior of these antimicrobial agents in VFs are needed.

UV disinfection generated FC and TC removal efficiencies of 85.0% and 84.9%, respectively. They were not significant and were lower than those achieved by the VF by 12.2% and 13.2%, respectively. González et al. [65] indicate that the presence of TSS (>26.7 mg/L) can influence the efficiency of disinfection treatment. Taking into account that in the present study the TSS concentration was 67.3 mg/L, this could be the reason that the UV treatment was hindered.

The FC and TC removals of the complete WWTP treatment were 99.4% and 98.9%, respectively, which were statistically significant ($p < 0.05$). However, the FC and TC concentrations in E2 were 6.2 ± 6.4 and 5.8 ± 5.8 $\log_{10}$(MPN/100mL), respectively. According to the WHO, the maximum allowable limit in effluents before discharge into rivers is 3 $\log_{10}$(MPN/100mL) [16]. Therefore, the discharge of these effluents into the receiving water body could pose a risk to human and animal health due to the possibility of triggering gastrointestinal diseases produced by *E. coli, enterovirus, adenovirus, salmonella*, and *Crystosporidium* [66]. Tyagi et al. [67] in a study on the removal of pathogens in various WWTPs, determined that the removal of bacteria in sewage is associated with the removal of BOD_5 and TSS. Therefore, optimization of operating parameters and VF design is necessary. Several studies suggest that the removal of organic matter, TSS and pathogens is enhanced by synchronizing FVs with other treatment systems [17, 21, 39]. Because of this, further studies are suggested.

3.3.2 Antibiotic-resistant bacteria

Figure 4.7 shows the rates of bacterial resistance to AMX, CTX, and CIP in IN, E1, and E2 and the removal efficiencies obtained after the treatments. In IN the average rates of resistance to AMX, CTX, and CIP were 95.6%, 79.8%, and 71.5%, respectively. This result indicates high resistance rates in rural sewage which have been reported countless times by various authors [7, 68, 69]. This is attributed mainly to the direct consumption of antibiotics to treat infectious diseases and indirect consumption in the form of contaminated food [70-72]. As a result of this consumption, selective pressure on enteric bacteria can lead to the development of antibiotic resistance [5]. These bacteria are subsequently excreted into sewage together with traces of antibiotics that can continue to select for ARB in the environment [73].

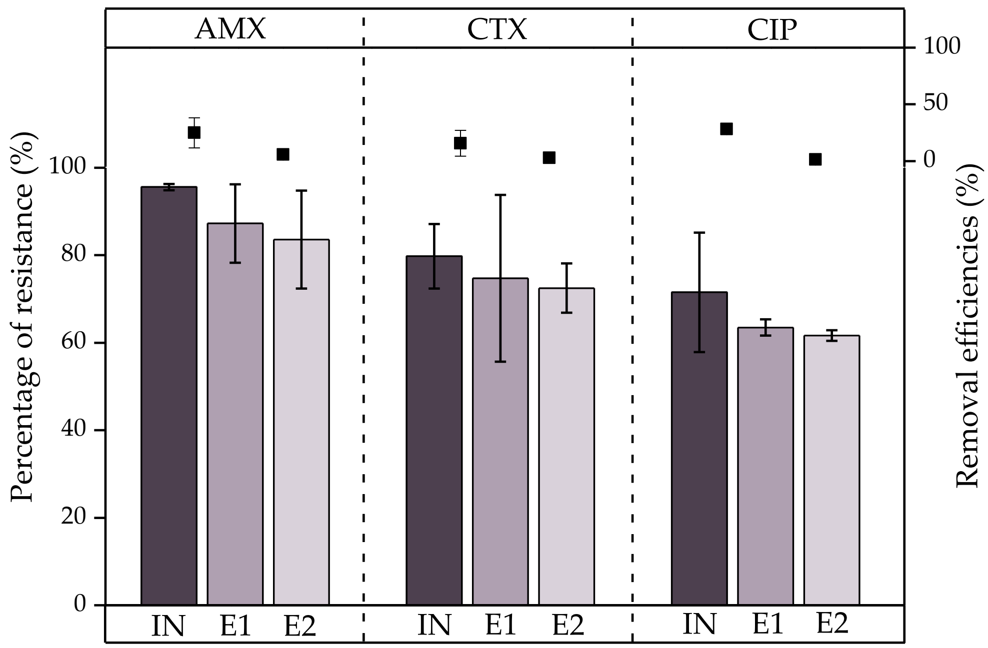

Figura 4.7. Bacterial rates of resistance (%) to amoxicillin (AMX), ceftriaxone (CTX) and ciprofloxacin (CIP) in IN (), E1 (), and E2 () and ARB removal efficiencies (%).

The resistance rates of E2 for AMX, CTX, and CIP were 87.3%, 74.7%, and 63.5%, respectively, with the VF generating ARB removal efficiencies of 25.0%, 15.7%, and 28.2%, respectively. However, these efficiencies were not significant ($p > 0.05$). Arora et al. [23] reported removal efficiencies of 100% for bacteria resistant to ampicillin, ticarcillin, gentamicin, and chloramphenicol. These analyses were conducted in a pilot-scale VF; therefore, further studies in a full-scale VF are suggested. ARG behavior can vary depending on environmental conditions, parameters, and operating period [74, 75]. Due to biofilm accumulation, ARB and ARG in a pilot-scale system will not have the same fate as in a larger system with a longer operating period.

As the ARB counting method is specific to enterobacteria, the detected ARB are considered to belong to the genera *Escherichia, Salmonella, Enterococcus, Shigella*, and *Klebsiella* [6, 76]. Considering that the VF significantly removed coliforms and not ARB, this result is associated with the increase in ARB in the system. The presence of selection agents such as antibiotics, heavy metals, and disinfection products raises the risk of an increase in antibiotic resistance, as their ability to select for ARB in the environment has been documented by various authors [77-79]. In addition, the greater oxygenation of the system and high organic load present could be favoring the transfer of ARG to previously susceptible bacteria [71,80]. The earthworms and microorganisms present form biofilms as a symbiotic association to degrade compounds and other organisms [9, 24]. Zhang et al. [81] and Engeman et al. [82] observed a rapid migration of *tet* genes from water columns with animal waste to biofilms. This indicates the capacity of ARG to progressively accumulate in biofilms; therefore, they could act as an ARG reserve. E2 presented bacterial resistance rates of 83.6%, 72.5%, and 61.7% for AMX, CTX, and CIP, respectively. The ARB removal efficiencies were 5.9%, 2.8%, and 1.4%, respectively. Similarly, it has been documented that disinfection treatments can cause pathogen reactivation, as they have developed DNA repair mechanisms when low doses of UV radiation are applied [65]. In addition, Stange et al. [83] reported average *E. coli* and *E. faecalis* reductions of 5.5 ulog, but insignificant ARG reductions by UV disinfection; therefore, ARG can be transferred to previously susceptible bacteria even after disinfection. Zhuang et al. [84], meanwhile, reported significant removals of *sul*1 and *tet*G; however, they state that the most effective doses of UV radiation are between 10 and 100 times more than those commonly used in WWTP, which would mean high energy consumption. These results indicate that E2 presents a high antibiotic resistance load, both with respect to the assessed ARB and the possible ARG that could be present; therefore, there is a high risk of transmission of antibiotic resistance through the discharge of these effluents into their receiving river. Bueno et al. [85] studied the dissemination of ARG after effluent discharge by a WWTP, finding a significant increase in 17 ARG downstream of a WWTP in Chile. Similarly, Proia et al. [86] observed increases in bla_{TEM}, *qnr*S, *tet*O, and *tet*W in the receiving river of two WWTPs in Belgium. To prevent this spread of antibiotic resistance to bodies water, authors have investigated the combination of disinfection methods and the use of advanced oxidation processes (AOP). Barancheshme et al. [87] and Zhang et al. [88] report better results using a sequential chlorination/UV disinfection treatment compared to treating each alone. While Zhang et al. [89] and Karaolia et al. [90] determined that AOPs such as UV/H_2O_2 and UV/TiO_2 can generate ARG reductions of up to 6 ulog, further investigations are recommended both at full-scale VF and with different sewage characteristics.

8. Conclusions

Considering the results of this study, it can be concluded that sewage treatment by means of a full-scale VF operated with 0.5 m3/m2d and 0.6 Kg COD/m2d:

1. Generates statistically significant removals ($p < 0.05$) of COD (77%), BOD5 (84%), TN (53%), and TP (36%). Seasonality is a factor that significantly influenced COD, BOD5, and TN removal. COD and BOD5 are eliminated at 9% and 11% higher rates in spring-summer, respectively, while TN is eliminated at a 21% higher rate in fall-winter. Because temperature is influential in the growth and activity of earthworms and microorganisms causing FV removal efficiencies.
2. The molecular weight distribution indicates that the organic matter (COD and TOC) percentage decreases by an average of 6% in the <1,000 Da fraction after the VF, while it increases by an average of 16% in the >10,000 Da fraction; therefore, the VF does not generate considerable changes in the molecular weight distribution of organic matter and NH4+-N due to processes of degradation, adsorption, and formation of aggregates with high molecular weights.
3. Coliform removal by the VF was statistically significant ($p < 0.05$) at 99.4% and 98.9% for FC and TC, respectively, although the concentrations in the effluents were 6.2 and 5.8 log10(MPN/100mL), respectively; therefore, the WWTP does not reduce coliforms to safe levels. In addition, ARB removal was not significant ($p > 0.05$); thus, ARB selection and ARG transfer processes are occurring within the system. The effluents discharged into the receiving river could lead to dissemination of antibiotic resistance in the environment.
4. Thanks to the results obtained, the projections of this research are focused on optimizing full-scale VFs in the elimination of organic matter, nutrients and pathogens. These include evaluating different operating and design parameters; determining the efficiency of VFs synchronized to other technologies and specifying and adequate cost-effective disinfection method for the efficient removal of ARB and ARG.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15086842/s1, Table S1, Physicochemical characteristic of the active layer with different earthworm density

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CAPÍTULO 5

HETEROTROPHIC ACTIVITY IN THE ACTIVE LAYER OF A VERMIFILTER USED FOR SEWAGE TREATMENT: EFFECTS OF WORM DENSITY AND SEASONALITY

Gutiérrez, V., Gómez, G., Rodríguez, D.C., Vidal, G. (2025). Heterotrophic activity in the active layer of a vermifilter used for sewage treatment: effects of worm density and seasonality. *J. Environ. Chem. Eng*. 13, 117639. https://doi.org/10.1016/j.jece.2025.117639.

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 5.1. 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.

Tabla 5.1. Summary of design and operating parameters of the vermifilter.

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

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 5.1 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].

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.

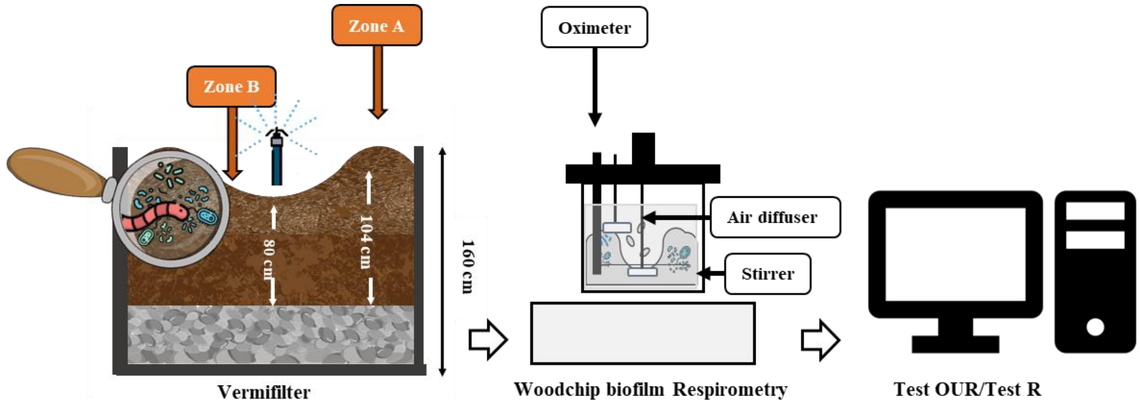

Figura 5.1. Schematic representation of a VF and respirometry test.

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 5.2 shows the main kinetic parameters related to heterotrophic activity that were calculated via BM-Advance, which is integrated into the device.

Tabla 5.2. Key kinetic parameters of the respirometry test.

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.

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 5.3 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.

Tabla 5.3. Kinetic parameters of heterotrophic biomasses in different WWTPs.

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.

3.1.1 Oxygen consumption (OURend and SOURend)

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 $mgO_2/L \cdot h$ to 2.61 $mgO_2/L \cdot h$. 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 5.4 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, $\mu_{max.}$

Tabla 5.4. Kinetic parameters of the heterotrophic biomass in a VF between seasons.

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.

3.3 Effects of earthworm density on heterotrophic activity

Figure 5.2 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].

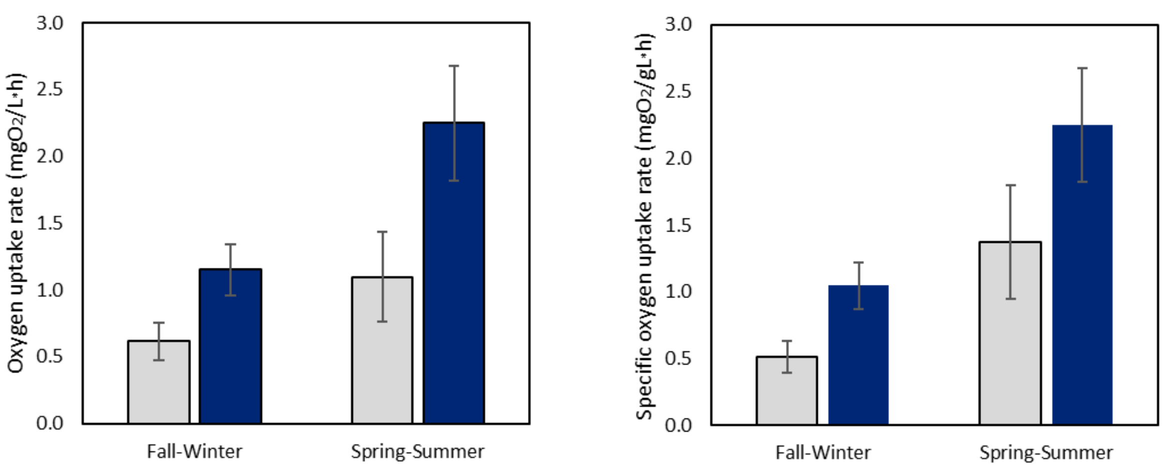

Figura 5.2. Respirometry test in different seasons and with different earthworm densities. a) OUR_{end} and b) $SOUR_{end}$. Where Zone A: 1105 ± 982 worms/m^3; Zone B: 7221 ± 1699 worms/m^3.

3.4. Organic matter removal and heterotrophic activity in the VF

Figure 5.3 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 $mgO_2/gL\cdot h$ in fall-winter to 1.6 $mgO_2/gL\cdot h$ 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.

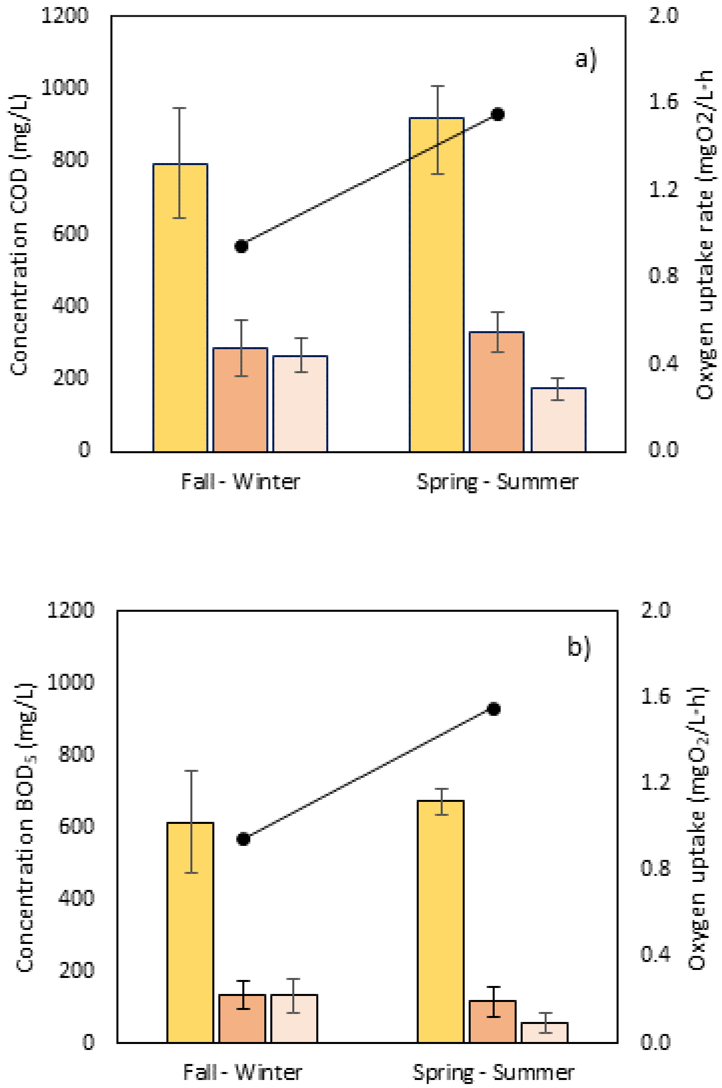

Figura 5.3. Concentrations (mg/L) of a) COD and b) BOD_5 in IN (■), E1 (■), and E2 (■) and OUR_{end} in fall-winter and spring-summer.

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 $m^3/m^2 \cdot d$ and an OLR of 0.54 kg $COD/m^3 \cdot d$, 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 $m^3/m^2 \cdot d$ and OLR between 0.2–0.6 kg COD/$m^3 \cdot d$ [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/$m^2 \cdot h$ of CO_2, 36.5 mg/$m^2 \cdot h$ of CH_4, and 12.3 mg/$m^2 \cdot h$ 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 YH value of 0.39-0.54 mgCODm/CODs and a μmax 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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CAPÍTULO 6

GREENHOUSE GAS EMISSIONS FROM A FULL-SCALE VERMITILFER FOR SEWAGE TREATMENT: EFFECTS OF SEASONALITY AND SEWAGE PARAMETERS

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Greenhouse gas emissions from a full-scale vermitilfer for sewage treatment: Effects of seasonality and sewage parameters

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Abstract

Vermifilters (VF) contain earthworms that interact with microorganisms to remove organic matter and nitrogen from wastewater. The biological processes in these wastewater treatment plants (WWTP) can generate carbon dioxide (CO_2), methane (CH_4) and nitrogen dioxide (N_2O). Although these emissions correspond to greenhouse gases (GHG), few studies have evaluated the impact of VF on the generation of these gases. The objective of this study was to evaluate the GHGs´emissions from a full-scale VF for sewage treatment regarding the effects of seasonality and sewage parameters. The study monitored the influent and effluent of the VF in a rural area. Emission fluxes were measured using the static chamber method in fall-winter and spring-summer. The results showed that comparing the emissions generated by one person per year (kgCO_2eq/cap·y), VFs generated less GHG than conventional and non-conventional WWTPs with CO_2, CH_4, and N_2O emissions ranged between 0.8 – 7.5 kg/cap·y, 0.1 – 0.5 kgCO_2eq/cap·y, and 5.7 – 9.5 kgCO_2eq/cap·y, respectively. Regarding the seasonality effect, CO_2 increases by 139% in spring-summer compared to fall-winter. N_2O increases by 139% in fall-winter compared to spring-summer. Positive correlation between influent COD concentrations and CO_2 emissions (r^2 = 0.7) was observed whereas influent carbon/nitrogen ratio (C/N) and N_2O emissions (r^2 = -0.6) reported negative correlation. These results evidenced that seasonality and sewage characteristics influenced the emissions of GHGs in a full-scale VF.

Keywords: Greenhouse gases; Vermifilter**;** Sewage treatment; Seasonality

1. Introduction

Climate change is the main problem society faces today [1]. It is generated by GHGs that alter the temperature of the planet, and it affects climate stability, society and ecosystems [2]. Among the 7 gases considered by the IPCC as the main emissions, CO_2, CH_4 and N_2O are the most studied since they represent 70%, 23% and 7% of GHGs respectively [3,4]. Regarding the warming potential, CO_2 generates a significant greenhouse effect on the planet; however, over a 100-year time horizon, CH_4 and N_2O have a warming potential 23 and 296 times greater than CO_2 [5]. In this context, GHGs from WWTP contribute 1.5% of CO_2 and 5% of non- CO_2 GHG emissions and are expected to reach 42% of waste-related emissions by 2030 [6,7], therefore, the operation of these facilities has a significant effect on climate [8]. Although these figures account for the impact of the wastewater treatment sector, most of these studies are based on estimates that consider conventional WWTPs as activated sludge (AS) and anaerobic sludge (AN) installed in urban areas, leaving aside what happens in rural areas [9].

In 2020, 45% of the wastewater generated in the world had no safe treatment and a large part is concentrated in rural areas with low population density and dispersion of inhabitants [10 11]. To avoid contamination in these areas, decentralized technologies exist that treat wastewater at the source or near the source of generation for small populations [12]. Constructed wetlands (CW), soil treatment units (STU) and vermifilters (VF) are decentralized technologies based on nature [13]. Particularly, VFs are systems that work with earthworms that, by interacting with microorganisms, change the physicochemical properties of wastewaters [10]. Earthworms are used in vermifilter to accelerate processes that occur in soil filtration during wastewater treatment [14]. The ingestion, excavation and excretion of earthworms reinforce this relationship and generate aerobic environments that promote the growth of microorganisms. Some studies have reported removal efficiencies of 83.4% of organic matter measured as chemical oxygen demand (COD), 54.1% of total nitrogen (TN), 96.6% of ammonium (NH_4^+) and 52.2% nitrate (NO_3^-), [15,16,17]. However, few studies have evaluated the impact of these systems on GHGs generation [18]. The role of earthworms in the generation of emissions is unclear. Some studies claim that earthworms could increase CO_2 and N_2O from soils by 33% and 42% [19]. Whereas the CO_2 is related with overall decomposition, the N_2O occurs during a particular type of decomposition (denitrification) that requires anaerobic conditions [20]. However, it can be produced by ammonia-oxidizing bacteria through nitrification [5]. In contrast, other studies show that earthworms could decrease CO_2 by increasing carbon storage through decomposition of organic matter and carbon stabilization by formation of microaggregates and macroaggregates [21]. The ingestion and subsequent peristalsis destroy the pre-existing microstructure. During gut transit, however, clay minerals and organic materials are mixed and become encrusted with mucus to create a new nucleus for microaggregate formation [22,23]. However, stabilization depends on the organic load and earthworm density of the medium [21]. Specifically in VF, Zhao et al. [24] and Huang et al. [25], showed that CO_2, CH_4 and N_2O emissions depend on operational conditions such as earthworm density and the C/N ratio of the influent. While decreasing C/N favors incomplete denitrification processes and generates N_2O, an increase in earthworm density can increase CO_2 and limit CH_4 generation to 3% of CO_2 [10]. Considering the above, the objective of this study is to evaluate the GHG emissions in full-scale VF and to determine the effect of seasonality and operating parameters on GHGs.

2. Materials and Methods

2.1 Design and operating conditions

This study was carried out in a rural WWTP in Copiulemu, Concepción, Biobío Region, Chile. This WWTP uses a sequential system that includes pretreatment for solids removal, followed by a VF and a radiation ultraviolet as a disinfection system. The VF is a vertical subsurface flow system. 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. According to Gutiérrez et al. [13], the VF operates with a flow (Q), hydraulic loading rate (HLR), and organic loading rate (OLR) of 160 m^3/d, 0.6 $m^3/m^2 \cdot d$, and 0.5 $kgCOD/m^2 \cdot d$, 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*, heterotrophic and autotrophic microorganisms responsible for biological reactions [26,27]. The monitored earthworm density was between 1105 – 7221 earthworms/m^3.

2.2 Monitoring strategy

The VF was installed in 2020; however, this study included monitoring during 2022 (Period I), 2023 (Period II), and 2024 (Period III), with a sampling frequency of two to three months during the fall-winter seasons (n=7) and spring-summer seasons (n = 8) of the southern hemisphere. The wood chip was replaced at the end of Period II. Figure 1 shows a schematic of the VF and the emissions monitoring strategy. GHG samples (CO_2, CH_4 and N_2O) between the active layer and the atmosphere were measured using the static chamber method, which corresponds to a cylinder with a lid 10 cm in diameter and 35 cm high, to enclose a volume of atmosphere from the topsoil [28]. The cylinder was buried 5 cm deep to collect samples in vials using a 60 ml syringe attached to the chamber by a hose. The sampling frequency was every 15 min for 1 hour. Solid samples of the active layer were analyzed at different times of use, considering Period II, and to determine the effect of replacing the wood chips on the VF, samples were taken at different times in Period III (0, 2, and 8 months). For the analysis, 5 subsamples were taken at different points until 5 kilograms were reached. Additionally, wastewater samples were taken from the influent (IN) at the pretreatment outlet and from the effluent (E) at the VF outlet. All samples were stored at a 4°C temperature for less than 24 h before analysis.

2.3 Analysis of gas samples

The sample vials were collected in the field in fall-winter and spring-summer of 2024. A gas chromatographic analysis was then performed [29]. The equipment used was GC Greenhouse, a Shimadzu with methanizer, a flame ionization detector and an electron capture detector. The flux of CO_2, CH_4, N_2O between the soil surface and the atmosphere inside the chamber was calculated using the Eq. (1) [30]:

$$f = \frac{\Delta CPV}{\Delta tRTA} \tag{1}$$

Where *f* represents the flux of CO_2, CH_4, N_2O ($mg \cdot m^2/h$) ΔC is the change in gas mass (mg) inside the chamber due to the change in the time (Δt), P is atmospheric pressure (atm) inside the chamber (assumed to be 1 atm), V is the volume of the chamber (L), R is the ideal gases constant (0.08205 at L/mol·K), T is the temperature (K) inside the chamber and A is the area of the chamber (m^2).

Table 6.1 shows the emission factors resulting from the static chamber measurements. By considering these factors and the removal efficiency of COD and TN in the VF influent, the GHGs were estimated for the I, II and III periods.

Tabla 6.1. Vermifilter emission factor in spring-summer and fall-winter (CO_2, CH_4, N_2O)

Season	kgCO_2/COD removed	kgCH_4/COD removed	kg N_2O /TN removed
Fall-Winter	$1.3 \cdot 10^{-1}$	$2.3 \cdot 10^{-5}$	$2.0 \cdot 10^{-2}$
Spring-Summer	$3.1 \cdot 10^{-1}$	$2.4 \cdot 10^{-4}$	$6.2 \cdot 10^{-3}$

2.4 Analysis of liquid samples

The liquid samples, IN and E, were filtered using the Whatman membrane with a pore size of 0.7 µm and analyzed based on the protocol described in the standard method [31]. The organic matter present in the samples was identified in the form of COD (colorimetric method, 5220-D), biological oxygen demand (BOD_5) (azide-modified Winkler method, 5210-B) and total organic carbon (TOC) (catalytic combustion oxidation and non-dispersive infrared detection method). The analyzed nutrients were measured in the form of NH_4^+-N, NO_3^--N, NO_2^--N (spectrophotometer UV–Vis Shimadzu UV 1800, Kyoto Japan) and total nitrogen (TN) (Spectroquant-Nova 60, kits Merck, Darmstadt, Germany).

2.5 Analysis of soil samples

Solid samples of the active layer were analyzed at different times of use. The organic carbon of the active layer woodchip was measured via calcination loss at 550°C [32]. The NH_4^+-N and NO_3^--N were measured with the KCl 2 mol/L, extraction method, colorimetry in Skalar autoanalyzer [33]. Total nitrogen was measured using the Kjeldahl method [34].

2.6 Mass balance analysis

Mass balances were calculated in terms of TOC and TN using the Eq.2 [36]:

$$Ci \cdot Qi - (Storage) - \ Emission = Ce \cdot Qe$$

Where Ci = influent concentration of COT or TN (kg/L), Qi: influent flow (L/d), Storage = biomass growth and retention, Emission: refers to the mass of carbon or nitrogen emitted in gaseous form, specifically as C-CO_2, C-CH_4, or N-N_2O (kg/d), Ce= effluent concentration of COT or TN (kg/L), Qe: effluent flow (L/d). Carbon and nitrogen concentrations were determined from liquid samples as described in Section 2.4. Gaseous emissions were quantified using gas chromatography, as detailed in Section 2.3. To express emissions in terms of carbon or nitrogen content, the molecular weights of CO_2 (44 g/mol), CH_4 (16 g/mol), and N_2O (44 g/mol) were used, converting them to C-CO_2, C-CH_4, and N-N_2O, respectively, based on the molar mass of the element within each compound [35].

2.7 Statistical analysis

To evaluate the effect of each period and seasonality on COD and TN storage, statistical analyses were carried out using RStudio software version 4.3.2, with a significance level of $p = 0.05$. The Shapiro-Wilk and Levene tests were performed to analyze the normality and homogeneity of variance, respectively. Subsequently, an ANOVA test was performed [36]. Furthermore, a principal component analysis (PCA) was used to determine the correlation between the physicochemical parameters of the wastewater influent and the GHG emissions of the VF (CO_2, N_2O, CH_4).

3. Results and Discussion

3.1 CO_2, N_2O and CH_4 formation in WWTP

Table 6.2 shows the kgCO_2eq/cap·y emissions in different WWTPs. While conventional technologies generate emissions ranging from 67.9 – 122.6 kgCO_2eq/cap·y, non-conventional technologies generate emissions that range from 1.1 – 75.5 kgCO_2eq/cap·y. For WWTPs the main source of gas emissions are biological transformations involving microorganisms [35]. Generally, in these systems, CO_2 is generated by oxidation in aerobic processes and CH_4 by reduction of organic matter in anaerobic processes [17]. N_2O is generated by nitrification and denitrification of nitrogen compounds in wastewater [8].

Tabla 6.2. CO_2 eq emissions (kg/cap·y) for different WWTP.

		Emissions (kgCO_2 eq/cap·y)				
Type	**Name**	**CO_2**	**N_2O**	**CH_4**	**Total**	**Reference**
Conventional	AO	14.6	3.3	64.2	82.1	[37]
Conventional	ANS	30.1	36.0	56.5	122.6	[8]
Conventional	AS	30.3	36.7	12.5	79.5	[8]
Conventional	A^2O	18.0	2.0	48.2	67.9	[38]
No-Conventional	CW	1.4	5.5	13.3	20.2	[38]
No-Conventional	CW	26.0	3.7	45.9	75.5	[39]
No-Conventional	STU	6.3	0.9	2.8	10.0	[9]
No-Conventional	SSTU	-2.8	0.6	2.7	3.3	[9]
No-Conventional	VF	0.8	9.5	0.5	10.8	[24]
No-Conventional	VF	0.6	-	0.5	1.1	[25]
No-Conventional	VF	7.5	5.7	0.1	13.1	This study

Note: AO: Anoxic-Oxic, ANS: Anaerobic Sludge, AS: Aerobic Sludge, A^2O: Anaerobic-Anoxic-Oxic, CW: Constructed Wetlands, STU: Soil treatment unit, SSTU: Secondary Treatment-Soil treatment unit, VF: Vermifilter.

CO_2 emissions in VF were lower than other WWTPs with 0.6 - 7.3 kgCO_2/cap·y. STU, in particular, was -2.8 kgCO_2/cap·y. However, Knapper et al. [9] suggests that this is due to low organic matter removal efficiency in the biofilter. Some VFs have reported, with other influents (i.e., dairy and swine waste), that CO_2 decreases as worm density increases [40,41]. Consequently, low CO_2 concentrations are linked to the role of the earthworm in mineralization, carbon stabilization and the generation of recalcitrant vermicast [21]. In the case of wetlands, although plants assimilate atmospheric CO_2 through photosynthesis and transfer carbon compounds into the soil, it returns to the atmosphere during respiration [5]. This explains the higher CO_2 concentrations ranging between 1.4 – 26 kgCO_2/cap·y. For conventional WWTPs such as ANS, AS, A^2O and AO, emissions ranged between 14.6 – 30.3 kgCO_2/cap·y. With these technologies, organic matter degradation is generated by aerobic and anaerobic processes, which explains the increase in CO_2 levels [37]. In aerobic processes, however, the heterotrophic microbial kinetics is higher in conventional plants [42], leading to increased emissions.

CH_4 emissions in VF were lower than other WWTPs with -0.04 - 0.5 kgCO_2eq/cap·y. The reduction of CH_4 is attributed to the action of earthworms and microorganisms that enhance the aeration of VFs [43]. These systems generate aerobic environments with ORP between 200 mV – 300 mV [13,44] that reduce CH_4 [24]. On the other hand, Luth et al. [40] reported emissions of -3.3 and -

9.7 mg/d CH_4 with another type of influent. Negative concentrations suggest the presence of methanotrophic microorganisms [39]. Wetlands had higher CH_4 than VFs, with 13.3 – 45.9 $kgCO_2eq/cap·y$. Unlike with the case of VFs [45], anaerobic microorganisms (i.e. hydrolytic, fermentative and acetogenic) are not inhibited and can degrade organic matter into simpler compounds (e.g. acetate, CO_2 and H_2). These compounds are subsequently transformed into CH_4 by methanogenic bacteria [46]. Conversely, when aerobic environments are favored, methanotrophic bacteria can reduce more than 50% of CH_4 levels [47]. Therefore, CH_4 generation depends on the quantity, composition, and activity of methanogenic and methanotrophic microorganisms impacted by the operational conditions, i.e. temperature, UV radiation, organic load [5]. In conventional WWTPs, such as ANS, A^2O and AO, emissions ranged between 48.2 – 64.2 $kgCO_2eq/cap·y$. In this type of technology, anaerobic digestion is part of the main processes and methane is generated as a by-product of methanogenesis [9]. For AS, methane is much lower at 12.5 $kgCO_2eq/cap·y$. In these systems, the O_2 injected by pumps is above 2 mg/L, which energetically favors aerobic degradation and generates a toxic effect on methanogenic microorganisms [37]. N_2O emissions in VF were between 5.7 - 9.5 $kgCO_2eq/cap·y$. Such emissions are generated by nitrification and denitrification processes, depending on the operational parameters of the VF [13]. Zhao et al. [24] observed increases in denitrification and N_2O generation when the influent C/N ratio drops from 10 to 2.5. In the present study, the influent C/N ratio was between 8 - 10. This fact, and the aerobic environment generated by the worms, inhibits the generation of N_2O via a denitrification process [48]. In the wetlands, N_2O was between 3.7 - 5.5 $kgCO_2eq/cap·y$. These systems have aerobic, anaerobic, and anoxic microbial sites, therefore, the source of N_2O corresponds to nitrification and denitrification processes [39]. Even though there is no consensus as to which is the primary source, similarly to the case of VFs, there are operational conditions that cause the increase of N_2O, i.e. increased nitrogen, low organic load, O_2 and pH [5]. In conventional WWTPs, such as ANS, AS, A_2O and AO, emissions were between 2 - 36.7 $kgCO_2eq/cap·y$. N_2O can be generated under aerobic or anoxic conditions. Under aerobic conditions, oxidation of ammonium and nitrite generates N_2O [37]. Under anaerobic conditions, only ammonium oxidation generates N_2O. In activated sludge, 90% of emissions are generated during sludge aeration and the remaining 10% in sludge storage tanks [49]. Factors that increase N_2O

generation refer to low pH levels, the presence of toxic compounds, or low oxygen concentrations [50].

3.2 Vermifilter performance and mass balance

Figure 6.1 shows the mass balance of TOC. In the effluent, TOC is reduced to 8.3 ± 3.4 kg/d, representing 33% of the influent. Additionally, the system emits 4.9 ± 3 kg/d of $C\text{-}CO_2$, $7.2 \cdot 10^{-3}$ ± $7.4 \cdot 10^{-3}$ kg/d of $C\text{-}CH_4$, which together account for 19% of the influent. $C\text{-}CO_2$ and $C\text{-}CH_4$ emissions are associated with the degradation of organic matter related to the action of earthworms and aerobic microorganisms [10]. Low $C\text{-}CH_4$ emissions ($7.2 \cdot 10^{-3}$ kg/d) indicate prevailing aerobic conditions and methanotrophic activity [5,24]. Therefore, the remaining 48% of TOC is likely retained as worm biomass, vermicast, microbial biomass and adsorbed carbon in the woodchip of the active layer [35].

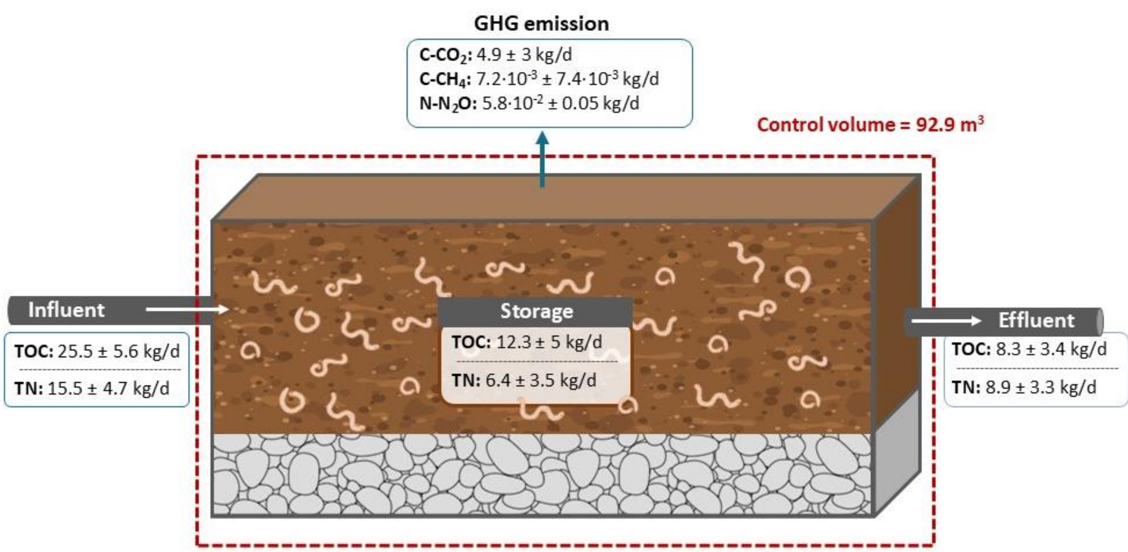

Figura 6.1. Mass balance in a full-scale vermifilter.

Table 6.3 shows the physicochemical characteristics of the active layer analyzed in period II and III. The results show that, during the first 8 months of period III with the new woodchip, there was a rapid decrease of TOC of 12 % (i.e. 23.074 kg to 20.482 kg). Decrease in TOC in the active layer has been seen in other VFs [51,33] and is related to an acclimatization of the VF and an increase in earthworm biomass [52]. Earthworms feed on organic matter, grow and reproduce [53]. This changes the microstructure and compacts the active layer with an increase of microbial enzymes in the biofilms [54] and a loss of some compounds present in the woodchip, such as lignin, cellulose, hemicellulose [55,56].

Tabla 6.3. Characterization of the active layer in the period of operation II and III.

Parameter	Unit	Period			
		II-48 month	**III-0months**	**III-2 months**	**III-8 months**
TOC	kg	17.138	23.074	22.364	20.482
TN	kg	835	84	217	534
NH_4^+-N	kg	0.8	0.5	13	8
NO_3^--N	Kg	41	0.1	12	12
N-org	Kg	793	83	192	514
C/N	-	21.9	243.5	109.1	38.9

The mass balance of TN can be observed in Figure 2. In the effluent, the TN is reduced to to 8.9 ± 3.3 kg/d, representing 58% of the influent. In addition, the system emits $5.8 \cdot 10^{-2}$ ± 0.05 kg/d N_2O. N_2O emissions depend on the removal of the nitrogen that is related to nitrification and denitrification processes [53]. In the cited study, the denitrification process is inhibited by the aerobic environment of the VF [57]. However, the earthworm gut functions as a bioreactor that generates N_2O by incomplete denitrification when the C/N ratio decreases [56], as evidenced in period III (i.e. after 48 months of active layer usage, see Table 3). N_2O emissions represent 0.4% of the TN in the influent. Thus, like TOC, the remaining 42% of TN is retained as worm biomass, worm vermicast, microbial biomass and nitrogen adsorbed on the woodchip of the active layer [35]. In Table 3, unlike TOC, it can be observed an increase from 84 kg to 534 kg of TN in the first 8 months of period III. This is related to the adsorption of organic nitrogen increasing from 83 kg to 514 kg and to the nitrification of NH_4^+-N increasing NO_3^--N from 0.1 kg to 12 kg. In period II-48 months, the biofilm is well developed, and these processes intensify reaching 793 kg of N-org and 41 kg of NO_3^--N.

3.3 Effect of seasonality

In Figure 6.2, when comparing the composition of emissions between seasons, it can be assessed that CO_2 in the fall-winter season was 4,177 ± 1,798 kg/y and in the spring-summer season, 9,992 ± 1,714 kg/y. The significant increase ($p < 0.05$) of 139% in spring-summer is related to the activity of earthworms and microorganisms. Earthworms are poikilothermic species; therefore, they are affected by temperature [14]. The optimal life range of earthworms is between 15°C and 20°C. In this study, the temperature range was between 11 ± 2 °C in fall-winter and 18 ± 4 °C in spring-summer. These differences affect the metabolism, growth, reproduction cycle and activity of earthworms and microorganisms that can degrade organic matter [58]. Considering the low CH_4 concentrations, the main mechanism of organic matter degradation in the VF corresponds to aerobic processes [57]. Although the optimal range of aerobic degradation is between 25-35 °C [59], it has been observed in environments with earthworms that exothermic processes of bioconversion of organic compounds can increase the temperature of 1-5 °C regarding the environment [60]. This is consistent with other publications that have recorded increases in heterotrophic activity responsible for organic matter degradation in the spring-summer season [58]. Furthermore, temperature can also affect air diffusion in the active layer, increasing the extent of biological reactions [59].

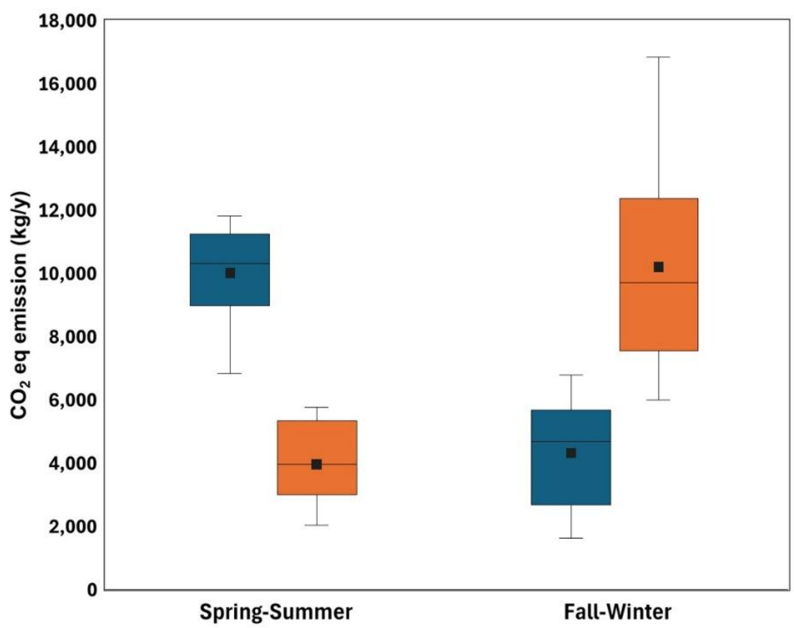

Figura 6.2. Effect of seasonality on CO_2 eq emissions due to CO_2 (■) and N_2O (■), in a full-scale vermifilter.

In Figure 6.2, when comparing the composition of emissions between seasons, it can be assessed that N_2O in the fall-winter season was 9,555 ± 3,763 kgCO_2eq/y and in the spring-summer season, 3,992 ± 1,279 kgCO_2eq/y. The significant increase ($p< 0.05$) of 139% in fall-winter may be related to variations in the C/N ratio [13]. In fall-winter, the C/N ratio was 8 ± 3, and in spring-summer, the

C/N ratio was 10 ± 2. These changes may be related to variations in TN concentrations generated by rain events that sweep along nitrogen compounds [61].

3.4 Effects of other parameters in GHG emissions

Figure 6.3 a) shows a PCA, in which 66.9% of the variance is explained by component 1 (PC1) and component 2 (PC2). Component 1 is represented by N_2O (0.43) y C/N (0.44) and component 2 by CO_2 (0.41), COD (0.44), and TN (0.51). Figure 4 b) shows the correlation matrix of the physicochemical parameters measured in the influent and GHG. Moreover, Table 3 presents a mass balance for different active layer usage times.

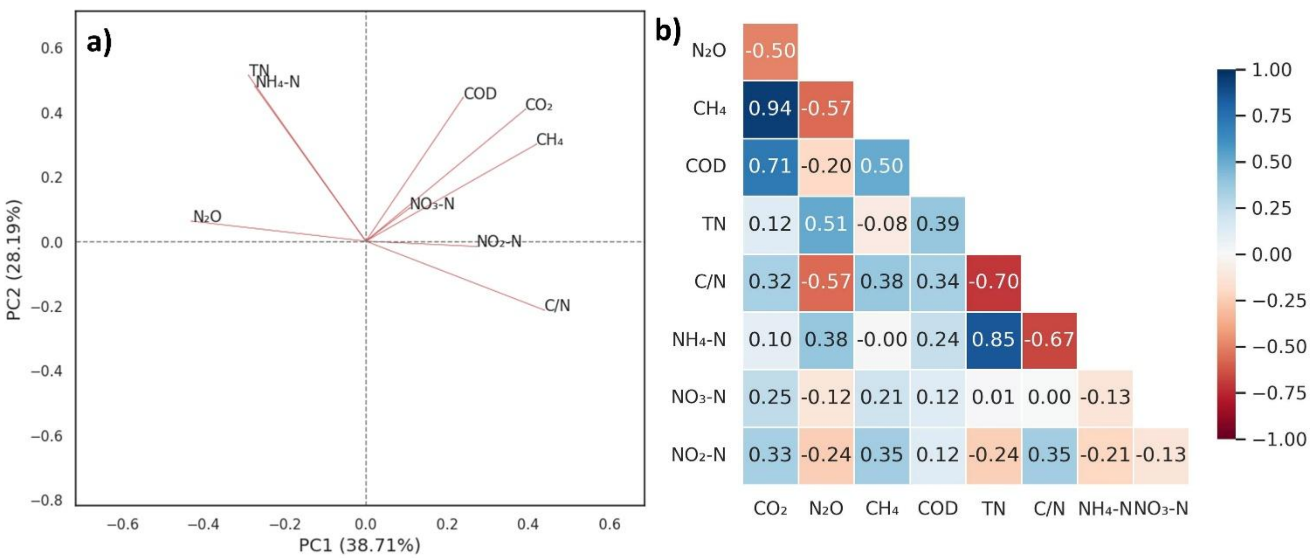

Figura 6.3. Relationship between physicochemical parameters and greenhouse gas emissions, a) principal component analysis and b) correlation matrix.

For CO_2, the PCA showed a positive relationship with COD. The correlation factor between COD and CO_2 was 0.71 (see Figure 4b). Generally, in all systems, the CO_2 is directly related to overall decomposition rates of organic matter [19]. In wetlands, Gagnon et al. [62] found that high organic matter concentrations influence the activity of bacteria, generating an increase in CO_2. In VF, the variations in organic loads favor earthworm activity and, therefore, affect the respiration of aerobic microorganisms [36,63]. Therefore, the organic matter concentration in sewage affects the activity of earthworms and microorganisms and induces an increase in CO_2 in a VF [13].

For N_2O, the PCA showed a positive relationship with TN and a negative one with C/N. The correlation factor of N_2O with TN and C/N was 0.51 and -0.57, respectively (see Figure 4b). The denitrification activity in earthworms can be 1000 times more than in the soil. This produces N_2 as an end product with the generation of N_2O [23]. However, when decreases the C/N ratio, enhanced incomplete denitrification in earthworms and releasing N_2O. On the contrary, when increase C/N ratio, enhanced complete denitrification and released N_2 [64]. Generally, denitrification is carried out by heterotrophic bacteria that grow when organic carbon is abundant in the system [20]. The mucus secreted by earthworms has gut microbes, including heterotrophs, mixed with the active layer [10]. Zhao et al. [24] found similar results when increasing the influent TN and reducing the C/N ratio. When the C/N ratio was 10, N_2O emissions were between 2.03 – 2.41 $mg/m^2 \cdot h$. However, when the ratio was 2.5 N_2O emissions increased to 12.24 – 12.34 $mg/m^2 \cdot h$.

Table 6.4 shows a significant increase in TOC storage ($p < 0.05$) from 40% in period II to 61% in period III. This is due to the change in woodchip of the active layer. The change of C-CO_2 from 4.0 to 4.2 kg/d was not significant ($p > 0.05$). Therefore, in the first year with new woodchips, the main processes may be related to woodchip adsorption and worm biomass increase [26,51]. The storage of TN is higher in period I with 47.4%. However, these differences are not substantial regarding period II and III. It is possible that the rainy seasons and changes in the C/N ratio could generate significant changes within the periods, thus explaining the lack of significance of the results [13].

Tabla 6.4. Mass balance in periods of operation I, II and III of the vermifilter.

Parameter	**Unit**	**Period**		
		I	**II**	**III**
Organic matter				
Influent TOC	kg/d	29.2 ± 3.5	24.7 ± 4.1	22.6 ± 6.8
Effluent TOC	Kg/d	9.7 ± 2.3	10.7 ± 2.7	4.6 ± 2.8
Storage TOC	Kg/d	13.2 ± 3.6	10.0 ± 5.0	13.8 ± 5.8
	%	45%	40%	61%
C-CO_2	Kg/d	6.3	4.0	4.2
Nitrogen				
Influent TN	Kg/d	17.8 ± 5.1	11.6 ± 2.3	17.0 ± 3.4
Effluent TN	Kg/d	9.3 ± 1.7	7.2 ± 2.1	10.6 ± 5.4
Storage TN	Kg/d	8.4 ± 4.5	4.3± 1.1	6.3 ± 2.7
	%	47.4	37.2	37.1
N-N_2O	Kg/d	$8.0 \cdot 10^{-2}$	$4.0 \cdot 10^{-2}$	$5.2 \cdot 10^{-2}$

Note: Period I: 2022; Period II: 2023; Period III: 2024. Storage: Mass retained in the VF.

4. Conclusions

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

- VFs generate lower GHGs in relation to other WWTPs. CO_2 ranges between 0.8 – 7.5 $kgCO_2eq/cap \cdot y$, CH_4, between 0.1 – 0.5 $kgCO_2eq/cap \cdot y$ and N_2O, between 5.7 – 9.5 kgCO2eq/cap·y.
- According to the mass balance, 19% of the influent TOC and 0.4% of the influent TN were converted into CO_2 and N_2O emissions, respectively, in the full-scale VF system.
- Seasonality affects GHG emissions of the VF. CO_2 in fall-winter was 4,052 ± 1,722 kg/y and in spring-summer was 9,630 ± 1,640 kg/y. N_2O in fall-winter was 11,423 ± 6,330 $kgCO_2eq/y$ and in spring-summer was 3,992 ± 1,279 $kgCO_2eq/y$.
- Influent COD concentrations and C/N ratio were factors that determined GHG production. COD concentrations were positively correlated with emissions which indicated that higher COD concentrations in the influent generated higher CO_2 emissions. On the contrary, the C/N ratio was negatively correlated with N_2O. In this case higher C/N ratios produced lower emissions of this GHG.
- This study is the first attempt to establish GHG emissions from a full-scale VF, providing a basis for future research in this area.

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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. **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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CAPÍTULO 7

DISCUSIÓN

7.1 CONDICIONES DE OPERACIÓN DE UN VERMIFILTRO, PARA ELIMINAR MATERÍA ORGANICA Y NUTRIENTES

7.1.1 Condiciones de operación

Para cumplir con el primer objetivo del proyecto, se realizó un estudio de 101 publicaciones en VF de aguas servidas. Por otro lado, se evaluó un VF a escala real para tratar las aguas servidas de Copiulemu, Región del Biobío. En el Capítulo III, se determinaron los parámetros que establecen las condiciones de operación para eliminar la materia orgánica y los nutrientes. Estos son: especie (S), densidad de lombrices (ED), medio filtrante (FM), altura del medio filtrante (FMH), carga hidráulica (HLRs), carga orgánica (OLRs) y temperatura (T). En el análisis bibliográfico, el 95% de las publicaciones utilizaron la especie *Eisenia foetida* por su alta capacidad reproductiva, ciclos de vida cortos y baja sensibilidad a compuestos tóxicos (Bhat et al., 2020). Respecto a la densidad de lombrices, el rango optimo está entre 3.000 y 6.000 lombrices/m^3, sin embargo, estos valores están determinados por HLRs y OLRs. HLRs bajo 2.5 $m^3/d \cdot m^2$ y OLRs entre 0.2- 0,4 kgCOD/m^2 favorecen el desarrollo del consorcio lombriz-microorganismo en la capa activa sin generar obstrucción en el sistema. Respecto a las características del FM, virutas de madera y alturas entre 0.6-1.2 generan las condiciones para desarrollar medio aeróbicos con capacidad de adsorción. Asimismo, el rendimiento del sistema depende de la temperatura ambiental, con un rango óptimo para las lombrices que esta entre 15 °C y 30 °C (Gutiérrez et al., 2023a). Estos valores deben ser considerados para el diseño y operación de un VF ya que incentivan la interacción de 3 componentes claves: Las lombrices, los microorganismos y la composición de la capa activa (Singh et al., 2021). La interacción de estos componentes genera diferentes proceso físicos – químico y biológicos que se complementan para eliminar contaminantes (Rajpal et al., 2014; Tejedor et al., 2020). Por ejemplo, aunque las lombrices favorecen el crecimiento de microorganismos aeróbicos, en su intestino existen desnitirifcadores anaeróbicos facultativos. Esto permite eliminar NH_4^+-N, NO_3^--N, NO_2^--N al completar el ciclo del nitrógeno (Chowdhury et al., 2021)

7.1.2 Operación del vermifiltro a escala real

El capítulo IV, V y VI, presentan los resultados de la evaluación del VF a escala real. Los procesos más relevantes descritos en el sistema corresponden a la degradación aeróbica, degradación anaeróbica, nitrificación, desnitrificación, mineralización del fosfato y adsorción (Kumar et al., 2024; Chowdhury et al., 2023; Tahar et al., 2022; Huang et al., 2014). La activación de estos mecanismos determina la eficiencia de eliminación de la materia orgánica (i.e DQO, DBO_5) y de los nutrientes (NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}–P). Esta investigación es una de las pocas que se han realizado en VF a escala real desde Liu et al. (2013).

La Tabla 7.1 compara las condiciones de operación y la eficiencia de eliminación de la DQO, NT y PT del VF de Copiulemu con los resultados reportados en la literatura. Los datos utilizados en esta tabla fueron obtenidos de los resultados expuestos en el Capítulo III y IV. La eficiencia de eliminación de la materia orgánica y los nutrientes del VF están dentro del rango obtenido en la literatura, que llega a 77%±11 para la DQO, 62%±16 para el NT y 53%±74 para el PT (Wang et al., 2013; Chicaiza et al., 2020; Tahar et al., 2022). Respecto de los parámetros operacionales, HLR con 0.5 m^3/m^2d y OLR 0.6 kgDQO/m^2d, están dentro de rangos óptimos, con valores leventemente elevados para el OLR que pueden ser explicados por incrementos estacionales de la carga orgánica. Sin embargo, DE y T registran valores fuera de los parámetros óptimos de funcionamiento. La DE presenta variaciones entre 1.300 y 7.000 lombriz/m^3 por la generación de parches que facilitan la operación y el mantenimiento del sistema. Los operarios estratifican la viruta de madera para optimizar el riego con agua servida por aspersión localizada. La estratificación limita la distribución homogénea del biofilm y disminuye la eficiencia de eliminación de la materia orgánica y nutrientes en zonas con baja DE. Respecto a T, aunque las temperaturas mínimas registradas en terreno estuvieron sobre los 10°C, la temporada de invierno pueden ir de 0°C a 13°C (Doussoulin- Guzmán et al., 2022). Estas temperaturas pueden reducir el metabolismo, el crecimiento, la reproducción y la actividad de lombrices y microorganismos afectando la eficiencia de eliminación de la materia orgánica (Bath, 2020). Por lo tanto, la DE y T, limitan el desempeño del VF estudiado.

Tabla 7.1. Condiciones de operación y eficiencia de eliminación de la materia orgánica y nutrientes

Parametros de diseño				Parametros de operación				Eliminación (%)			Referencia
Tiempo de operación (mes)	Volumen (m^3)	Capa activa	Densidad (Lombriz/m^3)	HLR (m^3/m^2d)	OLR (kg DQO/m^2d)	T°C	C/N	COD	NT	PT	
1-3	1.5	Compost / Suelo	5000-60000	2-3-4	0.37	-	7.0	60	-	-	*Ghasemi et al., 2020*
	0.01-0.06	Vermicompost / arena	10000	3-7	0.6-50	-	4-14	72	52-68	-	*Chowdhury et al., 2021*
	0.1	Suelo/ Heno de arroz	9000-33000	0.2	0.05	27	5	68-74	64-66	80-82	*Wang et al., 2013*
	0.3	Fracción orgánica / Vermicompost	7143	1	0.24	15-23	-	78			*Arora et al., 2016*
	0.02	Viruta de madera / Compost	8481	2.50	1.49	15-20	-	35-74	-	10-20	*Chicaiza et al 2020*
	0.1	Suelo /Aserrín	650-1300	0.2	0.05	28	5	59-86	39-63	69-92	*Wang et al., 2014*
	0.02	Vermicompost / Material de rivera	12000	2.50	1.04	-	9	46-72		-147	*Kumar et al., 2016a*
4-12	0.1	Suelo /Aserrín	25000	0.70	0.1	26	6	88	77.6	-	*Wang et al., 2016*
	0.3	Vermicompost	7143	1,3	0.8-1.8	25-30		66-71	-	-	*Kumar et al, 2016b*
	-	Viruta de madera	-	0.48			-	72-86	43-66	67-98	*Tompkins et al., 2019*
	-	Aserrín / Estiércol	-	0.02	0.04	24-42	6.5	82-83	-	-	*Adugna et al., 2019*
	0.01	Viruta de madera	5093-9458	0.48-0.96	0.34-0.68	9-13	4-7	77-82	44-66	48-76	*Tahar et al., 2022*
	0.02	Vermicompost	30000	1	0.45	2-16	-	74	-	-	*Arora and Kazmi, 2015*
	0.01	Ceramsita	64000	2	1	10-30	-				*Wang et al, 2017*
	0.5	Viruta de madera	13263	1.30	1.20	-	23	88-91	3 a -9	6-15	*Pous et al., 2021*
	0.45	Suelo / Viruta de madera	3061	0.06	0,01-0,02	7-36	2-5-10	77-87	68-88	77-83	*Zhao et al., 2014*
20	4.2	Ceramsita	16000	4.20	0.39	5-34	-	68	-	-	*Liu et al., 2013*
48	433	Viruta de madera	1300-7000	0.5	0.6	13°C-24°C	6-11	77	53	36	*Present study*

Para optimizar el VF, todos los parametros que determinan las condiciones de operación, deben estar en rangos óptimos. Por ejemplo, aunque Chicaiza et al. (2020) y Pous et al. (2021), presentaron DE entre 7.000 y 13.000, con eficiencias de eliminación para la DQO entre un 70% y 91%. Las eficiencias de eliminación del PT y NT fueron más bajas que el VF estudiado, con un 20% y 3% respectivamente. Esta disminución de la eficiencia puede ser explicada por incrementos en: HLR (1,3-2,5 m^3/m^2d) y OLR (1,2-1,5 kg DQO/m^2d). Cuando estos parámetros son altos, disminuye la retención hidráulica, la adsorción y el crecimiento de microorganismos autótrofos que participan en la nitrificación (Singh et al., 2021; Ghasemi et al., 2020). Por otro lado, Wang et al. (2016) diseñó un VF con DE de 25.000 lombrices/m^3, HLR de 0,7 m^3/m^2d, OLR de 0,1 $kgDQO/m^2d$ y T de 26 °C. En estas condiciones, el sistema alcanzó altas eficiencias de eliminación de 88% para la DQO y 77,6% para el NT. Si bien la OLR fue de 0.1 $kgDQO/m^2d$, el uso de sustratos orgánicos como el aserrín, mejoraron las condiciones de supervivencia de las lombrices. Si DE es alta con 25.000 lombrices/m^3, el PT puede incrementar, sin embargo, este parámetro no fue evaluado. Kumar et al. (2016) evaluó el PT y registro aumentos de PT con densidades de 12.000 lombrices/m^3. Dicho incremento está relacionado con microorganismos y enzimas presentes en las lombrices que mineralizan el PT y lo dejan disponible como PO_4^{3-}—P (Liu et al., 2012). Para contrarrestar este efecto, es necesario considerar medios filtrantes que favorezcan la adsorción o sorción física, como la viruta de madera utilizada en el VF estudiado (Zhao et al., 2014).

La relación carbono/nitrógeno (C/N) y el tiempo de operación de la capa activa (TOP) también son parámetros que pueden afectar el desempeño del VF. Se ha observado que C/N de 5, incrementan la eliminación del NT. Cuando C/N esta sobre 5, los microorganismos heterótrofos de crecimiento rápido inhiben la nitrificación llevada a cabo por microorganismos autótrofos de crecimiento lento (Chowdhury and Bhunia, 2021). Por otro lado, con un C/N por debajo de 5, la baja disponibilidad de carbono genera procesos de desnitrificación incompletos que generan N_2O (Zhao et al., 2014;; Wang et al., 2024). Respecto al TOP, los balances del Capítulo VI, indican que el tiempo de uso de la capa activa, tiene un efecto en el desempeño del sistema.

La Figura 7.1 muestra la eficiencia de eliminación de la DQO y NT relacionadas con el tiempo de operación. Cuando se agrupan los VF estudiados en la literatura, se pueden observar diferencias significativas ($P<0.05$). Mientras que las eficiencias de eliminación para VF con tiempos de operación cortos (1-3 meses) fueron de 71% ±13 para DQO y 56%±15 para NT, para tiempos de operación medio (4-12 meses) fueron de 81%±6 para DQO y 69%±14 para NT. Respecto al VF estudiado, el incremento en las eficiencias de eliminación solo se observó en la DQO. Con tiempos de operación cortos (1-3 meses) la DQO fue de 60% ±12.2 y NT fue de 43.1%±3 y con tiempos de operación medio (4-12 meses) la DQO fue de 85.1%±4.1 y NT 21.0%±9.8. Posteriormente cuando el VF es operado con tiempos más largos (13-48 meses), la DQO disminuye a 64.0% ±12.2 y NT incrementa a 45.3% ±12.8.

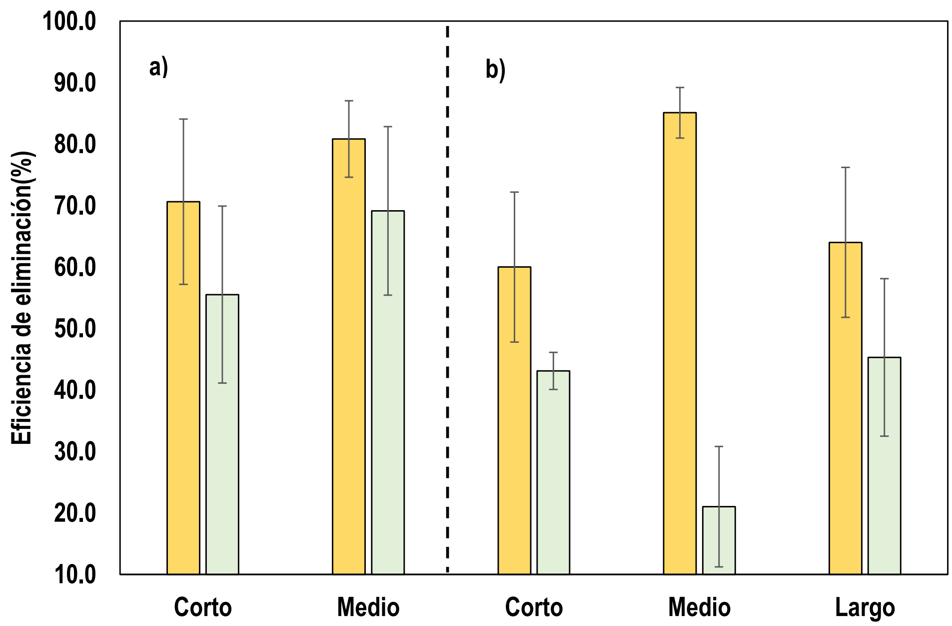

Figura 7.1. Eficiencia de eliminación de DQO (■) y TN (■) relacionado a los tiempos de operación del VF. Corto:1-3 meses, Medio: 4-12 meses, Largo: 13-48 meses. a) Resultados de la literatura, b) Vermifiltro estudiado.

Estas diferencias en las eficiencias de eliminación pueden ser explicadas por proceso físico – químicos y biológicos. Al ser reemplazado el medio filtrante, comienza una etapa de aclimatación de 30 a 90 días (Thompkins et al., 2020; Pous et al., 2021). Durante este periodo, las lombrices se reproducen, alimentan y excretan microorganismos y enzimas que permiten que el medio filtrante alberge procesos biológicos para desarrollar la capa activa (Tahar et al., 2022). Por lo tanto, en los primeros meses de un VF, la materia orgánica y el nitrógeno presente en forma de NH_4^+, son eliminados por adsorción (Samal et al., 2017). Posteriormente, cuando se desarrolla la capa activa, la materia orgánica se degrada por microrganismos aeróbicos y el NH_4^+ se transforma en NO_3^- incrementando las concentraciones de NT (Pous et al., 2022). Cuando los tiempos de operación de la capa activa son excesivos llegando a 48 meses, existe una disminución del área disponible para la adsorción y un incremento de la conductividad hidráulica (Tejedor et al., 2020), esto podría explicar la disminución en la eficiencia de la DQO. El incremento en la eliminación de NT a los 48 meses, puede ser explicado por microorganismos del intestino de la lombriz que generan procesos de desnitficación parcial o completa dependiendo de la disponibilidad de carbono en el medio (Chowdhury and Bhunia, 2023). Sin embargo, si no existe un recambio del medio filtrante, el incremento de la conductividad hidráulica del medio disminuirá la generación del biofilm y la capa activa dejará de albergar estos procesos disminuyendo la eliminación de la materia orgánica y los nutrientes.

7.2 EFECTO DE LAS LOMBRICES Y LA ESTACIONALIDAD SOBRE LA CAPA ACTIVA DE UN VERMIFILTRO

En el capítulo IV y V se observan los efectos de las lombrices y de la estacionalidad sobre la capa cativa. La capa activa, corresponde al medio que alberga los procesos biológicos llevados a cabo por las lombrices y por microorganismos (Tahar et al., 2022). Al analizar las distribuciones de las lombrices en el VF, fue posible constatar que los operarios durante los años de monitoreo mantuvieron estratificado el sistema. Esto generó zonas con alta densidad de lombrices (Zona A: 1105 ± 982 lombriz/m^3) y zonas con baja densidad de lombrices (Zona B: 7221 ± 1699 lombriz/m^3) que modifican las propiedades fisicoquímicas y biológicas de la capa activa (Kumar et al., 2024). Aunque no fue posible variar las concentraciones de DE en el VF de Copiulemu para evaluar el efecto sobre la eliminación de la materia orgánica, si fue posible estudiar las zonas estratificadas. Para esto, en el capítulo IV se presentan los resultados de las propiedades físico - químicas de la capa activa y en el Capítulo V los análisis de respirometria.

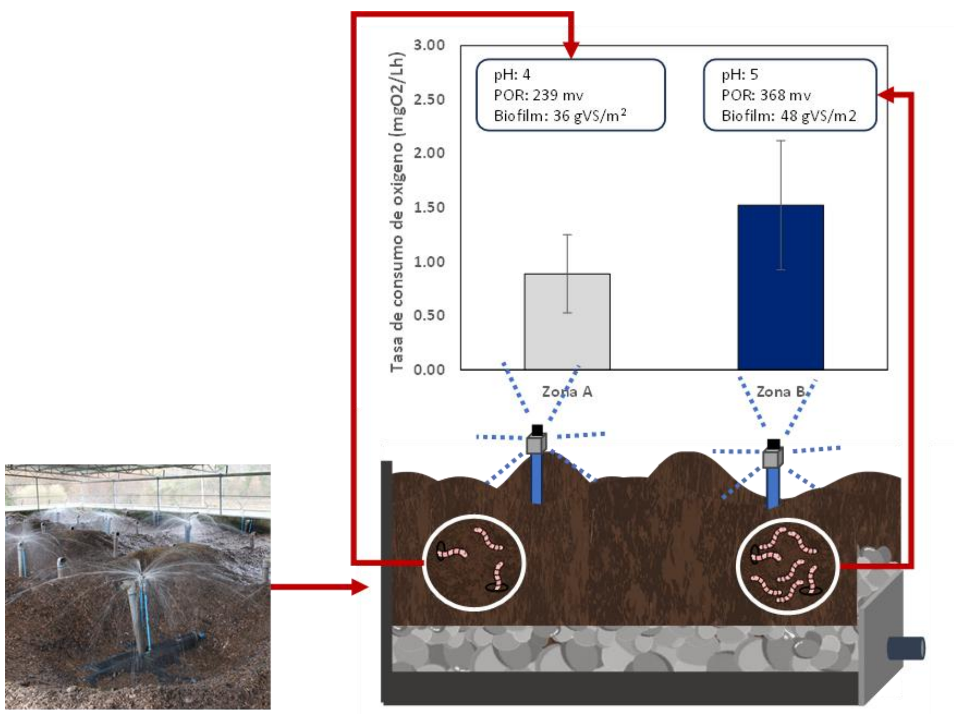

Figura 7.2. Características fisicoquímicas y biológica de la capa activa. Zona A: 1105 ± 982 lombriz/m^3; Zona B: 7221 ± 1699 lombriz/m^3.

La Figura 7.2, muestra las características fisicoquímicas y biológicas de la capa activa para cada zona. Al analizar los parámetros fisicoquímicos, se observó que el pH incrementó de 4 a 5 y el potencial oxido – reducción (POR) incrementó de 239 a 368 mv en las zonas con más DE (Zona B). Esto se corresponde con el incremento del biofilm de 36 a 48 gVS/m^2 y la tasa de consumo de oxígeno (OUR) que sube de 0.89±0.36 a 1.52±0.6. Las lombrices tienden a neutralizar el pH del medio entre 5-9 al incentivar procesos metabólicos de microorganismos que generan CO_2 y ácidos orgánicos que elevan el pH (Ghasemi et al., 2020; Hughes et al., 2007). En cuanto al POR, la Zona B fue de 368 mv. Estos valores son característicos de zonas aeróbicas, en las cuales los procesos de oxidación emplean O_2 como aceptor final de electrones, facilitando la degradación de la materia orgánica y otros compuestos reducidos (Szogi et al., 2004). De esta manera, la medición de parámetros fisicoquímicos como el pH y POR, pueden ser utilizados como pruebas iniciales de las condiciones de supervivencia de las lombrices en la capa activa del VF y el OUR como un indicador para evaluar la eficiencia de eliminación de la materia orgánica por microorganismos heterotróficos (Di Trapani et al., 2018). Por lo tanto, variaciones en la DE cambian las propiedades fisicoquímicas y biológicas de la zona A, esto favorece los procesos aeróbicos, incrementa la actividad de microorganismos, la actividad enzimática y mejora la eficiencia de eliminación de la materia orgánica.

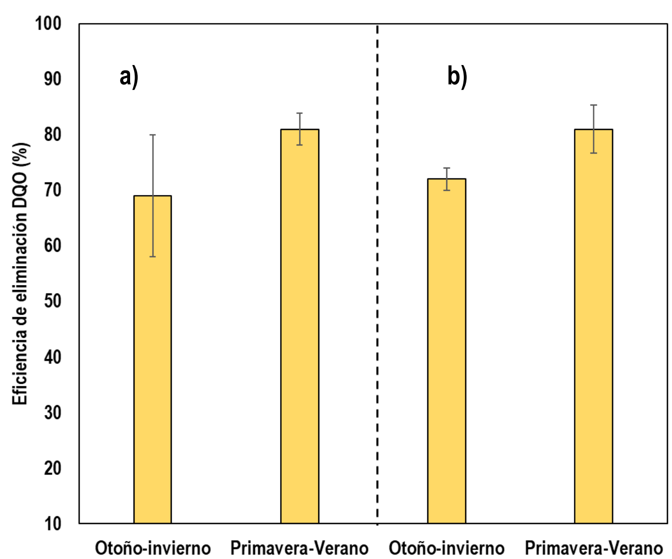

Figura 7.3. Eficiencia de eliminación de DQO (■) en diferentes estaciones. a) Resultados de la literatura, b) Vermifiltro estudiado.

Respecto a las estaciones, la Figura 7.3 compara la eficiencia de eliminación de la DQO del VF de Copiulemu con los resultados reportados en la literatura. Los datos corresponden a VF analizados en el Capítulo III y IV. En este análisis, se observó que existen diferencias significativas entre las estaciones cálidas y frías de un 12%. Esto se confirma en el VF estudiado, en donde la eficiencia de eliminación de la DQO incremento un 9% en primavera-verano. Por lo tanto, en el VF estudiado, DE y T están fuera de los rangos óptimos. Esto afecta negativamente el desempeño global del sistema. Particularmente estos resultados confirman la importancia de mantener la DE homogénea en toda la capa activa para mantener los procesos fisicoquímicos y biológicos que permiten eliminar contaminantes (Jian et al., 2016)

7.4 EMISIONES DE GASES DE EFECTO INVERNADERO EN UN VERMIFILTRO

En el Capítulo VI, están los resultados de las emisiones de gases de efecto invernadero en el (GEI) VF. En la literatura, pocas investigaciones han evaluado los GEI en estos sistemas. Huang et al. (2014) y Zhao et al. (2014), determinaron que los VF generan CO_2, N_2O y CH_4. Los principales mecanismos de generación pueden incluir procesos de degradación aeróbica, nitrificación, desnitrificación y procesos anaeróbicos. La evidencia disponible en la literatura no es concluyente respecto al papel de la lombriz en la generación de gases de efecto invernadero. Mientras que algunos estudios identifican los medios con lombrices como fuente emisoras (Lubbers et al., 2013), otros los describen como potenciales sumideros de carbono (Luth et al., 2011; Miito et al., 2022). Para comprender el papel que tienen las lombrices, debemos revisar los resultados del Capitulo V. Esta sección describe los parámetros cinéticos de los microrganismos. Aunque los VF alcanzan eficiencias de eliminación de la DQO de un 70 % y 90 % (Zhao et al., 2014), al comparar la cinética microbiana entre tecnologías, la tasa de crecimiento heterotrófica es baja y llega a un 0.05 (μ_{max}). Por lo tanto, es posible que la competencia y la selección de microorganismos de crecimiento lento en un VF, tengan un efecto en la generación de CO_2, N_2O y CH_4.

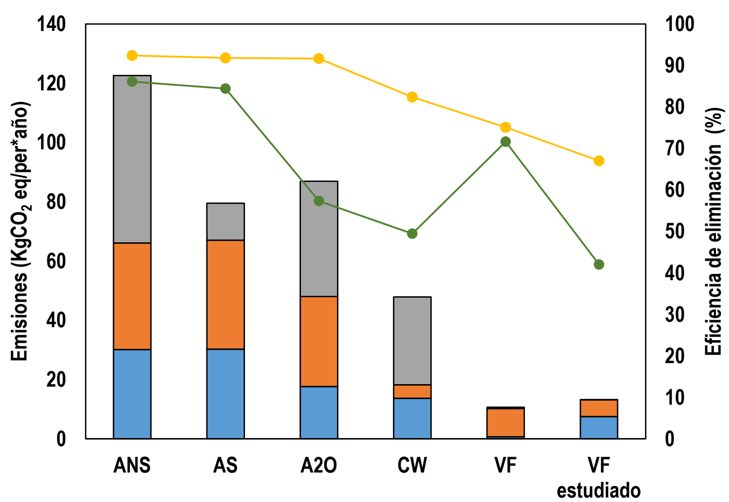

Figura 7.4. Emisiones de CO_2eq (kg CO_2eq /persona*año) con relación a la eliminación de DQO (-•-) y NT (-•-) para diferentes tecnologías de tratamiento de aguas servidas: CO_2 (■), N_2O_{eq} (■), CH_{4eq} (■). AO: anoxico - oxico, ANS: Lodo anaeróbico, AS: Lodo aeróbico, A2O: Anaeróbico-anoxico - oxico, CW: Humedal construido, VF: Vermifiltro.

La Figura 7.4 compara las emisiones de CO_2 eq/per·año y la eficiencia de eliminación de la DQO y NT con los resultados reportados en la literatura para tecnologías convencionales, vermifiltros (VF) y Humedales (CW). Los datos utilizados en esta tabla fueron obtenidos de los resultados expuestos en el Capítulo IV, V y VI. En esta Figura se observó que las emisiones del VF estudiado están dentro del rango de la literatura con 13.2 KgCO_2 eq/per·año. Esta cifra es un 72% más baja que los humedales y un 81% más baja que tecnologías convencionales como lodos aeróbicos, anaeróbicos y sistemas anoxico-oxico. Por otro lado, las eficiencias de eliminación de los VF, están entre un 67%-75% para la DQO y un 42%-72% para el NT. Estos valores son bajos respecto a tecnologías convencionales que están entre 91%-92% para la DQO y un 57%-86% para el NT.

Para explicar las eficiencias de eliminación obtenidas junto con las bajas emisiones biogénicas registradas, es necesario analizar los mecanismos de generación de las emisiones. En el VF estudiado, la generación promedio de CO_2 fue un 63 % inferior al de tecnologías convencionales y un 45 % menor que los humedales. Esto indica que, aunque los VF eliminan la materia orgánica por degradación aeróbica, también contribuyen con la estabilización del carbono, favoreciendo la formación de humus recalcitrante (Schon et al., 2020).

En cuanto al N_2O, la generación promedio fue un 81 % menor que en tecnologías convencionales, sin diferencias significativas respecto a los humedales. Tanto en los VF como en los humedales, el N_2O se produce a través de procesos de nitrificación y desnitrificación. En los VF, el ambiente aeróbico de la capa activa inhibe la desnitrificación. Por lo tanto, este proceso depende de bacterias facultativas desnitrificantes presentes en el intestino de las lombrices y del carbono disponible en el medio. Zhao et al. (2014) observo incrementos de N_2O por desnitrificación incompleta cuando la relación C/N del influente desciende a 2,5. En el VF estudiado, la relación C/N del influente fue de 8 ± 2, este valor no favorece la generación de N_2O. Sin embargo, según los resultados del Capítulo VI, la disminución de C/N por la acumulación de nitrógeno en la capa activa tras 48 meses de operación, puede favorecer incrementos en la producción de N_2O por desnitrificación incompleta.

7.5 OPTIMIZACIÓN DE VERMIFILTRO EN CHILE

7.5.1 Distribución de vermifiltros

En la Figura 7.5 se observa la distribución de 132 vermifiltros en Chile con una concentración del 80% en las siguientes regiones: General Bernardo O´Higgins (31%), La Araucanía (10%), El Maule (9%), Metropolitana (%), Biobío (8%), Los Lagos (8%), Ñuble (5%). Al comparar estos datos, con el último catastro realizado por la subsecretaría de desarrollo regional y administrativo (SUBDERE, 2012), esta cifra incremento un 175%. Este tipo de tecnologías no ha tenido un seguimiento y control adecuado. Aunque el 2012 el porcentaje de fallas era de un 48%, del 2020 al 2024, la superintendencia de servicios sanitarios (SISS) sólo realizó 18 fiscalizaciones que corresponden al 1,2% de las inspecciones.

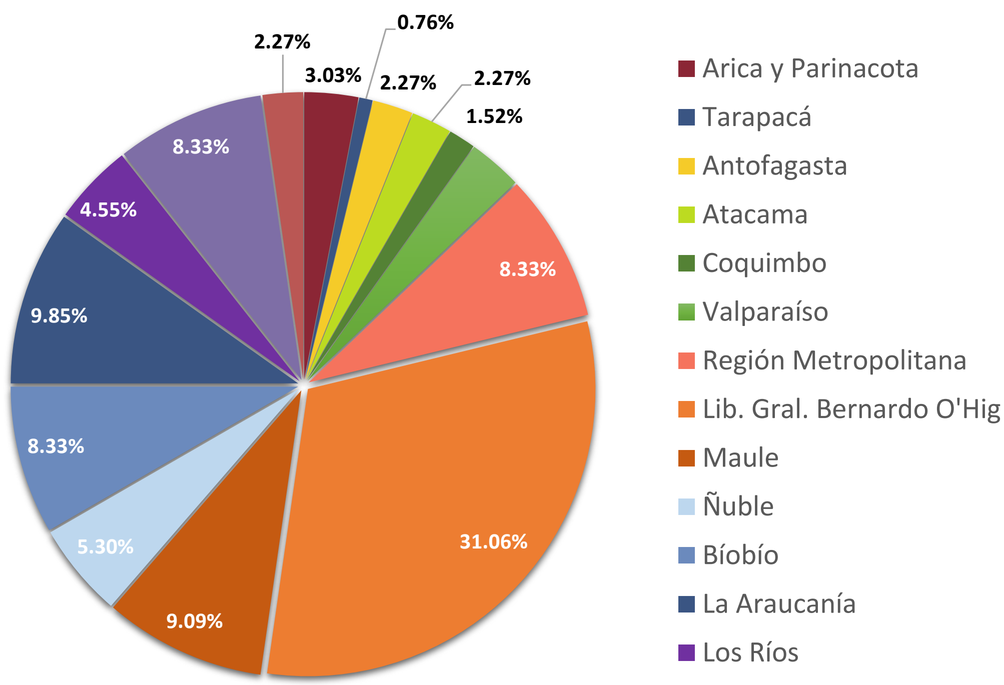

Figura 7.5. Distribución de Vermfiltros de aguas servidas en Chile (N= 132).

El D.S. N.º 90/2000, define los parámetros de calidad exigibles para las descargas en aguas continentales superficiales. En este contexto, la Tabla 7.2 compara los parametros de calidad del efluente del VF con la normativa legal de Chile y la Unión Europea (UE). En el VF estudiado, 3 parametros están bajo el límite permisible: NTK: -42%, PT: -20%, SST: -24% y 2 sobre el límite permisible: DBO_5: +67%, Coliformes fecales: +99%. Sin embargo, según la norma de la UE el VF no cumple con ningún parámetro de calidad, ya que todos superan los límites: DQO: +98%, DBO_5: +332%, PT: +300%, SST: +77%.

Tabla 7.2. Comparación de los parametros de calidad del efluente del VF estudiado con los límites permitidos en Chile y la Unión Europea.

Parametros	Unidad	Vermifiltro		Limite permisible	
		Promedio ± DS	Rango	Chile	UE
DQO	mg/L	247 ± 85	137 - 468	-	125
DBO_5	mg/L	108 ± 62	12 - 229	35	25
NTK	mg/L	29 ± 17	5 - 80	50	15
PT	mg/L	8 ± 2	4 - 13	10	2
SST	mg/L	61 ± 36	20 - 159	80	35
CF	NMP/100 ml	$4 \cdot 10^5$	$200 - 4 * 10^6$	1000	-

DS: Desviación estándar, UE: Unión Europea. NTK: Nitrógeno total de Kjeldahl, PT: Fosforo total, SST: Solidos suspendidos totales, CF: Coliformes fecales. Limite UE para descargas según Directiva 91/271/CEE, Limite UE para riego según Reglamento 2020/741, Límite de Chile según Decreto 90.

Aunque la Unión Europea no utiliza como indicador los coliformes totales, la normativa incorpora parámetros más específicos. Por ejemplo, el Reglamento (UE) 2020/741 establece como indicadores *Escherichia coli*, *Legionella*, Nematodos intestinales, colífagos y esporas de *Clostridium perfringens* cuando el análisis de riesgo lo requiere. Esto se debe a que dichos organismos son más representativos del riesgo sanitario real y más confiables para evaluar la calidad microbiológica de las aguas tratadas (Richiardi et al., 2023). Por otro lado, en Chile no se definen límites específicos para la DQO en el D.S. N.º 90/2000. La ausencia de este parámetro constituye una brecha regulatoria importante, ya que la DQO incluye fracciones recalcitrantes que no son evaluadas por la DBO_5 y que pueden persistir en el medio receptor, causando efectos sobre la calidad del agua. Estas fracciones pueden incluir ligninas, fenoles, compuestos húmicos y contaminantes emergentes como fármacos o pesticidas que, aunque no son biodegradables, mantienen una demanda de oxígeno que afecta el sistema (Pinto et al., 2022). A nivel internacional, tanto la Unión Europea como la EPA incluyen la DQO como parámetro básico de control, lo que permite caracterizar mejor la contaminación orgánica total y diseñar procesos de tratamiento más eficientes (Andreo-Martínez et al., 2017). En el Capítulo IV se monitoreó la resistencia bacteriana a los antibióticos en el VF. La presencia de antibióticos como amoxicilina, ceftriaxona y ciprofloxacina son contaminantes emergentes que no están regulados, a pesar de incrementar la resistencia bacteriana (Chen et al., 2014). La OMS advierte que la resistencia antimicrobiana es una de las principales amenazas para la salud pública global, y que el control de estos compuestos en las descargas es prioritario para prevenir su propagación (WHO, 2020).

7.5.2 Acciones de mejora para optimizar el VF

El VF es una solución basada en la naturaleza que aprovecha la actividad de lombrices y microorganismos para degradar materia orgánica, eliminar nutrientes y patógenos (Singh et al., 2021). Sin embargo, su desempeño es afectado por parametros de diseño y operación analizados en esta investigación. La Figura 7.6 presenta las propuestas de mejoras, orientadas a optimizar la distribución de la carga hidráulico, los procesos biológicos y la adsorción. Aquí se definen tres líneas de acción: i) Implementar un sistema de irrigación por aspersión aérea para evitar estratificar el sistema; ii) Mejorar el medio filtrante, y iii) Implementar tiempos de recambio y limpieza gradual de la capa activa. Estas acciones están enfocadas en mejorar los valores de los siguientes parametros de operación: La densidad de lombrices, volumen de la capa activa y la reducción de los tiempos de operación. A continuación, se describe en detalle cada acción:

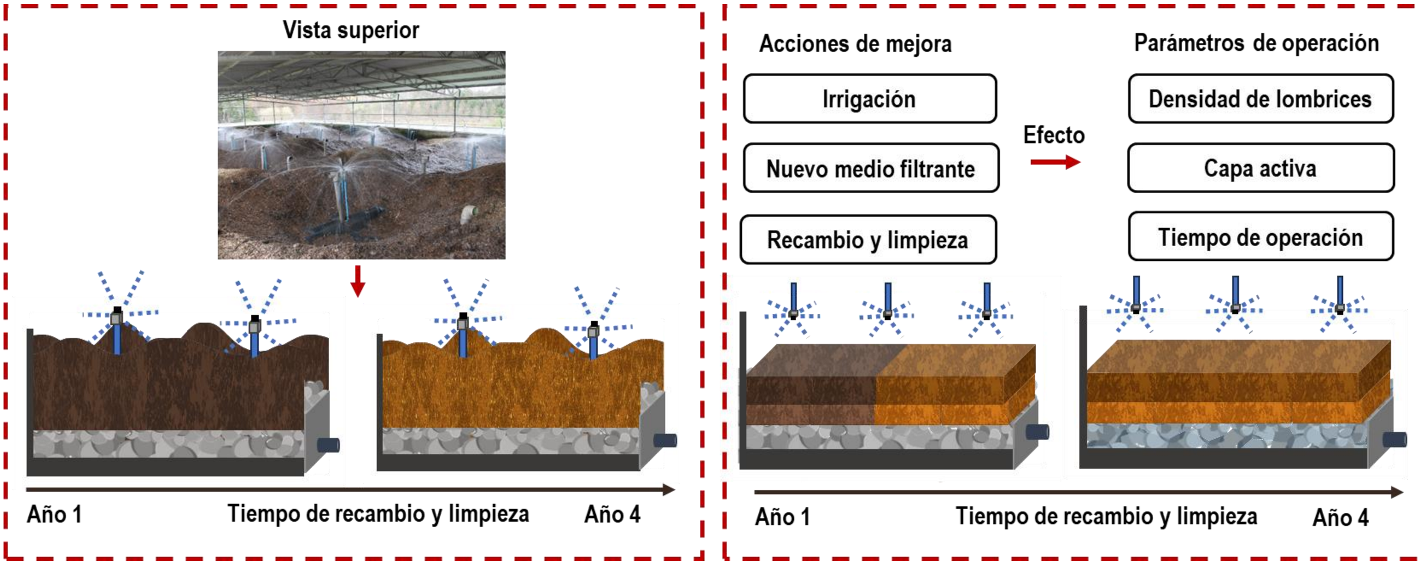

Figura 7.6. Propuestas de mejoras al sistema

a) Irrigación por aspersión aérea

La eficiencia de un VF no solo depende del caudal hidráulico, sino que también de la frecuencia y de la uniformidad con la que entra el influente (Chicaiza et al., 2021). La alimentación localizada del influente favorece la formación de canales preferenciales y zonas con diferentes niveles de humedad, lo que obliga al operario a estratificar áreas de la superficie para optimizar la irrigación de la capa activa. Esta distribución heterogénea afecta la DE y en consecuencia provoca variaciones en las propiedades fisicoquímicas de la capa activa (Singh et al., 2017). La implementación de un sistema de aspersión aéreo permite dispersar las aguas servidas de manera homogénea sobre la superficie del VF. De esta forma es posible evitar la estratificación de la capa activa y mejorar distribución de las lombrices (Pous et al., 2021; Thompkins et al., 2019).

b) Recambio y limpieza gradual de la capa activa cada dos años

La capa activa, formada por la interacción de lombrices, microorganismos y el medio de soporte, constituye los componentes principales del VF. Sin embargo, con el tiempo, la acumulación de vermicompost y las partículas finas reducen la porosidad, favorece la compactación y disminuye la capacidad de adsorción (Adugna et al., 2019; Singh et al., 2018b; Samal et al., 2017). Este fenómeno genera pérdidas en la eficiencia de eliminación de la materia orgánica que pueden llegar a un 12% según la Figura 7.1. Por otro lado, actualmente cuando existen recambios del medio filtrante, el sistema deja de operar y el influente es descargado directamente en los cuerpos de agua fluviales. Posteriormente, cuando la capa activa es retirada, se rellena el sistema con el nuevo medio filtrante. Por lo tanto, el proceso de limpieza genera contaminación, perdida de lombrices y perdida del biofilm. Para evitar esto, es recomendable que el VF tenga secciones independientes con recambios alternados cada 2 años para evitar la interrupción total del tratamiento y preservar las comunidades microbianas establecidas. Esta medida permite restablecer la capacidad de filtración y mantener la estabilidad de los procesos biológicos.

c) Mejora del medio filtrante

Los resultados presentados en el Capítulo III indican que la viruta de madera presenta un buen desempeño como medio filtrante y soporte para los procesos biológicos, alcanzando eficiencias máximas de eliminación del 77 % para DQO, 79 % para NT y 87 % para PT (Gutiérrez et al., 2023a). No obstante, con el tiempo, la acumulación de vermicompost y partículas finas reduce la porosidad, favorece la compactación y disminuye la capacidad de adsorción (Thompkins et al., 2022). Cuando esto ocurre, la capa activa puede liberar materia orgánica y sólidos suspendidos que atraviesan la única capa de piedra gruesa presente en el VF estudiado (piedras de 10–15 cm de diámetro y un espesor de 0–20 cm). Para prevenir el ingreso de materia orgánica al efluente, se propone una configuración mejorada del medio filtrante que mantenga, en su base, la capa de piedras de 10–15 cm y 0–20 cm de altura como drenaje primario y soporte estructural (Chowdhury et al., 2021). Sobre esta, una capa de piedras de 4–6 cm con una altura de 20–30 cm, que actúe como zona de transición y retención de sólidos gruesos (Pous et al., 2022). Finalmente, se recomienda mantener la viruta de madera como medio biológico principal, con una altura de 30–80 cm ya que en el VF estudiado, luego del recambio de la viruta y después del periodo de aclimatación, las eficiencias de eliminación de DQO alcanzaron un 85,1 % ± 4,1 durante el primer año de operación.

CAPÍTULO 8

CONCLUSIÓN Y RECOMENDACIONES

8.1 CONCLUSIONES

- La eliminación de la materia orgánica y los nutrientes de un VF, dependen de los parametros de diseño y operación descritos en el capítulo III y del tiempo de operación de la capa activa evaluado en el capítulo VI y VII (TOP). Respecto a la eliminación de la materia orgánica, las zonas con bajo DE (1105 lombriz/m^3), disminuyen la actividad heterotrófica en un 41% de OUR, TOP prolongados (48 meses) disminuyen la eficiencia de eliminación de la DQO en un 21%, estaciones con bajas T afectan la DQO en un 12%. Respecto a la eliminación del NT, depende de la relación C/N del influente, TOP prologados incrementan la eficiencia de eliminación en un 24%, sin embargo, incrementos de DE en la capa activa pueden incrementar las eficiencias en TOP medios. En un VF TOP medios asegura la estructura y porosidad del medio filtrante para mantener la capacidad de adsorción, la transferencia de oxígeno y la actividad biológica.

- La DE modifica significativamente las condiciones fisicoquímicas y biológicas de la capa activa. Las zonas con mayor densidad de lombrices (7.221 ± 1.699 lombrices/m^3) presentaron valores más altos de: pH (de 4 sube a 5), potencial óxido–reducción (368 mV), biomasa de biofilm (48 g·VS/m^2) y tasas de consumo de oxígeno (1,52 ± 0,6 mg O_2/L·h) en comparación con zonas de baja densidad (1.105 ± 982 lombrices/m^3). En cuanto a la estacionalidad, las temperaturas más altas de primavera–verano (15°C ± 20°C) incrementaron en un 9 % la eficiencia de eliminación de DQO respecto a otoño–invierno (10°C ± 1°C), mientras que la literatura revisada muestra diferencias cercanas al 12 % entre estaciones cálidas (26°C ± 3°C) y frías (16°C ± 2°C). Por lo tanto, mantener una capa activa homogénea con DE cercanas al rango optimo (6.000 lombrices/m^3) considerando los cambios estacionales, son determinantes para mantener el desempeño del VF.

- Respecto a las emisiones de gases de efecto invernadero, los VF emiten 4.9 ± 3 kg/d de CO_2, $7.2 \cdot 10^{-3} \pm 7.4 \cdot 10^{-3}$ kg/d de CH_4 y $5.8 \cdot 10^{-2} \pm 0.05$ kg/d de N_2O. Las emisiones medias de CO_2 y N_2O fueron inferiores en un 63 % y 81% respectivamente a las registradas en sistemas convencionales.

- Finalmente, con base en los resultados reportados, se rechaza la hipótesis planteada ya que la temperatura y la densidad de lombrices, no son los únicos parametros que condicionan el desempeño del vermifiltro. Por otro lado, las bajas temperaturas estacionales no produjeron diferencias estadísticamente significativas en las emisiones de CO_2 equivalente.

8.2 RECOMENDACIONES FINALES

- Los resultados de esta tesis evidencian que existen parámetros de operación como la T y TOP que afecta las eficiencias de eliminación de la materia orgánica y los nutrientes. En futuros estudios se recomienda evaluar el desempeño del sistema luego de: Modificar el sistema por Irrigación, generar secciones independientes con recambios alternados cada 2 años del medio filtrante y mejorar la capa de soporte del medio filtrante.
- Para monitorear el desarrollo de la capa activa con estas modificaciones, se debe medir el pH y POR. Si el medio es neutro y el POR supera los 300 mV, se recomienda realizar experimentos de respirometria para medir la tasa de consumo de oxígeno de microorganismos heterótrofos y autótrofos con el objetivo de tener bioindicadores que permitan evaluar la eficiencia de eliminación.
- Las acciones propuestas en esta investigación pueden mejorar la eliminación de la DQO, DBO_5, SST, NT para acercarse al cumplimiento de la norma de la Unión Europea. Aunque el proyecto no tenía por objetivo evaluar patógenos, los coliformes fueron monitoreados durante la investigación. Al respecto, el VF no es capaz de eliminar coliformes fecales en niveles seguros sin un tratamiento optimizado para la desinfección. En Copiulemu, el sistema corresponde a un UV. Al respecto es recomendable modificar el sistema para lograr la hermeticidad de las lámparas UV y evitar las fallas recurrentes que fueron observadas.
- Respecto a la variación de temperaturas por cambios estacionales, se recomienda monitorear la T del ambiente y la T interna de la capa activa. El incremento y la homogenización de la capa activa, puede generar un medio estable protegido de la intemperie y mejorar las eficiencias de eliminación de la materia orgánica y los nutrientes.
- En relación con la generación de gases de efecto invernadero, el muestreo se realizó después del recambio de la capa activa. Por lo tanto, es recomendable generar estudios que evalúen las emisiones de CO_2, CH_4 y N_2O durante el tiempo de operación del VF para evaluar si el

cambio de la tasa C/N en el medio filtrante en el tiempo, afecta significativamente la generación de N_2O.

CAPÍTULO 9

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(Introducción-discusión)

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ANEXO I

PORTADA DE LOS ARTÍCULOS PUBLICADOS

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Critical analysis of wastewater treatment using vermifilters: Operating parameters, wastewater quality, and greenhouse gas emissions

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ABSTRACT

Vermifiltration is a biooxidative process in which detritivorous worms interact intensely with microorganisms to eliminate contaminants present in the water. Although these systems have been presented as a plausible solution for the treatment of wastewater in rural areas, there are different parameters that can affect the removal efficiency and at a scientific level, there is no consensus on how to operate and design these systems, which It results in different alternatives and not very standardized forms of operation. This article presents a critical review of vermifiltration wastewater treatment, beginning with an analysis of related articles, followed by an evaluation of the influence of design and operational parameters, efficiency, and greenhouse gas generation. The main results of this review indicate that vermifiltration systems should use worm densities (DE) between 3000 and 6000 worms/m3, tributaries with hydraulic loading rates (HLR) below 2.5 m^3/dm^2, loading rates organic matter (OLR) not higher than 0.4 $kgCOD/m^2.d$, and filter material (FM) that includes wood chips to counteract the increase in NO3- and TP concentrations generated by nitrification and mineralization. Regarding the generation of greenhouse gases (GHG), more research is required in the area, since, although the HLR and OLR can affect the process, it has not been clearly defined whether the activity of the earthworms can generate carbon sinks or GHG sources. The results of this review article allowed us to define the most important operating parameters and the operating ranges to be considered in its application and operation in rural areas.

1. Introduction

Worldwide wastewater production reaches 2200 km^3/year. Around 40% of the world population lacks basic sanitation, and coverage is often much lower in rural areas than in urban areas. Twenty-five percent of urban inhabitants of developing countries lack access to sanitation services, while for rural populations in developing countries the figure reaches up to 82% [1]. The lack of adequate sanitation services leads to various diseases. The WHO estimates that 297,000 children under five die each year from diarrheal diseases caused by deficient sanitation, deficient hygiene, or unsafe drinking water [2]. Inadequate sanitation infrastructure and the elimination of phosphates, nitrates and organic matter is a challenging environmental issue [3] and a global problem that especially affects rural zones. These areas are characterized by low population densities and scattered homes; a decentralized wastewater management system is considered a feasible solution for rural wastewater treatment [3,4]. Nonconventional wastewater sanitation technologies are seen as alternatives to be considered for decentralized systems in small rural communities [4].

The vermifiltration is an example of nature-based solutions, to treat wastewater using vermiculture. The introduction of worms into the environment generated by the geo-microbial filter lends the system two main properties: a) ingestion of wastewater and b) degradation of soil pollutants [5]. The parameters under which a vermifilter can be operated have not yet been well studied and small modifications could alter the quality of the treated effluent and promote the generation of CH_4, CO_2, and N_2O emissions due to mineralization, nitrification, and denitrification processes that can occur in the system [6,7]. The objective of this work is to carry out a critical review of wastewater treatment using vermifilters. It will provide a comprehensive analysis of the state of the

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Article

Performance of a Full-Scale Vermifilter for Sewage Treatment in Removing Organic Matter, Nutrients, and Antibiotic-Resistant Bacteria

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Abstract: The vermifilter (VF) is regarded as a sustainable solution for treating rural sewage. However, few studies have investigated the performance of a full-scale vermifilter. The objective of this study is to evaluate the performance of a full-scale vermifilter in reducing organic matter, nutrients, and antibiotic-resistant bacteria contained in sewage. Influent and effluents were obtained from a rural sewage treatment plant using a VF and UV disinfection system. The results show a significant removal ($p < 0.05$) of chemical organic demand (COD) (77%), biochemical oxygen demand (BOD_5) (84%), total nitrogen (TN) (53%), and total phosphorus (36%). Seasonality is an influential variable for COD, BOD_5, and TN removal. In addition, the molecular weight distribution shows that the VF does not generate a considerable change in the distribution of organic matter (COD and total organic carbon (TOC)) and NH_4^+-N. The UV disinfection system eliminated 99% of coliform bacteria; however, they are not eliminated to safe concentrations. Therefore, it is possible to detect bacteria resistant to the antibiotics ciprofloxacin, amoxicillin, and ceftriaxone at 63.5%, 87.3%, and 63.5%, respectively, which were detected in the effluents. This study shows the potential of a system for the removal of pollution and the need to optimize the VF to be a safe treatment.

Keywords: sewage treatment; full-scale vermifilter; antibiotic-resistant bacteria; molecular weight distribution

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1. Introduction

In 2010, the World Health Organization (WHO) recognized access to drinking water and sanitation as a human right. However, in 2020, 45% of the sewage generated in the world was still discharged without safe treatment [1]. It has also been observed that sanitation coverage is often much lower in rural areas than in urban areas. The lack of adequate sanitation services can lead to environmental pollution, causing water quality deterioration, biodiversity loss, and changes in an ecosystem's structure and function [2]. In addition, because sewage contains a great variety of fecal microorganisms and pathogens, the creation of an unhealthy environment leads to the transmission of diarrheal diseases such as cholera, dysentery, and typhoid fever [1,3]. Meanwhile, a lack of sewage treatment contributes to the propagation of antibiotic resistance.

The emergence of antibiotic-resistant bacteria (ARB) and their dissemination through the environment—recognized as one of the main problems of the 21st century [4]—limit the treatment of infectious diseases and increase the probabilities of morbidity and mortality in the population [5]. ARB can survive and multiply in the presence of antibiotics thanks to antibiotic resistance genes (ARG), which encode proteins that participate in various resistance mechanisms and can be transferred to other bacteria to make them resistant [6].

Therefore, the search for decentralized technologies that can be applied in rural areas with low populations and scattered homes to minimize the risks of inefficiently treated

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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.

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 [7–9]. However, as with any biological treatment systems, performance

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ANEXO II

RESUMEN PARA DIFUSIÓN

RESUMEN PARA DIFUSIÓN

Chile es uno de los países más vulnerables a la escasez hídrica y a los impactos del cambio climático, situación que afecta especialmente a las zonas rurales donde aún existen más de 300 mil viviendas sin sistemas formales de saneamiento. En estas localidades, las familias dependen de pozos negros y fosas sépticas, tecnologías que no logran eliminar eficientemente la materia orgánica, nutrientes y microorganismos. En este contexto, los vermifiltros han sido propuestos como una alternativa sustentable para el tratamiento descentralizado de aguas servidas. Sin embargo, estos sistemas presentan fallas asociadas a parámetros de diseño y operación que no están estandarizados. La ausencia de criterios técnicos claros limita su aplicación y genera incertidumbre tanto en la eficiencia del tratamiento como en su contribución a las emisiones de gases de efecto invernadero (GEI), aspecto poco estudiado a escala real.

El objetivo del proyecto fue evaluar los parámetros de diseño y operación de un vermifiltro a escala real, para optimizar el diseño y la operación del sistema para eliminar la materia orgánica, nutrientes y controlar las emisiones de gases de efecto invernadero. Para ello, el estudio fue desarrollado en una planta real, en donde se realizaron mediciones de parámetros de calidad fisicoquímicos y biológicos de las aguas servidas. Adicionalmente, se emplearon técnicas de respirometria para evaluar la actividad de microorganismos heterotróficos en la capa activa (Medio que alberga las reacciones biológicas del sistema). Finalmente, se midieron emisiones de GEI para comparar el efecto que tiene la estacionalidad y la densidad de lombrices en el CO_2, CH_4, N_2O.

Los resultados permitieron identificar rangos operacionales óptimos para mejorar el desempeño del vermifiltro: densidades de lombrices entre 3.000 y 6.000 lombrices/m^3, cargas hidráulicas inferiores a 2.5 m^3/m^2 día, cargas orgánicas entre 0.2-0.4 kgDQO/m^2día, temperaturas entre 15 °C y 30 °C y tiempos de operación de la capa activa inferiores a 24 meses. Bajo estas condiciones, el sistema podría mejorar las eficiencias registradas de 77% para DQO, 53% para nitrógeno total y 36% para fósforo total. No obstante, los coliformes fecales solo disminuyeron desde 8,9 a 6,9 $\log_{10}$(MPN/100mL), lo que evidencia limitaciones para su aplicación en procesos de reúso. La estacionalidad tuvo un rol determinante, observando un aumento de 9% en la eliminación de DQO en primavera - verano y una disminución de 20% en la eliminación de nitrógeno total en el mismo periodo.

La actividad de microorganismos heterotróficos en la capa activa mostró variaciones marcadas entre estaciones y densidades de lombrices. La tasa de consumo de oxígeno (OUR_{end}) y la tasa específica de consumo de oxígeno ($SOUR_{end}$) fueron 95% mayores en primavera-verano respecto de otoño - invierno y 64% mayores en la zona con mayor densidad de lombrices. Estos resultados evidencian que tanto la temperatura como la densidad de lombrices afectan la capacidad microbiana para degradar materia orgánica. Respecto de las emisiones de GEI, los valores anuales por persona fluctuaron entre CO_2: 0.8 - 7.5 kg/persona·año, CH_4: 0.1 - 0.5 kgCO_2eq/ persona·año, y N_2O: 5.7 - 9.5 kgCO_2eq/persona·año, respectivamente. También se observaron efectos estacionales diferentes: el CO_2 aumentó 139% en primavera - verano y el N_2O incrementó 139% en otoño - invierno.

Finalmente, esta investigación aporta evidencia técnica en Chile sobre el funcionamiento de vermifiltros a escala real, entregando criterios para mejorar su diseño y operación. Los resultados permiten avanzar hacia sistemas de saneamiento rural más eficientes y sostenibles contribuyendo a la seguridad hídrica y a la toma de decisiones en políticas públicas y proyectos de infraestructura sanitaria en zonas rurales del país