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Optimization and sustainability assessment of a vermifiltration system for the treatment of greywater for low-income populations in sub-Saharan Africa

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DEDICATION

I dedicate this work to all my families!!!

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ABSTRACT

Greywater, representing a significant portion of domestic wastewater, originates from household activities such as bathing, dishwashing, and laundry. When discharged untreated into the environment, it poses serious health and environmental risks, particularly in sub-Saharan Africa, where treatment infrastructure is limited. However, greywater contains nutrients that can be valorized in agriculture. Among innovative solutions, vermifiltration stands out as an ecological and decentralized technology that uses earthworms and filtering materials to treat greywater while producing high-quality agronomic vermicompost. Despite its potential, adoption remains limited, and studies on optimizing its key parameters—Hydraulic loading rate, HLR, earthworm density, and organic load (COD)—are still insufficient. This study aimed to evaluate, predict and optimize the performance of a vertical flow vermifiltration system while assessing its agronomic and economic sustainability. Experiments were conducted under varying conditions of HLR (64 to 191 L/m²/day), organic loads (initial COD of 1000 to 4000 mg/L), and earthworm densities (0 to 200 worms or 0 to 10 000 worms/m^3). Results indicated that moderate hydraulic loads (64 L/m²/day) ensure optimal COD removal, even for high organic loads (4000 mg/L). Conversely, high HLR was found to reduce system efficiency, particularly under significant organic loads. Earthworm density significantly improved treatment performance, but its effectiveness depended on the optimization of hydraulic and organic parameters. Kinetic analyses revealed that Grau's second-order model best describes the experimental data. Additionally, response surface methodology (RSM) identified optimal conditions to maximize COD removal: an HLR of 133 L/m²/day, an influent COD of 1087 mg/L, and a density of 178worms (8900 worms/m^3), with a predicted efficiency of 91.51% and a residual COD of 92.29 mg/L, that complies with discharge standards. In terms of sustainability, the produced vermicompost was of high agronomic quality, nutrient-rich, pathogen-free, and met international standards. Economically, life cycle cost (LCC) analysis showed that the community-scale system was the most viable, with a minimal total cost of 237.56 XOF/m³, a net present value (NPV) of 71,360 XOF over 10 years, and a cost-benefit ratio of 1.03 for a community of 180 people. The study also highlighted the potential for large-scale replication of vermifiltration for greywater treatment. Although relatively unknown, this technology presented a promising solution to mitigate environmental impacts, improve hygiene, valorize agricultural resources, and address local population needs.

Keywords: Vermifiltration, Greywater, Modeling, Optimization, Sustainability.

RÉSUMÉ

Les eaux grises, représentant une grande part des eaux usées domestiques, proviennent des activités ménagères comme les bains, la vaisselle et les lessives. Leurs rejets non traités dans la nature entraînent des impacts sanitaires et environnementaux graves, notamment en Afrique subsaharienne, où les infrastructures de traitement sont limitées. Ces eaux contiennent toutefois des nutriments valorisables en agriculture. Parmi les solutions innovantes, la vermifiltration, technologie écologique et décentralisée, se distingue par son utilisation de vers de terre et de matériaux filtrants pour traiter les eaux grises tout en produisant un vermicompost de haute qualité agronomique. Cependant, son adoption reste marginale, et les études sur l'optimisation de ses paramètres clés – la charge hydraulique (HLR), la densité de vers et charge organique (DCO) – sont encore insuffisantes. Cette étude a pour but d'évaluer et d'optimiser les performances d'un système de vermifiltration gravitaire, tout en étudiant sa durabilité agronomique et économique. Les expériences ont été menées à différentes conditions de HLR (64 à 191 L/m²/jour), de charges organiques (DCO initiale de 1000 à 4000 mg/L) et de densité de vers (0 à 200 vers ou 0 à 10 000 vers/m^3). Les résultats ont montré que des charges hydrauliques modérées (64 L/m²/jour) permettaient une élimination optimale de la DCO, même pour des charges organiques élevées (4000 mg/L). À l'inverse, une HLR élevée réduisait l'efficacité du système, surtout avec une DCO initiale importante. La densité de vers a amélioré significativement les performances de traitement, mais leur efficacité dépend de l'optimisation des paramètres hydrauliques et organiques. Les analyses cinétiques ont montré que le modèle de second ordre de Grau décrit le mieux les données expérimentales. Par ailleurs, la méthodologie des surfaces de réponse (MSR) a identifié les conditions optimales pour maximiser l'élimination de la DCO : un HLR de 133 L/m²/jour, une DCO de l'influent de 1087 mg/L et une densité de 178 vers (8900 vers/m^3), avec une efficacité prédite de 91,51 % conduisant à une concentration résiduelle de 92,29mg/L respectant les normes de rejet et de réutilisation. En termes de durabilité, le vermicompost produit est de haute qualité agronomique, riche en nutriments, libre de pathogènes, et conforme aux normes internationales. Sur le plan économique l'analyse des coûts du cycle de vie (LCC) a montré que l'échelle communautaire était la plus économiquement viable, avec un coût total minimal de 237,56 XOF/m³, une valeur nette actualisée (NPV) de 71 360 XOF sur 10 ans et un ratio coût-bénéfice de 1,03 pour une communauté de 180 personnes. L'étude a également mis en avant le potentiel de réplication à grande échelle de la vermifiltration pour le traitement des eaux grises. Cette technologie, bien que peu connue, pourrait donc constituer une solution prometteuse pour réduire les impacts environnementaux, améliorer les conditions d'hygiène, valoriser les ressources agricoles et répondre aux besoins des populations locales.

Mots clés : Vermifiltration, Eaux grises, Modélisation, Optimisation, Durabilité.

LIST OF PUBLICATIONS, SCIENTIFIC COMMUNICATIONS AND AWARDS

Articles

1. **Saapi SSY, Andrianisa HA, Zorom M, et al. (2024)** New developments on vermifiltration as a bio-ecological wastewater treatment technology: Mechanism, application, performance, modelling, optimization, and sustainability. Heliyon 10:

https://doi.org/10.1016/j.heliyon.2024.e25795 (Impact Factor: 3.4)

2. **Saapi SSY, Andrianisa HA, Zorom M, et al. (2024)** Optimization of a Vermifiltration process for the treatment of high strength domestic greywater in hot climate area: A Response Surface Methodology approach. Water Res 122803.

https://doi.org/10.1016/j.watres.2024.122803 (Impact Factor 2024: 11.5)

Conference presentations

1. **Saapi SSY, Andrianisa HA, Zorom M, Mounirou LA,** Modeling of the Vermifiltration System for the Treatment of gof low-income poupulations of Sub-Saharan Africa, Salon International des Toilettes de l'Assainissement et de l'Hygiène de Ouagadougou (SITAHO), Ouagadougou (Burkina Faso), 18 – 21th November 2021.

2. **Saapi SSY, Andrianisa HA, Zorom M, Mounirou LA,** The use of a Vermifilter for Greywater treatment for low-income populations in Sub-Saharan Africa, 1st IWA Non Sewered Sanitation Conference, Johannesburg (South Africa), 15 – 18th October 2023.

3. **Saapi SSY, Andrianisa HA, Zorom M, Mounirou LA,** Utilisation d' Eudrilus Eugeniae dans un système de vermifiltration pour le traitement des eaux grises des populations à faibles revenus d'Afrique Subsaharienne, Journées scientifiques du CAMES, Abidjan (Côte d'ivoire), 11 – 14th March 2024.

4. **Saapi SSY, Andrianisa HA, Zorom M, Mounirou LA**, Optimisation du traitement par vermifiltration des eaux grises – approche statistique par la méthodologie de surface de réponse (MSR), Colloque international du RESCIF, Yaoundé (Cameroun), 18 – 19th April 2024.

5. Saapi SSY, Andrianisa HA, Zorom M, Mounirou LA, Traitement bio-ecologique des eaux grises par vermifiltration pour les populations à faibles revenus d'Afrique Sub-Saharienne Forum National de l'Eau et de l'Assainissement du Burkina Faso, Ouagadougou (Burkina Faso), 22 – 24th April 2024.

6. Saapi SSY, Andrianisa HA, Zorom M, Mounirou LA, Modeling of the Vermifiltration system for the treatment of greywater of low-income populations of sub-saharan Africa, Doctoriales 2iE, Ougadougou (Burkina Faso), 14 – 15th December 2021.

7. Sidesse SSY, Modeling of the Vermifiltration system for the treatment of greywater of low-income populations of sub-saharan Africa (Oral communication - MT180), Doctoriales 2iE, Ouagadougou (Burkina Faso), 17 – 18th December 2020.

List of the Awards

1. Saapi SSY, Andrianisa HA, Zorom M, Mounirou LA., FINISH Mondial Annual San Tech Hackathon: innovative solutions in Greywater Recycling for Sustainable Agriculture and Kitchen Gardening, 2023-2024, **3rd Price of jury and 1st Price of popular vote** for the most innovative project.

RESUME SUBSTANTIEL EN FRANÇAIS

Optimisation et évaluation de la durabilité d'un système de vermifiltration pour le traitement des eaux grises des populations à faibles revenus en Afrique subsaharienne

Les eaux grises, représentant une grande part des eaux usées domestiques, proviennent principalement des activités ménagères (bains, lessives, vaisselle, etc.). Leurs rejets non traités dans l'environnement engendrent d'importants risques pour la santé publique et les écosystèmes, en particulier en Afrique subsaharienne, où les infrastructures de traitement sont encore largement insuffisantes. Toutefois, ces eaux présentent un potentiel de valorisation, notamment en agriculture, grâce aux nutriments organiques qu'elles contiennent. Parmi les solutions émergentes, la vermifiltration se distingue comme une technologie écologique, peu coûteuse et adaptée à un contexte décentralisé. Cette approche exploite l'action des vers de terre et de différents matériaux filtrants pour traiter les eaux grises, tout en produisant un vermicompost riche en nutriments et en réduisant la charge en pathogènes. Cependant, malgré ses nombreux avantages, cette technique reste peu répandue en Afrique subsaharienne, et les études menées à ce sujet demeurent rares dans ce contexte, notamment celles visant à optimiser ses paramètres de fonctionnement.

Les technologies conventionnelles de traitement, souvent complexes et coûteuses, ne sont pas adaptées aux populations à faibles revenus. Les approches décentralisées et écologiques, comme les filtres plantés et les filtres à sable, souffrent d'une efficacité limitée, en raison notamment de problèmes tels que la surcharge hydraulique, les colmatages fréquents et une gestion complexe des boues générées. Dans ce contexte, la vermifiltration apparaît comme une solution prometteuse, mais ses performances dépendent fortement de trois paramètres principaux : la charge hydraulique (HLR), la densité en vers de terre (EWD) et la charge organique (concentration initiale en DCO). Ces paramètres interagissent de manière complexe et nécessitent une optimisation rigoureuse pour garantir une efficacité durable.

Peu d'études jusqu'ici offrent une analyse quantitative approfondie des effets des paramètres sur les performances des vermifiltres. En outre, les aspects économiques et environnementaux de la mise en œuvre à grande échelle, notamment les coûts du cycle de vie (LCC), restent peu documentés. Cette recherche se fixe donc pour objectif de combler ces lacunes par une approche intégrée, combinant des expérimentations, la modélisation et une évaluation de la durabilité. Les questions de recherche qui se dégagent sont les suivantes :

Questions de recherche

1. Comment les performances d'un système de vermifiltration varient-elles dans des conditions spécifiques de HLR, EWD et DCO initiale ?
2. Les effets de ces paramètres clés pourraient-ils être quantifiés, et les performances prévues à l'aide d'un modèle mathématique ?
3. Dans quelle mesure le système de vermifiltration peut-il être considéré comme durable ?

Objectifs

Les objectifs spécifiques de cette étude sont :

1. Évaluer les performances d'un système de vermifiltration dans des conditions variables (HLR, EWD, DCO initiale).
2. Modéliser, quantifier l'effet des paramètres clés et déterminer leurs valeurs optimales à l'aide de la méthodologie des surfaces de réponse (RSM) et de modèles cinétiques.
3. Évaluer la durabilité du système de vermifiltration.

Approche méthodologique

L'étude s'est appuyée sur une combinaison d'expérimentations et de modélisation. Les eaux grises brutes sont d'abord caractérisées, avant d'être traitées dans un système de vermifiltration vertical à flux gravitaire, fonctionnant sous différentes conditions expérimentales. Les performances du système sont évaluées en mesurant les paramètres dans l'effluent tels que : la concentration en DBO, en DCO, le pH, l'oxygène dissous, la conductivité électrique. La modélisation cinétique et la MSR sont utilisées pour optimiser les performances en identifiant les interactions entre les paramètres clés. Enfin, la durabilité du système est étudiée à travers une analyse agronomique et hygiénique du vermicompost, couplée à une évaluation économique.

Structure de la thèse

La thèse s'articule autour des axes suivants :

1. **Introduction** : Contexte, problématique et justification de l'étude.
2. **État de l'art** : Synthèse des recherches existantes sur la vermifiltration, mise en évidence des lacunes et opportunités.
3. **Conception expérimentale et évaluation des performances** : Description des matériaux et méthodes, résultats obtenus sur les performances du système.
4. **Modélisation et optimisation** : Utilisation de la RSM et de modèles cinétiques pour prédire les performances et déterminer les valeurs optimales des paramètres clés.
5. **Évaluation de la durabilité** : Analyse agronomique et économique du vermicompost produit, complétée par une étude des coûts du cycle de vie.
6. **Conclusion et perspectives** : Résumé des résultats, limites de l'étude et recommandations pour les recherches futures.

Dans le chapitre de l'état de l'art, nous mettons en lumière les recherches récentes dans le domaine du traitement par vermifiltration. Les études ont principalement porté sur les mécanismes d'élimination des polluants, les performances, le devenir des composants du filtre, y compris les vers de terre, ainsi que les sous-produits du système. Les méthodes de recherche appliquées par différents auteurs pour examiner le mécanisme de vermifiltration ont été présentées. Le chapitre aborde également les défis et les obstacles rencontrés dans ce domaine. Les études récentes se sont intéressées à la modélisation et à l'optimisation de la conception et des paramètres opérationnels des filtres. Une attention particulière a été portée à ces solutions d'optimisation afin de mieux orienter le choix des différents composants du système lors de sa mise en œuvre. En outre, nous avons mis en évidence les caractéristiques de durabilité de la vermifiltration.

D'après les résultats de recherche des dix dernières années, incluant ceux des études que nous avons précédemment menées, la vermifiltration (VmF) peut être considérée comme une bonne alternative pour le traitement et la réutilisation des eaux usées. Il apparaît que cette technologie possède un potentiel non négligeable pour répondre aux défis liés au traitement des eaux usées, notamment dans les zones confrontées à des contraintes financières et un manque de main-d'œuvre qualifiée. De plus, il a été démontré que la VmF est une technologie durable. L'analyse des performances d'élimination des polluants a montré que le vermifiltre (VF) peut éliminer divers contaminants présents dans les eaux usées, tout en produisant des sous-produits tels que de l'eau traitée et du vermicompost. Elle offre également un potentiel d'optimisation des paramètres opérationnels pour atteindre des performances maximales. Cependant, cette technologie présente encore certaines limites face aux normes de qualité de l'eau de plus en plus strictes pour le traitement et la réutilisation des eaux usées et face des difficultés souvent rencontrées au cours du processus, telles que le colmatage.

Le chapitre de la conception expérimentale et l'évaluation des performances des vermifiltres, évalue les performances des systèmes de vermifiltration et analyse l'influence de paramètres critiques sur leur efficacité, notamment la charge hydraulique (HLR), la densité des vers de terre (EWD) et la concentration initiale de la demande chimique en oxygène (Initial COD). Dans un premier temps, le chapitre détaille les matériaux et méthodes utilisés, incluant le contexte de l'étude, la collecte des vers de terre et des eaux grises nécessaires aux expériences, ainsi que la préparation méticuleuse des eaux grises synthétiques pour garantir la reproductibilité des résultats. Le protocole expérimental est également présenté, avec la configuration du vermifiltre, les méthodes d'échantillonnage et les techniques utilisées pour mesurer et enregistrer d'autres paramètres influents comme la température. Les vermifiltres (VFs) sont des colonnes en PVC d'un diamètre de 20 cm et d'une hauteur totale de 80 cm et hauteur utile de 60cm, dans lesquelles sont disposées différentes couches de matériaux filtrants et les vers de terre. Les réacteurs sont alimentés en eaux grises brutes par un système par lots. Ils sont alimentés 4 fois par jour avec des quantités régulières et à des intervalles de 4 heures, entre 08h00 et 20h00. Cela correspond aux HLR de 64, 127,5 et 191 L /m²/jour. Ensuite, les résultats concernant la caractérisation des eaux grises réelles et synthétiques sont discutés. L'analyse examine l'impact du HLR, de l'EWD et de la DCO initiale sur les performances des vermifiltres en analysant séparément les paramètres physiques et chimiques. Ces analyses permettent de déterminer dans quelle mesure ces facteurs améliorent ou détériorent les performances de filtration et de la biodégradation. Ce chapitre fournit ainsi une vue d'ensemble empirique de l'efficacité des vermifiltres. Les données et analyses présentées orientent davantage l'optimisation de cette technologie.

Les résultats de l'étude révèlent qu'une charge hydraulique élevée (191 L/m²/jour) augmente généralement la conductivité électrique des eaux grises traitées, bien que cet effet ne soit pas statistiquement significatif. Toutefois, une augmentation du HLR à 191 L/m²/jour réduit l'efficacité de filtration, parfois de plus de 35 %, particulièrement sous des charges organiques élevées (4000 mg/L de DCO initiale). En revanche, une HLR plus faible (64 L/m²/jour) assure une meilleure élimination de la DCO et de la DBO, même en présence de charges organiques élevées. Cela souligne l'importance d'optimiser le HLR en fonction de la charge organique pour améliorer les performances du traitement. La présence de vers de terre améliore significativement l'élimination de la DCO et de la DBO, avec une augmentation de 30 % de l'efficacité d'élimination (de 60 % à 90 %). Les vers de terre stabilisent également les performances du filtre, surtout sous des charges organiques élevées et des conditions de faible HLR. Cependant, les vers de terre seuls ne suffisent pas ; il est nécessaire d'optimiser à la fois les charges hydrauliques et organiques pour des performances optimales. Par ailleurs, des niveaux initiaux élevés de DCO entraînent une variabilité accrue du pH et réduisent les niveaux d'oxygène dissous (OD), ce qui affecte négativement l'efficacité du traitement, notamment sous des conditions de HLR modérée ou élevée. Ce chapitre met en évidence l'influence significative des niveaux initiaux de DCO, du HLR et de l'EWD sur le traitement des eaux grises par vermifiltration, leurs effets combinés étant encore plus marqués. Il est donc essentiel de développer un modèle du système pour prédire les performances et déterminer la combinaison optimale de paramètres pour un traitement efficace. Une telle modélisation permettrait également de quantifier et de représenter les effets individuels et synergiques de ces facteurs afin de mieux comprendre les mécanismes impliqués dans le processus de traitement. Ces aspects ont été approfondis dans le chapitre suivant.

Le chapitre de la modélisation et optimisation présente une analyse détaillée de la modélisation et de l'optimisation du système. Il est structuré en deux sections interdépendantes : la modélisation cinétique et l'optimisation des paramètres clé à travers la méthodologie de surface de réponse (MSR), chacune contribuant à une meilleure compréhension des performances du système et à son amélioration pour des applications pratiques.

La première section s'intéresse à l'application des modèles cinétiques pour décrire la dégradation de la matière organique, notamment l'élimination de la DCO. Trois modèles cinétiques couramment utilisés (modèle cinétique du premier ordre, modèle du second ordre de Grau et le modèle de Stover-Kincannon) sont appliqués pour évaluer le comportement du système sous différentes conditions opérationnelles. Ces modèles fournissent des informations sur les taux d'utilisation du substrat, l'activité microbienne et l'influence de la charge organique sur l'efficacité du système. En comparant les coefficients cinétiques dérivés, cette section établit un cadre théorique solide pour caractériser les processus de traitement du vermifiltre. La seconde section porte sur l'optimisation des paramètres clés : HLR (A), concentration initiale de DCO (B) et EWD (C). En utilisant la RSM couplée au design de Box-Behnken (BBD). Cette section explore les effets individuels et interactifs de ces paramètres sur l'efficacité d'élimination de la DCO. Le BBD permet d'identifier les conditions de fonctionnement optimales tout en minimisant le nombre d'expériences, offrant une approche économique et précise pour l'optimisation des processus.

La modélisation cinétique a révélé des différences notables dans l'adéquation des modèles. Le modèle cinétique du premier ordre s'est avéré inadéquat pour représenter la dégradation de la DCO dans le système de vermifiltration (R^2 = 0,3148, constante cinétique K_1= 0,583 d^{-1}). En revanche, le modèle cinétique du second ordre a montré un ajustement supérieur (R^2 = 0,9978, paramètres a = 0,105, b = 1,111 et K_2 = 0,938 d^{-1}), reflétant le rôle significatif de l'activité microbienne et de la relation symbiotique entre les vers de terre et les micro-organismes. Le modèle de Stover-Kincannon s'est avéré inadapté, les valeurs négatives de U_{max} et K_S révélant des incohérences. La modélisation et optimisation par la MSR a démontré que les facteurs HLR, DCO initiale et EWD influencent de manière critique les performances du système. Les interactions entre ces variables sont également significatives. Le modèle développé est le suivant :

L'efficacité de l'élimination du COD (%) = 88,8167 - 11,9113 A + 10,8225 B + 9,23875 C - 11,7675 AB - 8,055 AC - 4,0125 BC - 6,80333 A² - 4,59083 B² - 20,9733 C²

A = HLR, B = Concentration initiale de DCO et C = EWD

Les conditions optimales pour l'élimination de la DCO ont été identifiées : DCO initiale de 1087 mg/L, HLR de 133 L/m²/j et EWD de 178 vers, avec une efficacité prédite de 91,51 % et une DCO résiduelle de 92, 29 mg/L respectant les normes de rejet et de réutilisation. Les tests de confirmation ont validé ces prédictions. En combinant modélisation cinétique et optimisation paramétrique, ce chapitre établit un lien entre la compréhension théorique et les stratégies pratiques de conception et d'exploitation. Les résultats orientent le développement pratique de systèmes de vermifiltration efficaces.

Le chapitre sur la durabilité des VFs offre une évaluation de la durabilité du système de vermifiltration en abordant deux dimensions essentielles : la qualité agronomique et hygiénique du sous-produit qu'est le vermicompost, et la faisabilité économique du traitement des eaux grises grâce à cette technologie.

Les analyses de la qualité agronomique et hygiénique examinent le potentiel du vermicompost en tant qu'amendement de sol de haute qualité. La teneur en nutriments, la composition en matière organique et les paramètres de sécurité, tels que la présence de pathogènes et d'œufs d'helminthes, sont évalués selon les normes internationales NFU et FAO. Des techniques de détection des pathogènes comme Salmonella spp. et des œufs d'helminthes, basées sur les méthodes établies par l'AFNOR et l'US-EPA, garantissent une évaluation fiable de sa qualité hygiénique, tandis que des protocoles de coloration valident la viabilité des œufs d'helminthes détectés. Parallèlement, la faisabilité économique du système de vermifiltration est examinée à travers une analyse des coûts du cycle de vie (LCC). Cela inclut une évaluation des coûts d'investissement, d'exploitation et de maintenance, mise en balance avec les bénéfices potentiels du système, comme la commercialisation de l'eau traitée, des matériaux usagers, et du vermicompost. L'étude intègre des indicateurs tels que la valeur nette actualisée (NPV) et le ratio coût-bénéfice pour fournir une compréhension globale de la durabilité du système.

En combinant des perspectives agronomiques, hygiéniques et économiques, ce chapitre met en évidence le potentiel de la technologie de vermifiltration en tant que solution intégrée pour une gestion durable des eaux grises.

Les échantillons de vermicompost répondent aux normes internationales, présentent de bons profils nutritifs et favorisent la germination des semences sans effets nocifs. Bien que certains niveaux de nutriments, comme le phosphore et le calcium, soient inférieurs aux seuils recommandés, des suppléments ciblés peuvent répondre aux besoins des cultures. Le compost respecte les normes d'hygiène, avec une présence minimale de pathogènes et aucune trace de Salmonella spp., bien qu'un œuf d'helminthes détecté dans un échantillon indique la nécessité d'une désinfection supplémentaire pour certaines applications. L'analyse des systèmes de vermifiltration à l'échelle domestique et communautaire met en lumière des avantages et des limites distincts. Le système communautaire s'est avéré plus rentable à long terme grâce au partage des ressources et à de meilleures performances financières. L'analyse des coûts du cycle de vie (LCC) révèle que le scénario 2 option 2 (échelle communautaire) est le plus économiquement viable, avec un coût total le plus faible de 237,56 XOF/m^3, une NPV de 71 360 XOF sur une période de 10 ans, et un ratio coût-bénéfice proche de 1,03 pour une communauté de 180 personnes. Au-delà des aspects financiers, ces systèmes offrent des bénéfices qualitatifs significatifs. L’eau grise traitée peut être utilisée pour l’irrigation dans les régions arides, le vermicompost améliore la productivité agricole, et le traitement des eaux grises réduit la prolifération des moustiques, répondant ainsi à des enjeux critiques de santé publique au Burkina Faso. Ces bénéfices environnementaux et sociaux, bien qu’indirects, augmentent considérablement la valeur globale du projet et justifient une exploration plus large pour des applications étendues.

En conclusion, cette thèse a exploré le potentiel de la vermifiltration comme une technologie innovante, durable et économique pour le traitement et la réutilisation des eaux usées. En étudiant des paramètres clés de conception et de fonctionnement, elle a permis de mieux comprendre les performances, les limites et les applications de cette technologie, notamment dans les régions confrontées à des défis économiques, environnementaux et de santé publique.

Les études expérimentales ont montré que des paramètres clés que sont la HLR, EWD et la concentration initiale en DCO influencent fortement l’efficacité du système. L’évaluation de la durabilité confirme la qualité agronomique et hygiénique du vermicompost produit, bien que des compléments soient nécessaires pour compenser certaines carences en nutriments. L'absence de pathogènes majeurs valide son utilisation agricole. Enfin, l’analyse économique démontre que les systèmes à l’échelle communautaire sont plus rentables. Les bénéfices environnementaux et sociaux, comme la réduction des moustiques et l’amélioration de la productivité agricole, renforcent la valeur globale du système, notamment dans des régions arides comme le Burkina Faso.

Limites de l'étude :

1. **Colmatage du système :** Malgré la présence de vers, le colmatage reste un problème qui fait qu'une grande quantité d'eau ne pourra pas être traitée à la fois
2. **Variabilité des eaux grises :** Les concentrations élevées nécessitent un HLR faible, limitant les volumes d'eau traitables, ce qui pose également des contraintes pour des applications industrielles.
3. **Sensibilité des vers :** Les vers sont vulnérables aux variations climatiques, à la qualité des eaux grises et à la présence de substances inadaptées, ce qui pourrait affecter la durabilité du système.

Perspectives :

1. **Affinement des modèles mathématiques :** Des recherches sont en cours pour modéliser la dynamique des vers, des micro-organismes, des matériaux et des eaux grises. Cela permettra d'évaluer de La durée de vie exacte du filtre et l'établissement d'intervalles de nettoyage optimaux pour lutter efficacement contre le colmatage et maintenir les performances du filtre.
2. **Mise à l'échelle des filtres :** Concevoir et tester des filtres à grande échelle, avec des études à long terme pour valider leur efficacité, leur acceptabilité et leur évolutivité.

Ces efforts contribueront à promouvoir la vermifiltration comme une solution efficace, adaptable et durable pour la gestion des eaux grises, en particulier dans les régions à ressources limitées.

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LIST OF ACRONYMS AND ABBREVIATIONS

2iE	:	International Institute for Water and Environmental Engineering
AFNOR	:	Association Française de Normalisation
ANOVA	:	Analysis of Variance
BBD	:	Box Behnken Design
BOD5	:	Biochemical Oxygen Demand
COD	:	Chemical Oxygen Demand
DN	:	Nominal Diameter
DO	:	Dissolved Oxygen
E. coli	:	Escherichia coli
EB	:	Economic benefits
EC	:	Electrical Conductivity
EWD	:	Earthworms Density
FAO	:	Food and Agriculture Organization
FC	:	Fecal Coliforms
GI	:	Germination Index
HLR	:	Hydraulic Loading Rate
HRT	:	Hydraulic Retention Time
LEHSA	:	Water Hydro Systems and Agriculture Laboratory
NH4+-N	:	Ammonium-Nitrogen
NO3-N	:	Nitrate-Nitrogen
NPV	:	Net Present Value
OS	:	Organic Strength
PBP	:	Payback Period
PVC	:	Polyvinyl Chloride
RSM	:	Response Surface Methodology
SF	:	Streptococcus
TOC	:	Total Organic Carbon
TOM	:	Total Organic Matter
TSS	:	Total Suspended Solids
US EPA	:	United States Environmental Protection Agency
VF	:	Vermifilter

GENERAL INTRODUCTION

1. Context of the study

Greywater, the wastewater derived from domestic activities such as bathing, laundry, and dishwashing, constitutes a significant proportion of household wastewater in many parts of the world. The discharge of non-treated or partially treated, contaminated greywater to the environment poses risks of spreading various diseases to human beings. Moreover, the breakdown of organic contaminants found in greywater can rapidly deplete the dissolved oxygen (DO) levels in aquatic environments, rendering them inhospitable for the survival of aquatic organisms [1,2]. In Sub-Saharan Africa, the management of greywater presents a critical challenge, particularly in low-income communities where access to conventional wastewater treatment facilities is limited. Addressing these challenges is imperative to mitigate environmental degradation and enhance water security. Moreover, Greywater contains some organic nutrients with high agricultural potential [3].

2. Problem Statement

Our previous studies have demonstrated that greywater in contexts similar to Ouagadougou, Burkina Faso are highly polluted, and they are not treated but directly dropped into soils [4]. Intensive wastewater treatment systems can be solutions, but small communities cannot access them due to high installation and operation costs. It is assumed that by adapting decentralized wastewater treatment technologies, reliable and effective long-term solutions can be introduced [3]. While eco-friendly treatment technologies such as filtration [5,6], aerobic biological treatment units [7], constructed wetlands [8,9], aquatic plant restoration [10], oxidation ponds [11], ecological floating beds [12,13], and microbial remediation [14] are currently employed for various domestic and industrial wastewater treatments, their efficiency in removing contaminants is not optimally designed [15]. Additionally, in the middle of the treatment process, various problems can occur including overloading, and high sludge production. It has been identified that vermifiltration holds remarkable potential to effectively overcome these limitations [16].

Vermifiltration (VmF) has emerged as a promising, low-cost, and sustainable alternative. By employing earthworms in combination with filter bed materials such as compost, sawdust, and soil, vermifiltration systems enhance the decomposition of organic matter, reduce pathogen levels, and remove nutrients [16–19]. During the vermifiltration process, earthworms are used to increase and diversify the microbial communities living in soil-based biofilters[20].

Earthworms have been around for about 600 million years and have adapted to toxicity. They can help clean wastewater by devouring microorganisms[21]. Earthworm-based technologies are self-promoting, self-improving, and self-reinforcing, requiring very little energy and producing no waste. In addition, they are simple to design, use and to maintain. Vermifiltration is a technology that is known to be self-sustaining, natural, and environmentally friendly with an integrated mechanism to remove not only organic matter but also nutrients and pathogens [22]. Additionally, these systems produce vermicompost, a valuable by-product that can be used as an organic fertilizer. The technology is easily adaptable in developing countries due to its simplicity and the fact that it treats water to acceptable standards [23].Despite its potential, the application of vermifiltration in Sub-Saharan Africa remains limited due to a lack of comprehensive research addressing local operational challenges and optimization.

While existing studies demonstrate the potential of vermifiltration for treating greywater, several issues persist. These include variability in greywater composition, challenges in system design and a limited understanding of how critical operational parameters—such as Hydraulic Loading Rate (HLR), Earthworm Density (EWD), and organic strength—affect performance. Furthermore, the economic feasibility and agronomic value of the vermicompost by-product require detailed evaluation to support large-scale adoption.

3. Research Justification

Over the past several decades, numerous studies have been conducted under various conditions and with different types of water to better understand the vermifiltration process and to identify the factors that influence its operation and performance, with the aim of optimizing them [24]. Following multiple observations and experiments, several elements have been identified as factors influencing the operation and performance of the vermifilter. These include, among others : wastewater type, earthworm inoculation density (EWD), Hydraulic loading rate (HLR), hydraulic retention time (HRT), C/N ratio, Vermibed media type, height of active vermibed, feeding mode, organic strength (OS), and temperature [2,25].Of all these factors, EWD, OS, and HLR have particularly been reported as the primary factors influencing organic removal performance [2,26]. The OS of the influent provides the substrate for the microbes living and thriving within the system, whereas EWD contributes to the introduction of dissolved oxygen (DO) and gut microbes into the system, thereby enhancing the COD removal [20]. It suffices to note that the rise in HLR leads to increased infiltration rate, thereby reducing the contact time between pollutants, filter bed, and microorganisms. It is desirable to maximize the HRT, though too low hydraulic loading ratio leads to a build-up of untreated wastewater, resulting in wasted time and space, and higher treatment costs. Therefore, it is essential to achieve an optimum HRT/HLR ratio to effectively treat wastewater in the VF system, striking a balance between performance and cost-effectiveness [25].

However, to our knowledge, very few studies to date have delved deeply into providing a quantitative explanation of the influence of key factors on pollution removal by vermifiltration, through mathematical models. Such models would not only enable predictions of system performance but, more importantly, would allow for the optimization of key influencing factors for more precise and optimal system design, and treatment's cost evaluation.

Some authors, notably Samal et al. [27], Samal and Dash [15] have used Response Surface Methodology (RSM) to model and predict the performance of vermifilters for organic pollution removal from dairy wastewater. They optimized the HLR, the height of active vermibed, and the OS. The only study by [28] employed RSM to optimize the three key parameters in the treatment of brewery wastewater by vermifiltration. Indeed, RSM allows for the simultaneous evaluation of both the individual effects of different parameters and their interaction effects on system performance [29]. However, to our knowledge, none of the study went further to reach the design and a life cycle cost (LCC) assessment of a full-scale system.

This study therefore seeks to contribute to a deep understanding and quantification of the individual and combined effects of HLR, Initial COD, and EWD on COD removal from domestic greywater in a vermifilter, through RSM and Kinetic modelling. It also aims to assess the sustainability of the system by determining the vermicompost suitability and proposing a design and LCC assessment of a household vermifilter system. Thus, the research questions are stated below.

4. Research questions

Research questions

1. How do the performances of a vermifiltration system vary under specific conditions of HLR, EWD, and initial COD?
2. How do the key parameters vary during grey water treatment by the vermifiltration system and how can the performance be mathematically modeled?

3. To what extent can the vermifiltration system be considered as sustainable?

5. Objectives of the research

The overarching aim of this research is to optimize the performance and evaluate the sustainability of a vermifiltration system for greywater treatment, specifically targeting low-income populations in Sub-Saharan Africa. This aim is broken down into the following specific objectives:

1) To evaluate the design and the performance of a vermifiltration system under varying conditions, including HLR, EWD, and initial COD concentrations.
2) To quantify the interactive influence of key parameters on vermifiltration performance and identify the optimal conditions for achieving high efficiency in the vermifilter system
3) To assess the sustainability of the system.

6. Methodological Approach

The methodology adopted in this study combines experimental, analytical, and modeling techniques. First, a literature review identifies knowledge gaps and informs the experimental design. Greywater samples have been characterized for key parameters such as COD, BOD. Experimental trials have been conducted using a vertical flow vermifilter under controlled conditions, with systematic variations in HLR, EWD, and initial COD levels. Data from these experiments have been analyzed using statistical and kinetic modeling approaches to identify optimal operating conditions. The sustainability assessment has involved evaluating the agronomic properties of vermicompost and conducting a life cycle cost analysis to estimate the economic and environmental benefits of the technology.

7. Structure of the Thesis

This thesis is organized as follows:

Introduction

The introduction provides an overview of the research context, highlighting the challenges associated with greywater management, particularly in resource-limited settings like Sub-Saharan Africa. It also defines the problem statement and justifies the need for this research by emphasizing its relevance to environmental protection and public health. The objectives of the study, both general and specific, are outlined, alongside the general methodology adopted to achieve them.

Chapter 1: State of the Art

This chapter reviews the vermifiltration technology, covering its principles, operational mechanisms, and advantages compared to conventional methods. It also identifies research gaps in the field, particularly concerning the optimization of operational parameters and the sustainability of vermifiltration systems.

Chapter 2: Experimental Design and Performance Evaluation

This chapter details the experimental setup and methodology used to evaluate the performance of the vermifiltration system. It includes descriptions of the greywater characterization, system configuration, and operational parameters such as Hydraulic Loading Rate (HLR), initial COD and Earthworm Density (EWD). The chapter discusses the system's effectiveness in reducing organic loads and assesses its stability under varying conditions.

Chapter 3: Modeling and Optimization of the Vermifiltration System

In this chapter, mathematical models are used to analyze the relationship between critical parameters and system performance. The kinetic models are used to understand the substrate utilization rates, microbial activity, and the influence of organic loading on system efficiency and The Response Surface Methodology (RSM) is employed to identify optimal operational conditions for maximum efficiency in Chemical Oxygen Demand (COD) reduction. The findings provide valuable insights into the mechanisms governing the vermifiltration process.

Chapter 4: Sustainability Assessment

This chapter examines the broader implications of implementing vermifiltration systems. It evaluates the agronomic quality and hygienic safety of the vermicompost produced, considering its potential as a soil amendment. Additionally, a life-cycle cost analysis is conducted to assess the economic feasibility of the system in low-income settings. The environmental and social sustainability of vermifiltration technology is also discussed.

Conclusion

The conclusion synthesizes the key findings from the study, highlighting their practical and theoretical implications. It discusses the limitations encountered during the research and proposes recommendations for future studies to enhance the adoption and scalability of vermifiltration systems in similar contexts.

CHAPTER 1: STATE OF THE ART

1. Introduction

In this chapter, we aim to highlight the latest research in the field of vermifiltration treatment. We focused on the pollution removal mechanism, performance, fate of filter components including earthworms, and system by-products. The research methods applied by the different authors to study the vermifiltration mechanism have been presented. The chapter also presents the challenges and bottlenecks in the field. Recent studies have addressed the modeling and optimization of filter design and operating parameters. Particular attention is paid to these optimization solutions, to allow a better choice of the different components of the system, in the implementation. In addition, we brought out vermifiltration sustainability features.

2. General information on vermifiltration: definition and mechanism

2.1. Definition and types of vermifilters

Vermifiltration is a wastewater filtration process in which epigeic earthworms interact with microorganisms for the removal of wastewater pollution. It encompasses all forms of treatment, including primary (removal of sand, silt, etc.), secondary (biological degradation), and tertiary (removal of pathogens) in one unit [30]. The wastewater treatment system that is used for vermifiltration is the vermifilter (VF). It includes an active zone in which earthworms live, and a filter bed in which microorganisms grow [31]. It is also called microbial-earthworm ecofilter [32]. Different filters configurations have been implemented in recent years. They are vertical subsurface flow vermifilters (VVFs) horizontal subsurface flow vermifilters (HVFs) and two-stage vermifilters (VVFs + HVFs) (Figure 1).

2.1.1. Vertical subsurface flow vermifilters

The bed materials are stacked in vertical layers, and more often coarse gravel is placed in the lowest layer as a support ([33–35]). The wastewater flows vertically through the filter material and the treated effluent is received at the filter outlet. According to Ilyas and Van Hullebusch [36], the distribution of wastewater over the filter surface is better, allowing a higher oxygenation capacity. This favors the development of aerobic bacteria responsible for the degradation of pollutants. The high oxygenation also allows the complete degradation of organic matter (OM) in the wastewater. Carbon dioxide (CO_2) is thus released as the only gaseous emission. Environmental sustainability is therefore ensured by negotiating the emissions of greenhouse gases such as methane (CH_4) and nitrous oxide (N_2O) [37].

2.1.2. Horizontal subsurface flow and two stages flow vermifilters

In contrast to the VVFs, in the HVFs, the bed materials are stacked horizontally and the gravel layer is located right at the outlet [38]. Here, the wastewater flows horizontally through the different layers of the filter and the treated effluent is received at the outlet. The coexistence of aerobic and anaerobic conditions is higher in this configuration. Due to the combined effect of horizontal subsurface flow and earthworms, nitrification is facilitated in the upper layer due to earthworm activities and denitrification in the anoxic lower layer [39]. According to Singh et al. [38], one advantage of horizontal flows is that they allow a longer contact time compared to deep beds. In the two-stages flow vermifilter, the water is filtered in two stages as the name suggests [40,41]. Generally, the first stage consists of a VVF. The treated effluent from the VVF is reintroduced as influent into the HVF and the final effluent from the system is received at the outlet of the HVF (**Figure 1**). The advantages of both VVF and HVF configurations are thus combined here. In addition, the path length of the wastewater through the treatment system is higher, increasing the contact time between earthworms, microorganisms, and pollutants, which increases the treatment efficacy.

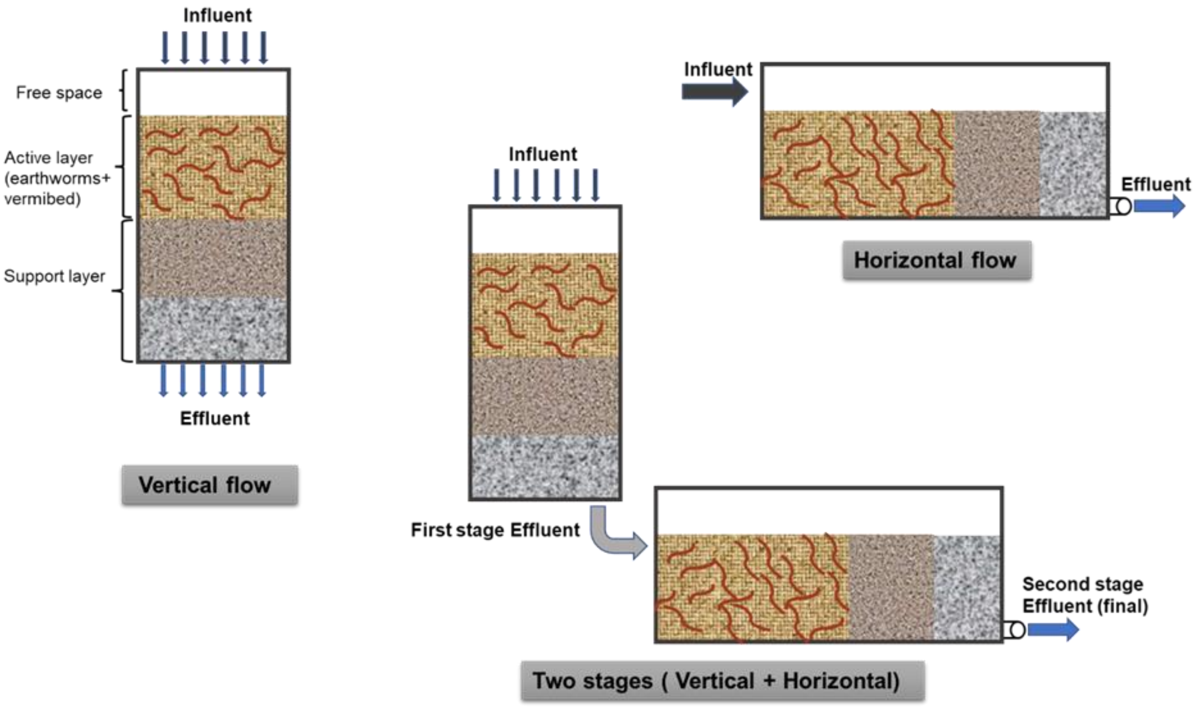


Figure 1. Schematic diagrams of different types of vermifilter configurations

To conduct a comprehensive comparative analysis, it is necessary to further explore several factors. These include scalability, cost-effectiveness, maintenance requirements, and potential challenges associated with each vermifiltration configuration. Additionally, it is important to evaluate the effectiveness of each configuration in removing specific types of pollutants and assess their suitability for different wastewater treatment scenarios. By considering these aspects, a more thorough and well-rounded comparison can be made between the vermifiltration configurations.

2.2. Mechanism of vermifiltration

2.2.1. Symbiosis earthworms - microorganisms in the degradation of pollutants

According to several studies, earthworms and microorganisms work synergistically to remove pollution from wastewater [42,43]. In that synergy, the microorganisms biochemically degrade the matter while the earthworms by muscular actions also allow a degradation but especially a homogenization of the matter. Through the investigation of bacterial and protein characteristics, it has been demonstrated by Arora et al. [44] that the feeding of earthworms and the interactions between earthworms and micro-organisms are responsible for the removal of pollutants during the vermifiltration process.

Figure 2. Synergy between earthworms and microorganisms in the degradation of OM

Indeed, the digestion of organic matter (OM) in the digestive tract of earthworms is carried out within the framework of mutualism existing between the earthworm and the microorganisms ingested in the environment. In the digestive tract, the earthworm secretes water and mucus which will reactivate microorganisms in the environment. Under the action of this « priming effect, the stimulated microorganisms will decompose and mineralize a part of the OM (**Figure 2**).

The earthworms will then be able to assimilate some of the carbon and nutrients released by microbial activity. The passage through the gastrointestinal tract of earthworms has a qualitative and quantitative influence on the microbial community of the biofilm in the vermifilter. It is important to understand the synergistic relationship between earthworms and microorganisms for the degradation of pollutants and to highlight the respective actions of each entity on the outcome. The study conducted by Arora et al. [44], reveals that only 40% of the bacterial species that were investigated in the system did not originate from the worm gut, so less than half. Adugna et al. [45] noted a five-fold increase in the microbial population of the vermifilter compared to the control filter, without the earthworms. It was also observed that there was a more extensive variety within the population present in the vermifilter [46]. The higher the density of earthworms in the system, the greater the diversity of microorganisms [47].

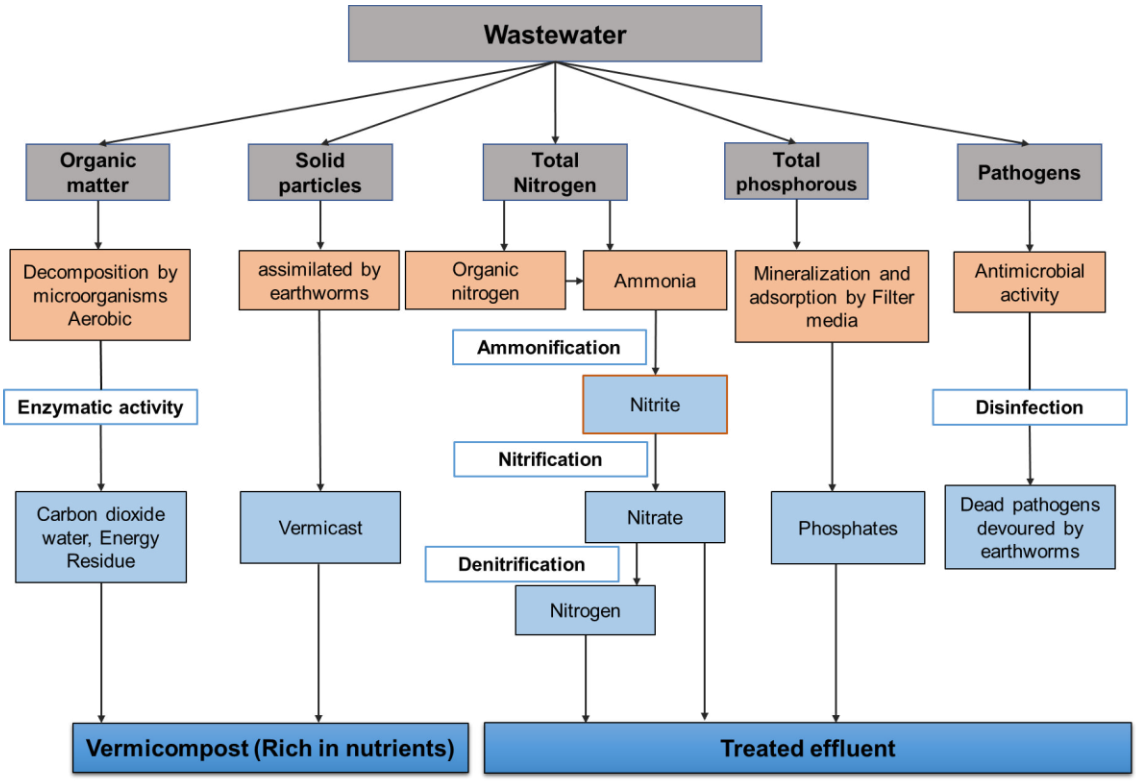


Figure 3. Schematic diagram of the vermifiltration mechanism

The different stages of degradation are the following:

First, the larger organic and inorganic solid particles are broken down by earthworms into finer particles. In this way, the surface area of the particles increases, thus facilitating microbial degradation [48,49]. The finer particles are trapped within the pores of the vermifilter bed media and subsequently undergo degradation by microorganisms [43,45]. Beyond his physical function, the bed media serves as an essential component by offering a habitat conducive to the growth of beneficial microorganisms. Additionally, the media is effective in adsorbing specific contaminants, including heavy metals and pathogens.

Nutrients undergo a kind of recycling and are added to the vermicompost and treated effluent which are the by-products of the vermifiltration mechanism (**Figure 3**). The processes of ammonification, nitrification, and denitrification have been listed in the literature as the origin of nitrogen elimination in the vermifilter [48,50,51]. One part of the organic phosphorous is mineralized to inorganic phosphate. The remaining portion is adsorbed by the filtering media.

2.2.2. Role of earthworms in degrading pollutants

The significant role of earthworms in the decomposition of pollutants has been well-established over the years. Chao et al. [52] conducted a comprehensive meta-analysis to assess the extent of earthworms' impact on the breakdown of organic pollutants. The findings from this analysis reveal a statistically significant average effect size, indicating a 128.5% increase in organic pollutant degradation ($p < 0.05$). The increase in the concentration of dissolved organic matter resulting from earthworm treatment, promotes the proliferation of soil bacteria involved in decomposition [53]. Furthermore, earthworms, by their burrowing activity create galleries i.e., many small holes in the system, thus promoting the development of aerobic bacteria for wastewater treatment [54–56]. The intestinal system of earthworms plays a crucial role in the processes of nutrient solubilization, bioremediation, and the strengthening of advantageous microbial populations. Analysis of microbial diversity reveals the presence of various microorganisms in vermicast, including Azotobacter, Rhizobium, Nitrobacter, nitrogen-fixing bacteria, phosphate-solubilizing bacteria, as well as Basidiomycetes and Ascomycetes, among others [57]. An investigation of the type of bacteria in the system shows a higher concentration of aerobic bacteria than facultative and anaerobic ones [58]. In addition, Arora et al. [54] were able to detect 26 species of bacteria in the VF with *Bacillus* as the dominant genus, compared to only 11 in the filter without earthworms. It is known that *Bacillus* produces metabolites that have antimicrobial and cytotoxic or antifungal properties. Moreover, typical biofilm macrographs and micrographs showed that the feeding and dejections of the earthworms changed the bacterial population in the VF. The use of Automatic Testing Bacteriology (ATB) expression and sequencing data also reveals that the presence of earthworms leads to an increase in microbial diversity and metabolic activities in the biofilm [59]. This observation underscores that the presence of earthworms significantly amplifies the bacterial population within the system. The research of Wang et al. [60] on the biofilms revealed a significantly higher pore number in the VF than in a filter without earthworms or non-vermifilter (NVF). The total organic carbon (TOC) was also higher in the VF. Dehydrogenase activity (DHA) and adenosine triphosphate (ATP) are better in VF than in the NVF. This activity is improved by more than 16%. The burrowing activities of the earthworms can also increase parameters including the hydraulic conductivity of the system, the specific surface of the substrate, the nutrient exchange rate and the aeration of the system [61]. Another role of earthworms in the vermifilter is the consumption of the bacterial population that competes for substrates with the microorganisms responsible for the degradation of pollutants. Earthworms secrete a viscous fluid that traps these harmful bacteria. The microbes left behind by the earthworms, which are not harmful, also compete with the pathogens for nutrients [62]. It has also been shown that earthworms accumulate heavy metals in their gut, leading to a substantial decrease in pollution levels in the final treatment products [63].

3. Benefits and limitations of vermifiltration

3.1. Benefits

The benefits of vermifiltration are numerous, including:

- Low methane emissions: From the study of Dore et al. [33] it was observed that in the course of the vermifiltration process, methane emissions were significantly diminished in comparison to those from an anaerobic lagoon, showing a remarkable reduction ranging from 97% to 99%.The methane emissions are minimal at about 0.7kgCH_4/m^2/year.
- Odor-free process: lombrifiltration systems operate in a predominantly aerobic state, meaning an oxygen-rich environment for earthworms and micro-organisms. This state favors the decomposition of organic matter without generating foul-smelling anaerobic by-products. The system also ensures a continuous flow, avoiding water stagnation which can lead to the build-up of odorous gases [64].
- Little or no sludge production: vermifiltration systems have the advantage of not producing sludge requiring treatment [64,65]. Sludge yield is generally less than 0.2 kg of suspended solids per kg of chemical oxygen demand (COD) [66].

- High performance in pollutant removal: vermifiltration excels in organic matter removal from wastewater due to the earthworms' ability in breaking down and digest organic matter. The treated effluent from the VF can easily be reused in many industrial plants for production processes and various secondary uses. Oils and fats are also removed at a rate of approximately 84 - 89% and surfactants at a rate of 95 - 99% [67].
- Low Operating Costs: the vermifiltration approach proves cost-effective by taking advantage of natural processes and eliminating the need for substantial energy consumption or expensive equipment. Unlike conventional wastewater treatment methods, vermifiltration is less energy-intensive, making it a sustainable choice [68]. It is possible to have a cost reduction of about 60 - 70% compared to other systems [69].
- Adaptability and environmentally friendly: vermifiltration is an environmentally friendly method that promotes the recycling of organic matter while reducing the environmental impact associated with wastewater disposal. Also, vermifiltration can be used flexibly in a variety of environments, including rural areas and decentralized wastewater treatment systems.

3.2. Limitations

- Limited performance in the removal of certain pollutants: for the optimal removal of nitrates and phosphorus, it is necessary to combine other processes or techniques with vermifiltration [34]. Nie et al. [70] also concluded that the VF has a poor performance in the removal of these two elements. For pathogen removal also, additional disinfection steps may be required for safe reuse of wastewater. Furthermore, the concentrations of heavy metals in VF effluent often do not meet acceptable thresholds, especially for industrial waters [71].
- Environmental sensitivity: The effectiveness of vermifiltration is sensitive to environmental conditions, including temperature and humidity levels, which can have an impact on earthworm activity. Temperature in particular, is a critical factor, as earthworms thrive in a specific range, generally between 15°C and 25°C, as pointed out by Edwards and Arancon [72]. Earthworms cannot survive at temperatures below 10°C or above 35°C, making vermifiltration unviable in such extreme temperature conditions. Some toxic constituents present in the wastewaters to be treated could also be fatal to earthworms.
- Clogging problems: Over time, vermifiltration systems can become clogged with solids and organic matter. Clogging can be triggered by a range of factors including the hydraulic

loading rate, precipitation on the bed, salinity, exposure to sunlight, and the characteristics of the filter bed. Clogging can diminish the effectiveness of the vermifiltration system and escalate maintenance expenses [35,73,74].

- Capacity limitations: Lombrifiltration may not be well-suited for handling large volumes of wastewater, making it more appropriate for small-scale applications, as highlighted by Suhaib and Bhunia [74].

4. Different factors to consider in the implementation of a Vermifilter

4.1. Wastewater type and organic loading rate

In the last few years, vermifiltration has been used in the treatment of different types of wastewaters and not only for domestic wastewater as in its early stage of testing. Water from dairies, palm oil processing plants [46], the textile industry [75], piggeries [76] , from feedlots, to hospital water, and several other sources (listed in **Table 1**) have been treated. This showcases the versatility and potential of vermifiltration as a viable option for handling different types of wastewaters. Moreover, the organic loading rate (OLR) admissible by the system, ranges from low levels of the order of 100 - 200 mg/L of chemical oxygen demand (COD) to higher levels (**Table 1**). Vermifiltration can be used as a treatment process for highly polluted waters. Manyuchi et al. [77] have worked on distillery waters with COD values of more than 92,000 mg/L and biochemical oxygen demand (BOD) values of more than 25,000 mg/L, with removal rates of about 89% and 91% respectively. Other authors have also had similar results (Table1). Despite the fluctuation of the organic load at the inlet, the VF can work over long periods [78]. However, wastewater can have varying characteristics that can affect VF performance. For example, industrial wastewater may contain toxic chemicals that can inhibit earthworm activity, reducing treatment efficiency [71]. Earthworms are well-known for their ability to withstand various types of contaminants, including herbicides, heavy metals, and organic pollutants that often originate from industrial, factory, and hospital wastewater as well as sewage sludge [79]. They possess the capacity to decrease the concentrations and toxicity of wastewater contaminants by storing them in their body tissues [64]. However, when wastewater containing these contaminants is applied, it can result in the accumulation of these substances within the earthworm’s body, which in turn could induce physiological and biochemical harm. This can result in growth abnormalities, enzyme inhibition, and toxicity, which may extend to genetic levels [79].

Table 1. Different types of wastewaters treated by vermifiltration

Type of wastewater	Place of experiment	Influent COD (mg/L)	COD Removal (%)	Influent BOD (mg/L)	BOD Removal (%)	References
University campus	India	325-400	92	180–250	98	[44]
Cattle feedlot	USA	300-550	61.4-69.1	-	-	[80]
Clinical Laboratory	India	390-420	75 - 80	200-250	80-85	[48]
Distillery	Trichy, Inde	54400	90	18100	95	[81]
Domestic greywater	Burkina Faso	1075-3520	83	800-2100	97.6	[45]
Urban (Sewage and rainwater)	-	450±10	87.6	210±10	91.3	[82]
Swine wastewater	Portugal	1997	-	149	83	[34]
Slaughterhouse wastewater, synthetic	USA	2100-2400	70-85	-	-	[80]
Synthetic brewery wastewater	-	2250-11250	92-96	-	-	[56]
Side-stream of dairy wastewater	USA	2100-3400	45±4.1	-	-	[35]
Textile dye effluent	India	5800	85-89	1933	76-80	[83]
Urban sewage	Spain	706±407	88±7	392±262	85±19	[84]
Domestic septic tank sewage	Zimbabwe	-	-	49.9	68-97	[85]
Hospital wastewater	Iran	227-461	75	145-300	93	[78]
Municipal wastewater	China	240-320	83.5±2.1	120-200	81.3±2.9	[60]
Dairy wastewater	India	2560	67	-	-	[86]
Domestic wastewater		92.2±18	67.6	39.1±10.2	78	[66]
Synthetic Dairy Wastewater	India	1734± 110	80.7	1103.6 ± 82	88.4	[87]
Sewage sludge	China		53.01±10.53	9900-20000	61.06±13.87	[88]
Organized industrial zone			80			[71]

4.2. Hydraulic loading rate (HLR) and Hydraulic retention time (HRT)

The hydraulic loading rate (HLR) is the amount of water passing through the filter per unit area and per unit time. It is one of the most important parameters in water filtration. It has been observed that the treatment performance and the clogging of the VF are closely dependent on the administered HLR.

Table 2. HLR an HRT applied in vermifiltration and their impact on removal performance.

Wastewater	HLR (m^3/m^2/day)	HRT (Hours)	Removal performance (%)							References
			COD	BOD	TSS	TDS	TN	TP	NO^{3-}	
University campus	1	4-6	92	98	90				-50	[44]
Cattle feedlot parc wastewater	0.5		61.4-69.1	-	-	-	34.4-38.8	48.0-54.0	-	[80]
Clinical Laboratory	1	7-8	78 - 85	-	-	-	-	-	-	[48]
Urban (Sewage and rainwater)	0.89	6	87.6	91.3	98.4	-	-	-	-	[82]
Synthetic Dairy wastewater	0.6	10	83.2	-	-	-	-	-	-	[89]
Side-stream of dairy wastewater	0.48	4	45±4.1		68±10		77±8.4	48±6	74 ±9.5	[35]
Textile dye effluent		8	85-89	76-80	73-77	71-76	-	-	-	[83]
Hospital wastewater	1	-	75	93	89	-	-	-	-	[78]
University campus wastewater	2.5	-	-	88	78	75	-	-	-	[90]
Municipal wastewater	2	-	83.5±2.1	81.3±2.9	93.7±2.6	32.4±10.6	-	38.6±3,6	55.6 ±11.6	[60]
Synthetic wastewater	1	7.8	74	92	-	-	-	-	-	[54]
Synthetic domestic wastewater	0.2	-	76.6±5.18		-	-	63.8±2.75	81.0 ±2.25	-	[59]
Domestic wastewater	4.2	-	67.6	78	-	89	-	-	-	[66]
Synthetic Dairy Wastewater	0.6	-	80.7	88.4	-	-	61.7	77.8	-	[87]
Domestic greywater	0.16		83	97.6	99.4			31.3	62.2	[45]

The natural activities of earthworms, such as feeding and burrowing, help improve the porosity of the filter bed. However, when the HLR is increased, earthworm activity tends to decrease, which can result in the accumulation of clogging materials in the initial section of the filter bed, leading to severe clogging problems [73,74].

According to Suhaib and Bhunia [91], the instability of earthworm casts within the filter bed at higher HLRs can further reduce the beds' permeability. Moreover, the studies conducted by the same authors [92] revealed that a filter bed at an HLR of 2.34 m^3/m^2/day causes an increase in organic and suspended solid loads on the filter bed compared to a (lower) HLR of 0.66 m^3/m^2/day, resulting in significant accumulation of clogging materials like biofilm biomass and suspended solids at the inlet end of the filter bed.

On the other hand, Samal et al. [91] concluded that the performance of the VF decreased with increasing HLR. A too-high HLR decreases the contact time of the wastewater with the filter media, the hydraulic retention time (HRT). The HRT represents the duration of the interaction between wastewater and the active layer of the VF. It is mainly dependent on the HLR, the porosity of the bedding materials, and the volume of the filter profile. It is a very important parameter because it represents the time that the worms and microorganisms will spend in contact with wastewater. Indeed, they need enough time to be able to degrade and stabilize solids, organic matter (OM), and nutrients.

According to Rajpal et al. [93], a sufficient HRT allows the rapid mineralization of nitrogen into nitrate. It is therefore preferable that the HRT is as long as possible, which would mean reducing the HLR as much as possible. However, a too-low HLR means that a large quantity of wastewater will not be treated at the same time. This represents a loss of time, space and therefore, an increase in the treatment cost.

It is thus essential to have an optimal HRT/HLR ratio to allow an efficient and effective treatment of the wastewater in the VF with a good balance between performance and cost. Singh et al. [38] concluded to an optimal HLR of 1.84 m^3/m^2/day for brewery wastewater treatment and Adugna et al. [45] to a 64L/m^2/day HLR for domestic greywater. Various HLR and HRT with associated performances are presented in **Table 2**. Their impact on the removal of different wastewater pollution parameters can be noted.

4.3. Earthworms' species and density of application

The earthworms used in vermifiltration are epigeic. Also called surface-dwelling earthworms, they live in the upper layer of the soil and deposit wormcasts on the surface. Their involvement in the breakdown of organic matter is widely recognized as crucial. Experiments have been conducted so far on the species including *Eisenia fetida*, *Lumbricus rubellus, Lumbricus terestris*, *Eudrilus eugeniae*, *Perionyx sansibaricus*, *Eisenia Hortensis*, and *Eisenia andrei*. In recent years the species that have been used the most are *Eisenia fetida* and *Eudrilus Eugeniae* as shown in **Table 3**.

Table 3. Species used and inoculation rate of earthworms in vermifilter.

Species	Inoculation rate	Wastewater type	COD removal performance Influent(mg/L)	Removal (%)	References
Eisenia fetida	10000 worms / m^3	University campus	325-400	92	[44]
Lumbricus terestris	1000 worms / m^3	Cattle feedlot	-	61.4-69.1	[80]
Eisenia fetida	1000 worms / m^3	Clinical Laboratory	-	78 - 85	[48]
Eisenia fetida	20g/ L	Urban (Sewage and rainwater)	-	87.6	[82]
Eisenia fetida	10-12g/L	Swine wastewater	1997	83	[34]
Eisenia fetida	10,000 worms/m^3	Synthetic Dairy wastewater	1758.6 ± 144	83.2	[89]
Eudrilus eugeniae	1000-1500worms/m^3	Textile dye effluent	5800	85-89	[83]
Eisenia fetida	15000 worms / m^3	Sewage	706 ± 407	88 ± 7	[84]
Eisenia fetida	10000 worms /m^3	Hospital wastewater		75	[78]
Eisenia fetida	32g/L	Municipal wastewater	300	83.5 ± 2.1	[60]
Eisenia fetida	10000 worms /m^3	Synthetic wastewater	456 ± 32	74	[54]
Eisenia fetida	8g/L	Domestic wastewater	92.2	67.6	[66]
Eisenia fetida	10 000/ worms /m^3	Synthetic sewage		73.9	[30]
Eisenia fetida	40g/L	Piggery wastewater	2960±88.8	99.2	[76]
Eisenia fetida	10 000 worms /m^3	Synthetic dairy Wastewater	1734± 110	80.7	[87]

Eisenia fetida is however the most used because it can withstand a wide range of temperatures [60,85,94]. It also has a high reproduction rate and an optimum operating temperature of 25-27^{o}C [95]. It is a specie of epigeic earthworms that contains digestive enzymes such as alkaline, protease, phosphates, and cellulose. They possess an exceptional microflora in their intestine that allows the development of a very varied microbial community in their gut. They use their body as a filter, like other species used [85]. The effects of inoculation rate of earthworms have also been investigated in the context of wastewater treatment by vermifiltration. It was shown that the variation of the inoculation rate affects the treatment [96]. In general, the more earthworms there are, the higher the rate of water pollution removal. This is perfectly justified, considering the role of earthworms in the VF that was presented above.

The more earthworms there are, the more the burrowing activity, allowing the aeration of the system to increase, and also the microbial activity to be enhanced. Earthworm stocking density also regulates microbial community structure and fatty acid profiles during vermicomposting of lignocellulosic waste [97]. The higher application rate would therefore allow for the treatment of more highly loaded wastewater (**Table 3**). However, as with the HLR, it is important to maintain an optimal rate.

4.4. Vermibed media and height of active vermibed

The bedding materials and especially the active layer are very important parameters to consider when implementing a vermifiltration system. Indeed, the time that the wastewater will spend in the filter closely depends on these parameters, given the different porosities, permeability, and other characteristics of the filter materials. Singh and Kaur [98] have shown that the growth, reproduction, and performance of earthworms in the degradation of OM depend on the type of active layer. One of the most frequent signs of the discomfort of earthworms in an environment is that they try to escape and or die [99]. Some materials of the active vermibed can even harm earthworms' bodies. The type of filter material also has a great impact on the structuring of the microbial community responsible for the decomposition of OM [55]. The rate of clogging of the filtration system is also closely related to the porosity and permeability of the filter material. They control hydraulic conductivity. Low hydraulic conductivity leads to rapid clogging [100]. Materials with good porosity also provide a larger surface area for biofilm development and wastewater treatment [101]. The porous nature of the bed media permits aeration, creating a controlled environment conducive to microbial activity. Most of the active vermibed materials used in recent years are listed in **Table 4**. Vermicompost is the most used and its purification performance is superior to that of sawdust. In addition, according to the research of Kumar et al. [55], river bed material has been found to be better than wood coal, glass balls, and mud balls both in terms of pollution removal performance (**Table 4**), and increase in earthworm biomass. The COD removal rate for example is 72.3% for riverbed material versus 64.6%; 61.5%; and 59.8% for wood coal, glass ball and mud balls respectively.

As mentioned above, the earthworms used for vermifiltration are epigeic and they generally live and develop in the first centimeters of the soil, between 10 and 15 cm. It is therefore essential to consider this information in the design of the VF. **Table 4** shows that in most cases, the depth of the active layer is between 10 and 30 cm.

Table 4. Impact of bedding material and vermifilter bed height on treatment.

Wastewater type	HLR ($m^3/m^2/day$)	HRT (Hours)	Bedding material	Bed height (cm)	Removal performance (%)							References
					COD	BOD	TSS	TN	TP	NO3-	Nh4+ -N	
University campus wastewater	1	4-6	Cow dung+vermigratings	20	92	98	90			-50		[44]
Clinical Laboratory	1	7-8	Cow dung +vermigratings	30	78 - 85							[48]
Distillery		8-10	Garden soil	7	90	95	80					[81]
Domestic greywater	0.16		Sawdust	40	83	97.6	99.4		31.3	62.2	75	[45]
Urban (Sewage and rainwater)	0.89	6	Vermicompost	16	87.6	91.3	98.4				76.5	[82]
		6	Sawdust	16	79.7	90.5	98.4				63.4	
Domestic sewage	2		Lombricompost	20	83					60		[102]
Commercial dairy		4	Woodchips	30				84+-8%				[33]
Dairy wastewater, synthetic	0.6		1:3 mixture Garden soil and vermicompost	30	83.2			57.3				[89]
Side-stream of dairy wastewater	0.48	4	Wood shaving and chips	20	45+-4.1		68+-10	77+-8.4	48+-6	74+-9.5		[35]
Synthetic Dairy wastewater	0.3		vermicompost and garden soil on the ratio of 1:3by volume	30	83.2			57.3				[91]
Textile dye effluent		8	garden soil	7	85-89	76-80	73-77					[83]
hospital wastewater	1		garden soil	30	75	93	89					[78]
Synthetic wastewater	1.5		River bed material+ vermicompost	15	72.3	81.2	75					[55]
			Wood coal+ vermicompost		64.6	74.5	64					
			Glass balls+ vermicompost		61.5	72.7	59					
			Mud balls+ vermicompost		59.8	70.9	55					
Synthetic domestic wastewater	0.2		Artificial soil +padding soil+rice straws	35	76.6+-5.18			63.8+-2.75	81.0+-2.25			[103]
Synthetic sewage	1.3		Gravel+ mature vermicompost	30	73.9	84.8						[30]
Synthetic Dairy Wastewater	0.6		1:3 mixture of vermicompost and garden soil	25	80.7	88.4		61.7	77.8			[87]

The research of Nie et al. [70] on rural synthetic wastewater, showed that the majority of pollution removal takes place in the first 40 cm of the filter bed. An anaerobic zone can develop in the lower zones of the filter bed if the depth of the bed is too great. This will have a negative impact on earthworms and biofilm microorganisms [104]. Wang et al. [105] showed that variation in VF height had a significant effect on COD and total phosphorus (TP) removal rates, earthworm population, and *actinomycete* numbers, but had no effect on total nitrogen (TN) and ammonia nitrogen (NH_3-N) removal rates, and on bacterial and *fungi* numbers.

4.5. Planted vermifilters

Macrophyte VFs are a very promising technique to remove organic and mineral matter from wastewater. In the literature, it is reported that the average removal rates of planted VFs are better than those of simple VFs. A theory put forward by Singh et al. [80], is that the root exudates from the plants in the planted VF make the microbial activity more intense by promoting the growth of microorganisms. In addition, the increase in aeration due to the roots would also increase the population of microorganisms [106]. Huang et al. [107] showed a significantly higher growth rate of earthworms in the planted VFs compared to the ones without plants. The increase in the number of earthworms was also significantly higher in the planted VFs, by about 76.5%. For the growth of their biomass, the plants would absorb the phosphates as nutrients. With the plants in the VF, there is also a decrease in clogging due to the fact that the roots of the plants by their growth, create cracks in the filter bed [50]. Plants also enhance the denitrification potential of the system [102]. The most widely used macrophyte species according to the literature is *Canna indica*. It is a specie of herbaceous flowering plants in the *Cannaceae* family. It is sometimes called *Indian canna*. Compared to other macrophytes such as *Saccharum spontaneum* and *Typha angustifolia* the percentage of COD removal is 3.9 % higher than the former, 7.3% higher than the latter, and 13.4% higher than the VF without plants [56].

4.6. Temperature

Earthworms are sensitive to temperature fluctuations. Fecundity, cocoon production, and maturation of earthworms are some parameters that may be affected by temperature [72]. Therefore, the temperature has a significant impact on the performance of the VF. specifically on the removal of total ammonia nitrogen (TAN) and COD [35]. The effect of temperature was also noted on the removal of NH_3-N by Wang et al. [32]. Indeed, the diversity and composition of the NH3-N oxidizing *Betaproteobacteria* community in the different layers of the VF over the year were probably influenced by temperature changes. Arora and Kazmi [95], also revealed that variations in ambient temperature had a significant effect on the reduction of COD, biochemical oxygen demand (BOD), and pathogens.

5. Modeling and optimization of the vermifiltration process

Various authors have worked on the modeling and optimization of the vermifiltration process. Mathematical models of vermifiltration can be used not only to design and optimize the operation of the system but also to predict its behavior and to control it [108]. Numerous authors used the response surface methodology (RSM) and the Box-Behnken design (BBD) to evaluate both the individual effect of the different factors and their simultaneous interaction on the system performance [38,41,108–110]. Samal and Dash [108] worked to optimize biochemical oxygen demand (BOD) removal in the treatment process of a synthetic dairy wastewater. Hydraulic loading rate (HLR), biodegradable organic strength (BOS) and depth of the active layer (ALD) of the Vermifilter unit were considered as the main parameters influencing the VF performance. According to the developed model, the optimal conditions to achieve maximum BOD removal for the designed system were at an influent concentration of 1701.00 mg/L, HLR of 0.39 m^3/m^2/day, and ALD of 34.40 cm. The experimental BOD removal of 86.45% was obtained against a predicted value of 87.08% under optimal conditions. Singh et al. [38] sought to optimize the chemical oxygen demand (COD) removal from a synthetic brewery wastewater, with HLR and BOS, as the influencing parameters, but this time earthworm density (EWD) instead of ALD. The optimal conditions to achieve maximum COD removal for the designed system were at an influent concentration of 3 542 mg/L, HLR of 1.84 m^3/m^2/day, and EWD of 9 661 earthworms/m^3. Under the optimal conditions, a COD removal of 94.99% was obtained against the predicted value of 95.85%. Model verification on real brewery wastewater also showed minimal error from the predicted COD removal. The COD removal index (CRI) a mathematical tool has also been developed by Singh et al. [109], to predict the organic removal performance of vermifilter. The authors defined the BOS, the EWD but the HRT this time as the influencing parameters. The R^2 value of the plot between the calculated value of the CRI and the obtained removal was found to be 0.8, showing that the tool can be well used to predict the removal of organic matter in a VF. Samal et al. [41] optimized the removal of COD and TN from a synthetic dairy wastewater with HRT, BOS, and ALD as influencing factors. They obtained maximum removal percentages of 83.2% for DOC and 57.3% for TN. Concerning the kinetic model, the Stover-Kincannon model was found to be the most suitable for the degradation of pollutants by vermifiltration, rather than the first-order model and the Grau second-order model with the best regression coefficient both for COD. R^2 = 0.9961 and for TN, R^2 = 0.9353 against COD R^2= 0.5212 for the first-order model and R^2 for COD and TN, 0.9817 and 0.8395, respectively for the Grau second-order model. It has been shown by Singh et al. [73] that the clogging of the VFs can also be minimized by optimizing the HLR, EWD, and COD concentration still by the RSM method. They were able to find that for brewery wastewater, the minimum clogging is obtained with HLR of 1.84, EWD of 9475 earthworms/m^3, and 3701 mg/L of COD, with insignificant errors. Mathematical models and response surface methodology (RSM) are thus, important to better understand the behavior of the vermifiltration system. They aid understanding and enable accurate predictions, facilitating more efficient system design and operation. Research highlights the adaptability of vermifiltration to various wastewater treatment scenarios. Performance can be optimized by fine-tuning variables such as HLR, BOS, EWD and ALD. By optimizing these parameters, specifically HLR, EWD and COD, clogging problems can also be minimized. CRI has proved invaluable in assessing the organic removal efficiency of vermifiltration systems. This index can be useful for monitoring and improving system efficiency over time.

6. Fate of wastewater parameters and performance of vermifilter

6.1. pH

Various studies have shown that VF acts as a buffer. One passes respectively from acidic pH to neutral pH and from basic pH to neutral pH. The vermifilter would thus allow neutralization of the pH of the effluents [34,48]. In the experiments conducted by Arora et al. [48] concerning the application of vermifiltration for treating clinical wastewater, the initial influent pH levels exhibited considerable variability within the range of 4.2 to 9.3. However, at the vermifilter outlet, the pH levels stabilized and remained relatively constant, falling within the range of 6.7 to 7. In their study, Ghasemi et al. [102] noticed an increase in pH at the beginning of the treatment and stabilization at a neutral pH until the end of the treatment. Thus, one can go from pH 8.5 to about pH 7 [44], and from pH 4 to pH 7 [77]. This tendency to neutralization and stabilization of pH can be explained by the elimination of bio-contaminants contained in the influents, and the decrease of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) concentrations also [77]. It could also be attributed to the aerobic degradation of organic matter generating carbonic acid [51]. Another explanation is that *VTpase*, a protein upregulated by earthworms in response to stressful wastewater conditions, may be responsible for stabilizing pH.

6.2. Electrical conductivity (EC)

No significant difference was observed between the electrical conductivity (EC) of the effluent and the influent in most of the studies. But in the work of Kannadasan et al. [81], a significant difference was noted between the EC of distillery effluents treated by a NVF and those treated by vermifiltration. The EC values of the simple filter effluent were in the range of 2.03 ± 0.04 mS/cm for the 50% diluted effluent while that of the vermifiltration treated effluent were in the range of 5.41 ± 0.01 mS/cm. The increase in EC in the vermifilter effluent could be due to the release of minerals from the earthworm gut. Other authors also noticed an increase in EC [34].

6.3. Dissolved oxygen (DO)

Authors have reported on the behavior of DO during the treatment by vermifiltration. A clear increase of this parameter is noted at the outlet of the VF [48,90]. However, on closer observation, Rustum [86] observed that the DO of the water is reduced at the beginning of the treatment, but as the treatment progresses, the levels begin to increase until the end. According to him, this phenomenon is attributed to the initial consumption of DO by microorganisms during the initial stages of treatment, which is essential for breaking down the organic matter. With the aeration of the system during the treatment, this DO is replaced by oxygen from the atmosphere. At this point, the dissolved oxygen concentration starts to increase [86].

6.4. Organics and solids removal

The different mechanisms that occur during the degradation of organic matter during wastewater treatment are: dehydrogenation, oxidation, stabilization of OM, and transformation of unsaturated structures into saturated structures [111].

The degradation and stabilization of the OM in the vermifilter is possible by the enzymatic activity of the micro-organisms. It allows the decomposition of starch, proteins, and cellulose. More specifically, it would be the activity of cellulase, amylase, and protease [54]. The high COD removal rates due to the enzymatic activity of the microorganisms would not have been possible without the presence of earthworms [54,90]. According to Wang et al. [32], the diversity of bacteria in the VF could influence COD removal. The high COD removal rates is also attributed to the symbiotic action of earthworms and microorganisms. The different burrowing actions of the earthworms keep the environment aerated and allow the oxidative work of the aerobic bacteria [38]. A correlation has been observed between COD removal in the VF and the activity of antioxidase enzymes and oxygen reactive species in the earthworm tissues and also the length of the earthworm burrow [112]. Microorganisms are responsible for the degradation of OM and dissolved, suspended, organic and inorganic solids in the VF. The degradation of the solid matter is done by catabolic activity. However, to facilitate the removal of total suspended solids (TSS) and total dissolved solids (TDS), the earthworms perform grinding, dispersal, ingestion, and digestion activities of these TSS and TDS [42]. Earthworms then release finer particles to be trapped in the pores of the VF for direct removal from the wastewater. Quantitative analysis using δ15N showed that earthworm feeding and earthworm-microorganism interaction were responsible for approximately 21% and 79%, respectively, of the highly volatile suspended solids removal [113].

6.5. Nutrient removal

Nitrogen compounds are among the most present elements in wastewater. They are also the main cause of water eutrophication. As mentioned earlier, ammonification, nitrification, and denitrification are the processes through which nitrogen is eliminated from wastewater. Further analysis of the transformation of nitrogen speciation by the δ15N-NO^{3-} isotope dilution method also confirmed the occurrence of nitrification and denitrification processes [114]. First, the organic nitrogen is converted to ammonium. Then ammonium is converted to nitrates, and nitrites during nitrification. The heterotrophic bacteria that enhance the mineralization of nitrogen come largely from the gut of earthworms [82]. Finally, as a result of the last reaction, denitrification, nitrates and nitrites are converted into nitrogen gas by the action of denitrifying bacteria [33]. *Comamonadaceae*, from the *Betaproteobacteria* family, were identified as involved in the denitrification process. Analysis of 16S rRNA gene profiles in influent and effluent from the VF concluded that these bacteria were increased during vermifiltration [115]. VF is mainly dominated by *proteobacteria*. More precisely the *γ-proteobacteria* followed by the *Acidobacteria*, the *Bacteroidetes,* and the *planctomycetes* [66]. Besides these types of bacteria, another community in a lower number but functional has been detected from the study. These are *Gemmatimonadetes*, *Verrucomicrobia, Actinobacteria, and Chloroflexi*. It is found that the dominant bacteria at more than 76-92% of the microbial community of the earthworm gut are the *gammaproteobacteria* [113]. It is also reported in the literature that, the low ammonium content of treated effluent may be due to the rapid mineralization of nitrogen. An increase in nitrification genes has also been noticed by Lai et al. [115], suggesting that nitrogen removal is due to ammonium conversion. The results of Kannadasan et al. [81] show that the residues from the treatment of distillery water diluted to 50% by vermifiltration are 1.81% richer in nitrogen than the water treated with the NVF. According to the authors, the dead earthworm tissues are a cause of the increase in Total Nitrogen (TN). Nitrification is accelerated with oxygen and therefore denitrification is slowed down, so the denitrifying bacteria are anaerobic. To have a favorable performance for nitrate removal, the conditions must be modified by creating anoxic conditions for the microorganisms to limit the penetration of air in the lower parts of the system and by forcing the microorganisms in these sections to use nitrate instead of oxygen [51,67].

The phosphate contained in wastewater is rapidly mineralized to inorganic phosphate PO_4^{3-}. This would be due to the enzymatic activities [116]. For this purpose, Lourenço and Nunes [82] observed that the removal efficiencies of TP in wastewater were negative. The leaching of vermicasts along the filter to the outlet can also be an explanation for the increase of TP in the effluents treated by vermifiltration, these being rich in nutrients including phosphorus [90]. The mineralization process is more pronounced in VFs than in NVFs. According to Kannadasan et al. [83], Total Calcium and Total Magnesium concentrations almost quadrupled from VF effluent to NVF effluent. Values go from 1.64 and 1.05 respectively to 4.1 and 4.92.

6.6. Pathogens removal

The pathogens would undergo the effect of antibiotic and toxic secretions of the earthworms - the coelomic liquid - and of the microflora of the system [43,85]. This would therefore lead to an inhibition of the activity and growth of these pathogens. The mucus shed by the earthworm sticks to the surface of the bedding material and traps microbes that may be harmful in nature by competing with favorable microbes. The trapped pathogens are then killed being unable to move and also due to the lack of food and oxygen nearby [117]. This would be a possible reason for the elimination of pathogens in the VF. River bed materials and mud balls have been reported to be very suitable filter media for pathogen removal [118]. They also observed that there is a large diversity of microorganisms that have the ability to prevent the growth of other pathogens. The research of Arora et al. [30] also showed antibacterial activity of the microorganisms isolated from the VF *against gram-positive Staphylococcus aureus* (*ATCC 29213*) and *gram-negative E. coli* (*ATCC 25922*), further confirming the pathogen elimination mechanism.

6.7. Heavy metals and antibiotics remediation

The bioavailability of some heavy metals such as Cd, Ni, Pb, Cu, Cr, and Zn decreases significantly during vermifiltration [83]. Heavy metals are accumulated in the organisms of earthworms [63,119]. *Metallothioneins* are proteins in the gut of worms that have the ability to bind heavy metals and make them biologically inactive [64,89]. It is more precisely the *chloragogens* that accumulate these metals. Vermicast also offers good adsorption sites for these metals and chemical pollutants in wastewater [103]. The vermifilter is also capable of reducing the ecotoxicity of certain antibiotics. Even if this alone cannot be considered as a full-fledged treatment of hospital wastewater, because of its high concentration of various antibiotics, it can nevertheless be a tertiary or refining treatment [120].

6.8. Stabilization of sewage sludge

VFs allow for the stabilization of sewage sludge. Research showed that VF has clear advantages over NVFs in the stabilization of sewage sludge [121]. Xu et al. [122] reported an average decrease of 0.05 in the settled sludge volume (SSV)/SSV ratio, along with an enhanced SSV reduction efficiency of 13.86% as compared to reduction efficiencies of 14.5% [123] and 14.7% [124]. The Volatile Suspended Solids (VSS)/Suspended solids (SS) ratio can reach 0.65 ± 0.02, and a VSS reduction of 48.09 ± 2.21% in the VF, which is consistent with the 40% sludge stabilization level required for anaerobic and aerobic digestion. These results suggest a close relationship between stabilization performance and earthworms. Chen et al. [125] also found that the addition of earthworms to a sludge treatment system improved sludge stabilization remarkably with a decrease in the volatile solids VS/TS ratio from 49% to 18% in the accumulated sludge. A vermireactor was also employed for the co-treatment of organic fraction of municipal solid waste (OFMSW) and sewage. The presence of earthworms in the treatment process resulted in the removal of various components, with removal percentages reaching up to 75% (TOC), 86% (Total COD), 87% (BOD5), 59% (ammonia nitrogen), and 99.9% (coliforms) [126]. These findings suggest that earthworms are also well-suited for co-treating OFMSW and municipal sewage on-site.

7. Fate of the VF system

7.1. The vermibed

Clogging is a recurrent problem in deep filtration, especially in wastewater filtration due to high pollution loads. It results from a decrease in the porosity of the filtering materials, due to the accumulation of organic and inorganic solid matter in the pores of the filtering materials [45]. Comparative studies of vermifilters (VFs) with non- vermifilters (NVFs) filters have shown that VFs are slower to clog. The parameters often used to judge clogging in the filter are hydraulic conductivity and Headlosses. Singh et al. [51] showed a decrease in hydraulic conductivity of 10.5 m/day for the NVFs and a decrease of 4.7 m/day for the VF, for the same period of operation. Also, an increase of 0.41 cm of the headloss was observed in the VF against an increase of 0.97 cm in the NVF, representing more than half of the precedent value. According to these results, the presence of earthworms in the filter delays the clogging even if it is not completely eliminated. Adugna et al. [45] confirm these results. The burrowing, tunnelling and ingestion activities of earthworms are responsible for this decrease in clogging. The strong aeration of the filter bed due to these activities leads to the destruction of the solids contained in the pores so that the VF can work smoothly and uninterruptedly for a long time. Furthermore, in a normal biological treatment system, suspended solids accumulate on top of the filter. With time these forms sludge which is responsible for clogging, as it chokes the system. In the VF, these suspended solids are permanently consumed by the earthworms and rejected in the form of vermicompost [127]. The system can operate for 12 months without clogging, despite high concentrations of pollutants Adugna et al. [45].

7.2. Fate of the microorganism's community

The Shannon index (H), which expresses the diversity of a studied population, has been determined in various studies to judge the diversity of the microbial population in the VF. It appears that compared to a NVF this index is always higher. Xu et al. [49] obtained an H = 2.58 for the VF and H = 1.99 for the NVF. They were also able to detect the presence of *Actinobacteria* and *Acidobacteria phyla* only in VF and not in NVF. Other authors like Zhao et al. [123] found respectively HVF = 3.77 and HNVF = 3.49, i.e. 16% higher for the VF.

7.3. Fate of earthworms

Research on the earthworm population and their enzymatic activity in the VF area indicates that: during the first week, which often represents the acclimatization phase to their new environment, signs of discomfort are noticed in the earthworm community.

Weight loss, swelling of the clitellum area, curling up and some deaths can be noticed [128]. But after this acclimatization phase, the earthworms start to show signs of comfort and well-being. They grow in number and stature as they go along. **Table 5** shows the variation in the density of earthworms in the filter during the treatment. Indeed, earthworms develop defense strategies against the hostile environment that wastewater often offers with several types of waste, organic matter, nutrients, heavy metals, etc. Some differential proteins such as *V-*

ATPase, *actin*, and *tubulin* are up-regulated and contribute to cell motility and stress response [42].

Table 5. Fate of earthworms during vermifiltration

Start of the experiment	End of the experiment	Duration of the experiment (days)	References
	Number of earthworms		
200	214	77	[80]
200	206	77	[80]
480	670	133	[78]
800	950	120	[54]
800	1000	91	[62]
	Density of earthworm's biomass (g/L)		
39.05	47.64	77	[80]
32	43	60	[129]
50	86.5		[55]
50	73.5		[55]
50	64.5		[55]
50	65		[55]
	Percentage of increase (%)		
28.3 – 31.5	100	[91]	
19.1 – 26.2	100	[91]	
20.29 – 27.82		[108]	
11.4		[90]	
26-32.4		[41]	

8. By-products of vermifiltration

8.1. Treated effluent

Given the current challenges in accessing and managing conventional sanitation, it is essential to explore low-cost technologies and systems that provide a closed loop between sanitation and agriculture [130]. Several studies have concluded that vermifiltration treated effluents can easily be used without restriction in agriculture [90,131]**.** According to the results of Manyuchi et al. [77], distillery effluent treated by vermifiltration can be reused for crop irrigation and thus reduce the demand on other water sources which are sometimes insufficient. An important indicator in water that can be used to judge the usefulness for agriculture is the DO. The high amounts of DO in treated effluent are very promising for reuse in agriculture because they reduce the septicity of the water [38,40,132]. The amounts of DO in the study of Arora et al. [44] increased from 0-0.35 mg/L in the influent to 3 and 5mg/L in the effluent. Also, the mineralization of phosphate and nitrogen from wastewater with *polysaccharides* and proteins, secreted by earthworms, during vermifiltration produces bioavailable compounds for plants. The research of Arora et al. [44] also showed removal rates of total coliforms (TC), faecal coliforms (FC), and faecal sludge (FS) pathogens of more than 99% making the waters suitable for irrigation according to the 1989 World Health Organism (WHO) standards. There are essential plant nutrients, which make these vermifilter (VF) effluents highly nutritious for plants and potentially usable for crop irrigation. They are very rich in NPK (Nitrogen Phosphorus and Potassium). Having been tested for the irrigation of onions (*Allium cepa*), the effluents have demonstrated their performance for the increased germination of roots without any malformation or chromosomal anomaly [64].

8.2. Vermicompost

Vermicompost or lombricompost, a biofertilizer recognized for its high properties, rich in NPK, is a by-product of vermifiltration [77]. The earthworm gut acts as a bioreactor and can ingest solid and liquid organic waste from wastewater and expel them as vermicompost [78]. During the vermifiltration process the earthworms consume the organic matter contained in the wastewater and release castings [40]. These vermicastings are full of many enzymes and microorganisms good for soil fertility. The vermifilter would recover 20% of the initial nitrogen beneficial to the crops [33]. It provides plants with macro and micronutrients [133]. Medina-Sauza et al. [134] supported the idea that the work of microorganisms with earthworms leads to a privileged selection of certain bacteria in the vermifilter. Bacterial species contained in earthworms are mostly gram-negative and those present in the filter media are gram-positive. This confirms that the earthworms also purify the filter medium making it suitable for use as an organic fertilizer. Some *Pseudomonas sp*. are found to be plant growth promoters and are used as biological control and bioremediation agents and *Klebsiella* is said to fix nitrogen in the soil. Thus, their presence indicates an enriched quality of the compost formed because of the treatment. Also, the growth rate of earthworms in VF is a good indicator of the ecology of the vermifilter.

9. Sustainability of vermifiltration

To be identified as sustainable, technology must be so on the three main dimensions including environmental, social and economic. The technology must be environmentally sustainable, economically viable and socially acceptable. Without addressing these key aspects of sustainability, a treatment system will fail during its operational phase [135,136].

9.1. Environmental sustainability

The various aspects to consider for the environmental sustainability assessment of a technique include:

- **Life Cycle Assessment (LCA)**: LCA involves evaluating the environmental impacts of a product, process, or system throughout its entire life cycle. This assessment considers all stages, from raw material extraction and manufacturing to use, maintenance, and disposal or recycling. Regarding vermifiltration, it has been demonstrated that vermifiltration technology has a minimal dependence on fossil fuels, thereby contributing to the conservation of non-renewable natural resources. Researches of Passteni et al. [137] and Lourenço and Nunes [138] on this matter has shown significant variations in fossil fuel consumption between different wastewater treatment technologies. These variations include quantities of up to 0.04 kg/kg of BOD_5 removed for Activated Sludge Process (ASP), up to 0.03 kg/kg of BOD_5 removed for Aerated Lagoons (ALs), and 0 kg/kg of BOD_5 removed for Vermifiltration (VF). VF also does not require the use of fossil fuels during the construction phase, whereas ASP consumes slightly over 0.6 kg of fossil fuels per equivalent inhabitant during construction. In the operational phase, electricity consumption is 1,160,000 MJ per equivalent inhabitant for ASP compared to only 4,520 MJ for VF. In general, the use of vermifilters in domestic wastewater treatment is associated with relatively low greenhouse gas (GHG) emissions. This low emission can be partly attributed to the activity of earthworms, which promote an aerobic environment, thereby reducing the production of gases such as methane (CH4) and nitrous oxide (N_2O), both of which are considered potentially harmful GHGs. In comparison, other wastewater treatment technologies, such as anaerobic lagoons, macrophyte ponds, upflow Anaerobic Sludge Blanket Reactors (UASBR), and ASP, are often associated with significant GHG emissions, especially when their operation depends on the use of fossil fuels for electricity generation [139]. Furthermore, as mentioned earlier, VF generates very little sludge, with only 0.08 kg of suspended solids (SS) per kg of COD removed, primarily in the form of vermicompost. [66]. In comparison, conventional processes can produce up to 100 grams of sludge per cubic meter of treated domestic wastewater. Vermicompost has highly advantageous characteristics for agriculture [77].
- **Life Cycle Impact Assessment (LCIA)**: LCIA aims to assess the potential environmental impacts of a product, process, or system on the environment throughout its life cycle. Research has shown that VF is the most environmentally friendly method, with a reduced environmental footprint compared to other technologies. For example, VF has a climate change impact (CC) of 6.7 m^3/(kg CO2 eq./kg COD removed) and an eutrophication impact (EUT) of 10 984.1 m^3/(kg P eq./kg COD removed), while ASP has a climate change impact of 3.8 m^3/(kg CO2 eq./kg COD removed) and an eutrophication impact of 10 518.5 m^3/(kg

P eq./kg COD removed). ALs also show higher environmental impacts than VF. Therefore, VF is the best option in terms of environmental sustainability for domestic wastewater treatment [137].

- **Preserving the quality of water, air, soil and ecosystems:** inadequate treatment of domestic wastewater can harm water quality and the aquatic ecosystem due to the presence of pollutants such as pathogens, heavy metals, and emerging contaminants. However, as demonstrated earlier, VF can effectively remove them, thus preserving water quality and the balance of the aquatic ecosystem. Furthermore, the production of wastewater treatment sludge in other methods can pose environmental problems, such as greenhouse gas (GHG) emissions during their treatment. In contrast, VF uses earthworms to digest the sludge, producing nutrient-rich vermicasts that are beneficial for soils and terrestrial ecosystems. Therefore, VF has minimal impact on air quality. In comparison, other wastewater treatment methods, such as activated sludge, have shown higher environmental impacts, including significant GHG emissions. In summary, VF proves to be an environmentally friendly option for domestic wastewater treatment, with measurable benefits in terms of water quality, GHG emissions, and soil and ecosystem benefits [33,93].
- **Reuse of treated effluent:** the reuse of treated effluent depends on its level of contamination, with indicators such as Dissolved Oxygen (DO), organic matter (BOD and COD), pathogens, NH4+-N, and NO3-N. The effluent from domestic wastewater VF is clear, odorless, rich in DO, and has a neutral pH [140]. Therefore, it can be effectively used for various non-potable purposes such as soil irrigation, toilet flushing, cooling tower makeup in industries, and agricultural applications

9.2. Economic affordability

The economic feasibility assessment of technology considers investment costs, operational and maintenance costs, treatment efficiency, and waste management

- **Investment costs (ICs):** ICs encompass expenses related to land acquisition, raw materials, energy consumed during construction, transportation of raw materials, and the installation of various equipment. According to a study, the land area required for Small Infiltration at Reduced Flow (SRI), Constructed Wetlands (CWs), and Activated Sludge Process (ASP) in the treatment of domestic wastewater for small communities of 120, 120, and 500 inhabitants, respectively, was significantly reduced when these processes were replaced by VmF technology. VF requires a much smaller land area. Regarding raw materials, materials including sawdust, wood shavings, vermicompost, and others used in VF are generally locally available at a low cost, or even obtained as waste from other activities. They are, therefore, very cost-effective. Furthermore, acquiring earthworms at a very low cost is considered a one-time investment, as earthworms can be reused in new vermifilters. Compared to conventional methods, VF technology is decentralized and requires less space, making it suitable for use near the source of domestic wastewater. This reduces costs associated with transporting wastewater to treatment sites [138]. Furthermore, VF technology does not require heavy equipment, which contributes to its cost-effectiveness. Studies have shown that the capital costs of VF technology are significantly lower than those of other on-site domestic wastewater treatment systems. For example, the capital costs of systems such as septic tanks with percolation areas, membrane bioreactors (MBR), moving bed biofilm reactors (MBBR), sequential batch reactors (SBR), and constructed wetlands (CWs) were significantly higher than those of VF technology [141,142].

- **Operating and Maintenance Costs (OMCs):** OMCs of wastewater treatment methods encompass energy consumption, bed material renewal (especially for VF), equipment replacement or repair, skilled manpower, sludge management, and chemical requirements. Longevity also impacts costs. As demonstrated above, VF technology consumes significantly less energy during the operational phase, mainly due to earthworm-driven natural aeration. VF's simplicity eliminates the need for skilled manpower, further reducing operational costs [140]. Maintenance expenses are also lower compared to conventional WWTPs. VF technology boasts longevity, lasting up to 3-8 months for low-strength domestic sewage chemical requirements are lower, reducing costs compared to other methods [137]. **Table 6** provides a comprehensive overview of both capital costs and operational costs for each wastewater treatment method.
- **Treatment efficiency and by-products:** VF has demonstrated high effectiveness in removing various pollutants from domestic wastewater, including organics, nutrients, and pathogens [55,62]. Studies have indicated that VF-treated effluent meets stringent surface water discharge standards, particularly concerning pathogens [62]. As discussed above, VF also generates beneficial by-products. The vermicompost can contribute to a reduction in food production costs and enhance food safety by reducing the risk of chemical contamination [143]. Vermicompost offers additional advantages such as improved soil moisture retention, higher agricultural yields, and efficient resource utilization [143].

In summary, VF seems under certain conditions emerge as an economically viable and sustainable option for domestic wastewater treatment, delivering substantial environmental benefits while positively impacting agriculture and food security. However, it requires deep investment to make sure it is the case.

Table 6. Overview of both capital costs and operational costs for each wastewater

Wastewater Treatment Method	Capital Costs (€ per User)	Operational Cost (€/year or €/m^3/year)	References
Vermifilter (VF)	100 to 150	0.05 €/m^3/year	[141,142]
Conventional Sewage Treatment Plant	Up to 3,750,530	Varies (e.g., up to 3,750,530 $ in rural settings)	[142]
Septic Tank with Percolation Area	Approximately 1,132	14 €/user/year	[144]
Membrane Bioreactor (MBR)	1,800 to 2,000	50-70 €/user/year	[144]
Moving Bed Biofilm Reactor (MBBR)	Approximately 1,500	20-30 €/user/year	[144]
Sequential Batch Reactor (SBR)	620 to 900	4-7 €/user/year	[144]

9.3. Social acceptability

The acceptance of a specific wastewater treatment technology within society hinges on several key factors: safeguarding public health, engaging the public and fostering community development, and considering aesthetic aspects.

- **Safeguarding of public health:** given that Vermifiltration (VF) technology has the potential to substantially remove various pollutants, including organic matter, nutrients, and pathogens, waterborne disease outbreaks would decrease, thereby reducing the risk of human toxicity [62]. Furthermore, Kumar et al. [116] postulated that the concentration of NO_3-N in the effluent obtained from domestic wastewater VF was < 45 mg/L. Therefore, discharging the effluent from VF into surface water bodies would not cause blue baby syndrome [39]. Moreover, as previously mentioned, as an organic fertilizer, the application of vermicompost enables the production of healthy, chemical-free food, thus reducing the risk of harmful impacts on humans from food consumption. Since VF technology can reduce contaminant levels below permissible limits, it can be stated that the application of VF technology promotes public health protection.
- **Economic development of the local community** given that vermifiltration is a decentralized technology, it can be effectively used to treat domestic wastewater generated by individual households or small communities. Due to the ease of VF construction and operation, residents can decide how to construct and use the system for their economic growth. In other words, VF ensures public involvement, which is not possible with

centralized wastewater treatment methods. Vermicompost can be sold in the market as biofertilizer. Devkota et al. [145] reported that vermicompost was sold in the market at a price of 25 rupees/kg, with a net profit of 9.32 rupees/kg. Additionally, earthworms can also be sold to various farms as raw material. Thus, the VF process also helps boost the economy, attracting rural residents to the VF process. Since it is a decentralized method, all community members can also benefit from the VF facility. Moreover, the treated effluent can be used for various non-potable purposes by the beneficiaries. It also promotes social resilience and stability through the wise use of resources. Therefore, through the implementation of VF for domestic wastewater treatment, all community members will be able to prosper through appropriate natural resource-based development.

- **Aesthetic aspects:** VF is socially accepted due to its positive impact on environmental aesthetics and the absence of unpleasant odors. It is particularly suitable for small communities as it preserves aesthetics and avoids sludge production. Its effluent is clear and does not alter the color of the water, maintaining aesthetic value [64].

The vermifiltration sustainability features are summarized in **Table 7**. It can be concluded that vermifiltration is a sustainable alternative for the treatment of domestic and industrial wastewater. Almost all the criteria regarding the different dimensions are satisfied.

Table 7. Vermifiltrations' sustainability features.

Dimensions	Features	References
Environmental	No consumption of any fossil fuel during all the stage of the vermifiltration process.	[137]
	Protection the air quality, especially while treating the domestic sewage.	[61]
	Nutrient recovery - organic fertilizer.	[77]
	Reusability of treated effluent.	[38,40,132]
	Preservation of aquatic ecosystem.	[62]
	No chemical toxicity on the soil-based organisms.	[140]
	Air pollution - can potentially cut down the risk of GHG emissions and thereby minimizing the GWP to a great extent.	[137,138]
	No odor emission.	[64]
	No sludge production.	[64,65]
Economical	Does not necessitate the installation of the heavy-duty instruments, cost-effective alternative.	[138]
	No requirements of external aerators-energy efficiency.	[40]
	Efficient removal of pollutants, including organics, nutrients, and pathogens from various types of water.	[54,90].
	Value-added by-products linked to circular bio-economy.	[146]
Social	Public Health – drastic reduction of outbreak of the water-borne diseases, human toxicity risk reduction.	[55,62]
	Public involvement and community. Development - growth of local socio-economy.	[143]
	Cultural acceptance – no odor, no pungent small, no sludge, clear effluent.	[40,64]

10. Conclusion

From the research results of the last ten years on vermifiltration (VF), it can be concluded that the vermifilter can be considered as a good alternative for wastewater treatment and reuse. It appears from the literature that VF has a very high potential as a suitable treatment technology for wastewater from various sources, especially for countries facing severe challenges including insufficient investment costs and skilled labor. Furthermore, it has been demonstrated to be a sustainable technology. The analysis of the removal performance of vermifiltration showed its ability to remove various pollutants from the water, with by-products such as treated wastewater and vermicompost. Additionally, it demonstrates the potential for optimizing the operational parameters of the vermifilter to achieve peak performance. However, VF still has some limitations in a non-negligible number of aspects, due to the increasingly strict water quality standards for wastewater treatment and treatment issues.

CHAPTER 2: EXPERIMENTAL DESIGN AND PERFORMANCES

1. Introduction

This chapter aimed to explore in detail the performance of a vermifilter and analyze the influence of various critical factors on its efficiency. Specifically, we will focus on the impact of the HLR, EWD, and the initial COD on COD removal.

Firstly, this chapter presents the materials and methods used to conduct this study. Secondly the study describes the context, including the collection of earthworms and greywater required for the experiments, as well as the meticulous preparation of synthetic greywater to ensure the reproducibility of results. Thirdly, the experimental protocol is detailed, covering the setup of the vermifilter, sampling methods, and the techniques used to measure and record parameters. Next, the obtained results are discussed regarding the characterization of greywater, both real and synthetic. Then the analysis of how HLR, EWD, and initial COD influence the performance of the vermifilter is done by separately examining the physical and chemical parameters. These analyses helped determine to what extent these factors contribute to the improvement or deterioration of filtration and biodegradation performance.

Thus, this chapter provides an empirical overview of the vermifilters' efficiency. The data and analyses presented further guided the optimization of this technology and encourage its adoption in various greywater management applications.

2. Materials and Method

2.1. Description of the Study Area

To test the design and the performances of the vermifiltration process, this study made use of laboratory-scale reactors The reactors were installed indoor at the Water, Hydro-Systems and Agriculture Laboratory (LEHSA), Institute 2iE, Ouagadougou in Burkina Faso, with 12.38°N and 1.50°W (see **Figure 4)**. The region is characterized by a Sahelo-Sudanese climate, with a lengthy dry season from November to May, alternating with a short rainy season from May/June to October. The study was conducted from August to October, with recorded ambient temperatures ranging between 26°C and 31°C.

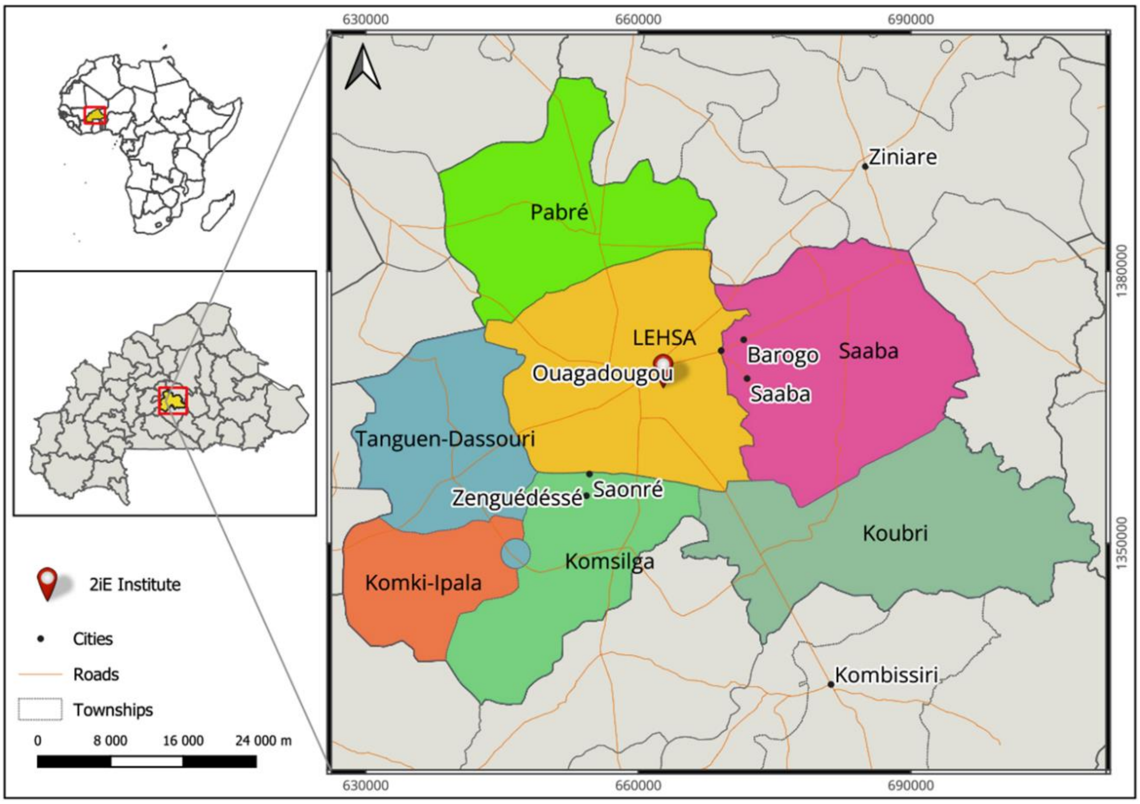


Figure 4. Localization of the study site

2.2. Reactor description

These are PVC columns with a diameter of 20 cm and a height of 80 cm, (with usable height of 60 cm) in which various layers of filtering materials are arranged (**Figure 5**). The reactors were fed with raw greywater by a batch system. They were supplied 4 times a day at regular amounts and intervals of 4 hours, between 08:00 and 20:00. To study the influence of HLR, three HLR were considered to corresponded to different hydraulic loads of 64, 127.5, and 191 L/m^2/day , i.e., 2x10^{-3}, 4x10^{-3}, and 6x10^{-3} m^3/day respectively [16].

Figure 5. Onsite reactor illustration

Based on previous studies, and elements including suitability, quality and availability of local materials [16,19], the following design was set for the reactors. At the bottom, the filter media was made up of two layers of gravel of 5 cm thick each: coarse and fine (3-5 cm and 1-2 cm particle size respectively). Then, a 20 cm thick layer of washed was completed with a 30 cm thick layer of sawdust, as shown in **Figure 6**. **Table 8** displays the characteristics of the filter materials used.

Table 8. Filter materials characteristics

Material characteristics	Sawdust	Sand
Absolute density (kg/m^3)	1666.67	2500.00
Apparent density (kg/m^3)	143.51	1422.59
Porosity	0.91	0.43
Total mass used (kg)	1.35	8.93
Effective size (mm)	0.23	0.22
Specific surface (m^2/kg)	6.00	10.29
Uniformity coefficient	1.96	2.18

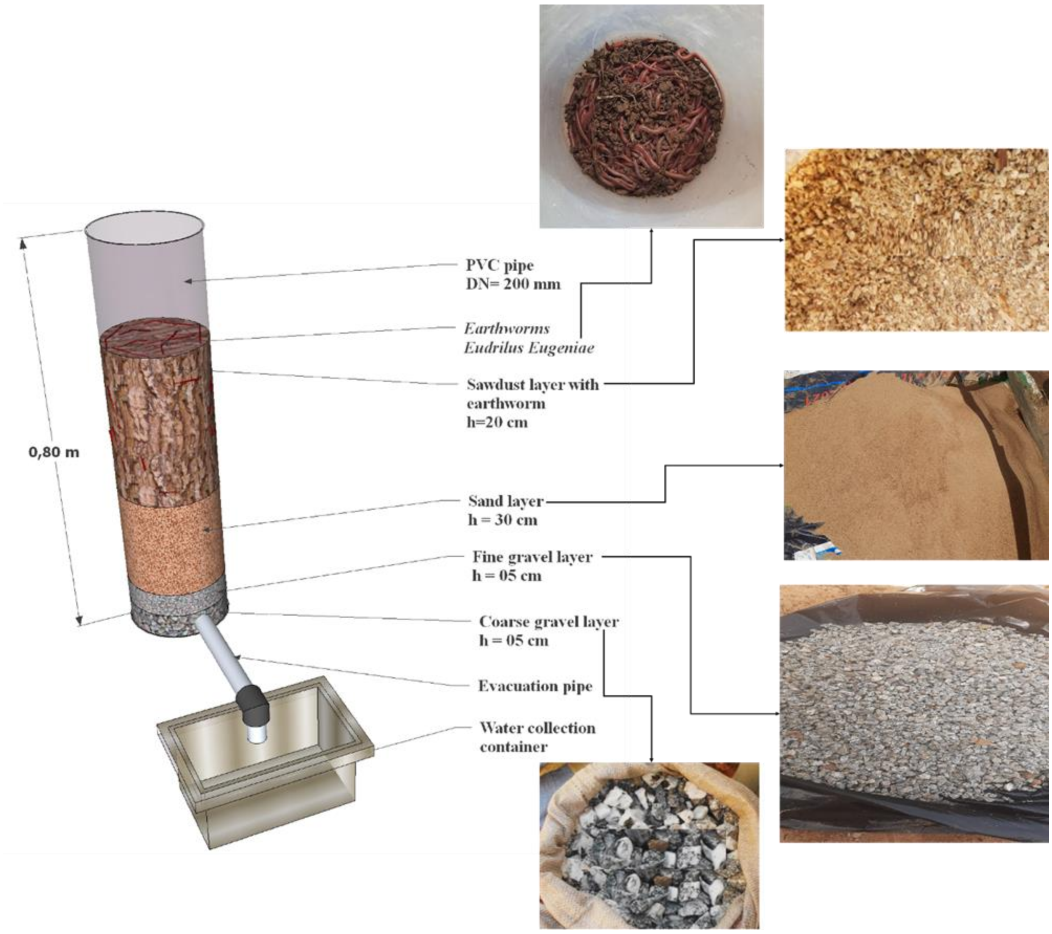


Figure 6. Detailed composition of a vermifilter

2.3. Collection of Earthworms

The species chosen for our study is *Eudrilus eugeniae*. Individuals of various sizes and ages of *Eudrilus eugeniae* were collected from the wetland area near the Tanghin dam in Ouagadougou and were cultivated in a humid environment with good protection against predators (ants, toads, lizards). Indeed *Eudrilus Eugeniae* commonly refers to as the "*west African night crawler*", is largely found in the region from Nigeria to Ghana and has been identified as a suitable specie for vermifiltration purposes [19,147]. The worms were counted, washed and weighed before being introduced into the active layer of the filters (**Figure 7**). The different densities of application were 0, 5000, and 10,000 worms/m^3 [4,148–150]. The densities correspond respectively to 0,100 and 200 Earthworms. Each experiment was conducted for 90 days, including a 28-days acclimatization period allowing the earthworms to adapt to their new environment. Treated greywater samples were collected at the outlet of the filters and analyzed once a week.

Figure 7. Introduction of the Eudrilus Eugeniae just in the filter

2.4. Collection of real greywaters

The greywater was collected in a 100-liter plastic bucket, placed for 24 hours at households near the 2iE campus. It was then transported to the experimental site in 20-liter jerry cans, mixed, and subsequently used for feeding the vermifilters and for analyses (see in **Figure 8**). Specifically, environmental samples consisting of a mixture of laundry and dish greywater were collected from three nearby households.

Figure 8. Collection of real greywaters in households of the neighborhood

2.5. Preparation of synthetic greywater

Synthetic greywater was prepared by adding (per liter of solution) molasses ($C_{12}H_{22}O_{11}$), wheat flour, urea (CH_4N_2O), potassium dihydrogen phosphate (KH_2PO_4), powdered soap, liquid soap, and 1% of environmental greywater [151]. **Table 9** provides the different concentrations of used elements for the 3 types of synthetic greywater A, B, and C. The mixture was then mixed with tap water, resulting in COD concentrations of 1000 (A), 2500 (B), and 4000 mg/L (C) i.e., 1 kg/m^3, 2.5 kg/m^3 and 4 kg/m^3 respectively.

Table 9. Concentrations of Synthetic greywater constituents

Elements	**A**	**B**	**C**
Molasses (g)	0,7	1,75	2,8
Urea (g)	0,1	0,25	0,4
wheat flour (g)	0,4	1	1,3
KH_2PO_4 (g)	0,01	0,025	0,04
Powdered soap (g)	0,07	0,07	0,07
Liquid soap (g)	0,03	0,03	0,03
Environmental greywater (L)	0,01	0,01	0,01

According to [152,153] and the conducted tests in the area of study, these values are within the range of the domestic greywater in the studied zone. The concentrations of the influent greywater were varied in different experimental runs, as indicated in **Table 10** to **Table 12**. The synthetic greywater was prepared two hours before application in the vermifilters. After preparation, the greywater was dropped into the active filtration layer to allow gravity flow through the filter until the outlet.

Table 10. HLR influence at different initial COD concentrations and earthworm densities.

Experimental runs	COD	Worms	HLR
VF1	1000	100	64
VF2	1000	100	191
VF3	4000	100	64
VF4	4000	100	191
VF7	2500	200	64
VF8	2500	200	191

Table 11. Worms' influence at different initial COD concentrations and HLRs

Experimental runs	COD	Worms	HLR
VF11	1000	200	127.5
NVF9	1000	0	127.5
NVF10	4000	0	127.5
VF12	4000	100	127.5
VF7	2500	200	64
NVF5	2500	0	64

Table 12. Initial COD influence different earthworm densities and HLRs

Experimental runs	COD	Worms	HLR
VF2	1000	100	191
VF4	4000	100	191
VF11	1000	200	127.5
VF12	4000	200	127.5

2.6. Treated effluent sampling

Samplings were conducted once a week at the outlet of each filter before the first supply of 8 a.m. Greywater was also collected on the same day to evaluate the treatment capacity of each filter. The samples were collected in borosilicate glass bottles for microbiological analyses and polyethylene bottles for physicochemical analyses, respectively. The collected samples are then stored at 4°C until laboratory analyses are performed.

2.7. Analysis Methods for greywater

The raw greywater from households and the treated water collected at the filter outlets were analyzed to study various physical and chemical parameters.

2.7.1. Physical parameters

- **Hydrogen Potential (pH)**

The potentiometric method was used to determine the pH using a portable pH meter. The pH was measured using a WTW pH meter, (WTW 3310), in accordance with AFNOR T 90-08 and AFNOR 90-008 standards

- **Conductivity**

Conductivity was determined by using a portable WTW 3310 conductometer, according to the AFNOR 90-031 standard. The technique involved immersing the electrode in the water sample to be analyzed, followed by reading the value in μS/cm.

- **Temperature**

The ambient temperature of the room was measured using a thermometer which was securely mounted on a metallic surface within the room using a magnet affixed to the back of the device. This setup ensured stable positioning and reliable readings of the room's temperature over time.

- **Dissolved oxygen**

The potentiometric method was used to determine the dissolved oxygen (DO) using a portable DO meter. The DO was measured using a WTW Oxi 3310 meter, in accordance with AFNOR T 90-106 and AFNOR T 90-103 standards.

2.7.2. Chemical parameters

- **BOD5 (Biochemical Oxygen Demand):**

BOD was determined using a BOD meter (WTP Oxitop). The principle is based on the respirometry method. An appropriate amount of each sample is placed in the Oxitop, ensuring airtight sealing, and incubated in a BOD incubator (B83650) at 20°C for 5 days. The difference between the initial and final dissolved oxygen values represents the BOD of the sample. The integrated memory of the measurement values automatically records a BOD value every 24 hours. It is expressed in milligrams of oxygen per liter of water (mg/L).

- **COD (Chemical Oxygen Demand)**

It involved the use of specific kits or chemical reagents to assess the amount of oxygen required to oxidize the organic substances present in a water sample. First, a volume of 0.2 mL of the sample is introduced into an LCK 1014 cuvette containing a reagent. The cuvette is then placed in a heating block set at 150°C for 2 hours. After heating, the cuvette is cooled and placed in a DR 3900 molecular absorption spectrometer for the determination of COD.

- **TSS (Total Suspended Solids)**

To determine the TSS (Total Suspended Solids), the procedure followed was based on the AFNOR NFT 90-105 standard. It involves passing a volume of the sample through a pre-weighed Whatman GF/C filter with a diameter of 47 mm to obtain its initial weight. The filter is then removed from the filtration apparatus and placed in an oven at 105°C until a constant mass is achieved. After this, the filter is cooled in a desiccator for 30 minutes before being weighed again. The concentration of suspended solids in the sample, expressed in mg/L, is calculated from these measurements as shown in **Eq. (1)**:

$$TSS = \frac{(MF - MI) * 1000}{\mathrm{VS}} \quad \textbf{(1)}$$

TSS: Total suspended solids in mg/L,

MF: Final mass of the membrane + suspended solids in g,

MI: Initial mass of the membrane in g,

VS: Volume of sample used in L.

- **Nitrogen Compounds (NO_3^-, NH_4^+) and Phosphorus (Ptotal):**

The concentration of these elements is determined using the direct-reading HACH DR/3900 spectrophotometer under the conditions listed in Table below.

2.8. Data processing and analysis

The data obtained are analyzed using Excel 2019. The various treatment efficiencies for the parameters were determined using the following formula (**Eq. (2)**):

- Calculation of Treatment Efficiency (chemical parameters):

$$R(\%) = \frac{[GW]-[Filter]}{[GW]} \times 100 \quad \textbf{(2)}$$

With R (%), the greywater treatment efficiency, [GW], the concentration in the greywater, and [Filter], the concentration in the water leaving the filter.

Statistical software R 4.3.1 and SPSS were also used for plotting the graphs and determining significancy of the results and he treatment performances of the filters were compared using a Mann-Whitney test with a significance level of p = 0.05.

3. Results and discussion

3.1. Greywater characteristics

This characterization was crucial for establishing a baseline for comparison before and after treatment and for accurately assessing the vermifilters' efficiency.

3.1.1. Real greywater

The following **Table 13** shows the characteristics of the real greywater used for the experiments and analyses throughout the processes.

Table 13. Greywater used characteristics

Parameters	Real domestic greywater with standard deviation
T°(C)	25.00
pH	6.81 ± 0.09
DO (mg/L)	2.04 ± 0.05
EC (mS/cm)	1.19 ± 0.26
TSS (mg/L)	2123.33 ± 50.33
COD (mg/L)	3506 ± 64.37
BOD_5 (mg/L)	2286 ± 29.46
NO^-_3 (mg/L)	36.67 ± 3.51
NH^+_4 (mg/L)	30.90 ± 2.14

3.1.2. Synthetic greywater

Various parameters, temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), chemical oxygen demand (COD), biological oxygen demand (BOD_5), nitrate (NO_3^-), ammonium (NH_4^+), and total phosphorus (TP) were measured in the three types of greywater A, B and C. The measurements were performed all the weeks with those of treated water. The results are recorded in **Table 14**. The values are presented with both the mean and standard deviation for each parameter across the samples.

Table 14. Parameters' measurements in synthetic greywater type A to C with standard deviation

Parameters	A	Std. deviation	B	Std. deviation	C	Std. deviation
T°(C)	25,25	0,51	25,33	0,52	25,15	0,51
pH	5,29	0,89	5,16	0,83	4,84	0,69
DO (mg/L)	2,39	0,36	1,48	0,46	1,37	0,23
EC (µS/cm)	327,29	85,13	525,29	84,64	840,67	77,96
COD (mg/L)	1028,88	83,16	2495,44	164,71	3923,13	179,34
BOD5 (mg/L)	613,50	189,83	1256,75	116,90	1939,50	291,39
NO^-_3 (mg/L)	11,50	0,99	26,75	2,90	28,70	0
NH+4 (mg/L)	16,65	3,05	48,38	0,88	85,75	3,18
TP (mg/L)	14,80	1,94	15,55	1,91	18,27	1,91

The measurements were made to assess the quality of these synthetic greywaters and verify that the parameter concentrations align with the expected values. It can be seen from the results that, temperature is quite similar across the samples, ranging from 25.15°C to 25.33°C, which suggests that the system operates under relatively stable thermal conditions. The pH shows some variation, with sample C having the lowest average (4.84), indicating a more acidic condition compared to samples A and B (5.29 and 5.16, respectively) the range of values are slightly lower than for real greywater and previous study by Amare et al. [152]. This could be due to the reactions occurred during the preparation of the synthetic greywater. However, the Dissolved oxygen levels are lower in sample B (1.48 mg/L) and sample C (1.37 mg/L) compared to sample A (2.39 mg/L). Low DO levels may indicate that oxygen consumption by microorganisms is higher, which could influence the biodegradation process. It has been proven that High DO levels induced shift in bacterial population in a filtering media[154]. Electrical conductivity (EC) shows significant differences between the samples, with sample C having the highest EC (840.67 mS/cm), which might indicate higher salinity or dissolved solids in the system.

COD values were much higher in samples B and C, with sample C being the highest (3923.13 mg/L) as expected, suggesting more organic matter that can be degraded. BOD_5 followed the same trend, with sample C having the highest value (1939.50 mg/L) also as expected. This indicates a higher biological oxygen demand, showing a potentially more biodegradable organic load. Nitrate and ammonium concentrations are also significant, with sample C having the highest levels of NH_4^+ (85.75 mg/L), indicating possible contamination or higher nutrient loading in this sample. The nitrogen forms present can affect the biodegradation rate, particularly in nitrogen cycle-related processes. Total phosphorus concentrations were higher in sample C (18.27 mg/L), which might suggest a greater level of eutrophication potential.

Based on the biodegradability index (BI), the BOD/COD ratio, all the samples were found to be easily biodegradable, with BI ranges from 0.494 for "C" to 0.596 for "A", which are higher than 0.3.

In conclusion, the results met the expectations and felt within the range of values for greywater in the area of study[153]. They were also consistent with those obtained in our previous studies [19,45,152].

3.2. Evaluation of Vermifilter Performance and influence of HLR

The impact of HLR was analyzed by varying both the HLR and the initial COD concentration of the filters. Specifically, the influence of HLR was evaluated at initial COD levels of 1000, 2500, and 4000 mg/L as shown in **Table 10 (section 2.5)**. This approach aimed to identify whether the effect of HLR is also influenced by another factor, namely the initial COD. As mentioned earlier, the P-values were determined to assess the significance of the differences throughout the tests. The calculated p-values are recorded in **Appendix A1.**

3.2.1. Physical parameters

- **pH**

Figure 9 compares the pH levels of the raw water or influent (Inf.) and the treated water or effluent (Eff.) for vermifilters (VFs) 1, 2, 3, 4, 7 and 8.

As shown in **Figure 9 (a)**, for VF2 (HLR of 191 L/m²·h), the pH levels of treated water fluctuated slightly around 7 to 8.0, with values generally higher than those of the influent's pH. In the case of VF1 (HLR 64 L/m²·h), there's a noticeable variation in pH, ranging from 6 to 8.25. However, the difference between the values of VF1 and VF2 was found to be non-significant, with a p-value of 0.406 higher than 0.05. The HLR did not appear to have an impact on pH behavior at an initial COD concentration of 1000 mg/L. Overall, the pH of the effluent tended to stabilize around a neutral value, transitioning from its initial acidic state. This confirmed the assumption that the vermifilter contributes to pH regulation in greywater[19,155,156].

In **Figure 9 (b)**, VF3 (HLR of 64 $L/m^2 \cdot h$), the treated water pH followed a trend like that of VF2, with values (around 6.5 to 8.0) slightly fluctuating and maintained higher than values of the influent's pHs until week 8, where a peak was observed. For VF4 (HLR of 191 $L/m^2 \cdot h$), the pH fluctuated more, with values reaching as high as 9.25, indicating an impact of a higher HLR on pH variation. With a p-value of 0.018, that impact was found to be significant at 4000mg/L.

Finally, from **Figure 9 (c)**, it can be said that for the effluent in VF7 (HLR of 64 $L/m^2 \cdot h$), the pH was relatively stable, ranging from around 6 to 7.3. In VF8 (HLR 191 $L/m^2 \cdot h$) pH of the effluent shows more significant variability, with pH values ranging from 6 to little more than 8.75. The differences in values of VF7 and VF8 were significant at an initial COD of 2500mg/L. with a p-value of 0.041. Based on the observations from Figure 9(b), it can be concluded that HLR negatively influences the pH behavior in the vermifilter at high organic strength of the influent. This finding aligned with the literature, which suggests that high organic strength combined with high HLR is not ideal for the overall performance of the vermifilter [140], even though the specific impact on pH is rarely discussed.

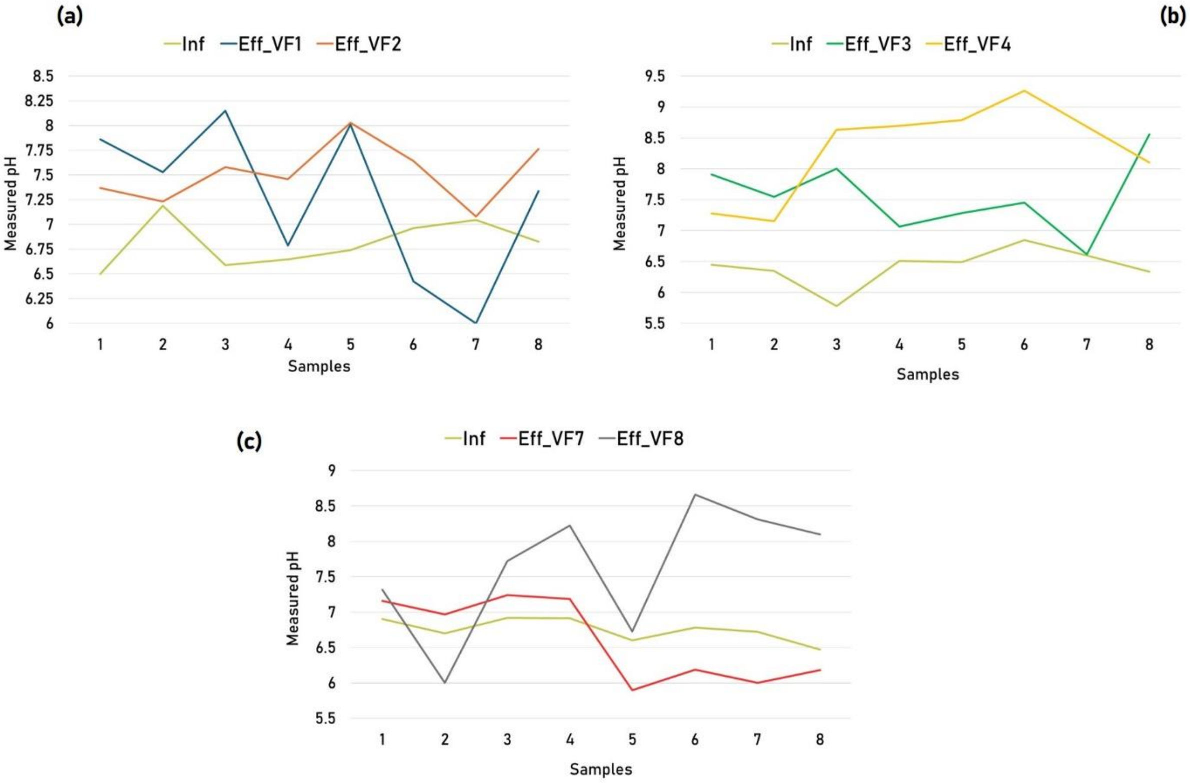


Figure 9. pH levels of influent and effluent (influence of HLR). (a) VF1 vs. VF2. (b) VF3 vs. VF4. (c) VF7 vs. VF8

- **Electrical conductivity (EC)**

Table 15 gives the results of the influence of HLR on the electric conductivity in the studied filters.

VF1 (HLR 64 L/m²·d) vs. VF2 (HLR of 191 L/m²·d)

For VF1, the EC of the influent ranged from 220 to 422 µS/cm, while for the effluent, it ranged from 574 to 748 µS/cm, meaning that the difference in EC is positive in most cases (except in week 8), indicating an increase in EC after treatment, with the last point showing a slight decrease. In VF2, EC for the effluent ranged from 411 to 613 µS/cm, showing also a positive which this time was consistent throughout the period of experiment. Here, the key observation is that although both VF1 and VF2 generally show positive Δ_{EC}. The increase in EC at the outlet of the vermifilter has been attributed in the literature, to the release of the minerals from the earthworms gut [156,157]. The values for VF1 and VF2 didn't show any significant difference (P-value = 0.142).

VF3 (HLR 64 L/m²·d) vs. VF4 (HLR of 191 L/m²·d)

The EC of the influent for both VF3 and VF4, ranged from 733 to 976 µS/cm. For the effluent of VF3, values rangd from 641 to 1812 µS/cm, showing a consistently positive difference which indicate a significant increase in EC after treatment; meanwhile, effluent of VF4 show an EC of between 566 and 1818 µS/cm meaning a similar pattern to VF3 but with higher EC values overall. The key observation here was that VF4, with a higher HLR, showed a more pronounced increase in EC (positive Δ_{EC}) compared to VF3, suggesting that a higher HLR combined with the high COD seemed to exacerbate the increase in EC, possibly due to more dissolved substances being retained. But once again the P value is higher than 0.05 (0.371).

VF7 (HLR 64 L/m²·d) vs. VF8 (HLR of 191 L/m²·d)

In VF7 and VF8 both the EC for the influent ranges from 407 to 630 µS/cm, while that of the effluent ranges from 462 to 1260 µS/cm for VF7 and from 632 to 1510 µS/cm. The difference was consistently positive, showing an increase in EC after treatment in VF7. VF8 consistently exhibits positive values, with greater increases in EC compared to VF7. The P-value is 0.848.

Table 15. Electric conductivity of influent and effluent in µS/cm (influence of HLR)

Experimental runs	Type of water	Samples							
		1	2	3	4	5	6	7	8
	Inf.	220	248	259	252	431	281	398	422
VF1	Eff.	574	639	747	748	671	633	532	411
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↓
VF2	Eff.	613	441	464	522	535	450	411	600
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
	Inf.	733	499	779	762	807	870	976	850
VF3	Eff.	1528	641	1257	1285	1238	1145	1039	1812
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
VF4	Eff.	1572	566	1302	1374	1588	1748	1818	950
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
	Inf.	515	407	586	552	630	566	416	520
VF7	Eff.	1014	600	1134	1052	1260	1066	462	986
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
VF8	Eff.	1102	632	880	670	825	1510	1127	874
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑

The summary of observations here suggests that a higher HLR (191 L/m^2.d) generally resulted in a more increase in electrical conductivity after treatment, as seen by consistently larger positive Δ_{EC} values. But statistically the difference was not significant. We could thus assume that the HLR doesn't have an impact on the EC fate in the vermifiltration system.

Dissolved Oxygen (DO)

The analysis focuses on the variations in DO (Δ_{Do}) effluent compared to Influent for different filters with varying HLR and other filter characteristics (see **Table 16**).

VF1 (1000 mg/L COD, 100 worms, 64 L/m²·d) vs. VF2 (1000 mg/L COD, 100 worms, 191 L/m²·d)

For both VF1 and VF2, DO ranged from 1.18 to 2.95 mg/L for the influents, whereas for the effluents, in VF1 DO ranged from 3.06 to 7.94 mg/L while in VF2 DO ranged from 2.85 to 7.01 mg/L. VF1 showed a higher increase in DO, reaching 7.94 mg/L at sample 6, while VF2 showed a less pronounced increase, with a maximum of 7.01 mg/L (also sample 6). Key observation here included the fact that increasing the HLR from 64 to 191 L/m²·d (from VF1 to VF2) seems to slightly reduce the filter's ability to increase DO in treated water, though both filters show a greater increase in DO compared to raw water. There was not found a significance difference between the Δ_{Dos} from VF1 and VF2 (P-value = 0.2). The increase in DO at the outlet of the filters could be attributed to the fact that, with the aeration of the filter, the DO is replaced by the oxygen from the atmosphere[158].

VF3 (4000 mg/L COD, 100 worms, 64 L/m²·d) vs. VF4 (4000 mg/L COD, 100 worms, 191 L/m²·d)

For both VFs DO ranged from 1.42 to 1.69 mg/L for the influents. In VF3, DO of the effluent ranged from 2.62 to 5.34 mg/L, while in VF4 DO ranged from 0.35 to 2.52 mg/L. On the differences, VF3 showed significant increases in DO across all samples, with a maximum value of 5.34 mg/L at sample 6, whereas VF4 showed limited increases, and even decreases for samples 5 and 7, with a minimum of 0.35 mg/L. Here, the observation made was that for high COD levels (4000 mg/L), a lower HLR (VF3) is more effective at increasing DO in treated greywater compared to a higher HLR (VF4). Like pH, it can be concluded that a higher HLR may reduce the efficiency of DO treatment. This could be attributed to clogging observed in VF4, caused by the combination of high HLR and high initial COD. Clogging prevents atmospheric oxygen from entering the filter, leading microorganisms to consume the DO within the media, thereby reducing DO levels at the filter outlet. This assumption was also suggested by Rustum et al. [156].

VF7 (2500 mg/L COD, 200 worms, 64 L/m²·d) vs. VF8 (2500 mg/L COD, 200 worms, 191 L/m²·d)

For both VFs, DO ranged from 1.40 to 1.93 mg/L for the influent. In VF7 DO ranges from 2.45 to 6.12 mg/L for the effluent, while for VF8 the effluent's DO ranged from 1.44 to 4.80 mg/L. For differences between DOs, VF7 shows a great increase in DO across all samples, with a maximum value of 6.12 mg/L (sample 6), while VF8 shows a weaker increase in DO, with even a decrease for samples 6 and 7. Here it can be said that as with VF3 and VF4, a lower HLR (VF7) is more effective at increasing DO in treated water, while a higher HLR (VF8). The same conclusions as in the previous comparison can be drawn.

Table 16. Dissolved oxygen of influent and effluent in mg/L (influence of HLR)

Experimental runs	Type of water	Samples							
		1	2	3	4	5	6	7	8
	Inf.	1.18	2.13	2.4	2.11	2.16	2.95	2.18	2.06
VF1	Eff.	3.06	4.63	5.03	4.74	3.26	7.94	3.55	5.37
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
VF2	Eff.	2.85	4.59	4.90	3.15	3.23	7.01	3.81	4.36
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
	Inf.	1.42	1.48	1.60	1.59	1.30	1.69	1.64	1.45
VF3	Eff.	2.62	4.08	4.62	4.10	2.43	5.34	3.30	3.75
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
VF4	Eff.	1.82	2.52	1.61	2.38	0.35	1.83	0.93	2.37
	Δ_{DO}	↑	↑	↑	↑	↓	↑	↓	↑
	Inf.	1.40	1.80	1.95	1.89	1.87	1.84	1.93	1.70
VF7	Eff.	2.45	3.70	4.44	4.01	2.91	6.12	3.03	5.16
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
VF8	Eff.	2.24	3.16	4.20	4.80	2.57	1.52	1.44	2.24
	Δ_{DO}	↑	↑	↑	↑	↑	↓	↓	↑

In summary, increasing the HLR seems to negatively impact the filtration efficiency for increasing DO in treated water, when COD levels are high.

3.2.2. Chemical parameters

- **COD**

Figure 10 and Figure 11 give us the results of the influence of HLR on chemical parameters, especially here the COD removal. From **Figure 10**, we can observe the distribution of COD values, while **Figure 11** illustrates their evolution throughout the treatment.

VF1 (1000 mg/L COD, 100 worms, 64 L/m^2·d) vs. VF2 (1000 mg/L COD, 100 worms, 191 L/m^2·d)

In conditions of low organic load, the median COD removal achieved by VF1 is 83%. VF2, with a higher HLR of 191 L/m^2/d, attained a median COD removal of 87%. The P-value of 0.674 shows there was not a significative difference between their COD removal percentage. However VF1 exhibits slighly lower variability in its performance (**Figure 10a**), which could indicate a more stable and consistent treatment efficiency. However from **Figure 11a** , the evolution of the treatment seems to be the same with more stable values starting from the 4th week. The vermifilter has effectively been found to be more stable after 25-28 days, which corresponds to the acclimatization time of earthworms to their new environment [27,28]. The high removal percentage were consistent with our previous study. We found COD removals around 83% [16,19,128]. The high removals has been attributed to the synergic action between the microorganisms and the earthworms in the degradation of pollution [25].

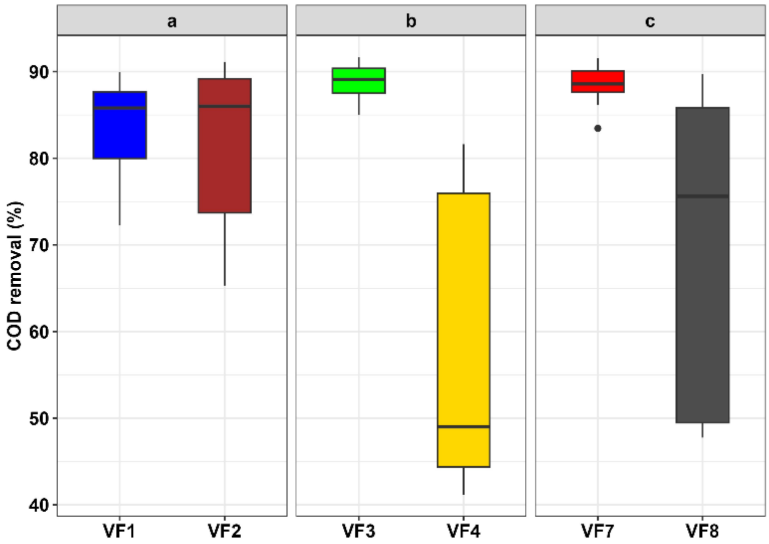


Figure 10. Data distribution for analyzing HLR influence on COD removal performances. (a) VF1 vs. VF2. (b) VF3 vs. VF4. (c) VF7 vs. VF8

VF3 (4000 mg/L COD, 100 worms, 64 L/m²·d) vs. VF4 (4000 mg/L COD, 100 worms, 191 L/m²·d)

Under high organic load conditions, VF3 operating at an HLR of 64 L/m²/day achieved a median COD removal of 88%. In contrast, VF4 with an elevated HLR of 191 L/m²/day records a significantly lower median COD removal of 60% (P-value=0.001). Additionally, VF4 demonstrated very high variability, highlighting substantial instability and a pronounced decrease in efficiency over time (**Figure 11b**). Over time, VF4 became saturated and began to clog. This clogging slowed down worm activity and reduced the filter's access to aeration, ultimately leading to a decline in performance.

VF7 (2500 mg/L COD, 200 worms, 64 L/m²·d) vs. VF8 (2500 mg/L COD, 200 worms, 191 L/m²·d)

For medium organic load scenarios, VF7 at an HLR of 64 L/m²/d achieved a median COD removal efficiency of 87% (**Figure 10c**). On the other hand, VF8 operating at a higher HLR of 191 L/m²/d resulted in a reduced median COD removal of 67%. This performance is further characterized by high variability in VF8, suggesting a notable decline in consistency and overall treatment effectiveness.

The analysis across varying organic load conditions consistently indicated that a lower HLR of 64 L/m²/d facilitates higher and more stable COD removal efficiencies. Increasing the HLR to 191 L/m²/d appeared not to have a significant impact on the COD removal at low initial COD, but leads to significant reductions in both efficiency and stability under medium and high organic load conditions. These findings underscored the critical importance of optimizing HLR settings according to the specific organic load to achieve optimal greywater treatment performance [28].

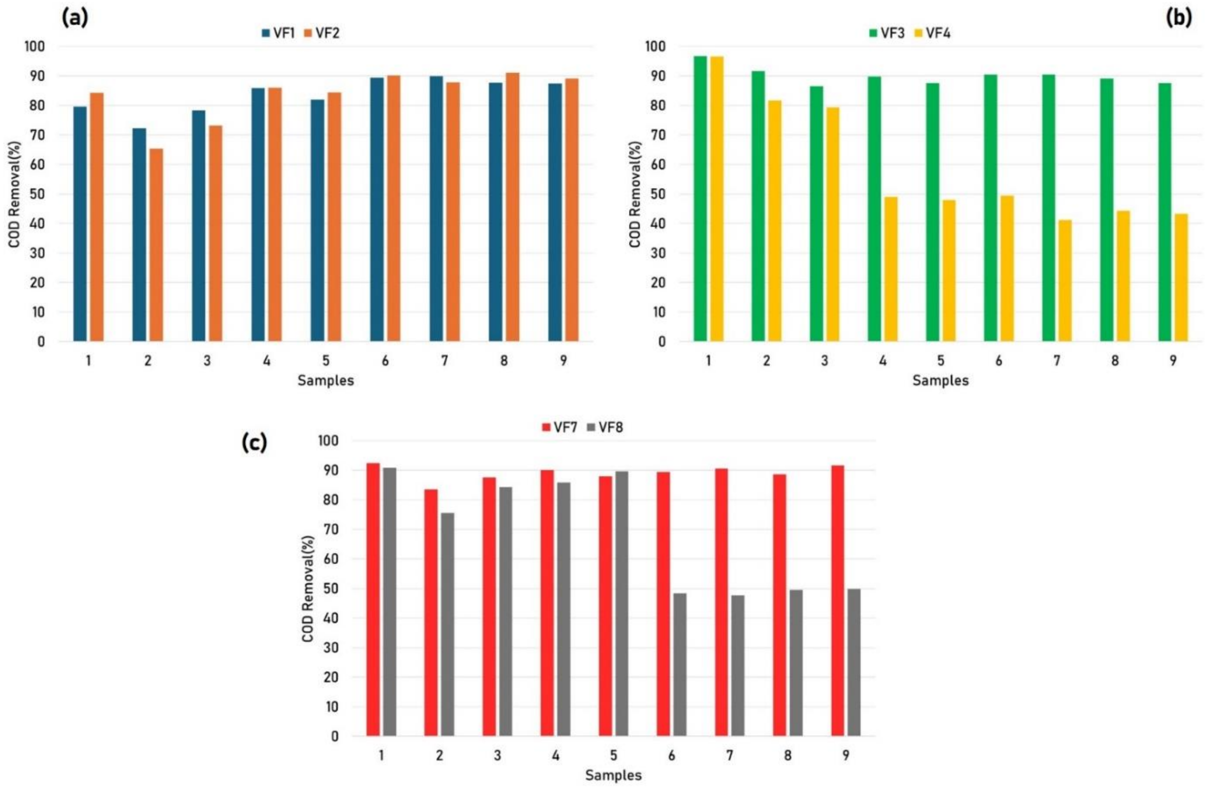


Figure 11. HLR influence on COD removal. (a) VF1 vs. VF2. (b) VF3 vs. VF4. (c) VF7 vs. VF8

- **BOD**

Figure 12 and Figure 13 give us the results of the influence of HLR on the BOD removal.

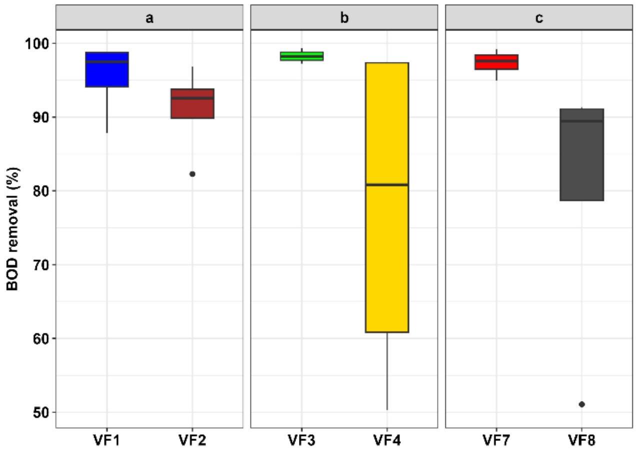


Figure 12. Data distribution for analyzing HLR influence on BOD removal performances. (a) VF1 vs. VF2. (b) VF3 vs. VF4. (c) VF7 vs. VF8

VF1 (1000 mg/L COD, 100 worms, 64 L/m²·d) vs. VF2 (1000 mg/L COD, 100 worms, 191 L/m²·d)

In conditions of low organic load, the median BOD removal achieved by VF1 operating at an HLR of 64 L/m²/d is 96%. Comparatively, VF2, with a higher HLR of 191 L/m²/day, attained an inferior median BOD removal of 92% (**Figure 12a**). It can be noticed that VF1 and VF2 exhibits lower variability in their performance, This may indicate a more stable and consistent treatment efficiency at the increased HLR. However, no significant differences were observed between the values of the two runs (P-value = 0.248).

VF3 (4000 mg/L COD, 100 worms, 64 L/m²·d) vs. VF4 (4000 mg/L COD, 100 worms, 191 L/m²·d)

Under high organic load conditions, VF3 operating at an HLR of 64 L/m²/day achieved a median BOD removal of 97%. In contrast, VF4 with an elevated HLR of 191 L/m²/day recorded a significantly lower median BOD removal of 79%. Additionally, VF4 demonstrated very high variability, highlighting substantial instability and a pronounced decrease in treatment efficiency when subjected to a higher HLR under heavy organic loading. Concerning the evolution of the treatment, **Figure 13b** showed that the removal start decreasing considerably with the 3rd sample. The same conclusions can be drawn as those for COD.

VF7 (2500 mg/L COD, 200 worms, 64 L/m²·d) vs. VF8 (2500 mg/L COD, 200 worms, 191 L/m²·d)

For medium organic load scenarios, VF7 at an HLR of 64 L/m²/d achieved a median BOD removal efficiency of 98%. On the other hand, VF8 operating at a higher HLR of 191 L/m²/d resulted in a reduced median BOD removal of 86.5%. This performance was further characterized by higher variability in VF8 compared to VF7, suggesting a decline in consistency and overall treatment effectiveness at the increased HLR level in medium organic load condition. There was a significative difference between the values of the two runs.

The analysis across varying organic load conditions consistently indicated that a lower HLR of 64 L/m²/day facilitates higher and more stable BOD removal efficiencies. Increasing the HLR to 127 L/m²/day and 191 L/m²/day appeared to lead to significant reductions in both efficiency and stability. The same conclusions can be drawn as those for COD.

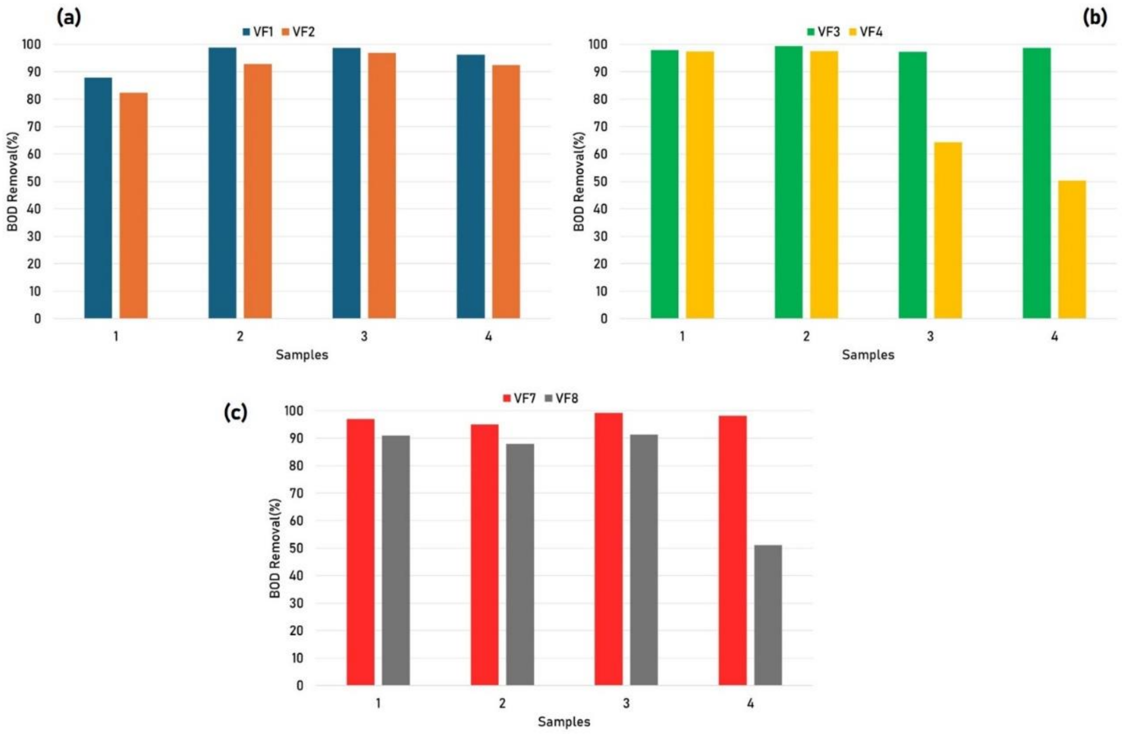


Figure 13. HLR influence on BOD removal. (a) VF1 vs. VF2. (b) VF3 vs. VF4. (c) VF7 vs. VF8

3.3. Evaluation of Vermifilter Performance and influence of EWD

3.3.1. Physical parameters

- **pH**

Figure 14 (a) compares the trend of the pH in the different samples for NVF5 (without worms) and VF7 (with worms). We can see that in VF7 the pH remained relatively stable, with a slight fluctuation between 6 and 7.5, while in NVF5, the absence of worms leads to a much wider pH variation, ranging from 7.5 to 9 (P-value = 0.003). This indicated that earthworms helped to stabilize the pH of the system [25,155].

Figure 14 (c) compares the evolution of the pH in the samples for NVF10 (without worms) and VF12 (with worms). In NVF10: The pH showed consistent variability across the weeks, with a range of pH of between 7 and 9. For VF12 while there is some variability in pH, especially from week 1 to 2, it is more stable compared to the other worm-influenced filters, ranging from 6 to 9. There was not significant difference between the values of the two runs.

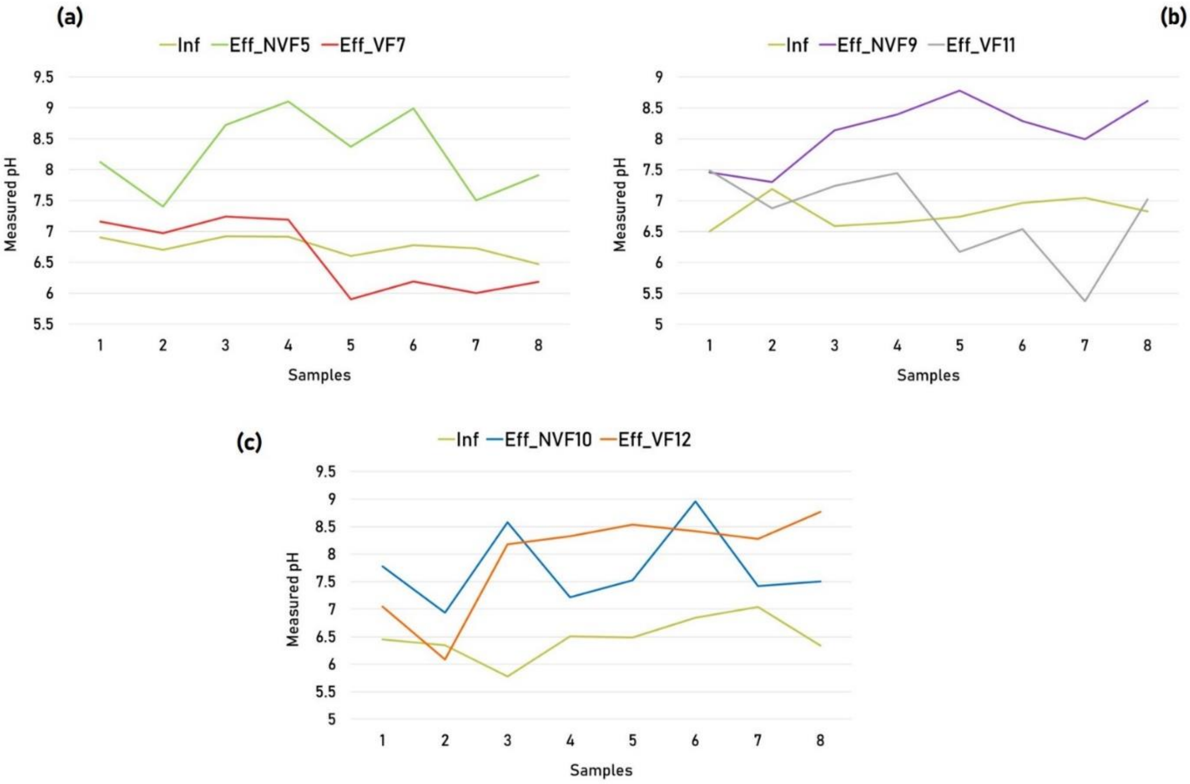


Figure 14. pH levels of influent and effluent (influence of worms). (a) NVF5 vs. VF7. (b) NVF9 vs. VF11. (c) NVF10 vs. VF12

In summary, across all comparisons, the presence of worms generally leaded to a less pH variability.

- **Electrical conductivity (EC)**

Table 17 below gives the results and discussion of the influence of EWD on the physical parameters, especially the pH here.

Table 17. Electric conductivity of influent and effluent in µS/cm (influence of EWD)

Experimental runs	Type of water	Samples							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	Inf.	220	248	259	252	431	281	398	422
VF11	Eff.	808	561	811	625	579	500	513	566
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
NVF9	Eff.	503	584	439	619	583	522	454	593
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
	Inf.	733	499	779	762	807	870	976	850
NVF10	Eff.	1424	1020	1147	1209	1794	1775	1938	1744
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
VF12	Eff.	1244	743	1166	1056	875	812	889	1113
	Δ_{EC}	↑	↑	↑	↑	↑	↓	↓	↑
	Inf.	515	407	586	552	630	566	416	520
VF7	Eff.	1014	600	1134	1052	1260	1066	462	986
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
NVF5	Eff.	746	497	661	1215	1211	1044	780	411
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↓

NVF9 (1000 mg/L COD, 0 worms, 127.5 L/m². d) vs. VF11 (1000 mg/L COD, 200 worms, 127.5 L/m².d)

For the two runs, the EC for the influent ranged from 220 to 422 µS/cm, whereas those of the effluent ranged from 500 to 808 µS/cm for VF11 and 454 to 619 µS/cm for NVF9. All differences (Δ_{EC}) positive across all measurements, showing an increase in EC after treatment. The key observation here is that the presence of worms likely contributed to more significant organic matter breakdown and ion release, leading to higher EC in treated water, as said before.

NVF10 (4000 mg/L COD, 0 worms, 127.5 L/m². d) vs. VF11 (4000 mg/L COD, 200 worms, 127.50 L/m².d)

The EC for both NVF10 and VF12 influents, ranged from 733 to 976 µS/cm regarding the influent. For the effluents in NVF10 and VF12, the ECs ranged respectively from 1020 to 1938 µS/cm, and from 743 to 1244 µS/cm.

NVF5 (2500 mg/L COD, 0 worms, 64 L/m².d) vs. VF7 (2500 mg/L COD, 200 worms, 64 L/m². d).

In both filters, EC ranges, for the influent, from 407 to 630 µS/cm. For the effluents' EC, in VF7, it ranges from 462 to 1260 µS/cm, while it ranges from 411 to 1215 µS/cm for NVF5. Positive differences were noted across all measurements for all filters, except a slight decrease for the last measurement of NVF5. Therefore, the presence of worms seemed to contribute to greater increases in EC. But once again, no significant differences were found.

To sum up all, the presence of worms generally resulted in greater increases in electrical conductivity (Δ_{EC}) in treated water, as previously explained. Worms seem to enhance the breakdown of organic matter, potentially releasing more ions into the water and thereby increasing EC. However, no significant difference was observed between the Δ_{ECs} of filters with worms and those without. This study did not establish a conclusive influence of worms on the electrical conductivity of greywater.

- **Dissolved Oxygen (DO)**

Table 18 focuses on comparing the effect of the presence or absence of worms on DO in effluent for various experimental runs, keeping the hydraulic loading rate (HLR) constant within pairs.

VF11 (1000 mg/L COD, 200 worms, 127.5 L/m²·d) vs. NVF9 (1000 mg/L COD, 0 worms, 127.5 L/m²·d)

For both filters the DO ranged from 1.18 to 2.95 mg/L. In VF11 the DO of the effluent ranged from 2.23 to 7.76 mg/L, while that of NVF9 ranged from 2.32 to 4.66 mg/L. About the differences, VF11 showed a significant increase in DO, especially in samples 6, 7, and 8, where DO reached up to 7.76 mg/L, whereas NVF9 shows a more moderate increase in DO, with the highest value of 4.66 mg/L at sample 3. There was a significant difference. The key observation here is the presence of worms in VF11 resulted in a greater and more consistent increase in DO compared to NVF9 without worms. The worms will contribute to aerating the systems, and the microorganisms will consume oxygen from the atmosphere, instead of that from the system.

NVF10 (4000 mg/L COD, 0 worms, 127.5 L/m²·d) vs. VF12 (4000 mg/L COD, 100 worms, 127.5 L/m²·d)

Both for NVF10 and VF12, the influent DO stood in the range of 1.42 - 1.69 mg/L. For NVF10 the effluents' DO ranges from 0.30 to 3.77 mg/L, while that of VF12 ranges from 1.10 to 5.69 mg/L. In terms of difference, NVF10 showd a decline in DO for some samples (5, 6, and 8), with a minimum of 0.30 mg/L, whereas VF12, with worms, generally showed a higher DO, reaching up to 5.69 mg/L at sample 6. However, VF12 did not show any decrease in DO in samples 7 and 8, suggesting that worms may not fully counteract the effects of high COD levels on DO. Due to the clogging of the filter caused by the high organic load and moderate HLR, earthworms cannot survive in such conditions. Consequently, the state of VF12 will ultimately resemble that of a filter without earthworms [159].

Table 18. Electric conductivity of influent and effluent in mg/L (influence of EWD)

Experimental runs	**Type of water**	**Samples**							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	Inf.	1.18	2.13	2.4	2.11	2.16	2.95	2.18	2.06
VF11	Eff.	2.78	2.23	4.63	4.09	4.08	7.76	4.44	5.70
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
NVF9	Eff.	2.82	4.18	4.66	2.78	2.32	3.61	3.14	2.41
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
	Inf.	1.42	1.48	1.60	1.59	1.30	1.69	1.64	1.45
NVF10	Eff.	2.34	2.81	3.77	2.38	0.85	1.20	1.91	0.30
	Δ_{DO}	↑	↑	↑	↑	↓	↓	↑	↓
VF12	Eff.	2.41	2.52	3.21	2.28	2.12	5.69	1.10	1.41
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↓	↓
	Inf.	1.40	1.80	1.95	1.89	1.87	1.84	1.93	1.70
VF7	Eff.	2.45	3.70	4.44	4.01	2.91	6.12	3.03	5.16
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
NVF5	Eff.	2.80	3.52	3.92	2.40	0.49	1.75	3.43	2.06
	Δ_{DO}	↑	↑	↑	↑	↓	↓	↑	↑

VF7 (2500 mg/L COD, 200 worms, 64 L/m²·d) vs. NVF5 (2500 mg/L COD, 0 worms, 64 L/m²·d)

The DO of the influent ranged from 1.40 to 1.93 mg/L in both VF7 and NVF5. For VF7, the DO ranges from 2.45 to 6.12 mg/L for the effluent, while in NVF5, the DO ranges from 0.49 to 3.92 mg/L. VF7 showed a consistent increase in DO across all samples, with a peak of 6.12 mg/L at sample 6, while NVF5 showed a decline in DO for samples 5 and 6, with a minimum of 0.49 mg/L at sample 5. The presence of worms in VF7 significantly improved DO levels compared to NVF5 without worms, as expected. The P-value is 0.025.

In summary, worms generally enhanced the increase in dissolved oxygen (DO) during greywater treatment, particularly at lower HLRs. Filters with worms and low HLRs, and low initial COD (VF11 and VF7) exhibited higher and more consistent DO levels in effluent compared to those without worms (NVF9, NVF10, NVF5). VF12, which contains worms but operates under moderate HLR and high initial COD, was finally found to function like a filter without earthworms due to clogging caused by the high initial COD and moderate HLR. The influence of worms is evident, highlighting the crucial role of their biological activity in the oxygenation processes within the filtration system. However, it can be concluded that the presence of worms alone does not guarantee effective oxygenation. HLR and influent COD levels must also be carefully controlled, as demonstrated by other studies [41] and [110].

3.3.2. Chemical parameters

- **COD**

Figure 15 and Figure 16 give the results of the influence of EWD on the COD removal.

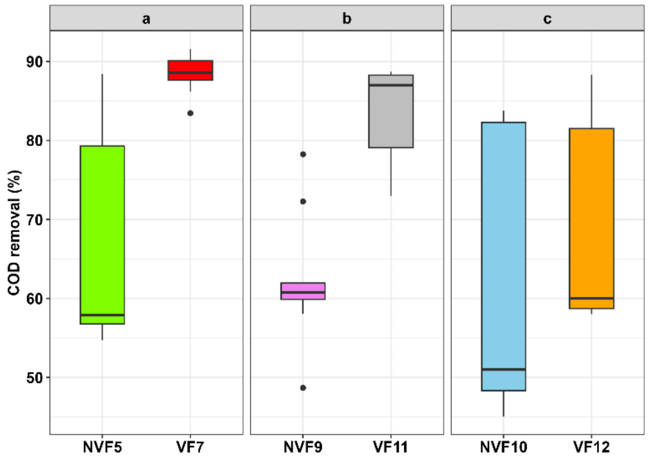


Figure 15. Data distribution for analyzing worms influence on COD removal performances. (a) NVF5 vs. VF7. (b) NVF9 vs. VF11. (c) NVF10 vs. VF12.

NVF9 vs. VF11 (Moderate HLR: 127.5 L/m²/day)

For NVF9 (No Worms) the median COD removal was little higher than 60%, with variability in performances. For VF11 (With Worms): Median COD removal was far higher, at 83%, with also slightly more variability in performance (**Figure 15b)**. From **Figure 16b**, the time evolution of NVF9 showed that its performance started at 87% but remained inconsistent, dropping to 60% by the end of the treatment. In contrast, VF11 exhibited a more stable performance over time. NVF9 became saturated and clogged, aligning with the observations discussed earlier. This suggests that the presence of worms at this HLR level significantly enhances the median COD removal and improves overall treatment performance

NVF5 vs. VF7 (Low HLR: 64 L/m²/day)

For NVF5 (No Worms), the median COD removal was around 70%, with a higher variability in performance with respect to VF7. For VF7 (With Worms), the median COD removal was very high at 89%, with lower variability, showing stable and efficient performance (**Figure**

15a). The time evolution of NVF5 and VF7 (**Figure 16a**) revealed similar observations to those noted for NVF9 and VF11 respectively.

NVF10 vs. VF12 (High Organic Load at HLR: 127.5 L/m²/d)

For NVF10 (No Worms), the median COD removal is around 67%, with a higher variability in performance with respect to VF12, which contains worms. For VF12 (With Worms), the median COD removal is a little higher at 70%, with slightly lower variability. at medium HLR also, the presence of worms seemed not to have a good effect on the performance of the filter even with moderate HLR. The impact of EWD on vermifilter performance demonstrated that the presence of worms generally enhanced COD removal across various HLR levels. Worms contributed to more stable and efficient performance, particularly under conditions of high organic loads and low HLR. Additionally, their presence reduced variability in COD removal, resulting in more consistent and reliable treatment outcomes.

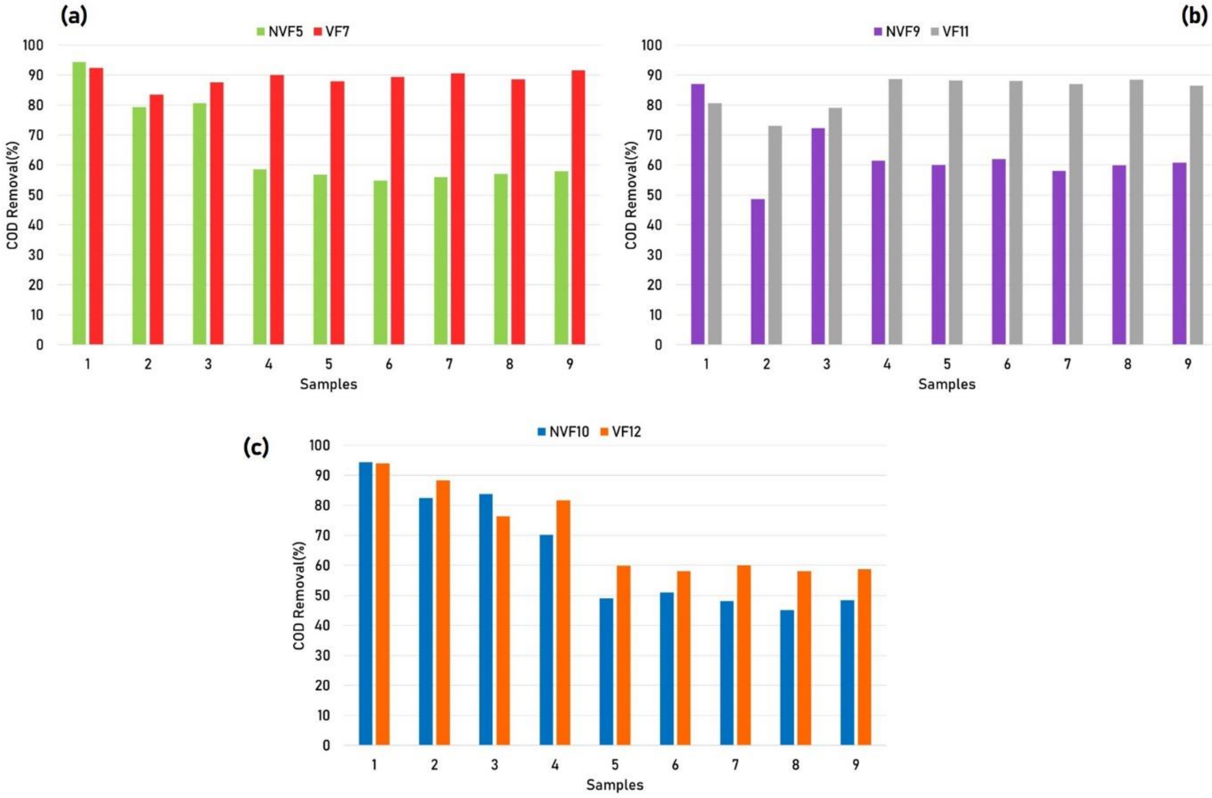


Figure 16. Worms influence on COD removal. (a) NVF5 vs. VF7. (b) NVF9 vs. VF11. (c) NVF10 vs. VF12

- **BOD**

Figure 17 and Figure 18 give the results of the influence of EWD on chemical parameters, especially here the BOD removal.

NVF5 vs.VF7 (Low HLR: 64 L/m²/d)

Samples 1 and 2 showed consistently high BOD removal efficiency for both filters (over 90%). However, starting from sample 3, NVF5 (without worms) showed a notable decrease in efficiency, while VF7 (with 200 worms) maintaind consistent efficiency (**Figure 17a**). From a temporal evolution analysis NVF5 showed a decline in BOD removal efficiency over time till 60%, while VF7 remained stable and in high range removal performance. suggesting that the presence of worms helped maintain long-term performance under low hydraulic load conditions.

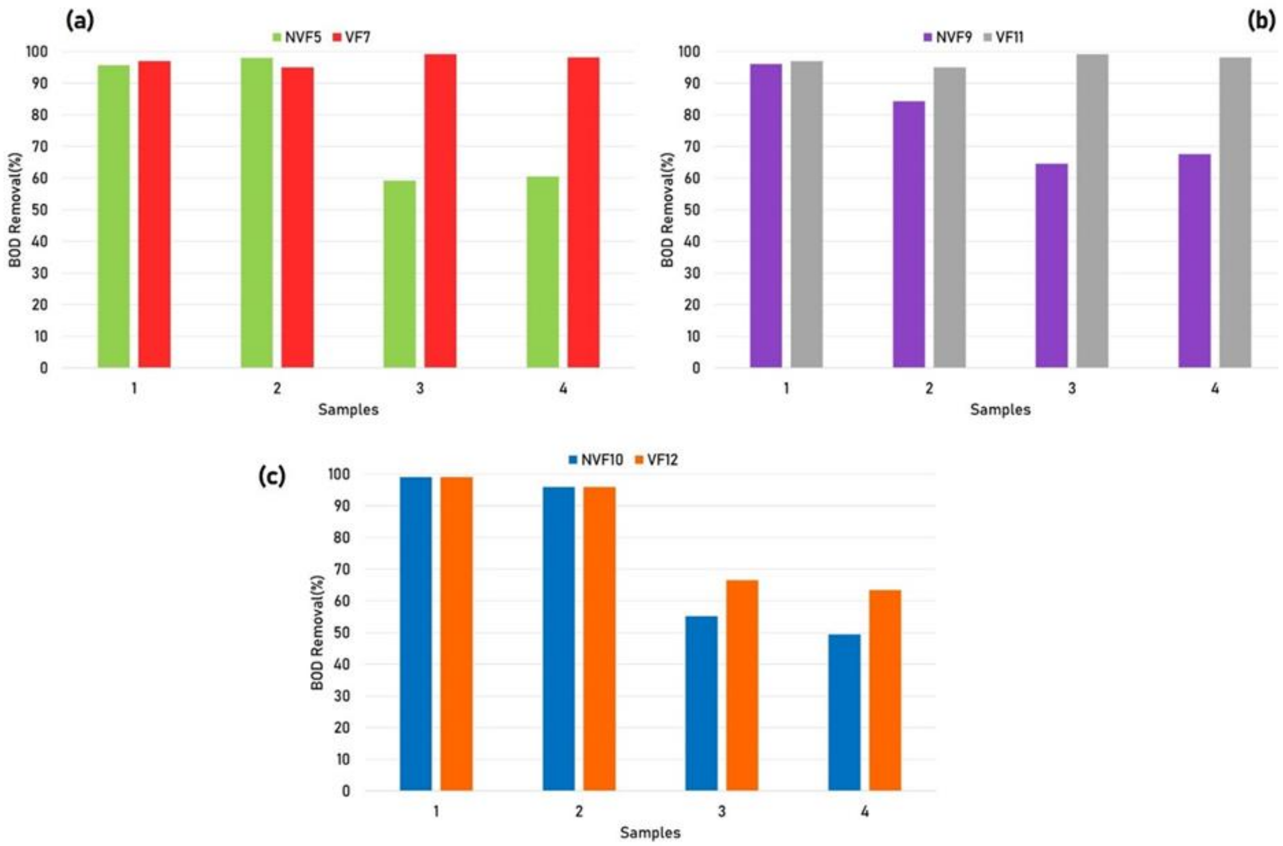


Figure 17. Worms influence on BOD removal. (a) NVF5 vs. VF7. (b) NVF9 vs. VF11. (c) NVF10 vs. VF12.

NVF9 vs. VF11 (Moderate HLR: 127.5 L/m²/d)

Both VF9 (without worms) and VF11 (with 200 worms) exhibited high efficiency in the initial samples. However, NVF9 showed a significant drop in efficiency starting from sample 3, whereas VF11 remains consistent throughout the period. The same observations have been made as for the previous couple of filters. (**Figure 17b and Figure 18b).**

NVF10 vs. VF12 (High Organic Load at HLR: 127.5 L/m²/day)

Initially (samples 1 and 2), both filters showed very high BOD removal efficiency, of little less than 100%. However, from sample 3 onwards, a drop was observed in both cases, but it was more pronounced for NVF10 (without worms). Such analysis can be stated as this: VF12 (with worms) showed better initial performance, but both filters exhibited a decline over time, indicating that even with the aid of worms, high COD load conditions may limit long-term treatment efficiency at moderate HLR. under high COD load conditions.

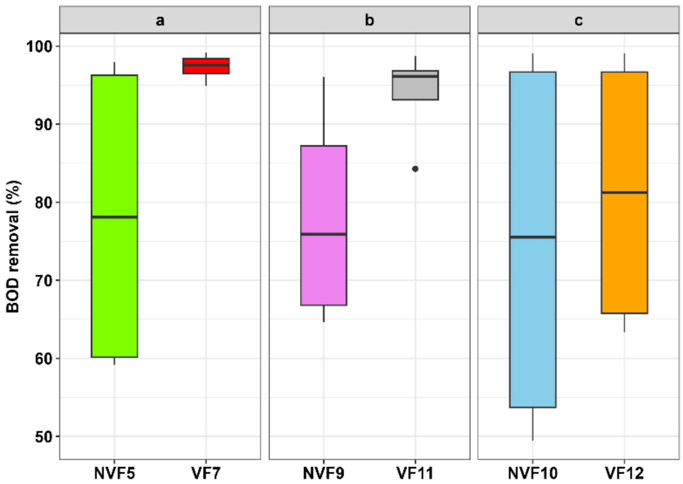


Figure 18. Data distribution for analyzing worms influence on BOD removal performances. (a) NVF5 vs. VF7. (b) NVF9 vs. VF11. (c) NVF10 vs. VF12.

In summary**,** the presence of earthworms had a demonstrably positive effect on BOD removal in vermifilters, contributing both to treatment stability and performance. The performance is enhanced by more than 30% with the presence of earthworms, aligning with findings of our previous studies and those reported in the literature [108,152]. This improvement was attributed not only to their burrowing activity, which promotes system aeration and prevents clogging, but also to the proliferation of aerobic bacteria in their intestines, which are responsible for BOD degradation [102,146,150]. However, the presence of earthworms alone was found not sufficient; hydraulic and organic loading rates must also be controlled and maintained within the optimal operating conditions of the filter.

3.4. Evaluation of Vermifilter Performance and influence of Initial COD

3.4.1. Physical parameters

- **pH**

Figure 19 compares the pH levels of the influent (Inf.) and effluent (Eff.) for vermifilters 11, 12, 4 and 2. As shown in **Figure 19 (a)**, in the one hand, for both VF4 (4000mg/L) and VF2 (1000mg/L) (HLR of 191 L/m²·h), whether it was low or high initial COD, pH in the influent seemed to be more stable, with minor fluctuations (values evolving between 5.5 and little more than 7). On the other hand, effluents' pH showed more variability, with VF4 demonstrating the biggest values, between 7 and 9.5.

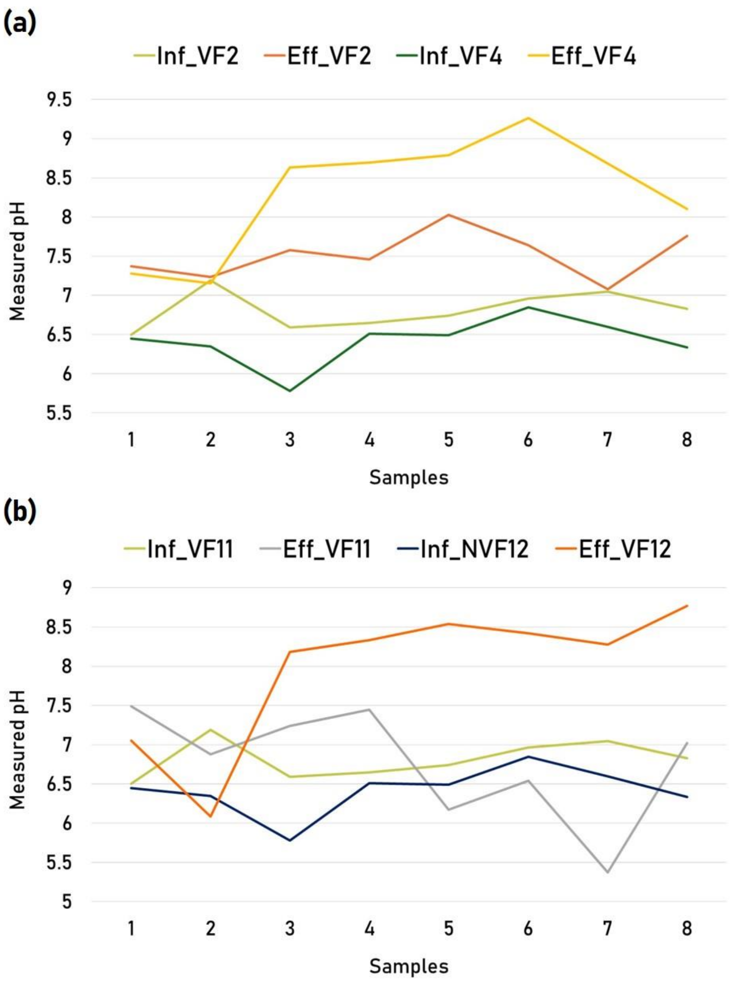


Figure 19. pH levels of influent and effluent (initial COD influence). (a) VF2 vs. VF4. (b) VF11 vs. VF12.

In **Figure 19 (b)**, as noticed for VF2 and VF4, it can be noticed that whether it is low or high initial COD, pH in the influent seemed to be more stable, with minor fluctuations (values evolving between 6.5 and 7.5 and between 5.5 and 7). On the other hand, effluents' pH showed more variability, with VF12 (4000mg/L) demonstrating the biggest values, between 6 and 9. There was a significant difference between both the couple VF2vs VF4 and VF11 vs VF12 with P-value = 0.018.

In summary, higher initial COD levels generally resulted in higher pH values and greater variability in treated water compared to lower COD levels (1000). This suggests that the initial COD significantly impacts the pH during the vermifiltration process, with higher COD leading to more pronounced changes in pH.

- **Electrical conductivity (EC)**

VF2 (1000 mg/L COD, 100 worms, 191 L/m²·d) vs.VF4 (4000 mg/L COD, 100 worms, 191 L/m². d)

On the one hand, in VF2 and VF4, the EC for influent ranged from 220 to 431 µS/cm and from to respectively 499 to 976 µS/cm. On the other hand the EC of the effluent ranged from 411 to 613 µS/cm in VF2, and from 566 to 1818 µS/cm respectively for VF2 and VF4 (**Table 19)**. On the differences, VF2 showed positive across all measurements, showing an increase in EC after treatment, whereas VF4 also showed positive across all measurements. The differences between the Δ_{EC} of the two, have not been found significant (See **Appendix A2**)

VF11 (1000 mg/L COD, 200 worms, 127.5 L/m²·d) vs. VF12 (4000 mg/L COD, 200 worms, 127.5 L/m²·d)

For both the runs, VF11 and VF12, the EC for influent ranged from 220 to 431 µS/cm and from to respectively 733 to 976 µS/cm. On the other hand, the EC of the effluent ranges from 411 to 613 µS/cm in VF2, and from 566 to 1818 µS/cm respectively for V11 and V12 (**Table 19**). On the differences, for VF11, positive differences were recorded across all measurements, showing an increase in EC after treatment. No significant differences in ΔEC were observed between the two. The initial COD didn't therefore seems to have an impact on the treatment of EC in the vermifilter.

Table 19. Electric conductivity of influent and effluent in µS/cm (influence of Initial COD)

Experimental runs	Type of water	Samples							
		1	2	3	4	5	6	7	8
VF2	Inf.	220	248	259	252	431	281	398	422
	Eff.	613	441	464	522	535	450	411	600
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
VF4	Inf.	733	499	779	762	807	870	976	850
	Eff.	1572	566	1302	1374	1588	1748	1818	950
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
VF11	Inf.	220	248	259	252	431	281	398	422
	Eff.	808	561	811	625	579	500	513	566
	Δ_{EC}	↑	↑	↑	↑	↑	↑	↑	↑
VF12	Inf.	733	499	779	762	807	870	976	850
	Eff.	1244	743	1166	1056	875	812	889	1113
	Δ_{EC}	↑	↑	↑	↑	↑	↓	↓	↑

- **Dissolved Oxygen (DO)**

VF11 (1000 mg/L COD, 200 worms, 127.5 L/m²·d) vs. VF12 (4000 mg/L COD, 200 worms, 127.5 L/m²·d)

For VF11 and VF12 the DO ranged respectively from 1.18 mg/L to 2.95 mg/L and from 1.42 mg/L to 1.69 mg/L (**Table 20)**. In VF11 the DO of the effluent ranged from 2.23 mg/L to 7.76 mg/L, while that of VF12 ranges from 1.10 mg/L to 5.69 mg/L. About the differences, in VF11 there was an increase in DO for all samples, with the maximum increase at Sample 6 (Δ_{Do} = +4.81 mg/L), while for VF12 there was generally an increase in DO, Sample 7 (Δ_{DO} = -0.54 mg/L) and Sample 8 (Δ_{DO} = -0.24 mg/L) showed decreases in DO. The DO here reflected the decline in filter performance.

Table 20. Dissolved Oxygen of influent and effluent in mg/L (influence of Initial COD)

Experimental runs	Type of water	Samples							
		1	2	3	4	5	6	7	8
VF2	Inf.	1.18	2.13	2.4	2.11	2.16	2.95	2.18	2.06
	Eff.	2.78	2.23	4.63	4.09	4.08	7.76	4.44	5.70
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
VF4	Inf.	1.42	1.48	1.60	1.59	1.30	1.69	1.64	1.45
	Eff.	1.82	2.52	1.61	2.38	0.35	1.83	0.93	2.37
	Δ_{DO}	↑	↑	↑	↑	↓	↑	↓	↑
VF11	Inf.	1.18	2.13	2.4	2.11	2.16	2.95	2.18	2.06
	Eff.	2.78	2.23	4.63	4.09	4.08	7.76	4.44	5.70
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↑	↑
VF12	Inf.	1.42	1.48	1.60	1.59	1.30	1.69	1.64	1.45
	Eff.	2.41	2.52	3.21	2.28	2.12	5.69	1.10	1.41
	Δ_{DO}	↑	↑	↑	↑	↑	↑	↓	↓

VF2 (1000 mg/L COD, 100 worms, 191 L/m²•d) vs.VF4 (4000 mg/L COD, 100 worms, 191 L/m².d)

For VF2 and VF4 the DO ranged respectively from 1.18 mg/L to 2.95 mg/L and from 1.42 mg/L to 1.69 mg/L. In VF2 the DO of the effluent ranged from 2.23 mg/L to 7.76 mg/L, while that of VF4 ranged from 0.35 mg/L to 2.52 mg/L (**Table 20)**. About the differences, in VF2 there was an increase in DO for all samples, with the maximum increase occurring at Sample 6 (Δ_{DO} = +4.81 mg/L). For VF4 there was generally an increase in DO, except for Sample 5 (ΔT = - 0.95 mg/L) and Sample 7 (Δ_{DO} = - 0.71 mg/L). The same conclusion can be drawn as for the VF11-VF12 pair. P-value = 0.002 (**Appendix A3**)

With regard of all the observations made, we could say that initial COD levels have a significant bad influence on the DO levels in treated greywater

3.4.2. Chemical parameters

- **COD**

Figure 20 and Figure 21 give us the results of the influence of initial COD on the COD removal.

VF2 (1000 mg/L and 191 L³/m²/d) vs. VF4 (4000 mg/L and 191 L/m²/d) Figure 20a.

From **Figure 20a,** we can see that, for VF2, a median COD removal was recorded of around 86%, with a narrower interquartile range (IQR) of about 5-10%, indicating consistent performance with respect to VF4 (4000 mg/L), for which the median removal was about 30% lower compared to that of VF2, and which also shows a wider IQR of 20%, suggesting more variability in treatment efficiency. When considering the evolution of the treatment performance over time, VF2 was also more stable than VF4 (**Figure 21a**).

VF11 (1000 mg/L and 127 m³/m²/d) vs. VF12 (4000 mg/L and 127 L/m²/d) Figure 20b.

From **Figure 20b,** VF11 showed high median COD removal (~ 87%) with a narrow IQR of 5-10%, suggesting consistent performance like VF2. For VF12 (4000 mg/L), the median removal was around 62%, with a wider IQR of about 25%, indicating greater variability similarly to VF4. The evolution of the treatment was also more stable for VF11 (**Figure 21b**).

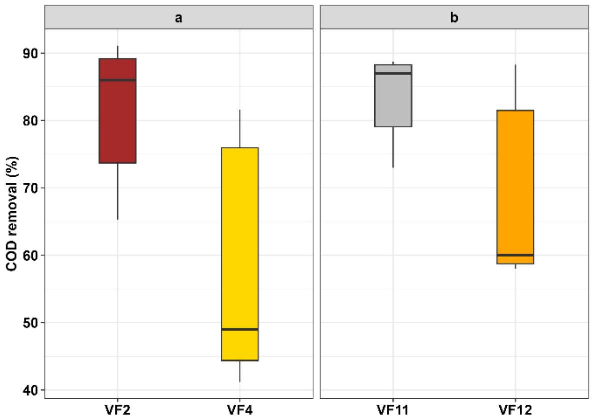


Figure 20. Data distribution for analyzing initial COD influence on COD removal performances. (a) VF2 vs. VF4. (b) VF11 vs. VF12

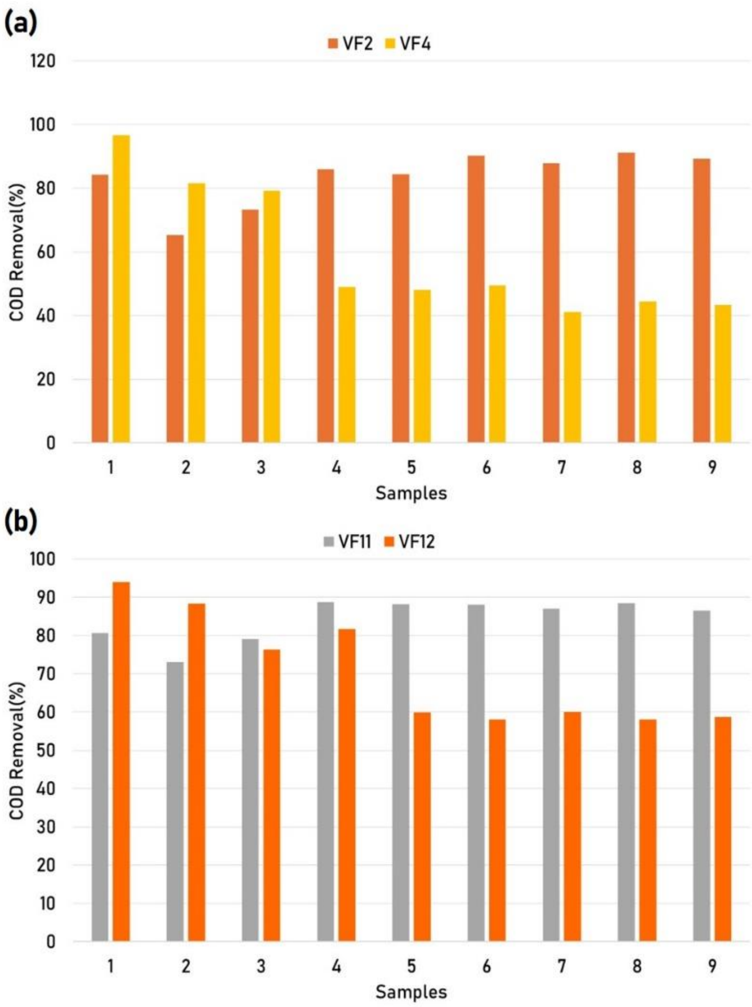


Figure 21. Initial COD influence on BOD removal. (a) VF2 vs. VF4. (b) VF11 vs. VF12

In conclusion, it can be stated that at moderate to high HLR levels, an increase in initial COD adversely affects COD removal efficiency.

- **BOD**

Figure 22 and Figure 23 give us the results of the influence of initial COD on chemical parameters, especially here the BOD removal.

VF2 (1000 mg/L and 191 L/m²/d) vs. VF4 (4000 mg/L and 191 Lm²/d).

For VF2, the BOD removal efficiency was consistently high across all the sample especially after week 1 (**Figure 22a**). This suggests a stable treatment process over time. For VF4, the treatment process was not stable. From the **Figure 23a,** VF2 showed high and consistent BOD removal (~93% median, minimal variation), while VF4 had lower efficiency (~81% median) and greater variability (~60–100%). VF2 surpassed VF4 in both performance and stability.

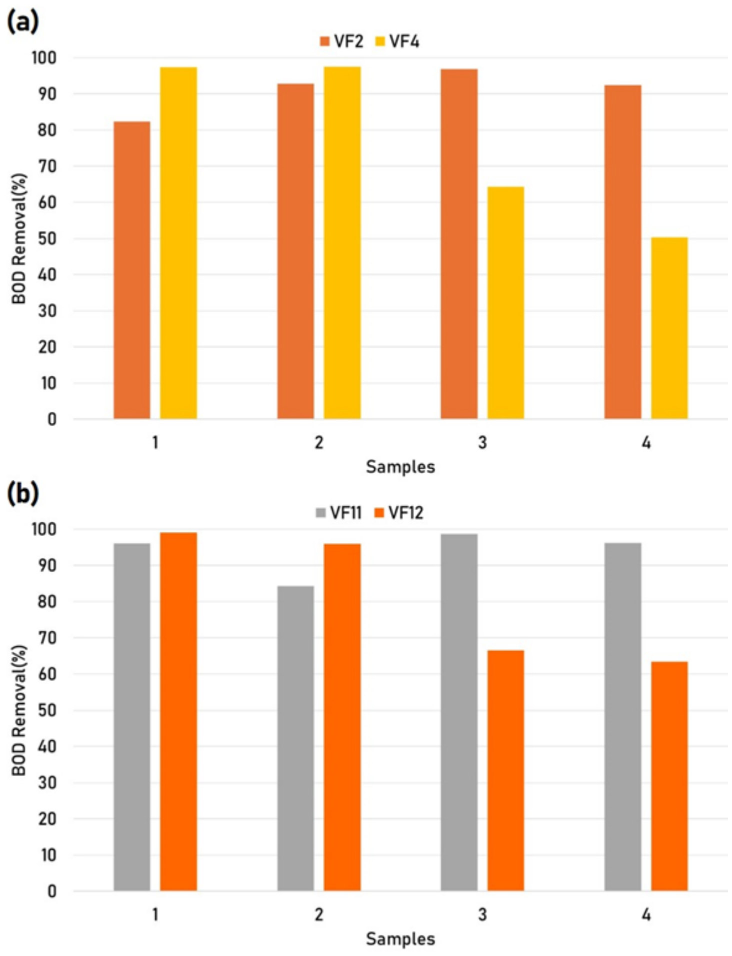


Figure 22. Initial COD influence on BOD removal (%). (a) VF2 vs. VF4. (b) VF11 vs. VF12

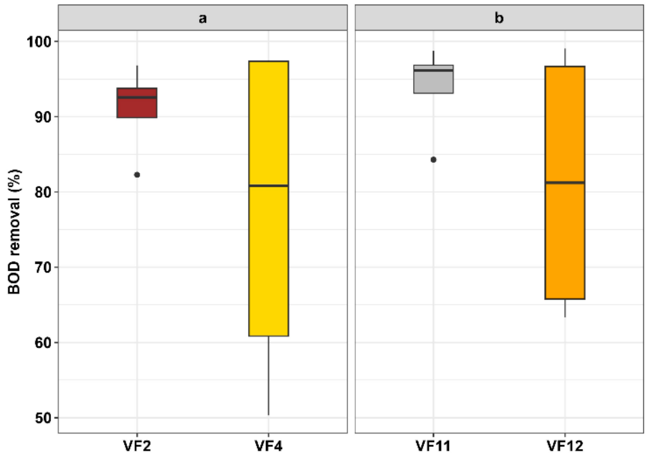


Figure 23. Data distribution for analyzing initial COD influence on COD removal performances. (a) VF2 vs. VF4. (b) VF11 vs. VF12

VF11 (1000 mg/L and 127 L/m²/d) vs. VF12 (4000 mg/L and 127 L/m²/d).

Like VF2, VF11 showed consistently high BOD removal across all samples, generally maintaining values between 92% and 100%. VF12 exhibits more variability, reaching around 60 - 70% (**Figure 22a**). VF11 showed consistent performance (~95% median, narrow distribution), like VF2. VF12 had greater variability (60–100% range) and lower median removal, reflecting the instability seen in VF4 due to higher COD concentrations **(Figure 23b)**

The conclusion for the effect of initial COD increase, on BOD removal was the same as for the COD removal.

4. Conclusion

This chapter examined the performance of vermifilters and the influence of key parameters such as Hydraulic Loading Rate (HLR), Earthworm Density (EWD), and initial COD concentration on their efficiency. The study revealed that a higher HLR (191 L/m²/day) generally increased electrical conductivity in treated greywater, although this effect was not statistically significant. However, increasing the HLR to 191 L/m²/day resulted in a decrease in filtration efficiency, sometimes by more than 35%, particularly under higher organic loads (4000 mg/L). In contrast, a lower HLR (64 L/m²/day) ensured better and more stable removal of COD and BOD, even under conditions of high organic load. This highlights the importance of optimizing the HLR based on organic load for improved greywater treatment performance. The presence of earthworms significantly enhanced COD and BOD removal, contributing to a 30% increase in removal efficiency at times, from 60% to 90%. Earthworms have also been shown to improve the stability and efficiency of filters, especially under high organic loads and low HLR conditions. However, the presence of earthworms alone was found to be not sufficient; both hydraulic and organic loading rates should be optimized for optimal performance. The study also indicated that higher initial COD levels lead to increased pH variability and negatively affect dissolved oxygen (DO) levels, suggesting that elevated COD reduces treatment efficiency, particularly under high or moderate HLR conditions.

This chapiter highlighted the significant influence of initial COD levels, HLR, and EWD on greywater treatment through vermifiltration, with their combined effects proving even more impactful. It is therefore essential to develop a model of the system to determine the optimal parameter combination for efficient treatment. Such modeling would also serve to quantify and represent both the individual and synergistic effects of these factors and allow a better understanding of the mechanisms involved in the treatment process. These aspects will be explored in greater detail in the next chapter.

CHAPTER 3: MODELING AND OPTIMIZATION OF THE VERMIFILTRATION SYSTEM

1. Introduction

This chapter presents a comprehensive analysis of the modeling and optimization of a vermifilter system for treating domestic greywater. It is structured into two distinct but interrelated sections: kinetic modeling and parametric optimization, each contributing to a deeper understanding of the system's performance and its enhancement for practical applications.

The first section focuses on the application of kinetic models to describe the degradation of organic matter, specifically Chemical Oxygen Demand (COD) removal. Three widely used kinetic models—First-Order, Grau Second-Order, and Stover-Kincannon—are employed to evaluate the system's behavior under various operational conditions. These models provide insights into substrate utilization rates, microbial activity, and the influence of organic loading on system efficiency. By deriving and comparing kinetic coefficients, this section establishes a robust theoretical framework to characterize the vermifilter's treatment processes.

The second section shifts focus to the optimization of key operational parameters: Hydraulic Loading Rate (HLR), Initial COD concentration, and Earthworm Density (EWD). Using the Box-Behnken Design (BBD) approach coupled with Response Surface Methodology (RSM), this section investigates the individual and interactive effects of these parameters on COD removal efficiency. The BBD enables the identification of optimal operating conditions while minimizing experimental runs, providing a cost-effective and precise approach to process optimization.

The methodology underpinning this chapter includes standard procedures for analyzing influent and effluent parameters such as COD, BOD, and TSS, alongside advanced modeling and statistical techniques. The kinetic analysis begins with deriving rate equations, plotting key relationships, and calculating constants for each model. The optimization analysis follows with a detailed experimental design, encompassing statistical validation and response surface generation to identify optimal parameter settings.

By combining kinetic modeling and parametric optimization, this chapter bridges theoretical understanding with practical design and operation strategies. The findings are expected to contribute to the advancement of sustainable wastewater treatment technologies and guide the development of efficient, scalable vermifilter systems.

2. Materials and Methods

2.1. Kinetic Models' methodology

2.1.1. First order model

The substrate concentration variation rate in the system, assuming the application of a first-order removal model, can be represented as follows (**Eq.(3)**) [160] :

$$-\frac{dC}{dt} = \frac{QC_i}{V} = -\frac{QC_e}{V} = -K_1 C_e \qquad \textbf{(3)}$$

With the elements of the equation given below:

C_i: Substrate concentration of the influent (g.L^{-1}) C_e: Substrate concentration of the effluent (g.L^{-1})

Q: Influent flow rate (L.d^{-1}) V: Volume of the reactor (L)

K_1: First-order kinetic constant (d^{-1})

Under conditions of pseudo-steady state, the substrate concentration remains virtually constant, with any changes in its rate being so minimal that they can be considered insignificant. (i.e., $-\frac{dC}{dt}$ = 0). In this scenario, the first equation (**Eq. (4)**) can be expressed as follows:

$$\frac{C_i - C_e}{HRT} = K_1 C_e \qquad \textbf{(4)}$$

Where HRT represents the hydraulic retention time, which is equal to V/Q.

Thus, the value of K_1 can be determined from the slope by plotting the ratio of $\frac{C_i - C_e}{HRT}$ against C_e.

2.1.2. Grau second-order model

The general expression for the second-order multicomponent kinetic model is given in **Eq. (5)** as follows: [161].

$$-\frac{dC}{dt} = K_2 X \left(\frac{C_e}{C_i}\right)^2 \qquad \textbf{(5)}$$

Such an equation is expressed in its integrated and linearized form as follows (**Eq. (6)**):

$$\frac{C_i}{C_i - Ce} HRT + \frac{C_i}{K_2 X} \qquad \textbf{(6)}$$

The elements of the equation are given as: K_2: Second-order kinetic constant (d^{-1}). It refers to the rate at which the substrate is removed per unit of microbial biomass, and X: Average biomass concentration in the system ($g.VSS.L^{-1}$)

The converted form of such equation (**Eq. (7**is given below:

$$\frac{V}{Q(C_i - C_e)} = \frac{1}{K_2 X} + \frac{V}{QC_i} \qquad \textbf{(7)}$$

When assuming the constancy of the second term of the right-hand side of the equation (**Eq. (8)**), the following equation is obtained then:

$$\frac{C_i}{C_i - C_e} HRT = a + bHRT \qquad \textbf{(8)}$$

Where the coefficients of equation are given as follows: ***a*** = ${C_i}/{K_2 X}$ expressed in (d^{-1}), ***b*** is the constant for the Grau second-order model, and

The ratio $E = C_i - C_e/C_i$ is known as the substrate removal efficiency. In these conditions, we can write the **Eq. (8)** in a more simplified form as shown in (**Eq. (9)**):

$$\frac{HRT}{E} = a + bHRT \qquad \textbf{(9)}$$

The kinetic coefficients (***a*** and ***b***) can be obtained from the graph consisting of a plot with HRT as the x-axis and $\frac{HRT}{E}$ as the y-axis. The intercept and slope of the plot correspond to the values of "***a***" and "***b***" respectively. K_2 can be calculated from the expression given for the coefficient a (a $= Ci/K_2X$). The coefficient "**b**" is given in **Eq. (8).** The value is nearly one, highlighting the practical challenges of achieving a COD value of zero. This indicates that while significant reductions in COD are possible, complete elimination is typically unattainable due to residual organic matter or inherent limitations in the process.

2.1.3. Stover-Kincannon model

The Stover-Kincannon model was originally developed to evaluate the performance of attached biomass growth in rotating biological contactors. Over time, it was adapted and extensively applied to predict and describe the performance of various other bioreactor systems. In this model, the substrate utilization rate is expressed as a function of the organic loading rate, making it highly relevant for systems with varying influent characteristics [162]. The foundational form of the model is given as **Eq. (10)**:

$$\frac{dC}{dt} = \frac{Q\ (C_i - C_e)}{V} \tag{10}$$

Where the expression of dc/dt is defined in **Eqs. (11Saturation constant) and (12)** as follow:

$$\frac{dC}{dt} = \frac{U_{max}\left(\frac{QSC_i}{V}\right)}{K_s + \left(\frac{QC_i}{V}\right)} \tag{11}$$

$$\frac{1}{\left(\frac{dC}{dt}\right)} = \frac{V}{Q\ (C_i - C_e)} = \frac{K_s\ V}{U_{max}\ QC_i} + \frac{1}{U_{max}} \tag{12}$$

If the expression $V/[Q\ (C_i -\ C_e)]$, It represents the inverse of the total substrate removal rate plotted against the reciprocal of the total substrate loading rate, (V/QC_i), this will result in a straight line, where the intercept and slope provide the respective values of key parameters in the model $1/U_{max}$, and $K_s\ /U_{max}$, respectively and from those the kinetic constants U_{max} and K_s can be obtained. U_{max} is the maximum substrate utilization rate and K_s is the saturation constant.

The effluent substrate concentration can be determined by solving this expression as follows:

The substrate balance for the reactor under steady-state conditions can be expressed as follows:

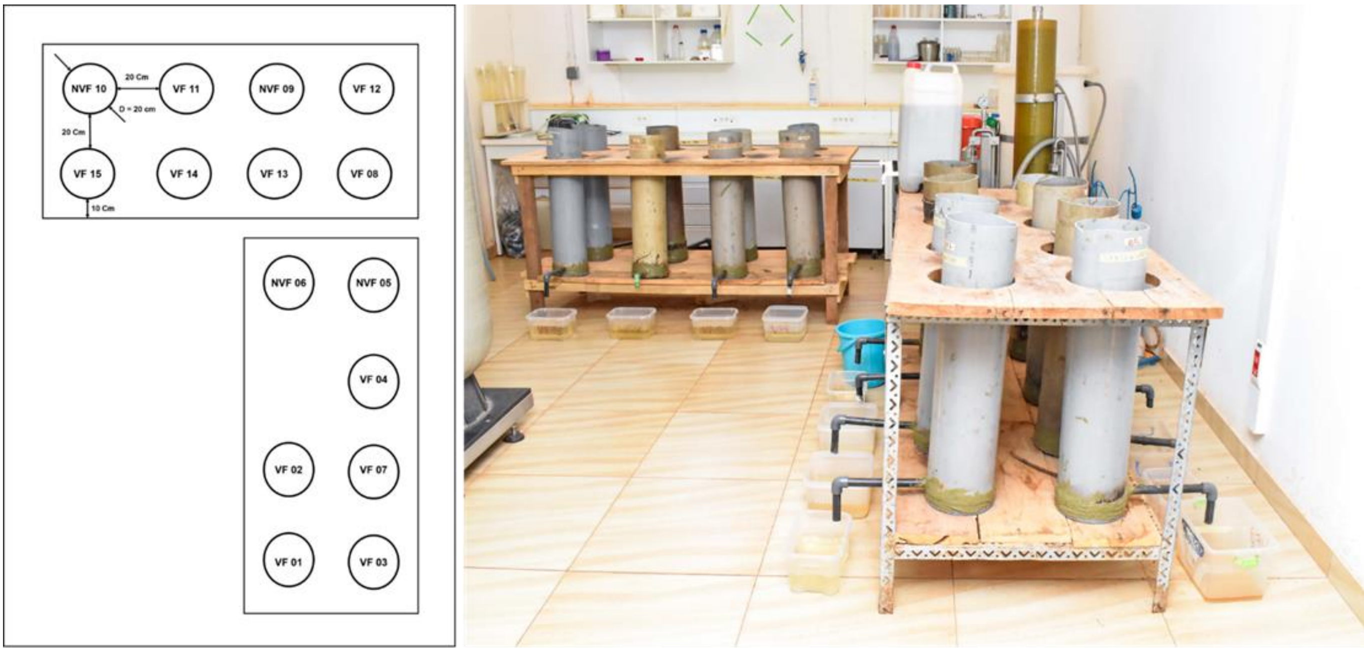
$$QCi = QC_e + V\frac{U_{max}\left(\frac{QC_i}{V}\right)}{K_s + \left(\frac{QC_i}{V}\right)} \tag{13}$$

$$C_e = C_i - \frac{U_{max}\ C_i}{K_s + \left(\frac{QC_i}{V}\right)} \tag{14}$$

2.2. Response surface methodology

2.2.1. Reactors arrangement

The reactors were arranged in the order given as follows in **Figure 24**. **Figure 24 (a)** presents the exact position of each experimental run, and **Figure 24 (b)** shows an overview of all the reactors.



(a) (b)

Figure 24. Arrangement of reactors on the study area (a) and onsite illustration (b)

2.2.2. Analytical procedure

Greywater and filtrate samples were analyzed for COD (Barcode Method), BOD_5 (Acid modification method), TSS (Gravimetric method), NH_4^+ (Nessler method), NO_3^- (Sodium salicylate method), DO, EC and pH were measured using a portable meter. These were determined according to standard methods for examination of water and wastewater. The pollutant removal efficiency was calculated using **Eq. (15)**:

$$Removal\ efficiency = \frac{Ci - Ce}{Ci} \times 100 \quad \textbf{(15)}$$

Where *Ci* and *Ce* represent the influent and effluent concentration values, respectively

- **Measurement of Total Organic Matter (TOM)**

The total organic matter (TOM) is also known as volatile solids (VS). The measurement of volatile solids (VS) is performed according to the FANOR NFT 90029 standard. The filter previously used for suspended solids (SS) is placed in a furnace at 550°C for 2 hours. After this, the filter is transferred to a desiccator to cool down, and then it is weighed. The VS content of the sample is calculated using the following formula:

$$\mathbf{VS = (M2 - M1) / V \times 1000} \quad \textbf{(16)}$$

M_2 is the mass in grams of the filter paper after ignition in the furnace,

M_1 is the mass before ignition, and V is the volume of the sample.

2.2.3. Experimental Design - The Box-Behnken Design (BBD)

The experimental setup used the software Design Expert 11.0.0 to provide the experimental runs and perform analysis of variance (ANOVA). The Box-Behnken Design (BBD) technique was employed to determine the combinations of influencing factors (experimental runs) for the removal of COD. The Box- BBD is a statistical method for experimental design that assesses the interaction effects between multiple factors and identifies optimal conditions for desired outcomes. It is especially valuable for examining the impact of several factors on a response variable while minimizing the number of required experimental runs. The method is widely used in fields like chemistry, engineering, and manufacturing [163]. The advantages of BBD plans include their spherical nature and the fact that they require only three factors. Additionally, the plans are rotatable and offer orthogonal blocking. [164]. It offers excellent precision and predictive capability within the experimental framework [41]. The BBD is also recognized as a cost-effective design, requiring only three levels for each factor, typically configured as -1, 0, and 1. [29].

For our study, the treatment process focused on three primary independent variables: hydraulic loading rate [HLR] (A) COD Initial Concentration [Initial COD] (B), and Earthworms density [EWD] (C), with their actual values and coded values presented in **Table 21**.

Table 21. Factors and their BBD levels

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	HLR	$L/m^2/d$	Numeric	64	191	-1 ↔ 64	+1 ↔ 191	127.5	48.00
B	Initial COD	mg/L	Numeric	1000	4000	-1 ↔ 1000	+1 ↔ 4000	2500	1133.89
C	EWD	Number	Numeric	0	200	-1 ↔ 0	+1 ↔ 200	100	75.59

The response variable chosen was COD removal. The range of actual value was decided based on previous studies' results as underlined previously. A total of 15 experimental runs were conducted during the design process, incorporating 12 different combined coded levels and 3 central coded levels. For a BBD the required number of experiments (N) can be calculated using the following equation **Eq. (17)** [163,165]:

$$N = 2k(k-1) + n_0 \quad \textbf{(17)}$$

With, n_0: the number of central points, and k: the number of factors.

The central point helps to estimate the pure error as well as enabling intermediate levels calculation of the response function. Hence, a system performance estimation within the studied range is possible following the replication of the central point [166].

The comprehensive design matrix is outlined in **Table 22**. A second-order polynomial model might be suitable for establishing the mathematical correlation between the response and the factors in a 3-factor, 3-level experimental design. The response surface regression was used to perform the evaluation of the experimental data. The corresponding equation, **Eq. (18)** is given as follows:

$$B = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad \textbf{(18)}$$

Where B represents the removal efficiency (%), and b_0 the response value obtained from the fitted model at the center point of the experimental design, the intercept. $b_{1\ldots3}$, $b_{11\ldots33}$ and $b_{12,13,23}$ are respectively linear, quadratic and interaction terms. $X_{1\ldots3}$ are dimensionless coded variables. The adequacy of the model was evaluated through Fisher's F-test and probability - p-value - at a 5% significance level. Model adjustment was expressed in terms of determination coefficients (R^2, adjusted R^2, and predicted R^2).

3. Results and discussion

3.1. Kinetic Modelling

3.1.1. First-order model

Reaction orders can differ when there is a variation in the microorganisms, substrates and environmental conditions. The removal of organic pollutants has traditionally been described using a continuous first-order reaction model, where the degradation rate is initially rapid but gradually slows down as the organic material is consumed [167,168]. The first-order kinetic constant K_1 was calculated by plotting $(C_i - C_e)/HRT$ versus C_e COD, see **Figure 25**. **Table 22** presents the various elements involved in defining the first-order model. Only the filters containing earthworms were considered. Furthermore, the data for the evaluation of the kinetic models were collected after the acclimatization phase, i.e., after the stabilization of the filters. The filters VF8 (HLR 191 L/m²/d, 2500 mg/L, EWD 200) and VF12 (127.5 L/m²/d, 4000 mg/L, EWD 200) were therefore not considered, despite initially containing earthworms, as the earthworms did not survive to the filter conditions. Data from three campaigns were collected. From that table and **Figure 25**, The kinetic coefficient K_1 was calculated to be 0.583 d^{-1}, with a corresponding R^2 value of 0.3148. This low R^2 value suggests that the first-order kinetic model does not adequately describe the COD degradation process in the VF system. The weak correlation could indicate that the first order model fails to capture the complexity of the degradation dynamics. Indeed, the first-order kinetic model, links the substrate removal rate to its concentration, shows variation across systems depending on wastewater type and concentration [169]. The weakness of the first order model is potentially due to additional factors influencing the process, such as non-linear interactions, variability in substrate availability, or the role of earthworm activity [2,143]. As such, alternative kinetic models may be more appropriate for accurately representing the COD removal behavior in this system. The results are similar to those of Samal et al. [41] who obtained a kinetic coefficient of 2.0314 d^{-1} and a R^2 of 0.5212 while treating dairy wastewater in a macrophytes assisted vermifilter. Although their kinetic constant is higher than ours, indicating a faster degradation according to this model, they also got to the conclusion that the first order kinetic model could not be used to model the degradation of organic matter through a hybrid macrophyte vermifilter. **Table 22** shows the kinetic coefficient values for different models. From the table, It can be noticed that the first-order kinetic model, links the substrate removal rate to its concentration, shows variation across systems depending on wastewater type and concentration [169]. Regarding the value of the R^2, it can be assumed that the kinetic of the COD removal in the VF is not directly related to the influent concentration. Furthermore, COD removal rate in our study, lower than that reported for synthetic wastewater treated in UAASFF systems by [170]. In their study, the less K_1 is equal to 12.09 that the system is highly efficient in breaking down COD.

Table 22. Experimental data obtained under steady state conditions – First order model

Experimental runs	Influent Concentration (C_i)	Effluent concentration (C_e)	Reactor volume (L)	Flowrate (L/J)	Hydraulic Retention Time (HRT)	$y=C_i-C_e/HRT$	K_1 (First order kinetic constant)
VF1	1.016	0.102	18.840	1.960	9.612	0.095	
VF2	1.016	0.124	18.840	5.600	3.364	0.265	
VF3	3.853	0.371	18.840	1.680	11.214	0.310	
VF7	2.408	0.226	18.840	1.720	10.953	0.199	
VF11	1.016	0.132	18.840	3.280	5.744	0.154	
VF13	2.408	0.260	18.840	3.680	5.120	0.420	
VF14	2.408	0.355	18.840	3.760	5.011	0.410	
VF15	2.408	0.296	18.840	1.960	9.612	0.220	
VF1	0.890	0.110	18.840	1.800	10.467	0.075	
VF2	0.890	0.079	18.840	5.720	3.294	0.246	
VF3	3.560	0.388	18.840	1.600	11.775	0.269	
VF7	2.225	0.254	18.840	1.640	11.488	0.172	
VF11	0.890	0.102	18.840	3.520	5.352	0.147	0.583
VF13	2.225	0.181	18.840	3.600	5.233	0.391	
VF14	2.225	0.274	18.840	3.400	5.541	0.352	
VF15	2.225	0.212	18.840	1.760	10.705	0.188	
VF1	1.161	0.146	18.840	1.880	10.021	0.101	
VF2	1.161	0.126	18.840	5.720	3.294	0.314	
VF3	4.028	0.500	18.840	1.760	10.705	0.330	
VF7	2.640	0.223	18.840	1.840	10.239	0.236	
VF11	1.161	0.156	18.840	3.120	6.038	0.166	
VF13	2.640	0.278	18.840	3.680	5.120	0.461	
VF14	2.640	0.345	18.840	3.600	5.233	0.439	
VF15	2.640	0.243	18.840	1.800	10.467	0.229	

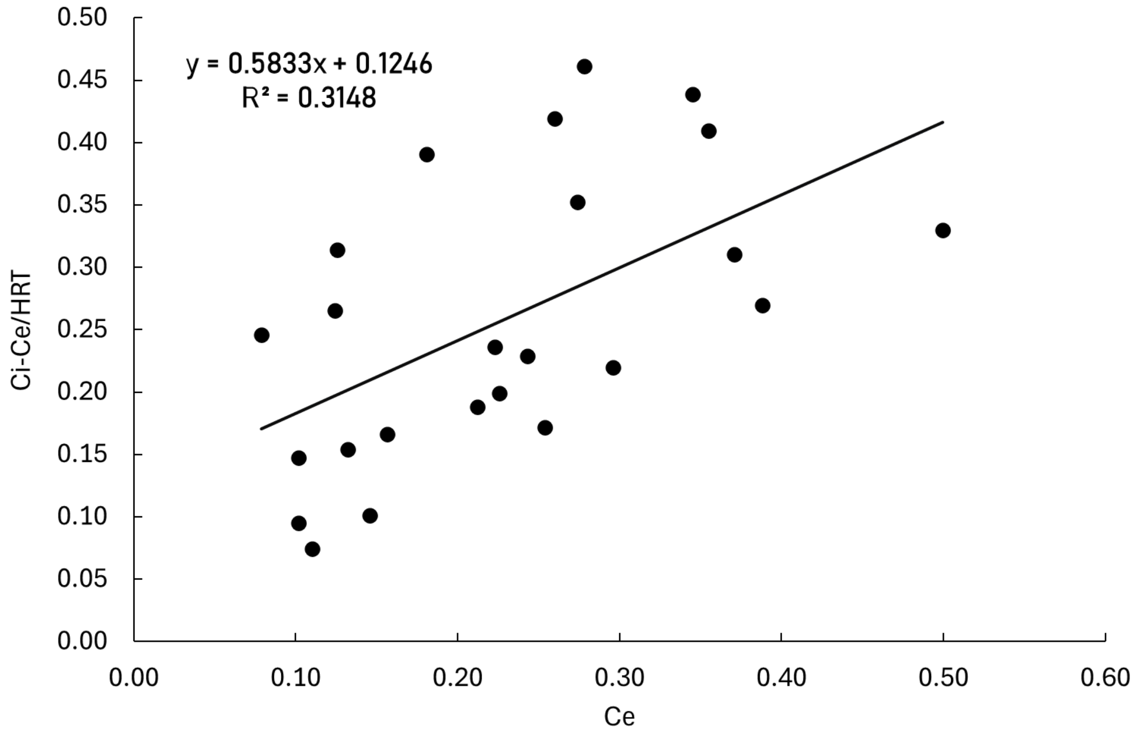


Figure 25. Plot of first-order model

3.1.2. Grau second-order model

The second-order model was also employed to assess the biodegradation rate within the VF systems. To find out the kinetic coefficients (a, b and K_2), **Figure 26** shows the plot of the model. The kinetic coefficient values were determined from the interception and slope of the linear plot on the graph. The coefficients "a" and "b" were estimated to be 0.105 and 1.111, respectively, for COD removal. The coefficient of correlation R^2 was found to be 0.9978. The concentration of substrate in the effluent can be calculated by rearranging **Eq. (19)** and substituting the values of "a" and "b in the **Eq. (8)**. Based on the **Eq. (19)** the relationship between effluent and influent COD concentration and HRT is given as follows:

$$C_e = C_i \left[1 - \frac{HRT}{0.105 + 1.11\, HRT}\right] \quad \textbf{(19)}$$

The average value of K_2 was calculated as 0.938 d^{-1} using the equation: $a = C_i / K_2.X$, which reflects the substrate removal rate for each unit of microorganism. A high R^2 value of 0.9978 suggests a strong correlation between the experimental data and the model's predictions, indicating that the model effectively describes the kinetics of COD in the vermifiltration system. The Grau model is a reliable representation of the degradation process, accurately capturing the microbial dynamics and substrate consumption in the system. The estimated coefficients “a” and “b” for COD removal were found to be 0.105 and 1.111, respectively. These values reflect the efficiency of substrate degradation and the interaction between microbial activity and substrate concentration over time. This further demonstrates that COD degradation in the VF is facilitated by microbial activity, which is significantly enhanced by the presence of earthworms [149,171,172]. Thus, the symbiotic relationship between the earthworms and the microorganism in the system is key to the successful degradation of COD.

Additionally, the average value of the second-order rate of 0.938 d^{-1}, further supporting the high efficiency of the system in degrading organic matter. Samal et al. 2018 [41] obtained the values of 0.0977, 1.195, 0.16 and 0.9817 respectively for a, b, K_2 and R^2. Their results are quite like that of our study, except for kinetic constant. In our study, the kinetic constant (K_2) for COD removal is found to be higher than the corresponding value reported in their study, dealing with dairy wastewater treatment. This result suggests that the greywater treatment system may be more efficient in terms of microbial degradation under the conditions tested. This could be due to the fact that greywater typically contains lower concentrations of complex organic compounds and fats compared to dairy wastewater [173]. The organic matter in greywater, primarily composed of detergents, soaps, and food residues, is generally more readily biodegradable than the proteins and fats prevalent in dairy wastewater, which require more specific microbial activity for degradation. Therefore, the microbial communities involved in the treatment of greywater may be more efficient at processing the available organic load, resulting in a higher second-order kinetic constant. This could also be the reason why Isik and Sponza [174] presented the values of kinetic coefficients a, b and K_2 as 0.562, 1.095 and 0.337 d^{-1}, respectively for synthetic textile wastewater treatment.

Compared to synthetic wastewater treated in UAASFF systems see **Table 23**, where K_2 reached 5.950 d^{-1} with a ‘b’ value of 0.928 [170], the VF system demonstrates lower kinetic efficiency, likely due to the complex composition of greywater. Interestingly, the hybrid UASB treating synthetic coal wastewater reported a K_2 of 1.720 d^{-1} with a ‘b’ value of 0.964 [175] which is not far from the VF. This similarity indicates that the vermifilter system, though simpler in design, competes effectively with more complex technologies for greywater treatment.

Table 23. Experimental data obtained under steady state conditions - Model of Grau second order model

Experimental runs	Influent concentration (C_i) (g/L)	Effluent concentration(C_e) (g/L)	Reactor volume (L)	Flowrate (L/d)	x = Hydraulic retention time (HRT)	Biomass (X) (g/L)	y=(HRT*C_i)/(C_i-C_e)	a (Intercept)=C_i/K_2*X	b (Slope)	K_2 (Second order kinetic constant)
VF1	1.016	0.102	18.840	1.960	9.612	30.800	10.685	0.105		0.315
VF2	1.016	0.124	18.840	5.600	3.364	30.340	3.832	0.105		0.320
VF3	3.853	0.371	18.840	1.680	11.214	17.083	12.409	0.105		2.155
VF7	2.408	0.226	18.840	1.720	10.953	22.830	12.088	0.105		1.008
VF11	1.016	0.132	18.840	3.280	5.744	29.170	6.602	0.105		0.333
VF13	2.408	0.260	18.840	3.680	5.120	19.360	5.739	0.105		1.188
VF14	2.408	0.355	18.840	3.760	5.011	18.130	5.877	0.105		1.269
VF15	2.408	0.296	18.840	1.960	9.612	25.560	10.959	0.105		0.900
VF1	0.890	0.110	18.840	1.800	10.467	74.787	11.943	0.105		0.114
VF2	0.890	0.079	18.840	5.720	3.294	29.540	3.615	0.105		0.288
VF3	3.560	0.388	18.840	1.600	11.775	16.358	13.215	0.105		2.079
VF7	2.225	0.254	18.840	1.640	11.488	23.688	12.968	0.105	1.111	0.897
VF11	0.890	0.102	18.840	3.520	5.352	29.714	6.045	0.105		0.286
VF13	2.225	0.181	18.840	3.600	5.233	18.521	5.697	0.105		1.148
VF14	2.225	0.274	18.840	3.400	5.541	18.855	6.319	0.105		1.127
VF15	2.225	0.212	18.840	1.760	10.705	22.342	11.832	0.105		0.951
VF1	1.161	0.146	18.840	1.880	10.021	52.793	11.459	0.105		0.210
VF2	1.161	0.126	18.840	5.720	3.294	29.940	3.694	0.105		0.370
VF3	4.028	0.500	18.840	1.760	10.705	16.720	12.220	0.105		2.301
VF7	2.640	0.223	18.840	1.840	10.239	23.259	11.184	0.105		1.084
VF11	1.161	0.156	18.840	3.120	6.038	29.442	6.979	0.105		0.377
VF13	2.640	0.278	18.840	3.680	5.120	18.940	5.723	0.105		1.332
VF14	2.640	0.345	18.840	3.600	5.233	18.492	6.020	0.105		1.364
VF15	2.640	0.243	18.840	1.800	10.467	23.951	11.528	0.105		1.053

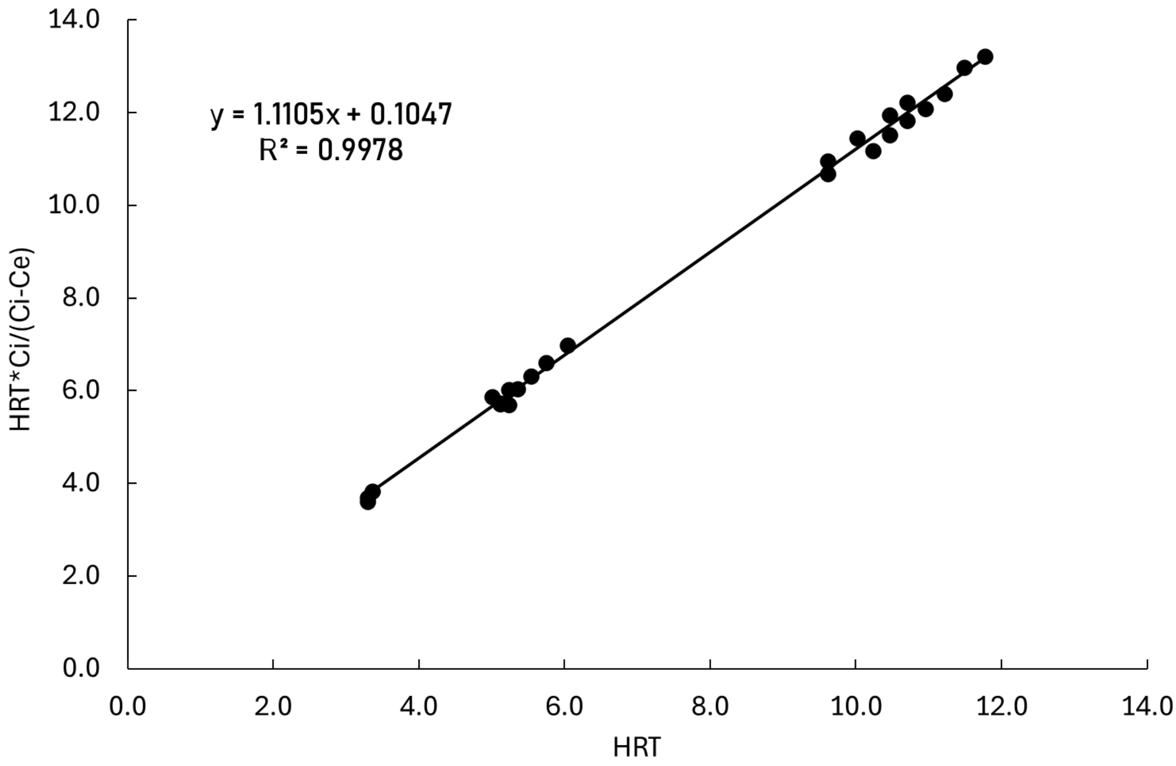


Figure 26. Plot of the model of Grau second order model

3.1.3. Stover-Kincannon model

Stover-Kincannon model was also applied for kinetic modelling of COD removal. **Figure 27** was plotted between the reciprocal of organic loading removal rate and reciprocal of the total organic loading rate for determining the values of maximum utilization rate (U_{max}) and saturation value constant (K_S). Intercept and slope were determined by using linear regression methods. K_S and U_{max} for COD removal was -27.332, and -24.049 respectively. The correlation coefficient (R^2) was 0.999. The negative sign of U_{max} and K_S seems to show the unsuitability of the model to explain the kinetic of degradation of COD by vermifiltration. When considering the variables plotted to obtain the constants of the model, as defined earlier, we have X = V/QC$_i$ versus Y = V/Q (C_i - C_e). Given the high removal percentages, reaching up to 90.25%, the values of C_i and C_i - C_e are very close (see **Table 24**). Under these conditions, when considering the equation of the line Y = aX + b, the term aX will easily exceed Y, leading to a negative intercept. As U_{max} is the inverse of the intercept, this will result in a negative Umax, and consequently, a negative K_S value. This suggests that the Stover-Kincannon model does not adequately describe systems with high degradation rates, where the concentration difference between C_i and C_e becomes very small.

Table 24. Experimental data obtained under steady state conditions – Stover Kincannon model

Experimental runs	Influent concentration (C_i) (g/L)	Effluent concentration (C_e)	Reactor volume reacteur (L)	Q= Flowrate (L/d)	x = V/Q*C_i	Hydraulic Retention Time (HRT)	Y = V/Q(C_i-C_e)	1/Umax (Intercept)	K_S/Umax (Slope)	U_{max}	K_S (Stover Kincannon kinetic constant)
VF1	1.016	0.102	18.840	1.960	9.461	9.612	10.517				
VF2	1.016	0.124	18.840	5.600	3.311	3.364	3.772				
VF3	3.853	0.371	18.840	1.680	2.911	11.214	3.221				
VF7	2.408	0.226	18.840	1.720	4.549	10.953	5.020				
VF11	1.016	0.132	18.840	3.280	5.653	5.744	6.498				
VF13	2.408	0.260	18.840	3.680	2.126	5.120	2.383				
VF14	2.408	0.355	18.840	3.760	2.081	5.011	2.441				
VF15	2.408	0.296	18.840	1.960	3.992	9.612	4.551				
VF1	0.890	0.110	18.840	1.800	11.760	10.467	13.419				
VF2	0.890	0.079	18.840	5.720	3.701	3.294	4.061				
VF3	3.560	0.388	18.840	1.600	3.308	11.775	3.712				
VF7	2.225	0.254	18.840	1.640	5.163	11.488	5.828				
VF11	0.890	0.102	18.840	3.520	6.014	5.352	6.792	-0.042	1.137	-24.049	-27.332
VF13	2.225	0.181	18.840	3.600	2.352	5.233	2.560				
VF14	2.225	0.274	18.840	3.400	2.490	5.541	2.840				
VF15	2.225	0.212	18.840	1.760	4.811	10.705	5.318				
VF1	1.161	0.146	18.840	1.880	8.632	10.021	9.870				
VF2	1.161	0.126	18.840	5.720	2.837	3.294	3.182				
VF3	4.028	0.500	18.840	1.760	2.658	10.705	3.034				
VF7	2.640	0.223	18.840	1.840	3.878	10.239	4.236				
VF11	1.161	0.156	18.840	3.120	5.201	6.038	6.011				
VF13	2.640	0.278	18.840	3.680	1.939	5.120	2.168				
VF14	2.640	0.345	18.840	3.600	1.982	5.233	2.280				
VF15	2.640	0.243	18.840	1.800	3.965	10.467	4.367				

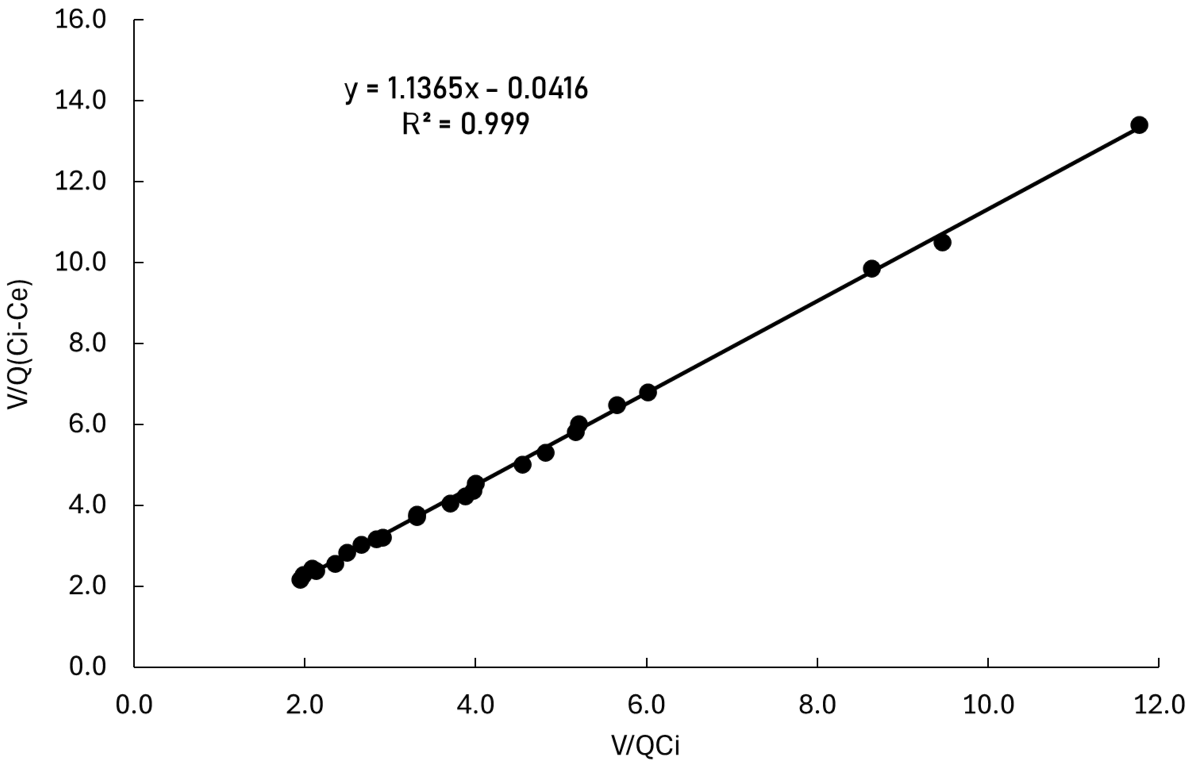


Figure 27. Stover Kincannon model plot

In the study by Samal et al. [27] on the MAVF system, the highest COD removal rates percentages reached approximately 83%. According to the conclusion of their study, the Stover-Kincannon model would be ideal for explaining the degradation kinetics, with Umax, Ks, and R^2 values of 17.39g/L, 20.4, and 0.9961, respectively. The model could be used in the design of the MAVF reactor. These high values would indeed support their conclusion. **Table 25** also shows studies with high Stover-Kincannon kinetic constants.

Table 25. Comparison of kinetic constants across various models

Models	Substrate	COD (g. L^{-1})	HRT (d)	Kinetic parameters			References
First-order				**K_1 (d^{-1})**			
	Dairy wastewater	1.2 – 2.8	0.53 – 1.79	2.0314			[27]
	Synthetic wastewater	1	0.083 – 0.271	12.09 – 30.71			[170]
	Synthetic greywater	1-4	3.294 -11.775	0.583			Our study
Grau second order				**K_2 (d^{-1})**	**a**	**b**	
	Synthetic coal wastewater	-	0.75 – 1.5	1.720	0.078	0.964	[175]
	Synthetic wastewater	1	0.083 – 0.271	5.950	0.042	0.928	[170]
		0.75 – 4.5	0.5 – 1.0	3.582	0.047	1.007	[176]
	Formaldehyde-containing wastewater	-	0.42 – 1.0	0.133	0.64	9.36	[167]
	Dairy wastewater	1.2 - 2.8	0.53 – 1.79	0.16	0.0977	1.195	[27]
	Synthetic greywater	1-4	3.294 -11.775	0.938	0.105	1.111	Our study
Stover-Kincannon				**U_{max} ($L^{-1}d^{-1}$)**	**K_S ($L^{-1}d^{-1}$)**		
	Poultry slaughterhouse	1.6 – 9.1	-	12.148	130.28	-	[177]
	Fruit canning wastewater	-	0.5	109.9	53.5	-	[178]
	Synthetic wastewater	1	0.083 – 0.271	38.46	37.88	-	[170]
	Simulated textile wastewater	4.21	-	7.501	8.211	-	[179]
	Synthetic wastewater	0.75 – 4.5	1	8.3	9.45	-	[180]
	Molasses	2.0 – 15.0		83.3	186.23	-	[181]
	Soybean wastewater	7.5 – 11.45	1 – 1.45	83.3	85.5	-	[168]
	Dairy wastewater	1.2 – 2.8	0.53 – 1.79	17.39	20.4	-	[27]
	Synthetic greywater	1-4	3.294 -11.775	-24.049	-27.332	-	Our study

3.2. RSM model design for COD Removal

The individual and interactive effects of the variables were analyzed through BBD-based modeling to assess COD removal efficiency. Predicted COD removal rates ranged from 42.96 to 90.25%, as outlined in **Table 26**. They closely matched the actual values. Singh et al. [28] obtained slightly higher predicted values for COD removal in the range of 54.4 and 96.67% when optimizing brewery wastewater treatment using vermifiltration. The values also closely matched to the actual values of their experimentations. The high COD removal is largely attributed to the enzymatic activity of microorganisms, which is greatly enhanced by the presence of earthworms. This enzymatic activity facilitates the breakdown of proteins, starch and cellulose, primarily through enzymes such as amylase, cellulase, and protease, enabling the degradation and stabilization of organic matter in the vermifilter [150,171]. The symbiotic interaction between microorganisms and earthworms is crucial to the treatment process. The burrowing actions of earthworms keep the environment aerated, supporting the oxidative processes of aerobic bacteria [28]. Additionally, a correlation has been identified between COD removal in VFs and the activity of antioxidant enzymes, the presence of reactive oxygen species in earthworm tissues, and the length of their burrows [182].

Table 26. Experimental design for optimizing COD removal

Standard order	Run order	Independent variables			COD Removal Efficiency (%)		
		A:HLR ($L/m^2/d$)	B:Initial COD (mg/L)	C:EWD (number of earthworms)	Actual	Predicted	Residuals
11	1	127.5	1000	200	87.36	87.33	0.034
7	2	64	2500	200	90.25	90.25	0.005
15	3	127.5	2500	100	89.66	88.82	0.843
4	4	191	4000	100	42.96	42.92	0.039
8	5	191	2500	200	49.02	50.31	-1.290
3	6	64	4000	100	89.02	90.28	-1.260
2	7	191	1000	100	89.36	88.10	1.260
1	8	64	1000	100	88.35	88.39	-0.039
10	9	127.5	4000	0	47.17	47.20	-0.034
9	10	127.5	1000	0	59.57	60.82	-1.250
13	11	127.5	2500	100	90.17	88.82	1.350
6	12	191	2500	0	47.94	47.95	-0.005
14	13	127.5	2500	100	86.62	88.82	-2.200
12	14	127.5	4000	200	58.91	57.66	1.250
5	15	64	2500	0	56.95	55.66	1.290

The equation for COD removal efficiency (%) is given in **Eq. (20)** below, in terms of coded values. A positive sign (+) indicated a synergistic effect, whereas a negative sign (-) signified an antagonistic effect.

$$\text{COD removal efficiency (\%)} = 88.8167 - 11.9113\,A + 10.8225\,B + 9.23875\,C - 11.7675\,AB - 8.055\,AC - 4.0125\,BC - 6.80333\,A^2 - 4.59083\,B^2 - 20.9733\,C^2 \quad \textbf{(20)}$$

The intercept here is 88.82, meaning that the COD removal measured when not including any of the factors will be 88.82%. The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant (**Table 27.** Regression analysis' results for the COD removal efficiency. So here, on the individual effects we can see on the one hand that factors including HLR and Initial COD have a negative effect on COD removal, i.e., the more HLR and the more Initial COD, the less the COD removal. The results of Singh et al. [28] and Samal et al. [27] lead to same conclusion. On the other hand, EWD has a positive effect on the COD removal, meaning that the more EWD, the higher the COD removal. [28] came to the same conclusion with their model. In terms of magnitude, increase in HLR and in Initial COD by one unit, will lead to a drop of COD removal respectively by 11.91 and 10.82% respectively whereas increase in EWD by one unit will lead in increase of COD removal by 9.24%.

All other considerations have negative effects on the COD removal, including the double effect of one factor on the COD removal, and the combined effects of factors on COD. For example, with other factors holding constant, the combined effect of HLR and initial COD suggests a negative relationship with COD removal. An increase in both factors by one unit while holding EWD constant will lead to a drop in COD removal of 11.77%. In this same way, the combined effect of HLR and EWD on COD removal efficiency is negative, an expected drop in COD removal efficiency by 8.06% as each of the parameters increase by one unit. The lowest effect recorded was that of the combined action of Initial COD and EWD on the removal efficiency, with a magnitude of 4.01 recorded suggesting drop in COD removal efficiency by 4.01% as the two parameters increase by one unit each. The highest recorded effect was that of the double effect of the EWD, with a coefficient of 20.97%, indicating a potential drop in COD removal as effect of the EWD.

Table 27. Regression analysis' results for the COD removal efficiency

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	88.82	1	1.07	86.08	91.56	
A-HLR	-11.91	1	0.6524	-13.59	-10.23	1.0000
B-Initial COD	-10.82	1	0.6524	-12.50	-9.15	1.0000
C-EWD	9.24	1	0.6524	7.56	10.92	1.0000
AB	-11.77	1	0.9227	-14.14	-9.40	1.0000
AC	-8.06	1	0.9227	-10.43	-5.68	1.0000
BC	-4.01	1	0.9227	-6.38	-1.64	1.0000
A^2	-6.80	1	0.9604	-9.27	-4.33	1.01
B^2	-4.59	1	0.9604	-7.06	-2.12	1.01
C^2	-20.97	1	0.9604	-23.44	-18.50	1.01

On the accuracy of the coefficients, we can see that standard errors stand in the range of 0.6524 to 0.9604, indicating accurate results when considering the 95% interval confidence values given by the model (**Table 27**). For example, on the combine effect of HLR and EWD, it can be seen a magnitude of - 8.06 and a standard error of 0.9227, meaning a range of -7.14 and -8.89, which falls within the given 95% interval confidence of -5.68 and -10.43. All considered computed values fitted with all the given 95% CI, suggesting accuracy of the model.

Finally, here the VIF are very close to 1, so factors are orthogonal. As VIFs greater than 1 are very less and departure from 1 very less (1.01), multi-collinearity has been avoided in data distribution. Indeed, all VIF large than 1 are less than 10, making them tolerable.

The significance of the developed model was assessed using ANOVA, as detailed in **Table 28**. ANOVA helps to determine the relationship between the response and the independent variables.

Table 28. ANOVA for Quadratic model

Source	Sum of Squares	degrees of freedom	Mean Square	F-value	p-value	
Model	5382.29	9	598.03	175.61	< 0.0001	significant
A-HLR	1135.02	1	1135.02	333.30	< 0.0001	
B-Initial COD	937.01	1	937.01	275.15	< 0.0001	
C-EWD	682.84	1	682.84	200.51	< 0.0001	
AB	553.90	1	553.90	162.65	< 0.0001	
AC	259.53	1	259.53	76.21	0.0003	
BC	64.40	1	64.40	18.91	0.0074	
A^2	170.90	1	170.90	50.18	0.0009	
B^2	77.82	1	77.82	22.85	0.0050	
C^2	1624.17	1	1624.17	476.94	< 0.0001	
Residual	17.03	5	3.41			
Lack of Fit	9.66	3	3.22	0.8740	0.5727	not significant
Pure Error	7.37	2	3.68			
Cor Total	5399.32	14				
		Model Summary Statistics				
Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	15.50	0.5102	0.38	0.1170	4767.69	
2FI	14.86	0.6728	0.43	-0.0451	5642.59	
Quadratic	**1.85**	**0.9968**	**0.9912**	**0.9683**	**171.12**	**Suggested**
Cubic	1.92	0.9986	0.99			Aliased

The higher F-value (175.61) indicates the model's significance. The variance explained by the model is substantially greater than the variance within the data. The model is thus adequate in explaining the variation in the response derived from it. The 'Prob > F' is less than 0.05 (95% confident level) suggesting the significance of each term in the model. In this model, terms *A, B, C, AB, AC, BC,* A^2, B^2, and C^2 are deemed significant. The p-value for lack of fit was found to be 0.5727, indicating that lack of fit is insignificant. The model's significance and lack of fit insignificance confirm its accuracy. The R^2 and adj-R^2 values account for possible dissimilarities in results due to noise in the model. Pred-R^2 indicates the variability in predicted COD removal. The high values of R^2 (0.9968), adj-R^2 (0.9912) and Pred-R^2 (0.9683) obtained for this study, suggest that the model effectively explains the actual COD removals and the predicted values are reliable [183]. [28] obtained similar values of R^2 (0.99), adj-R^2 (0.99) and

Pred-R^2 (0.97) when optimizing the same three parameters for the removal of COD by vermifiltration. The "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 31.430 obtained for the developed model, indicates an adequate signal [184]. This model can be used to navigate the design space.

3.2.1. Variables interaction and the mutual effect on response

Figure 28 (a) represents the correlation between predicted and actual COD removal efficiencies.

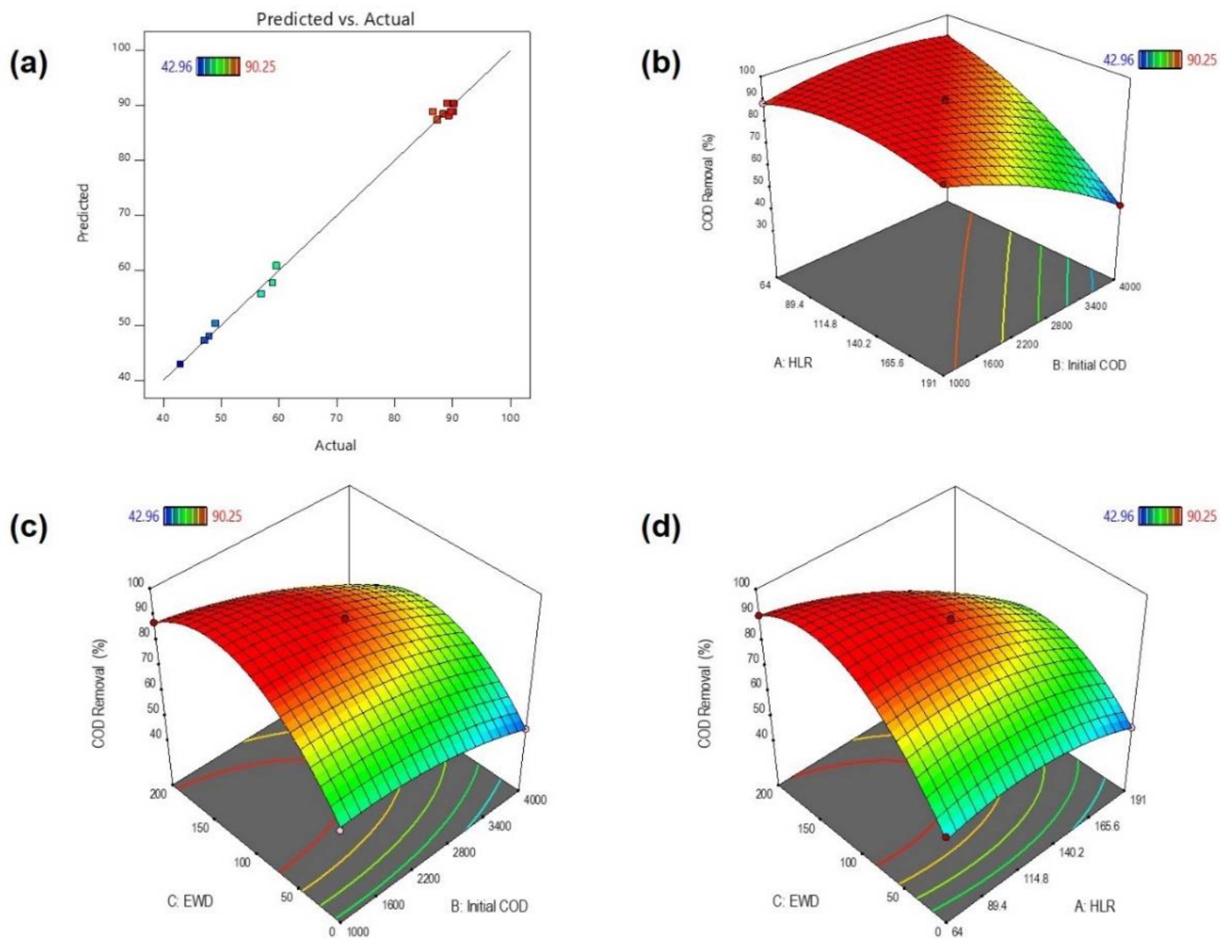


Figure 28. 3-D Response surface plot showing : (a) Predicted vs actual, (b) Effect of Hydraulic Loading rate (HLR) and Initial COD of greywater, (c) effect of Earthworms density (EWD) and Initial COD of greywater, (d) effect of HLR and EWD.

From the blue ones which represent low values of COD (at least 42.96%) to the red ones which correspond to the highest (up to 90.25%). We can see an alignment of the points on the straight line. This shows a good correlation between the model's predictions and the observed outcomes.

The interactions between independent variables and their influence on COD removal are presented in **Figure 28 (b, - d)**. The figure shows the response surface plots of Initial COD, HLR and EWD. The method to obtain the COD removal response involved varying two variables while holding the other variable constant.

3.2.2. Individual effect of EWD and cross effect with the other parameters

Concerning the effect of the EWD on the response, the systems without earthworms (Biofilters) were seen to yield highest COD removal efficiencies of 59.57%, whereas the COD removal efficiency in the systems with EWD (VFs) of 100 or 200 Earthworms were found to be the highest at 90.25%. For example, from **Figure 28 (d)**, at the EWD of 0 and 200 earthworms, the average observed COD removal rates were 58 and 77%., with HLR of 127.5 L/m^2/d, and Initial COD of 2500. When varying the HLR the same observations could be made. The same observations have been made by Singh et al., (2019a) while optimizing the treatment of brewery wastewater. They obtained 65% as the highest COD removal efficiency for biofilters and 97% as the highest for VFs. The difference between the two types of system is practically the same between our results and theirs, 30.55% and 32% respectively. The role of earthworms is thus crucial for the treatment, in both types of wastewaters. The high COD removals is largely attributed to the enzymatic activity of microorganisms, which is greatly enhanced by the presence of earthworms. This enzymatic activity facilitates the breakdown of proteins, starch and cellulose, primarily through enzymes such as amylase, cellulase, and protease, enabling the degradation and stabilization of organic matter in the vermifilter [150,171]. The symbiotic interaction between microorganisms and earthworms is crucial to the treatment process. The burrowing actions of earthworms keep the environment aerated, supporting the oxidative processes of aerobic bacteria [28]. An investigation taken by [185] on the bacteria in the VF system shows that is a greater abundance of aerobic bacteria compared to facultative and anaerobic bacteria. Additionally, a correlation has been identified between COD removal in VFs and the activity of antioxidant enzymes, the presence of reactive oxygen species in earthworm tissues, and the length of their burrows [182].

It can also be noticed on **Figure 28 (c)** that values of less than 70 worms lead to a significant drop in COD removal whatever the Initial COD values are. In addition, the perturbation graph represented by **Figure 29** demonstrates that the EWD is the factor that most influences the response. The curve representing the EWD is the most deviated from the horizontal line**.**

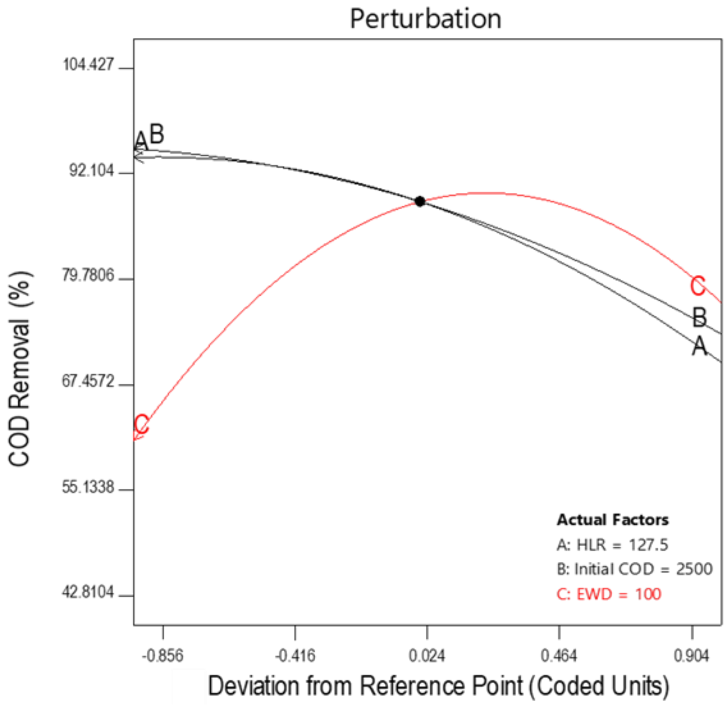


Figure 29. Perturbation plot, showing the trend of the effect of the factors (A-HLR, B-Initial COD, and C-EWD) on the response (COD removal).

3.2.3. Individual effect of HLR and cross effect with the other parameters

Figure 28 (b) reveals that while keeping EWD and Initial COD respectively at 100 earthworms and 2500 mg/L, the COD removal for HLRs of 64 and 191 L/m^2/d are respectively 94 and 70%. It shows that the increase in HLR leads to the decrease of COD removal efficiency. Samal et al. (2018a) and Singh et al. (2019a) had the same analysis while optimizing the vermifiltration treatment. For the first cited authors, when HLR value increases beyond the average value, COD removal efficiency started decreasing for the highest influent COD of 2800mg/L. For the second, there is a 21% difference between the removal efficiencies for their low and their high HLR with 2000 mg/L as initial COD and EWD of 100.

This could be explained by the fact that, as the HLR increases, especially with a high Initial COD, earthworm activity tends to decrease, potentially leading to the accumulation of clogging materials in the initial section of the filter bed. This accumulation can result in severe clogging issues [186]. **Figure 30 (a and b)** shows the views from the top of the experimental runs 4 (**Figure 30 (a)**) with HLR 191 L/m^2/d and Initial COD 4000 mg/L and experimental run 6 (**Figure 30 (b)**) with HLR 64 L/m^2/d and Initial COD 4000 mg/L. The accumulation of clogging materials can be observed on the top of the experimental run 4.

(a) (b)

Figure 30. View from the top of the filters: Experimental Run 4 (a) and Experimental Run 7 (b).

Suhaib and Bhunia (2022) also reported that the instability of earthworm casts within the filter bed at higher HLRs can further reduce the beds' permeability. A too-high HLR is also known to be reducing the hydraulic retention time, the contact time of wastewater with the filter media, hindering the adequate degradation and stabilization of organics by the earthworms [172].

However, when considering the mutual effect of HLR and Initial COD, on the COD removal, **Figure 28 (b)**, reveals that, even when increasing the HLR to the maximum value of 191 L/m^2/d. it does not have a bad effect on the COD removal when the Initial COD is kept at the lowest. **Figure 28 (b)** shows the top of the experimental run 7, HLR 191 mg/L and Initial COD 1000 mg/L with no accumulation. The COD removal rate observed for this combination is 89.36%. COD removal starts decreasing significantly, under 86%, at the maximum HLR when the Initial COD is beyond 1200 mg/L. However, it can be observed from the same figure that when progressing from lower to highest values of the parameters taken together, the effect on the response is worse even with a medium to high EWD. Indeed, it will be therefore judicious to keep the HLR at the lowest when dealing with medium to high-load greywaters.

3.2.4. Individual effect of initial COD and cross effect with the other parameters

From **Figure 28 (b)**, an increase in the initial COD of the greywater doesn't affect the COD removal, unless in the case of High HLRs. With the maximum initial COD of 4000 mg/L, the COD removal significantly reduced to below 86% for a HLR of over 89.4 L/m^2/d. For example, under a constant EWD of 100 and HLR of 70 L/m^2/d, the COD removal for a 4000 mg/L initial COD is 89% and is 90% for 2000 mg/L in the same conditions but the difference is just 1%. Meanwhile, the COD removal is 74% for 127 L/m^2/d at initial COD of 4000 mg/L. That is 15% lower than the one for 70 L/m^2/d. These results are not in good agreement with the those obtained by Singh et al., (2019a). The authors found that the variation in COD removal efficiency between influent organic strengths of 2000 and 4000 mg/L consistently remained within a narrow range of 2–3% across all combinations of HLR and EWD. The drop of the COD removal due to the increase of Initial COD, at High HLR could be explained by the same phenomenon of accumulation of clogging particles on the top of the filter. A higher concentration of organics could lead to an accumulation of organic matter, if microorganisms and earthworms are unable to consume it within an appropriate timeframe. This could then result in clogging issues, thus, impairing the treatment efficiency [187]. As mentioned previously, the time for degrading a high amount of organic matter, is shorter and insufficient when the HLR is higher. Mutual effect of EWD and Initial COD on the COD removal also shows similar trends as that of both EWD and HLR on COD removal. As illustrated in **Figure 28 (d)**, COD removal increases when high to moderate and low to moderate values are recorded respectively for the EWD and Initial COD parameters.

3.2.5. Model adequacy assessment based on residuals

In addition to R^2, adj-R^2, and pred-R^2, the developed models' acceptability was assessed using studentized residuals. For well-performing models with large sample sizes, minor deviations from normality might not significantly impact the outcomes. If the plots of normal probability exhibit a straight line, it suggests that errors are normally distributed. **Figure 31 (a)** depicts the plot of residuals against the normal distribution for COD removal performance. The trend follows a straight line, indicating minimal variation in residuals and validating the model. Moreover, the plot of residuals vs. predicted response displayed in **Figure 31 (b)** shows that residuals are distributed on both sides of the centerline within the boundaries of - 3 to + 3. This indicates that there is no discernible pattern or irregular structure, suggesting that the proposed model is appropriate and there are no apparent violations of the assumptions of constant variation or independence. **Figure 31 (c)** demonstrates how the residuals are distributed across their experimental runs. The trends and distribution of residuals for COD removal efficiency shown, conform adequately to a normal distribution. Similar observations was reported by Sharma et al., (2013) and Samal and Dash (2021), during their study respectively on characteristics of a wire electric discharge machine process parameters with RSM and the modelling of pollutants removal in integrated vermifilter.

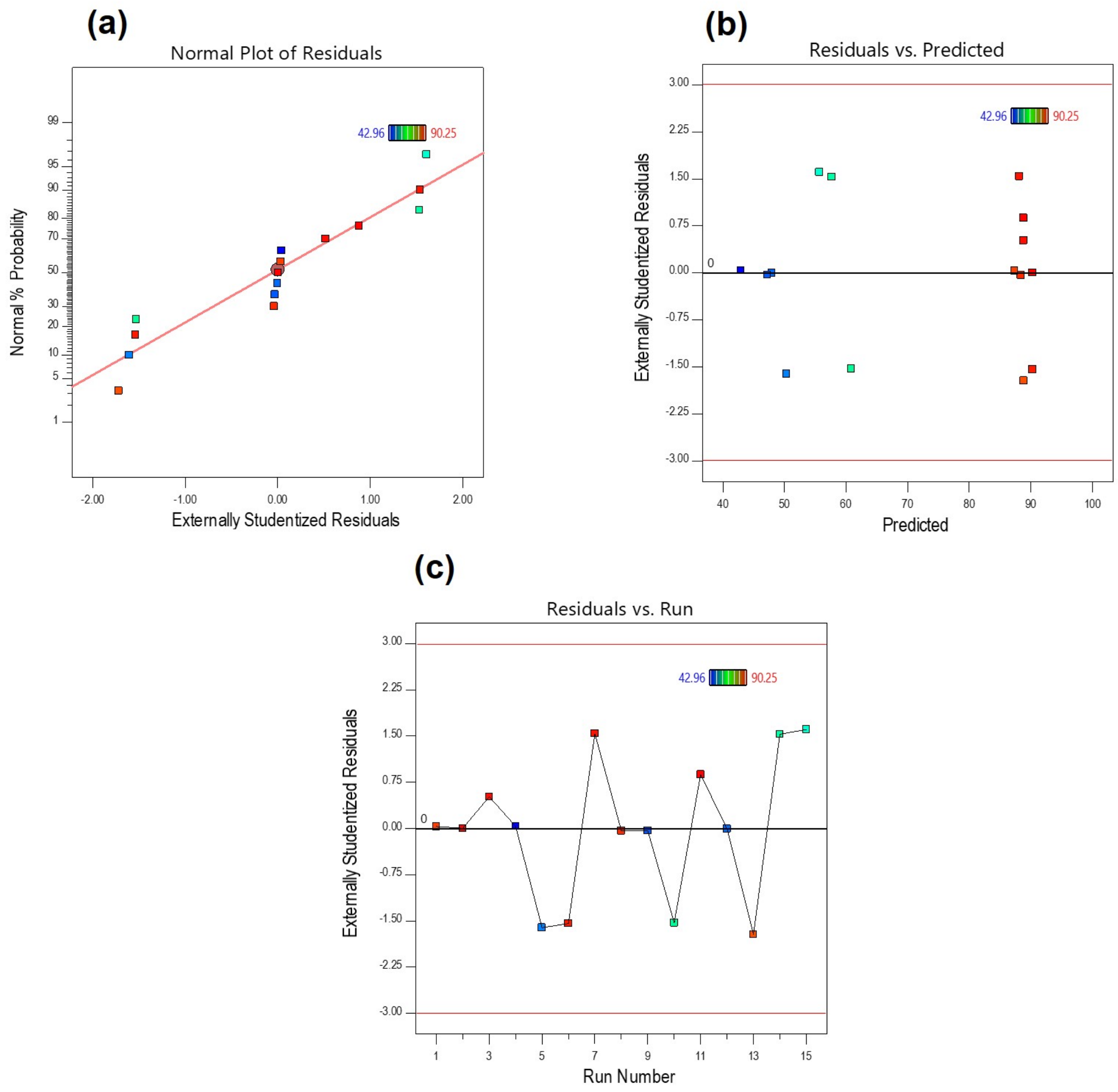


Figure 31. Residual plots of COD removal efficiency of VF reactor; (a) normal plot of residual, (b) residual versus predicted value (c) residuals versus run

3.2.6. Optimization and validation

Numerical optimization was conducted to determine the optimal point for achieving maximum COD removal. Three conditions were considered. Firstly, the optimum COD removal and all the independent factors, in range; secondly, the maximum COD removal for the maximum input of COD, and the other factors in range; and thirdly, the maximum COD removal for the maximum HLR and the other factors in range. The optimum COD removal, with all the factors in range, was found to be 91.51 % with 133 $L/m^2/d$ of HLR, 1087mg/L i.e.,1.087 kg/m^3 for initial COD and 178 Earthworms, achieving a residual COD value of 92.29 mg/L, that meet the requirements for the WHO discharge and reuse standards (World Health Organization, 2006). The other optimized solutions are found in **Table 29.** The solutions were chosen based on higher desirability. For the model validation purposes, experiments were conducted using both real domestic greywater and synthetic domestic greywater. The characteristics of the real greywater are shown in **Table 30.** The ratio BOD/COD also called the biodegradability index (BI) helps to indicate the toxicity of an effluent. The BI of real domestic wastewater falls at 0.652 which is over 0.3, suggesting effectively that the effluent is highly biodegradable. A large portion of the organic matter can be broken down by biological processes [190]. [191] also observed a high pollutant removal load for WWTPs when BI ranged from 0.4 to 0.6.

Table 29. Combinations of independent variables for validation experiments.

Type of greywater	Initial COD (mg/L)	EWD (Number of earthworms)	HLR ($L/m^2/d$)	Actual COD removal (%)	Predicted COD removal (%)	Residuals (%)
Real domestic greywater	3506	136	79	91.9	92.5	**0.6**
Synthetic domestic greywater	1087	178	133	88.54	91.51	**2.97**
Synthetic domestic greywater	1000	114	191	84.1	88.42	**4.32**
Synthetic domestic greywater	4000	130	64	91.75	92.38	**0.63**

Regarding the real greywater, the actual COD removal was 0.6 % lower than the predicted value (see **Table 29**). The results align well with those reported by [15] and [28], who found residuals of 2.08% and 3.11 % during the validation of their RSM model for optimizing BOD and COD removal in dairy wastewater and brewery wastewater, respectively. For synthetic greywater the actual COD removal was respectively 2.97%, 0.63 % and 4.32% lower than the predicted values. [15] and [28] found 0.63% and 0.86% respectively. For the real and synthetic greywaters, the difference between the actual and the predicted values of removals were within 5%. Such minimal residuals demonstrate the strength of Eq. (3) for its application in the design and implementation of the vermifiltration systems for domestic greywater treatment.

Table 30. Characteristics of real domestic greywater

Parameters	Real domestic greywater
T°(C)	25.00
pH	6.81 ± 0.09
DO (mg/L)	2.04 ± 0.05
EC (mS/cm)	1.19 ± 0.26
SS (mg/L)	2123.33 ± 50.33
COD (mg/L)	3506 ± 64.37
BOD_5 (mg/L)	2286 ± 29.46
NO^{-}_3 (mg/L)	36.67 ± 3.51
NH^{+}_4 (mg/L)	2.14

3.2.7. Earthworms' growth

Table 31 represents growth characteristics of earthworms during the experiment. The percentage of increase in the number of earthworms in all the vermifilter (VF) units were in the range of 17 – 52.5%. The results suggest that the filters were suitable for earthworms' growth. Similar observations were made by Samal et al. [27] . In some units such as the ones corresponding to the runs 4, 5 and 14, there was no earthworm left at the end of experiment. With the accumulation of clogging particles on the top of the VFs, clogging issues occurred. This allows for constant water stagnation above the filter, preventing the aeration of the filter and the respiration of the earthworms, thus causing their death.

Table 31 : Earthworms grow characteristics

Run order	Number of earthworms		Percentage of increase
	Initial	Final	
1	200	289	44.5
2	200	305	52.5
3	100	128	28
4	100	0	-
5	200	0	-
6	100	122	22
7	100	119	19
8	100	130	30
9	0	0	0
10	0	0	0
11	100	121	21
12	0	0	0
13	100	117	17
14	200	0	-
15	0	0	-

3.2.8. Implications of results

- **Understandings of mechanisms**

The importance of earthworms in a vermifiltration system has been demonstrated in this study as in many others on vermifiltration. However, it has also been demonstrated through this study that, the variation in the density, EWD at the beginning of the treatment is not as that important parameter in terms of varying in number, after reaching 70 earthworms equal to 3500 worms/m^3. Many authors worked with 10 000 worms/m^3 [149,150,192]. With regards to the obtained results, the EWD could be less than that at the beginning of the treatment and allow nevertheless an optimal treatment. Earthworms will multiply in the filter until the Biotic capacity. However, enough is necessary at the beginning to allow the treatment to occur with regard also to the deaths that can happen with the acclimatization period. The highest number didn't also have any negative impacts on the performance. On the other hand, one of the highest threats to the filter with a high HLR is clogging, which is one of the greatest problems of sand filtration. Although it has been reported that the action of earthworms significantly delays this problem [4], it was demonstrated in this study that with an inadequate HLR, the earthworms themselves become trapped in the filter. They will therefore be unable to breathe due to prolonged clogging, which can lead to their death, thus drastically reducing the filter's performance. Another reason is that with the continuous death of the earthworms, the burrowing activity will be increasingly reduced, further accelerating clogging. Additionally, the entire body of earthworms is made of organic matter [193]. Their death would lead to an excess of organic matter in the system, further contributing to clogging and increasing the residual organic matter content in the treated effluent. In this case, the more the EWD, the lower the efficiency of the system. Thus, the optimal filter HLR must be determined strictly with the regards to the initial COD. This will surely reduce the quantity of water that can be treated at the time but will allow the filter to be more efficient and last longer.

- **Engineering implications**

Considering the three parameters, the two parameters that must be defined for the design, are the HLR and the initial EWD. They must be set regarding the Initial COD. Regarding the results of our study, the HLR is the most sensible parameter when designing a vermifilter and must be set carefully.

The available results on greywater investigations, especially in our study area, show that initial COD concentrations are highly variable from day to day [4]. This is justified by the variability of domestic activities over the days. For optimal performance, given the significant variability in inlet concentrations, the engineer needs to design for the mean HLR that allows the system to operate continuously and maintain the same performance as described in the literature. It is also necessary for the engineer to design a cylindrical filter with layers of the same thickness as those in our study. The variability in influent concentrations means that the filter cannot be designed based on the optimal influent concentration of 1087 mg/L COD. Instead, the filter should be sized according to the average discharge values, which, according to [16,194], are around 2500 mg/L. Based on our model, an initial COD of 2500 mg/L would require an HLR of 122.96 and an EWD of 115 to achieve a COD removal efficiency of 90.63%. These values will be used to assessment cost for this domestic greywater treatment.

4. Conclusion

This chapter aimed at the modeling and optimization of COD removal through vermifiltration using two types of models: kinetic models and Response Surface Methodology (RSM).

Kinetic modeling revealed notable differences in the suitability of various approaches. The first-order kinetic model was found to inadequately represent COD degradation in the vermifiltration system, with a low correlation coefficient ($R^2 = 0.3148$) and a kinetic constant K_1 of 0.583 d^{-1}. This model's limitations stem from its inability to capture the complex interactions between earthworm activity, microbial processes, and substrate variability. Conversely, the second-order kinetic model provided a better fit, with a high correlation coefficient ($R^2 = 0.9978$) and parameters (a = 0.105, b = 1.111, and $K_2 = 0.938$ d^{-1}) that reflected the significant role of microbial activity and the symbiotic relationship between earthworms and microorganisms. The Stover-Kincannon model, however, was unsuitable for the high degradation rates observed in this study, as the resulting negative values for U_{max} and K_s indicated inconsistencies in its application.

Beyond kinetic modeling, RSM was employed to optimize operational parameters and assess their influence on COD removal. The analysis demonstrated that Hydraulic Loading Rate (HLR), Initial COD concentration, and Earthworm Density (EWD) were critical factors affecting system performance. The interactions between these variables were also significant, as indicated by the statistical model developed. Optimal conditions for COD removal were identified at an influent COD of 1087 mg/L, an HLR of 133 L/m²/d, and an EWD of 178 earthworms, yielding a predicted removal efficiency of 91.51%. Confirmatory tests closely matched the predictions, further validating the model.

The insights provided by RSM highlight its utility in optimizing the design and operation of vermifiltration systems. Combined with the evaluation of kinetic models, this chapter underscores the potential of vermifiltration as an effective, adaptable, and sustainable technology for greywater treatment.

CHAPTER 4: SUSTAINABILITY ASSESSMENT OF THE VERMIFILTRATION SYSTEM

1. Introduction

This chapter provides a comprehensive assessment of the sustainability of the vermifiltration system by addressing two critical dimensions: the agronomic and hygienic quality of the vermicompost by-product, and the economic feasibility of greywater treatment through this technology.

The agronomic and hygienic analyses evaluate the vermicompost's potential as a high-quality soil amendment. Nutrient content, organic matter composition, and safety parameters such as the presence of pathogens and helminth eggs are assessed against international standards NFU and FAO. Techniques for detecting pathogens like Salmonella spp. and helminth eggs, using methods established by AFNOR and the US-EPA, ensure reliable determination of its hygienic quality, while staining protocols validate the viability of any detected helminth eggs.

In parallel, the economic feasibility of the vermifiltration system is examined through a life cycle cost analysis. This includes an evaluation of investment, operational, and maintenance costs, balanced against the system's potential benefits, such as water reuse in regions vulnerable to water scarcity. The study incorporates indicators like net project value and benefit-cost ratio to provide a holistic understanding of the system's sustainability.

By combining agronomic, hygienic, and economic insights, this chapter underscores the potential of vermifiltration technology as an integrated solution for sustainable greywater management.

2. Materials and Method

2.1. Analysis methods for vermicompost

2.1.1. Sampling

The vermicompost samples (**Figure 32**) were collected at the end of the experiment, representing the active layer of the vermifilter without earthworms. For the analysis, 3 kg of compost was sampled and divided into three separate bags (1 kg per bag) From each bag, 50 g of compost was taken and diluted with distilled water to obtain a final volume of 500 ml. These solutions were then used to conduct the required analyses. The analyses were conducted to evaluate the agronomic and hygienic quality of the vermicompost. The results of the analysis where then compared to two norms from the National Farmers' Union NFU 44-095, and the Food and Agriculture Organization of the United Nations (FAO)

(a) **(b)**

Figure 32. Vermicompost (a) and vermicompost samples (b)

2.1.2. Agronomy quality

- **Phytotoxicity of vermicompost**

Phytotoxicity was assessed using tomato seed (Lycopersicon esculentum L.) germination tests. The seeds were immersed in a solution prepared from a compost extract diluted at a ratio of 1/10 with distilled water. The tests were conducted by placing 10 seeds on Whatman paper, each in separate Petri dishes. A group of seeds was exposed to distilled water as a control. All experiments were replicated. The Petri dishes were sealed with Parafilm to minimize water loss while allowing air circulation. They were then kept in the dark at a temperature of 26°C for 72 hours. The Germination Index (GI) was calculated using **Eq. (21)** described by Zucconi et al. [195]:

$$GI = \frac{nVSS \times RLS}{VSS \times RLC} \times 100 \quad \textbf{(21)}$$

Where **GI** represents the germination index, **nVSS**, the number of viable seeds in sample, **nVSC**, the number of viable seeds in control, **RLS**, the root length in sample, and **RLC**, the root length in control.

- **Evaluation of the nutrient content**

The nutrient content of the final compost was analyzed using the aqua regia digestion method. A 1 g dried and ground sample was digested with 1 ml of concentrated nitric acid (HNO_3) and 3 ml of concentrated hydrochloric acid (HCl). The mixture was boiled for 15 minutes, cooled, and filtered into a 100 ml volumetric flask. The filtrate was adjusted to the desired volume with demineralized water. Nutrient concentrations (calcium, magnesium, and potassium) were determined using the ICP-OES AVIO 220 Max spectrometer.

Total Organic Carbon (TOC)

TOC was estimated by dividing TOM by 2 (as shown in **Eq. (22)**), based on a conversion factor recommended for organic material.

$$\%TOC = \%TOM*0.58 \quad \textbf{(22)}$$

Kjeldahl Nitrogen (TKN)

TKN was measured in three steps:

- Mineralization: Samples were digested with sulfuric acid at 180°C, 250°C, and 340°C for 2 hours each.
- Distillation: Neutralized digests were distilled using a BUCHI K-355 distiller.
- Titration: The distillate was titrated with 0.04 mol/L sulfuric acid.

Nitrate, Nitrite, and Ammonium Nitrogen

Nitrogen forms (N- NO^{-}_{3}-, N-NO^{-}_{2}, N-NH4+) were analyzed using reagents and a spectrophotometer. Results were directly read from HACH DR 3900.

Derived calculations included:

- Organic Nitrogen: Norg = TKN - N(NH_4^+)
- Total Nitrogen: Ntotal = TKN + N (NO^{-}_{3}) + N(NO_2)

2.1.3. Hygienic quality

- **Pathogenic Microorganisms**

The quantitative detection (Microorganisms/g) of Salmonella in 10 g of compost samples was performed according to the AFNOR NF U44-051 (2018) standard in two stages using the following media: Rappaport Vassiliadis Soy Broth for enrichment and CHROMagar™ Salmonella for isolation. The search for helminth eggs was conducted following the method of the U.S. Environmental Protection Agency (US-EPA) (Schwartzbrod et al., 1998). The viability of helminth eggs was determined using the Safranin O staining method developed by De Victorica & Galván [196]

The conditions for the inoculation of pathogenic microorganisms are summarized in **Table 32** below.

Table 32. Inoculation conditions for hygienic quality

Germs	Method	Culture medium	Incubation temperature	Incubation time	References
Salmonella	Most probable number	Rappaport Vassiliadis Soy Broth + CHROMagar™ Salmonella	44.5 °C	24 h	AFNOR U44 – 051 (2018)
Helminths eggs	Separation method (two-phase sedimentation and flotation) followed by microscopic observation	Reagents: acetate-acetate buffer, di-ethilic ether, zinc sulfate, safranin O			[196,197]

2.2. Methodology for economic feasibility assessment

2.2.1. The life cycle cost

In the life of the vermifiltration system, different costs are involved as aforementioned. Evaluation of the cost throughout the life of the system can be computed with one indicator called the Life Cycle Cost (LCC) of the system. Here, the LCC of our system was computed using a methodology well know from the literature for the wastewater treatment systems [198–200]. Such a model computed the net present value (NPV) of all costs that can occur during the service life of the systems (t) accounting for yearly value changes due to the consideration of the real discount rate (r). **Eq. (23)** gives the model used for a define year (t).

$$LCC\ (\$/m^3) = \sum_{t=0}^{T} \frac{C_t}{(1+r)^t} \quad \textbf{(23)}$$

The yearly costs (C_t), with the *LCC* being their sum throughout the duration of the project or the system. The consideration of the real discount rate (r) is important as parameters including inflation and bank interest rates are most of the time included in projects.

About the computing of the yearly costs, the following equation was used. **Eq. (24)**:

$$C_t = C_I + C_{O\&M} \quad \textbf{(24)}$$

The investment cost (C_I) is only valid the year 0 (t = 0) where the system is constructed or implemented, while the cost of the operation and maintenance intervene at each year because of their nature. Indeed, operation and maintenance activities take place every year to keep the system in a good state. The operation and maintenance costs only represent the costs of material changes in the case of our system.

The following equations (**Eqs. (25 - 26)**) gives the two types of costs defined.

$$C_I = C_T + C_{OC} \quad \textbf{(25)}$$

$$C_{O\&M} = C_{mw} + C_{OC} \quad \textbf{(26)}$$

Where C_T corresponds to the cost of all tanks (C_T), C_{OC}, the cost of other components including cost of locally sourced external components including piping elements, sand, gravel, and sawdust, and C_{mw} the costs for material washing (after a time of use, the sand and gravel are washed and can be reused).

2.2.2. Economic Benefit

The economic benefit (*EB*) was computed thanks to the potential water savings that the treated water consists of along with the associated to the volume of that water reused by the treatment systems. The following equation (**Eq. (27))**:

$$EB = \sum_{t=1}^{n} \frac{W_r \times C_{ws}}{(1+r)^t} \quad \textbf{(27)}$$

Where *Wr* is the potential water reclaimed from the treating system, *Cws* is the current utility service unit price of water.

2.2.3. The Net present value (NPV)

Financial profitability was assessed by computing the net present value of the project for the studied sites, which consists in calculating the cash flow expected from the investment and summing it up throughout the overall life of the project (see **Eq. (28)**).

$$\mathrm{NPV} = -\mathrm{C_I} + \frac{\mathrm{CF_1}}{(1+\mathrm{i})} + \frac{\mathrm{CF_2}}{(1+\mathrm{i})^2} + \cdots + \frac{\mathrm{CF_n}}{(1+\mathrm{i})^\mathrm{n}} \quad \textbf{(28)}$$

Where $\mathrm{C_I}$, corresponds to the initial investment (year 0), $\mathrm{CF_n}$, to the annual saving or cash flow (annual income or benefit – annual expenditure), n is the life of the project (taken here as 10 years, corresponding to the life of the polyethylene tanks).

2.2.4. The Payback period (PBP)

PBP is the amount of time it takes to recover the cost of an investment and calculated using **Eq. (29)**

$$PBP = \frac{C_I}{CIF} \quad \textbf{(29)}$$

For the greywater system, the net cash inflow (CIF) is the balance between the generated benefit from the implementation of the greywater recycling system and the maintenance and operation costs. Benefits would detail several factors like water savings and other costs involved in the implementation of the greywater recycling system as shown before.

2.2.5. The cost-benefit ratio

The benefit-to-cost ratio, $R_{B/C}$, is useful to illustrate a cost-benefit comparison. The outcome is utilized as an economic feasibility criterion. Moreover, the benefit-to-cost ratio (RB/C) was calculated using **Eq. (30)** [201].

$$R_{B/C} = \frac{C_P}{B_P} \quad \textbf{(30)}$$

Where Cp and Bp are respectively the involved costs and benefits within the overall project in his lifetime. As a result, if benefit-to-cost ratio exceeds one ($R_{B/C} > 1$), then the project is considered as economically feasible whereas if benefit to cost ratio is equal to or less than one ($RB/C \leq 1$), then the project is considered economically unfeasible.

2.2.6. Initial conditions and scenarios considered

Two scenarios (Si) were considered for the vermifilter systems' economic performance assessment. The first scenario considered was a system designed for a single house while the second was a system design for a community. Again, for each scenario, two options depending on how operation and maintenance are carried out were also investigated (S_{i-1} and S_{i-2}). The options are described as follows:

- **Option 1: Vermifilter materials change and sell each year**

In the first option, the material including sawdust, sand and gravel are changed each year. Added to the selling of the byproducts including the vermicompost and the earthworms, sand and gravel also are sold.

- **Option 2: Vermifilter materials change each two years with washing each year**

In this second option, mentioned materials especially sand and gravel, are changed each two years (except the sawdust that is sold as vermicompost and therefore changed each year). In these conditions, such materials are washed after a year and sold at the year 2.

For the selling conditions, material will be washed (gravel and sand will be washed, with the same volume of water considered, and price of water chosen as that of the utility services 188 XOF/m^3).

A daily use and rejection of 15 L per person was considered. For scenario 1, a single household with 6 persons was considered, corresponding to the results of the last census of the country and the city of Ouagadougou [202]. For scenario 2 the community (level), a total number of 180 people was considered, with the same amount of greywater rejected per person. The required surface area was computed considering the determined optimal HLR as given in **Eq. (31)**. Finally, the radius of each cylinder, and the useful volume were computed respectively thanks to the use of **Eqs. (32) - (33)**.

$$Required\ area\ per\ system = \frac{Volume\ of\ greywater\ to\ treat}{optimal\ HLR} \quad \textbf{(31)}$$

$$Radius\ of\ systems\ cylinders = \sqrt{\frac{Required\ area\ per\ system}{\pi}} \quad \textbf{(32)}$$

$$Useful\ volume\ of\ system = \pi\, h\, r^2 \quad \textbf{(33)}$$

The costs were selected from the local market. To compute the benefits from the reused water, the considered price was computed at 40% of the price at which the water utility services sell the water. The duration of the project was spent 10 years considering the duration of the polyethylene tank, with a 10% discount rate. Also, the choice of a 10-year evaluation term and a 10% discount rate is likely to reflect a balance between the project's expected lifespan, financial considerations, risk assessment, and industry norms, providing a standardized framework for evaluating the project's cost-effectiveness and long-term viability.

3. Results and Discussion

3.1. Result and discussion for the vermicompost analysis

3.1.1. Agronomic quality of the vermicompost

The results of the agronomic quality analysis of the vermicompost are presented in **Table 33** The chemical analysis of the vermicompost samples (S1, S2, and S3) indicated that the nutrient content varies slightly among them but generally reflects good compost quality.

- **Total Nitrogen (N)**

The total nitrogen (N) content ranged from 1.014% to 1.70%, in the range of the FAO guideline of 1%-3% and the NFU 44-095 standard (<3%). This high nitrogen content was found to be beneficial for soil fertility and plant growth, highlighting the compost's suitability for agricultural use [203]. All plants absorb nitrogen primarily in the forms of NO_3^- and NH_4^+. Nitrogen is an essential element for proper plant growth and development, significantly boosting yield and improving quality by playing a crucial role in the biochemical and physiological processes of plants [204,205]. These values are similar to those reported by Rehman et al. [206] and Arancon et al. [207], who conducted vermicomposting using green waste and Farmyard Manure, and food waste, respectively, obtaining total TN contents of 1.86% and 1.3%, respectively.

- **Total phosphorus (P)**

The phosphorus (P) levels were extremely low, between 0.0042% and 0.0054%, which is significantly below the FAO recommendation of 0.5%-1%. This indicates that phosphorus supplementation may be necessary when applying this compost to phosphorus-demanding crops. These results are also lower than those reported by previous authors, such as Rehman et al. [206], who obtained 0.15% more. This low phosphorus content could be explained by the initially low phosphorus concentrations in the greywater being treated. Indeed, the phosphorus levels in the greywater used ranged from 14.39 to 18.27 mg/L.

- **Potassium (K)**

The K content, at 0.52%, aligned with the FAO guideline of 0.5%-1%, making it adequate for general fertilization needs. Compared to other studies, our results are slightly higher than those of Rehman et al.[206] (0.41%) and slightly lower than those of Gong et al.[208] (0.78%), who conducted vermicomposting using *C. erectus* leaves + FYM and green waste, respectively.

- **Magnesium (Mg) and Calcium (Ca)**

Concerning Mg content, it ranged from 0.384% to 0.48%, falling slightly below the FAO range of 0.5%-1%. Calcium (Ca) was consistent across samples at 0.96%, which is below the FAO threshold of >2%. This could suggest the need for additional calcium inputs for crops with high calcium requirements. These results are nevertheless higher than those reported by Ararcon et al. [207], who obtained 0.004% for Mg and 0.045% for Ca. However, they recorded an increased yield for both test plants, *Lactuca sativa and Solanum lycopersicum*, even at reduced concentrations (25% and 50%, respectively). Mg and Ca are indeed considered as secondary elements in the assessment of compost's agronomic quality [209]. Their presence, even in small amounts, is sufficient to ensure good yields for certain plants.

- **Total organic Matter (TOM) and Total organic carbon (TOC)**

The TOM levels, ranging from 25.42% to 25.86%, meet the NFU standard (>20%) but fell slightly below the FAO guideline of 30%-60%. While sufficient for soil conditioning, higher TOM levels would improve water retention and microbial activity in soils[203].
Total organic carbon (TOC) values (14.74%-14.99%) exceed the NFU minimum (>10%) and align well with the TOM content, indicating a good source of carbon for soil microbial activity.

- **Germination index (GI)**

The GI values were exceptionally high, ranging from 136.85% to 221.46%, far exceeding the FAO standard (>80%). This confirmed that our vermicompost is not phototoxic and can be used for agricultural applications [210], especially to enhance tomato seed germination and growth Haruna et al. [211] also came to a similar conclusion with their study. They produced vermicompost from poultry manure, cow dung, and rice bran, achieving germination and growth rates of 193.3%, 136.3%, and 127.9%, respectively.

Table 33. Results of the agronomic quality assessment for the vermicomposting

Samples	TN (%)	P (%)	Mg (%)	Ca (%)	K (%)	TOM (%)	TOC (%)	GI (%)
S1	4,584	0,0046	0,384	0,96	0,519	25,416	14,7417669	156,09
S2	5,7644	0,0054	0,384	0,96	0,519	25,559	14,8242135	221,46
S3	6,7716	0,0042	0,48	0,96	0,519	25,85835462	14,9978457	136,85
NFU 44-095	<3	<3	-	-	<3	> 20%	> 10%	
FAO	1%-3%	0,5-1	0,5-1	> 2%	0,5-1%	30%-60%	-	> 80%

3.1.2. Hygienic quality of vermicompost

The results of the Hygienic quality analysis of the vermicompost are presented in **Table 34**. The pathogen analysis shows that:

Fecal coliforms (FC) were absent in S1 and S2 but present at 600 UFC/kg in S3. This value was still below the WHO and NFU standards for market gardening (<10^3 UFC/kg), indicating minimal health risks.

Similarly, **E. coli** was absent in S1 and S2 but appears at 400 UFC/kg in S3, which remains within acceptable limits for market gardening (<10^3 UFC/kg).

Table 34. Pathogens analysis of the vermicompost in comparison to standards

Standards	Samples	FC (UFC/kg)	E. coli (UFC/kg)	SF (UFC/kg)	Salmonels	helmith eggs
	S1	0	0	0	0	0
-	**S2**	0	0	1 400	0	0
	S3	600	400	700	0	1
NFU 44-095 (2011)	**Market gardening**		<1000	<10 000	Absence in 25g	Absence in 25g
	All other crops	<1000	< 10 000	-	Absence in 1g	Absence in 1g
WHO	**-**	1000	-	-	-	Absence

Streptococcus (SF) was absent in S1 but present in S2 and S3 at 1400 UFC/kg and 700 UFC/kg, respectively. The results still fell under the NFU standards for market gardening (<10^5 UFC/kg).

Salmonella spp. is absent in all samples, meeting both NFU and WHO standards, which ensures the compost's safety for agricultural applications, especially for crops consumed raw. Helminth eggs are absent in S1 and S2 but present as one egg in S3, exceeding the NFU limit of absence in 25g for market gardening. This suggests that additional sanitization may be needed for the vermicompost if it is intended for more sensitive uses.

3.2. Results and discussion for economic feasibility assessment

3.2.1. Characteristics of the designed systems

With the given initial conditions for the two scenarios, the first step was to design the system in term of useful volume of units (tanks), radius of the units, number of the units, and volume or amount of each material or component. In the one hand, as given in **Table 35**, the system for scenario 1 (a household) only required one unit of vermifilter, while the system for scenario 2 (community of households) required 15 of them. About each unit of the systems for the two scenarios, it can be inferred that a unit of the system for scenario 2 has twice that of a unit of the system for scenario 1.

Table 35. Elements of design of the vermifilters for the two scenarios

Parameters	Unit	Scenario 1	Scenario 2
Grey Water per person	Liters	15	15
Total number of people	-	6	180
Total daily greywater to treat	Liters	90	2700
Total number of units	-	1	15
Water per unit	Liters/day	90	180
HLR considered	Liters/m²/day	123	123
Surface per unit	m²	0.73	1.46
Radius of each unit	m	0.48	0.68
Useful volume of unit	m^3	0.44	0.88
Volume of Sawdust per system	m^3	0.220	0.44
Volume of Sand per system	m^3	0.146	0.29
Volume of Gravel per system	m^3	0.073	0.15

Therefore, knowing that a common useful height of 0.6 m was designed for the systems for both scenarios, the volume of the materials (sand, sawdust, and gravel) in the units of system for scenario 2 are twice that of the materials in the units of systems for scenario 1.

3.2.2. Primary costs involved

Table 36 and Table 37 respectively give the summary of all the previously defined costs including mainly the investment or capital cost, the operation and maintenance cost, and the economic benefit for the two scenarios.

Table 36. Summary of the costs involved in the life cycle of the system for scenario 1

Type of cost	Designation	Quantity	Unit price (XOF)	Total price (XOF)	Total price (USD)
Capital costs	Polyethylene tank 0.5 m^3)	1	55 000	55 000	88.00
	Gravel (pack of 0.07 m3)	1	1 700	1 700	2.72
	Sand (pack of 0.15 m^3)	1	2 500	2 500	4.00
	Sawdust (packs of 0.22 m^3)	1	1 000	1 000	1.60
	Earthworms (1750/filter)	1	5 000	5 000	8.00
	Connection pipes + elbows + T	ff	5 000	5 000	8.00
	Total capital cost			**70 200**	**112.32**
O&M costs	Filter materials change	1	5 200	5 200	8.32
	Washing costs	1	41	41	0.07
	Total O&M yearly costs			**5 241**	**8.39**
Economic benefits	Water savings	365	6.77	2 470	3.95
	Vermicompost selling	1	4 192	4 192	6.71
	Watered sand and gravel	1	3 150	3 150	5.04
	Earthworms selling	1	5 000	5 000	8.00
	Economic Benefits			**14 813**	**23.70**

On the one hand, we can see the investment in scenario 1 was evaluated as 70 200 XOF, i.e., 112.32 US $, while in scenario 2 an investment of 1 808 200, i.e., 2 893. 12 $ US, is required to start the project. It can be inferred here that the individual system required more investment funds when compared on a unit-basis to the community system. In fact, the number of people in the community is 30 times that of the household, meanwhile the capital costs of the community level system are less than 30 times that of the household level system.

The same can be noticed for the yearly maintenance costs, while the opposite can be noticed for the benefits costs per year. It means here that the system at community level should be expected to have better financial performance with respect to the individual level system.

Table 37. Summary of the costs involved in the life cycle of the system for scenario 2

Type of cost	Designation	Quantity	Unit price (XOF)	Total price (XOF)	Total price (USD)
Capital costs	Polyethylene tank (1 m^3)	15	100 000	1 500 000	2 400.00
	Gravel (pack of 0.15 m3)	15	3 230	48 450	77.52
	Sand (pack of 0.29 m^3)	15	4 750	71 250	114.00
	Sawdust (pack of 0.44 m^3)	15	1 900	28 500	45.60
	Earthworms (3500/filter)	15	10 000	150 000	240.00
	Connection pipes + elbows + T	ff	10 000	10 000	16.00
	Total capital cost			**1 808 200**	**2 893.12**
O&M costs	Filter materials change	15	9 880	148 200	237.12
	Washing costs	15	83	1 238	1.98
	Total O&M yearly costs			**149 438**	**239.10**
Economic benefits	Water savings	365	203.04	74 110	118.58
	Vermicompost selling	15	8 385	125 768	201.23
	Watered sand and gravel	15	5 985	89 775	143.64
	Earthworms selling	15	10 000	150 000	240.00
	Economic Benefits			**439 653**	**703.44**

On the elements of the capital costs, **Figure 33** helps display their contribution to the total amount. It can be seen as illustrated that structural element, here the tanks are the main contributors in the capital costs for both systems in scenario 1 and 2 (78 and 85.79% respectively), followed by the components or the elements inside the filters (earthworms, sawdust, sand, gravels), and then by the accessories of the systems (pipes for connections etc.). Accessories are almost negligible in the case of scenario 2's system.

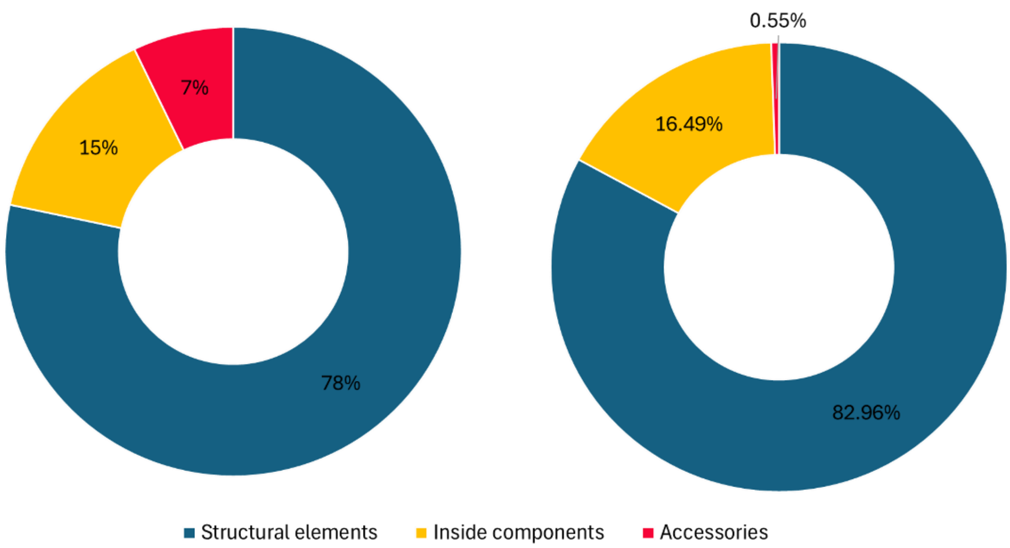


Figure 33. Breakdown of the capital costs of the systems for the two scenarios

3.2.3. Financial performance indicators

The following **Table 38** presents the results of the economic analysis of the project for the systems for the full life cycle of the project (10 years).

Table 38. Indicators of financial performance for the systems in each scenario

Scenario	Option	C_I (XOF)	$C_{O\&M}$ (XOF)	LCC (XOF)	EB (XOF)	LCC (XOF/m^3)	NPV (XOF)	PBP (years)	$R_{B/C}$
S1	$S_{1\text{-}1}$	70 200	32 205	102 405	91 017	311.74	- 11 388	11.9	0.89
	$S_{1\text{-}2}$	70 200	18 687	88 887	80 878	270.59	- 8 009	11.3	0.91
S2	$S_{2\text{-}1}$	1 808 200	918 232	2 726 432	2 701 477	276.65	- 24 956	10.1	0.99
	$S_{2\text{-}2}$	1 808 200	532 968	2 341 168	2 412 528	237.56	71 360	9.6	1.03

First, it can be noticed that LCC was computed as from 237.56 to 311.74 XOF/m^3, with scenario 2 option 2 showing the least cost obtain for the LCC. In such conditions, filter elements are washed in year one and sold in year two to be replaced by new elements. The advantage of such an option is that it limits the costs in terms of material purchase.

The next thing we can also notice is that the LCC breakdown (**Figure 34**) again showed the domination of capital or investment costs. For every option of each scenario, the capital costs were the most contributor during the life cycle of the systems. Scenario 1 Option 2 emerges here, with capital costs demonstrating the highest contribution, a total of 79%. This explains the best performance of that option with respect to that of option 1 for scenario 1 in terms of LCC.

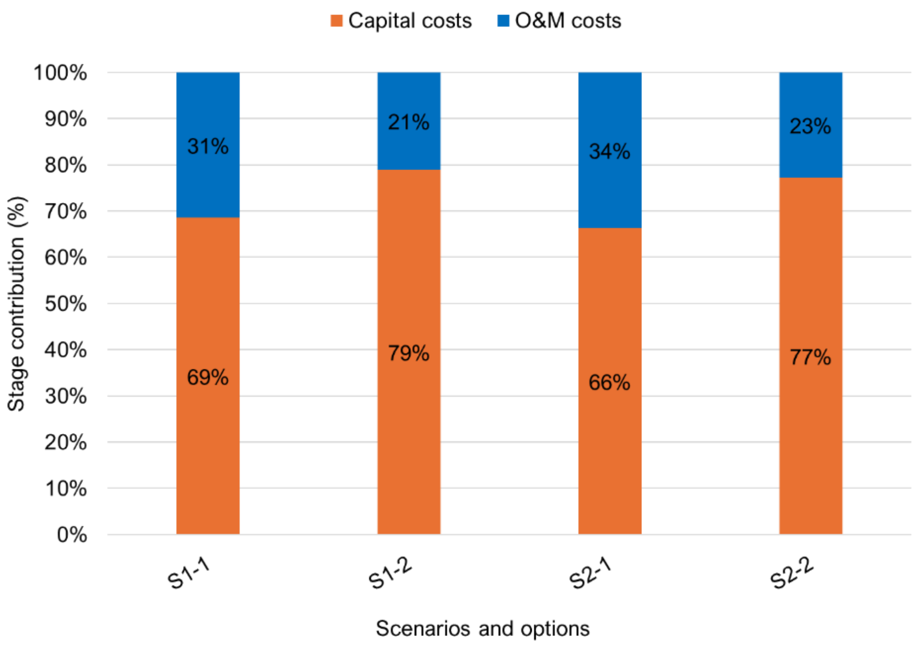


Figure 34. LCC breakdown per option for each of the two scenarios

As all options have the same investment per scenario, operation and maintenance costs become critical, and scenario 1 option 2 is the one that has the optimal operation and maintenance costs between the two options. The same can be noticed in scenario 2.

Finally, according to **Figure 35** it can be also noticed that even though some options of scenarios seem to give better LCC than others, none of them compare positively to the price of water from the utility service for the scale at which the system is (less than 8 m^3). It can be inferred that the investment in treating the greywater for our systems is higher than that to treat the water from the utility services. Also, such cost from the utility services doesn't consider other potential involved costs like the royalties, and the fixed premiums.

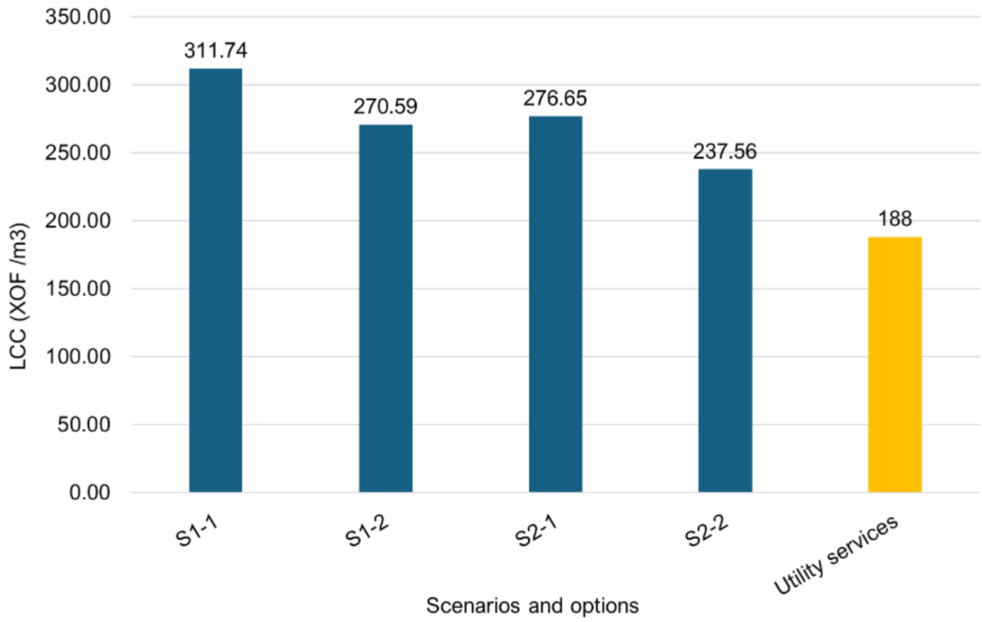


Figure 35. LCC for the scenarios and options compared to the price of water from utility services.

Else from the LCC, we can see that on a "cash flow" outlook, except scenario 2 option 2, the benefits were not sufficient to bear the investment and operation and maintenance costs for all the other options and scenarios considered in our study. Indeed, three indicators were computed to assess the profitability of the vermifiltration system. On the one hand, we can see from **Table 3** that the NPVs computed ranged from – 11 388 to + 71 360 XOF, i.e., – 18.22 to + 114.18 $ US, with scenario 2 option 1 showing the highest deficit in terms of NPV. Here, it is however important to underline that although the deficit of option 1 of scenario 2 seems to be larger than those of scenario 1, when it is brought to the same scale, the results are different: indeed, if the

NPV are divided by 30 to have it per 6 people, they become less than that of the individual scale system.

Overall, the values of the NPVs witness the non-viability of the project for the considered period, except for the scenario 2 option 2, and this information is backed up by the values of the payback periods. PBPs were estimated at between 9.6 years and 11.9 years, with only option 2 of scenario showing PBP less than 10 years. Benefit-cost ratios also confirm such a notice as they range from 0.89 for scenario 1 option 1 to 1.03 scenario 2 option 2. Those values compare very well with literature [199,212]. It is also important to notice that although the NPV, the benefit-costs ratios and the payback periods were demonstrated to be non-beneficial for the viability of the project, in some scenarios the benefit-costs ratios were somehow close to 1 than 0, showing that the "non-viables" systems just fall by little to be economically viable. This can help investigate in terms of other nature or type of benefit that the system can provide such as qualitative benefits.

3.2.4. Discussion

The life cycle costs of the project within the 10 years were evaluated at from 237.56 to 311.74 XOF/m^3 i.e., 0.380 to 0.499 \$ US/m^3, which from **Table 39** is like that of the study of Pryce et al. [198] and Abdelhay and Abunaser [213]. Indeed, depending on the type of system, the mentioned authors found that life cycle cost of the system can reach 0.310 \$ US/m3 and 0.390 \$ US/m3 respectively. Globally the life cycle cost of our system is similar to other studies except for that of Rodriguez et al [199] especially if we consider for example our scenario 2, option 2. This can be explained among other things by the difference in the amount of water treated by our system, and maintenance costs engaged in each system. For instance, in the one hand Rodríguez et al. [199] included systems with pumps for an activated carbon system. This generated electricity costs inside maintenance costs and therefore brought the LCC at higher values.

On the other hand, studies like that of Abdelhay and Abunaser [213] had moderate elements in the operation and maintenance stage, because the material change cost was not included in operation and maintenance cost. For our project, considering the high concentrations of influents, it is inevitable to replace the filter media to prevent clogging. Also, for Pryce et al. [198], Abdelhay and Abunaser [213], they treated a very huge amount of water at once per day, which brings their results like that of our results for scenario 2's option.

Table 39. Life cycle cost of the project in comparison to that of other studies.

Study	Type system	LCC of the project ($ US/m^3)
Burkina Faso (our study)	Vertical Vermifilter	0.380 to 0.499
Chile [199]	Activated carbon filters system	2.11 to 17.51*
Jordan [213]	Novel vertical-flow constructed wetland	0.391
India [198]	Integrated fixed film activated sludge (IFAS) system	0.310

*: depending on the scale of the installations (3 installations were investigated).

When comparing only the LCC, our system is like that of other low-cost systems. However, the vermifiltration system offers a significant added value: vermicompost. Based on the assessed cost-benefit analysis, vermicompost provides a considerable financial advantage.

3.2.5. Quantitative and quantitative benefits

Regarding the financial benefits of the system, the key elements considered are treated greywater, used filter materials, and vermicompost. For treated water, only 40% of the price of the public water supply service was applied, as the treated water is non-potable and suitable only for purposes such as market gardening, washing, and similar activities.

The second type of benefit is qualitative. The social advantages of treated greywater play a crucial role in evaluating the system. The availability of water for irrigation in arid or semi-arid regions can significantly impact the local population. Treated greywater can be used to irrigate crops, fruit trees, ornamental trees, and green spaces, which helps to lower local temperatures.

Additionally, vermicompost, being a bio-compost, can be safely used in agriculture, enhancing agricultural productivity. Lastly, domestic greywater treatment effectively reduces the presence of mosquitoes in the area, as noted in the introduction. This is particularly important in Burkina Faso, where mosquitoes transmitted dengue fever, causing 641 deaths last year. [214] .

4. Conclusion

The chapter on the sustainability assessment of vermifiltration for greywater treatment highlighted the overall effectiveness and potential of vermicompost derived from the system, demonstrating both agronomic and hygienic quality. The vermicompost samples meet international standards, showing good nutrient profiles and promoting seed germination without harmful effects. While some nutrient levels, like phosphorus and calcium, are below recommended thresholds, targeted supplementation can address crop demands. Compost adheres to hygiene standards, with minimal pathogen presence and no Salmonella, though a single helminth egg in one sample suggests the need for additional sanitization in certain applications.

The analysis of household- and community-scale vermifiltration systems highlights distinct advantages and limitations. The community system was proven more cost-effective in the long term due to resource sharing and better financial performance per person.

The life cycle cost (LCC) analysis shows that scenario 2 option 2 stands out as the most economically viable, with the lowest LCC of 237.56XOF/m^3, a NPV of 71360XOF on a 10 years project period, and a benefit-cost ratio close to 1.03, for a community of 180 people.

Beyond financial aspects, the systems offer significant qualitative benefits. Treated greywater can be used for irrigation in arid regions, vermicompost enhances agricultural productivity, and greywater treatment reduces mosquito breeding, addressing critical public health challenges in Burkina Faso. These environmental and social benefits, while indirect, significantly increase the project’s overall value and justify further exploration for broader applications, especially by integrating non-financial benefits.

GENERAL CONCLUSION

1. Conclusion

This thesis has explored the potential of vermifiltration as an innovative, performant and sustainable technology for greywater treatment and reuse. By addressing key operational and design parameters, this research has contributed to a deeper understanding of VF's performance, limitations and applications. The findings from the last decade's research and the experimental studies conducted provide a strong foundation for advancing this technology in regions facing economic, environmental, and public health challenges.

The review of existing literature highlighted the growing recognition of vermifiltration as a viable alternative for wastewater treatment, particularly in low-resource settings. VF demonstrates high adaptability across diverse wastewater sources and achieves significant pollutant removal, producing valuable by-products such as treated water and vermicompost. This dual-purpose functionality aligns with the principles of sustainable development, emphasizing resource recovery and environmental protection.

The experimental evaluation underscored the critical role of operational parameters, particularly HLR, EWD and initial COD concentration, in the efficiency of VF systems. Notably, a higher HLR of 191 L/m²/day increased electrical conductivity in treated greywater, although this effect was not statistically significant. However, the same higher HLR resulted in a decrease in filtration efficiency, sometimes by more than 35%, particularly under high organic loads of 4000 mg/L. In contrast, a lower HLR of 64 L/m²/day ensured better and more stable removal of COD and BOD, even under conditions of high organic load. The presence of earthworms significantly enhanced COD and BOD removal, contributing to a 30% increase in removal efficiency, from 60% to 90%. Earthworms also improved the stability and efficiency of filters, particularly under low HLR and high organic load conditions. However, both hydraulic and organic loading rates must be optimized to achieve peak performance. Furthermore, the research demonstrated the negative effects of elevated initial COD levels on DO and pH stability, reaffirming the need for precise control of influent characteristics. These insights provide actionable guidance for designing VF systems tailored to specific wastewater profiles and environmental conditions.

The integration of kinetic modeling and Response Surface Methodology (RSM) represents a significant advancement in the predictive and optimization capabilities of VF systems. While first-order kinetic models proved insufficient due to the complexity of interactions within the system, second-order models accurately captured the dynamics of COD removal, highlighting the symbiotic relationship between earthworms and microorganisms. The second-order model showed a high correlation coefficient ($R^2 = 0.9978$) and parameters ($a = 0.105$, $b = 1.111$, and $K_2 = 0.938\ d^{-1}$), reflecting the significant role of microbial activity and earthworm-microorganism interactions. Conversely, the Stover-Kincannon model was unsuitable for the high degradation rates observed, as it yielded negative values for Umax and Ks, indicating inconsistencies in its application.

From the study it is concluded that COD removal from greywater could be predicted by a mathematical model developed through the RSM combined with BBD design. Statistical analysis suggest that all the three factors have significant impact on COD removal within the tested range. The variation in EWD at the beginning of the treatment is not a significant parameter in terms of changes in numbers, once the population reaches 70 earthworms. When keeping the EWD under 70, it has a bad effect on the COD removal. The cross effects of the parameters on the response have also been found very significant. The model shows a maximum of 91.51% for COD removal at an influent COD of 1087 mg/L i.e.,1.087 kg/m^3, a HLR of 133 L/m^2/d, and a EWD of 178 earthworms. During confirmatory tests, actual COD removal was 4.32 and 0.59 % less than predicted value in case of synthetic domestic greywater and 3.05% less in case of real domestic greywater. Given the high variability in household greywater quality in the area, the system was designed at full scale for a COD concentration of 2500 mg/L, corresponding to an HLR of 123 L/m^2/d according to the model. These results suggest that the model can effectively support the design of field-scale vermifiltration systems with minimal variation.

The sustainability assessment demonstrated the good agronomic and hygienic quality of vermicompost derived from VF systems, reinforcing its utility as a valuable agricultural input. Although some nutrient levels, such as phosphorus and calcium, were below optimal thresholds, these can be supplemented to meet specific crop requirements. The overall compliance with hygiene standards, with minimal pathogen presence and no Salmonella, supports the safe use of VF by-products in agriculture, though the detection of a single helminth egg in one sample suggests the need for additional sanitization measures in certain contexts.

The economic evaluation revealed that community-scale systems are more cost-effective in the long term, with the lowest life cycle cost (LCC) recorded at 237.56 XOF/m^3 for a community of 180 people. This finding underscores the viability of VF systems in resource-constrained settings, where economies of scale can be leveraged to maximize benefits. The NPV of 71,360 XOF over a 10-year project period and a benefit-cost ratio of 1.03 further highlight the financial feasibility of such systems. Beyond financial metrics, the indirect environmental and social benefits—such as reduced mosquito breeding, improved agricultural productivity, and access to irrigation water—significantly enhance the overall value of VF systems, particularly in arid regions like Burkina Faso.

The modeling framework developed in this thesis, coupled with the sustainability assessment, provides a robust basis for scaling VF systems to household and community levels. RSM model for predicting the performance of the vermifilter was successfully developed and validated. The dynamics of earthworms were shown to be the most influential parameter, and optimizing the hydraulic load proved to be crucial for the proper functioning of the system. Furthermore, the evaluation of sustainability was conclusive.

This research reinforces the potential of vermifiltration as an effective, adaptable, and sustainable solution for wastewater treatment and reuse. By addressing key operational challenges, advancing modeling techniques, and demonstrating the multifaceted benefits of VF systems, this thesis contributes to the broader goal of sustainable water management. With targeted efforts to optimize system design and operation, vermifiltration can play a pivotal role in addressing global water challenges, particularly in regions with limited resources and urgent environmental needs.

2. Limits of study

Regarding the limitations of the technique for practical applications, three key points can be highlighted:

- Despite the presence of earthworms, clogging remains an issue for the system. Clogging can cause the system to malfunction entirely and result in the death of the worms, which is both economically and ecologically disadvantageous. with the clogging, a huge amount of greywater cannot be treated at once, if we want the system to perform sustainably
- Pressurized water could be considered to resolve the clogging issue, but the worms would need to be removed first to avoid harming them. This would cause further disruption to the system.
- The variability in greywater concentrations, especially under high loads, requires the Hydraulic Loading Rate (HLR) to be kept low to prevent clogging. This reduces considerably the volume of water that can be treated at a time, which could be a limitation for practical and industrial applications.
- Because the system relies on earthworms, which are living organisms and may be sensitive to significant variations in climate, greywater quality, or even certain unsuitable substances in the greywater, these factors could pose a threat to the long-term performance of the system and treatment efficacy.

As for the limitations of the study, it would have been more attractive to monitor the removal of pollutants through the filters by measuring additional parameters such as BOD5, turbidity, TSS, TN and others. This study focused solely on the consistent monitoring of COD. Future research should take all these parameters into account. It could also be relevant to properly address the management of earthworms in vermifiltration, focusing on safe disposal methods to prevent heavy metal leakage and investigating their growth and reproduction to optimize harvesting and ensure environmental safety.

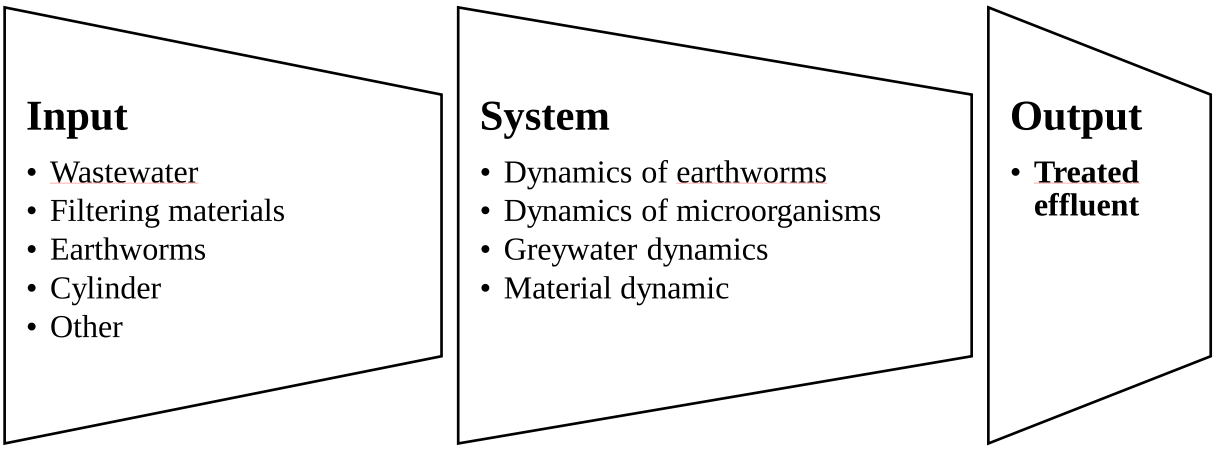
3. Perspectives

Future research should focus on refining mathematical models to gain a deeper understanding of the various dynamic components of the vermifiltration system. The development of an in-depth mathematical model is already underway to study these dynamics. This ongoing research aims to determine the exact operating lifespan of the filter and establish optimal cleaning intervals to maintain its performance.

The following dynamics are being studied as part of the model:

- Earthworm Dynamics: These can be modeled using a logistic growth model to capture their population behavior within the system.
- Micro-organisms Dynamics: Kinetic models are being used to describe and quantify microbial activity and interactions.
- Material Dynamics: Equations for heat and mass transfer are applied to explain the mechanisms of pollutant particle deposition on the surface of the filter media.
- Greywater Dynamics: Models are being developed to capture the mechanisms responsible for pollutant reduction in the treated water.

The diagram below illustrates the various components of the proposed model.



In addition to model refinement, it will also be necessary to:

- Design and implement optimized filters at field scale, followed by long-term studies to validate their performance, scalability, and community acceptance under diverse conditions.

Regarding the applicability, future studies should focus on exploring the practical aspects of implementing a vermifiltration system at the community level. In this thesis, we tested the household-level approach, where greywater was collected by households in containers, which we then retrieved and transported to the laboratory for analysis. The households faced no difficulty in collecting their greywater and were even pleased to dispose of it properly rather than discharging it into their environment. Scaling up this approach to a community-wide vermifiltration system would require addressing key practical considerations, such as organizing the collection of greywaters at the household level, transporting them to a centralized vermifiltration unit within the community, and ensuring proper operation and maintenance of the system. This would involve training individuals to manage and maintain the vermifiltration unit, defining clear roles and responsibilities, and developing cost management strategies to ensure the system's long-term functionality and sustainability. Addressing these questions is crucial to ensure the effective and sustainable implementation of vermifiltration units at a community level.

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APPENDIXES

Appendix A: Man Withney-U tests results for the physical and chemical parameters for the assessment of the performances of the VFs.

Table A.1. P-values recorded for the Man Withney-U tests for the PH

Influence of HLR					
VF1	VF2	VF3	VF4	VF7	VF8
0.406		0.018		0.041	
Influence of Worms					
VF9	VF11	VF10	VF12	VF5	VF7
0.003		0.406		0.002	
Influence of Initial COD					
VF2	VF4	VF11	VF12		
0.018		0.018			

Table A. 2. P-values recorded for the Man Whitney-U tests for the EC

Influence of HLR					
VF1	VF2	VF3	VF4	VF7	VF8
0.142		0.371		0.848	
Influence of Worms					
VF9	VF11	VF10	VF12	VF5	VF7
0.848		0.035		0.565	
Influence of Initial COD					
VF2	VF4	VF11	VF12		
0.110		0.406			

Table A. 3. P-values recorded for the Man Whitney-U tests for the DO

Influence of HLR					
VF1	VF2	VF3	VF4	VF7	VF8
0.200		0.002		0.085	
Influence of Worms					
VF9	VF11	VF10	VF12	VF5	VF7
0.125		0.406		0.025	
Influence of Initial COD					
VF2	VF4	VF11	VF12		
0.002		0.064			

Table A. 4. P-values recorded for the Man Whitney-U tests for the COD

Influence of HLR					
VF1	VF2	VF3	VF4	VF7	VF8
0.674		0.001		0.009	
Influence of Worms					
VF9	VF11	VF10	VF12	VF5	VF7
0.001		0.064		0.001	
Influence of Initial COD					
VF2	VF4	VF11	VF12		
0.003		0.016			

Table A. 5. P-values recorded for the Man Whitney-U tests for the BOD

Influence of HLR					
VF1	VF2	VF3	VF4	VF7	VF8
0.248		0.083		0.021	
Influence of Worms					
VF9	VF11	VF10	VF12	VF5	VF7
0.08		0.559		0.149	
Influence of Initial COD					
VF2	VF4	VF11	VF12		
1.00		0.386			