

DEVELOPMENT OF A NATURE-BASED SOLUTION TO ENABLE THE REUSE OF TREATED WASTEWATER IN AGRICULTURE

DÉVELOPPEMENT D'UNE SOLUTION FONDÉE SUR LA NATURE POUR PERMETTRE LA RÉUTILISATION DES EAUX USÉES TRAITÉES EN AGRICULTURE

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ABSTRACT

This study evaluated an aerated horizontal flow treatment wetland (20 m²) treating primary treated domestic wastewater to assess its potential for safe agricultural reuse under French regulations. Over one year, the system performance was monitored for conventional pollutants, physicochemical parameters, and multiple microbial indicators, including *Escherichia coli*, total coliforms, anaerobic sulfite-reducing bacteria spores, helminths, and phages. The wetland achieved high removal of organic matter and nutrients (93% COD, 99% NH₄-N, 58% TN) and notable pathogen reduction, with geometric mean log reduction values of 3.2 for *E. coli*, 3.7 for crAssPhages, and 1.8 for anaerobic sulfite-reducing bacteria spores. Pathogen removal was heterogeneous along the flow path, suggesting differing removal mechanisms for bacteria, viruses and protozoan indicators. Overall, the effluent mostly met Class B reuse requirements, with 20% of samples downgraded to Class C due to residual *E. coli*, highlighting the need for expanded monitoring of additional pathogens and molecular indicators to better characterize treatment reliability.

Keywords: Reuse of treated water; System performance; Effluent; Pathogen removal; molecular indicators.

RÉSUMÉ

Cette étude a évalué une zone humide de traitement à écoulement horizontal aéré (20 m²) traitant les eaux usées domestiques traitées primaires afin d'évaluer son potentiel de réutilisation agricole sûre selon la réglementation française. Pendant un an, la performance du système a été surveillée pour détecter les polluants conventionnels, les paramètres physicochimiques et de multiples indicateurs microbiens, notamment *Escherichia coli*, coliformes totaux, spores bactériennes anaérobies réductrices de sulfites, helminthes et phages. La zone humide a permis une élimination élevée de la matière organique et des nutriments (93 % de DCO, 99 % de NH₄— N, 58 % de TN) ainsi qu'une réduction notable des agents pathogènes, avec des valeurs de réduction logarithmique moyenne géométrique de 3,2 pour *E. coli*, de 3,7 pour les crAssPhages et de 1,8 pour les spores de bactéries anaérobies sulfite réducteur. L'élimination des agents pathogènes s'est avérée hétérogène le long du parcours d'écoulement, ce qui suggère des mécanismes d'élimination différents pour les indicateurs bactériens, viraux et protozoaires. Dans l'ensemble, l'effluent répondait majoritairement aux exigences de réutilisation de classe B, 20 % des échantillons ayant été reclassés en classe C en raison de la présence résiduelle d'*E. coli*, ce qui souligne la nécessité d'étendre la surveillance à d'autres agents pathogènes et indicateurs moléculaires afin de mieux caractériser la fiabilité du traitement.

Mots-clés : Réutilisation des eaux usées; Performance du système; Effluent; Elimination des agents pathogènes ; Indicateurs moléculaires.

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INTRODUCTION

To address the challenges of climate change, many countries are investigating the reuse of treated wastewater. However, the widespread development of reuse practices can have negative environmental impacts. Emissions from water and sanitation services account for 3 to 7% of cities' greenhouse gas emissions (Alix et al., 2022). Among non-conventional water sources, treated wastewater reuse — through tertiary treatment — is among the most energy-intensive processes after desalination. To prevent the expansion of reuse from driving an increase in greenhouse gas emissions, it is essential to focus on more energy-efficient approaches. Nature-based solutions for wastewater treatment have demonstrated their potential to contribute to water reuse and circular water management. However, the capacity of treatment wetlands to remove the pathogenic indicators defined by reuse regulations remains an open question.

Under French regulation, the requirements for reuse of treated water in agriculture set pathogen thresholds as follows: lower than 10/100/1,000/10,000 CFU per 100 mL respectively for Classes A/B/C/D for *Escherichia coli*, total coliphages, and *Clostridium perfringens* (or anaerobic sulfite-reducing bacteria spores). Physicochemical parameters must meet thresholds of below 5 NTU for turbidity and below 10 mg/L for TSS and CBOD₅. Some studies have shown that pathogen removal in passive horizontal flow treatment wetlands is limited (1 to 2 log per treatment stage for *E. coli*). The addition of aeration to treatment wetlands has been shown to improve *E. coli* removal, providing up to 4-log reduction (Nivala et al., 2019). However, *E. coli* removal may not be representative of other microbial groups, such as viruses and protozoa, even though it is widely used as a model organism.

MATERIALS AND METHODS

This study used a pilot system located on the REFLET experimental platform, northwest of Lyon, France. The system consisted of a 20 m² aerated horizontal flow treatment wetland, 4 m wide and 5 m long, saturated to a depth of 1 m (Figure 1). The filter medium consisted of 20–40 mm gravel with approximately 40% theoretical porosity. Aeration was continuous, and the hydraulic loading rate during the study was 1,220 L/d, corresponding to a theoretical hydraulic retention time (HRT) of 5 days.

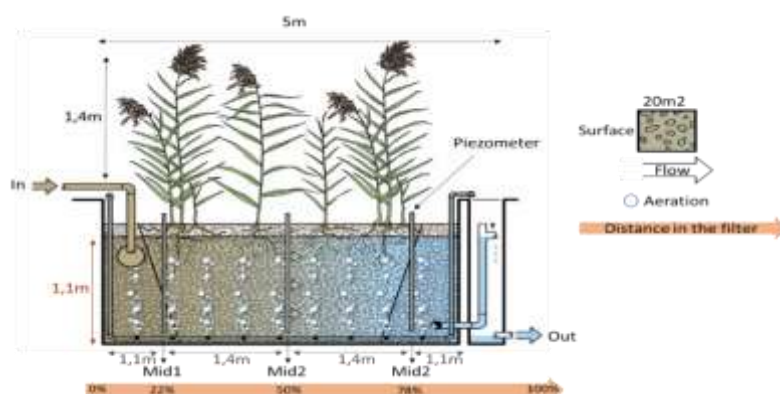


Figure 1: Schematic of the aerated horizontal flow treatment wetland on the REFLET experimental platform.

The system was fed with primary-treated domestic wastewater and monitored over one year (July 2024 to September 2025, $n = 25\text{--}52$ samples). Performance was assessed through monitoring of conventional pollutants — including dissolved chemical oxygen demand (COD), total suspended solids (TSS), total organic carbon (TOC), total nitrogen (TN), total dissolved nitrogen (TDN), and Kjeldahl nitrogen (TKN) — as well as physicochemical parameters (DO, pH, ORP, temperature) and pathogenic indicators (*Escherichia coli*, total coliforms, anaerobic sulfite-reducing bacteria spores, *Legionella pneumophila*, helminths, and phages). Samples were collected at multiple points along the filter: at the inlet (following primary settling), at 22%, 50%, and 78% of the treatment length, and at the outlet.

Samples were collected once or twice per week between July 2024 and September 2025 and analyzed for the following microorganisms:

- **Bacteria:** *Escherichia coli*, total coliforms, *Enterococcus faecalis*, *Legionella pneumophila*
- **Viruses:** RNA-F-specific bacteriophages, crAssPhages
- **Protozoa:** anaerobic sulfite-reducing bacteria spores
- **Helminths:** *Ascaris* eggs

E. coli and total coliforms were quantified using IDEXX colorimetric quantification (Colilert-18). Anaerobic sulfite-reducing bacteria spores were analyzed by a certified laboratory using membrane filtration following European standard NF EN 26461-2. CrAssPhage concentrations were determined by digital PCR using a QIAcuity instrument (Qiagen). Concentrations of *Legionella pneumophila* and helminth eggs were very low throughout the study and are not discussed further.

RESULTS

For carbon and solids removal, initial results showed 93% removal of COD, 95% removal of TOC, and 97% removal of TSS. For nitrogen, the system achieved 58% removal of TN and 99% removal of $\text{NH}_4\text{-N}$. Denitrification might be limited by lack of Carbon, as concentration of COD is very low in the second part of the filter. Physicochemical parameters were measured in accordance with standards NF EN ISO 15705 and NF EN 872 and are presented in Figure 2.

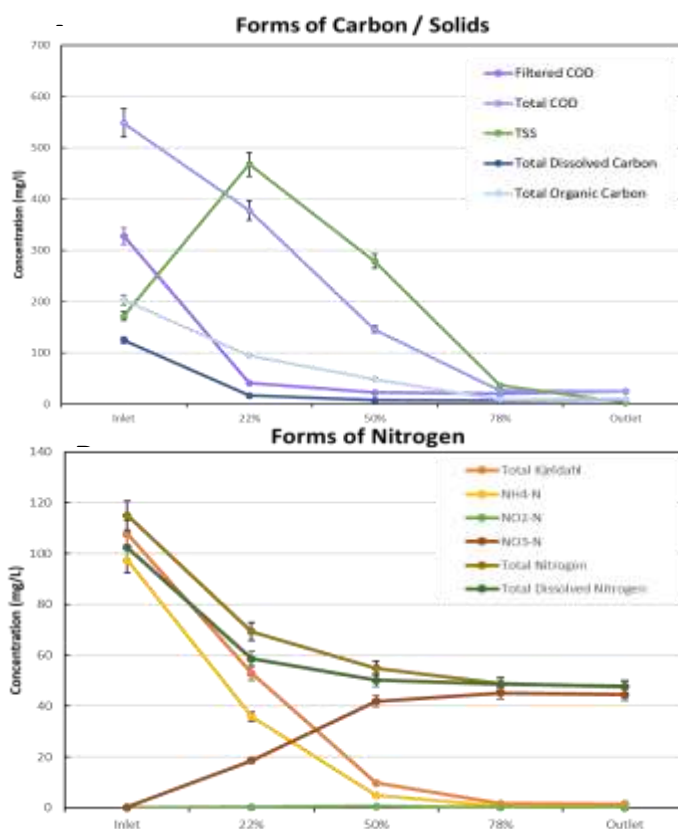


Figure 2: Concentrations of carbon and solids fractions from inlet to outlet (A). Nitrogen fraction concentrations along the treatment (B).

Over the course of the study, the system achieved geometric mean log removal values (LRV) of 3.2 for *E. coli*, 3.7 for crAssPhages, and 1.8 for anaerobic sulfite-reducing bacteria spores (Figure 3). Removal was not homogeneous across indicator types. *E. coli* and crAssPhages show the same pattern, but anaerobic sulfite-reducing bacteria spores showed different removal profiles; suggesting that distinct mechanisms govern their removal.

Spatially, approximately 75% of *E. coli* removal occurred in the first quarter of the filter (from a mean inlet concentration of 5.9 log to 4.5 log at 22% of treatment length), with slower removal thereafter. A similar pattern was observed for crAss Phage's (6.1 log at inlet, 5.1 log at 22%), with over 60% of removal occurring in the first quarter. In contrast, anaerobic sulfite-reducing bacteria spores were removed more gradually across the filter, declining from 5 log at the inlet to approximately 4 logs at 22%, 3.5 log at 75%, and around 2.5 log at the outlet.

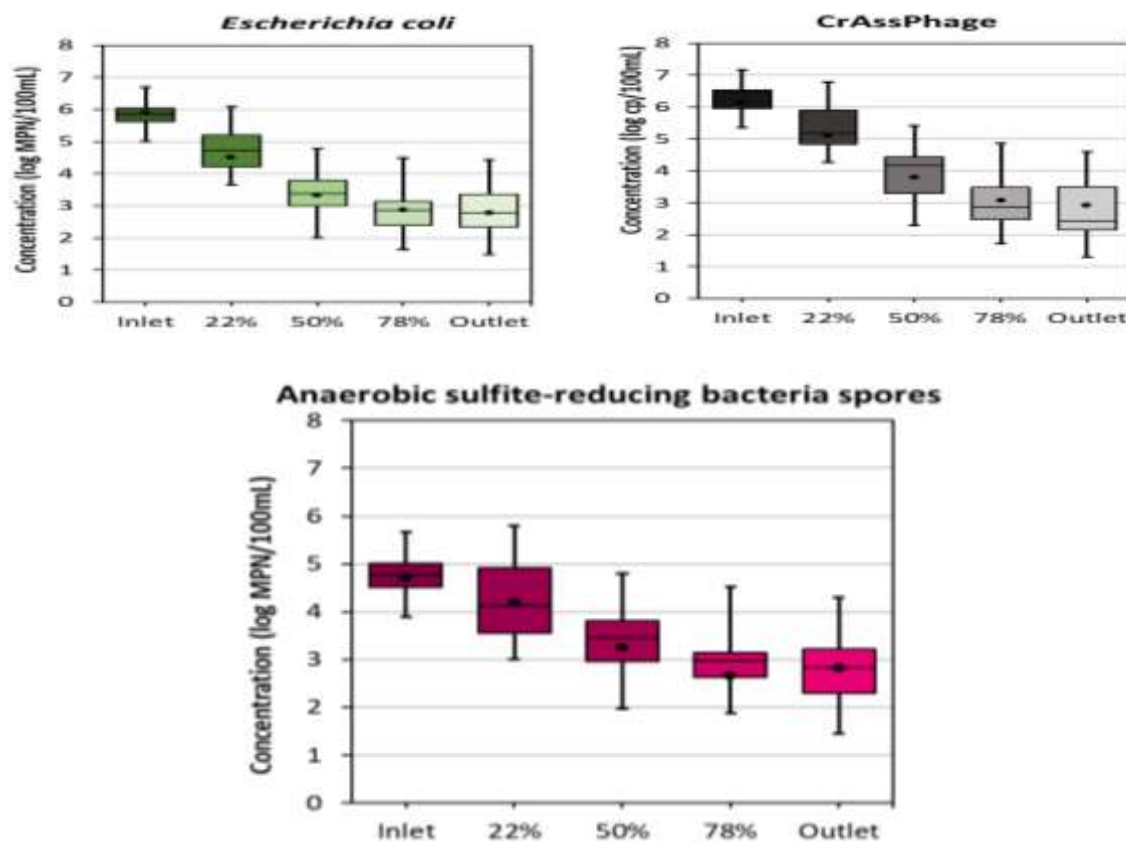


Figure 3: Pathogen concentrations at different positions along the aerated horizontal flow treatment wetland.

DISCUSSION

The system demonstrated strong performance for physicochemical parameter removal. TSS removal reached approximately 97%, with the largest reduction occurring in the first half of the filter. Organic matter removal was 93%, consistent with the elimination of both total and filtered COD. Total nitrogen was removed by approximately 70%, with complete nitrification observed. The incomplete nitrogen removal is likely attributable to limited carbon availability in the latter half of the filter. Phosphorus removal was approximately 5%, which is typical for treatment wetlands.

Results from pathogen monitoring confirm that removal performance is not homogeneous across indicator types. The spatial differences in removal along the filter suggest that distinct mechanisms are involved depending on the organism, particularly when comparing bacterial indicators with spore-forming organisms such as anaerobic sulfite-reducing bacteria spores.

CONCLUSION

Over the course of this study, treated effluent corresponded to Class B under French water reuse regulations in 80% of monitoring campaigns, in terms of both concentration and removal efficiency (Figure 4). The remaining 20% of campaigns resulted in Class C classification, primarily because *E. coli* concentrations did not consistently fall below the required threshold despite generally good removal performance.

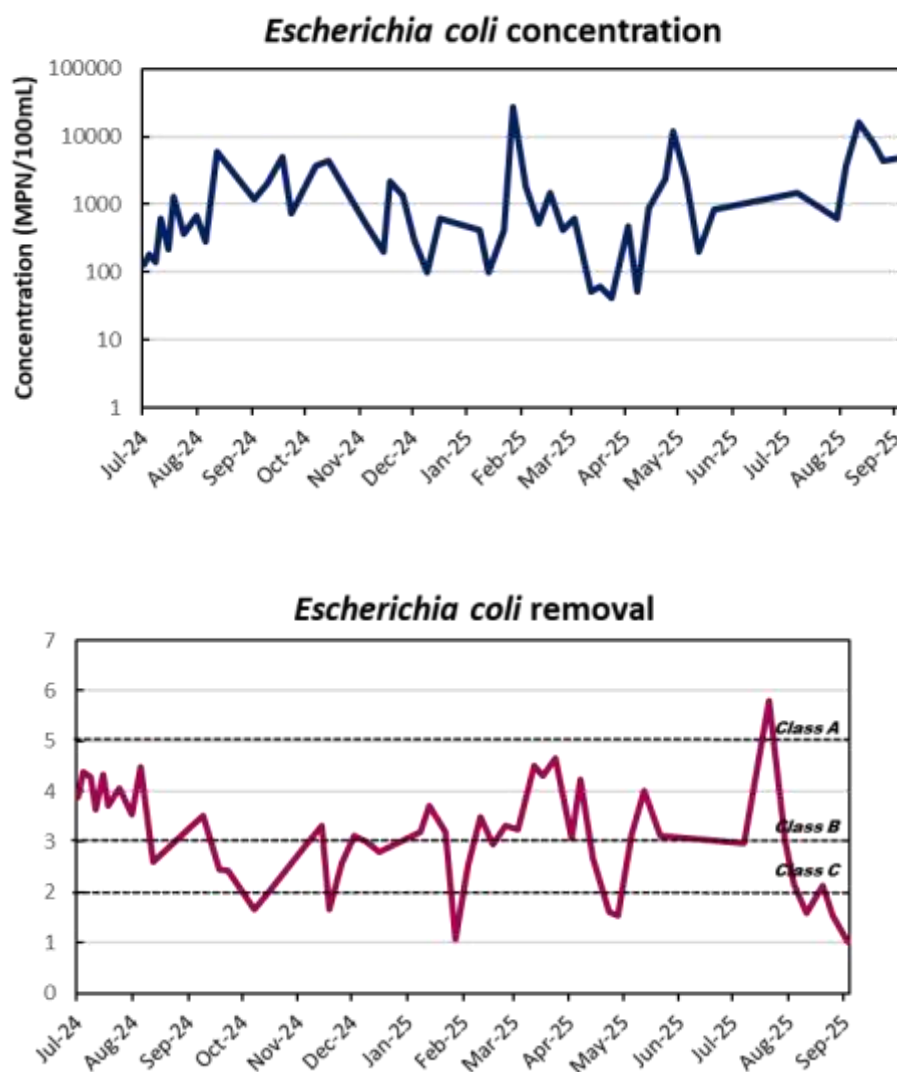


Figure 4: *Escherichia coli* concentration and removal across campaigns ($n = 52$) compared to different regulatory thresholds.

In accordance with European regulations, achieving Class A may be possible through the implementation of “barrier measures” — equipment, operational conditions, or procedures that limit contamination risk or further reduce microorganism concentrations in the treated effluent. A next step in this study will be to investigate the integration of such barrier measures with aerated treatment wetland technology and to assess their contribution to safe water reuse. To support the broader development of reuse and to better characterize treatment capabilities, it will also be important to analyze a wider range of microorganisms with differing biological properties from those currently used as indicators. Additionally, the observed differences in removal performance across organism types raise questions about the role of other factors, such as physical parameters (filter dimensions, hydraulic loading) and chemical parameters (e.g., nitrogen content).

REFERENCES

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