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CONSTRUCTED WETLAND IN MESOCOSM DESIGN FOR THE TREATMENT OF MUNICIPAL WASTEWATER EFFLUENT: PERFORMANCE OF NON-CONVENTIONAL ADMIXTURES

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ABSTRACT

One of the greatest global challenges is the rapid growth of the population. This exponential increase leads to a higher production and release of anthropogenic compounds, which, due to insufficient wastewater treatment systems, become pollutants, specifically micropollutants (MPs). Simultaneously, water is essential for life and sustainable development, yet it faces increasing contamination challenges, particularly from these micropollutants. Traditional wastewater treatment methods struggle to effectively remove these substances, thus leading to the exploration of advanced technologies such as constructed wetlands. CWs are promising due to their cost-effectiveness, simplicity, and environmental compatibility. This study evaluates the performance of non-conventional substrates - biochar from recovered cellulose and biochar from plant residues in CWs for micropollutant removal at the mesocosm scale.

The study utilized effluent from the Beringen wastewater treatment plant in Luxembourg, characterized by low of levels conventional pollutants. Two types of biochar, Emisûre-AC (from plant residues) and WOW-AC (from recovered cellulose), were activated biologically and used as substrates in mesocosm CW systems. Both units were configurated with gravel and substrate layers, planted with *Phragmites australis* and Iris Pseudacorus. Four operational scenarios varied feeding mode (continuous or batch) and load (low or high), with regular monitoring of physical parameters and pollutants concentrations.

Results showed that both biochar types effectively treated pollutants, with cellulose-based biochar outperforming plant residue-based biochar across all scenarios. Continuous mode with low load was identified as the optimal operational condition, preventing overloading, and maintaining high pollutant removal efficiency. Continuous low load operation provided ample interaction time between pollutants and microorganisms, enhancing micropollutant removal. Additionally, continuous mode offered greater operational stability compared to batch mode, sustaining the microbial community, and ensuring consistent efficiency.

The study concludes that CWs with non-conventional substrates, particularly cellulose-based biochar, are effective for wastewater post-treatment. Optimizing continuous low load operation is crucial for maximizing CW efficiency, contributing to sustainable and resource-efficient wastewater treatment technologies.

RESUME

L'un des plus grands défis mondiaux est la croissance rapide de la population. Cette augmentation exponentielle entraîne une production et une libération accrues de composés anthropiques qui, en raison de systèmes de traitement des eaux usées insuffisants, se transforment en polluants, notamment en micropolluants (MP). Simultanément, l'eau est essentielle à la vie, mais elle fait face à des défis croissants de contamination, notamment par ces micropolluants. Les méthodes traditionnelles de traitement des eaux usées ont du mal à éliminer efficacement ces substances, ce qui a conduit à l'exploration de technologies avancées telles que les zones humides construites. Les ZHs sont prometteuses en raison de leur rentabilité, de leur simplicité et de leur compatibilité environnementale. Cette étude évalue la performance de 2 substrats non conventionnels pour l'élimination des micropolluants à l'échelle des mésocosmes.

L'étude a utilisé les effluents de la station d'épuration des eaux usées de Beringen au Luxembourg, caractérisée par de faibles niveaux de polluants conventionnels. Deux types de biochar, Emisûre-AC (à partir de résidus végétaux) et WOW-AC (à partir de cellulose récupérée), ont été activés biologiquement et utilisés comme substrats dans les mésocosmes. Les deux unités étaient configurées avec des couches de gravier et de substrat, plantées de *Phragmites australis* et d'*Iris Pseudacorus*. Quatre scénarios opérationnels ont varié le mode d'alimentation (continu ou discontinu) et la charge (faible ou élevée), avec un suivi régulier des paramètres physiques et des concentrations de polluants.

Les résultats ont montré que les deux types de biochar traitaient efficacement les polluants, le biochar à base de cellulose surpassant le biochar à base de résidus végétaux dans tous les scénarios. Le mode continu avec une faible charge a été identifié comme la condition opérationnelle optimale, empêchant la surcharge et maintenant une efficacité élevée dans l'élimination des polluants. Le fonctionnement continu à faible charge a fourni un temps d'interaction suffisant entre les polluants et les micro-organismes, améliorant l'élimination des micropolluants. De plus, le mode continu offrait une stabilité opérationnelle supérieure par rapport au mode discontinu, soutenant la communauté microbienne et garantissant une efficacité constante.

L'étude conclut que les ZHs avec des substrats non conventionnels, en particulier le biochar à base de cellulose, sont efficaces pour le post-traitement des eaux usées. L'optimisation du fonctionnement continu à faible charge est cruciale pour maximiser l'efficacité des ZHs,

contribuant ainsi aux technologies durables et efficaces en ressources pour le traitement des eaux usées.

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LIST OF ABBREVIATIONS

AC: Activated Carbon

AOPs: Advanced Oxidation Processes

BOD: Biochemical Oxygen Demand

COD: Chemical Oxygen Demand

CWs: Constructed Wetlands

DO: Dissolved Oxygen

EC: Electrical Conductivity

EIB: European Investment Bank

HFCW: Horizontal Flow Constructed Wetland

HLR: Hydraulic Loading Rate

HRT: Hydraulic Retention Time

HSSF: Horizontal Sub Surface Flow

LECA: Light Expanded Clay Aggregate

LOD: Limit Of Detection

LOQ: Limit Of Quantification

MPs: Micropollutants

PAC: Powdered Activated Carbon

PE: Population Equivalent

PPCPs: Pharmaceuticals and Personal Care Products

RE: Removal Efficiency

SF: Surface Flow

SIDERO : Syndicat Intercommunal de Dépollution des Eaux Résiduaires de l’Ouest

SRT: Sludge Retention Tank

SSF: Sub Surface Flow

TSS: Total Suspended Solids

UN: United Nations

UWWTD: Urban Wastewater Treatment Directive

VF: Vertical Flow

VFCW: Vertical Flow Constructed Wetland

VSSF: Vertical Sub Surface Flow

WWTP: Wastewater Treatment Plant

INTRODUCTION

Water is essential for life (Goel, 2019). At the Rio+20 United Nations Conference on Sustainable Development, it was agreed that water is crucial for sustainable development, affecting social, economic, and environmental aspects (UN-Water, 2015). Over the past three decades, environmental concerns particularly regarding chemical and biological water contamination, have risen to prominence within both society and authorities. Various activities, like domestic, agricultural, and industrial processes, produce wastewater containing harmful substances, which can be dangerous for humans and animals (Crini & Lichtfouse, 2018). Major pollutants found in wastewater effluents include nutrients (such as nitrogen and phosphorus), heavy metals, organic matter, and micropollutants (Akpor et al., 2014). Micropollutants are substances that pose potential hazards and are typically present in water bodies at low concentrations, usually less than one microgram per liter. These pollutants originate from various sources, including industrial chemicals, pharmaceuticals, cosmetics, pesticides, and hormones, among others (*Microplastics and Micropollutants in Water*, 2023). Municipal wastewater exhibits low organic strength and high particulate organic matter (Sikosana et al., 2019). To avoid the negative effects of untreated and inadequately treated wastewater effluents, it is imperative to ensure effective and efficient treatment before discharge into receiving water bodies. Wastewater treatment involves enhancing the quality of wastewater to meet regulatory standards (Water Framework Directive, 2000).

Various techniques have been utilized to remove pollutants from wastewater., each with specific advantages and limitations. These methods encompass physical (e.g. screening, filtration, sedimentation coagulation-flocculation, membrane technologies), chemical (Ph neutralization, precipitation, oxidation/reduction), biological (aerobic/anaerobic degradation, bioremediation) processes (Michael Smarte Anekwe et al., 2022). Traditional Wastewater treatment plants (WWTPs) have long been employed as conventional methods for treating wastewater, aiming to remove contaminants (COD, BOD, suspended particles, nitrate, ammonium, phosphorus…) and ensure water quality. However, they are not designed to eliminating micropollutants which are present in low concentrations, and they can partially and not be eliminated at all. These micropollutants pose significant challenges due to their persistence and potential ecological impacts. Hence a post-treatment step is necessary. According to a study conducted by (Lauesen, 2022), innovative technologies are being developed to improve traditional wastewater treatment methods. These include Advanced Oxidation Processes (AOPs), which use strong oxidizing agents to break down harmful micropollutants, such as Ultraviolet light/hydrogen peroxide, Ozone/activated carbon, and Fenton processes. Other important techniques include Powdered Activated Carbon (PAC) and membrane filtration methods like microfiltration, ultrafiltration, nanofiltration, and reverse osmosis, which use special membranes to physically separate micropollutants from water. Adsorption techniques employ materials such as activated carbon, biochar, and ion exchange resins to capture and remove micropollutants from wastewater. Ozonation uses ozone to break down micropollutants into simpler compounds. Additionally, emerging technologies include advanced biological processes like membrane bioreactors and sequencing batch reactors, nanotechnology, electrochemical processes such as electrocoagulation and electrooxidation, and photocatalysis. These technologies often require significant investments and consume a lot of energy. Constructed wetlands emerge as promising alternatives for treating effluents from WWTPs in rural catchment. They offer several advantages for micropollutant removal compared to other technologies. They mimic natural ecosystems, using vegetation, soil, and water interactions to break down micropollutants. CWs are cost-effective to construct and maintain, requiring minimal or no energy and having lower operational costs. They blend well with the environment, support wildlife, and contribute to biodiversity. CWs are simple to operate and do not rely on complex machinery or chemicals. They effectively remove emerging micropollutants and can be integrated with other technologies to enhance their efficiency. Additionally, CWs are resilient to climate change, making them a sustainable and robust solution for long-term micropollutant removal (Gebru & Werkneh, 2024).

This study aims to evaluate the performance of non-conventional substrates used in constructed wetlands as post-treatment step. The study is conducted with an experimental set-up simulating a constructed wetland in vertical flow configuration at mesocosm scale. The main hypothesis are non-conventional biochars are suitable admixtures in constructed wetlands; operating mode can affect the efficiency of constructed wetlands; mesocosm design is a versatile tool to assess different parameters easily. From these hypotheses, the specific goals of the thesis were to assess the performance of cellulose-based biochar as admixture towards macropollutants and personal care products removal; to determine best operational parameters and modes contributing to the overall optimization; to propose a testing protocol for the mesocosm design to facilitate further investigation.

I. STATE OF THE ART

I.1 DEFINITION OF CONSTRUCTED WETLANDS

Constructed wetlands (CWs) are artificial systems that have been designed and constructed to utilise the natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in treating wastewater or mitigating environmental pollution (Ebie et al., 2013). Pollution is removed through the processes which are common in natural wetlands but, in constructed wetlands, these processes proceed under more controlled conditions (Vymazal, 2010).

Because of their natural composition, they are helping to conserve nature areas and they actively contribute to the enhancement of biodiversity, habitat creation and aesthetic improvement (Brunhoferová, 2022).

CWs are often labelled as extensive technology due to their low maintenance costs and better environmental, economic, and social sustainability, however, requiring higher area demands (Brunhoferová, 2022).

I.2 MAIN COMPONENTS IN CWs

A constructed wetland primarily consists of three main components: the support matrix (bed substrate), the vegetation and the microbial population (microorganisms). The synergy between those components allows for the effective removal of pollutants, making constructed wetlands efficient for wastewater treatment (Dordio & Carvalho, 2013).

Substrate: is an important component in CWs. Substrate does not serve only as a support for plant and microorganisms’ growth but also directly interacts with contaminants through sorption processes (Li et al., 2014). According to Dupoldt in the Handbook of CWs, substrates, sediments, and litter play crucial roles in wetland ecosystems for various reasons. They serve as habitats for numerous living organisms, supporting biodiversity within wetlands. Additionally, substrate permeability influences water movement throughout the wetland, affecting its hydrology and nutrient cycling. Within these substrates, numerous chemical and biological transformations occur, particularly driven by microbial activity, contributing to nutrient cycling and pollutant removal. Moreover, substrates act as storage sites for various contaminants, helping to mitigate their impact on water quality within the wetland environment. When selecting a substrate for a constructed wetland (CW), it's crucial to consider specific properties that can improve the removal of pollutants. The substrate choice directly impacts the pollutant removal process. The substrate needs to contain sufficient organic matter (dead plant material such as leaves, stems, roots; humic substances; peat; compost; wood chips…) to support both plant growth and microbial activity (*A Handbook of Constructed Wetlands*, s. d.). The effectiveness of the soil used for treatment depends on various factors, including its active surface area, surface charge (for better interaction with charged compounds), porosity, particle size, texture (which affects hydraulic retention time and thus pollutant removal), conductivity, and oxygen availability (Brunhoferová, 2022). Various materials have been employed as support matrix for constructed wetlands (CWs). Among the most common choices are gravel, crushed rock, river sand, mixtures of gravel and sand, and local soil (Dordio & Carvalho, 2013). Constructed wetlands using common substrates like sand and gravel can face issues such as clogging and limited pollutant removal efficiency. Furthermore, these traditional materials simply act as filters for retaining larger particles and as a support for the growth of biological organisms, while their adsorption capacity is limited compared to specialized adsorbents (Dordio & Carvalho, 2013). Because of this, there's been a rise in research on alternative materials, also called emergent materials, for constructed wetlands. Some examples of these emerging substrates include tire strips, construction waste, light expanded clay, and waste from the ceramic industry like clay bricks (Marcelino et al., 2020). Agricultural waste and similar residues can sometimes serve as beneficial components (be incorporated into the substrate) in the operation of a CW. In instances where the wastewater has low carbon content, these wastes act as biomass, introducing solid carbon into the system. This carbon aids in the denitrification process, enhancing the removal of nitrogen from the water (Fernández Ramírez et al., 2023). Laboratory batch experiments have shown that light expanded clay aggregate (LECA) effectively sorbs acidic pharmaceutical compounds (Li et al., 2014). Activated carbon has demonstrated its efficacy as an adsorbent for removing a broad range of organic pollutants from both aqueous and gaseous environments (Dordio & Carvalho, 2013). Clay minerals are abundant components of soils and are extensively studied for their adsorption properties, making them a natural choice for support matrix components in constructed wetlands (CWs). With a high potential for ion exchange and surface interactions, clays act as natural pollutant scavengers due to their large surface areas. However, their hydrophilic surfaces and charges make naturally occurring clays less effective for adsorbing anionic contaminants and hydrophobic organic pollutants (Dordio & Carvalho, 2013). Instead of chemically modifying clay surfaces, some studies have utilized lightweight clay materials derived from processed natural clays. These materials typically undergo thermal treatment, which causes rapid vaporization of interlayer hydration water and, sometimes, expansion of the sheet structure due to injected gases like CO2. This process creates highly porous materials with increased accessible surface area, enhancing sorbent capacity. Common processed clay materials include light expanded clay aggregates (LECA), expanded shale, expanded slate, and exfoliated vermiculite. The utilization of zeolites and other natural siliceous sorbents like perlite, diatomite, silica, and glass fiber for removing organic xenobiotics from wastewater is on the rise. This trend is driven by their abundance, availability, and cost-effectiveness (Dordio & Carvalho, 2013).

Nowadays, recent studies are testing the performance of certain non-conventional admixtures, among which biochar produced from different materials. Some of these non-conventional admixtures will be the case study of this thesis.

Plants: they also play a crucial role in the removal of pollutants. The primary criterion for selecting wetland plants is their ability to thrive in aquatic environments, which is why macrophytes, or aquatic plants, are commonly chosen for constructed wetlands (CWs). Additionally, plants must withstand the toxic effects of pollutants (MPs), and seasonal variations, and adapt to changing conditions within the wetland matrix, such as fluctuating nutrient and contaminant levels. A crucial factor is the plants' capability to absorb contaminants (metals, MPs, cyanotoxins) from the wastewater (Brunhoferová, 2022). Various plant species have been identified for pollutants and micropollutants wastewater treatment in wetlands, with popular choices including *Phragmites australis* and *species of Typha* such as *Typha angustifolia* and *Typha latifolia* (Li et al., 2014). According to Dupoldt, plants in constructed wetlands play crucial roles in wastewater and runoff treatment. They stabilize substrates, slow water flow, and aid in the settling of suspended materials. These plants uptake carbon, nutrients, and trace elements, transferring them into their tissues. They facilitate gas exchange between the atmosphere and sediments and create oxygenated microenvironments within the substrate. Additionally, their root systems provide attachment sites for microbes, and upon decomposition, they provide organic matter to the ecosystem. The movement of oxygen within the plant serves not only to meet the respiratory needs of the submerged tissues but also contributes oxygen to the rhizosphere through root leakage. This oxygen leakage from roots fosters oxidized conditions in the typically anoxic substrate, promoting aerobic decomposition of organic matter and the proliferation of nitrifying bacteria (*Hans Brix*, 2003). When pollutants are absorbed by plants, they undergo degradation through internal plant mechanisms, known as phytoremediation (Brunhoferová, 2022).

Microorganisms: In wetlands, diverse microorganisms play a crucial role in removing pollutants, including MPs, from wastewater. These microbes typically have initial access to dissolved compounds in water and can directly degrade them or work in symbiosis with plants to contribute to phytoremediation efforts (Brunhoferová, 2022). According to Stephen Norton, microorganisms in soil play a vital role in nutrient uptake and storage, but their metabolic functions are especially crucial in removing organic pollutants. Soil bacteria, predominant among these microorganisms, utilize carbon from organic matter as an energy source. In aerobic conditions, they convert it to carbon dioxide, while in anaerobic conditions, they produce methane. Microbial metabolism also significantly contributes to the removal of inorganic nitrogen. Dupoldt presents reliance on microorganisms and their metabolic processes for regulating functions as a key feature of wetlands. Microorganisms, including bacteria, yeasts, fungi, protozoa, and algae, play vital roles in wetland ecosystems. They serve as major sinks for organic carbon and nutrients, contributing to the transformation of various substances into harmless or insoluble forms. Microbial activity alters the redox conditions of the substrate, affecting the wetland's processing capacity and nutrient recycling. Some microbial transformations require oxygen (aerobic), while others occur in its absence (anaerobic). Many bacterial species are facultative anaerobes, capable of functioning under both aerobic and anaerobic conditions. Microbial populations adapt to changes in water quality, expanding rapidly in response to suitable energy sources. When conditions become unfavourable, microbes can enter a dormant state for extended periods. However, the microbial community in constructed wetlands can be affected by toxic substances like pesticides and heavy metals, necessitating precautions to prevent their introduction at harmful concentrations (*A Handbook of Constructed Wetlands*, s. d.).

I.3 OVERVIEW OF CWs DESIGNS AND CONFIGURATIONS

Constructed wetlands can be classified into various types depending on several factors, including their function, the type of vegetation, and their hydrological characteristics, such as water level and flow direction (Dordio & Carvalho, 2013).

I.3.1 Classification according to their function

(Stefanakis et al., 2014) classified CWs according to their function in three areas of application:

- **CWs for Habitat creation**: their key characteristics such as water presence and vegetation, make them ideal for creating ecological habitats, attracting various wildlife species, and establishing green areas. Moreover, these systems can provide sources of food and fiber, as well as serve as public recreation sites.
- **CWs for flood control**: they are constructed with the purpose of managing runoff during flood events. Their installation can enhance stormwater storage capacity and increase infiltration volumes, consequently reducing the amount of water flowing into sewer systems and treatments plants. These wetlands contribute to Integrated Urban Water Management and offer opportunities for recycling stored water volumes.
- **CWs for wastewater treatment**: they are designed to receive and purify wastewater through natural treatment processes. It is this category that can be classified based on vegetation type and hydrology.

I.3.2 Classification according to the hydrology

Hydrology is the most important design factor in constructed wetlands because it links all the functions in a wetland and because it is often the primary factor in the success or failure of a constructed wetland (*A Handbook of Constructed Wetlands*, s. d.).

Depending on the flow path in the system, there are two main types of constructed wetlands ((Sundaravadivel & Vigneswaran, 2001); (Stefanakis et al., 2014); (Verlicchi & Zambello, 2014)): Surface Flow constructed wetlands (SF), and Subsurface Flow constructed wetlands (SSF). The latest can be divided according to the flow direction, into Horizontal Subsurface Flow (H-SSF) and Vertical Subsurface Flow (V-SSF) (Vymazal, 2010).

- **Surface Flow constructed wetlands (SF)**: they are frequently used in North America ((Stefanakis et al., 2014); (Vymazal, 2010)) and they consist of shallow channels with a sealed bottom (impermeable surface layer) to prevent wastewater leakages to the underlying aquifer (Stefanakis et al., 2014). Due to the presence of a water surface, surface flow wetlands more closely mimic natural wetlands, thereby offering greater benefits for wildlife habitats (Stefanakis et al., 2014). The water level is above the ground surface, with rooted vegetation emerging above the water surface. The vegetation can be floating or submerged. The primary flow of water occurs above the ground (*A Handbook of Constructed Wetlands*, s. d.). The flow of water will facilitate various processes including sedimentation, filtration, oxidation, reduction, adsorption, and precipitation, which aid in the treatment of pollutants (Kochi et al., 2020). The concentration of dissolved oxygen in the water column fluctuates, with higher levels near the surface and nearly absent levels near the bottom. This implies that in surface flow wetlands, the upper layer is typically aerobic, while the deeper waters and substrate tend to be anaerobic (*A Handbook of Constructed Wetlands*, s. d.). In a study conducted by Stefanakis in 2014, it has been proven that Surface flow wetlands are effective in the removal of suspended solids and BOD, removal of nitrogen, pathogens, and other pollutants (such as heavy metals) is high, while phosphorous removal is limited.

- **Horizontal Subsurface Flow constructed wetlands (H-SSF)**: those systems consist of gravel or soil beds typically planted with emergent macrophytes. The key distinction from SF CWs is the absence of a water surface exposed to the atmosphere. Instead, wastewater is fed into the wetlands at the inlet zone and flows horizontally along the bed below the substrate surface, passing through the pores of the porous media and the plant roots (Li et al., 2014). In such systems, the fed is continuous (Verlicchi & Zambello, 2014). As a result, health risks for wildlife habitat and humans are minimized (Stefanakis et al., 2014). Pollution is eliminated in the filtration beds through microbial degradation, as well as chemical and physical processes occurring within a network of aerobic, anoxic, and anaerobic zones. Most of the beds remain anoxic or anaerobic due to permanent saturation of the bed (Yoshitaka et al., 2013). Aerobic zones are limited to areas adjacent to roots, where oxygen seeps into the substrate (Vymazal, 2010). In these systems, there's not enough oxygen to break down waste properly. So, anaerobic processes become important in HSSF CWs enabling removal of BOD (Biochemical Oxygen Demand) and enhancing denitrification (Yoshitaka et al., 2013; (Brunhoferová, 2022)). Suspended solids are mainly trapped through filtration and settling, resulting in very high removal rates. In HSSF CWs, denitrification is the main process for removing nitrogen. However, the lack of oxygen in the filtration bed due to constant waterlogging limits the removal of ammonia. Phosphorus removal primarily occurs through ligand exchange reactions, where phosphate replaces water or hydroxyls on the surface of iron and aluminium hydrous oxides. Without special materials, phosphorus removal is typically low in HSSF CWs (Vymazal, 2010).
- **Vertical Subsurface Flow constructed wetlands (V-SSF)**: The flow also occurs through a porous medium, but the feed is distributed in large batches over the bed's surface, allowing water to percolate down through the medium ((Verlicchi & Zambello,

2014); (Vymazal, 2010)). The new batch is introduced only once all the water has percolated down and the bed is free of water: the feed is intermittent; thus this allows oxygen from the air to diffuse into the bed (Verlicchi & Zambello, 2014). Consequently, VSSF CWs are much more aerobic than HFCWs and offer favourable conditions for nitrification. However, VFCWs do not facilitate denitrification (Vymazal, 2010). Another type is the upflow vertical subsurface wetland, where wastewater is introduced at the bottom of the wetland. The water then percolates upward and is collected either near or on the surface of the wetland bed. Recently, "fill and drain" or "tidal" constructed wetlands have emerged. In tidal flow systems, wastewater percolates upward until the surface is flooded. Once the surface is fully flooded, feeding stops, and the wastewater is held in the bed. After a designated time, the wastewater is drained downward. Once the water has drained from the filtration bed, the treatment cycle concludes, allowing air to diffuse into the voids in the filtration material (Vymazal, 2010). VFCWs are also highly efficient in removing organic matter and suspended solids. However, phosphorus removal is typically limited unless media with high sorption capacity are employed (Vymazal, 2010).

- **Hybrid constructed wetlands**: Hybrid systems combine different types of constructed wetlands, mainly VFCWs and HFCWs, to enhance overall efficiency (Stefanakis et al., 2014). The concept is to capitalize on the strengths of one type to mitigate the weaknesses of the other. For example, while HSSF systems may have limited nitrification capacity due to oxygen transfer constraints, this can be compensated for by VFCWs, which are more effective in nitrification due to higher oxygen transfer capacity (Vymazal, 2010). Conversely, HFCWs provide favourable conditions for denitrification, unlike VFCWs. Hybrid systems are typically arranged in two or three stages to enhance treatment efficiencies (Li et al., 2014).

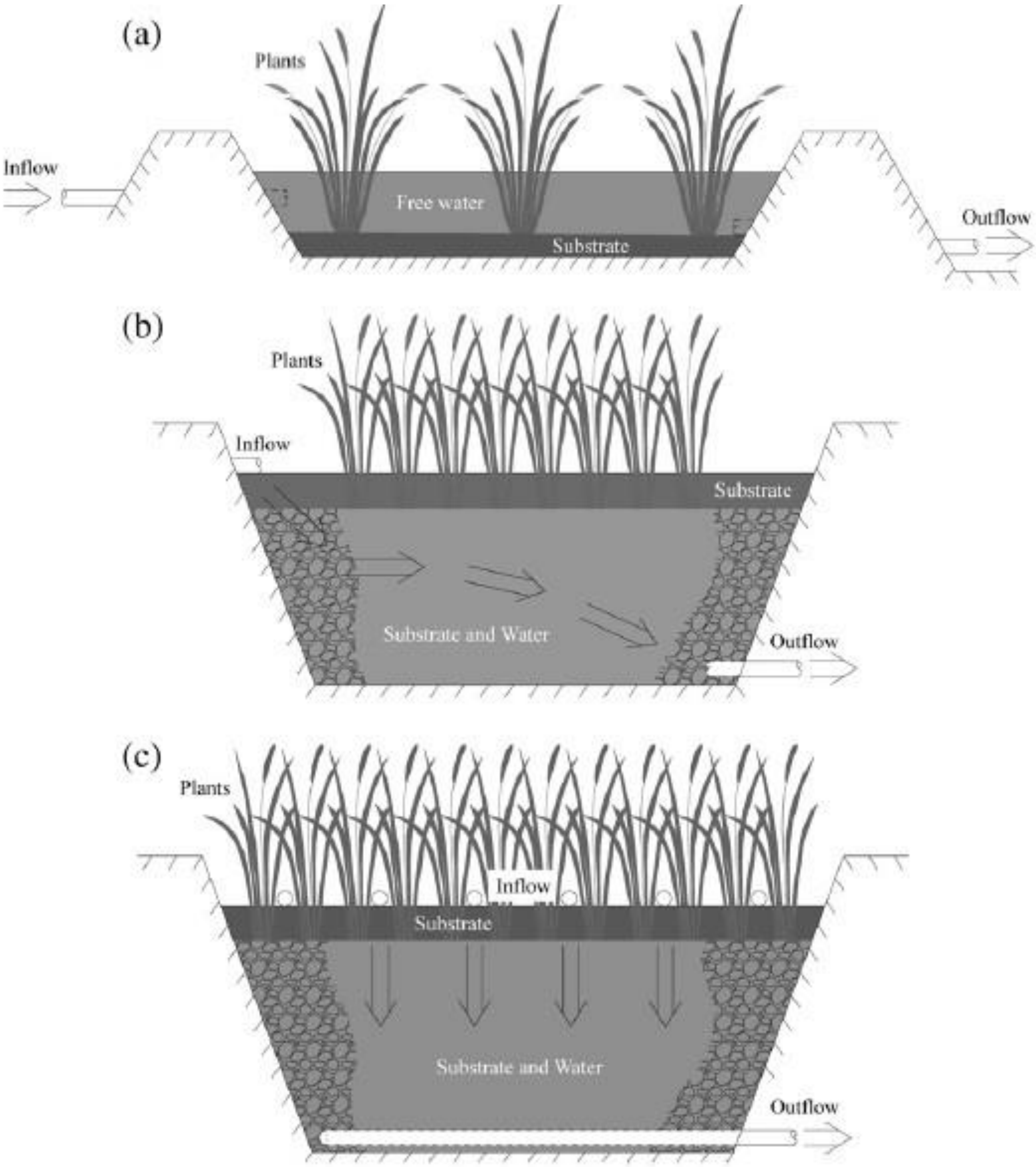

Figure 1: Structure of constructed wetlands. (a) SF CWs; (b) HSSF CWs; (c) VSSF CWs (Li et al., 2014)

I.3.3 Classification according to the vegetation type

Wetland vegetation is crucial within wetland ecosystems, playing a significant role in wastewater treatment processes. It decelerates water flow, creates microenvironments within the water column, and serves as attachment sites for microbial communities (*A Handbook of Constructed Wetlands*, s. d.). The primary parameter for selecting wetland plants is their ability to grow in aquatic environments. Additionally, other important factors include the plants' resilience to the toxic effects of pollutants, their ability to withstand seasonal variations, and their adaptability to changing environmental conditions such as fluctuating nutrient and contaminant levels. Furthermore, the capability of plants to uptake contaminants from wastewater is another crucial consideration (Brunhoferová, 2022).

One classification system defines four types of CWs based on dominant plant species**: floating macrophytes (free floating plants**); **floating leaf macrophytes (floating leaves plants); submerged macrophytes (submerged plants) and emerged macrophytes (emergent plant)** (Dordio & Carvalho, 2013). Generally, this classification based on vegetation type is commonly found in surface flow constructed wetlands, where the water level is above the substrate level (Kochi et al., 2020).

- **Emergent macrophytes**, which grow within a water table range from 50 cm below the soil surface to depths of 150 cm or more, are the dominant life forms in wetlands. They develop aerial stems, leaves, and an extensive root and rhizome system. Common species include Phragmites Australis, Glyceria, Eleocharis, Typhas, Scirpus, Iris, and Zirania aquatica (Hans Brix, 2003).
- **Floating leaf aquatic plants** encompass species rooted in the substrate (e.g., Nymphae spp, Nuphar) as well as those freely floating on the water surface (e.g., Eichhornia crassipes, Pistia stratiotes, Lemna, and Spirodella) (Hans Brix, 2003).
- **Submerged macrophytes** have their photosynthetic tissue entirely submerged, while their flowers are typically exposed to the atmosphere. Two recognized types include the elodeid type (e.g., Elodea, Myriophyllum, Ceratophyllum) and the isoetid type (e.g., Isoetes, Littorella, Lobelia) (Hans Brix, 2003).

In Europe, common macrophytes include *Phragmites australis, Typha*, and various species of *Scirpus*. These plants are preferred due to their well-documented ability to uptake pollutants from water (Brunhoferová, 2022).

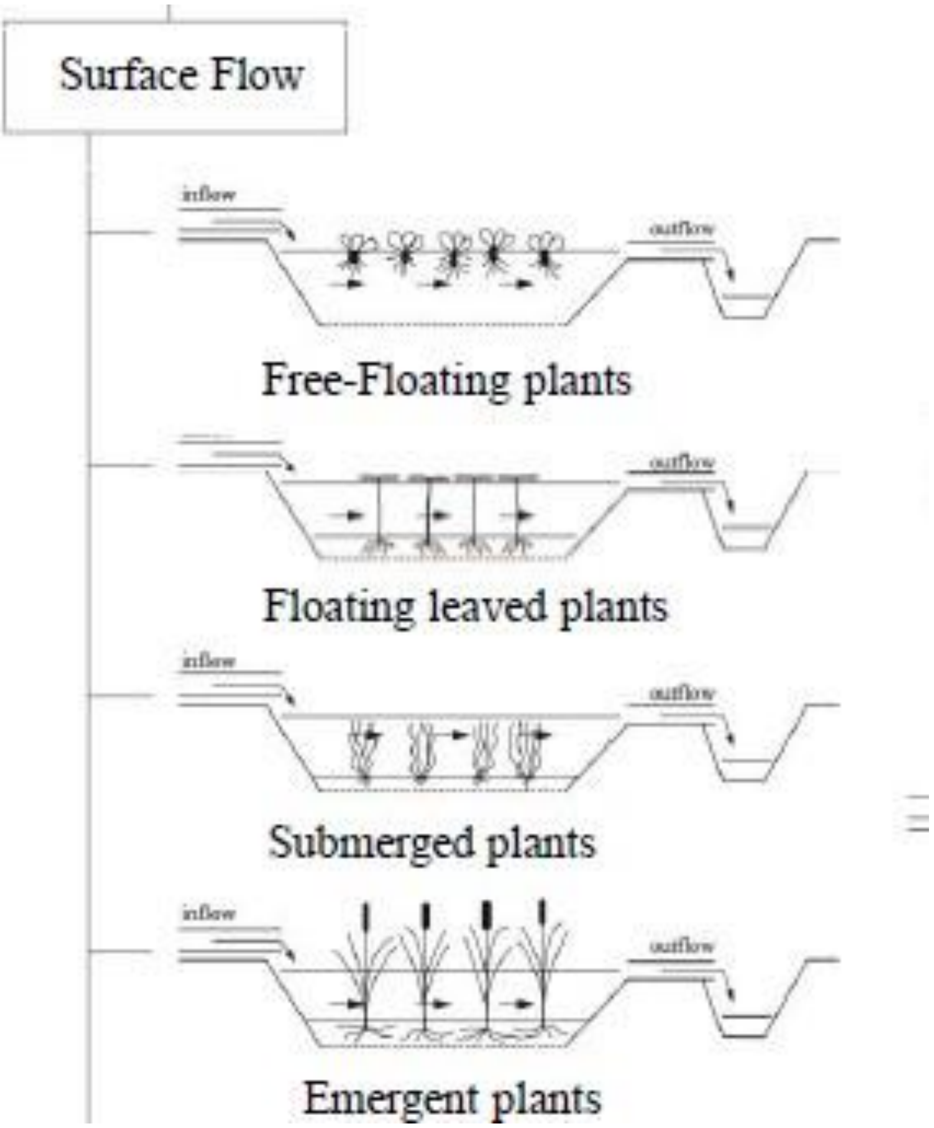

Figure 2: plants species in constructed wetland

I.4 REMOVAL MECHANISMS IN CONSTRUCTED WETLANDS

The main mechanisms of pollutant removal in CWs can be summarized as sorption onto the soil of the wetland, phytoremediation facilitated by plants, photodegradation through the action of the sun, and bioremediation carried out by microbes (Brunhoferová, 2022).

Sorption: The sorption of pollutants onto the substrate surface occurs through various mechanisms, including hydrophobic partitioning, electrostatic interaction, ion exchange, and surface complexation (Li et al., 2014). Non-polar pollutants are more likely to stick to materials with lots of organic matter, like soil, compost, and agricultural waste, through hydrophobic interaction. On the other hand, polar or ionic pollutants mainly stick to materials like certain types of clay through electrostatic interactions or ionic exchange (Li et al., 2014).

Phytoremediation: Pollutants can enter plants through roots or leaves, depending on their properties like size or solubility. The phytoremediation process involves different ways in which plants interacts with pollutants in the environment such as rhizodegradation, phytostabilization, phytovolatilization and phytoaccumulation (Singh et al., 2024). Rhizodegradation occurs when microorganisms in the rhizosphere (soil area close to plant roots where interactions occur between the roots, soil, and microorganisms) break down pollutants in the soil, while phytostabilization involves roots binding with contaminants to prevent their movement in the soil. Additionally, certain plants can release pollutants into the air through phytovolatilization, while others accumulate contaminants in their tissues through phytoaccumulation (Singh et al., 2024). Dordio and Carvalho (2013) observed that in constructed wetlands, most plant species can release oxygen from their root tips and young lateral roots. This oxygen release in the rhizosphere contributes to the oxidation of contaminants in wastewater, as well as the proliferation of aerobic microorganisms in the root zone, enhancing the efficiency of biodegradation processes.

Photoremediation: the mechanism of pollutants elimination occurred through the action of sun; thus, seasonal variation could strongly influence the removal of pollutants. It has been observed that, this process is not very effective in subsurface flow CW especially in vertical configuration where there is less surface area exposed to sunlight (Brunhoferová, 2022).

Bioremediation: This mechanism involves microorganisms and their activities. According to Dupoldt, microorganisms (including bacteria, yeasts, fungi, protozoa, and algae) help to break down both organic and inorganic substances into harmless or insoluble forms. They also alter the redox conditions of the environment, influencing substance processing in the wetland. Some microbial processes require oxygen, while others occur without it, and many bacteria can adapt to both aerobic and anaerobic conditions (Li et al., 2014). Microbial populations can quickly grow when provided with suitable energy sources, but they can also enter a dormant state when conditions become unfavourable. However, the microbial community in constructed wetlands is vulnerable to disruption by harmful substances like pesticides and heavy metals, highlighting the importance of careful management to maintain ecological balance and effective bioremediation.

I.5 CHARACTERISTICS OF A MESOCOSM DESIGN

A mesocosm is a controlled experimental setup designed to mimic natural environmental conditions, allowing researchers to study ecological processes and interactions (Wiersma, 2022). It is larger than laboratory-based microcosms but smaller than full-scaled field studies.

Mesocosms offer a middle ground, providing more realism than lab experiments while maintaining greater control than field studies (Perceval et al.,*MR2011_Mesocosms, 2009*). Typically, large tanks or enclosures are used, lined with natural substrates like soil, sand, and aquatic plants to create a realistic habitat. These mesocosms are populated with representative flora and fauna, including primary producers, consumers, and decomposers, while environmental factors such as water quality, temperature, light, and humidity are carefully managed (Ganesh et al., s. d.). Important design considerations include the appropriate scale of the mesocosm, replication for statistical accuracy, the inclusion of control and treatment groups, and the duration of the study to observe ecological processes fully (Gamble, s. d.). Regular monitoring and data collection are crucial for tracking changes over time. Mesocosms are employed in various research areas, including pollutant impact assessments, biodiversity and species interaction studies, climate change research, and testing ecological restoration methods (Wiersma, 2022). According to a study conducted by Wiersma in 2022, mesocosms offer several advantages for ecological research. They allow for precise control and environmental factors such as temperature and nutrients, enabling researchers to manipulate conditions accurately. The ability to setup multiple mesocosms facilitates replication, enhancing the reliability results. Additionally, it is often simple to meet the criteria for good experimental design, including control, replication, and randomisation.

II. MATERIALS AND METHOD

II.1 Characteristics of selected municipal wastewater

The wastewater used in the experiment comes from the Beringen wastewater treatment plant in Mersch commune, operated by SIDERO (Syndicat Intercommunal de Depollution des eaux Résiduaires de l'Ouest). This plant, with a capacity of 70,000 Population Equivalents (PE), treats an average of 11,373 m³/day. It was chosen for this study due to its high-quality effluent, with low levels of COD, BOD5, Total Nitrogen, nitrate nitrogen, ammonia nitrogen, and total phosphorus. The Beringen plant is one of the largest in Luxembourg. Its conventional activated tank has a volume of 14,800 m^3, with a sludge retention time (SRT) of 40 days and a hydraulic retention time (HRT) ranging from 12 to 41 hours. The plant also includes a screen, sand and grease trap, primary clarifier, digester, and dewatering facilities.

II.2 Characteristics of the substrates (admixtures)

The two types of biochar were produced using a low temperature pyrolysis at 750°C, an effective for capturing non-polar compounds. After carbonization, minerals (such as phosphate and carbonate salts), bacteria (including Lactobacillus, Rhodopseudomonas, and Saccharomyces) and yeast, were mixed with the biochar. This mixture was then left to ferment at temperatures between 25 and 35°C for four weeks, a process known as biological activation.

The difference between the two types of biochar is:

- Emisûre-AC: purely produced from plant residues, it has been widely used in previous studies, often compared to more traditional substrates like zeolite.
- WOW-AC: this biochar is produced from recovered cellulose, a by-product of the wastewater treatment process. The cellulose is separated during the mechanical treatment step to filter out cellulosic material from the influent stream. The pressed material is dried at 60°C to produce a raw cellulose material with 65–70% dry content. It is then converted into pellets with a diameter of 6 mm and further dried at 120°C to reach a final 90% dry content, mixed with 50% straw before pelletisation.

II.3 Configuration of mesocosm CW system

Two mesocosm CW systems (M1 and M2) were built with tanks of 27 cm length, 25 cm width and 30 cm height with 675 cm^2 of surface area and 20.25 l volume.

Figure 3: Mesocosm M1 and M2 configuration

Both units were planted with *Phragmites australis and Iris Pseudacorus* combining the benefit of having long and short roots respectively. The systems were filled with layers of gravel and substrate. The bottom layer, 10 cm thick, consisted of coarse gravel (4-8 mm) followed by fine gravel (2-8 mm). On top of this, there was a 20 cm layer of substrate, which is the most active part of each unit. Both units had the same support matrix structure, made of naturally occurring sand (95% Liapor sand, Germany) containing 55% SiO_2, 24% Al_2O_3, 14% Fe_2O_3, and 5% CaO. Additionally, 5% of activated biochar admixtures from different sources were added: Emisûre-AC (plant residues) for unit M1, and WOW-AC (50% cellulose/ 50% straw) for unit M2.

The two units are placed in the Urban Waste Management Laboratory (Campus Kirchberg, Luxembourg), where all analyses took place. LED lamps are installed on the top of both mesocosms to mimic sunlight and enable photodegradation.

The mesocosms are fed from a two headed pump which takes water from the tank cooled at 4°C. The hydraulic loading rate (HLR) is a critical parameter in the design and operation of constructed wetlands as it affects the efficiency of the treatment process by influencing the contact time between microorganisms and pollutants, which is crucial for effective biodegradation. HLR is inversely related to the hydraulic retention time (HRT), which is the time the wastewater spends in the treatment unit. HLR is defined with reference to both surface and volume of substrate:

- **HLRs with reference to surface** is the ratio of flow to the surface area of the wetland. $\boldsymbol{HLRs = \frac{Q}{A}}$ (m^3/ m^2d), where Q is the influent flow rate (m^3/d), and A is the wetland surface area (m^2).
- **HLRv with reference to the volume:** when considering volume, HLR is related to the volume of wastewater applied to the surface of the wetland unit per time. It involves the volume of the unit and the flow rate into the unit. $\boldsymbol{HLRv = \frac{Q}{V}}$ (m^3/ m^3d), with V the volume of the wetland.

NB: for mesocosm design, HLR is relevant if measured based on the volume of soil because the volume is reduced compared to other designs (lysimeters…) since the depth is smaller. The reduced soil volume in mesocosms means that the water moves through the system more quickly than in larger – scale systems. Therefore, the HLR becomes an important measure as it influences the contact time between the soil and water, affecting processes such as nutrient cycling, pollutant degradation, and overall ecosystem functioning.

Figure 4: Laboratory configuration of the mesocosm unit

II.4 Operation and sampling strategies

As initial conditions, the two units were left to dry for three days before the start of the investigation. Four operational scenarios were implemented for the mesocosm units: i) continuous mode with low load (lasting for one month), ii) batch mode with low load (lasting for two weeks), iii) continuous mode by doubling the load of the first scenario (lasting for one month) and finally iv) batch mode with high load (lasting for one week). The experiment started the 25th of March 2024 and latest for 11 weeks. The first scenario was the continuous mode with low load.

- **In the continuous mode with low load (Scenario 1) and high load (Scenario 3)**, the units were fed twice daily for 30 minutes each time. The applied continuous volumes were 30 ml/min per cycle, resulting in 1800 ml/day per unit for Scenario 1 and 60 ml/min per cycle, resulting in 3600 ml/day per unit for Scenario 3.
- **In the batch mode low and high load (Scenario 2 and 4 respectively)**, the units were fed only twice a week, with two days of drying followed by one day of feeding so that each unit received in one day, the total load it would have received in three days under the continuous mode of Scenario 1 and 3. Each feeding session lasted for 30 minutes. The applied batch volumes were 90 ml/min per cycle, resulting in 5400 ml/day per unit (Scenario 2) and 180ml/min per cycle, resulting in 10,800 ml/day per unit (Scenario 4).

After initiating the pump to supply the units, the effluent volume exiting each mesocosm was monitored every 15 minutes (percolation test) to measure the infiltration rate and retention volume.

The operational conditions for all four scenarios are summarized in the table below:

	SCENARIO 1	**SCENARIO 2**	**SCENARIO 3**	**SCENARIO 4**
Characteristics	Continuous	Batch	Continuous	Batch
Feeding mode or number of cycles	2 times every day	2 times every three days	2 times every day	2 times every three days
Test duration (weeks)	4 weeks	2 weeks	4 weeks	2 weeks
Setting pump (mL/min)	30	90	60	180
HLRs (L/m2day)	26,67	80	53,33	160
HLRv (L/m3day)	133,33	400	276,67	800
Number of samples	7	5	5	4

Table 1: Summary of Operational Conditions for the four Experimental Scenarios

II.5 Selection of the target compounds to be monitored

To assess the performance of the wetland units, 21 samples were collected from the outlet of each mesocosm and analysed for

1) various **physical parameters** such as electroconductivity (EC), dissolved oxygen (DO), redox potential (Redox), pH, temperature (T) and total suspended solids (TSS). Conventional WTW (Xylem, UK) probes are employed for measuring redox potential, pH, temperature, dissolved oxygen and electroconductivity.
2) **Macropollutants** such as soluble chemical oxygen demand (CODs), TOC, TC, IC, TN, NO_3-N, NH_4-N, PO_4-P were analysed. Hach Lange cuvette tests (Hach^{TM} LCK) are used for measuring CODs, NO_3-N, NH_4-N, PO_4-P, while a TOC analyzer (Shimadzu,B) is used for TOC, TC, IC, TN. TC=TOC+IC while COD is normally 3 times TOC.

Figure 5: Measurements of physical parameters

Figure 7: Hach tests for COD, NO3, NH4, PO4 measurements

Figure 6: TOC analyzer for TN, TOC, TC, IC measurements

3) **Personal care products** were analysed in one campaign only to better assess how performant is the whole treatment process from the conventional WWTP to the post-treatment (CW) in the removal of pollutants and the suitability of the studied substrates. The selected 23 target compounds were grouped into 7 categories:

- **Pharmaceuticals (4)**: Diclofenac, Clotrimazole, Fluconazole, Miconazole
- **Antiseptics (1)**: Triclosan
- **Fragrances (2)**: Galaxolide, Tonalide
- **Insecticides (1)**: DEET
- **Preservatives (4)**: Methylparaben, Ethylparaben, Propylparaben, Butylparaben
- **Surfactants (8)**: Diethylexylphthalate, Monoethylexylphthalate, 5-hydroxy-ethylexylphthalate, 5-oxo-ethylexylphthalate, Nonylphenol, Nonylphenol ethoxylate, Octylphenol, Octylphenol ethoxylate
- **UV-filters (3)**: Avobenzone, Octocrylene, Oxybenzone

All these target compounds are presented in Table 2. Logkow and Logkoc are essential parameters for environmental risk assessment and predicting the behavior and fate of chemicals in various environmental compartments, such as water, soil, air, and biota. The octanol-water partition coefficient (kow) indicates how a compound distributes between a hydrophobic phase (octanol) and a hydrophilic phase (water). A higher Logkow value means the compound has a stronger tendency to associate with octanol (or other organic phases) rather than water. The koc represents a compound's tendency to adsorb to organic carbon in soil or sediment, with higher koc values suggesting that the compound is more likely to bind to soil or sediment organic matter (Sipma et al., 2010). Hence in the table, compounds with high koc (Logkoc>3) are expected to be strongly adsorbed to the soil particles.

The analysis of the personal care products was performed externally by the Luxembourg Institute of Science and Technology (LIST). The quantification of PPCPs was performed by Liquid Chromatography and Tandem Mass Spectometry (LC-MS/MS) on an Infinity II U-HPLC coupled to 6495 triple-quadrupole MS (Agilent). The MS was operated in positive and negative Electrospray Ionisation (ESI). The analytical column was a Phenomenex Kinetex Biphenyl 150x2.1 mm (2.6 µm particle size). The mobile phases used in positive mode were DI-Water and methanol, both buffered with 0.1% Formic acid, and 5 mM ammonium Acetate and Methanol in negative mode. The flow rate was 300 µL/min, and the column temperature was 40°C.

The injection volume was 100µL, allowing the direct analysis of water samples without prior preconcentration. The quantification of target compounds was obtained by matrix-matched internal calibration, based on standards and quality check samples.

Group	Category	Class	Substance (INCI)	CAS number	Abbreviation	Molecular formula	Average mass (Da)	Logkow	Logkoc	Henry's law constant (atm-m3/mole)
PPCPs	Pharmaceuticals (4)	Anti-inflammatories (1)	Diclofenac	15307-86-5	DCF	C14H11Cl2NO2	296,149	4,51	2,921	4,73E-12
		Antimycotics (3)	Clotrimazole	23593-75-1	CLOT	C22H17ClN2	344,837	6,26	6,215	3,24E-08
			Fluconazole	86386-73-4	FCZ	C13H12F2N6O	306,271	0,25	4,722	3,51E-12
			Miconazole	22916-47-8	MIZ	C18H14Cl4N2O	416,129	6,25	4,788	8,70E-09
	Antiseptics (1)	Antiseptics (1)	Triclosan	3380-34-5	TCS	C12H7Cl3O2	289,542	4,76	4,265	3,83E-07
	Fragrances (2)	Fragrances (2)	Galaxolide	1222-05-5	HHCB	C18H26O	258,398	5,43	3,799	1,75E-04
			Tonalide	21145-77-7	AHTN	C18H26O	258,398	5,8	3,716	2,28E-04
	Insecticides (1)	Insecticides (1)	DEET	134-62-3	DEET	C12H17NO	191,27	2,18	2,73	1,25E-06
	Preservatives (4)	Parabens (4)	Methylparaben	99-76-3	MeP	C8H8O3	152,147	1,96	2,099	2,86E-08
			Ethylparaben	120-47-8	EtP	C9H10O3	166,174	2,47	2,365	1,07E-08
			Propylparaben	94-13-3	PrP	C10H12O3	180,201	3,04	2,631	1,38E-07
			Butylparaben	94-26-8	BuP	C11H14O3	194,227	3,57	2,896	4,03E-07
	Surfactants (8)	Phthalates (4)	DiethylhexylPhthalate	117-81-7	DEHP	C24H38O4	390,556	7,6	5,219	9,22E-03
			Monoethylhexylphthalate	4376-20-9	MEHP	C16H22O4	278,344	4,73	2,666	2,00E-07
			5-Hydroxy-EthylHexyl Phthalate	40321-99-1	5-OH-MEHP	x	x	x	x	x
			5-oxo-EthylHexylPhtalate	40321-98-0	5-oxo-MEHP	x	x	x	x	x
		Non-ionic surfactants (4)	Nonylphenol	84852-15-3	NP	C15H24O	220,35	5,84	4,651	2,67E-05
			Nonylphenol ethoxylate	9016-45-9	NPE	C17H28O2	264,403	5,58	3,44	5,63E-08

			Octylphenol	1806-26-4	OP	C14H22O	206,324	5,5	4,528	8,51E-06
			Octylphenol ethoxylate	9002-93-1	OPE	x	x	x	x	x
	UV-filters (3)	UV-filters (3)	Avobenzone	70356-09-1	AVO	C20H22O3	310,387	4,51	3,232	3,66E-07
			Octocrylene	6197-30-4	OC	C24H27NO2	361,477	6,88	5,614	3,94E-07
			Oxybenzone	131-57-7	OXY	C14H12O3	228,243	3,79	3,103	2,90E-08
Total	23					**23**				

Table 2: Target PPCPs to be monitored

II.6 Methodological Approach for Calculating Removal Efficiencies

To calculate the removal efficiencies of each pollutant, the concentrations of each pollutant were measured in the influent (in this case, the Beringen effluent) and in the effluents (both mesocosms effluent). The removal efficiency was then calculated for each sample by using the formula:

$$\text{Removal efficiency } \mathrm{RE} = \frac{Cinfluent - Ceffluent}{Cinfuent} \times 100\%$$

Where Cinfluent represents the pollutant concentration in the influent, and Ceffluent represents the pollutant concentration in the effluent.

After calculating the removal efficiency for each sample, the mean of these efficiencies was determined. This approach provides a comprehensive assessment of the overall effectiveness of the treatment process in removing pollutants from the influent.

III. RESULTS AND DISCUSSION

III.1 Characterisation of the WWTP effluent (Beringen effluent)

Prior to interpreting and discussing the experiment's findings, it is essential to provide an in-depth characterization of the influent (equivalent to the WWTP effluent). This preliminary step is crucial for understanding the baseline pollutant concentrations and contextualizing the effectiveness of the treatment process. To achieve this, table 3 summarises the mean values of pollutant concentrations alongside their respective standard deviations.

Although there is some variability in pollutant concentrations, the mean values of most compounds show a degree of stability over time. This implies a certain level of consistency in influent characteristics, with fluctuations likely attributed to operational factors, seasonal changes, or sporadic external inputs, rather than substantial alterations in the composition of the source. It is also evident that the mean concentration of CODs is 9.88 mg/L, which is significantly below the restriction limit of 125 mg/L, indicating compliance with organic pollutant levels. The average TC concentration is 57.08 mg/L, with a COD to TC ratio of about 0.17, indicating that TC levels are approximately 5.78 times higher than COD levels. This differs from findings in some research where TC levels are approximately 3 times COD levels, possibly due to sample characteristics. Furthermore, the mean TN concentration is 6.78 mg/L, which is below the regulatory limit of 10 mg/L (this value represents the threshold applicable to WWTPs sized between 10.000 and 150.000 PE as it is the case of Beringen WWTP), indicating compliance with Total Nitrogen standards. However, the standard deviation indicates some variability in TN levels, emphasizing the need for consistent monitoring and control strategies. Overall, the wastewater treatment processes at the Beringen plant effectively manage pollutant levels, including organic matter and nitrogen compounds, within regulatory limits.

BERINGEN EFFLUENT SAMPLES (mg/l)											
Compounds	25/03/2024	12/04/2024	18/04/2024	29/04/2024	14/05/2024	22/05/2024	29/05/2024	06/06/2024	**Mean concentration**	Standard deviation	**Limits from legislation (UWWTD 2024)**
CODs	8,83	8,91	10,50	9,81	13,90	9,81	7,96	9,33	**9,88**	1,80	**125**
TC	59,48	52,63	60,13	72,96	63,53	42,33	63,23	42,33	**57,08**	10,70	**-**
IC	58,99	52,70	60,00	51,67	55,92	40,54	58,41	60,40	**54,83**	6,63	**-**
TN	10,77	7,42	7,89	5,39	8,53	4,01	6,24	4,01	**6,78**	2,34	**10**
NO3-N	6,69	5,61	5,65	4,22	6,78	3,06	4,63	7,37	**5,50**	1,46	**-**
NH4-N	2,44	1,24	1,51	0,82	0,77	0,50	0,67	0,98	**1,12**	0,62	**-**
PO4-P	0,28	0,23	0,25	0,32	0,48	0,28	0,38	0,42	**0,33**	0,09	**0,70**

Table 3: Characterisation of the WWTP effluent, equivalent to mesocosm influent

III.2 Physical parameters

The objective was to understand the significance of physical parameters such as electroconductivity, redox potential, and dissolved oxygen on the performance and removal mechanisms of the wetlands. After analysing the mean values of these parameters for each scenario (see annex), the following observations were made:

- In Scenario 1, the average EC in M1 and M2 effluents is lower compared to the Beringen effluent. M1 and M2 effluents exhibit slightly higher redox potential values and similar pH ranges that are slightly higher than those of the Beringen effluent. Additionally, M1 and M2 have higher levels of DO than the Beringen effluent.
- In Scenario 2, M1 and M2 effluents have similar EC values, slightly lower than those of Beringen effluent. The redox potential in M1 and M2 effluents is higher than in Beringen effluent. The pH values of M1 and M2 effluents are slightly lower compared to Beringen effluent. DO levels in M1 and M2 effluents are similar but slightly lower than those in Beringen effluent.
- In Scenario 3, M1 and M2 effluents have lower EC values than Beringen effluent. The redox potential in M1 and M2 effluents is like that of Beringen effluent. The pH values of M1 and M2 effluents are slightly higher than those of Beringen effluent. The average temperatures in M1 and M2 effluents are higher than in Beringen effluent, and the DO levels in M1 and M2 effluents are like those in Beringen effluent.
- In Scenario 4, M1 and M2 effluents have lower EC values than Beringen effluent. The redox potential in M1 and M2 effluents is like that of Beringen effluent. The pH values of M1 and M2 effluents are slightly higher than those of Beringen effluent. The average temperatures in M1 and M2 effluents are like those in Beringen effluent, while the DO levels in M1 and M2 effluents are lower than those in Beringen effluent.

Overall, the wetland treatment processes lower the concentration of dissolved ions, by reducing electrical conductivity, thus improving water quality. Additionally, the mesocosms exhibit higher redox potential values than the Beringen effluent, indicating conditions that favour oxidation processes and the removal of organic matter and pollutants. However, the diminution of DO levels is observed, suggesting the consumption of oxygen by microorganisms in the substrates.

III.3 Percolation test

The objective of this test was to 1) determine the volume of water retained from the mesocosms; 2) measure the contact time; 3) assess the influence on the performances after watering cycles under different flow rates, feeding methods, and soil conditions. In each test scenario, the percolation test required monitoring the water draining from each mesocosm every 15 minutes to determine the retained water volume considering the pump's flow rate and the duration of each cycle. In parallel, the hydraulic retention time (HRT) could be calculated by recording when the pump started and when the first water drop from each mesocosm was observed. HRT provide crucial information about whether the water stays in the system long enough for pollutants to be removed effectively by different processes. A longer HRT typically facilitates these processes, improving the overall treatment performance of the wetland system.

III.3.1 Scenario 1: continuous mode with low load

It is important to recall that both units were left for drying more than three days before the start of the experiment. After conducting the percolation test on both mesocosms and analysing the seven samples, it's observed that M2 exhibits faster drainage than M1, retaining more water **with 13% of retained volume average compared to M1's 11% average.**

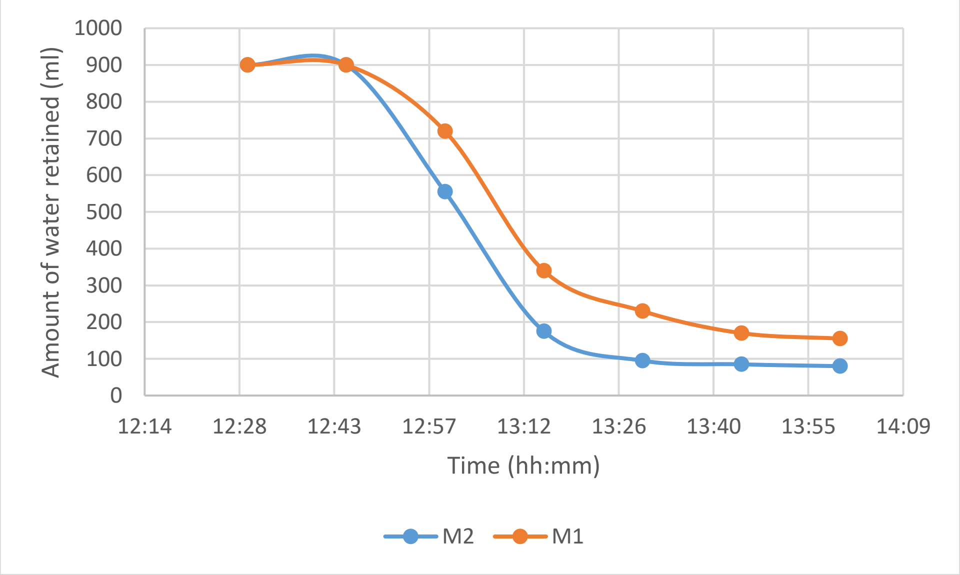

Figure 8: Percolation test initial of Scenario 1

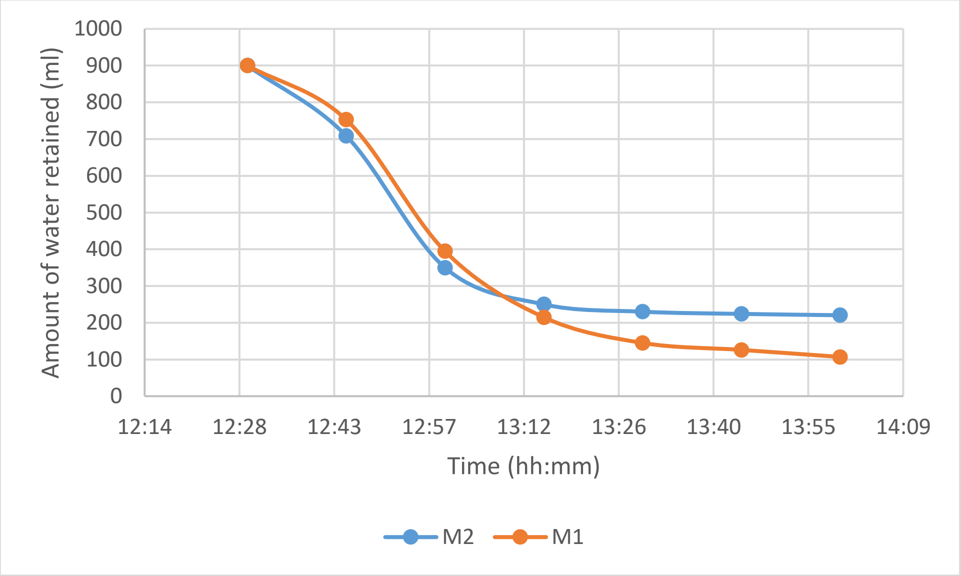

Figure 9: Percolation test final of Scenario 1

Initially, both units have high hydraulic retention times (HRT) 17 minutes for M1 and 14 minutes for M2, which gradually decrease over time to an average of 9 minutes for M1 and 7 minutes for M2 as it can be observed in table 4, while the retained volume increases over time for each mesocosm.

	Hydraulic retention time (minutes)		Retained water (%)	
	M1	M2	M1	M2
Sample of 25/03/2024	17	14	9%	0%
Sample of 26/03/2024	9	7	17%	9
Sample of 03/04/2024	9	4	9%	16%
Sample of 12/04/2024	9	7	9%	16%
Sample of 18/04/2024	9	7	12%	16%
Sample of 19/04/2024	6	7	12%	14%
Sample of 22/04/2024	9	8	12%	24%

AVERAGE	**9,71**	**7,71**	**11%**	**13%**

Table 4: Changes in Hydraulic Retention Time and Retained Water Over Time in Mesocosms scenario 1

This difference in water retention between M1 and M2 may be due to the distinct properties of each substrate like permeability or particle size distribution. M2 appeared to have a higher water retention capacity, although M1 retained more water than M2 during the first two days of the experiment. The decrease in HRT over time could be because the substrates become saturated in response to continuous water feeding allowing water to pass through the system more quickly. This increase in retained volume might affect the system's performance by reducing contact time between water and treatment media, potentially decreasing the effectiveness of pollutant removal processes such as adsorption, and microbial degradation. **As a result, shorter hydraulic retention times could compromise the overall treatment efficiency of the system**.

III.3.2 Scenario 2: Batch mode with low load

In scenario 2, where the feeding mode of the units was changed to batch mode, the results show a different pattern compared to the continuous mode of scenario 1.

- The HRT initially decreased in both units compared to the first scenario which was the beginning of the experiment, with M2 showing a slightly faster percolation than M1. However, the mean HRT are around **6.3 minutes** for M1 and **5.3 minutes** for M2, indicating a quick water flow through the M2 system.
- The retained volume increased significantly in both units, with M2 showing a slightly higher average retained volume (**38%**) compared to M1 (**36%**). These values are considerably higher than those observed in continuous mode (Scenario 1). This suggests that the batch mode with a load at once (Scenario 2), allowed for better water absorption and retention in the substrate.

	Hydraulic retention time (minutes)		Retained water (%)	
	M1	M2	M1	M2
Sample of 25/03/2024	6	5	17%	19%
Sample of 12/04/2024	6	5	48%	45%

Sample of 18/04/2024	7	6	43%	50%
AVERAGE	**6,33**	**5,33**	**36%**	**38%**

Table 5: Changes in Hydraulic Retention Time and Retained Water Over Time in Mesocosms scenario 2

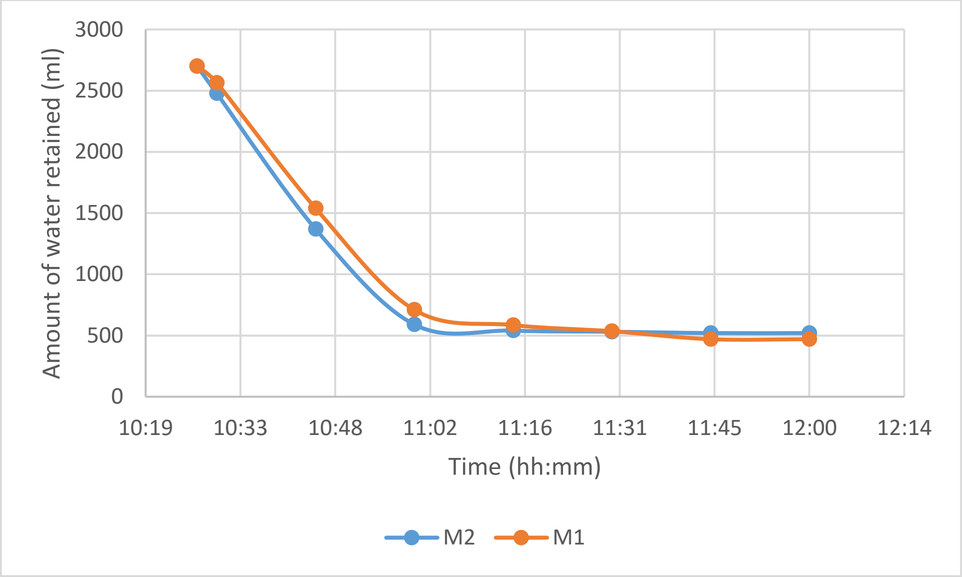

Figure 10: Percolation test initial of Scenario 2

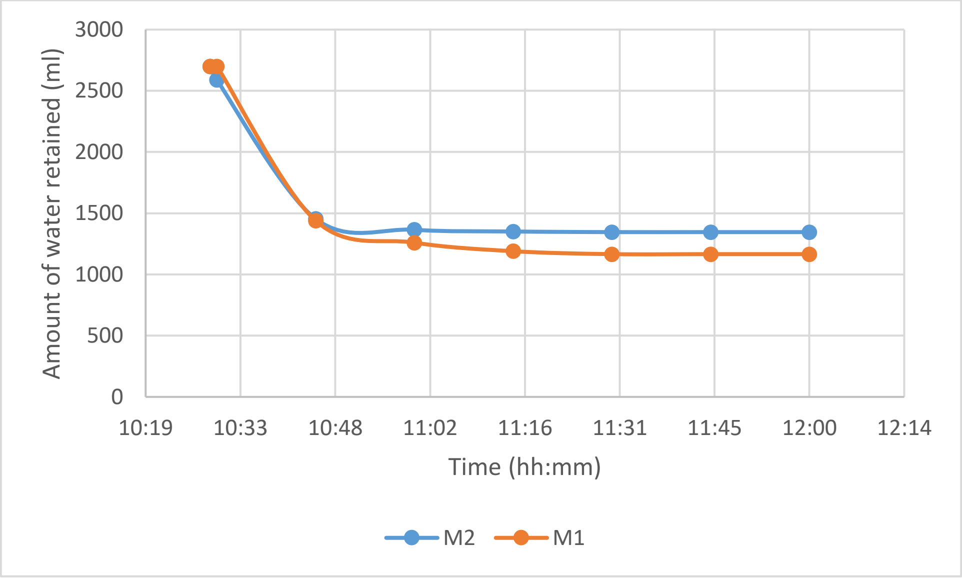

Figure 11: Percolation test final of Scenario 2

III.3.3 Scenario 3: continuous mode doubling load of scenario 1

As it was in Scenario 1 and 2, M2 still exhibits the highest retained volume and the lowest HRT. Compared to Scenario 1, Scenario 3 reveals significant differences in hydraulic performance. In Scenario 3, both M1 and M2 retained more water, with retained volumes increasing to 15% and 17% respectively, compared to 11% and 13% in Scenario 1. However, the hydraulic retention time (HRT) decreased in Scenario 3, with M1 dropping from 9.71 minutes to 7.2 minutes, and M2 from 7.71 minutes to 4.8 minutes.

	Hydraulic retention time (minutes)		Retained water (%)	
	M1	M2	M1	M2
Sample of 14/05/2024	14	11	22%	26%
Sample of 17/05/2024	6	3	11%	15%
Sample of 22/05/2024	5	4	12%	10%
Sample of 29/05/2024	6	3	15%	17%
Sample of 30/05/2024	5	3	14%	19%
AVERAGE	**7,2**	**4,8**	**15%**	**17%**

Table 6: Changes in Hydraulic Retention Time and Retained Water Over Time in Mesocosms scenario 3

The increase in retained volume and decrease in hydraulic retention time (HRT) in Scenario 3 compared to Scenario 1 can be attributed to several factors. Firstly, the higher flow rate in Scenario 3 likely led to more water being retained within the mesocosms due to the treatment media reaching its saturation point more quickly, resulting in increased water retention. Conversely, the increased flow rate naturally reduced the time water spends in the system, leading to a lower HRT. This quicker percolation once the retention capacity is met, results in faster water discharge. Consequently, while Scenario 3 optimized for higher initial water retention, it allowed for quicker passage, thereby increasing the retained volume but shortening the overall retention time. These dynamics could potentially affect pollutant removal efficacy for certain parameters.

III.3.4 Scenario 4: Batch mode doubling load of Scenario 2

In this last scenario, M2 still exhibits higher retained volume than M1 respectively 17% and 15%, while both present the same HRT 3,5 minutes.

	Hydraulic retention time (minutes)	Retained water (%)

	M1	M2	M1	M2
Sample of 03/06/2024	3	3	4%	11%
Sample of 06/06/2024	4	4	26%	22%
AVERAGE	**3,5**	**3,5**	**15%**	**17%**

Table 7: Changes in Hydraulic Retention Time and Retained Water Over Time in Mesocosms scenario 4

On the first day of this scenario, the retained water volume was lower compared to the following day of the experiment, this can be explained by the fact that the four-day drying period after scenario 3 likely allowed the mesocosms to desaturate a bit, which would indeed facilitate easier water percolation initially. By the second day of this scenario, the mesocosms substrates could have become more saturated, leading to increase water retention. As the substrate becomes more saturated, its capacity to retain additional water rises until it reaches a saturation point. Beyond this point, the substrate may become waterlogged, which can negatively impact treatment efficiency by reducing oxygen levels and affecting processes like aerobic degradation of pollutants and clogging. Thus, while increased saturation initially improves water retention, excessive saturation can hinder the system's overall treatment performance. The observed shorter HRT for both mesocosms could be a result of the high flow rate of water being injected, which forces water through the system more quickly.

Overall, the performance of constructed wetlands in a mesocosm setup is greatly affected by the feeding mode and Hydraulic Loading Rate (HLR). Batch feeding, as demonstrated in Scenario 2 and 4, lead to higher retained volumes. In contrast, continuous feeding appears to result in the longest HRT, which could be beneficial for the removal of micropollutants. Therefore, optimizing these parameters is essential for maximizing both water retention time and treatment efficiency in constructed wetlands. Achieving a balance between retention time and volume is crucial for effective contaminant removal. These findings can help guide the design and operational strategies for constructed wetlands to achieve specific water treatment objectives.

III.4 Macropollutants removal efficiency

III.4.1 Scenario 1: continuous mode with low load

Comparing the performance of the two different substrates (mesocosm 1 and 2), we observed contrasting results for the removal of different macropollutants. Both units showed similar performances for total carbon removal, with average removal rates of 38%. However, the results were more divergent for CODs, where M2 displayed superior performance with a removal rate of 69%, while M1 recorded a rate of 48%. Regarding total nitrogen, M2 demonstrated better performance with a removal rate of 24% compared to 16% of M1. Additionally, both units achieved 100% removal for NH4-N, suggesting a high efficiency of biological processes in nitrification. However, performance for NO3 were negative, indicating possible inefficiency of denitrification processes and conversion of ammonium into nitrates. Regarding PO4-P, both units showed high performance with removal rates of over 90%. Overall, mesocosm 2 demonstrates superior performance in macropollutants removal. This could be attributed to the distinct properties of the substrates used in each unit, which may positively influence pollutant treatment processes. It's also noteworthy that almost all the carbon is primarily in the inorganic form since the mean value of the TC and IC are close.

Measuerd concentrations scenario 1 (mg/l)					**Removal efficiency (%)**	
		Beringen effluent	**M1 effluent**	**M2 effluent**	**M1**	**M2**
CODs	Min	8,83	2,49	0,00	48%	69%
	Max	10,50	6,50	5,65		
	Mean	9,41	4,97	2,93		
	Standard deviation	*0,94*	*1,71*	*2,05*		
TC	Min	52,63	23,58	23,66	38%	38%
	Max	60,13	43,70	43,55		
	Mean	57,41	36,34	36,42		
	Standard deviation	*4,16*	*8,39*	*8,72*		
IC	Min	52,70	22,91	23,75	38%	37%
	Max	60,00	43,66	43,80		
	Mean	57,23	36,08	36,58		
	Standard deviation	*3,96*	*8,85*	*8,80*		
TN	Min	7,42	4,74	4,29	24%	0%
	Max	10,77	9,75	8,36		
	Mean	8,69	7,27	6,55		
	Standard deviation	*1,81*	*1,85*	*1,57*		
NO3-N	Min	5,61	4,34	3,96	-11%	-3%
	Max	6,69	8,73	7,78		
	Mean	5,98	6,64	6,15		
	Standard deviation	*0,61*	*1,61*	*1,50*		
NH4-N	Min	1,24	0,00	0,00	100%	100%
	Max	2,44	0,01	0,01		
	Mean	1,73	0,00	0,00		
	Standard deviation	*0,63*	*0,00*	*0,00*		

PO4-P	Min	0,23	0,00	0,00	92%	92%
	Max	0,28	0,10	0,04		
	Mean	0,25	0,02	0,02		
	Standard deviation	*0,02*	*0,04*	*0,01*		

Table 8: Measured Concentrations and Removal Efficiency of Various Pollutants in Scenario 1

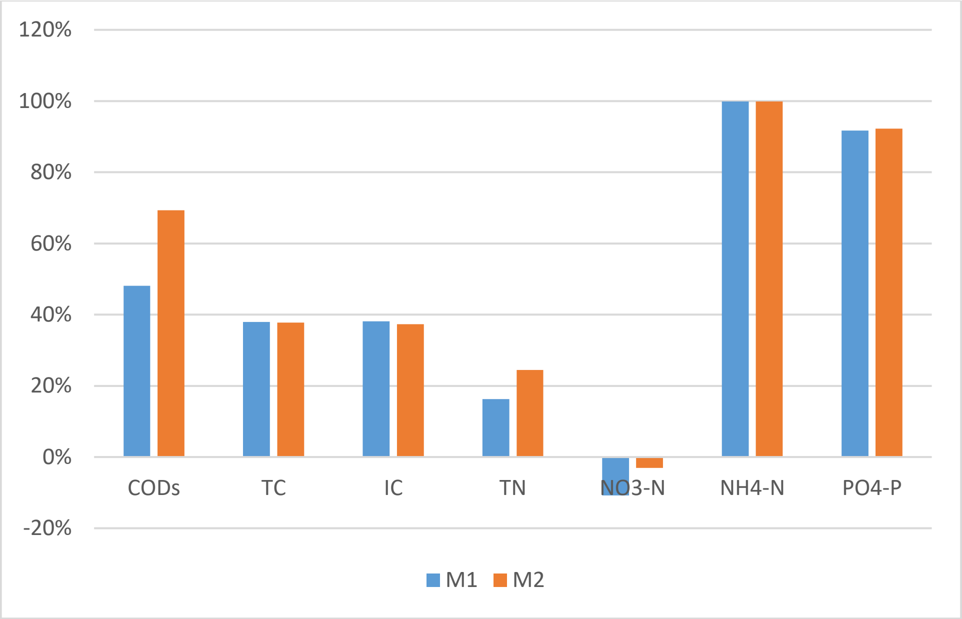

Figure 12: Macropollutants mean removal efficiency scenario 1

The negative performance for NO3-N removal in both units could be attributed to factors such as incomplete denitrification (vertical flow CWs are not favourable for denitrification because aerobic) and the conversion of ammonia into nitrates in the units. Denitrification, the process by which nitrates are converted into nitrogen gas, may not occur efficiently under certain conditions, leading to incomplete removal of NO3-N. As for the excellent performance in NH4-N removal, it could be due to the favourable conditions for nitrification within the constructed wetlands since the vertical flow configuration of the mesocosm provides ample oxygenation (aerobic conditions) that is favourable for nitrification. Nitrification, the process by which ammonia is converted into nitrates, is typically facilitated by aerobic conditions and the presence of nitrifying bacteria.

The moderate removal of CODs could be explained by the fact that CODs consist of both biodegradable and non-biodegradable organic compounds. In constructed wetlands, the breakdown of organic compounds can be influenced by factors like time contact within the substrate, substrate composition, and microbial activity. If conditions aren't ideal for biodegradation, it may result in incomplete removal of CODs. In our case, the hydraulic retention time is not high enough to allow the complete removal of CODs. Furthermore, it is important to note that the Hach Lange cuvettes used to measure COD concentrations have a range of 5-60 mg/L O2. Given that the COD concentrations in the mesocosm effluents are very low, the accuracy of the Hach Lange cuvettes may be limited.

Regarding the relationship with TC removal, it's noteworthy that both units show similar performance for TC removal, despite variations in COD removal. This suggests that the composition of carbon compounds in the effluents may differ, and it has been observed that while conducting the TOC analyzer test, almost all the carbon was in the inorganic form, which could be released from the media. It's generally not easy to eliminate inorganic carbon through biological processes. Inorganic carbon is primarily present in the form of mineral compounds such as carbon dioxide (CO2) or carbonates. These compounds are not easily degraded by microorganisms of constructed wetlands. Biological processes are more effective at degrading organic compounds, rather than inorganic compounds.

Regarding the removal efficiency of mesocosm 2, for example, we observe that in scenario 1 (continuous mode), the mesocosm shows high performance in pollutant removal at the beginning of the experiment (dry conditions). However, this performance decreases over time as the soil becomes saturated with water due to continuous feeding, except for the removal of NH4-N and PO4-P, which remain consistently high. This can be explained by the fact that at the start of the experiment, the biochar substrate is dry, providing ample space for water and pollutants to be absorbed and interact with the biochar and microbial communities. This led to high initial removal efficiencies as the pollutants are effectively captured and broken down. Over time as the mesocosm is continuously fed with water, the substrate becomes saturated. This reduces the available space for new water and pollutants, leading to a decrease in overall performance. The saturation can limit oxygen diffusion, which is necessary for aerobic microbial processes that break down organic pollutants. The removal of NH4-N and PO4-P is an exception, as they are removed through mechanisms that remain effective even under saturated conditions.

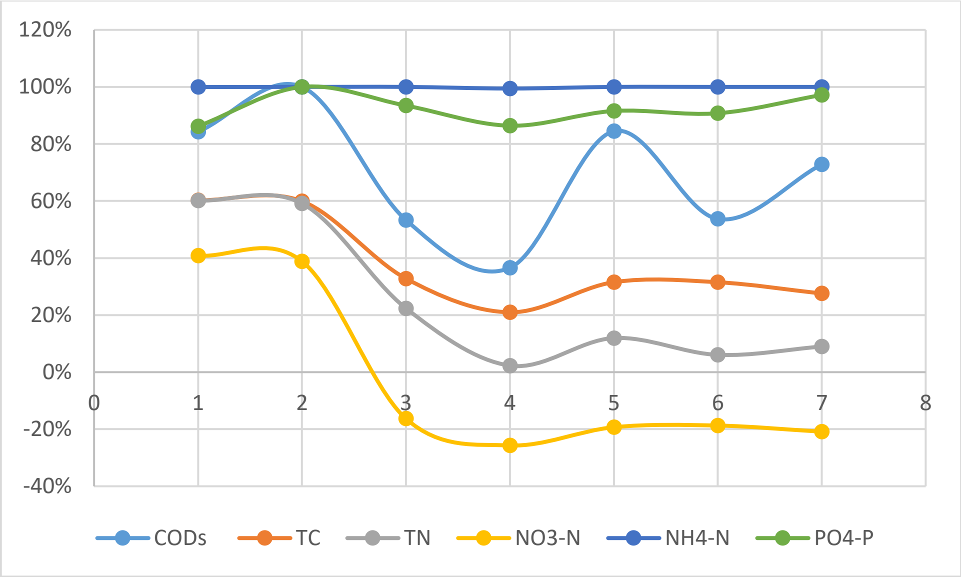

Figure 13: M2 removal efficiency in scenario 1

III.4.2 Scenario 2: Batch mode with low load

In Scenario 2, operating in batch mode, M1 exhibits lower mean removal efficiencies compared to its performance in continuous mode, with CODs at 31%, TC at 30%, and negative values for TN at -19% and NO3-N at -37%. Despite this, NH4-N and PO4-P removal remains high. Similarly, M2 shows a decrease in performance in batch mode compared to continuous mode, but still outperforms M1 with CODs at 48%, TC at 36%, and less negative values for TN at -15% and NO3-N at -46%. In terms of quantitative comparison, M2 is superior in CODs removal in both modes, although both units experience a notable decrease in batch mode. For TC removal, both units perform similarly in continuous mode, but M2 maintains higher efficiency in batch mode. TN removal is better in M2 during continuous mode, but both units struggle in batch mode, showing negative values. NO3-N removal yields negative values in both modes for both units, worsening in batch mode. However, NH4-N and PO4-P removal show excellent performance in both modes for both units.

Measuerd concentrations in scenario 2 (mg/l)					**Removal efficiency (%)**	
		Beringen effluent	**M1 effluent**	**M2 effluent**	**M1**	**M2**
CODs	Min	9,81	5,04	2,90	31%	48%
	Max	9,81	7,40	6,65		
	Mean	9,81	6,73	5,11		
	Standard deviation	*0,00*	*0,98*	*1,37*		

TC	Min	72,96	40,23	28,05	30%	36%
	Max	72,96	63,70	68,57		
	Mean	72,96	50,74	46,82		
	Standard deviation	*0,00*	*9,52*	*15,93*		
IC	Min	51,67	37,15	29,45	22%	30%
	Max	51,67	44,99	44,13		
	Mean	51,67	40,47	36,05		
	Standard deviation	*0,00*	*2,94*	*5,33*		
TN	Min	5,39	5,43	4,98	-19%	-15%
	Max	5,39	7,37	8,20		
	Mean	5,39	6,40	6,21		
	Standard deviation	*0,00*	*0,73*	*1,21*		
NO3-N	Min	4,22	4,52	5,85	-37%	-46%
	Max	4,22	6,83	6,69		
	Mean	4,22	5,77	6,18		
	Standard deviation	*0,00*	*0,82*	*0,32*		
NH4-N	Min	0,82	0,00	0,00	99%	100%
	Max	0,82	0,04	0,01		
	Mean	0,82	0,01	0,00		
	Standard deviation	*0,00*	*0,01*	*0,00*		
PO4-P	Min	0,32	0,02	0,02	91%	92%
	Max	0,32	0,04	0,03		
	Mean	0,32	0,03	0,02		
	Standard deviation	*0,00*	*0,01*	*0,00*		

Table 9: Measured Concentrations and Removal Efficiency of Various Pollutants in Scenario 2

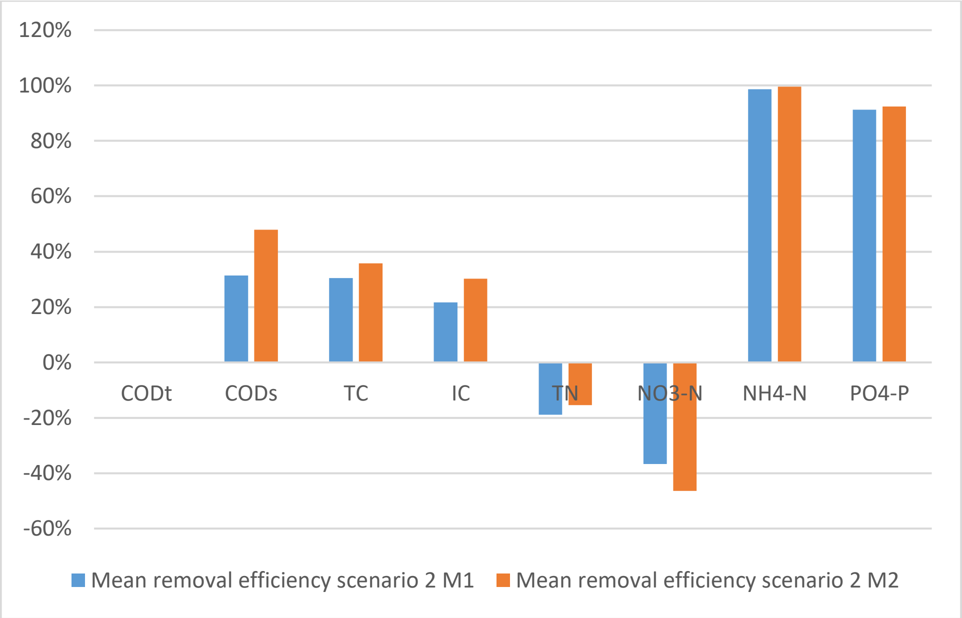

Figure 14: Macropollutants mean removal efficiency scenario 2

The observed decrease in efficiency for the removal of macropollutants from continuous mode to batch mode, except for NH4 and PO4, can be attributed to several factors. In continuous mode, the consistent low hydraulic loading rate (HLR) allows for more contact time between the water and the substrate (HRT is greater in continuous mode than in batch mode), enhancing macropollutants removal. In contrast, batch mode involves higher loading rates over a shorter period, insufficient for effective treatment processes. Continuous feeding maintains substrate saturation and moisture levels beneficial for microbial activity (avoiding the extreme of saturation and drying seen in batch mode), whereas batch mode's drying periods cause moisture fluctuations, which can negatively impact microbial populations. Microbial dynamics are also disrupted by intermittent feeding in batch mode, while continuous mode provides a stable environment for these processes. The organic load in batch mode is delivered in larger quantities at once, challenging the system's efficiency compared to the smaller, frequent loads in continuous mode (in batch mode, the units become stressed because they must handle larger quantities of load all at once). Additionally, nutrient competition in batch mode due to sporadic feeding can affect removal efficiencies, whereas continuous mode ensures balanced nutrient availability.

For NH4-N and PO4-P, the removal mechanisms differ:

- NH4-N removal, primarily through aerobic nitrification, remains unaffected by the drying periods in batch mode, maintaining high removal efficiencies.

- PO4-P removal, through adsorption onto the substrate or plant uptake, is less impacted by the feeding mode, ensuring consistent high removal efficiencies.

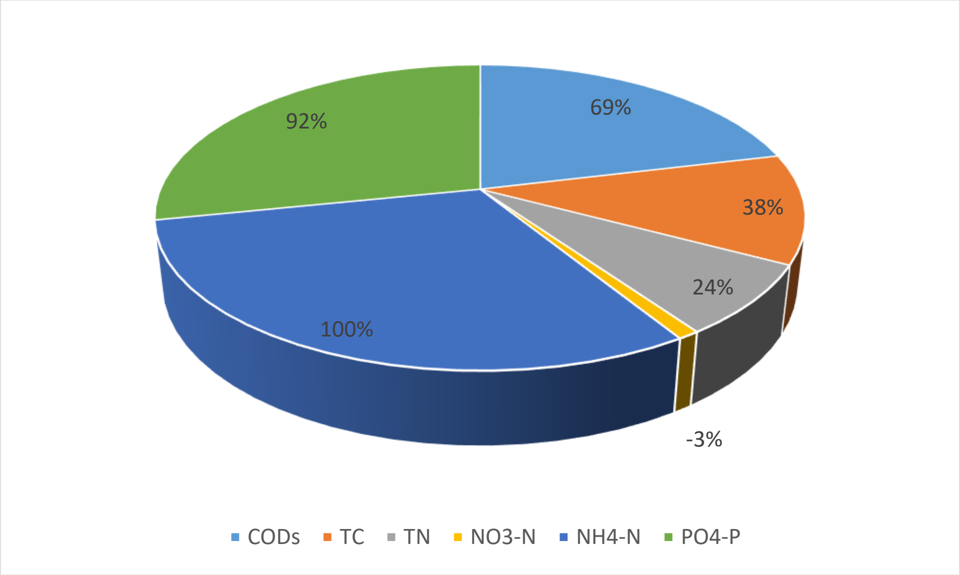

Figure 15: M2 mean removal efficiency scenario 1

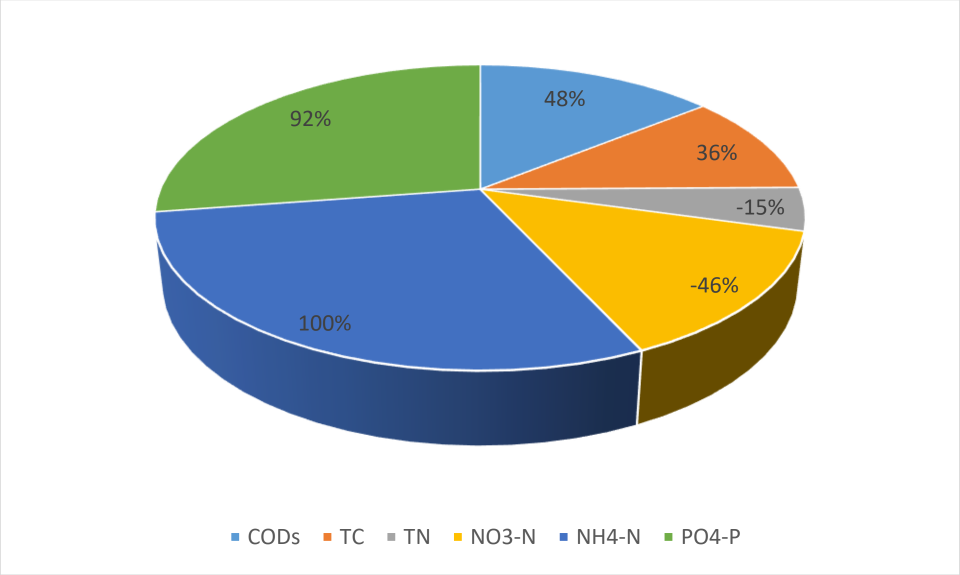

Figure 16: M2 mean removal efficiency scenario 2

In conclusion, the decrease in macropollutants removal efficiencies in batch mode is likely due to the intermittent feeding and higher loads which created altered environmental conditions affecting microbial activity and treatment processes, while NH4 and PO4 removal remains efficient due to their lesser dependency on these conditions. Overall, **M2 in continuous mode appears to be the most suitable substrate for macropollutants removal**.

III.4.3 Scenario 3: Continuous mode with high load

One initial observation is that the overall removal efficiencies for both mesocosms are lower than those of Scenario 1.

Measured concentrations scenario 3 (mg/l)					**Removal efficiency (%)**	
		Beringen effluent	**M1 effluent**	**M2 effluent**	**M1**	**M2**
CODs	Min	7,96	5,43	4,79	35%	44%
	Max	13,90	7,62	6,71		
	Mean	10,56	6,67	5,80		
	Standard deviation	*3,04*	*0,79*	*0,75*		
TC	Min	42,33	36,06	35,08	23%	4%
	Max	63,53	47,85	89,89		
	Mean	56,36	44,38	55,62		
	Standard deviation	*12,15*	*4,89*	*20,76*		
IC	Min	40,54	34,66	32,32	22%	25%
	Max	58,41	47,16	45,40		
	Mean	51,62	41,08	38,88		
	Standard deviation	*9,68*	*5,86*	*5,95*		
TN	Min	4,01	4,25	3,73	-6%	0%
	Max	8,53	8,17	7,98		
	Mean	6,26	6,53	6,10		
	Standard deviation	*2,26*	*1,67*	*1,84*		
NO3-N	Min	3,06	4,05	3,99	-25%	-20%
	Max	6,78	7,52	7,19		
	Mean	4,82	5,90	5,62		
	Standard deviation	*1,87*	*1,68*	*1,51*		
NH4-N	Min	0,50	-	-	99%	99%
	Max	0,77	0,03	0,03		
	Mean	0,65	0,01	0,01		
	Standard deviation	*0,14*	*0,01*	*0,01*		
PO4-P	Min	0,28	0,02	0,02	83%	90%
	Max	0,48	0,09	0,07		
	Mean	0,38	0,06	0,04		
	Standard deviation	*0,10*	*0,03*	*0,02*		

Table 10: Measured Concentrations and Removal Efficiency of Various Pollutants in Scenario 3

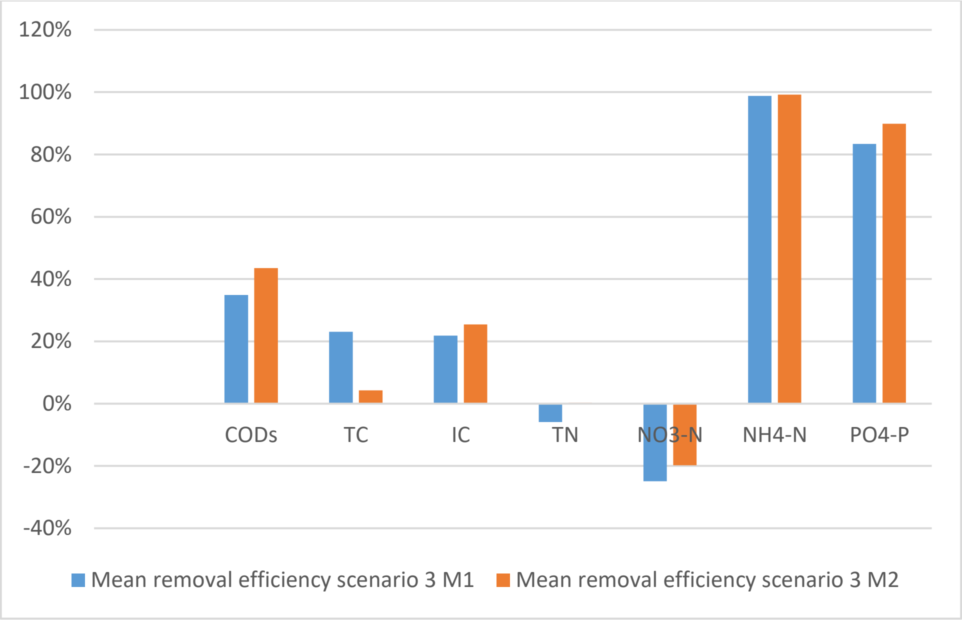

Figure 17: Macropollutants mean removal efficiency Scenario 3

Specifically, CODs removal in Scenario 1 was significantly higher, with M1 at 48% and M2 at 69%, compared to Scenario 3's 35% and 44%, respectively. Total carbon (TC) removal in Scenario 1 was 38% for both M1 and M2, whereas in Scenario 3, it was lower at 23% for M1 and just 4% for M2. Total nitrogen (TN) removal was also better in Scenario 1, with M1 at 16% and M2 at 24%, while Scenario 3 exhibited negative removal efficiencies, indicating increased TN concentrations, with M1 at -6% and M2 at 0%. Nitrate nitrogen (NO3-N) removal was consistently negative in both scenarios, but Scenario 1 had slightly better performance with M1 at -11% and M2 at -3%, compared to Scenario 3's -25% and -20%. Both scenarios achieved high removal efficiencies for ammonium nitrogen (NH4-N) and phosphate phosphorus (PO4-P), with Scenario 1 and Scenario 3 both showing 99% to 100% NH4-N removal, and PO4-P removal at 92% in Scenario 1 versus 83% for M1 and 90% for M2 in Scenario 3.

The difference in performance between the scenarios (1 and 3) can be primarily attributed to **the flow rate**. In Scenario 3, the higher flow rate causes the wastewater to move through the mesocosms more quickly, reducing the contact time necessary for effective adsorption and microbial degradation. This increased flow rate results in a shorter hydraulic retention time (HRT), giving pollutants less time to interact with the substrate and microbial community, thereby decreasing removal efficiency. Additionally, the higher flow rate can lead to substrate saturation with pollutants more rapidly, diminishing its effectiveness, especially for macropollutants that depend on the substrate's capacity to adsorb contaminants. Furthermore, increased flow rates can limit oxygen levels within the mesocosm, negatively affecting processes like nitrification and aerobic degradation that require sufficient oxygen. In summary, it can be argued that reduced contact time, shorter HRT, potential substrate saturation, and oxygen limitation due to the higher flow rate in Scenario 3 contribute to its decreased performance compared to Scenario 1.

III.4.4 Scenario 4: Batch mode with high load

Scenario 4 reveals some differences in treatment performance. CODs' removal efficiency was lower in this scenario, with M1 at 19% and M2 at 33%, indicating less effective organic matter reduction compared to Scenario 3. For TC, M1 showed a removal efficiency of 38%, while M2 had 11%, suggesting M1 was more effective in removing total carbon under higher loading conditions. TN removal was more effective in Scenario 4, with M1 achieving 33% and M2 at 15%, showing better nitrogen removal compared to Scenario 3. NO3-N removal was neutral for M1 (0%) and slightly negative for M2 (-6%), like the results in Scenario 3, indicating persistent issues with nitrate removal. NH4-N removal remained high at 96% for M1 and 100% for M2, consistent with Scenario 3. PO4-P removal efficiency was 67% for M1 and 84% for M2, slightly lower than in Scenario 3 but still indicating effective phosphate removal.

Measured concentrations in scenario 4 (mg/l)					**Removal efficiency (%)**	
		Beringen effluent	**M1 effluent**	**M2 effluent**	**M1**	**M2**
CODs	Min	7,96	5,42	4,17	19%	33%
	Max	9,33	8,65	7,61		
	Mean	8,65	6,98	5,80		
	Standard deviation	*0,97*	*1,33*	*1,42*		
TC	Min	61,40	0,58	46,78	38%	11%
	Max	63,23	55,07	68,79		
	Mean	62,32	38,61	55,75		
	Standard deviation	*1,29*	*25,59*	*9,32*		
IC	Min	58,41	40,00	41,55	20%	19%
	Max	60,40	53,91	53,52		
	Mean	59,41	47,83	48,25		
	Standard deviation	*1,41*	*6,14*	*5,55*		
TN	Min	6,24	0,03	5,34	33%	15%
	Max	9,30	8,82	7,94		
	Mean	7,77	5,46	6,48		
	Standard deviation	*2,16*	*3,78*	*1,08*		
NO3-N	Min	4,63	4,76	4,95	0%	-6%
	Max	7,37	7,81	7,21		

	Mean	6,00	5,91	6,09		
	Standard deviation	*1,94*	*1,34*	*1,07*		
NH4-N	Min	0,67	0,00	0,00	96%	100%
	Max	0,98	0,08	0,02		
	Mean	0,82	0,03	0,00		
	Standard deviation	*0,22*	*0,04*	*0,01*		
PO4-P	Min	0,38	0,10	0,02	67%	84%
	Max	0,42	0,16	0,10		
	Mean	0,40	0,13	0,06		
	Standard deviation	*0,03*	*0,03*	*0,03*		

Table 11: Measured Concentrations and Removal Efficiency of Various Pollutants in Scenario 4

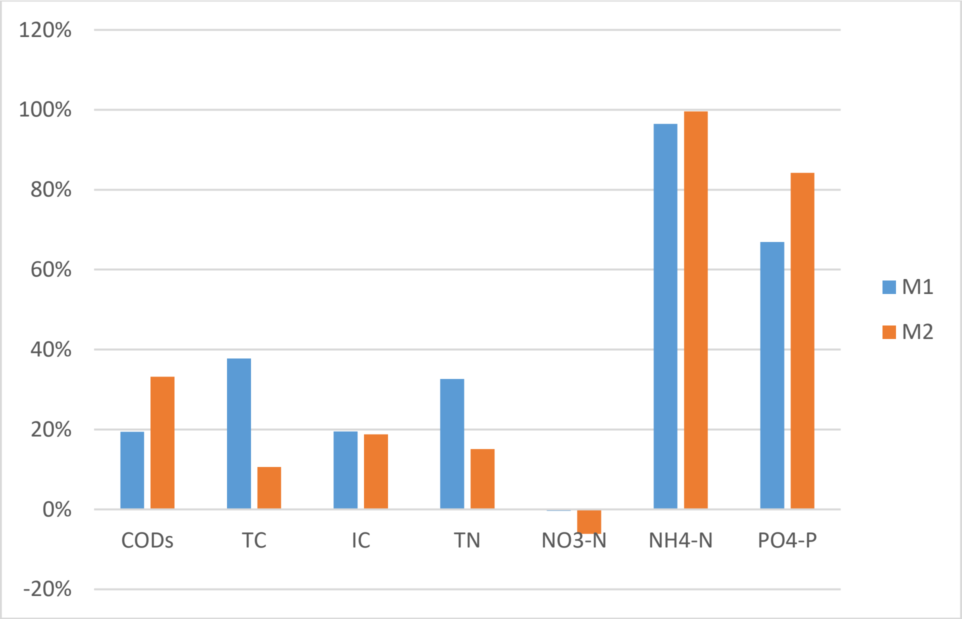

Figure 18: Macropollutants mean removal efficiency Scenario 4

III.5 Comparison of the Four scenarios

This section aims to make a comprehensive comparison to determine which scenario is the most suitable for macropollutant removal. The removal efficiencies of M2 were used, as it proved to be more effective than M1 in almost all scenarios. After evaluating these different scenarios, it was considered appropriate to compare the two units by first varying the feeding mode (continuous or batch mode) under low load conditions (scenario 1 and 2), and similarly for high load conditions (scenario 3 and 4). In the second step, by varying the load (low or high) for both continuous and batch modes. A careful interpretation of these results has revealed that:

III.5.1 Assessing treatment efficiency under Varied feeding mode (Continuous and Batch)

When comparing the feeding modes at low load, Scenario 1 (continuous) outperforms Scenario 2 (Batch) in the removal of CODs, TC, and Total Nitrogen TN, with both scenarios performing similarly well for NH4-N and PO4-P, achieving efficiencies close to 100% for NH4-N and high efficiencies for PO4-P. For NO3-N, both scenarios exhibit negative removal efficiency, but Scenario 1's performance is slightly better (less negative) than Scenario 2.

For high load conditions, Scenario 3 (Continuous) stands out for its better performance in removing PO4-P and CODs. On the other hand, Scenario 4 (Batch) is more effective for removing TC, TN, and NO3-N. Both scenarios exhibit high and similar performance in removing NH4-N.

III.5.2 Assessing treatment efficiency under varied load conditions (low and high)

When comparing the flow rate in continuous mode, Scenario 1 (low load) outperforms Scenario 3 (high load) in the removal of CODs, TC, and Total Nitrogen TN, with both scenarios performing similarly well for NH4-N and PO4-P. for NO3-N, both scenarios exhibit negative removal efficiency, but Scenario 1's performance is slightly better (less negative) than Scenario 3.

For batch mode, Scenario 2 (low load) stands out for its better performance in removing CODs and TC. On the other hand, Scenario 4 (high load) is more effective for removing TN, and NO3-N. Both scenarios exhibit high and similar performance in removing NH4-N and PO4-P.

In summary:

1) PO4-P, NH4-N removal is independent from the feeding mode;
2) overall, Scenario 1 appears to be the most performant for the treatment of the municipal wastewater effluent among the other scenarios. A low load helps to prevent overloading of the units, thus avoiding saturation and maintaining efficiency in macropollutant removal. This continuous low load provides ample time for macropollutants to interact with the microorganisms in the substrate, promoting better elimination. Additionally, continuous mode offers greater operational stability compared to batch mode, as it minimises fluctuations in load and environmental conditions between feeding cycles. By avoiding drying periods inherent in batch mode, continuous mode sustains the microbial community, ensuring consistent efficiency in micropollutant removal and overall system stability;

3) Denitrification (elimination of NO3-N) is ineffective in all four scenarios because the CW system is used as a post-treatment step and employs a vertical VF configuration. According to a study by (Cheng et al., 2022), denitrification is often challenging in secondary treatment wetlands (and most wastewater treatment systems) because nitrification must first convert influent ammonia into nitrate. This process cannot occur until sufficient organic carbon is consumed, often leaving too little residual organic matter for denitrification. The high oxygenation potential of VF systems makes them inefficient for denitrification since the process requires anoxic conditions to produce nitrogen gas. In contrast, most HF systems can fully denitrify the nitrate produced during secondary treatment. Consequently, VF wetlands generally remove little TN and have high nitrate concentrations in the effluent. HF wetlands can remove some TN, but the effluent may still contain significant ammonium nitrogen.

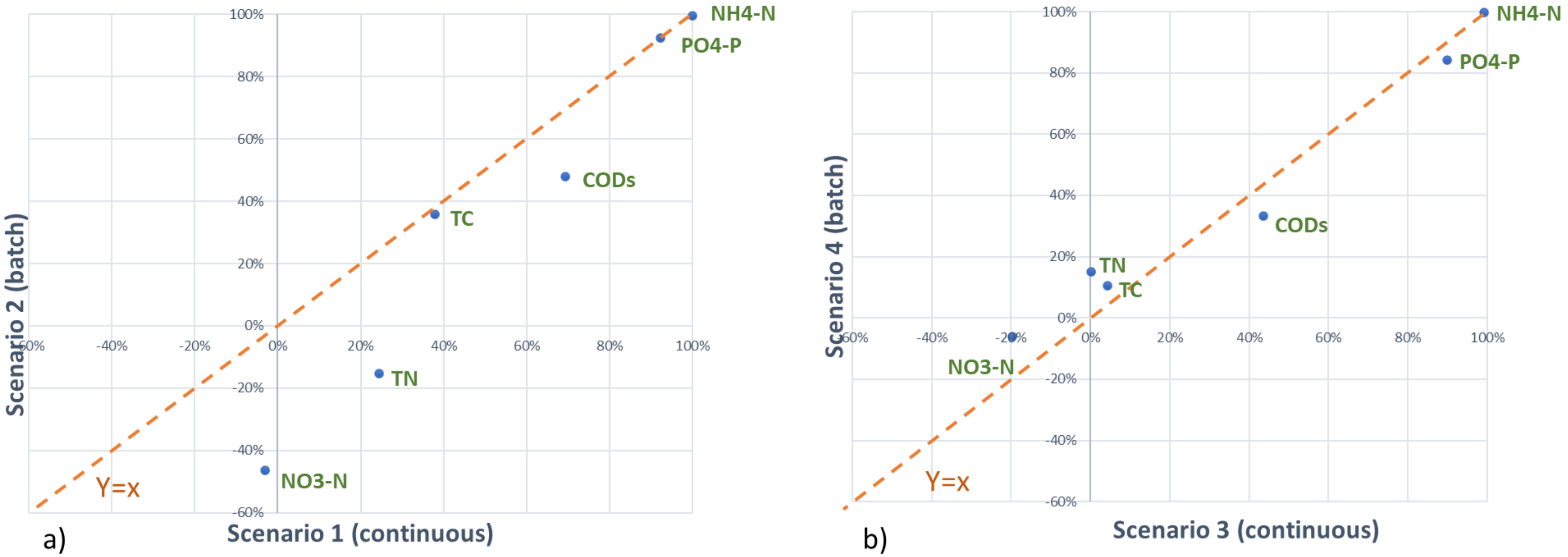

Figure 19: Assessing treatment efficiency under Varied feeding mode a) low load; b) high load

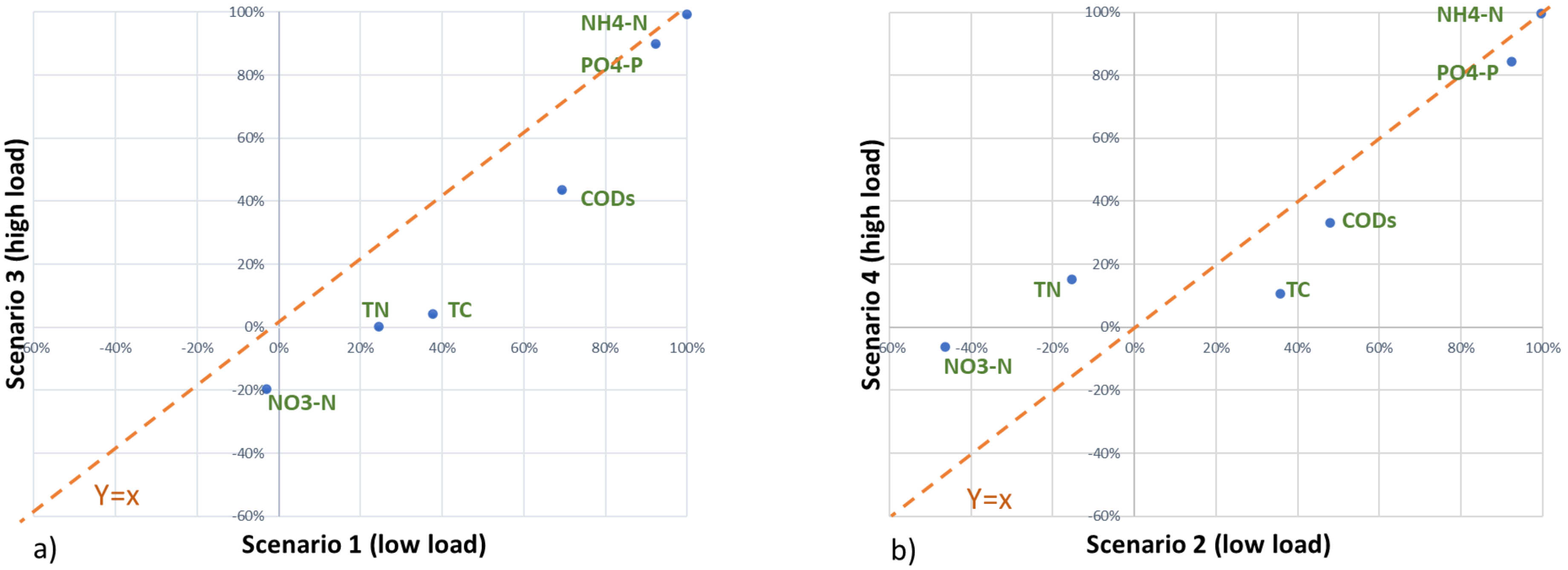

Figure 20: ***Assessing treatment efficiency under varied load conditions a) continuous mode; b) batch mode***

III.6 Performance of Constructed wetlands with respect to PPCPs

Among the 23 PPCPs listed in the table 2, only 6 were detected in the analyses conducted by the LIST laboratory: Diclofenac, Fluconazole, Octocrylene, Nonylphenol, Tonalide and Galaxolide. The others were not detected because their concentrations were below the limit of quantification. The limit of quantification (LOQ) of a micropollutant is the minimum concentration that can be reliably and accurately measured using standard analytical methods. It is important in environmental analysis because it defines the smallest amount of a micropollutant that can be detected and precisely quantified in a sample. The LOQ is usually higher than the limit of detection (LOD), which is the lowest concentration that can be detected but not necessarily quantified. Determining the LOQ is crucial for monitoring trace contaminants and ensuring that measurements are both dependable and meaningful (Mutzner et al., 2022).

Table 12 presents the concentrations of these detected micropollutants along with their removal efficiencies at each step of the treatment process.

- **Diclofenac and Fluconazole**: The concentrations of the Beringen WWTP effluent were higher than those of the Beringen WWTP influent, resulting in a negative removal efficiency of the plant. Consistent with findings from other studies, this negative removal is assumed 0%. However, after treatment in both mesocosms M1 and M2, the effluent concentrations were below the limit of quantification. This indicates that the mesocosms were highly effective, achieving 94% removal efficiency for diclofenac.
- **Octocrylene**: This compound was completely removed by the Beringen treatment plant, which translates to a 100% removal efficiency.
- **Nonylphenol**: There was no removal in the Beringen plant or mesocosm M1, but mesocosm M2 achieved a 7% removal efficiency. The overall process efficiency was very low, with 0% for M1 and only 1% for M2, suggesting that this compound is not effectively removed by the entire process. A definitive conclusion cannot be drowned because the LOQ (206,4 ng/l) is very high and comparable with the analysed concentrations.
- **Tonalide and Galaxolide**: Their removal is accomplished by both the WWTP and the CW, with each contributing to the overall removal efficiency, thereby enhancing the elimination of these pollutants.

For Tonalide, the Beringen plant removed 64% of the compound, and the mesocosms further reduced its concentration, with M1 removing an additional 40% and M2 removing 60%, resulting in total process efficiencies of 79% with M1 and 86% with M2. For Galaxolide, the Beringen plant achieved a 22% removal, while the mesocosms significantly enhanced this, with M1 removing 63% and M2 removing 76%, leading to total process efficiencies of 72% with M1 and 82% with M2. It can also be observed that on the second day (19/04/2024), as shown in figure 21, the removal efficiencies of both mesocosms remained high.

In summary, the mesocosms, particularly M2, were generally effective at removing the micropollutants that were not removed by the Beringen treatment plant. The results suggests that the substrate in M2 might be more effective for micropollutant removal compared to M1, especially for Nonylphenol, Tonalide, and Galaxolide. However, for Diclofenac, both mesocosms performed equally well. As shown in Table 12, the Beringen plant is ineffective at removing diclofenac. However, the constructed wetland performs well in eliminating this compound, with a removal efficiency of 94%. This aligns with the fact that diclofenac has a LogKoc value greater than 3, making it favourable for binding to the solid media in constructed wetlands. Furthermore, the removal efficiencies of all these micropollutants exceed the 80% threshold set by the 2024 UWWTD legislation, highlighting the importance of constructed wetlands (CWs) in eliminating micropollutants as a post treatment step. It’s important to note that “<XXX” values indicate that the concentration was below the limit of quantification, which is considered as complete removal or 100% efficiency for that specific step.

Compounds	Beringen influent (ng/l)	Beringen effluent (ng/l)	Beringen removal efficiency	M1 effluent (ng/l)	M2 effluent (ng/l)	M1 removal efficiency	M2 removal efficiency	Efficiency of the whole process with M1	Efficiency of the whole process with M2
Diclofenac	158,5	262,9	0%	<10,01	<10,01	94%	94%	94%	94%
Fluconazole	27,3	40,94	0%	<10,35	<10,35	100%	100%	100%	100%
Octocrylene	128,9	<112,38	100%	<112,38	<112,38	100%	100%	100%	100%
Nonylphenol	213,46	226,61	0%	279,57	210,75	0%	7%	0%	1%
Tonalide	100,23	35,8	64%	21,41	14,48	40%	60%	79%	86%
Galaxolide	737,76	572,99	22%	210,06	135,44	63%	76%	72%	82%

Table 12: Concentrations and Removal Efficiencies of Detected Micropollutants

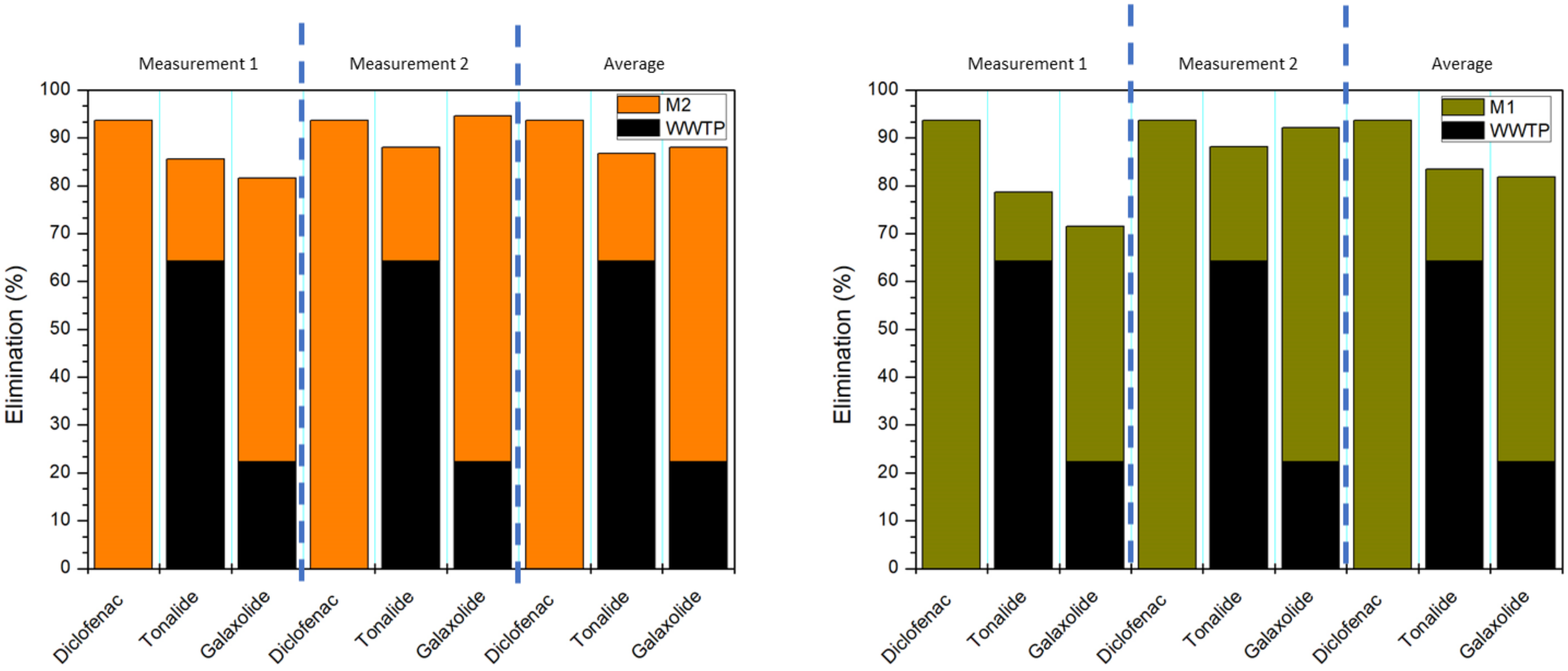

Figure 21: Removal efficiency of the whole process

CONCLUSION AND RECOMMENDATIONS

Constructed wetlands have shown great promise as effective alternatives for post-treatment of effluents from WWTPs, particularly for the removal of macropollutants and personal PPCPs. This study aimed to assess the performance of non-conventional substrates, specifically biochar produced from recovered cellulose and biochar produced from plant residues, used in CWs. The findings revealed that while both substrates are effective in treating these pollutants, the biochar produced from cellulose demonstrated superior performance across all four operational scenarios. This not only highlights its efficacy but also promotes a circular economy by valorising paper toilet residues into useful biochar for wastewater treatment.

The study also underscored the significant impact of operating mode conditions on the efficiency of CWs. Among the tested scenarios, the continuous mode with a low load was found to be the most appropriate for pollutant treatment. A low load prevents overloading and saturation of the units, thus maintaining efficiency in micropollutant removal. Continuous low load conditions allow ample time for micropollutants to interact with microorganisms in the substrate, leading to better elimination. Additionally, continuous mode offers greater operational stability compared to batch mode, minimizing fluctuations in load and environmental conditions between feeding cycles. By avoiding the drying periods inherent in batch mode, continuous mode sustains the microbial community, ensuring consistent efficiency in micropollutant removal and overall system stability. Scenario 1, although more effective, requires twice the surface area of scenario 3 to treat an equivalent daily volume of water. Despite its superior performance, this larger area needed could pose limitations based on space constraints or associated installation and maintenance costs.

Despite the overall positive results, both units showed poor efficiency in eliminating nitrate (NO3-N). Therefore, impacting the TN removal efficiency. As per the Revised Urban Wastewater Treatment Directive 2024, which limits TN concentrations to 10 mg/l, the mesocosm system's effluent remains below this threshold, ensuring safety. This can be attributed to the vertical flow design of the constructed wetlands, which does not provide favourable conditions for denitrification as it supports primarily aerobic processes. However, the removal efficiencies for phosphate (PO4-P) and ammonium (NH4-N) remained high across all scenarios, indicating that these pollutants are less dependent on operational mode conditions for effective removal.

In conclusion, this study demonstrates the potential of using constructed wetlands with biochar substrates, particularly those derived from recovered cellulose, as a viable post-treatment step for removing macropollutants and PPCPs from wastewater effluents. The continuous mode with low load emerges as the optimal operational condition, offering both high removal efficiency and operational stability. These findings contribute to the advancement of sustainable wastewater treatment practices and highlight the importance of integrating circular economy principles in environmental management.

However, it is important to note that the conclusions of these experiments are not exhaustive. For instance, modifying the depth of the substrate could provide insight into whether this change would affect the removal efficiencies of the mesocosms. Increasing the depth would enhance the hydraulic retention time (HRT), allowing pollutants more time to interact with the media. Future studies could explore varying the substrate depth to determine its impact on treatment performance.

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ANNEX

Annex 1: Scenario 1

Samples of the 25/03/2024				
Flow rate: 30ml/min				
Total feeding volume per mesocosm		900		
Start of the pump	12:33		Retained water (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:47	12:50		
12:30	0	0	900	900
12:45	0	0	900	900
13:00	410	210	490	690
13:15	800	590	100	310
13:30	885	740	15	160
13:45	915	790	-15	110
14:00	925	820	-25	80
Retained volume (%)	-3%	9%		
HRT (min)	00:14	00:17		

Samples of the 26/03/2024				
Flow rate: 30ml/min				
Total feeding volume per mesocosm		900		
Start of the pump	12:40		Retained water (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:47	12:49		
12:30	0	0	900	900
12:45	0	0	900	900
13:00	345	180	555	720
13:15	725	560	175	340
13:30	805	670	95	230
13:45	815	730	85	170
14:00	820	745	80	155
Retained volume (%)	9%	17%		
HRT (min)	00:07	00:09		

Samples of the 03/04/2024		
Flow rate: 30ml/min		

Total feeding volume per mesocosm		900		
Start of the pump	12:28		Retained water (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:32	12:37		
12:30	0	0	900	900
12:45	297	173	603	727
13:00	640	500	260	400
13:15	730	680	170	220
13:30	742	740	158	160
13:45	748	770	152	130
14:00	754	820	146	80
Retained volume (%)	16%	9%		
HRT (min)	00:04	00:09		

Samples of the 12/04/2024				
Flow rate: 30ml/min				
Total feeding volume per mesocosm		900		
Start of the pump	12:28		Retained water (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:35	12:37		
12:30	0	0	900	900
12:45	260	184	640	716
13:00	650	530	250	370
13:15	735	710	165	190
13:30	744	760	156	140
13:45	748,6	781	151,4	119
14:00	755	821	145	79
Retained volume (%)	16%	9%		
HRT (min)	00:07	00:09		

Samples of the 18/04/2024				
Flow rate: 30ml/min				
Total feeding volume per mesocosm		900		
Start of the pump	12:29		Retained water (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:36	12:38		

12:30	0	0	900	900
12:45	236	152	664	748
13:00	630	480	270	420
13:15	730	680	170	220
13:30	750	745	150	155
13:45	754,4	765	145,6	135
14:00	759,4	792	140,6	108
Retained volume (%)	16%	12%		
HRT (min)	00:07	00:09		

Samples of the 19/04/2024				
Flow rate: 30ml/min				
Total feeding volume per mesocosm		900		
Start of the pump	12:30		Retained water (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:37	12:36		
12:30	0	0	900	900
12:45	222	152	678	748
13:00	640	480	260	420
13:15	740	680	160	220
13:30	760	745	140	155
13:45	764,2	765	135,8	135
14:00	769,6	792	130,4	108
Retained volume (%)	14%	12%		
HRT (min)	00:07	00:06		

Samples of the 22/04/2024				
Flow rate: 30ml/min				
Total feeding volume per mesocosm		900		
Start of the pump	12:29		Retained water (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:37	12:38		
12:30	0	0	900	900
12:45	191	147	709	753
13:00	550	505	350	395
13:15	650	685	250	215
13:30	670	755	230	145
13:45	676	774	224	126

14:00	679,6	793	220,4	107
Retained volume (%)	24%	12%		
HRT (min)	00:08	00:09		

Retained volume (%) scenario 1		
	M1	M2
Sample 1	9%	-3%
Sample 2	17%	9%
Sample 3	9%	16%
Sample 4	9%	16%
Sample 5	12%	16%
Sample 6	12%	14%
Sample 7	12%	24%
mean	11%	13%

HRT (minutes) scenario 1		
	M1	M2
Sample 1	17,00	14,00
Sample 2	9,00	7,00
Sample 3	9,00	4,00
Sample 4	9,00	7,00
Sample 5	9,00	7,00
Sample 6	6,00	7,00
Sample 7	9,00	8,00
mean	9,71	7,71

Samples of the 25/03/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt	12,00		0,00	0,00	0,00
CODs	8,83	3,13	1,39	65%	84%
TC	59,48	23,58	23,66	60%	60%
IC	58,99	22,91	23,75	61%	60%
TN	10,77	4,74	4,29	56%	60%
NO3-N	6,69	4,34	3,96	35%	41%
NH4-N	2,44	0,00	0,00	100%	100%
PO4-P	0,28	0,00	0,04	100%	86%

Samples of the 26/03/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt	12,00			0,00	0,00
CODs	8,83	2,49	0,00	72%	100%
TC	59,48	24,92	23,84	58%	60%

IC	58,99	23,65	23,83	60%	60%
TN	10,77	4,75	4,40	56%	59%
NO3-N	6,69	4,57	4,09	32%	39%
NH4-N	2,44	0,00	0,00	100%	100%
PO4-P	0,28	0,00	0,00	100%	100%

Samples of the 03/04/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt	12,00	6,48	5,88	46%	51%
CODs	8,83	5,96	4,12	33%	53%
TC	59,48	38,77	39,96	35%	33%
IC	58,99	38,97	40,60	34%	31%
TN	10,77	9,75	8,36	10%	22%
NO3-N	6,69	8,73	7,78	-30%	-16%
NH4-N	2,44	0,00	0,00	100%	100%
PO4-P	0,28	0,00	0,02	100%	93%

Samples of the 12/04/2024 (effluent from 04/04/2024)					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt				0,00	0,00
CODs	8,91	6,50	5,65	27%	37%
TC	52,63	41,19	41,58	22%	21%
IC	52,70	41,41	42,08	21%	20%
TN	7,42	7,76	7,25	-5%	2%
NO3-N	5,61	7,45	7,05	-33%	-26%
NH4-N	1,24	0,01	0,01	99%	99%
PO4-P	0,23	0,10	0,03	56%	86%

Samples of the 18/04/2024 (effluent from 18/04/2024)

Parameter	Beringen influent 17/04/2024	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency	Overall removal efficiency M1	Overall removal efficiency M2
CODt	70,9				0,00	0,00		
CODs	40,4	10,50	6,26	1,63	40%	84%	85%	96%
TC	83,93	60,13	40,91	41,16	32%	32%	51%	51%
IC	73,7	60,00	41,23	41,17	31%	31%	44%	44%
TN	17,37	7,89	7,99	6,94	-1%	12%	54%	60%
NO3-N	0,959	5,65	7,24	6,74	-28%	-19%	-655%	-603%
NH4-N	13,1	1,51	0,00	0,00	100%	100%	100%	100%
PO4-P	1,28	0,25	0,02	0,02	94%	92%	99%	98%

Samples of the 19/04/2024 (effluent from 18/04/2024)					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt				0,00	0,00
CODs	10,50	4,01	4,86	62%	54%
TC	60,13	41,33	41,17	31%	32%
IC	60,00	40,73	40,85	32%	32%
TN	7,89	8,02	7,41	-2%	6%
NO3-N	5,65	6,76	6,66	-20%	-19%
NH4-N	1,51	0,00	0,00	100%	100%
PO4-P	0,25	0,01	0,02	96%	91%

Samples of the 22/04/2024 (effluent from 18/04/2024)					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt				0,00	0,00
CODs	10,50	6,44	2,85	39%	73%
TC	60,13	43,70	43,55	27%	28%
IC	60,00	43,66	43,80	27%	27%
TN	7,89	7,90	7,18	0%	9%
NO3-N	5,65	7,41	6,78	-31%	-21%

NH4-N	1,51	0,00	0,00	100%	100%
PO4-P	0,25	0,01	0,01	97%	97%

Mean removal efficiency scenario 1		
	M1	M2
CODt		
CODs	48%	69%
TC	38%	38%
IC	38%	37%
TN	16%	24%
NO3-N	-11%	-3%
NH4-N	100%	100%
PO4-P	92%	92%

Physical parameters				
		Beringen effluent	**M1 effluent**	**M2 effluent**
EC (uS/cm)	Min	899,00	414,00	427,00
	Max	1027,00	957,00	944,00
	Mean	980,00	776,14	779,29
	Standard deviation	90,51	237,42	240,11
Redox (mV)	Min	119,00	164,00	153,00
	Max	180,00	230,00	225,00
	Mean	154,67	192,00	188,00
	Standard deviation	31,79	28,39	26,76
pH	Min	7,42	7,74	7,78
	Max	8,32	8,16	8,33
	Mean	7,83	7,97	8,04
	Standard deviation	0,46	0,13	0,21
T (deg C)	Min	11,10	12,70	12,60
	Max	16,60	20,10	19,90
	Mean	13,40	18,31	18,17
	Standard deviation	2,86	2,58	2,57
DO (mg/l)	Min	5,02	7,52	7,13
	Max	10,12	8,64	8,23
	Mean	7,88	8,00	7,48
	Standard deviation	2,61	0,37	0,40

Annex 2: Scenario 2

Samples of the 29/04/2024

Flow rate: 90ml/min				
Total feeding volume per mesocosm		2700		
Start of the pump	10:22		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	10:27	10:28		
10:27	0	0	2700	2700
10:30	222	136	2478	2564
10:45	1330	1160	1370	1540
11:00	2110	1990	590	710
11:15	2160	2115	540	585
11:30	2169	2165	531	535
11:45	2180	2230	520	470
12:00	2180	2230	520	470
Retained volume (%)	19%	17%		
HRT (min)	00:05	00:06		

Samples of the 02/05/2024				
Flow rate: 90ml/min				
Total feeding volume per mesocosm		2700		
Start of the pump	09:50		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	09:55	09:56		
09:55	0	0	2700	2700
10:00	400	195	2300	2505
10:15	1390	1125	1310	1575
10:30	1460	1295	1240	1405
10:45	1473	1350	1227	1350
11:00	1483	1405	1217	1295
11:15	1483	1405	1217	1295
11:30	1483	1405	1217	1295
Retained volume (%)	45%	48%		
HRT (min)	00:05	00:06		

Samples of the 06/05/2024				
Flow rate: 90ml/min				
Total feeding volume per mesocosm		2700		
Start of the pump	10:23		Retained volume (ml)	

Time (hh:mm)	M2	M1	M2	M1
First drop	10:29	10:30		
10:29	0	0	2700	2700
10:30	110	0	2590	2700
10:45	1245	1260	1455	1440
11:00	1335	1440	1365	1260
11:15	1349	1510	1351	1190
11:30	1354	1534	1346	1166
11:45	1354	1534	1346	1166
12:00	1354	1534	1346	1166
Retained volume (%)	50%	43%		
HRT (min)	00:06	00:07		

Retained volume (%) scenario 2		
	M1	M2
Sample 1	17%	19%
Sample 2	48%	45%
Sample 3	43%	50%
mean retained volume	36%	38%

HRT (minutes) scenario 2		
	M1	M2
Sample 1	6,00	5,00
Sample 2	6,00	5,00
Sample 3	7,00	6,00
Mean HRT	6,33	5,33

Samples of the 29/04/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	9,81	5,04	5,26	49%	46%
TC	72,96	57,31	68,57	21%	6%
IC	51,67	40,25	35,22	22%	32%
TN	5,39	6,80	6,25	-26%	-16%
NO3-N	4,22	6,83	6,69	-62%	-59%
NH4-N	0,82	0,00	0,00	100%	100%
PO4-P	0,32	0,02	0,02	93%	94%

Samples of the 30/04/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					

CODs	9,81	7,06	5,52	28%	44%
TC	72,96	40,23	37,01	45%	49%
IC	51,67	38,82	34,34	25%	34%
TN	5,39	7,37	8,20	-37%	-52%
NO3-N	4,22	4,52	6,10	-7%	-45%
NH4-N	0,82	0,04	0,01	96%	99%
PO4-P	0,32	0,03	0,03	91%	91%

Samples of the 02/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	9,81	6,75	2,90	31%	70%
TC	72,96	45,72	28,05	37%	62%
IC	51,67	37,15	29,45	28%	43%
TN	5,39	6,08	4,98	-13%	8%
NO3-N	4,22	5,74	6,23	-36%	-48%
NH4-N	0,82	0,00	0,00	100%	100%
PO4-P	0,32	0,02	0,02	92%	93%

Samples of the 03/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	9,81	7,39	5,22	25%	47%
TC	72,96	63,70	56,15	13%	23%
IC	51,67	44,99	44,13	13%	15%
TN	5,39	5,43	5,71	-1%	-6%
NO3-N	4,22	5,94	6,02	-41%	-43%
NH4-N	0,82	0,01	0,00	98%	100%
PO4-P	0,32	0,04	0,03	88%	92%

Samples of the 06/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency

CODt					
CODs	9,81	7,40	6,65	25%	32%
TC	72,96	46,76	44,34	36%	39%
IC	51,67	41,15	37,10	20%	28%
TN	5,39	6,32	5,94	-17%	-10%
NO3-N	4,22	5,80	5,85	-37%	-39%
NH4-N	0,82	0,01	0,01	99%	99%
PO4-P	0,32	0,02	0,02	92%	92%

Mean removal efficiency scenario 2		
	M1	M2
CODt		
CODs	31%	48%
TC	30%	36%
IC	22%	30%
TN	-19%	-15%
NO3-N	-37%	-46%
NH4-N	99%	100%
PO4-P	91%	92%

Physical parameters scenario 2				
		Beringen effluent	**M1 effluent**	**M2 effluent**
EC (uS/cm)	Min	1083,00	972,00	953,00
	Max	1083,00	1039,00	1048,00
	Mean	1083,00	1013,00	1011,20
	Standard deviation	0,00	23,38	32,25
Redox (mV)	Min	0,00	244,00	246,00
	Max	0,00	250,00	255,00
	Mean	#DIV/0!	247,00	250,50
	Standard deviation	#DIV/0!	4,24	6,36
pH	Min	8,36	7,98	7,75
	Max	8,36	8,31	8,24
	Mean	8,36	8,17	8,08
	Standard deviation	0,00	0,12	0,17
T (deg C)	Min	14,40	18,50	18,60
	Max	14,40	20,90	20,80
	Mean	14,40	19,64	19,62
	Standard deviation	0,00	0,77	0,72
DO (mg/l)	Min	10,59	6,60	6,56
	Max	10,59	8,30	8,99
	Mean	10,59	7,21	7,31
	Standard deviation	0,00	0,61	0,89

Annex 3: Scenario 3

Samples of the 14/05/2024				
Flow rate: 60ml/min				
Total feeding volume per mesocosm		1800		
Start of the pump	12:00		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:11	12:14		
12:11	0	0	1800	1800
12:15	300	130	1500	1670
12:30	1010	870	790	930
12:45	1310	1260	490	540
13:00	1327	1340	473	460
13:15	1332	1375	468	425
13:30	1335	1400	465	400
14:00	1337	1410	463	390
Retained volume (%)	26%	22%		
HRT (min)	00:11	00:14		

Samples of the 17/05/2024				
Flow rate: 60ml/min				
Total feeding volume per mesocosm		1800		
Start of the pump	12:02		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:05	12:08		
12:05	0	0	1800	1800
12:15	400	200	1400	1600
12:30	1200	1040	600	760
12:45	1490	1440	310	360
13:00	1520	1520	280	280
13:15	1530	1570	270	230
13:30	1532	1590	268	210
14:00	1532	1600	268	200
Retained volume (%)	15%	11%		
HRT (min)	00:03	00:06		

Samples of the 22/05/2024		
Flow rate: 60ml/min		

Total feeding volume per mesocosm		1800		
Start of the pump	12:02		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:06	12:07		
12:06	0	0	1800	1800
12:15	415	250	1385	1550
12:30	1205	1120	595	680
12:45	1490	1490	310	310
13:00	1515	1575	285	225
13:15	1565	1585	235	215
13:30	1595	1588	205	212
14:00	1615	1589	185	211
Retained volume (%)	10%	12%		
HRT (min)	00:04	00:05		

Samples of the 29/05/2024				
Flow rate: 60ml/min				
Total feeding volume per mesocosm		1800		
Start of the pump	12:02		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:05	12:08		
12:05	0	0	1800	1800
12:15	390	250	1410	1550
12:30	1185	1080	615	720
12:45	1465	1390	335	410
13:00	1481	1470	319	330
13:15	1487,5	1510	313	290
13:30	1491,3	1528,5	309	272
14:00	1493,3	1536,5	307	264
Retained volume (%)	17%	15%		
HRT (min)	00:03	00:06		

Samples of the 30/05/2024				
Flow rate: 60ml/min				
Total feeding volume per mesocosm		1800		
Start of the pump	12:02		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	12:05	12:07		
12:05	0	0	1800	1800

12:15	380	250	1420	1550
12:30	1150	1080	650	720
12:45	1430	1390	370	410
13:00	1455	1470	345	330
13:15	1461,5	1520	339	280
13:30	1464,2	1536	336	264
14:00	1466,4	1546	334	254
Retained volume (%)	19%	14%		
HRT (min)	00:03	00:05		

Retained volume (%) scenario 3		
	M1	M2
Sample 1	22%	26%
Sample 2	11%	15%
Sample 3	12%	10%
Sample 4	15%	17%
Sample 5	14%	19%
mean	15%	17%

HRT (minutes) scenario 3		
	M1	M2
Sample 1	14,00	11,00
Sample 2	6,00	3,00
Sample 3	5,00	4,00
Sample 4	6,00	3,00
Sample 5	5,00	3,00
mean	7,20	4,80

Samples of the 14/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	13,90	6,83	6,20	51%	55%
TC	63,53	36,06	35,08	43%	45%
IC	55,92	34,97	34,45	37%	38%
TN	8,53	6,82	6,85	20%	20%
NO3-N	6,78	6,31	6,11	7%	10%
NH4-N	0,77	0,03	0,03	96%	97%
PO4-P	0,48	0,02	0,02	95%	96%

Samples of the 17/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	13,90	7,62	6,71	45%	52%
TC	63,53	46,09	45,46	27%	28%
IC	55,92	45,00	45,40	20%	19%
TN	8,53	8,17	7,35	4%	14%
NO3-N	6,78	7,52	6,76	-11%	0%
NH4-N	0,77	0,01	0,00	99%	100%
PO4-P	0,48	0,03	0,02	93%	95%

Samples of the 22/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	9,81	6,73	5,36	31%	45%
TC	42,33	44,15	50,78	-4%	-20%
IC	40,54	43,60	44,75	-8%	-10%
TN	4,01	7,96	7,98	-98%	-99%
NO3-N	3,06	7,41	7,19	-142%	-135%
NH4-N	0,50	0,00	0,00	100%	100%
PO4-P	0,28	0,07	0,04	74%	87%

Samples of the 29/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	7,96	6,76	5,94	15%	25%
TC	63,23	47,85	56,88	24%	10%
IC	58,41	34,66	32,32	41%	45%
TN	6,24	4,25	3,73	32%	40%
NO3-N	4,63	4,21	3,99	9%	14%
NH4-N	0,67	0,00	0,00	99%	100%
PO4-P	0,38	0,09	0,05	77%	88%

Samples of the 30/05/2024					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	7,96	5,43	4,79	32%	40%
TC	63,23	47,77	89,89	24%	-42%
IC	58,41	47,16	37,46	19%	36%
TN	6,24	5,44	4,58	13%	27%
NO3-N	4,63	4,05	4,06	13%	12%
NH4-N	0,67	0,00	0,00	100%	100%
PO4-P	0,38	0,09	0,07	77%	83%

Mean removal efficiency scenario 3		
	M1	M2
CODs	35%	44%
TC	23%	4%
IC	22%	25%
TN	-6%	0%
NO3-N	-25%	-20%
NH4-N	99%	99%
PO4-P	83%	90%

Physical parameters scénario 3				
		Beringen effluent	M1 effluent	M2 effluent
EC (uS/cm)	Min	661,00	715,00	694,00
	Max	1076,00	1043,00	1052,00
	Mean	877,00	915,60	913,80
	Standard deviation	208,02	131,67	160,82
Redox (mV)	Min	240,00	235,00	218,00
	Max	269,00	265,00	279,00
	Mean	257,33	243,40	246,00
	Standard deviation	15,31	12,46	23,84
pH	Min	7,37	8,21	8,12
	Max	7,72	8,32	8,21
	Mean	7,49	8,25	8,15
	Standard deviation	0,20	0,04	0,04

T (deg C)	Min	11,20	20,80	20,70
	Max	17,00	21,90	21,60
	Mean	13,50	21,26	21,16
	Standard deviation	3,08	0,47	0,38
DO (mg/l)	Min	6,18	5,89	5,62
	Max	7,54	7,62	7,65
	Mean	6,72	6,66	6,79
	Standard deviation	0,72	0,64	0,75

Annex 4: Scenario 4

Samples of the 03/06/2024				
Flow rate: 180ml/min				
Total feeding volume per mesocosm		5400		
Start of the pump	09:15		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	09:18	09:18		
09:18	0	0	5400	5400
09:30	1530	1500	3870	3900
09:45	3685	3790	1715	1610
10:00	4705	4960	695	440
10:15	4755	5060	645	340
10:30	4767,5	5120	633	280
10:45	4774,5	5160	626	240
11:00	4779,5	5183	621	217
Retained volume (%)	11%	4%		
HRT (min)	00:03	00:03		

Samples of the 06/06/2024				
Flow rate: 180ml/min				
Total feeding volume per mesocosm		5400		
Start of the pump	09:21		Retained volume (ml)	
Time (hh:mm)	M2	M1	M2	M1
First drop	09:25	09:25		
09:25	0	0	5400	5400
09:30	660	440	4740	4960
09:45	2270	1940	3130	3460
10:00	4170	3850	1230	1550
10:15	4207	3935	1193	1465

10:30	4210,9	3975	1189	1425
10:45	4213,3	3996	1187	1404
11:00	4215,5	4007,5	1185	1393
Retained volume (%)	22%	26%		
HRT (min)	00:04	00:04		

Retained volume (%) scenario 4		
	M1	M2
Sample 1	4%	11%
Sample 2	26%	22%
mean	15%	17%

HRT (minutes) scenario 4		
	M1	M2
Sample 1	3,00	3,00
Sample 2	4,00	4,00
mean	3,50	3,50

Samples of the 03/06/2024 (day)					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	7,96	5,42	4,17	32%	48%
TC	63,23	0,58	68,79	99%	-9%
IC	58,41	40,00	41,55	32%	29%
TN	6,24	0,03	5,34	100%	14%
NO3-N	4,63	5,27	4,95	-14%	-7%
NH4-N	0,67	0,00	0,00	100%	100%
PO4-P	0,38	0,10	0,06	73%	84%

Samples of the 03/06/2024 (night)					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	7,96	7,15	5,97	10%	25%
TC	63,23	52,14	52,70	18%	17%
IC	58,41	51,30	52,02	12%	11%
TN	6,24	6,51	6,19	-4%	1%
NO3-N	4,63	4,76	6,76	-3%	-46%
NH4-N	0,67	0,04	0,00	94%	100%
PO4-P	0,38	0,15	0,02	59%	94%

Samples of the 06/06/2024 (day)					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	9,33	6,70	5,44	28%	42%
TC	61,40	46,65	46,78	24%	24%
IC	60,40	46,12	45,89	24%	24%
TN	9,30	6,50	6,46	30%	31%
NO3-N	7,37	5,81	5,44	21%	26%
NH4-N	0,98	0,00	0,00	100%	100%
PO4-P	0,42	0,11	0,08	75%	82%

Samples of the 06/06/2024 (night)					
Parameter	Beringen effluent (mg/l)	M1 effluent (mg/l)	M2 effluent (mg/l)	M1 removal efficiency	M2 removal efficiency
CODt					
CODs	9,33	8,65	7,61	7%	18%
TC	61,40	55,07	54,72	10%	11%
IC	60,40	53,91	53,52	11%	11%
TN	9,30	8,82	7,94	5%	15%
NO3-N	7,37	7,81	7,21	-6%	2%
NH4-N	0,98	0,08	0,02	92%	98%
PO4-P	0,42	0,16	0,10	61%	77%

Mean removal efficiency scenario 4		
	M1	M2
CODt		
CODs	19%	33%
TC	38%	11%
IC	20%	19%
TN	33%	15%
NO3-N	0%	-6%
NH4-N	96%	100%
PO4-P	67%	84%

Physical parameters scenario 4				
		Beringen effluent	**M1 effluent**	**M2 effluent**
EC (uS/cm)	Min	894,00	896,00	849,00
	Max	1062,00	1043,00	1038,00
	Mean	978,00	973,25	963,75
	Standard deviation	118,79	60,96	81,32
Redox (mV)	Min	238,00	218,00	194,00
	Max	269,00	238,00	244,00
	Mean	253,50	232,75	215,50
	Standard deviation	21,92	9,84	25,01
pH	Min	7,72	8,23	8,10
	Max	7,95	8,56	8,54
	Mean	7,84	8,40	8,29
	Standard deviation	0,16	0,16	0,21
T (deg C)	Min	17,00	20,10	20,10
	Max	18,30	21,30	21,20
	Mean	17,65	20,45	20,43
	Standard deviation	0,92	0,57	0,53
DO (mg/l)	Min	7,54	5,03	5,01
	Max	7,73	7,01	5,66
	Mean	7,64	5,75	5,36
	Standard deviation	0,13	0,90	0,30