Hydro Nation Scholars Programme

Understanding how microbial community contributes to wastewater treatment performance in constructed wetlands within Scotch whisky distilleries

Policy Brief

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Overview

The research conducted focused on improving biological wastewater treatment processes in **Constructed Wetlands (CWs)**, particularly for treating whisky distillery byproducts. It explored various techniques for isolating and analysing bacterial biofilms from wetland substrates, with an emphasis on optimising DNA extraction and microbial community profiling using advanced sequencing technologies. The study also evaluated different wetland substrates, such as LECA, gravel, foamed ceramic, and biochar, for their effectiveness in removing pollutants like dissolved copper (dissCu) and organic carbon (OC).

Additionally, the research investigated seasonal variations in wetland performance, providing insights into how microbial communities and treatment efficiency fluctuate throughout the year. Another key aspect of the study was the use of biochar, including modified forms, as a pretreatment for acidic, copper-rich wastewater. Ecotoxicology tests further confirmed biochar’s potential to reduce wastewater toxicity.

Overall, this research contributes to improving the design and operation of CWs and highlights the sustainable application of biochar for industrial wastewater treatment, particularly in the whisky distillation industry.

Introduction

The Scotch whisky industry is a significant sector in Scotland, producing large volumes of wastewater, particularly from the second distillation phase, known as "spent lees." Managing this wastewater effectively is crucial to avoid environmental damage, meet regulatory standards, and ensure the industry's sustainability. CWs have emerged as an environmentally friendly, cost-effective solution for treating this wastewater by mimicking natural ecosystems to purify water. However, optimising CW performance for specific pollutants, like dissCu and organic compounds, is essential to enhance their effectiveness and ensure regulatory compliance.

The performance instability of CWs, which can lead to inconsistent treatment outcomes. Factors such as seasonal variations, substrate type, and microbial community dynamics contribute to fluctuations in the removal efficiency of pollutants like dissCu, chemical oxygen demand (COD), dissolved organic carbon (DOC), and pH neutralisation. These inconsistencies highlight the need for optimising CW design and treatment methods to ensure reliable performance year-round.

If left untreated or inadequately treated, whisky distillery wastewater can lead to a variety of environmental issues. Dissolved copper and organic pollutants can cause aquatic toxicity, leading to biodiversity loss and ecosystem imbalances in surrounding water bodies. The acidic nature of the wastewater can further lead to soil and water acidification, disrupting local pH balances. Non-compliance with environmental regulations can result in costly fines, operational restrictions, and potential reputational damage for whisky distilleries.

Addressing these challenges is vital not only for environmental protection but also to ensure the whisky industry's long-term sustainability. This research highlights the importance of improving wastewater treatment through innovations in CW design, substrate selection, and the addition of biochar. Biochar, in particular, has shown promise in enhancing CW efficiency by improving the removal of pollutants like dissolved copper and organic contaminants. Additionally, understanding and optimising microbial community dynamics within CWs can help sustain better long-term performance, making these systems more reliable year-round. By continuing to refine these approaches, the whisky industry can reduce its environmental impact, ensuring cleaner water and meeting global sustainability goals.

Research Undertaken

The research involved several studies aimed at improving the treatment of whisky distillery wastewater and understanding the role of microbial communities in CWs. The first objective was to optimise the isolation of bacterial biofilms from real wetland samples, which are crucial in biological water treatment processes. To achieve this, different techniques were tested, including adjusting agitation times, temperatures, and using enzymatic treatments to enhance DNA yield. This approach aimed to improve the recovery of microbial DNA for subsequent analysis. Data generation was performed using two sequencing technologies—Illumina MiSeq and Oxford Nanopore MinION—to analyse the bacterial communities present in the biofilms. These methods allowed the team to characterise microbial diversity, using 16S rRNA gene sequencing to identify bacterial species. The study also compared the effectiveness of different sequencing platforms and analysis methods for identifying microbial communities in wetlands.

In addition to biofilm isolation, the research examined how different substrates used in CWs—such as pea gravel, LECA (lightweight expanded clay aggregate), and Alfagrog (Foamed ceramic material)—affected the treatment of whisky distillery wastewater, particularly the byproduct "spent lees." Small-scale wetland models called mesocosms were used to evaluate the substrates, some of which were combined with biochar to potentially enhance pollutant removal capabilities. Data on pollutant levels and microbial diversity were collected over time to assess the impact of these substrates on wastewater treatment.

The research also included a study of three horizontal subsurface flow constructed wetlands (HSF CWs) located at whisky distilleries in Scotland. These wetlands were examined for their ability to treat wastewater from the distillation process, with data collected across different seasons to assess how the treatment performance varied. The researchers monitored key water quality parameters and microbial community dynamics to better understand the seasonal influences on wetland efficiency and microbial activity.

Additionally, the research explored biochar-based materials as a potential pretreatment for whisky distillery waste before it enters CWs. Several types of biochar, including modified forms treated with sodium hydroxide

(NaOH) and heat, were tested for their ability to neutralise acidity and remove pollutants like dissCu. Column studies were conducted to evaluate the biochar's performance, and surface analysis techniques were used to investigate how pollutants interact with the biochar material.

To complement the wastewater treatment research, ecotoxicology tests were conducted using *Lepidium sativum* (garden cress) and *Escherichia coli* to assess the impact of biochar treatments on reducing the toxicity of distillery wastewater. These tests provided additional insights into the potential environmental benefits of using biochar-based methods for sustainable wastewater treatment.

This research was mainly conducted by HNSP scholar Ilgaz Cakin, who combined fieldwork, laboratory experiments, and DNA sequencing to advance the understanding of biological wastewater treatment processes in CWs.

Key Findings

Bacterial Biofilm Detachment & Sequencing Methods:

- No significant difference in species richness was observed across different bacterial biofilm detachment techniques.
- Enzyme treatment enhanced DNA concentration.
- MinION sequencing with the EPI2ME pipeline showed poor correlation with Illumina MiSeq and DADA2 (SILVA132) at the genus level. However, the correlation improved when the same database (SILVA132) was used across both sequencing technologies.

Biochar and Substrate Performance in Wastewater Treatment:

- LECA + biochar and gravel + biochar were the most effective substrates, showing superior dissCu reduction, COD removal, OC reduction, and pH modulation.
- Biochar reduced bacterial alpha diversity, indicating a potential short-term negative effect on microbial diversity in wetlands.
- Microbial diversity dropped substantially after 2 months of spent lees treatment. Moderate bacterial differentiation and high fungal differentiation were observed among mesocosms.
- Bacterial classes Gammaproteobacteria, Bacteroidia, and Alphaproteobacteria, and fungal class Sordariomycetes, are likely to play key roles in treatment performance.

Seasonal Wetland Performance in Pollutant Removal:

- CWs consistently removed dissCu, but other pollutant removal varied seasonally.
- CW-A showed year-round effectiveness in suspended solids, COD, and DOC removal but struggled with ammonium and SRP (Soluble Reactive Phosphorus) especially during warmer months.
- CW-B and CW-C performed well in autumn but faced challenges in spring, particularly with ammonium and suspended solids.
- Significant differences in microbial composition were observed across wetlands, and seasonal changes had minimal impact on bacterial diversity.
- Bacterial classes Alphaproteobacteria, Gammaproteobacteria, and Planctomycetacia are hypothesised to play crucial roles in treatment performance.

Metal-Modified and Biochar-Mineral Composite Efficacy:

- The composite materials elevated pH and effectively removed dissCu, correlating with biochar dosage.
- DissCu precipitation was driven by increased alkalinity rather than surface complexation.
- Ecotoxicology tests using *Lepidium sativum* and *Escherichia coli* showed reduced toxicity of treated spent lees, with higher biochar doses (6-8 g/L) required for some samples.

Modified Biochar in Treating Spent Lees:

- NaOH-treated biochar showed effective treatment across spent lees samples with varying characteristics, demonstrating significant dissCu removal (5.72 mg Cu per g of biochar).
- This dissCu removal capacity compared favorably to other wood-derived biochars (2.75-7.44 mg/g).
- Surface characterization (EDX, XPS, FTIR, XRD) confirmed microprecipitation of Cu ions on biochar, highlighting its potential for copper remediation.

Conclusions

Microbial Community and DNA Isolation: There is no significant difference between biofilm detachment protocols in terms of bacterial richness in CWs. However, enzyme treatments increase DNA concentration. While MinION sequencing has limitations at the genus level, it can be a viable alternative to MiSeq at the phylum and class levels. Streamlined DNA isolation protocols, improved sequencing methods, and inclusion of fungi, algae, and protozoa are crucial for better understanding microbial community functions in wetlands.

Constructed Wetland Substrate Efficiency: Biochar-enhanced substrates like LECA and gravel were more effective in reducing dissCu, removing organic pollutants, and buffering pH in mesocosms treating spent lees. Biochar's impact on bacterial diversity was evident, with mesocosms incorporating biochar showing lower alpha diversity, and moderate differentiation in microbial communities was observed based on substrate type. Further research on anaerobic layers and long-term substrate performance is needed.

Seasonal and Operational Variability in CWs: CWs used to treat spent lees in whisky production exhibited variable pollutant removal efficiencies based on season and system design. Dissolved copper was consistently removed, but other pollutants like ammonium and SRP remained problematic, particularly in warm seasons. Issues with oxygenation and aeration systems may contribute to decreased treatment performance. Despite minimal seasonal changes in microbial alpha diversity, specific bacterial groups like Alphaproteobacteria and Gammaproteobacteria warrant further exploration due to their pollutant removal capabilities.

Biochar-Based Treatment for Spent Lees: Modified biochars, particularly metal-modified and biochar-mineral composites, proved effective in elevating pH and removing dissCu from spent lees. Point of zero charge analysis supports the hypothesis that copper ions microprecipitated onto the surface of the biochar, validating its remediation potential. This treatment method also reduced the toxicity of the effluent, demonstrating biochar’s potential as a sustainable pretreatment solution.

Industrial Biochar Modification: NaOH-modified biochar derived from whisky cask cooperage waste significantly enhanced the removal of dissolved copper from spent lees. The surface modifications created by alkaline treatments improved the biochar’s buffering capacity and adsorption efficiency. Field-scale trials are recommended to validate these findings and assess the process's economic feasibility at an industrial scale.

Policy Implications or Recommendations

The research findings underscore the crucial role of microbial communities in improving wastewater management, particularly in the context of CWs used for treating industrial effluents, such as whisky distillation byproducts. While biochar shows potential as a treatment material, the focus here is on understanding how microbial dynamics drive pollutant removal and how these communities can be optimised to enhance the overall efficiency of CW systems.

Microbial communities, including bacteria, fungi, and other microorganisms, play a key role in breaking down pollutants like dissCu, organic carbon, and nitrogen compounds. The study highlights that factors such as substrate type, seasonality, and aeration conditions can influence these microbial populations. Bacterial classes like Alphaproteobacteria and Gammaproteobacteria are identified as critical to pollutant degradation, suggesting that optimising wetland conditions to support these microorganisms could enhance treatment performance. In addition, substrate choices like LECA or gravel, alongside proper aeration, help maintain more consistent pollutant removal across seasons.

Potential aeration issues, as noted in some systems, can lead to suboptimal conditions for microbial activity, further emphasising the need for improved wetland operation. The findings recommend regular monitoring of microbial communities to ensure systems remain effective year-round, as well as exploring microbial dynamics in anaerobic layers, which could provide insights into deeper pollutant breakdown processes.

Furthermore, the study highlights the potential of advanced microbial profiling techniques, such as metagenomics and RNA analysis, to identify the most active microorganisms responsible for effective wastewater treatment. Such detailed microbial analysis could enable targeted interventions to enhance the performance of CWs, leading to more efficient removal of specific pollutants.

On a broader scale, these findings have significant implications for industries relying on CWs for effluent treatment. Policymakers and industry leaders should consider incorporating microbial monitoring as a standard practice, ensuring that CW systems maintain a healthy and diverse microbial ecosystem. This could be complemented by further research into the role of specific microbial groups, with the aim of improving CW design and operational strategies for better handling of complex waste streams.

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