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Dear Colleagues and Friends,

As part of the 65th International Scientific Conference of Riga Technical University section "Bioenergy Technologies and Biotechnologies", it is my great pleasure and anticipation to introduce this collection of abstracts, showcasing the latest advancements and research findings in the fields of bioresources, wastewater treatment, environmental engineering, and biotechnologies.

In today's world, where the pursuit of sustainable solutions is paramount, environmental engineering and biotechnologies have emerged as crucial pillars in our quest for a cleaner, greener future. These innovative approaches offer not only opportunities for energy generation but also promise significant benefits for resource recovery, wastewater treatment, and environmental conservation.

Together, let us harness the power of bioenergy technologies and biotechnologies to drive positive change for our planet and future generations.

Assoc. Prof. Linda Mezule
Director of Water Systems and Biotechnology Institute

CONTENTS

WOOD-DECAYING FUNGI FOR LIGNOCELLULOLYTIC ENZYME PRODUCTION AND BIOREMEDIATION PROCESSES

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INTRODUCTION

Wood-decaying fungi are well-known for their ability to degrade complex plant materials through the production of lignocellulolytic enzymes, such as cellulases, laccases, and peroxidases [1]. Fungal cellulases break down cellulose into fermentable sugars [2], while laccases and peroxidases play crucial roles in lignin degradation and pollutant removal [3]. Therefore, these enzymes have versatile potential applications in biofuel production [4], textile processing [1], paper and pulp industries [5], as well as waste and wastewater treatment [6], [7]. Currently, the global enzyme market is heavily dominated by fungal species like *Aspergillus*, *Trichoderma*, and *Penicillium* [8], and the demand for novel, cost-effective enzyme producers continues to grow.

In this study, we screened indigenous fungal isolates from temperate forests, which are rich in fungal diversity but underexplored as sources of novel enzyme producers. Using indicators like Congo Red for cellulases, ABTS for laccases, and Azure B for laccases and peroxidases, qualitative and quantitative analyses of enzyme activity were performed.

METHODS

Twenty fungal strains showing wood-decaying abilities were collected from Latvian boreal coniferous and nemoral deciduous forests in September 2023. The isolation of harvested fungal species was performed by cultivating the samples on Potato Dextrose Agar (PDA) (Oxoid Ltd., Basingstoke, Hants, UK) at 25 °C and 80 % rH in a climate chamber (BINDER GmbH, Tuttlingen, Germany). DNA-sequence identification of the isolates was performed by the Belgian Coordinated Collections of Microorganisms (UCLouvain, Belgium). In addition to the environmental isolates, commercial cultures of *Irpex lacteus* DSM 9595, *Pleurotus dryinus* (Pers.) P. Kumm, *Pleurotus ostreatus* DSM 1020, *Bjerkandera adusta* DSM 23426, *Trametes versicolor* DSM 6401, *Pycnoporus cinnabarinus* (Fr.) P. Karst, *Phanerochaete chrysosporium* DSM 9620, and *Trichoderma reesei* DSM 768 were used. All strains were maintained on PDA at 2–6 °C.

For the screening tests, agar media containing 0.8 g KH_2PO_4, 0.4 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2 g NH_4NO_3, 2 g yeast extract, 10 g glucose, and 15 g agar (pH 5.5 ± 0.2) per L of distilled water was prepared. For cellulolytic enzyme detection, 0.5 % carboxymethylcellulose and 0.1 % Congo Red was used, and 0.1 % ABTS and 0.01 % Azure B were added for ligninolytic enzyme detection. After the agar media preparation, ~ 1 cm^2 mycelial disk of each fungal species was placed on each type of agar and incubated for 336 hours at 25 °C and 80 % rH in a constant climate chamber.

RESULTS

During the qualitative screening, *C. unicolor*, *T. pubescens*, and *P. cinerea* showed strong oxidation of ABTS, producing dark green to dark purple colors over 99–100 % of the agar, indicating high laccase activity (Fig. 1). *C. unicolor* and *T. pubescens* also decolorized 100 % of Congo Red agar, suggesting significant cellulolytic enzyme secretion. *F. pinicola* decolorized 100 % of Congo Red and 96.15 % of Azure B agar, indicating cellulase and peroxidase activity. *P. subcorticalis*, T. *pubescens*, *C. unicolor*, and *C. pseudocladosporioides* also decolorized Azure B, indicating ligninolytic enzyme production. *T. pubescens* showed the most intensive lignocellulolytic enzyme activity, affecting 100 % of ABTS and Congo Red agar and decolorizing 69.57 % of Azure B after 168 hours.

Among commercial strains, *I. lacteus*, *P. dryinus*, *P. ostreatus*, *B. adusta*, and *T. versicolor* showed rapid ABTS oxidation, affecting 96.93–100 % of the medium after 336 hours (Fig. 1). *I. lacteus*, *P. dryinus*, *B. adusta*, *T. versicolor*, and *P. chrysosporium* decolorized 87.20–98.44 % of Congo Red agar, indicating intensive cellulolytic enzyme secretion. *T. versicolor* showed the strongest ligninolytic activity, decolorizing 95 % of Azure B agar and achieving 96.90 % decolorization of Congo Red and 100 % oxidation of ABTS, marking it as the most efficient commercial strain for lignocellulolytic enzyme production.

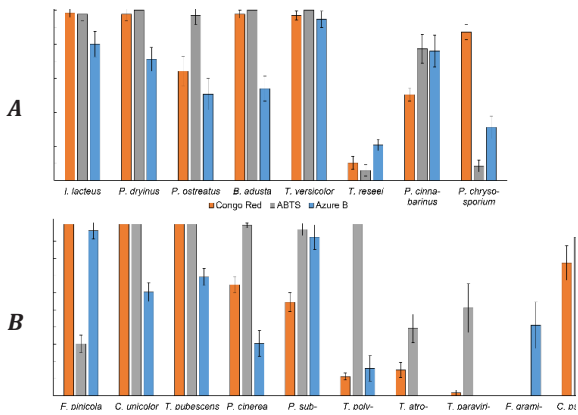

Fig. 1. *The percentage of oxidation zone formed from the total agar area after 336 hours of cultivating* ***A)*** *fungal commercial strains and* ***B)*** *wood-decaying environmental isolates. Standard deviation represents the average value from three independent repeats.*

DISCUSSION & CONCLUSIONS

This study has found a substantial richness in terms of lignocellulolytic enzyme-producing fungi in the boreal coniferous and nemoral summer green deciduous forests. Six isolates exhibited signs of pronounced enzyme-producing capability. While *C. unicolor* and *T. pubescens* are recognized as ecologically and biotechnologically significant wood-degrading basidiomycetes with high lignocellulose-degrading abilities [9], [10], *P. cinerea* is less commonly studied. Nonetheless, several studies suggest that this fungus may exhibit notable lignocellulolytic activity [11]. *P. subcorticalis* and *C. pseudocladosporioides*, which previously have not been studied or described as lignocellulolytic enzyme producers, also demonstrated intense oxidation of the enzyme activity indicators. These environmental isolates, together with active enzyme-producing white rot fungi like *B. adusta*, *T. versicolor*, and *I. lacteus*, have the potential not only in lignocellulose biomass conversion but also proved to be efficient candidates for complex chemical removal in waste and wastewater treatment, particularly in the textile industry. Furthermore, the findings of this study will be helpful for the set-up of new lignocellulolytic enzyme producers to advance the in-house enzyme production systems in temperate climatic zones. The results can also be used as a base for in-depth characterization of novel enzyme mechanisms in fungi previously not related to lignocellulolytic enzyme production.

ACKNOWLEDGEMENTS

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HARNESSING MIXED METAL OXIDEELECTRODES FOR ENHANCED ELECTROCHEMICAL DEGRADATION OF DAIRY WASTEWATER

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INTRODUCTION

The growing global population is driving undeniable expansion in the food production sector [1]. Among all the food industries, dairy is considered to be one of the major food industries playing an important role in the country's economy [2]. However, dairy industries consume a large volume of water for several processes like cleaning the equipment, sanitization, heating–cooling, floor washing, etc., and generate a huge amount of wastewater. There are several technologies available for dairy wastewater treatment, such as coagulation, adsorption, biological, membrane filtration, electrochemical, advanced oxidation process, photocatalysis, ozonation, etc. Several physicochemical processes are effective in the removal of emulsified compounds but need reagent addition which increases the treatment costs [3]. The biological method is a conventional yet very efficient method; however, if the wastewater contains heavy metals, biological treatment will often not be effective because heavy metals are not biodegradable and are toxic to many bacteria.

Recently, electrochemistry has been used in several environmental applications such as drinking water treatment, treatment of wastewater from various sources, soil remediation, treatment of gaseous streams, membrane fouling detection, etc. [4]. The main objective of the study was to remove dissolved and suspended contaminants from the dairy wastewater by electrochemical degradation method using mixed metal electrodes (MMO). In this regard, two different kinds of electrodes (Ti-Ru and Ti-Ru-Ir) were used. The surface morphology of the MMO was investigated by SEM and XPS before and after the treatment process.

METHODS

Electrochemical degradation of dairy wastewater experiments was performed in a titration vessel (150 mL) containing raw dairy effluent as the reactant and Na_2SO_4 (0.5 M) as the supporting electrolyte. Within this titration vessel, Ag/AgCl was used as a reference electrode, graphite rod as a counter electrode and two different MMOs were used as working electrodes to determine the best electrode combination for dairy effluent degradation, as shown in Fig. 1. The electrochemical performance of the two different MMOs was investigated using an electrochemical workstation (Bio-Logic Science Instruments SP-300 potentiostat) with EC-Lab software in a three-electrode cell configuration. All the electrochemical experiments were run under a nitrogen atmosphere with a stirring speed of 350 rpm controlled by a magnetic stir bar. During the reaction process, samples were taken with syringes at designated time intervals for COD and TOC analysis. All of the experiments were repeated at least three times. The presence of organic components in dairy effluents was analyzed by 1H nuclear magnetic resonance (NMR) spectroscopy.

RESULTS

The dairy wastewater contains various organic and inorganic contaminants. Table 1 represents the physicochemical parameters of the raw dairy wastewater. The colour of the dairy effluent was milky white with an unpleasant odour, which may be due to microbial activity along with the decomposition of organic matter. The pH of dairy wastewater depends on the nature of the final products. The studied dairy effluent was slightly acidic, which may be due to the breakdown of milk sugar (lactose) into lactic acid. The conductivity, salinity and turbidity value give the information about higher amount of dissolved solid present in the dairy effluents. The COD value determines the level of pollution in water, and from the present investigation, the COD value was found to be 1669 mg L^{-1}, which shows the higher amount of organic matter in dairy effluents, which could be detrimental to aquatic organisms. Total nitrogen count (inorganic and organic) is essential due to its harmful effects on the environment. Many bacteria convert the organic nitrogen into the ammonia, nitrite and nitrate ions. From the present study, the total nitrogen value was found to be 56 mg L^{-1} (inorganic and organic nitrogen), which is beyond permissible limits set by the EU legislation. The reactive phosphate value (17 mg L^{-1}) reveals the increased risk of eutrophication. The trace amount of iron content was also detected and found to be 1.2 mg L^{-1}.

Table 1. *Physicochemical parameters of raw dairy wastewater collected from dairy industry situated in Latvian*

Parameters	Average ± standard deviation
Colour	Milky
pH	6.1 ± 0.2
Conductivity ($\mu S \cdot cm^{-1}$)	108.2 ± 15.4
Salinity (ppt)	1.30 ± 0.08
Turbidity (NTU)	65.6 ± 13.7
Chemical oxygen demand ($mg\ L^{-1}$)	1669 ± 20.7
Total Nitrogen ($mg\ L^{-1}$)	56 ± 10.3
Nitrogen Ammonia ($mg\ L^{-1}$)	35 ± 9.7
Nitrite (mg^{-1})	3.5 ± 2.3
Reactive Phosphate (mg^{-1})	17 ± 4.5
Total Iron ($mg\ L^{-1}$)	1.2 ± 0.1

DISCUSSION & CONCLUSIONS

The present study focuses on the environmental problems associated with dairy industries. It emphasizes the potential of electrochemical degradation, particularly employing mixed metal oxide (MMO) electrodes, as a promising solution for dairy wastewater treatment. Through a complete investigation, the efficiency of MMO electrodes, including materials such as ruthenium oxide (RuO_2), titanium dioxide (TiO_2), and iridium oxide (IrO_2), and their combinations were explored in treating dairy effluents. These electrodes exhibited excellent electrocatalytic activity in degrading complex organic pollutants and removing nutrients from the wastewater.

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ENZYMATIC HYDROLYSIS OF WASTE STREAMS ORIGINATING FROM WASTEWATER TREATMENT PLANTS

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INTRODUCTION

In the European Union, various climate-related targets have been set for the future. By 2030, there are plans to restrict the landfilling of any waste material that is suitable for recycling or energy recovery, along with increasing the share of renewable energy to 42.5 %. Additionally, the wastewater sector aims to achieve energy neutrality by 2045, meaning that wastewater treatment plants (WWTPs) will need to produce the energy they consume [1]. Waste streams originating from WWTPs could help achieve these goals, particularly as a source material for bioenergy production. In 2022, 85 % of screenings and 40 % of sewage grit generated in Latvian WWTPs were sent to landfill [2]. Sewage-related wastes are not contaminated with other waste streams and contain organic matter suitable for biological processing. For example, enzymatic hydrolysis can be applied to wastes to produce sugars which can be fermented to bioethanol [3]. The production of sludge hydrolysate and further bioethanol production has been explored before [4]. Meanwhile, the use of non-commercial fungal enzymes for hydrolysis has been investigated on various lignocellulosic substrates [5]. In this study, for the first time, enzymatic hydrolysis was performed on waste streams originating from WWTPs using a laboratory-prepared *Irpex lacteu*s enzyme mixture.

METHODS

Primary, secondary and digested sludge, screenings and sewage grit were collected from various WWTPs in Latvia. Protein-free and lipid-free sludge samples were obtained after the extraction of proteins or lipids from secondary or primary sludge, respectively. Enzymatic hydrolysis was performed on all waste substrates for 48 h at 30 °C and 150 rpm using either a commercial enzyme (*Cellic CTec2, Sigma Aldrich*) or a laboratory-made fungal enzyme mixture produced by *Irpex lacteus*. Enzyme loading was 4 FPU/g dry substrate. Samples were taken at 0, 24 and 48 h, and the concentration of reducing sugars was determined using the dinitrosalicylic acid method [6].

RESULTS

After 48 h hydrolysis with the commercial enzyme, the sugar yield was 101.1 mg/g for screenings, 13.6 mg/g for secondary sludge and less than 3 mg/g for primary and digested sludge (Fig. 1). This corresponded to 90 %, 15 %, 3 % and 1 % of carbohydrates converted to reducing sugars, respectively.

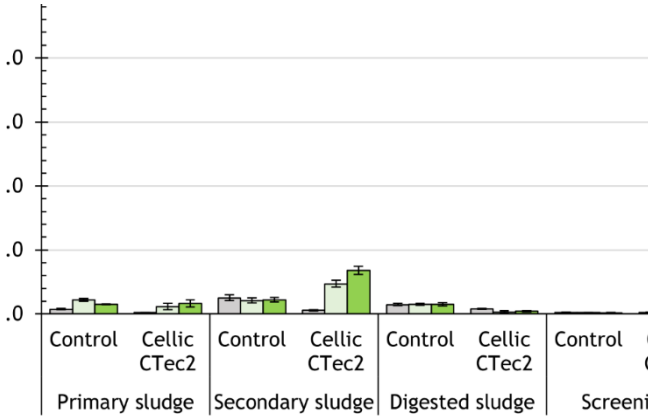

Fig. 1. *Sugar yield after 48 h of enzymatic hydrolysis with commercial enzyme.*

After 48 h hydrolysis with the *Irpex lacteus* enzyme preparation, the sugar yield was 31.2 mg/g for screenings, 17.2 mg/g for secondary sludge, 12.8 mg/g for primary sludge, 10.2 mg/g for sewage grit and less than 10 mg/g for digested, protein-free and lipid-free sludge (Fig. 2). The highest degree of saccharification was 28 % for screenings.

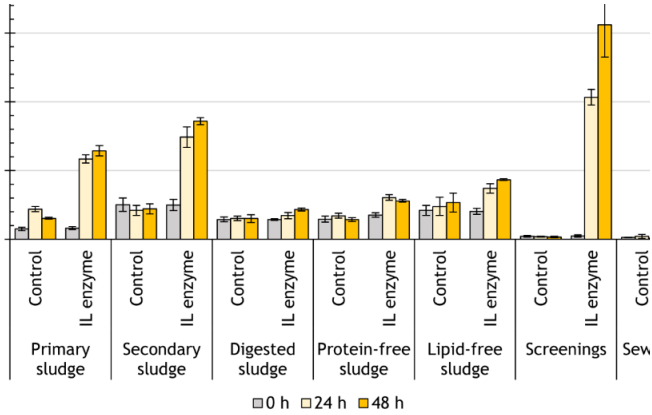

Fig. 2. *Sugar yield after 48 h of enzymatic hydrolysis with Irpex lacteus enzyme mixture.*

DISCUSSION & CONCLUSIONS

Enzymatic hydrolysis was performed on several waste streams originating from WWTPs, such as primary sludge, secondary sludge and screenings, to evaluate their potential for sugar production. After 48 h hydrolysis with either enzyme preparation, the highest sugar yields were demonstrated by screenings, showing the valorization potential of this waste material that is currently largely disposed of in a landfill. When using the *Irpex lacteus* enzyme mixture, all sewage-related substrates showed an increase in sugar release, demonstrating the versatility of this enzyme source. Improvements in sample preparation, fungal enzyme preparation and hydrolysis conditions should enable efficient bioethanol production in the future and move the wastewater sector closer to the climate neutrality targets.

ACKNOWLEDGEMENTS

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COST EVALUATION OF FUNGAL-BASED WASTEWATER TREATMENT

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INTRODUCTION

The detection of pharmaceutically active compounds (PAC) in various aquatic environments has raised ecological concerns about their possible harmful effects on different life forms in the ecosystem [1]. Effluents from wastewater treatment plants (WWTPs) can be a source of PACs releasement in aquatic systems, stressing the inefficiency of conventional WWTPs in eliminating PACs [2]. Typically, conventional WWTPs are not designed to remove PACs. Therefore, advanced treatment methods are needed to improve treatment efficiency for emerging pollution removal from wastewater. Nowadays, the introduction of fungal biofilm-based bioreactors into wastewater treatment has been a focus area of research [3], [4]. Filamentous fungi possess a unique ability to secrete an extracellular enzymatic complex during their metabolism, making them capable of degrading complex and resistant polymers, including PACs [2]. This makes fungi ideal candidates for removing pharmaceuticals from wastewater [5]. However, the

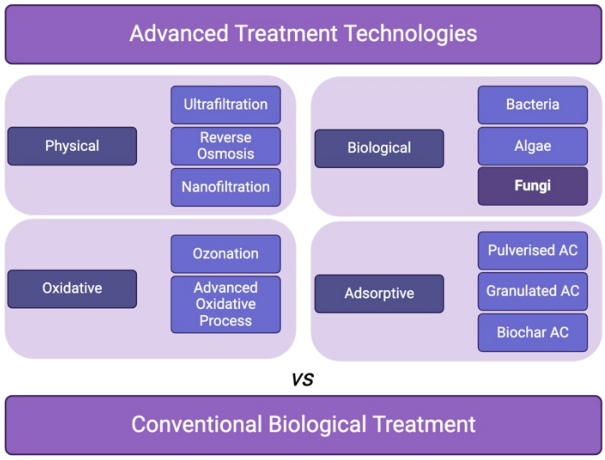

Fig. 1. Schematic characterization of advance treatment technologies versus conventional biological treatment technology (adapted by the Swedish Environmental Protection Agency, 2017 [6]).

implementation of fungal bioaugmentation in WWTPs requires additional costs. To accurately assess the cost of fungal-based wastewater treatment, the author of the study conducted a literature review to estimate the expenses. The literature study shows that the fungal treatment costs highly depend on fungal growth requirements (temperature, incubation time, electricity of shaking, composition of media). By understanding the specific costs, decision-makers can make informed choices when considering the integration of fungal treatment in WWTPs. Moreover, the results of this literature review can serve as a useful guide for decision-makers and researchers in the field of wastewater treatment, providing a comprehensive analysis of the costs associated with fungal bioaugmentation. By understanding the economic factors involved in fungal treatment, authorities can work towards creating sustainable and efficient solutions for removing PACs from water sources.

RESEARCH OBJECTIVE

The main objective of the study is to determine the cost-effectiveness of using fungal biofilm-based bioreactors for the removal of PACs from wastewater compared to different wastewater treatment technologies such as advanced treatment methods (physical, oxidative, adsorptive, and biological) to conventional biological treatment technology. This will involve analyzing the factors that impact the cost of fungal treatment, such as fungal growth requirements, and understanding how these costs compare to the potential ecological benefits of implementing this technology.

METHODS

The study was conducted in two stages. The first stage involved performing critical literature searches to identify the latest publications on the use of fungal-based treatment for wastewater. This involved creating a list of keywords that were used in the search. As the search progressed, the keyword list was updated to reflect the initial results and factors that may affect the search. A thorough literature review was conducted, focusing on the selected topic and any related variations. This review used databases such as SCOPUS, ScienceDirect, and PubMed. The second stage of the study involved acquiring and interpreting collected data, with the aim of formulating potential hypothesis cases.

ACKNOWLEDGEMENTS

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POTENTIAL FOR *LEGIONELLA* CONTROL THROUGH PHOSPHORUS LIMITATION MEASURES IN MULTISTOREY RESIDENTIAL BUILDINGS

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INTRODUCTION

Drinking water leaving treatment facilities is generally of high quality. However, secondary contamination often occurs during distribution. Compared to external networks, internal networks feature lower disinfectant residuals, elevated temperatures, extended residence times, a wider variety of pipe materials, and significantly smaller pipe diameters [1]. The high surface-to-volume ratio, along with other parameters, fosters conditions conducive to biofilm development, which can lead to deterioration in water quality.

Opportunistic premise plumbing pathogens (OPPPs) are a significant concern in internal networks, especially for individuals with compromised immune systems. These OPPPs are natural inhabitants of drinking water that have adapted to survive and thrive within potable water plumbing systems [2], with Legionella pneumophila being the most well-known representative. In the EU/EEA in 2021, there were reported 19 outbreaks and 137 confirmed cases [3]. The most affected group was males over 65, with an incidence rate of 8.9 cases per 100,000 population [3]. Some studies suggest that only about 10 % of Legionnaires' disease cases are diagnosed [4], indicating potential underreporting.

To enhance drinking water safety, several water utilities in countries such as the Netherlands, Switzerland, Denmark, Germany, and Belgium are increasingly focusing on providing biologically stable water [5]. Biostable water is defined by its inability to support microbial growth during distribution, thereby protecting consumer health, preserving aesthetic water quality, and preventing technical failures at all stages of the distribution process [1], [6], [7]. Limiting growth-promoting nutrients is essential in preparing biostable water. Although nutrient limitation to control bacterial growth in water supply systems has been widely studied, phosphorus-specific research is still limited. This may partly be due to the localised nature of growth-promoting nutrients, which can vary significantly even within a single distribution network [8], [9].

The fraction of phosphorus that is readily usable by microorganisms, termed microbially available phosphorus (MAP), is quantified through bacterial bioassay [10]. Reducing phosphorus, particularly the MAP fraction, could help limit biofilm formation and microbial growth, including that of Legionella spp. By restricting nutrient availability, phosphorus reduction has the potential to curb the proliferation of both planktonic microorganisms and biofilms, which could, in turn, support more effective *Legionella* control within water distribution networks.

METHODS

The pilot study was conducted in two adjacent five-storey residential buildings, approximately 100 metres apart, with a 22-year-old polypropylene internal water supply network delivering municipal groundwater. To standardise conditions, both buildings underwent centralised chemical flushing and disinfection of the internal water pipes: the domestic hot water (DHW) network was initially treated with an acidic, phosphate-free reagent containing formic acid to remove deposits (ALBILEX®-KALK-EX, Germany), followed by disinfection using hydrogen peroxide and silver ions (ALBILEX®-SUPER-des, Germany) to sanitise both cold and hot water systems. Hot water was heated by plate exchangers in basement heating substations, with temperature settings at 57 °C for weeks 0–14; from mid-October 2022, this was adjusted to a dynamic regime as an energy-saving measure, alternating between 48 °C at night, 52 °C during the day, and 57 °C on weekends. One building, equipped with a point-of-use (POU) filtration device at the inlet to reduce microbially available phosphorus (MAP), was designated the "POU-device building," while the other served as a "Reference building" for comparison. Sampling took place at four distinct points within each building, including kitchen taps (domestic cold water), showerheads, the DHW circulation return, and the inlet post-entry. Additional samples were taken after POU filtration in the POU-device building. Apartment residents collected first-draw samples according to a set protocol, with five apartments participating in the POU-device building (three on the 5th floor, one on the 2nd floor, and one on the 1st floor) and three in the Reference building (two on the 5th floor, one on the 1st floor).

RESULTS

Legionella counts were similar in both buildings during constant DHW temperature setpoint. However, when the dynamic setpoint was in place, *Legionella* spp. numbers increased more than tenfold in the MAP-limited building but did not change much in the building without additional MAP removal.

The only *Legionella* spp. detected were *L. pneumophila*. The most prevalent sero-group in both buildings was *L. pneumonia* SG 2, which was found in 51 % of samples taken from DHW sampling points in the POU-device building and 40 % of DHW samples in the Reference building (Fig. 1). SG 1 was detected more frequently in the Reference building.

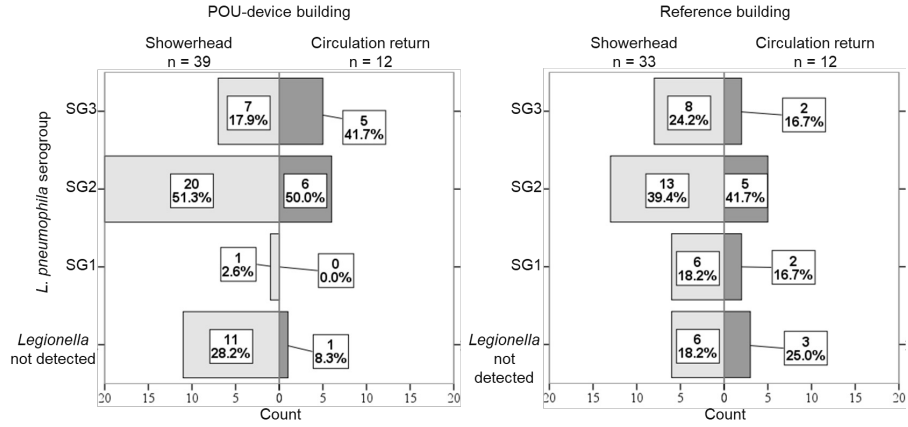

Fig. 1. *Frequency of detected Legionella pneumophila serogroups in samples collected from both DHW sub-systems.*

DISCUSSION & CONCLUSIONS

The centralised chemical flushing and disinfection temporarily eliminated cultivable Legionella bacteria. However, regrowth was observed within a week, with contamination detected in 36 % of samples and exceeding the EU Directive limit of 1000 CFU l^{-1} in both buildings after two months.

The MAP removal unit did not significantly lower Legionella spp. concentrations under standard temperature conditions; however, during dynamic temperature settings, it was associated with nearly a tenfold increase. This was likely influenced by factors including:

- nutrient-rich influent water (with higher levels of total organic carbon, magnesium, and intact cells compared to the Reference building),
- altered microbial competition due to limited nutrient availability,
- and DHW temperature fluctuations, which fostered a mesophilic environment conducive to *Legionella* growth.

The introduction of the POU sorption filter may have shifted the *Legionella pneumophila* species distribution, favouring non-SG1 strains, which appeared more frequently in the Reference building. This shift suggests that, while the filter might reduce certain *Legionella* strains commonly linked to clinical cases, the effects of nutrient limitation on the prevalence of potentially pathogenic strains warrant further study.

The limited number of sampling sites and buildings constrained the dataset's statistical robustness, reducing trend identification and the ability to compare across varied settings. Additionally, infrequent sampling and a narrow range of microbiological and chemical analyses restricted insights into temporal microbial dynamics and nutrient interactions.

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EXPLORING BIOFILM BEHAVIOUR ON MODIFIED MEMBRANES: ROLE OF ZNO IN BIOFILM FORMATION

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INTRODUCTION

Membranes are becoming more popular for their effectiveness in separation processes, especially in water treatment. However, fouling is still a major problem that reduces membrane performance. Particularly, biofouling is considered the most problematic type of fouling. For many years, researchers have been focused on finding ways to solve the fouling issue and improve membrane efficiency. Various modifications to membrane processes exist, including membrane modifications where additives are incorporated into or applied onto the membrane surface to inhibit the growth of microorganisms and reduce biofouling [1].

Despite extensive research into membrane modifications for biofouling mitigation, most evaluations still rely on basic microbiological methods such as cultivation and plate count techniques. Such methods are insufficient to conclusively demonstrate whether membranes possess true antimicrobial properties that effectively reduce biofouling [2].

Moreover, since most microorganisms in natural environments exist in biofilms, the protective matrix of the extracellular polymeric substances (EPS) surrounding the biofilm shields the cells from harsh conditions [3]. This matrix can also reduce the effectiveness of membrane antibacterial surfaces in real-life conditions, as the biofilm structure limits the direct interaction between the antimicrobial agents and the microorganisms. As a result, while antibacterial membrane surfaces may show high efficacy in controlled lab settings, their performance against biofilm-forming organisms in practical applications can be significantly reduced. Therefore, biofilm formation ability needs to be studied.

Several methods can be used to quantify EPS in biofilms, which are composed of various components such as proteins, polysaccharides, and nucleic acids, along with microorganism cells. Particularly, single species quantification assays can be used. However not all such methods are suitable for complex biofilms. For instance, the traditional Lowry method for protein quantification can produce inaccurate results due to interactions with other EPS components. In such cases, fluorescence assays provide a more reliable alternative [3].

In this study, the modified membranes were evaluated for their antibacterial properties using traditional methods, supplemented with bacterial viability tests to better understand the differences between these approaches. Additionally, the ability of the bacteria to form biofilm EPS on the membrane surface was assessed.

METHODS

Multiple samples of modified membranes were prepared using the non-solvent-induced phase separation (NIPS) technique. The base of the membranes consisted of typical polyethersulfone (PES, BASF, Germany). The modified membranes were made using a novel approach of dual-layer co-casting technique, where the surface layer consisted of PES and ZnO nanoparticles (GetNanoMaterials, France) mixture in different concentrations, similarly as described in [2].

The antibacterial properties were evaluated using a static method where *Escherichia coli* ATCC 25922 suspension was put on membrane coupons, incubated overnight and cultivated on agar plates. Additionally, the microscopy method was involved in evaluating the viability of the bacteria after contact with the membranes by treating the incubated suspension with 5-cyano-2,3-ditolyltetrazolium chloride (CTC, Sigma-Aldrich, MA, USA).

For biofilm evaluation, we focused on quantifying the protein content within the extracellular polymeric substances (EPS) of the biofilm. In this study, *Pseudomonas aeruginosa* ATCC 27853, a well-known biofilm-forming strain, was grown overnight in microplate wells containing the membrane coupons. Following the incubation, the samples were treated with SYPRO Ruby (Invitrogen, USA) and quantified using a microplate reader (CLARIOstar® Plus, BMG LABTECH, Germany).

RESULTS

Surface antibacterial tests using the cultivation method demonstrated high efficiency in *E. coli* inactivation, achieving reductions of more than 3 log (> 99.9 %) and up to 5 log (99.999 %) on the modified membranes, while significant growth was observed on the pure PES membrane, used as a negative control. However, bacterial viability assays revealed that only about half of the bacteria were killed, with the remaining population still metabolically active. Despite this, the total bacterial count on the ZnO-modified membranes was similar to the initial count, indicating that bacteria did not proliferate on these membranes as they did on the pure PES membranes. Interestingly, in one sample with the lowest ZnO concentration, the cultivation test showed over 99.9 % *E. coli* inactivation. However, the bacterial viability assay revealed no significant difference in the number of viable bacteria between this sample and the pure PES membrane.

Biofilm evaluation tests showed that none of the ZnO-modified membranes inhibited the growth of *P. aeruginosa* biofilm in the nutrient-rich medium used for surface tests, as the fluorescence intensity was similar to that of the pure PES membrane, with an average absolute fluorescence value approximately 2.3 times higher than the background. As a result, subsequent tests were conducted in a 0.1 % peptone solution instead of the nutrient medium. In the peptone solution, a significant difference was observed between the negative controls and the ZnO-modified membrane samples.

The fluorescence intensity in the wells containing the negative controls was the highest, while the intensity in the wells with ZnO-modified membranes varied depending on ZnO concentration, with an average reduction of approximately 35 %.

DISCUSSION & CONCLUSIONS

Our study demonstrates that the choice of evaluation methods can significantly influence experimental results, even in simple static tests using the same microorganism. While cultivation tests can indicate high antibacterial efficiency, in some cases, bacteria may still proliferate. The difference between results in *E. coli* inactivation efficiency observed in our study can be explained by the bacteria entering a viable but non-culturable (VBNC) state, as mentioned in our previous research [2].

Our biofilm evaluation tests successfully demonstrated distinguishing between control and experimental samples. The lower amount of fluorescence measured in ZnO-modified membranes here can be explained by the lower amount of protein produced by bacteria in contact with the membranes. In general, this confirms the ability of the ZnO membranes to reduce biofilm formation and corresponds to previous studies [4]. However, the study results still require further calibration for detailed quantitative biofilm assessment.

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MESOPHILIC AND THERMOPHILIC BACTERIA IDENTIFIED BY NEXT-GENERATION SEQUENCING DURING COMPOSTING OF TEXTILE WASTE

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INTRODUCTION

Composting is a self-heating process involving microbial metabolism, which increases the temperature beyond 50 °C [1]. This highly dynamic process comprises three phases: mesophilic, thermophilic and maturation [2]. Mesophilic and thermophilic alternating microbial consortia undertake the bio-decomposition of organic matter including cellulose, lignocellulose and proteins contained in the raw material, thereby generating a product that can be applied as a plant fertilizer or as a soil improver [3].

Moroccan textile industries are responsible for approximately 4 tons of solid waste per year. This waste is discharged into the environment completely untreated and therefore threatens the vitality of many ecosystems [4]. This waste is a valuable source of organic matter, potentially recoverable biologically by composting into bio-fertilizer, providing the nutrients needed for plant growth.

Composting is also recognized as a highly attractive source of new mesophilic and thermophilic micro-organisms, particularly those that contribute to biomass degradation [1]. In this respect, although a number of studies have focused on the identification of mesophilic and thermophilic microorganisms during composting, a more detailed insight into specific taxonomic and functional groups is needed [2], [3]. Various micro-organism species are associated with the composting process [5]. Bacteria are generally recognized as the most dominant species in processing due to their greater thermal tolerance [6]. Many studies have investigated compost maturity, but few have explored the interaction between mesophilic and thermophilic microbial communities. As previously mentioned, the main purpose of this study was to examine the relationships between bacterial communities and composting phases (mesophilic and thermophilic) through next-generation sequencing.

METHODS

The composting experiment was carried out using an in-silo composter of approximately 200 L (with an effective size of 0.58 m × 0.58 m × 0.92 m). A mixture was established for composting shredded waste, labelled 'Mix A' (with a 40 %/30 %/30 % ratio of textile/green/paper and cardboard waste). After the appropriate components were mixed, the silo was turned at least three times per week for 44 weeks. Samples were collected according to the four cardinal positions (north, east, south, and west) from three depths (0–20 cm, 30–40 cm, and 50–70 cm) in triplicate each week (in weeks 1, 6, 9, 18, 24, 28, 36, 40, and 44), and were then placed into polythene bags and stored at 4 °C until further analysis was conducted.

The temperature was monitored using an all-sun ETP109B Digital Thermometer. The C/N ratio for the initial mix and the end products was calculated by analyzing the total C and N percentages using a TOC analyzer (Shimadzu-V CSN) [7]. The total ammonium and nitrate ion (NH_4^+/NO_3^-) ratios for the pre-composted mix and the final products were calculated from the percentage of NH_4^+ and NO_3^- [8].

DNA was extracted from compost samples which were taken from the silo during both the mesophilic (week 9) and thermophilic phases (week 28) of the process. This was done using the PureLink Microbiome DNA Purification Kit following the manufacturers' instructions and was stored at –20 °C until use. Next-generation sequencing of 16S rRNA gene amplicons. The sequencing and amplification of 16S rRNA was performed at the Genomic Analysis Platform Macrogen (Republic of Korea), using the Illumina sequencing platform. Enzymatic activities were predicted using the UniProt (Universal Protein) database.

RESULTS

Changes in the C/N and $NH4^+/NO3^-$ ratios depended on temperature (Fig. 1) and were followed throughout the composting of biomaterial wastes. The evolution of the C/N and NH_4^+/NO_3^- ratio is inversely proportional to that of the temperature. According to several authors, a C/N ratio between 15 and 20 and NH_4^+/NO_3^- ratio below 1 reflects good organic matter degradation, thus proving a very advanced degree of textile waste compost maturation [8], [9].

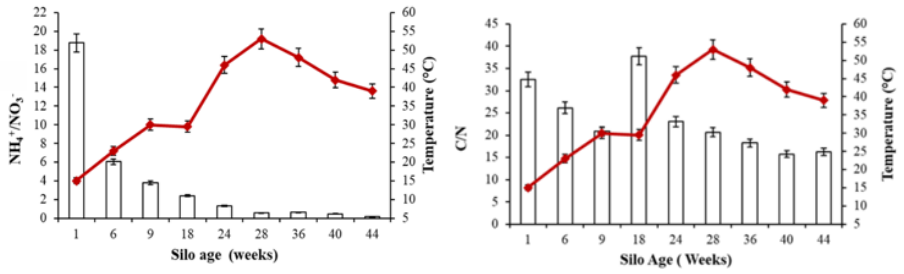

***Fig. 1**. Change in the NH_4^+/NO_3^- ratio (bars) and C/N ratio (bars) according to temperature (red line) in compost. Values of standard deviation based on three samples.*

Table 1 illustrates that all bacterial phyla were significantly impacted by the composting stage. It should be pointed out that the bacterial composition of the mesophilic stage is considerably different from that of the thermophilic stage. Sequences linked with *Cellulomonas* (Actinobacteria), *Steroidobacter* (γ-Proteobacteria), *Mycobacterium* (Actinobacteria), *Streptomyces* (Actinobacteria) and *Paenibacillus* (Firmicutes) showed the greatest abundance throughout the mesophilic phase. It is noteworthy that in the thermophilic phase, there is a significant decrease in all genera except for *Cellulomonas*, which was missing during the thermophilic phase (Table 1). Additionally, *Devosia* (α-Proteobacteria), *Flavobacterium* (Bacteroidetes), *Pseudoxanthomonas* (Proteobacteria), *Pseudomonas* (γ-Proteobacteria) and *Achromobacter* (β-Proteobacteria), remained the most abundant genera throughout the thermophilic phase. The abundance of these genera was significantly below that of the mesophilic phase. Furthermore, the UniProt database was employed to accurately identify the most prevalent enzymatic activities in the mesophilic and thermophilic phases, demonstrating that cellulase, hemicellulase, xylanase, pectin depolymerase and phosphatase (acid and alkaline) activities were found in both phases (Table 2).

Table 1. *Contribution of the most abundant bacterial genera during composting of textile waste*

Phylum	Family	Genus	% Contribution to the total number of sequences	
			Mesophilic phase	Thermophilic phase
Actinobacteria	Cellulomonadaceae	*Cellulomonas*	60	0
Proteobacteria	Sinobacteraceae	*Steroidobacter*	29	10
Proteobacteria	Xanthomonadaceae	*Pseudoxanthomonas*	26	33
Proteobacteria	Hyphomicrobiaceae	*Devosia*	23	56
Actinobacteria	Mycobacteriaceae	*Mycobacterium*	23	20
Bacteroidetes	Flavobacteriaceae	*Flavobacterium*	26	41
Actinobacteria	Streptomycetaceae	*Streptomyces*	16	4
Firmicutes	Paenibacillaceae	*Paenibacillus*	13	7
Proteobacteria	Pseudomonadaceae	*Pseudomonas*	10	30
Proteobacteria	Beijerinckiaceae	*Chelatococcus*	10	2
Proteobacteria	Alcaligenaceae	*Achromobacter*	9	25
Firmicutes	Clostridiaceae	*Clostridium*	8	10
Proteobacteria	Nitrobacteraceae	*Nitrobacter*	8	9
Actinobacteria	Nocardioidaceae	*Nocardioides*	3	9
Proteobacteria	Enterobacteriaceae	*Enterobacter*	2	0.2
Actinobacteria	Micromonosporaceae	*Micromonospora*	0.8	4
Bacteroidetes	Bacteroidaceae	*Bacteroides*	0.2	0.1
Actinobacteria	Bifidobacteriaceae	*Bifidobacterium*	0.05	0
Proteobacteria	Burkholderiaceae	*Burkholderia*	0	0.4
Bacteroidetes	Chitinophagaceae	*Chitinophaga*	5	0.07
Proteobacteria	Nitrosomonadaceae	*Nitrosomonas*	9	11

Table 2. *Enzymatic profile detected in bacterial communities involved in organic matter degradation throughout the composting phases (mesophilic and thermophilic), according to the UniProt database*

UniProt entry	Gene	Protein type	Mesophilic/ thermophilic phase	Bacteria
P10476	*celA*	Cellulase	Mesophilic and thermophilic	*Pseudomonas*
P23665	*endA*	Cellulase	Mesophilic	*Bacteroides*
P49424	*manA*	Hemicellulase (β-Mannanase)	Mesophilic and thermophilic	*Pseudomonas*
P51529	*gmuG,*	Hemicellulase (β-Mannanase)	Mesophilic and thermophilic	*Streptomyces*
P07986	*cexX*	Xylanase (hemicellulase)	Mesophilic and thermophilic	*Cellulomonas*
P17137	*xynB*	Xylanase (hemicellulase)	Mesophilic and thermophilic	*Clostridium*
C6CRVO	*xynA1*	Xylanase (hemicellulase)	Mesophilic and thermophilic	*Paenibacillus*
Q59219	*asdII*	Hemicellulase	Mesophilic	*Bacteroides*
P94552	*abf2*	Hemicellulase	Mesophilic	*Bacillus*
P20041	*pglA*	Pectin depolymerase	Mesophilic and thermophilic	*Pseudomonas*
Q05205	*phoA*	Alkaline phosphatase	Mesophilic and thermophilic	*Lysobacter*
A1YYW7	*phoK*	Alkaline phosphatase	Mesophilic and thermophilic	*Sphingomonas*
O53361	*sapM*	Acid phosphatase	Mesophilic and thermophilic	*Mycobacterium*
Q841V6	*abfB*	Hemicellulase	Mesophilic	*Bifidobacterium*

DISCUSSION & CONCLUSIONS

Organic matter biodegradation by micro-organisms is the primary factor in the processing of organic waste through composting. NGS analysis revealed that the studied mixture contained a wide range of microbial genera, confirming that compost derived from textile waste is an optimal environment for a variety of novel mesophilic and thermophilic microbes.

This investigation describes a comprehensive study of the textile waste composting process from the point of view of molecular microbiology. These results enable us to establish a list of the bacteria and their enzymes involved in the decomposition of lignocellulosic materials. The remarkable diversity of bacterial micro-organisms and metabolic pathways throughout the mesophilic and thermophilic phases of composting explains the results achieved in composting textile waste and demonstrates that the resulting compost reaches the necessary level of maturity.

Finally, it may be concluded that textile waste compost is a wealth of novel microorganisms and enzymes that are effective in breaking down organic matter. Interestingly, this study could potentially serve as a database for the prevalent microorganisms responsible for the degradation of organic matter.

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CHALLENGES OF DRINKING WATER TEMPERATURE IN DRINKING WATER DISTRIBUTION NETWORKS

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INTRODUCTION:

The World Health Organization has indicated that effective monitoring of drinking water quality is necessary to ensure the safety of water supply systems from contamination [1]. Since the year 2000, at least 24 large-scale microbiological contamination incidents in water supply systems have been identified in developed countries [2]. It can be inferred that cases of drinking water quality deterioration have been detected not only in developing but also in developed countries. Water supply system operators face challenges in their operations due to urbanization and population changes. In Riga, in 2023, around 29 % of the total water network length was older than 60 years [3], whereas in Europe, the average annual asset replacement rate is around 1 % [4]. A large portion of water supply networks was designed and built at a time when the population size and urbanization in areas were significantly different, meaning that water supply systems may not meet modern requirements. One of the solutions for identifying and reducing losses is the creation of pressure zones, which can reduce water flow to a specific zone and promote stagnation both inside and outside the zone if dead ends need to be created to isolate the area [5].

The impact of drinking water temperature on drinking water quality is a complex issue which can affect the microbiological quality of drinking water in different ways across various phases of water extraction, preparation, and distribution. For example, in biological filters used for drinking water preparation, no significant impact of temperature or seasonality on cell count and composition has been detected, while temperature can influence cell growth rates after filter backwashing [6]. Drinking water stagnation in the water supply network and the resulting temperature increase interact with emerging contaminants, such as microplastics. It has been shown that in stagnant water, the presence of microplastics accelerates the breakdown of disinfectants, and the presence of microplastics may influence the microbiological composition [7].

It has been proven that the temperature in the water supply system affects microorganisms' ability to form biofilms, as well as the types of microorganisms found in the water flow [6]. In a study [8]microbiota profiling using culture-free 16S rRNA gene next-generation sequencing (NGS, it was found that some microorganisms may only be present in certain seasons. Another study [9] concluded that seasonality, including temperature, determines the composition of the biofilm microbiome in water pipes. Elevated drinking water temperatures also cause [10]:

- increased chlorine breakdown rates;
- increased risk of turbidity;
- Iicreased growth rates of *L. Pneumophilia*;
- influence on trihalomethane formation.

Water shortages have repeatedly affected Central and Western Europe due to climate change and drought periods [11]. It is expected that climate change will reduce water availability by 40 % in Southern European regions [12]. The creation of reduced pressure zones in water supply systems can promote both more efficient detection of losses and a reduction in the volume of losses [5]. It is known that the creation of water supply zones can negatively impact drinking water quality by forming dead ends where valves are closed to separate the zone [13], but the impact of changes in both recorded and unrecorded consumption on drinking water quality and the resulting effects on water stagnation and temperature are not clear. To adapt to climate change, it is necessary to assess the negative impact of widely used solutions to improve the water supply system zone management plans and increase the safety of drinking water quality.

RESEARCH OBJECTIVE:

The objective of the study is to describe the effect of a drinking water distribution system district metering area with a pressure reduction zone on drinking water consumption, drinking water temperature and drinking water microbiological quality in a real-world scenario. It is hypothesized that the installation of a pressure reduction zone would reduce both the recorded and unrecorded water in the zone. It can also be hypothesized that the installation of a pressure-reducing zone increases drinking water stagnation and temperature in a drinking water distribution network and deteriorates the drinking water microbiological quality.

METHODS:

Firstly, the number of live cells and colony-forming units from flow cytometry and grab sample measurements are described before and after the installation of the pressure reduction zone. Then, the volume of recorded and unrecorded water in the zone before and after installing a pressure reduction zone is described. Afterwards, the impact of the pressure zone on water stagnation, temperature and the microbiological quality of drinking water is evaluated.

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