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WATER RESEARCH & BIOTECHNOLOGY

NoviCo

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BIOENERGY TECHNOLOGIES AND BIOTECHNOLOGIES

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PREFACE

Dear Colleagues and Friends,

With the EU Green Deal and the UN Sustainable Development Goals (SDGs) setting ambitious targets for reducing greenhouse gas emissions, increasing energy efficiency, and transitioning towards renewable energy sources, the development and implementation of innovative biotechnologies and bioenergy solutions have become more important than ever. These two crucial topics that are at the forefront of efforts to achieve a sustainable and low-carbon future.

On behalf of Water Systems and Biotechnology Institute, I cordially welcome you to our 4th event on "Bioenergy Technologies and Biotechnologies" that is organized as a subsection of Riga Technical University 63rd International Scientific Conference and supported by EEA Grants Project "Novel biorefinery concepts for valorisation of lignocellulosic residues" (NoviCo). In this context, this year we are covering topics on bioresources, technologies for lignocellulose conversion into high value materials, municipal wastewater treatment, monitoring and extraction of high-value chemicals from sewage sludge.

This book brings together the latest research and developments in biotechnologies and bioenergy, covering a range of topics including biofuels, bio-based materials, reuse of waste, and more. The contributions in this book offer valuable insights into how biotechnologies and bioenergy can contribute to achieving the EU Green Deal and the UN SDGs, as well as how they can help address some of the most pressing challenges facing our planet today.

Please enjoy the science behind these presentations and hope to have your contribution in our forthcoming event!

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

CONTENTS

Efficient lignin fractionation from Scots pine (*Pinus sylvestris*) using ammonium-based protic ionic liquid: Process optimization and characterization of recovered lignin

Sharib Khan[1], Daniel Rauber[2], Sabarathinam Shanmugam[1*], Christopher W.M. Kay[2,3], Alar Konist[4], Timo Kikas[1*]

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ABSTRACT: Lignin-based chemicals and biomaterials are feasible alternatives to their fossil fuel-based counterparts once their breakdown into constituents is economically viable. The existing commercial market for lignin remains limited due to its complex heterogenous structure and lack of extraction/depolymerization techniques. Hence, in the present study, a novel low-cost ammonium-based protic ionic liquid (PIL), 2-hydroxyethyl ammonium lactate [N11H(2OH)][LAC], is used for the selective fractionation and improved extraction of lignin from Scots pine (*Pinus sylvestris*) softwood biomass (PWB). Optimization of three process parameters, viz., incubation time, temperature, and PIL: biomass ratio, was performed to determine the best pretreatment conditions for lignin extraction. Under the optimal pretreatment conditions (180℃, 3 h, and 3:1 PIL: biomass ratio), [N11H(2OH)][LAC] yielded 61% delignification with a lignin recovery of 56%; the cellulose content of the recovered pulp was approximately 45%. Further, the biochemical composition of recovered lignin and pulp was determined and the recovered lignin was characterized using ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectros-copy, quantitative, quantitative ^{31}P NMR, gel permeation chromatography (GPC), attenuated total reflectance –fourier transform infrared spectroscopy (ATR-FTIR), and thermal gravimetric analysis (TGA) analysis analysis. Our results reveal that [N11H(2OH)][LAC] is significantly involved in the cleavage of predominant *β–O–4′* linkages for the generation of aromatic monomers followed by the in situ depolymerization of PWB lignin. The simultaneous extraction and depolymerization of PWB lignin favor the utilization of recalcitrant pine biomass as feedstock for biorefinery schemes.

KEYWORDS: Lignin; Protic ionic liquid (PIL); Lignin extraction; Depolymerization; Nuclear Magnetic Resonance (NMR) spectroscopy.

ACKNOWLEDGEMENTS: This study was supported by ERDF and the Baltic Research Programme project No. EEA-RESEARCH-173 "Novel biorefinery concepts for valorization of lignocellulosic residues (NoviCo)" under the EEA Grant of Iceland, Liechtenstein and Norway (Agreement No. EEZ/BPP/VIAA/2021/7) and Saarland University and German Research Foundation DFG (project number 4772985087).

Application of fungi for lignocellulosic biomass treatment and biofuel production

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INTRODUCTION: Lignocellulosic biomass (LCB) is considered as a potential source of biofuel since it is not a food competitive source of biomass [1-4]. Moreover, it has a wide range of sources, such as agriculture residues, forest residues, municipal waste from landfills, and otherwise unusable crops [1]. Lignocellulosic biomass resources are abundant and readily available all around the world and thus are sustainable for use in energy sector.

As of today, relatively little bioenergy originates from lignocellulosic biomass as compared to feedstock such as starch and sugarcane, primarily due to high cost of production encompassing biomass pre-treatment steps to biomass components (i.e., lignin, cellulose, and hemicellulose) and to disrupt the natural structure of these rigid polymers [2]. To design and develop sustainable technology, it is urgent to understand the composition, characters, and the refinery process of lignocellulosic biomass. Biological treatment and enzymatic hydrolysis of lignocellulosic biomass is a promising and well researched way of biomass treatment and the focus of this research is to find widely available sources of enzymes for this process and develop a technology for biofuel production from lignocellulosic biomass.

METHODS: Carbohydrate-degrading enzymes were produced from white-rot basidiomycetes *Irpex lacteus* (Fr.) Fr. IBB 104 that were obtained from Durmishidze Institute of Biochemistry and Biotechnology, Georgia and from 8 different fungi harvested in Latvian forests. Cultures were maintained on potato dextrose agar (Oxoid Ltd., Basingstoke, Hants, UK) at 2–8 °C.

To evaluate the efficiency of hydrolysis with the produced enzymes, hay was used as a substrate. Hay (dry weight (DW): 92.8 ± 1.3%; ash 6.03%) was collected from semi-natural grassland in Latvia in 2019.

For the enzyme production pieces of the fungal culture were inoculated in 250 mL flasks 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 and 10 g glucose per L for 3–4 days in an orbital shaker (150 rpm, 30 °C, New Brunswick™Innova®43, Eppendorf Austria GmbH, Wien, Austria). pH was adjusted to 5,3 – 5,5. The grown liquid culture was homogenized (1 min, 400 rpm, Retsch GM 200, Haan, Germany) and used for inoculation in the described liquid broth with glucose replaced with 4% of hay. All flasks were incubated in an orbital shaker at 150 rpm for 4 days at 30 °C.

After incubation, the obtained liquid was treated with ultrasound (ultrasonication at 30 or 50 Hz for 2 min), after that the liquid fraction was centrifuged for 10 min at 8500 RCF to remove smaller solids. The proteins were precipitated with 50% w/v ammonium sulfate and stored at 4 °C for 24 h. The obtained sediments were extracted with centrifugation for 10 min at 8500 RCF. After centrifugation, equal volumes of 0.05 M sodium citrate buffer were added and the obtained enzyme was stored at 4 °C.

Biomass hydrolysis was performed in 20 mL bottles containing 3% w/v of hay dry biomass and 10 mL of 0.01 M sodium citrate buffer (pH ~5.5). The test was performed in 3 independent repetitions. Obtained mixture was boiled for 5 min (1 atm) to reduce indigenous microorganisms and avoid lignification. After cooling, 100 µL of the produced enzyme was added. All bottles were incubated on an orbital shaker at 150 rpm at 30 °C for 24 h. Samples for sugar analyses were collected before enzyme addition and after incubation. All samples that were not processed immediately were stored in −18 °C.

Reducing sugar content after hydrolysis was measured using the dinitrosalicylic acid (DNS) method [5] and was expressed as total reducing sugar concentration. Absorption was measured with a spectrophotometer at 540 nm (GENESYS 150, Thermo Fisher Scientific Inc., Waltham, MA, USA).

The fungal enzyme activity was determined according to the Filter Paper Assay and Carboxymethyl Cellulase protocols described by Ghose et al. [6].

RESULTS: Fungi samples from Latvian forests were collected to test their abilities to degrade lignocellulose in comparison with *Irpex lacteus*. 8 fungi samples were tested – enzymes of these fungi were produced and enzymatic hydrolysis of hay biomass took place. An enzyme mix with similar FPU, CMC activity and ability to convert lignocellulose to *Irpex lacteus* was obtained from two fungi, which were identified as *Fomitopsis pinicola* (Fig. 1, X3) and *Trichoderma atrobrunneum* (Fig. 1, X6).

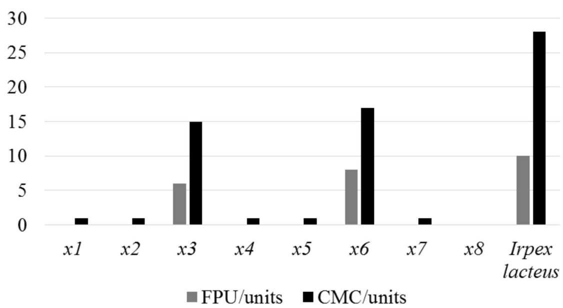

Fig. 1. FPU and CMC value of fungi harvested in Latvia comparing to I. lacteus, where X3 is Fomitopsis pinicola and X6 is Trichoderma atrobrunneum

It was determined that the enzyme activity of *Fomitopsis pinicola* is 6 FPU units/ml and 15 CMC units/ml and *Trichoderma atrobrunneum* 8 FPU units/ml and 17 CMC units/ml, respectively (an average enzyme activity of *Irpex lacteus* is 10 FPU units/ml and 28 CMC units/ml).

It was also found that using *F. pinicola* and *T. atrobrunneum* for the hay biomass hydrolysis can yield up to 200.24 and 171.85 mg fermentable sugar per g of dry biomass, respectively.

Hydrolysate as well as solid phase of this mixture can be used to produce liquid biofuels, biogas, pharmaceuticals and other high-value chemicals as well as paper of pulp. Liquid biofuel production from hydrolysate from *Irpex lacteus* enzymes has produced up to 6 g/L of biobutanol. This technology has to be further researched to improve production process and increase the productivity even though the initial results are promising.

DISCUSSION & CONCLUSIONS: The hydrolysate obtained after biomass enzymatic hydrolysis rich in reducing sugars as well as process residues can be further in various applications that include biofuel and biogas production, composting, high value chemical production and pulp and paper production [7]. In this study the main focus is on biofuel production, which is almost absent in Latvia since government limited funding for biofuel companies. In 2009 8 biofuel companies were established with funding from Latvian government and 9 years later only 3 were operational [8]. Most of biofuels are imported [9] and used for mixing with fossils, but as of June 2022, the obligatory component in fuels was cancelled to lower the fuel prices in Latvia [10]. This is another challenge for biofuel producers in Latvia. Therefore, the development of low-cost biofuel production technology is particularly important.

From all biofuels biobutanol is more superior due to its physical and chemical properties. Biobutanol has a higher flash point, higher energy density than biodiesel and bioethanol, it is also less corrosive and has a higher mixing rate with fossil fuels [7], which makes the use of biobutanol more promising.

Further research to develop a biorefinery concept for biobutanol production will be continued using 7 *Clostridium* spp. and hydrolysates produced using fungal enzymes described in this study.

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Enzymatic oxidation of hydrolysis lignin – A potential biorefinery approach

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ABSTRACT: Lignin is an abundant and renewable source capable of replacing different raw materials in the chemical industry. It can be obtained from various lignocellulosic biomass (LCB) via different pre-treatment methods. One such possibility is the thermo-mechanical-chemical pre-treatment in combination with enzymatic hydrolysis of cellulose and thus producing hydrolysis lignin (HL). In this study HL from the Sunburst pre-processing technology was utilized for enzymatic conversion. The completely soluble HL was obtained via alkali solubilization followed by the acid precipitation to obtain acid precipitated lignin (APL). APL was tested with three different bacterial laccases to identify the best optimal conditions for its conversion. Among the tested laccases, *Streptomyces coelicolor* A3(2) (*Sc*Lac) was found to display highest rate of APL conversion i.e., 50g/l in 0.25M NaOH solution. The size and structure of APL was characterized before and after the enzymatic oxidation by size exclusion chromatography (SEC) and two-dimensional heteronuclear single quantum correlation nuclear magnetic resonance (2D HSQC NMR) methods.

Keywords: biorefinery; lignocellulosic biomass; hydrolysis lignin; acid precipitated lignin; laccases.

Funding: This research was supported by ERDF and Estonian Research Council via project RESTA22 "Technologies for chemical and enzymatic valorization of lignin: products from lignin and its phenolic fragments – starting compounds for synthesis of materials, resins, adhesives, plastics, building materials".

Zymomonas mobilis high resistance against antimicrobial peptides.

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INTRODUCTION: Nowadays antimicrobial drug resistance is widespread and well identified worldwide problem, therefore there is a great interest in the discovery of novel therapies and their production possibilities to tackle this issue. As antimicrobial peptides (AMPs) are less prone to the evolution of bacterial resistance, they have been suggested as a viable class of novel antimicrobials[1]. *Zymomonas mobilis* is an facultatively anaerobic Gram-negative bacterium with a very efficient ethanologenic catabolism and other desirable industrial characteristics.

In a comparative evaluation of the antimicrobial activity of 12 different AMPs against a range of Gram-positive and Gram-negative bacteria, it was surprisingly revealed that *Z. mobilis* possesses hyper resistance against the tested AMPs.

METHODS: *Quantification of the antimicrobial resistance.* Quantification of the antimicrobial resistance against various AMPs was carried out by quantification of growth in 96-well plates in a microplate reader Infinite® M200 PRO Multimode Microplate Reader (Tecan, Maennedorf, Switzerland) according to our recent reports[2]. All bacterial strains and 3T3c/BALB eukaryotic cell lines were incubated at 32 ºC for 13–18 h at 200 rpm. Optical density measurements (λ = 600 nm) were taken automatically at 10.5 min intervals. Antimicrobial resistance activity is quantified as the minimum inhibitory concentration (MIC) – the lowest concentration of a peptide which prevents growth of bacterial cells.

Design of Antimicrobial Peptides. Seven of the AMP amino acid sequences were published earlier, while five sequences were novel peptides designed by project team members (Table 1). All peptides were synthesized in 1mg scale by ProteoGenix (France) using solid-phase method. Peptide purity was >96% as assayed by high performance liquid chromatography and mass spectroscopy.

Therapeutic Index. Therapeutic index is defined as the ratio of concentration at which the specific AMP is active against the prokaryotic pathogen without inducing cytotoxic damage to the 3t3c/BALB cells and is expressed as the ratio cytotoxicity/MIC.

Table 1. AMP sequences and sequence origin.

Given name	Sequence	Sequence origin
R_1	FAKKFAKKFKKFAKKFAKFAFAF	[3]
R_2	RWCFKVCYKGICYKKCK	[3]
R_3	VKRWKKWWRKWKKWV	[4]
R_4	GFCWYVCARRNGARVCYRRCN	[5]
R_5	KIAKRIWKRIWKILRRR	[6]
R_6	FRRWWWRRF	[7]
R_7	FAKALKALLKALKAL	Commercial oligopeptide 10
R_10	KKIAKKFWKKFWKFWKIFKK	This work
R_11	KFCLKFCFKGFCFKACGK	This work
R_12	IAKKFWKKFWKFWKIFKKIA	This work
R_13	IAKKFWPKFWKFWKIFKKIA	This work
R_14	KKIAKKFWKKFWKFWPKFWKIFKK	This work

RESULTS: *Antimicrobial resistance.* Comparative evaluation of the antimicrobial activity of 12 different antimicrobial peptides against library of ESKAPE pathogens, *E. coli* and *Z. mobilis* revealed that, with exception of R_6 and R_7, *Z. mobilis* bears significantly higher resistance against all examined peptides (Fig. 1).

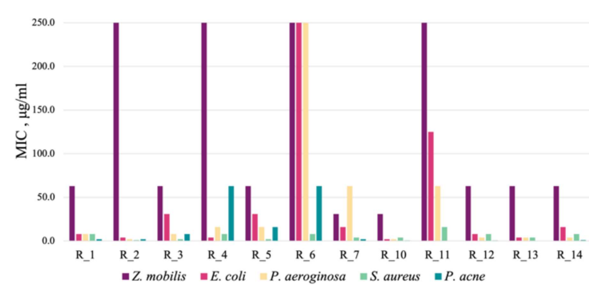

Fig. 1. MIC of AMPs against Z. mobilis, E. coli and ESCAPE pathogens (not all data shown).

Therapeutic index. In order to reveal the most promising peptide candidates for treatment of topical applications related to ESCAPE pathogens peptide cytotoxicity against 3t3c/BALB cells was examined. Afterwards the therapeutic indexes were calculated and graphically shown in Fig 2. Overall, *Z. mobilis* consistently shows lower therapeutic index values for the examined AMPs.

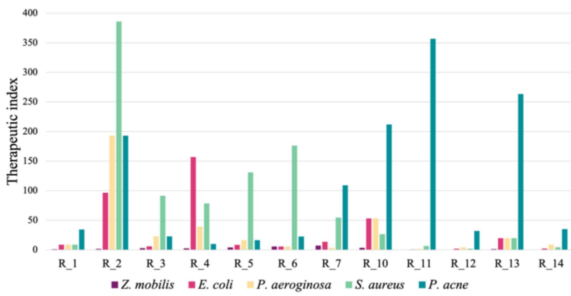

Fig. 2. Therapeutic index of the examined AMPs (not all data shown).

DISCUSSION & CONCLUSIONS: Based on the obtained results, peptides with lowest therapeutic index will be considered for treatment of topical infections related to examined microorganisms and production via heterologous expression in bacterial hosts. Unusually high *Z. mobilis* resistance against AMPs compared to other bacteria with previously described defense systems might indicate the presence of a novel resistance mechanisms in *Z. mobilis.* Based on the principal mode of action of AMPs we propose that *Z. mobilis* resistance most likely is either based on peptide cleavage, specific properties of cytoplasmic membrane, or related to the uncoupled energy metabolism of this bacterium, not involving transmembrane protonmotive force in the energy conversion. Understanding of this mechanism would allow to increase its resistance even further, thus facilitating the design of improved *Z. mobilis* AMP production strains capable of unfused AMP production at the scale beyond what is reported at the present. Additionally, possible implementation of *Z. mobilis* resistance mechanisms to other AMP bacterial production strains e.g., *E. coli,* might open options for innovative bioprocess design platforms on an even broader scale.

ACKNOWLEDGEMENTS: Study was funded by Latvian ERDF project 1.1.1.1/19/A/097: Novel anti-microbial peptides from *Zymomonas mobilis* for treatment of skin and soft tissue infections.

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Advantages and Challenges of Composting Reactors for Household Use: Smart Reactor Concept

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INTRODUCTION: In accordance to available data in 2009 61% of globally generated food waste was produced by households [1]. Meanwhile in the European Union (EU) every year around 502 kg of food waste is produced per person [2, 3], which is accounts for about 6% of total EU greenhouse gas emissions [4]. To increase the recycling of bio-waste, The EU has set up the target to recycle 65% of municipal waste and to reduce the amount of biodegradable municipal waste going to landfills to 10% by 2035 [5]. Nevertheless, most of the municipal waste generated in the EU is still landfilled (24%) or incinerated (27%), with less than half recycled (31%) and composted (17%) [6].

Composting mostly is associated with windrows; passively [14] or forced aerated windrows [15], in-vessel composting [16], and vermicomposting [17]. Commercially available composting systems for household use usually do not have or some limited smart features, as well as some of them are energy intensive.

The aim of this study is to create an affordable and same time efficient smart composting reactor concept for household use, equipped with specially selected sensors to monitor process quality, that is suitable for in-house use and do not require excess energy input. Construction, design, and component selection has been studied using multi-criteria decision-making analysis for available composting solutions to evaluate the sustainability and public acceptance of the proposed concept.

METHODS: The smart composting reactor prototype was developed (Fig. 1.). To create and operate a remote monitoring system for smart composting device, a set of sensors was selected together with data transmitting and receiving modules. All were either installed or connected to the reactor to collect and transmit information about the ongoing processes and to evaluate the composting efficiency and predict further actions of the composting process. Furthermore, data receiving and transmitting set-up was produced.

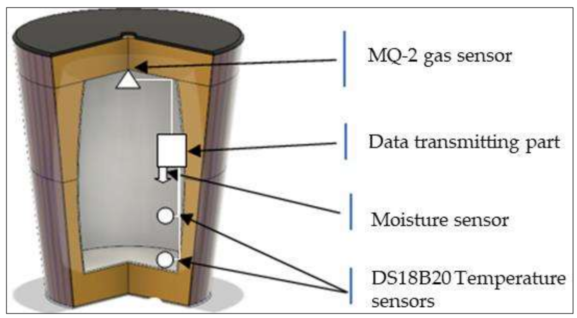

Fig. 1. Sensor placement inside the composting reactor

Created new smart composting prototype has been compared to the commercially available and at laboratory created composting devices using TOPSIS MCDM in pre-defined stages. Sensitivity analysis is used to monitor changes in TOPSIS results related to each indicator weight.

RESULTS: All initially set tasks were performed, the system behaved stably and provided all required parameters throughout the test period.

In order to evaluate the created reactor and its operation, several tests were carried out, including with leftover food (cabbage).

In the meals waste composting take a look at the compost produced heat with the aid of using itself, and the warmth become growing for the primary 3 days to attain 35°C. Further, for the following four days the temperature retained stable (~1-2°C decrease).

A slight decrease was observed in later stages of the experiment. However, it was still 3-4 degrees above ambient temperature. Based on the results, it can be concluded that new food residues should be introduced after 7 days to maintain vigorous thermogenesis. Also, you can adjust the aeration time for better results. Average moisture was 20-25%. Gas parameters showed that the gas in the compost reactor exceeded ambient parameters three times, mostly associated with unpleasant odors.

A weighting calculation using the AHP method was performed for 14 socioeconomic sustainability indicators. Experts concluded that technical indicators were more important than other indicators (14%), followed by energy consumption (12%) and off-machine pretreatment (11%). Economic and social indicators each account for less than 5% of the overall metric.

A sensitivity analysis of technical factors allowed us to determine the optimal capacity of the composting equipment (30-45 liters). Storing waste in landfills and reducing emissions annually had a similar impact on the stability of the devices we tested. Most of the composting solutions evaluated were research-level devices manufactured for laboratory testing and modeling of composting processes. A sensitivity analysis of technical factors therefore suggests that the prototype research paper is more stable than the commercial one. Economic, ecological and social aspects devalue TOPSIS for these alternatives. Unfortunately, most of the devices evaluated do not sell well to consumers due to device price or impractical device specifications (workload, energy consumption, etc.). Large commercial composters and domestic composting equipment were identified as the most stable alternatives and prototypes were developed in this study. Both solutions were developed to meet the needs of potential customers, and were therefore expected to be more stable than experimental lab equipment. The results of TOPSIS and the sensitivity analysis of the smart composting system developed as part of this study allow us to compare this solution with commercial and other laboratory instruments, highlighting shortcomings and potential improvements.

CONCLUSIONS: The distance control monitoring system performed consistently in all high humidity tests with water and the 14-day acceptance test with compost. The temperature and relative humidity sensors provided 45 hours of uninterrupted data logging for the entire test period. Compared to conventional cooling in uninsulated systems, the temperature in the reactor is 5-10°C lower and heat transfer is reduced by 32%. A multi-criteria analysis of the Alternate Hierarchical Process (AHP) method and the Order Prioritization Technique by Similarity to the Ideal Solution (TOPSIS) showed that the constructed system was 58% closer to the ideal solution.

ACKNOWLEDGEMENTS: The authors thank Janis Snucitis for technical assistance with the reactor construction and Ernests Kancevics for support in electronics. We also thank RTU Verticaly Integrated Project team for bringing us all together.

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Habitation of waterborne pathogenic bacteria in oligotrophic waters at different phosphorus concentrations

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INTRODUCTION: Pathogenic bacteria, such as *Escherichia coli*, may survive and even grow in oligotrophic environments, such as drinking water systems, and therefore possess a risk to human health. [1] However, the optimal conditions for growth both in terms of organic substrate and inorganic nutrients are not conclusive. Thus, more of such knowledge would allow to enhance microbial risk assessment as well as improve drinking water treatment design and operation [2]. In this study, the effect of phosphorous on growth of pathogenic bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) in oligotrophic tap water, were evaluated. In addition, we evaluated the influence of different types of media on the viability and reproduction of *Escherichia coli* and *Pseudomonas aeruginosa*, compared to indigenous drinking water bacteria, using flow cytometry. The results provide insight into the habituation of pathogens and could be used to further the research on their requirements for proliferation in such conditions.

METHODS: *Bacterial strains and pre-cultivation.* *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were grown overnight on Tryptone Soy Agar (TSA; Oxoid) plates at 37°C and stored in a refrigerator at +5°C until further use. Before the tests, one eyelet of bacteria was transferred into 10 ml Tryptone Soy Broth (TSB; Oxoid) media and incubated for 24 h at 35°C, 150 RPM. Afterwards, 100 µl of the suspension was transferred to 10 ml of fresh TSB medium, diluted 1:100 with 0.1 µm filtered tap water, and incubated for 24 h at 30°C, 150 RPM. Lastly, 1000 cells/ml were inoculated into 1:10 000 diluted TSB for 5 days at 30°C, 150RPM.

Preparation of samples. 0.1 µm filtered drinking water from three distantly located taps from two university buildings (further denoted Tap water 1 and Tap water 2a and 2b), 0.1% peptone and 0.9% NaCl solutions were used as experimental growth mediums. Sodium thiosulfate was added to the tap water to neutralize chlorine, which could interfere with bacterial growth.

An additional step of tap water pasteurization (60 min in a water bath at 60 °C) was added to all tap water-diluted growth media, as 0.1 µm filtration passed enough bacteria to facilitate indigenous bacteria regrowth (data not shown).

Phosphorus impact on bacterial growth. To test the possible limitation of carbon source and the effect of phosphate on the growth of bacteria, phosphorus in the form of Na_2HPO_4 was added to samples to achieve concentrations of 1 and 10 µg/l. To ensure that it was not a limiting element, carbon and salt stocks were added to the samples. Carbon was amended to a concentration of 0.5 mg/l acetate carbon and 0.5 mg/l ethanol carbon. From the salts stock samples were amended with 0.7 ppm NH_4NO_3, 0.1 ppm $MgSO_4 \times 7H_2O$, 0.1 ppm $CaCl_2 \times 2H_2O$, 0.1 ppm KCl and 0.1 ppm NaCl.

Flow cytometry measurements. After 5 days of incubation, the bacteria had reached the stationary phase and bacterial abundance was measured using flow cytometry (Sysmex Europe GmbH, Germany), equipped with a blue laser (488nm).

To ensure inoculation with undamaged or intact cells, samples were co-stained by nucleic acid-targeting stain SYBR Green I (Invitrogen, Switzerland) and Propidium Iodide dye, which penetrates the damaged cell membrane.

The emission signal was detected and plotted as a scatter by green (x-axis) and long-pass (red; y-axis) emission filters.

RESULTS: Tap water from three different taps of the university campus, peptone solution and sodium chloride solution was used in the growth experiments. Only *E. coli* bacteria were used as the test organisms, as they require more nutritious growth conditions.

The peptone solution provided the highest bacterial count (Table 1), which was expected due to higher levels of nutrients. There was a 3-log difference between tap water taken from one building in comparison to the other two tap sources from another building. The addition of sodium thiosulphate did not enhance bacterial growth, indicating that chlorine was not the cause limiting bacterial re-growth.

Table 1. E. coli bacterial count in different experimental growth mediums.

Experimental growth medium	Intact Cell Count, cells/ml	Standard deviation (triplicate samples)
Tap water 1	1.00E+06	1.70E+06
Tap water 2a	1.58E+03	4.52E+02
Tap water 2b	2.29E+03	1.30E+03
Tap water 2b + $Na_2S_2O_3$	2.68E+03	5.56E+02
0.9% NaCl	9.87E+06	7.67E+06
0.1% peptone	8.88E+08	6.43E+07

Further tests with an amendment of additional carbon source, salts (or nitrogen source) and phosphate did not show a change in the results (Figure 1).

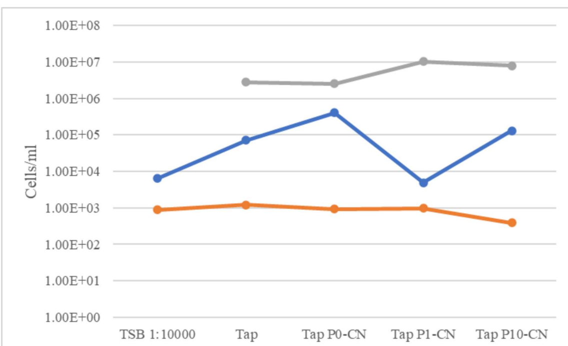

Fig. 1. Bacterial cell count depending on the phosphorus concentrations and carbon/ nitrogen (or salts) supplements.

Legend and abbreviations: Orange shows *Ps. aeruginosa*, blue *E. coli*, and grey – indigenous bacteria concentrations after reaching the stationary phase in diluted TSB (TSB 1:10000), tap water (Tap), and water amended with different phosphate concentrations (P0 – no P added, P1 – sample contains 1µg/l P, P10 – 10 µ/l P); CN stand for the addition of carbon and nitrogen (or salts stock).

DISCUSSION & CONCLUSIONS:

A study of *E. coli* adjustment to oligotrophic conditions and further study of possible limitation of carbon source and nutrients to the multiplication of *E. coli* and *Ps. aeruginosa* in comparison to tap water indigenous bacteria in drinking water media did not provide clear results.

In a similar study it was concluded that although carbon concentrations influence the growth of pathogens, it can only somewhat explain their growth. It appears that the type of organic substances [2] and other yet unknow factors are also important. It means that results might be unambiguous because of the changes in tap water composition, or other factors not considered in this study. This affects whether the bacteria would multiply, die, or enter the viable but non-culturable phase. Further studies in more controlled conditions are needed.

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Antibacterial coatings for membranes of water treatment

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INTRODUCTION: More than 2 billion people live in water-stressed areas and due to climate change it is expected for situation to be exacerbated. [1]. Therefore, there is an urgent need to provide the access to microbiologically safe and good quality drinking water in rural areas of both developed and developing regions. Currently, membrane filtration technologies are gaining popularity as being cheaper and more effective alternative than conventional water treatment methods. In addition, use of brownmillerite ($Ca_2Fe_2O_5$) – abundant, cheap and non-toxic mineral — in membrane manufacturing is demonstrated as an antibacterial material for surface coatings to prevent biofouling of the membranes [2][3].

METHODS: Ultrafiltration (UF) flat sheet membranes were prepared from polyethersulfone (PES, Ultrason E 6020 P, BASF, Germany) polymer and N-Methyl-2-pyrrolidone (NMP, Merck, Germany) solution by using immersion precipitation technique. 20 weight % (wt%) PES was prepared as a control membrane sample. For antibacterial coating integration brownmillerite powder was mixed into polymer solution in different concentrations. In this study, two coated membranes were evaluated: 1) 20 wt% PES + 3 wt% $Ca_2Fe_2O_5$ in single layer; 2) and double layer composite membrane of 20 wt% as a base and 10 wt% + 3 wt% $Ca_2Fe_2O_5$ as a top antibacterial layer.

Membrane filtration properties were evaluated by using self-assembled laboratory scale filtration unit. The unit was equipped with flat sheet membrane crossflow filtration cell (CF042A, Sterlitech, USA) with active area of 42 cm^2, peristaltic pump (WT600-2J, LongerPump, China), generic manometer, control valve and feed tank. The feed solution was prepared from pre-filtered tap water of public drinking water distribution system in Riga (Latvia). The effectivity of bacterial rejection was assessed by determining the rejection rate of faecal indicator *Escherichia coli.*

Approximately $2{\cdot}10^6$ cells/ mL of *E. coli* (ATCC®25922) were inoculated into 2 L of the feed solution of pre-filtered tap water (0.22 µm pore size filter, (Millipore, Merck, Germany)). Before the experiment, UF membranes were compacted for 24 hours with deionised water until stable permeate flux was achieved. All the permeability experiments were performed at constant feed flowrate at 40 L/h and pressure - 1.5 bar, at room temperature (20°C ± 2°C) for 4 hours. Both permeate and retentate were recirculated back into feed tank. During the experiments, permeate flowrate was recorded by using a graduated cylinder and timer. Feed and permeate water samples were evaluated with plate count technique.

RESULTS: The polymer solution with brownmillerite was successfully casted, and coated membranes were prepared. Due to chosen preparation method most of $Ca_2Fe_2O_5$ occurred to be inside of the polymer but not on the surface of the membrane. In filtration experiments both single layer membranes – uncoated control and coated with brownmillerite – had similar permeate flux and calculated permeability initially was 137 and 109 $L/[h{\cdot}m^2{\cdot}bar]$ (Fig. 1).

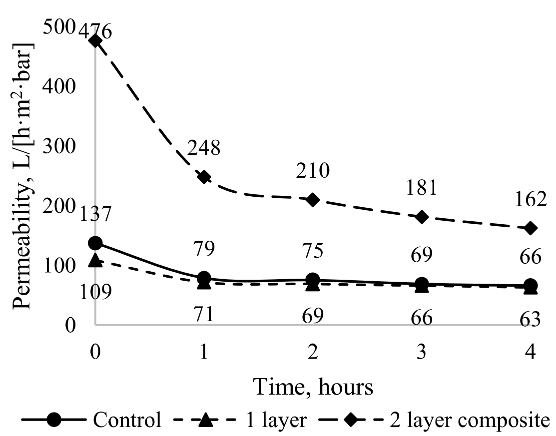

Fig. 1. Permeability of membranes

The flux of composite membrane was 476 L/[h·m^2·bar] which was much higher than was expected considering that the base layer was made of the same polymer solution that was used for control membrane manufacturing. Most of bacterial fouling on the membrane surface occurred in the first hour of filtration where the permeability decreased the most. The permeate flux after 4 hours against initial for each membrane was 48.2, 57.8 and 34.0 % respectively, which means that temporary fouling was 51.8, 42.2 and 66.0 % for each.

During experiment, the number of cultivable *E. coli* decreased in both feed and permeate samples. The log reduction of cultivable *E. coli* in permeate samples was calculated against *E. coli* concentration in feed sample of each hour (Fig. 2).

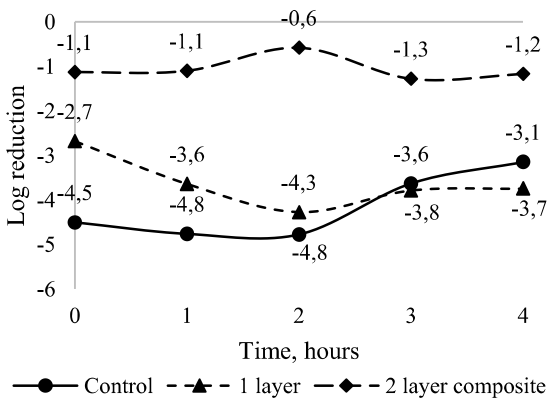

Fig. 2. Rejection of E. coli in permeate samples

The results showed that $Ca_2Fe_2O_5$ coated membrane in single layer had better bacterial rejection performance than the double layer composite membrane. The first had similar bacterial rejection to uncoated control membrane, which on average was -4.2 ± 0.7 logs.

DISCUSSION & CONCLUSIONS: The $Ca_2Fe_2O_5$ coated membrane in single layer showed acceptable filtration properties, which correspond to the control membrane. Since it showed the permeability and bacterial rejection almost as good as the control, this type of membrane is more likely to be used in further experiments. Membrane manufacturing process should be improved to prepare feasible membranes that can be practically used in water treatment. There is a concern about the possible defects of double layer composite membrane as it showed worse bacterial rejection and higher fouling which could be caused by microscopic holes. As it seems that such manufacturing process led to defective membranes, such type of membranes probably will not be used in further research. Nonetheless, antimicrobial properties of coated membrane surfaces still need to be investigated.

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Engineering Aspects of Wastewater-Based Epidemiology: Research Review Abstract

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BACKGROUND: According to the World Health Organisation more than 600 million cases of COVID-19 were reported in the world by October of 2022. More than 6.5 million died. Thus, there is a need to find new tools to predict spreading of infections and to make preventive decisions that mitigate possible impacts caused by the epidemiological factors to the society and economy. Wastewater-based epidemiology (WBE) has been recognised as an efficient approach for early warning of infectious disease transmission. However, this method has several drawbacks that limits its wider application.
Polo et al. [2] has found several factors that lead to over estimation of viral levels in wastewater: larger incidence rates in the community and lower temperatures. On the contrary, dilution due to infiltration of precipitation or from surface water leads to under estimation. Detailed modelling of viral decay and wastewater flow rates through the system are therefore required to accurately relate viral concentration at the point of sampling to the presence of virus within the catchment population. In addition, sampling SARS-CoV-2 in wastewater from, e.g., hospitals may be compromised by the wide range of disinfectants and detergents co-entering the sewage network.
Challenges of the WBE are quite wide, starting with sampling point selection, sampling technique, sample preservation, sample preparation (concentration), selection of proper analytical methods and continuing with factors that may impact interpretation of the results and development of the epidemiological prediction models: normalisation of the analytical data, composition of wastewater, temperature, pH, conductivity, detention time in the sewer system, density of population, mobility of population, life style of the population (working days vs weekends, etc.), type and condition of the sewerage system – whether it is separate or combined one and how extensive impact to the system is from industrial wastewater, stormwater and infiltration.

RESEARCH OBJECTIVES: The main objective of the study is to identify main factors impacting WBE prediction model accuracy.

METHODS: It is planned to implement study in several steps. The first step of the study will be to perform critical literature review to identify relevant publications about WBE. It is known that WBE was extensively used since 2008. It is planned to use search criteria for publications that are later than year 2012. A list of keywords will be prepared, which will be screened using selected eligibility and inclusion criteria. A literature researching keywords and its combinations will be updated considering preliminary search results scrutinising search factors. A comprehensive literature review will be prepared focusing on the selected topic and its possible variations. It is planned that for literature search will be used such searching engines and services: SCOPUS, ScienceDirect, PubMed, Google Scholar.
The second step will be data acquisition, treatment and interpretation, preparing basis for drafting of possible hypothesis cases and their screening. It is planned that there will be data used which acquired during wastewater monitoring on COVID-19 and other factors monitoring in Latvian towns performed by RTU in cooperation with Institute of Food Safety, Animal health and Environment "BIOR" (Latvia) and Latvian Biomedical Research and Study Centre (Latvia).
The final step will be scrutinising of the hypothesis and preparation for analytical experiments to evaluate the stated hypothesis, selecting proper case studies, acquiring additional data, influencing factors, analytical methods, data treatment and interpretation.

CONCLUSIONS: There is limited research about effect of engineering aspects on accuracy of WBE. The solutions require a multidisciplinary approach, taking look at the engineering, chemical, biological and other factors.

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Comparison of Membrane Cleaning Methods of Sodium Hypochlorite and Citric Acid for Municipal COVID-19 Wastewater Concentration Device

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INTRODUCTION: Wastewater-based epidemiology (WBE) and monitoring of the SARS-CoV-2 virus has been presented as a promising tool for health officials and researchers to predict outbreaks and provide additional surveillance data, contributing to community-level screening and prevention efforts. Among all the challenges, the most urgent one is concentration and detection of the relatively low viral particle loadings in large volumes of wastewater. Therefore, the sampling design, including the sample concentration, is a crucial step for detecting RNA of SARS-CoV-2 in municipal wastewater. The concentration device typically consists of ultrafiltration membrane. The previous researchers have shown that one of the most essential of the sampling process is membrane cleaning for sustainable operation of the device for further usage. The main reason for this issue is that the wastewater particles accumulate on the surface of membrane and can cause a decrease in flux. Furthermore, this can also decrease the efficiency of the monitoring device and increase the cost of maintenance. The main objective of this study is to tests two cleaning methods and compare the change in pressure, flow, and energy consumption between sodium hypochlorite with sodium hydroxide and citric acid cleaning methods.

METHODS: In this study, the concentration device was tested with two methods, sodium hydroxide and sodium hypochlorite mixture cleaning (Method I), and citric acid cleaning (Method II) [1], following the operation manual of ultrafiltration membrane in the device. Both methods had the same sequence of steps. Mixtures were made with deionized water, sodium hypochlorite method was adjusted to pH 10-11 with sodium hydroxide, while citric acid method was adjusted to pH 2-3, following the description of the membrane manual. Before the cleaning process 2L of wastewater was concentrated and filtered through the wastewater concentration device. All the experiments were carried out in triplicate.

At the start of each circulation pressure was adjusted to 1,5 Bars.
The sequence of steps is following:
Step 1 Flushing with tap water 3 times, each for 10 min
Step 2 Circulating mixtures I and II for 60 min
Step 3 Soaking the device for 30 min
Step 4 Flushing 20% of the I and II mixtures
Step 5 Circulating mixtures I and II for 60 min
Step 6 Flushing with tap water 3 times, each for 10 min

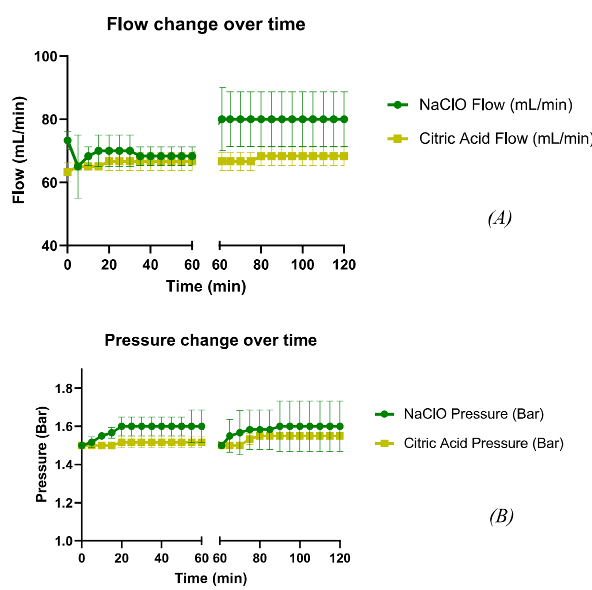

Figure 1. *Graphs of flow change over time (A) and pressure change over time (B) with both circulation cycles (1st circulation in time frame from 0 to 60 min and 2nd from 60 to 120 min)*

RESULTS: The study demonstrated that for membrane cleaning both methods can be used. In Fig. 1, both circulation cycles, i.e., flow and pressure averages can be seen. Method I showed an increase in flow over time from 65 mL/min to 80 mL/min, whereas Method II did not show any improvements in flow, stayed at 65 mL/min, despite the repeated experimental process of wastewater use. The results showed that there was no significant difference in energy usage between the two cleaning methods. The previous studies for ultrafiltration membrane cleaning have used cleaning methods such as enzymatic cleaning and hydrogen peroxide without sodium hypochlorite [2, 3], but not from wastewater fouling. For further optimization, more investigation and testing of selected ultrafiltration membrane cleaning methods must be done.

DISCUSSION & CONCLUSIONS: The literature study showed that ultrafiltration membrane cleaning from fouling is needed for sustainable use. For this study, two methods of membrane cleaning mixtures: citric acid and sodium hypochlorite with sodium hydroxide were applied and compared. In the further study, more methods will be tested and investigated, also a study on mixing cleaning methods together in one sequence of steps should be done.

ACKNOWLEDGEMENTS: Project "Platform for the Covid-19 safe work environment" (ID. 1.1.1.1/21/A/011) is founded by European Regional Development Fund specific objective 1.1.1 «Improve research and innovation capacity and the ability of Latvian research institutions to attract external funding, by investing in human capital and infrastructure». The project is co-financed by REACT-EU funding for mitigating the consequences of the pandemic crisis.

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Opportunities for resource recovery from Latvian municipal sewage sludge

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INTRODUCTION: As the European Union moves towards a circular economy, novel solutions are needed for waste and wastewater management. Sewage sludge is an inevitable by-product of biological wastewater treatment. In 2020, 23.1 thousand tons of dry sewage sludge were produced in Latvian wastewater treatment plants (WWTPs) [1]. Current sludge management approaches include land application, composting and anaerobic digestion which all utilize sludge as an organic fertilizer [1], [2]. Due to the diverse chemical composition of sewage sludge, there are opportunities to recover energy and materials from this waste biomass. Carbohydrates (including cellulose), proteins and lipids are candidates for resource recovery from sewage sludge. This assessment of Latvian municipal sewage sludge will enable selecting the most suitable biochemical resource recovery strategy for each type of sewage sludge.

METHODS: Sewage sludge samples were collected from 13 Latvian municipal WWTPs in January-March 2022. Different types of sludge were collected: excess secondary sludge (n=13), dewatered secondary sludge (n=12), primary sludge (n=1), anaerobically digested sludge (digestate, n=1) and dewatered digestate (n=1). Dry weight, volatile and fixed solids were determined gravimetrically. Protein content was determined by Lowry assay [3]. Carbohydrate content was determined by phenol-sulphuric acid method [4]. Lipid content was determined by sulfo-phospho-vanillin assay [5]. Cellulose content was determined by Schweitzer method [6].

RESULTS: The amount of valuable biochemicals was quantified in five types of sewage sludge (Fig 1). Primary sludge contained 10.0% carbohydrates, 23.9% proteins and 9.1% lipids. Dewatered secondary sludge contained 8.7% carbohydrates, 18.5% proteins and 9.8% lipids. Anaerobically digested sludge contained 3.7% carbohydrates, 8.6% proteins and 8.0% lipids. The content of cellulose in Latvian sewage sludge is presented in Figure 2. Primary sludge had the highest cellulose content (7.1%), while the other sludge types contained between 1 and 3% cellulose.

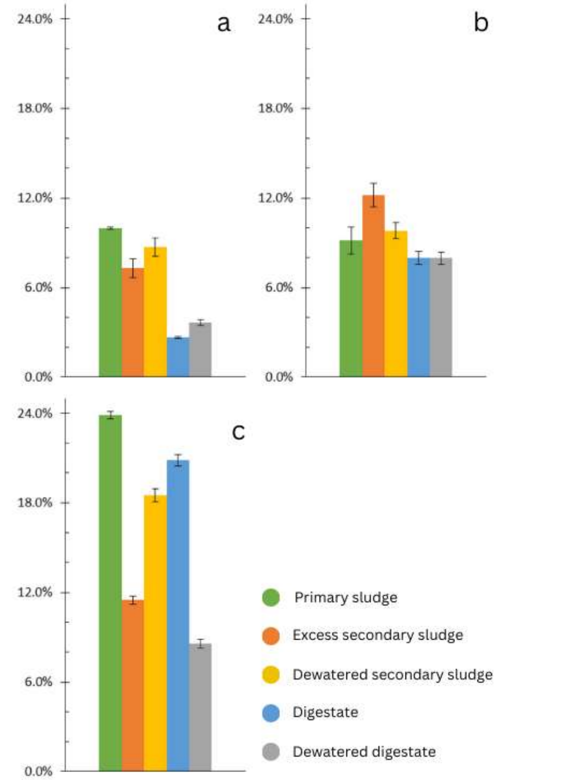

Fig. 1. Macromolecule content (%TS) in Latvian municipal sewage sludge. a – carbohydrates, b – lipids, c – proteins.

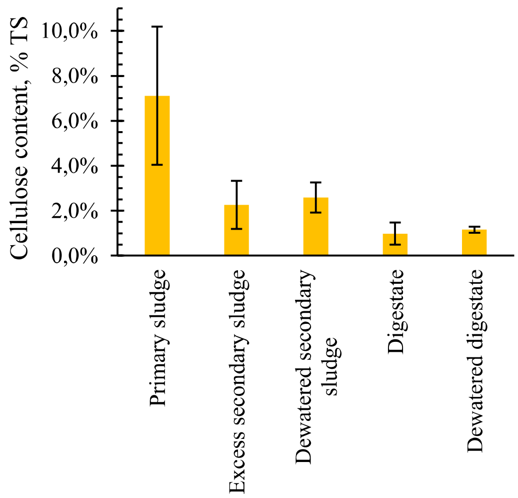

Fig.2. Cellulose content in Latvian municipal sewage sludge.

DISCUSSION & CONCLUSIONS: Out of all sludge types assessed, primary sludge was the richest in carbohydrates, cellulose and proteins, thus making it a suitable material for resource recovery. Its chemical composition suggests that valuable products, such as bioethanol and surfactants, could be produced [7], [8]. Dewatered secondary sludge is the most common type of sludge available in Latvian WWTPs. Chemical analysis showed that dewatered sludge is rich in proteins and, to a lesser extent, in lipids and carbohydrates. Given the widespread availability and favourable chemical content of dewatered sludge, recovery of valuable resources could be practiced at every Latvian WWTP. Anaerobically digested sludge has already undergone resource recovery via biogas production, however, the residue still contains proteins and lipids which could be recovered and valorized [9].

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Extraction and Characterization of Lipids from Sewage Sludge

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INTRODUCTION: Recovery of energy or value-added material from waste is becoming an essential practice to make the environment sustainable [1]. In this direction, sewage sludge can be used as raw materials for several applications, as it contains considerable amounts of organic compounds. The average concentration of organic matter in sewage sludge is around 38.4%, with highest carbohydrates percent at 55%, followed by proteins 20%, and lipids 20% [2]. As we move toward to circular economy, there are several options for sewage sludge to generate heat/electricity/biofuel and other direct/derived value-added products such as pharmaceuticals, ingredients for cosmetics, animal feeds, building materials etc. Several steps have been taken to extract the raw materials for the biodiesel production from sewage sludge. Conventionally, biodiesel is made from vegetable oils, and animal fats. In comparison to fossil fuels, biodiesel is safer and renewable which has good combustion performance [3]. Nevertheless, several research showed that 80% of the cost of biodiesel production is spent on the raw materials, including their cultivation and harvest. The high costs involved in harnessing biodiesel inhibit industrial production of biodiesel. To address this issue, cheaper raw materials like sludge has been used with no monetary cost with environmental benefits. Apart from raw materials for biodiesel applications, there are several other value added lipid products that can be extracted from the sewage sludge such as ceramide (Cer), phosphatidylcholine (PC), cardiolipin (CL), phosphatidylethanolamine (PE), etc. which has applicability in pharmaceutical and cosmetic industries. The current study focuses on the extraction of lipids by different organic solvents and characterization of the lipids though proton and carbon nuclear magnetic resonance spectroscopy (NMR).

METHODS:

Soxhlet extraction of lipids

The lipids extraction from the municipal sewage sludge was conducted in soxhlet extraction apparatus using several organic solvents such as hexane, toluene, chloroform, and methanol. Approximately 1 g of sludge was taken in a cellulose timber, and then the extraction was conducted with varying temperature of 40–80 °C, and treatment time of 2–8 h. After extraction, the extracted lipids were separated by evaporating the solvents at a temperature of 40 °C using a rotary evaporator. The percentage of extracted lipids was calculated by following equation.

$$\boldsymbol{Lipids}\ (\%) = \frac{\boldsymbol{Mass\ of\ oil\ (g)}}{\boldsymbol{Mass\ of\ dry\ sludge\ (g)}} \times \mathbf{100}$$

Lipid extraction using ionic liquid

Lipid extraction experiment was carried out by using 10 mL tetrakis(hydroxylmethyl) phosphonium chloride ionic liquid with 1 g dry sludge sample and the mixture was heated up to 100 °C for 8 h with a magnetic stirrer. After completion of the experiment, the mixture was allowed to cool down to room temperature followed by the addition of methanol (10 mL). The obtained mixture was then placed in a separating funnel by adding 5 mL hexane. The upper hexane phase containing lipids, then washed with distilled water (10 mL) to remove trace polar compounds. The hexane phase was dried with anhydrous sodium sulfate and evaporated in rotary evaporator. The resultant solution was stored in a desiccator for 24 h and weighed afterwards.

RESULTS & DISCUSSIONS: Fig. 1 showed the Percentage of lipids extracted from different organic solvents such as hexane, toluene, methanol, chloroform, mixture of methanol-chloroform and ionic liquid. It can be visualized from the figure that, the most efficient solvent for the extraction of lipid is the ionic liquid, which could extract about 12%. The ILs are the green replacements for harmful volatile organic solvents due to their non-volatile character, excellent chemical and thermal stability, potential recoverability, and design possibilities. The higher lipid yield by IL might be due to the fact that, direct dissolution of sludge in ionic liquids could lead to the recuperation of all lipid components such as cellular lipids, free fatty acids, and wax/gum.

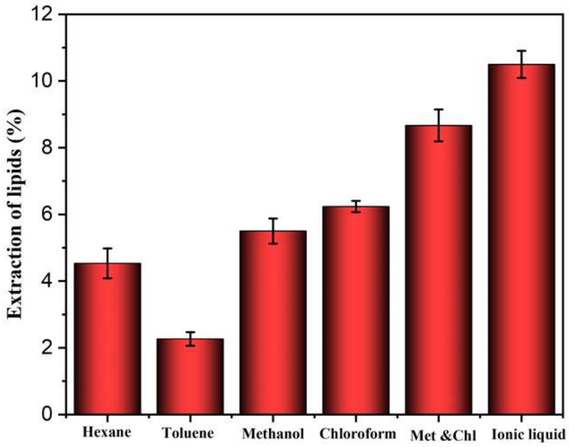

Fig. 1. Percentage of lipids extracted from different organic solvents and IL.

After extraction of lipids by hexane and toluene, we have characterized the left over sludge by FTIR spectroscopy to check the functional groups present on it. Fig. 2. Shows the FTIR spectrum of sewage sludge after lipid extraction by hexane. From the figure many sharp and broad peaks are visible which corresponds to the several functional groups like O-H, C=O, C-C, C-H, and N-H. The peak at 3304 cm^{-1} correspond to the stretching vibration of O-H group which may be due to the presence of polyalcohol and saccharides. The strong band at 3079 cm^{-1} is due to the N–H stretching vibration of protein and peptide groups. The duplet band at 2921 cm^{-1} referred to the stretching vibration of C–H bond which might be due to the polyalcohols, saccharides, and oils. The small peak at 1716 cm^{-1} corresponds to the C=O vibration in carboxyl group. In region 1650 cm^{-1}, signal reflects stretching, asymmetrical vibrations of COO− which is the characteristic peaks for peptides and proteins. Additionally, the band at 1540 cm^{-1} corresponds to symmetrical vibrations of NH^+ bond, characteristic for proteins. There can also be observed absorption bands near 1465 cm^{-1}: monosaccharides (deforming C–H bond vibration), alkenes (C–H functional group), and amides (N–H group). The last analysed peak at 1033 cm^{-1}, is a characteristic peak for C–O stretching vibration in glycerol. This peak confirms the presence of fats and fatty acids in the sample even after extraction of lipids through hexane. The results from FTIR suggests that hexane is not capable for complete lipid extraction from the sewage sludge.

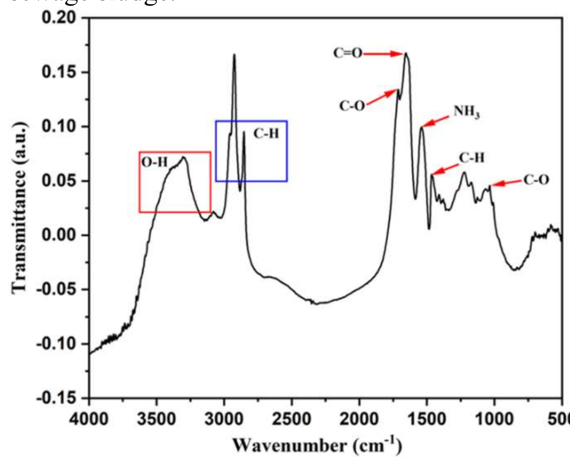

Fig. 2. FTIR spectrum of sewage sludge after lipid extraction by hexane.

CONCLUSIONS: The purpose of the present study was to quantify and qualify the lipids extracted from sewage sludge by several organic solvents. Most of the extracted lipids were cellular lipids, free fatty acids, wax and gum, accounting for more than 90% of the total lipids. The ionic liquid [tetrakis(hydroxylmethyl) phosphonium chloride] was able to extract higher amount of lipids as compared to the other organic solvents (hexane, toluene, methanol and chloroform) used in standard Soxhlet method.

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Comparing different physical-alkali and acid organic mass and protein extraction methods from municipal wastewater treatment sludge

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INTRODUCTION: It is a real and pressing problem to handle sewage sludge coming from municipal wastewater treatment facilities (WWTPs). Biological wastewater treatment methods result in the solid, semisolid, or slurry residual material known as waste activated sludge (WAS). Data from 2019 presented in the Eurostat website [1] the yearly sludge production into the EU-28 countries in 2017 was estimated to be between 9.0 and 9.5 million tons of dry solids based on the published estimates and the interpolation of the missing data. This research is focusing more on verification of known and used methods for the extraction and hydrolyses of the organic matter, protein and nitrogen from WAS and the comparison of the results obtained for one source of sludge. The sludge was subjected to different physicochemical treatment – thermos- chemical, ultrasonic-chemical, cavitation-chemical and microwave-chemical treatment in acidic and alkali environments performing a twostep treatment.

METHODS: WAS from the municipal WWTP with water treatment capacity of 5000 m^3/day where taken with following parameters: dry mater 14.8±0.3%, ash 3.8±0.2% and volatile solid content of 11.0±0.2%, Kjeldahl nitrogen content from organic mater 6,3±0.2% and in the second treatment round the supernatant form the first treatment step is taken. Sludge where treated using physical-chemical treatment using 3 M H_2SO_4 and 2.8 M NaOH as solvents and physical methods like heating at 80 °C for 3 h, 150 rpm, mechanical cavitation at 33000 rpm, ultrasound radiation with 79 W power at 40 kHz frequency for 2 hours and microwave pretreatment before chemical treatment at 650 W power. Each physical-chemical treatment was done in two steps first with acid or alkali reagent and selected physical treatment and then the supernatant where further treated with vice versa chemical reagent keeping the same physical treatment. For each procedure same amount of sludge where taken and treatment algorithm is shown in the Table 1.

Gained hydrolyses mass from each experiment where centrifugated at 3600 rpm and both precipitates, supernatant and hydrolyses mass were analysed with following methods.

Table 1. Sludge treatment algorithm and abbreviation.

Sample name	Physical treatment	1st step chemical	2nd step chemical
TCH	Thermal	Alkali	Acid
		Acid	Alkali
UCH	Ultrasound	Alkali	Acid
		Acid	Alkali
M+CH	Microwave	Alkali	Acid
		Acid	Alkali
CCH	Cavitation	Alkali	Acid
		Acid	Alkali

Extracted protein concentration were determined with Modified Lowry method using Bovine serum albumin as reference material [2], but total nitrogen extraction where analysed by measuring the change in Kjeldahl nitrogen [3] in participates referring to non-treated sample, but protein hydrolyses state where detected by comparing the Formol titration results [4] Organic mass (OM) extraction where detected by change in volatile solid content in sludge with gravimetry [5]. But in the same time

RESULTS: *Organic mass transfer.* in the first and second WAS hydrolyses step organic compounds pass into solution better with physical-alkaline hydrolysis techniques used reaching on average respectively 48.8% and 53.5% higher organic matter extraction comparing to the physical-acid treatment. In the same time total organic mass extraction efficiency is varying and there is no exact tendency between the physical-alkali-acid and physical-acid-alkali treatment pattern all thou it is necessary to admit that most of the times the highest organic mass is extracted in alkali step. UCH-alkali treatment is showing only 6.9% advantages over the UCH-acid treatment this may be explained with fact that ultrasonication manly leaves and influence on cell wall lyses in in such a degree that it compensates the low temperature disadvantage because acid/base hydrolyses process is energy consuming to reach effective extraction. [6] In the same time the most effective mass transfer where possible by using CCH-alkali-acid treatment.

Protein concentration in hydrolyses mass. On average physical-alkali WAS treatment is showing for 80 to 89 % better protein extraction comparing to the physical-acid extraction at first and second step respectively**.** Therefore, study shows that even though there is still protein left after the first extraction step it is not useful to perform the repeated protein extraction. Protein analyses shows that the biggest amount of protein is extracted by M+CH-alkali hydrolyses technique 23 046 mg/L of protein. Other researches using only alkali thermal method (120 ºC, pH 12 - 4 h) where ablet to extract 21 024 mg/L at slightly higher temperatures and longer exposure time. (26) M+CH -alkali extraction method is followed by TCH-alkali and CCH-alkali treatment methods which show 15 375 and 15 178 mg/L protein extracted respectively and can be counted as similar by their efficiency. In this case using M+CH-alkali single step hydrolyses it have been shown that WAS organic fraction contains not less than 31.4 % protein, which corresponds to literature data [7] and extracted organic matter consists of at least 52.0 % of protein knowing that in M+CH-alkali process it was possible to extract up to 60.4% of organic matter.

DISCUSSION & CONCLUSIONS: By combining the physical-alkali and acid physical treatment it is possible to gain the best organic mass extraction reaching 80.6 % and 90.2 % extraction using TCH alkali-acid and CCH alkali-acid extraction techniques, but the vast amount of organic fraction is getting extracted by physical-alkali treatment in the first step showing the organic mass transfer as high as 79.0% for CCH-alkali treatment and 74.1 % for CTH alkali treatment.

WAS physical-alkali treatment have shown better organic mass and protein extraction efficiency for first and second step treatment and Kjeldahl nitrogen extraction efficiency for the first step treatment with every physical treatment method used. Best performing nitrogen hydrolyses have been achieved when ultrasound and cavitation is used for secondary treatment. The protein extraction from the WAS is more efficient with any physical alkali treatment but data show that protein hydrolyses itself is more efficient when UCH -acid and M+CH-acid techniques are used.

The most efficient way to extra the nitrogen compounds including the protein and nitrogen reaching 52% from the OM extracted and 31.4 % from the total OM loaded in reaction - is by single step microwave radiation for 15 minutes at 650 W power and 30 min post mixing using NaOH as chemical extraction agent.

The two-step sludge treatment has to be evaluated for further researches because the vast amount of protein and organic matter is getting extracted already after first physical-alkali treatment and the further energy consumption may not be economical.

ACKNOWLEDGEMENTS: Research was supported by ERDF Project “Waste to resource technology development using sewage sludge as a raw material” (No. 1.1.1.1./20/A/041).

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Using of natural mineral material for phosphorus recovery from biological wastewater treatment plants

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INTRODUCTION: Phosphorus (P) is a crucial element for life. Therefore, it is commonly used in agriculture as a fertilizer. Often unnecessary high concentrations of fertilizers are used leading to phosphorus compounds runoff from the fields. These compounds later enter water bodies and promote eutrophication. Recently technologies allowing not only removal of P but also recovery gain more popularity.
The objective of this research is to develop an affordable technology that recovers P from wastewater in small and medium size municipal and industrial WWTPs that enables the use of it directly as a fertilizer in agriculture.

METHODS: In the preliminary experiments, the synthesis of sorbent/coagulant materials based on srebrodolskite or clay minerals was performed. The sorption capacity of developed materials against phosphate ions in batch experiments and untreated wastewater samples was determined.
The selected material was tested for possible use as a coagulant for wastewater. Experiments were made using simple Jar Test PB-700 (Phipps & Bird). Real or synthetic wastewater was used. The test included: 1 min of rapid mixing (313 rpm), 30 min of slow mixing (41 rpm), 1 h sedimentation.

RESULTS: The results of comparing different mineral-based materials indicated that the synthesized materials can reduce the amount of phosphates up to 30 times (Fig.1).
CaFe oxide (srebrodolskite) with a size <300 µm was selected for further tests due to good performance and acceptable size.

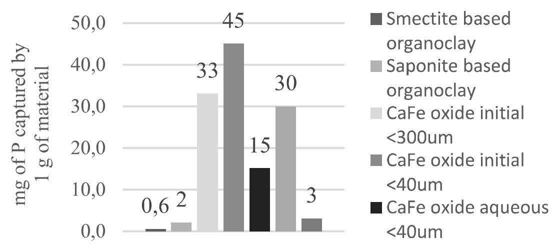

Fig. 1 Adsorption of phosphorus from wastewater by different mineral-based material.

The coagulation tests were carried to evaluate the possible use of selected material as a coagulant for wastewater (Fig.2). The coagulation process is utilized at wastewater treatment plants to remove suspended particles and organic matter from wastewaters.

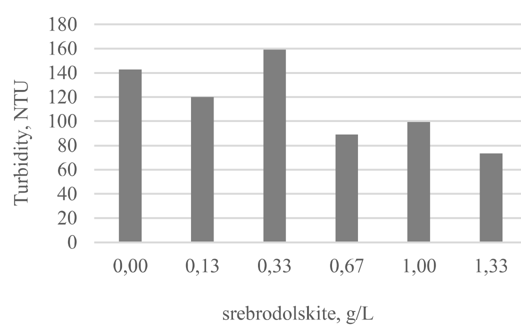

Fig. 2 Turbidity of real wastewater depending on the amount of srebrodolskite used as coagulant.

The initial turbidity of wastewater obtained from Daugavgrīva WWTP was 196 NTU. After 1 h of plain sedimentation (without added reagents) it decreased to 143 NTU. Even addition of 0.13 g/L of srebrodolskite reduced the turbidity by 20 units after 1h. The best results were obtained using the highest concentration of mineral (1.33 g/L). After the sedimentation the turbidity was 73.5 NTU.
Similar tendency occurred with UV absorption tests. UV absorption at wavelength of 254 nm

indicated decrease from 0.987 cm^{-1} to 0.967 cm^{-1}.

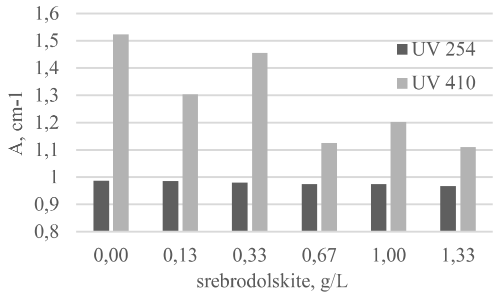

Fig. 3 UV light absorption depending on the concentration of srebrodolskite.

The initial UV absorption of wastewater at 254 nm was 1.793 cm^{-1}. UV absorption at wavelength of 410 nm indicated decrease from 1.523 cm^{-1} to 1.109 cm^{-1}. These results indicate that srebrodolskite has a good potential as a coagulant for wastewater treatment.

CONCLUSIONS: The natural mineral-based material (srebrodolskite) showed promising results to be used as the coagulant for wastewater treatment reducing organics and the turbidity of the real wastewater.

Continuing the research, it is planned to develop an environmentally friendly and cost-effective phosphorus recovery technology suitable for small and medium-sized WWTPs without the construction of additional infrastructure.

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