UNIVERSITY OF NOVI SAD
Technical Faculty "Mihajlo Pupin"
Zrenjanin, Republic of Serbia

XIII International Conference

Industrial Engineering and Environmental Protection IIZS 2023

PROCEEDINGS

Zrenjanin, Serbia, October 5-6, 2023.

University of Novi Sad
Technical Faculty “Mihajlo Pupin”
Zrenjanin, Republic of Serbia

XIII International Conference - Industrial Engineering and Environmental Protection (IIZS 2023)

Proceedings

Zrenjanin, October 5-6, 2023.

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Proceedings of the XIII International Conference - Industrial Engineering and Environmental Protection (IIZS 2023)

Conference organizer:

Technical Faculty “Mihajlo Pupin”, Zrenjanin, University of Novi Sad, Republic of Serbia

Publisher:

Technical Faculty “Mihajlo Pupin”, Zrenjanin, University of Novi Sad, Đure Đakovića bb, 23101Zrenjanin, Republic of Serbia

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Organization of this Conference is supported by the Ministry of Education, Science and Technological Development, Republic of Serbia and The Provincial Secretariat for Higher Education and Scientific Research

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CIP Classification

CIP - Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

62:005.3(082)(0.034.4)
502/504(082)(0.034.4)

INTERNATIONAL Conference Industrial Engineering and Environmental Protection (13 ; 2023 ; Zrenjanin)
Proceedings [Elektronski izvor] / XIII International Conference Industrial Engineering and Environmental Protection (IIZS 2023), Zrenjanin, 5-6th October 2023. - Zrenjanin : Technical Faculty "Mihajlo Pupin", 2023. - 1 elektronski optički disk (CD-ROM) ; 12 cm

Nasl. sa naslovnog ekrana. - Bibliografija uz svaki rad.

ISBN 978-86-7672-368-3

а) Индустријско инжењерство - Зборници б) Животна средина - Заштита - Зборници

COBISS.SR-ID 129354761

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Ministry of Education, Science and Technological Development, Republic of Serbia, and
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INTRODUCTION

Department of Mechanical Engineering and Department of Environmental Protection of Technical Faculty "Mihajlo Pupin" Zrenjanin have organized the XIII International Conference Industrial Engineering and Environmental Protection – IIZS 2023. The first international conference IIZS was organized in October 2011, and since October 2017, two departments have jointly participated in organizing this event. The topics of the scientific conference cover the fields of Industrial engineering and Environmental protection: Mechanical engineering, Energetics and Process Technique, Design and Maintenance, Oil and Gas Engineering, Health and Environmental Protection, Environmental Management, Occupational Safety, and Engineering management.

This year, IIZS was organized in a hybrid manner. Received and accepted papers were presented orally on the premises of the Technical faculty "Mihajlo Pupin" Zrenjanin and online using the Zoom platform. A specific number of papers was presented through posters. The Proceedings of IIZS 2023 contains 69 papers from 198 participants, among whom 49 are foreign authors. Besides Serbia, the authors come from 12 countries: Croatia, Bosnia and Herzegovina, Romania, India, Iran, Turkey, Bulgaria, Denmark, China, Montenegro, Slovenia, Austria, and Slovakia.

The main objectives of the IIZS 2023 conference are to innovate and expand engineering knowledge from industry and environmental protection, provide support to researchers in presenting their research results, establish new contacts with leading national and international institutions and universities, popularize the faculty and its leading role in our society and its immediate environment, draw the attention of diligent young researchers to study at our faculty, cooperate with other organizations, public companies, and industry, initiate collection of new ideas in solving specific practical problems, introduce professional and business organizations to results of scientific and technical research, present scientific knowledge and exchange experiences regarding the topics of the conference program.

We wish to express our gratitude to our long-term partners of the conference – „Aurel Vlaicu" the University of Arad, Faculty of Engineering, Arad, Romania, University "St. Kliment Ohridski," Technical faculty, Bitola, Macedonia, University Politehnica Timisoara, Faculty of engineering, Hunedoara, Romania, University of East Sarajevo, Faculty of mechanical engineering East Sarajevo, B&H, Republic of Srpska, and University of Giresun, Faculty of Engineering, Giresun, Turkey for supporting the organization of IIZS 2023. We are also grateful to all the authors who have contributed with their papers to organizing the scientific meeting IIZS 2023.

We want to extend our special thanks to the Technical faculty "Mihajlo Pupin" Zrenjanin and Dean Prof. Ph.D. Milan Nikolic for their active support concerning the organization of IIZS 2023. Also, our gratitude goes to the Ministry of Education, Science and Technological Development, Republic of Serbia, for providing financial support to organize this event.

The IIZS Conference became a traditional meeting of researchers from all over the world every year. We are open to and thankful for all valuable suggestions that could contribute to the next International Conference on Industrial Engineering and Environmental Protection organizationally and technically.

Chairman of the Organizing Committee
Assist. Prof. Mića Đurđev, PhD

Zrenjanin, October 5-6, 2023.

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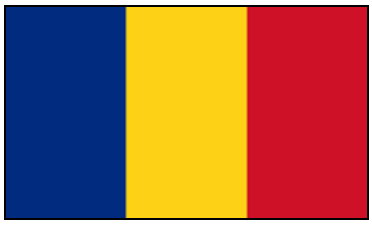

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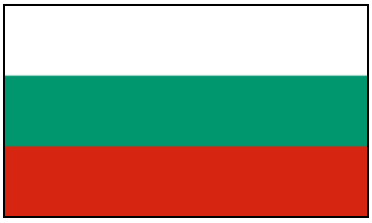

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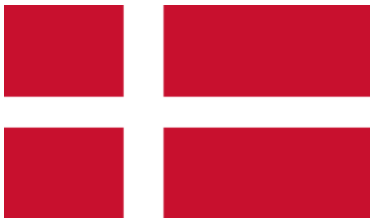

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FRESH HYPERALKALINE GROUNDWATERS CONTAIN POLYURETHANE-DEGRADING *FLAVOBACTERIUM*

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Abstract: The examined groundwaters, orginating from the fractured type of aquifer formed by Pre-Middle Jurassic ultramafic rocks, i. e. harzburgites, dunites, lherzolites and serpentinites, belong to the fresh hyperalkaline cold groundwaters of OH, Cl – Ca, Na + K genetic type. Studied occurrence was characterized by the presence of aerobic cultivable bacteria with ability to grow in a pH range of 7-12. Bacterial isolates were screened using plastic,cellulosic and hemicellulosic polymeric substrates as predominantcarbon source to assess their biotechnological potential. All 16 screened isolates (100%) demonstrated growth and/or activity on at least one tested substrate. One isolate belonging to genus *Flavobacterium*, produced prominent zone of clearing on Impranil® DLN-SD, a model substrate for polyurethanes (PU). Plastic-degrading potential has been previously documented for members of genus *Flavobacterium*. However, this is the first report of their PU-degrading activity. Accumulation of PU in the environment is of special concern, due to its toxicity, high production rates and lack of efficient recycling strategies. In the future, biotechnological potential of alkalophilic bacteria from groundwater environments should be explored more intensly, especially from the aspect of their application in the management of plastic waste and bioremediation treatments of plastic-polluted groundwater resources.
Key words:fresh hyperalkaline groundwaters, 16S rDNA sequencing, polyurethane-degrading Flavobacterium

INTRODUCTION

Terrestrial subsurface contains the largest reservoir of fresh water on the Earth and about 2.5 billion people worldwide depend exclusively on groundwater resources [1]. Hyperalkaline groundwaters (HAGW), with a pH value above 11.0[2], are globally extremely rare and are only recorded in several localities worldwide so far[3, 4]. Their zones of discharge, on the territory of Serbia, are within the framework of peridotite rocks [2]. The investigated HAGW occurrence (43.793721 N, 19.527435 E) orginated bellow the local erosion base from the fractured type of aquifer formed by Pre-Middle Jurassic ultramafic rocks [3], i. e. harzburgites, dunites, lherzolites and serpentinites of Zlatibor massif [5], which belong to central Dinarid Ophioltic Belt terrane [4]. The point of emergence of the studied HAGW is ascendant from the zone of large faults, fault zones and cracks, as a result of free gasses (such as CH_4, N_2, H_2 and O_2) and hydrostatic pressures. Based on the values of total dissolved solids (380 mg/L), pH (11.5) and temperature (17.9°C), the examined HAGW belong to the fresh hyperalkaline cold groundwaters of OH, Cl – Ca, Na + K genetic type [3], which are captured by a dug well “Sveti Jovan Krstitelj“ in the Kamišna River valley.
The genesis of HAGW is, in general, linked with the exothermic processes of serpentinization [6]and contemporary serpentinization processes in the area of investigated HAGW take place through the metamorphosis of primary anhydrous minerals (olive and pyroxene: diopside and enstatia) in the presence of water [3]. These geochemical processes provide a potential source of organic carbon for alkaliphiles [6], which represent a group of biotechnologically significant bacteria [7] that thrive at pH values above 9 [8]. Bacterial diversity of non-saline alkaline environments is not yet well documented, considering that these habitats are much rarer compared to saline alkaline habitats [6] and HAGW are characterized by less rich and diverse bacterial communities than those inhabited by waters with circumneutral pH values [9]. These habitats, as well as subterranean/groundwater

ecosystems in general, are rarely accessible to study and represent still largely unexplored resource of useful, stable enzymes [7] with biotechnological potential [10].
As a result of the growing global plastics production, estimated at 390.7 million metric tons in 2021 [11] and expected to double over the next 20 years [12] and its inefficient end-of-life management, with millions of tons accumulating in the environment annually [13], plastic waste has become one of major environmental burdens. Plastic particles, so called micro- and nano-plastics are also widespread in groundwaterenvironments. Identification of novel plastic-degrading bacteria and enzymes from unexplored resources, such as subterranean ecosystems, is of fundamental importance for a circular plastic economy [14]. For this reason, the plastic-degrading potential of HAGW isolateswas tested on several plastic polymers. In addition, their role in the degradation of one cellulosic and one hemicellulosic substrates was also examined, as there is a documented overlap in enzymatic activity on biomass and some of the plastic synthetic polymers [15, 16].

MATERIAL AND METHODS

The examined HAGW occurrence was sampled in September 2022 and both direct groundwater sample and pellet obtained from groundwater sample were cultivated on different solid media: Horikoshi-I [17]with pH = 12 and 3 media with pH in a 7-7.2 range: Luria Agar (LA), Yeast Extract-Malt Extract (ISP-2) and Mannitol-Soy Flour (MSF) at room temperature for 96 h. Cultivated isolates representative of distinct observed morphologies were screened for growth and/or enzymatic activity using plastic, cellulosic and hemicellulosic polymeric substrates as C source to assess their biotechnological potential. The Minimal Salt Medium (15 g/L agar, 9 g/L Na_2HPO_4 x 12 H_2O, 1.5 g/ L KH_2PO_4, 1 g/L NH_4Cl, 0.2 g/L $MgSO_4$ x 7H_2O, 0.2 g/ L $CaCl_2$ x 2H_2O, 0.1% trace elements solution and 0.025% N-Z amine) agar was supplemented with different plastic polymers: 6 g/L Impranil® DLN-SD (IMP-SD), 9 g/L Impranil® DL 2077 (IMP-DL), model substrates for polyurethanes (PU) and 6 g/L polycaprolactone diol (PCL), a model for polyester-based biodegradable plastics, as well as with 0.5% (w/v) carboxymethyl cellulose (CMC) and arabinoxylan (AXYL) and screened isolates were incubated for 4-8 weeks at room temperature. Solid media used for functional screening had a pH in a 7-7.4 range. Zones of clearance, suggesting substrate degradation is taking place, were visualized directly for plastic substrates and upon staining with 0.1% (w/v) Congo red for CMC and AXYL. Screened isolates with confirmed degrading activity on tested substrates were identified on genus level using 16S rDNA sequencing.

RESULTS AND DISCUSSION

The examined occurrence was characterized by the presence of aerobic cultivable bacteria with ability to grow at pH 7-12. Plate counts were in the range of 4-110 CFU/mL, which is at the lower end of the range documented for groundwaters, i. e. 10^2 – 10^8 CFU/mL [18]. This was expected, taking into account that alkaliphilic bacteria that that can survive in versatile pH conditions are rare [19]and that strict anaerobic communities prevail in the bacterial flora of HAGW [9]. Pigmented colonies with glistening circular appearance were predominant on all solid media.
In total, 16 HAGW isolates were screened for growth and/or enzymatic activity on plastic, cellulosic and hemicellulosic polymeric substrates. All isolates (100%) demonstrated growth and/or activity on at least one tested polymeric substrate and 1 isolate (6.25%), belonging to genus *Flavobacterium*, had the ability to degrade IMP-SD substrate, as shown in Fig. 1. Thirteen isolates in total had the ability to grow on IMP-SD substrate, while 2 and 10 isolates had the ability to grow on IMP-DL and PCL substrates, respectively. Seven isolates were capable to use CMC and 12 isolates to use AXYL as sole carbon source. Apart from *Flavobacterium*, no other tested HAGW isolate demonstrated zone of clearing on either plastic or cellulosic and hemicellulosic substrates.

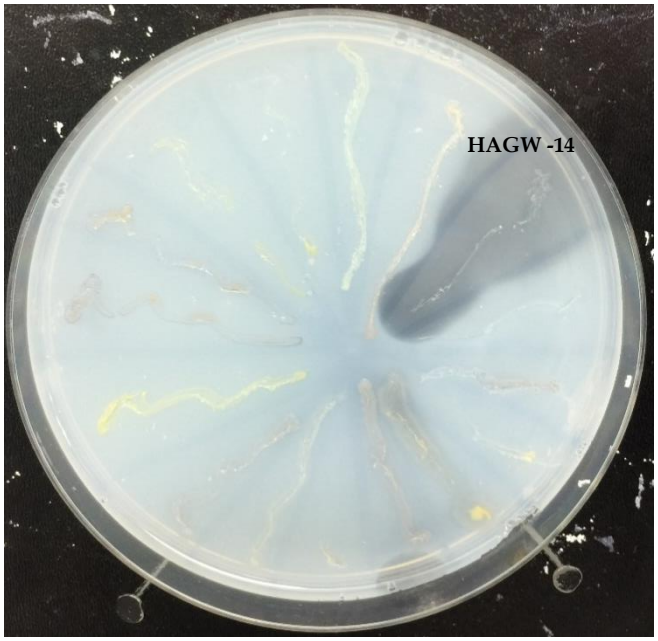

Fig. 1. *Production of zone of clearing on Impranil® DLN-SD substrate for groundwater Flavobacterium isolate HAGW-14.*

The presence of genus *Flavobacterium*was previously documented in serpentinization-driven groundwaters [20], and the HAGW occurrence examined in this work, in which *Flavobacterium* with IMP-SD degrading potential was detected, also belong to the same group of groundwaters [3]. The presence of *Flavobacterium* has also been detected previously by means of metagenomics in brackish weakly alkaline groundwater occurence (TDS – 7440 mg/L; pH – 7.6) [21]. Another isolate with IMP-SD degrading activity, with its 16S sequence closely clustering with 16S sequence from the HAGW-14 isolate, was detected by functional screening from brackish weakly acidicgroundwater [22], suggesting a wide range of conditions in which this genus persists in environment.

The role of alkaliphiles in the degradation of plastic polymers is known, with most of the documented alkaliphilic plastic-degrading bacteria belonging to the group of obligate alkaliphiles [8]. The plastic-degrading potential of *Flavobacterium* has been previously documented for members of genus *Flavobacterium*[23]and we confirmed the IMP-SD degrading potential of isolate belonging to this genus (Fig. 1), orginally isolated from LA cultivation medium with pH value of 7. This is, to our knowledge, the first report of PU-degrading activity for *Flavobacterium*.

Activity on the IMP-SD substrate, a model substrate for PU, is especially notable. PU, a heterogenous group of polymers widely used in the form of thermoplastics, thermosets or foams, are among the more commonly produced plastic polymers,with an annual production of more than 27 million tons, leading to their rapid accumulation in the environment [12, 13]. Pollution with this polymer is of special concern, as PU plastics and its microplastics have been identified as one of the most toxic types of plastic [24, 25]and, due to a lack of efficient recycling strategies, present an ongoing issue. Plastic particles are leaching in groundwaterfrom various sources[26, 27],including landfills, surface water, urban infrastructure, atmosphere, agricultural land [28], wastewater effluent [29] and fragmentation of fishing nets [30]. Prolonged consumption of groundwate contaminated with plastic particles may have a negative impact on human health [29].Therefore, it is particularly important to invest efforts in the sustainable use of this natural resource and management of microplastic contamination in groundwater.

CONCLUSION

The investigated HAGW were characterized by the presence of aerobic cultivable bacteria with the ability to persist in a range of pH values (7-12). HAGW *Flavobacterium* demonstrated PU-degrading potential, and this genus should be further explored for its applications in bioremediation treatments of PU-polluted groundwaters. In the future, biotechnological potential of alkalophilic bacteria from groundwater environments should be explored more intensly, especially from the aspect of their application in the management plastic waste.

ACKNOWLEDGMENT

This work was supported by the European Union’s H2020 Research and Innovation Programme under grant agreement No. 870292 (BioICEP) and by the Ministry of Science, Innovation and Technological Development of the Republic of Serbia (Agreement No. 451-03-47/2023-01/200042). HAGW-14 16S rDNA sequence is available in the NCBI GeneBank database under accession number OR116172.

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