N1-0452 **Vrednotenje stranskih produktov na osnovi hitina in odpadne biomase gliv za razvoj trajnostnih sorpcijskih biomaterialov za odstranjevanje PFAS in težkih kovin iz odpadnih voda v industriji galvanskega oblaganja (FunghiChi)**
Valorisation of chitin-based by-product and fungi waste for the development of sustainable sorption biomaterials, to remove PFAS and heavy metals from wastewater in the electroplating industry (FunghiChi)

Vodja projekta / Project leader: Enrico Daniso

1. VSEBINSKI OPIS RAZISKOVALNEGA PROJEKTA / DESCRIPTION OF THE RESEARCH PROJECT

FunghiChi projekt uvaja v okviru krožnega gospodarstva pionirsko rešitev - remediacijo industrijskih odpadnih voda z uporabo okolju prijaznih sorpcijskih biomaterialov pridobljenih iz kmetijskih in industrijskih odpadkov.
Za obravnavo obstojnih onesnažil, kot so per-in polifluoroalkilne snovi (PFAS) in težke kovine iz industrije galvanizacije bodo znotraj projekta ekstrahirani hitinolitični encimi (hitinaze in deacetilaze) iz izrabljenih substratov za gojenje gob vrste *Pleurotus ostreatus* in njenih delov, ki se ne uporabljajo za hrano (del beta in nožnične lupine). Ti encimi iz trajnostnih virov se bodo nato uporabili za biotehnološko predelavo surovih stranskih produktov hitina iz industrije ribje krme v prilagojene adsorbcijske materiale iz fragmentov hitina in hitozana. S pomočjo naprednih analiznih tehnik projekt preučuje ključna razmerja med strukturo in lastnostmi, ki določajo zadrževanje onesnažil. Razviti adsorbcijski materiali iz fragmentov hitina in hitozana se bodo nato vgradili v biofiltre. Razviti biofiltri bodo uporabljeni kot potrditev koncepta (*angl.* proof of concept) za remediacijo industrijskih odpadnih voda zbranih v sodelovanju z malimi in srednjimi podjetji (SME). Projekt FunghiChi združuje zeleno biotehnologijo, analizno kemijo in medresorski prenos znanja, da bi razvil postopek remediacij odpadnih voda brez uporabe kemikalij, kar je usklajeno z Zelenim dogovorom EU.

FunghiChi project pioneers a circular-economy solution for industrial wastewater treatment by transforming agricultural and industrial waste into eco-friendly sorption biomaterials. To tackle persistent pollutants like per- and polyfluoroalkyl substances (PFAS) and heavy metals from electroplating industries, within the project chitinolytic enzymes (chitinases and deacetylases) are extracted from the spent *Pleurotus ostreatus* mushroom substrates and parts of *Pleurotus ostreatus* that are not eaten (volva and the part of the stipe (steam)). These sustainably sourced enzymes are subsequently used to bio-engineer raw chitin by-products from the aquafeed industry into tailored chitin- and chitosan-fragment adsorption materials.
Through advanced analytical techniques, the project investigates key structure-property relationships governing pollutant retention. The optimized chitin and chitosan fragment adsorption materials are then packed into a proof-of-concept biofilters to validate real industrial wastewater collected in collaboration with small and medium-sized enterprises (SME). Ultimately, FunghiChi project integrates green biotechnology, analytical chemistry, and inter-sectoral knowledge transfer to develop a zero-chemical wastewater remediation aligned with the EU Green Deal.

2. OSNOVNI PODATKI GLEDE FINANCIRANJA / BASIC DATA ABOUT FINANCING

Projekt financira: Javna agencija za znanstvene raziskave in inovacije Republike Slovenije (v nadaljevanju: ARIS) v okviru razpisa »(So)financiranje raziskovalnih projektov Marie Skłodowska-Curie – Znak odličnosti«
Trajanje financiranja: 01/09/2026 – 31/08/2028

Javna agencija za znanstvenoraziskovalno in inovacijsko dejavnost Republike Slovenije

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3. SODELUJOČE ORGANIZACIJE / PARTICIPATING RESEARCH ORGANISATIONS

Kemijski inštitut / National Institute of Chemistry (Slovenia)

4. SESTAVA PROJEKTNE SKUPINE S POVEZAVAMI NA SICRIS (LE ZA SLOVENSKO SKUPINO) / PROJECT TEAM MEMBERS WITH LINKS TO THE SICRIS DATABASE (FOR SLOVENIAN TEAM)

Ime in priimek / Name and surname	**Raziskovalna organizacija / Research organisation**
Enrico Daniso	Kemijski inštitut / National Institute of Chemistry (Slovenia)

5. FAZE PROJEKTA IN NJIHOVA REALIZACIJA / PROJECT PHASES AND REALIZATION

DS1: Ekstrakcija in čiščenje encimov: Prva naloga delovnega sklopa WP1 je zbiranje vzorcev izrabljenih substratov za gojenje gob vrste *Pleurotus ostreatus* in njenih delov, ki se ne uporabljajo za hrano (del beta in nožnične lupine), ki jih bo brezplačno zagotovilo podjetje Consorzio Funghi di Treviso S.c.a, s katerim smo uspešno sodelovali v okviru (nacionalni italijanski projekt INEST). Ekstrakti vzorcev bodo analizirani za identifikacijo in kvantifikacijo prisotnih hitinolitičnih encimov (hitinaze in deacetilaze). Izveden bo celovit kemijski profil ekstraktov z uporabo kromatografskih in sklopljenih tehnik, da se odkrijejo morebitne dodatne bioaktivne spojine (predvsem sekundarni metaboliti), ki bi se lahko dolgoročno uporabile za proizvodnjo izdelkov z dodano vrednostjo. Glavni cilj delovnega paketa 1 je najti najučinkovitejše in okolju najprijaznejše analizne metode (↑izkoristek ekstrakcije encimov v primerjavi z ↓uporabo nevarnih kemikalij), ki se bodo lahko uporabljale kot standardni protokol za prihodnje študije in aplikacije. To bo vključevalo testiranje različnih zelenih ekstrakcijskih topil, tehnik (npr. maceracija, ekstrakcija z ultrazvokom, pospešena ekstrakcija s topilom) in inkubacijskih časov, da se doseže ravnovesje med količino ekstrahiranih snovi, časom, potrebnim za ekstrakcijo, in okoljskim vplivom tehnologije. Stopnja čistosti encima in zlasti njegova encimska aktivnost bosta ocenjeni s pomočjo encimskih testov. Na koncu tega delovnega sklopa bodo izbrani encimi prečiščeni, vzorcih pa bodo identificirane morebitne druge bioaktivne spojine.

DS2: Izdelava adsorbcijskih materialov iz fragmentov hitina in hitozana: Hitin, pridobljen kot stranski proizvod pri prodajalcu ribje krme (HALIEUTICA, Beaucouzé, Francija), bo okarakteriziran s pomočjo FTIR in NMR Z obema tehnikoma bosta ocenjeni čistost in stopnja deacetilacije hitina, ki sta pomembna parametra za nadaljnje encimske obdelave. Po potrebi bodo izvedeni postopki čiščenja za odstranitev nečistot, ki bi lahko motile encimski proces in ogrozile učinkovitost fragmentacije in deacetilacije. Hitin bo zmlet na različne velikosti delcev, s pomočjo nano-krogličnega mlinčka Retch 500. Zmlet hitin bo nato podvržen izoliranim in prečiščenim encimom, pri čemer dodo testirane različne koncentracije hitinaz in deacetilaz, inkubacijski časi in temperature. S tem postopkom bodo nastali adsorbcijski materiali iz fragmentov hitina in hitozana z višjo stopnjo deacetilacije in različnimi velikostmi (molekulska masa ≥ 20 kDa), kar naj bi povečalo absorpcijsko sposobnost izhodiščnega hitina. Pridobljeni adsorbcijski materiali iz hitina in hitozana bomo okarakterizirani. Preizkusili bomo njihovo adsorpcijsko zmogljivost z uporabo modelnih vzorcev, ki vsebujejo znane količine določenih težkih kovin (kot so arzen, kadmij, svinec, živo srebro, nikelj, krom, berilij in talij) in PFAS-ov (kot so perfluorooktansulfonska kislina, perfluorooktanojska kislina, perfluorodekanojska kislina, perfluorononononanojska kislina, perfluoroheksansulfonska kislina). Analize bodo izvedene s kromatograskimi in sklopljenimi tehnikami npr. z ICP-MS, pri čemer bodo ustrezne metode razvite in validirane s komercialnimi standardi.

DS3: Razvoj biofiltra za potrditev koncepta (POC biofilter): Podjetje za galvanizacijo bo priskrbelo različne vzorce odpadne vode. Vzorci bodo analizirani z metodami (razvitimi v DS2) za onesnažila (PFAS in težkih kovin). V drugem delu DS3 bo izdelan biofilter za dokaz koncepta namenjen industrijski končni uporabi. Za razvoj biofiltra za potrditev koncepta bo uporabljen filter za plavalni bazen z dodanim peskom. V filtru bo pesek v celoti ali delno nadomestil najučinkovitejši adsorbcijski material iz fragmentov hitina in hitozana. Po potrebi bodo dodani drugi adsorbenti, kot so aktivno oglje v prahu ali zeoliti. Modelni vzorci in vzorci, pridobljeni iz industrije (galvanizacijskega sektorja) bodo uporabljeni za oceno absorpcijske zmogljivosti POC-biofiltra s frontalno analizo. Pripravljeno bo tudi poročilo o razvitem POC biofiltru.

DS4: Razširjanje in komuniciranje: Ugotovitve projekta bodo razširjene in posredovane na različnih konferencah, delavnicah in predavanjih.

DS5: Upravljanje projekta in prenos znanja: Administracija projekta in upravljanje raziskovalnih podatkov bosta usklajena z načeli FAIR in skladno z GDPR. Uveden bo okvir za dvosmerni prenos znanja, ki bo združeval strokovno znanje Kemijskega inštituta (gostiteljska inštitucija)– na področju analizne kemije in znanjem vodje projekta na področju imunoencimskih testov in njihove uporabe v krožnem gospodarstvu. Strokovni razvoj bo podprt s ciljno usmerjenimi usposabljanji o podjetništvu, pripravi novih projektov in upravljanju intelektualne lastnine, ki bodo izvedene v sodelovanju s pisarno za prenos znanja in tehnologij Kemijskega inštituta. Poleg tega bodo uvedene prakse odprte znanosti, da se zagotovi učinkovito

razširjanje neobčutljivih ugotovitev prek revij z odprtim dostopom in javnih repozitorijev, hkrati pa se bo skozi celoten čas trajanja projekta ohranjalo tesno sodelovanje z industrijskimi in akademskimi deležniki.

WP1: Extraction and purification of enzymes: The first task of WP1 is the collection of the spent *Pleurotus ostreatus* mushroom substrates and parts of *Pleurotus ostreatus* that are not eaten (volva and the part of the stipe (steam)), which will be provided free of charge by Consorzio Funghi di Treviso S.c.a., thanks to a previous collaboration (national Italian project INEST). Subsequently, extracts of the obtained samples will be analysed to identify and quantify the present chitinolytic enzymes (chitinases and deacetylases). A complete chemical profiling of extracts will be conducted by using chromatographic and hyphenated techniques to reveal any additional potent bioactive compounds (mainly secondary metabolites), which could be used to produce added-value products in the long term. The main goal of WP1 is to find the most efficient and environmentally friendly methods (↑enzyme extraction yield vs ↓use of hazardous chemicals), which can be used as a standard protocol for future studies and applications. This will involve testing different green extraction solvents, techniques (e.g. maceration, ultrasound assisted extraction, accelerated solvent extraction), and incubation times to strike a balance between the amount of analyte extracted, the time it takes to extract it and the environmental impact of the technology. The degree enzyme's purity and especially its enzymatic activity will be assessed by means of enzymatic assays. At the end of this WP, selected enzymes will be purified, and possible other bioactive compounds will be identified in the extracts.
WP2: Production of chitin- and chitosan-fragment adsorption materials: The chitin obtained as a by-product from aquafeed reseller (HALIEUTICA, Beaucouzé, France) will be characterised using FTIR and NMR. These analytical techniques will assess the purity and the deacetylation degree of chitin, which are important parameters for subsequent enzymatic treatments. Purification steps will be implemented if necessary to eliminate impurities that could interfere with the enzymatic process and compromise the effectiveness of fragmentation and deacetylation. Subsequently, the chitin will undergo a physical grinding process, where different particle sizes will be produced using a Retch 500 nano ball grinder. Pulverised chitin will then undergo an enzymatic treatment using the purified enzymes, where different incubation times and temperatures, and different concentrations of the chitinases and deacetylases will be evaluated. This process will produce chitin- and chitosan-fragment adsorption materials of higher deacetylation grade and of different sizes (m.w ≥ of 20 kDa), which would increase the absorption capacity of the original chitin. The different chitin- and chitosan-fragment adsorption materials obtained will be characterised and tested for their adsorption capacity using model samples, containing known amounts of specific heavy metals (such as arsenic, cadmium, lead, mercury, nickel, chromium, beryllium and thallium) and PFAS (such as perfluorooctanesulphonic acid, perfluorooctanoic acid, perfluorodecanoic acid, perfluorononononanoic acid, perfluorohexanesulphonic acid). Analyses will be carried out by means of chromatographic and hyphenated techniques e.g. ICP-MS where appropriate methods will be developed and validated according to the commercial standards.
WP3: Proof-of concept (POC) biofilter: Different samples of wastewater will be collected at a galvanization company, and the samples will be analysed with methods for PFAS and heavy metals developed in WP2. In the second part of WP3, a POC biofilter will be produced for industrial end-use. A swimming pool sand filter will be modified by substituting the sand with the most potent chitin- and chitosan-fragment adsorption materials, totally or partially, and adding (if necessary) other adsorbents, such as powdered activated carbon or zeolites. Model samples and the samples obtained from the galvanization industry will be used to assess the absorption capacity of the POC biofilter by frontal analysis. At the end of these implementations and validation processes, a POC biofilter will be obtained and a report on the developed POC will be written.
WP4: Dissemination and communication: The project's findings will be disseminated and communicated through participation in conferences, workshops and lectures.
WP5: **Project management and transfer of knowledge**: The overall administration and Research Data Management of the project will be coordinated to align with FAIR principles and strict GDPR compliance. A two-way knowledge transfer framework will be executed, combining the expertise of National Institute of Chemistry (host institution) in advanced analytical chemistry and commercial exploitation with the researcher's expertise in immuno-enzymatic assays and circular economy applications. Professional development will be supported through targeted training sessions on entrepreneurship, grant writing, and intellectual property management delivered in collaboration with the host's Technology Transfer Office. Furthermore, open science practices will be implemented to ensure the effective dissemination of non-sensitive findings via high-impact open-access journals and public data repositories, while maintaining close engagement with industrial and academic stakeholders throughout the project lifecycle.

6. BIBLIOGRAFSKE REFERENCE, KI IZHAJAJO NEPOSREDNO IZ IZVAJANJA RAZISKOVALNEGA PROJEKTA / BIBLIOGRAPHIC REFERENCES ARISING DIRECTLY FROM THE IMPLEMENTATION OF THE RESEARCH PROJECT