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BioRemid2019

2nd International Meeting on New Strategies in Bioremediation Processes

BOOK OF ABSTRACTS

PORTO | 24-25th October 2019

BOOK OF ABSTRACTS

2nd International Meeting on New Strategies in Bioremediation Processes

BioRemid2019

PORTO :: 24-25TH OCTOBER 2019

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FOREWORD

Welcome to BioRemid2019

Dear Colleagues,

It is with great pleasure that we welcome you to the second edition of the International Meeting on New Strategies in Bioremediation Processes, BioRemid2019, hosted by the Faculty of Engineering of the University of Porto (FEUP), with the support of the Portuguese Foundation for Science and Technology (FCT), and held at Porto during October 24-25, 2019.

The purpose of this meeting is to gather researchers and industry professionals in a friendly environment to share the latest developments and innovations in the Bioremediation area. In addition, in this edition we will host an open-to-the-public event. For this, we will count on the expertise of Prof. Kenneth Timmis, who will talk on the Microbiology Literacy Initiative.

This abstracts book comprises all presentations of the conference, including two plenary lectures, seven keynote lectures, and 111 studies, presented in either oral or poster sessions. The abstracts are sorted according with the sessions or the topics of the conference.

We would like to thank you for your participation at the conference, our sponsors for their support, as well as to the scientific committee, for their invaluable contribution on the evaluation and selection process.

Enjoy your attendance at the BioRemid2019!

The Organizing Committee

ORGANIZING INSTITUTIONS

BioRemid2019 was financially supported by:

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SCIENTIFIC PROGRAM

Wednesday, 23rd October 2019

15:00 – 17:30 Registration

Thursday, 24th October 2019

09:00 – 09:30 **Late Registration**

09:30 – 10:00 **Opening session (Organizing Committee and the Dean of FEUP)**

10:00 – 10:40 **Plenary lecture (Chair: Olga Nunes)**

Concepción Calvo, Department of Microbiology, Institute of Water Research, University of Granada, Spain

PL 01 – An innovative technology for the treatment of wastewater polluted with hydrocarbons based on adsorption-biodegradation processes

10:40 – 11:00 ***Coffee break***

Session 1. Degradation of contaminants of emerging concern (Chairs: Philippe Corvini and Clementina Pozo)

11:00 – 11:30 Keynote speaker: Philippe Corvini, Institute for Ecopreneurship, School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Switzerland

KL 01 – Bacteria feeding on antibiotics – eating the poisonous

11:30 – 12:15 **Oral communications**

11:30 – 11:45 **OC 01** – Degradation of climbazole and fluconazole in intermittently loaded constructed wetlands (Adam Sochacki, Czech Republic)

11:45 – 12:00 **OC 02** – Fate of tetracyclines and sulphonamides in vegetable crops irrigated with wastewater after mycoremediation (mycoaugmentation) treatment (Raquel Camacho-Arévalo, Spain)

12:00 – 12:15 **OC 03** – Strain *Rhodococcus* sp. ED55 isolated from a WWTP in Macao degrades β-estradiol and removes toxicity of treated effluents (Irina Moreira, Portugal)

12:15 – 14:00 ***Lunch break/Poster session***

Session 2. New technologies in biotreatment (Chairs: Federico Aulenta and Hervé Bercovier)

14:00 – 14:30 Keynote speaker: Federico Aulenta, Water Research Institute (IRSA), National Research Council (CNR), Italy

KL 02 – Novel electrobioremediation strategies for cleaning up sediments contaminated by petroleum hydrocarbons

14:30 – 15:15 **Oral communications**

14:30 – 14:45 **OC 04** – Organic carbon source effect over the performance and the microbial community in a groundwater denitrifying granular sludge bioreactor (Alejandro Gonzalez Martinez, Spain)

14:45 – 15:00 **OC 05** – Electrokinetic technology for the remediation of contaminants of emergent concern in soil: feasibility assessment (Nazaré Couto, Portugal)

15:00 – 15:15 **OC 06** – Application of ultrasounds for membrane cleaning: Study of the microbial biofilm community (Alfonso Rodríguez Calvo, Spain)

Session 3. Anthropogenic impacts on the microbial communities (Chair: Ana García Ruiz)

15:15 – 15:45 Keynote speaker: Balbina Nogales, Department of Microbiology, University of the Balearic Islands, Spain

KL 03 – Marine microbial communities facing hydrocarbons: victims, survivors and opportunists

15:45 – 16:15 ***Coffee break/Poster session***

Session 4. New biodegraders and consortium-based strategies: consortia (Chairs: Angeles Prieto and Balbina Nogales)

16:15 – 16:45 Keynote speaker: Angeles Prieto-Fernández, Galician Institute of Agrobiological Research of the Spanish National Research Council, Santiago de Compostela, Spain

KL 04 – Phytotechnologies for metal rich and contaminated soils: potential role of plant associated bacteria

16:45 – 17:50 **Oral communications**

16:45 – 17:00 **OC 07** – Adaptive wastewater microbiome evolution towards hydrocarbon and lipid enhanced bioremediation (Pedro D. Teixeira, Portugal)

17:00 – 17:15 **OC 08** – Proposal of a biotechnological alternative to the use of cassava wastewater generated in flour production in Southern Brazil (Rafaela G. Corrêa, Brazil)

17:15 – 17:30 **OC 09** – Wood pellets as a sustainable organic subproduct to enhance denitrification in constructed wetlands treating oligotrophic nitrate polluted leachates from plant nurseries (Marc Viñas, Spain)

17:30 – 17:50 **OC 10** – Surfing in the storm: From biochemical networks of bacteria to deal with pollutants to microbial dynamics during bioremediation processes (Michael Seeger, Chile)

18:00 – 20:00 ***Meeting point for social program (bus) - Visit to the Cálem Port wine cellar***

20:30 – 23:00 ***Conference dinner***

__Friday, 25th October 2019__

09:00 – 09:40 **Plenary lecture (Chair: Kenneth Timmis)**

Maria Reis, Department of Chemistry, Faculty of Sciences and Technology, New University of Lisbon, Portugal

PL 02 – Prevention of environmental pollution through the conversion of organic wastes into bioplastics

Session 5. Waste recovery (Chairs: Cristina Queda and Joana Dias)

09:40 – 10:10 Keynote speaker: Cristina Queda, School of Agriculture, University of Lisbon, Portugal

KL 05 – The challenges of composting bioresources aiming soil amendment

10:10 – 10:40 **Oral communications**

10:10 – 10:25 **OC 11** – Simultaneous pretreatment and bioremediation with white-rot fungal isolates for improving saccharification of grape stalks (Joana M.C. Fernandes, Portugal)

10:25 – 10:40 **OC 12** – Bioresources from contaminated matrices for the biocontrol of hydrogen sulfide emissions and the recovery of sulphur (Simona Di Gregorio, Italy)

10:40 – 11:00 ***Coffee break***

Session 6. Bioremediation of priority pollutants (Chairs: Elisabet Aranda and Michael Seeger)

11:00 – 11:30 Keynote speaker: Elisabet Aranda, Department of Microbiology, Institute of Water Research, University of Granada, Spain

KL 06 – Degradation of pharmaceutical active compounds in fluidized batch bioreactors by xenobiophilic ascomycetes and microbial community dynamic associated

11:30 – 12:35 **Oral communications**

11:30 – 11:45 **OC 13** – Full-scale bioremediation of soil and groundwater contaminated with heat transfer fluid (Cynthia Alcántara, Spain)

11:45 – 12:00 **OC 14** – Integration between mycoaugmentation, bacterial metabarcoding and culturomics: designing bio-based approaches to the decontamination of total petroleum hydrocarbons contaminated soil (Ilaria Chicca, Italy)

12:00 – 12:15 **OC 15** – Bioremediation of oil spills in seawater using lyophilized native hydrocarbon-degrading bacteria (Rafaela P. Mendes, Portugal)

12:15 – 12:35 **OC 16** – Bioaugmentation and Biostimulation; Learnings from the Mining Industry (Lee W. John, Zimbabwe)

12:35 – 14:00 ***Lunch break/Poster session***

Session 7. New technologies & biodegraders in biotreatment (Chairs: Madalena Alves and Jesus Gonzalez-Lopez)

14:00 – 14:30 Keynote speaker: Madalena Alves, Centre of Biological Engineering, University of Minho, Portugal

	KL 07 – A wide perspective of carbon materials as catalysts for bioremediation of micropollutants and methanogenesis
14:30 – 15:00	**Oral communications**
14:30 – 14:45	**OC 17** – Innovative strategy for the treatment of landfill leachate: Ascomycetes to deplete contamination and toxicity (Giovanna Siracusa, Italy)
14:45 – 15:00	**OC 18** – Aerobic granular sludge bioreactor performance under different hydrodynamic regimens with or without silver nanoparticles regarding abatement of textile wastewater toxicity (Sofia Sousa, Portugal)
15:00 – 16:15	***Short Oral Communications***
16:15 – 16:30	***Coffee break***

Open session. OPEN ACCESS – YOUTUBE (Chair: Milton S. da Costa)

16:30 – 16:55	Keynote speaker: Kenneth Timmis, Institute of Microbiology, Technical University of Braunschweig, Germany
	KL 08 - The urgent need for microbiology literacy in society
16:55 – 17:15	***Closing session: Conference take home messages***
	Milton S. da Costa (Department of Life Sciences, University of Coimbra, Portugal); Kenneth Timmis (Institute of Microbiology, Technical University Braunschweig, Germany)
17:15 – 17:30	***Coffee break***
17:30 – 18:00	**Best young researcher communication awards (Ana M. García Ruiz, BBB; Benedita Chaves, Lipor; Sérgio Silva, Adventech)**
	Fairwell of the Organizing Committee

LIST OF COMMUNICATIONS

PLENARY LECTURES

KEYNOTE LECTURES

ORAL COMMUNICATIONS

SHORT ORAL COMMUNICATIONS

POSTER COMMUNICATIONS

TOPIC 1 – Degradation of contaminants of emerging concern

TOPIC 6 – Bioremediation of priority pollutants

TOPIC 7 – Identification and monitoring of pollutants

TOPIC 8 – Mathematical models for bioremediation processes

TOPIC 9 – Other

PLENARY LECTURES
(PL 01 – PL 02)

An innovative technology for the treatment of wastewater polluted with hydrocarbons based on adsorption biodegradation processes

PL 01

Alfonso Rodríguez-Calvo [1], Gloria Andrea Silva-Castro [1], Elisabet Aranda [1], Tatiana Robledo-Mahón [2], Jesús González-López and **Concepción Calvo** [1]

[1] *Department of Microbiology, Institute for Water Research, University of Granada.*
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Key words: Bioremediation, industrial wastewater, hydrocarbon pollution, biofilm, biosorbent.

Abstract
Pollution of waters with hydrocarbons is an environmental problem that causes ecological damage with negative effects in different areas, and therefore is of main concern at scientific, social and authorities' level. Bioremediation has been reported as useful strategy for remediation of hydrocarbon polluted sites [1]. For wastewater treatments, microorganisms attached to carriers have been successfully used since biofilms have better chance than planktonic microorganisms for adapting and surviving. This study aimed to develop an innovative treatment system for remediation of hydrocarbon polluted industrial wastewater based on adsorption-biodegradation processes that take place in bioreactors that contain carrier adsorbents on which a microbial biofilm of autochthonous microorganisms was formed. Granulated cork (CorkSorb®-03025, "CorkSorb®-01025") and polypropylene fibre (Pad-Sentec®", Cord-Sentec®)" were tested for their ability of adhering microorganisms [2]. The process was evaluated by monitoring heterotrophic and degrading bacterial growth, biofilm formation by scanning electron microscopy, and evolution of hydrocarbon content by gravimetric and gas chromatography/mass spectrometry analyses. Also, the predominant bacterial taxa forming the biofilm were studied using+ Illumina Miseq sequencing technologies. According to the results obtained at lab scale, a pilot plant was built with 4 columns of stainless steel, with vertical flow and Pad Sentec© as sorbent carrier installed inside the bioreactors. *Bacteroidales*, *Rhodobacterales* and *Desulfobacterales* were the predominant taxa identified in carrier samples while *Pseudomonadales*, *Burkolderiales* and *Flavobacteriales* showed the highest relative frequency in wastewater samples. The system showed high biodegradation rates inside bioreactors (60-80% of TPH) confirming that this technology could be considered as a suitable solution for hydrocarbon-polluted waters treatment.

Acknowledgments
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Bibliography
[1] Silva-Castro GA, Rodríguez-Calvo A, Laguna J, González-López J and Calvo C. (2016). Int. Biodeter. Biodegr., 108, 91-98.
[2] Rodríguez-Calvo A, Silva-Castro GA, Robledo-Mahón T, González-López J and Calvo C. 2018. Water Air Soil Poll., 229 (6), 175-188.

Prevention of environmental pollution through the conversion of organic wastes into bioplastics **PL 02**

Maria Reis

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Key words: Biopolymers, mixed microbial cultures, pilot plant, photofermentation.

Abstract

Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers that can be synthesized by several microorganisms and internally accumulated as carbon and energy reserves. In the last years, PHA production has received increased attention due to the diverse application possibilities of this polymer, such as its use as raw material for biodegradable plastics production. The main bottleneck for a better penetration of these polymers in the market is the higher cost compared with synthetic plastics. To overcome this restriction, efforts have been applied in the development of more cost-effective processes to produce these polymers consisting on use of less energy intensive technologies and low-cost feedstocks.

This work presents the new advances achieved on processes based on the use of open mixed microbial cultures (MMCs) and cheap wastes/by-products. The PHA production process with MMCs commonly requires the selection of a PHA accumulating culture by applying a feast and famine (FF) strategy. This strategy consists in the intermittent feeding of the substrate, where the external carbon is taken up and accumulated intracellularly as PHA (feast phase), followed by phases without substrate addition that favors cell growth on storage products (famine phase), thus creating a selection pressure for organisms capable of storing PHA. Traditionally, this process consists on using high intensive aeration, which contributes to increase the polymer cost. In order to minimize this cost factor, a new photosynthetic PHA producing system has been proposed and a feast and famine regime was also used as the selection strategy to enrich a photosynthetic mixed culture (PMC) in PHA accumulating organisms. Phototrophic organisms can draw energy from sunlight and by not requiring oxygen to produce ATP, aeration is nonessential, and the high costs associated with system's aeration can be eliminated. Optimization of the aerobic and photosynthetic processes the microbial population dynamics for each technology and the polymer characterization will be presented.

KEYNOTE LECTURES
(KL 01 – KL 08)

Bacteria feeding on antibiotics – eating the poisonous KL 01

Philippe Corvini

Institute for Ecopreneurship, School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Switzerland
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Key words: Sulfonamides, *ipso*-substitution, flavin dependent monooxygenase, wastewater treatment.

Abstract
Micropollutant removal from wastewater is of high concern in Europe and is regulated by the EU "Water Framework Directive" (WFD) and the "Swiss Water Protection Law". It has been shown that the presence of antibiotics in the environment contributes to the formation and spread of resistance genes among bacterial strains. Especially wastewater treatment plants (WWTP) are proposed to be a hubs for the emergence of resistant bacterial strains and one of the major sources for the input of bactericidal micropollutants into the environment. Among these substances, sulphonamide antibiotics are the second most used antibiotics worldwide in human and in veterinary medicine with a release of ~20,000 tons year^{-1}. The photo- and thermally stable sulfamethoxazole (SMX) as one representative of this chemical group, is often detected in significant concentrations reaching several µg/L. The biodegradation pathways are poorly understood and several studies report insufficient sulphonamide removals by conventional sewage treatment.
We report here on the isolation of bacterial strains, which are not only resistant to the sulphonamide antibiotics, but also degrade and mineralize them. One of these isolates, namely *Microbacterium* sp. strain BR1 is able to feed on SMX as sole carbon and energy source. In this bacterium, the degradation of SMX and structurally related compounds is initiated by an *ipso*-substitution, catalysed by a flavin-dependent monooxygenase acting in concert with a FMN reductase. The resulting *p*-aminophenol enters the central metabolism through a second monooxygenase activity, which leads to products amenable to ring opening. The cluster of genes involved in this degradation process was identified and each of these three enzymes could be heterologously expressed in *E. coli*. The presence of this gene cluster might represent an additional, yet unknown resistance mechanism for bacteria against sulfonamides. Even though the classic *sul1* gene is present as well in *Microbacterium* sp. strain BR1, its additional capacity to feed on SMX might represent a superior mechanism conferring to the bacterium clear advantages over a modified protein target especially in nutrient limited environments but also in case of human infection. Finally, we discuss the relevance of these findings addressing a series of questions arising from this research. Is the biodegradation of sulphonamides by *Microbacterium* BR1 a single case? Can the catabolism of sulphonamides be considered as a novel resistance to sulphonamides? What is the significance of *ipso*-substitution during wastewater treatment? What if bacteria like *Microbacterium* infects human? Can bacteria feed on other antibiotic families? Do catabolic genes involved in the biodegradation of a given antibiotic impact the propagation of genes determining the resistance to this antibiotic and reciprocally?

Novel electrobioremediation strategies for cleaning up sediments contaminated by petroleum hydrocarbons

KL 02

Federico Aulenta

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Key words: Electrobioremediation, electrochemical snorkel, electrolysis, marine sediments, petroleum hydrocarbons.

Abstract

Marine sediments represent an important sink of harmful petroleum hydrocarbons (PH) after an accidental oil spill. A number of different chemical, physical, and microbiological processes, contribute to the sinking of PH from the water column down to the seafloor, including weathering, adsorption onto sinking particulate matter (e.g., marine snow), and the addition of chemical dispersants. Upon sedimentation, PH penetrate the upper sediment layers whereby they persist due to the prevailing anoxic conditions that drastically limit the occurrence of oxidative biodegradation processes. In recent studies, we have presented the proof-of-concept of novel electrobioremediation approaches, which were found to accelerate PH bioremediation by overcoming electron acceptor limitations in contaminated sediments [1-3]. Electrobioremediation hold promise since it allows stimulating biodegradation processes with no need for adding chemicals and with little to no energy consumption [4,5]. This presentation will highlight recent trends in the development of sustainable electrobioremediation technologies for the clean-up of oil-contaminated marine sediments.

Bibliography

[1] Cappello, S. et al., (2019). Water Res 157, 381-395.
[2] Cruz Viggi, S. et al., (2017). Water Res 127, 11-21.
[3] Bellagamba, M. et al., (2017). N. Biotechnol 38, 84-90.
[4] Mapelli, F. et al., (2017). Trends Biotechnol 35(9), 860-870.
[5] Daghio, M. et al., (2017). Water Res 114, 351-370.

Marine microbial communities facing hydrocarbons: victims, survivors and opportunists

KL 03

Balbina Nogales [1,2], Antonio Busquets [1], Catalina Maria Alejandro-Marín [1], María Mas-Lladó [1], Rafael Bosch [1,2]

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Key words: Marine, pollution, hydrocarbons.

Abstract

Marine environments, particularly coastal areas, are under constant pressure and hydrocarbon pollution is one of the multiple stressors. Our perception about marine hydrocarbon pollution is biased towards accidental oil spills. Accordingly, we have detailed information on the effect of spills on microbial communities. These studies have shown the role of a group of marine gammaproteobacteria (HCB) specialised in hydrocarbon degradation (opportunists) which proliferate in events of acute pollution. However, most of the sea pollution is due to everyday practices (i.e. navigation), implies lower amounts of hydrocarbons and a constant or temporary, but often recurring, presence in the water. We can hypothesize that microbial communities facing this type of low-level pollution will have fewer problems to deal with hydrocarbon toxicity, although we also see the loss of certain populations (victims and survivors). In environments with chronic presence of low concentrations of hydrocarbons we can find stable microbial communities where HCB are rare or undetectable. This poses the question on which microorganisms might be degrading hydrocarbons in such environments. In the context of this low-level pollution we can consider hydrocarbons as usual components of the pool of carbon sources available to microorganisms in marine environments. Populations able to exploit this resource, even if they do it slowly and their degradation pathways are not performing efficiently, have a competitive advantage if hydrocarbons are present in the water. This means that we have to expand the list of opportunist degraders to include marine microbial groups not previously considered as hydrocarbon degraders.

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Phytotechnologies for metal rich and contaminated soils: potential role of plant associated bacteria

KL 04

Ángeles Prieto-Fernández [1], Vanessa Álvarez-López [1], Cristina Becerra-Castro [1], M. Isabel Cabello-Conejo [1], Andrea Cerdeira-Pérez [1], María Touceda-González [1], Carmela Monterroso [2], Beatriz Rodríguez-Garrido [1], Petra Kidd [1]

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Key words: Phytoremediation, phytomining, soil contamination, substrates enriched in trace elements (TE), plant-growth promoting bacteria.

Abstract

Soil contamination with organic compounds and/or trace elements (TE) is a worldwide serious problem. Nowadays, different plant-based technologies have been recognized as viable options for the treatment of contaminated soils (phytoremediation). In the last years, there is also a growing interest in the use of plants able to hyperaccumulate TE for extracting elements of high economical value (phytomining) from metalliferous soils or other TE enriched substrates. Contaminated and metalliferous soils and substrates constitute hostile environments for the development of a vegetation cover and numerous strategies for improving plant establishment and growth have been tested and applied. It is well known that numerous bacteria that live in close contact with plants (rhizospheric, epiphytes and endophytes) are able to promote plant growth (PGP bacteria) through different mechanisms, and some strains are successfully used as biofertilisers in agriculture. Plant associated bacteria showing PGP characteristics or able to modify the availability of organic contaminants and TEs are potentially very useful for the improvement of phytoremediation and phytomining technologies. The presentation reviews the main results obtained by our research group in the last years, working with plants from ultramafic areas and from other TE enriched substrates, as well as with plants from a lindane polluted site.

The challenges of composting bioresources aiming soil amendment KL 05

Ana C. Cunha-Queda

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Key words: Composting, soil, organic matter, bio-waste, circular bioeconomy.

Abstract
The recycling of bio-waste is an important step for the recovery of organic matter and production of soil organic amendments contributing for the circular bioeconomy. One bioenergetics process for this purpose is composting, which can be applied from home to industrial level. Composting is a controlled aerobic bioconversion of mixed organic bio-waste into a valuable product, the compost. During process raw organic materials such as food wastes, green residues, and organic fraction of municipal wastes are transformed to humic-like substances. To obtain high quality products is essential the collection of separate bio-waste and apply an efficient treatment to stabilise organic matter. New challenges in waste management have been reported by several authors and are promising for the use of composts. Bernal [1] refers that five main challenges have been identified in waste management in agroecosystems: to improve nutrient availability and soil cycling; to develop technologies for nutrient re-use; to reduce contaminants and improve food safety; to mitigate environmental emissions; and to enhance soil health and function. Another possibility is the recovery of substances from wastes or/and composts such as the recovery of humic-like substances from low quality composts [2]. This work aims to present the most important aspects of composting process, their impacts on the quality of compost as well as the biotechnological use of products as soil organic amendments or as a resource to produce high value substances.

Bibliography

[1] Bernal, M.P., 2017, Front Sustain Food Syst 1(1).

[2] Silva, M.E.F., Lemos, L.T., Bastos, M.M., Nunes, O.C., Cunha-Queda, A.C., 2013, Bioresour Technol 128, 624-32.

Degradation of pharmaceutical active compounds in fluidized batch bioreactors by xenobiophilic ascomycetes and microbial community dynamic associated KL 06

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Key words: Pharmaceutical active compounds, fungal community, bacterial community, Illumina MiSeq, Phylochip.

Abstract

Pharmaceutical active compounds (PhACs) represent an increasing problem of human concern in the wastewater around the world. PhACs encompass a variety of aromatic compounds considered emerging contaminant, since they are not regulated by the current legislation. Traces of these compounds are frequently found in water sources, starting to cause long term effects on aquatic organisms and the acquisition of antibiotic bacterial resistances, which could have serious implications for public health [1]. In this study we use a batch bioreactor inoculated with the xenobiophile ascomycete fungus *Penicillium*, isolated from a hydrocarbon polluted place. In this bioreactor, the capacity of removal of different non-steroidal anti-inflammatory compounds from a non-sterile real wastewater from a hospital was monitored by UPLC/MS-MS. The microbial community shifts over time was analyzed using two high-throughput molecular approaches, Illumina MiSeq sequencing platform and PhyloChip, a phylogenetic microarray that allows better detection of minority populations. The results showed *Penicillium* ability to remove the majority of the analyzed PhACs, including diclofenac, paracetamol, ketoprofen or mephenamic acid, in 24 hours. Also in the first 24 h, *Penicillium* outcompeted all the native fungal populations present in the wastewater, including several fungal human pathogens species such as *Mycosphaerella* or *Drechslera*. Bacterial community diversity decreased too along the treatment, with the displacement of some bacterial human pathogens belonging to *Clostridiaceae* and *Brucellaceae* families. Groups of bacteria which include important degraders such as *Pseudomonadaceae* remained in the system, which could involve a possible natural consortium formed during the degradation. These results indicate the possibility to use this system for the removal of PhACs under real conditions.

Bibliography

[1] aus der Beek, T., Weber, F. A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A. (2016) Pharmaceuticals in the environment—Global occurrences and perspectives. Environ. Toxicol. Chem. 35, 823-835.

A wide perspective of carbon materials as catalysts for bioremediation of emerging pollutants and methanogenesis

KL 07

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Key words: Carbon materials, methanogenesis, redox mediator, bioremediation.

Abstract

Biotransformation of emerging pollutants under anoxic conditions can be accelerated by carbon materials (CM) acting as redox mediators. CM have been also extensively reported as facilitating external electron transfer in methanogenic processes. Here, different CM including magnetic carbon materials (C@MNP), were prepared, characterized and applied as RM on the biological reduction of Acid Orange 10 (AO10) and ciprofloxacin (CIP). CIP could be biologically removed in the presence of CNT and CNT@2%Fe, and AO10 decolourisation rates were 79-fold higher in the assays with CNT@2%Fe. The effect of carbon nanotubes (CNT) on the activity of several pure cultures of methanogens was also investigated, demonstrating that CNT could accelerate up to 17-fold the methane production rate. It is evident from this work that carbon materials with different chemical and textural characteristics can accelerate significantly bioremediation and methanogenic processes. The fact that concentrations as low as 0.1 g/L were used with positive effects, is remarkable in terms of economic feasibility of using CM as efficient catalysts in both processes.

The urgent need for microbiology literacy in society **KL 08**

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Key words: Education, Microbiology Literacy Initiative, child-centric experience-relevant microbiological activity/global issues evidence-based decisions, grand challenges, sustainable development goals, world citizens-stakeholders.

Abstract

Microbiology literacy in society is absolutely essential if decision makers are to make informed policy decisions about the Grand Challenges facing humanity, including global warming, feeding the growing human population, exploding healthcare costs, the soil crisis, environmental pollution, and so forth. But it is also crucial for evidence-based, rather than fake news-based/social media-biased, personal decisions on everyday activities having a microbial component (the, by now, classical example being of course vaccination). The only way for microbial literacy to be achieved is through the incorporation of relevant topics into basic (pre)school curricula (and of course of continuing education offerings). Given the current exceptional level of excitement in microbiology, and the pervasive relevance to, and impact of its breathless pace of progress on, everyday lives, microbiology teaching in schools could become one of the most fascinating and inspiring topics in curricula, both for teachers to teach and for pupils to learn!

The Microbiology Literacy Initiative was launched with an Editorial that documents the need for microbiology literacy in society and presents a concept and roadmap for its achievement. Phase 2 of the initiative involves development of a series of ca. 100 lesson frameworks, each of which will deal with a selected microbiology topic relating to everyday child experiences (e.g. acquisition of a pet dog), in order to capture pupil interest at the outset of the lesson. Towards the end of the lesson, the relationship of the topic to global Grand Challenges and Sustainable Development Goals will be explained, thereby exposing and emphasising the triangle of personal activity/experience-relevant microbiological activity-the wider context of global issues. Raising awareness of Grand Challenges and global issues, and their relationship to our personal and local-regional activities, is absolutely essential for the transition of children to responsible citizens. Microbial activities are almost unique in enabling the linking of these elements in easily comprehensible narratives.

The topic frameworks, formulated in simple language, standardised to a template, and generic to enable them to be adapted to different age groups, will be conceived where possible to be stand-alone, to enable teachers to mix and match, according to their specific teaching objectives. In addition to the series of topic frameworks, there will be a series of class experiments targeted at different age groups, and a series of suggestions for excursions (e.g. to industries, public agencies, academic institutions, etc.), where pupils can experience professional microbiology at first hand, according to what is available nearby (and thereby also acquire insight into one type of potential future career options). All of these resources will be freely available to teachers and anyone else who has an interest.

ORAL COMMUNICATIONS
(OC 01 – OC 18)

Degradation of climbazole and fluconazole in intermittently loaded constructed wetlands

OC 01

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Key words: Antifungals, organic micropollutants, wastewater treatment.

Abstract

The goal of this study was to evaluate the removal and transformation of antifungal drugs: fluconazole and climbazole in constructed wetlands (CWs). The removal of these compounds (at a concentration of 10 µg/L) was studied in a model CW system treating artificial domestic wastewater. The system consisted of 4 types of columns with various water saturation levels and with or without vegetation. The removal of fluconazole was negligible and the removal of climbazole was 56%-96%. Fluconazole was not susceptible to retention in CWs. The removal of climbazole was enhanced in the unsaturated conditions and in the presence of plants. No transformation products of fluconazole were found in the effluents or in the plants. The transformation products of climbazole in the effluents were: hydrogenated (reduced) climbazole as the predominant intermediate, and hydroxylated (oxidized) climbazole. The amount of the reduced climbazole was comparable in the saturated (anoxic) and unsaturated (oxic) columns, but was noticeably lower in the partially saturated columns that provided variable oxic-anoxic conditions. The analysis of the below-ground and the upper-ground parts of the plants indicated the presence of climbazole and its reduced intermediate. The transformation of climbazole was observed to be an enantioselective process dependent on the water saturation level of the CWs. The ratio between the two pairs of diasteroisomers of the reduced climbazole in the effluent was <0.2 for the saturated (anoxic) columns; 0.6 for partially saturated columns (oxic-anoxic) and 1.3 for unsaturated columns.

Fate of tetracyclines and sulphonamides in vegetable crops irrigated with wastewater after mycoremediation (mycoaugmentation) treatment **OC 02**

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Key words: Sulfonamides, tetracyclines, mycorremediation, ligninolytic fungi, wastewater.

Abstract

The presence of low doses of antibiotics in the environment is generating multiresistant bacteria, which represent a threat to human health. The inefficiency of wastewater treatment plants and the low rate of metabolization by cattle are the main reason why they end polluting soils, waters and crops [1].

Mycoremediation is an ecological alternative to dissipate antibiotics from the environment due to the enzymatic system of some kind of fungi already used to degrade other kind of organic compounds [2]. During this work, several biofilters with ligninolytic fungi were developed to remove antibiotics from wastewater. Afterwards, their efficiency was tested by irrigating lettuce plants with the wastewaters before and after biofilter treatment. Tetracyclines (TCs) and sulfonamides (SAs) were chosen due to their high use in veterinary practices.

Spent mushroom substrate (SMS) based on wheat straw was sterilized and inoculated with different fungi of genus *Pleurotus*. After two weeks, the wastewater was introduced in the biofilters and aliquots were taken at different times to analyze the antibiotic concentrations by UPLC-MS and fungal extracellular activities spectrophotometrically [3].

After two weeks of irrigation, biofilters effectively removed antibiotics from wastewater. It was a combination between adsorption on SMS and fungi degradation. Antibiotics reached the leaves of the lettuce (edible part), however, the ones watered with the biofilter treatment showed between 65% and 70% less concentration of TCs and SAs, respectively. Meanwhile in the root the decrease was between 60% (SAs) and 80% (TCs). Consequently, mycoremediation techniques reduce the presence of TCs and SAs in wastewaters.

Bibliography

[1] Grenni, P., Ancona, V., Barra Caracciolo, A. (2018). Microchem J 136, 25-39.

[2] Yang, S., Hai, F.I., Nghiem, L.D., Price, W.E., Roddick, F., Moreira, M.T., Magram, S.F. (2013). Bioresour Technol 141, 97-108.

[3] García-Delgado, C., Yunta, F., Eymar, E. (2015). J Hazard Mater 300, 281-288.

Strain *Rhodococcus* sp. ED55 isolated from a WWTP in Macao degrades β-estradiol and removes toxicity of treated effluents

OC 03

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Key words: β-estradiol, biodegradation, *Rhodococcus* sp. ED55, wastewater, toxicity.

Abstract

β-estradiol (E2) is an endogenous steroid hormone excreted by humans and animals. Due to incomplete removal on wastewater treatment plants (WWTP) and animal waste disposal, this micropollutant reaches the environmental compartments, being detected in WWTP effluents, surface water, soil and sediments [1]. This is an issue of great concern due to its endocrine disruption potential. The main objective of the present study was to evaluate the biodegradation of E2 by a bacterial strain – *Rhodococcus* sp. ED55 - isolated from the sediments of a discharge point of a WWTP in Coloane, Macau. Biodegradation experiments were performed in synthetic mineral medium and in wastewater from a municipal WWTP (Parada, Maia – Portugal). Strain ED55 was able to completely degrade the supplied amount of E2 in few hours, both in synthetic medium and in municipal wastewater. Estrone (E1) was identified as intermediate degradation metabolite, by comparison with a commercial standard. The detection and identification of other biodegradation intermediates by UPLC-QTOF/MS/MS is ongoing, aiming at elucidation of the metabolic pathway of degradation. Moreover, the bioaugmentation with E2 significantly improved the natural attenuation of the compound in municipal wastewater. The acute test with luminescent marine bacterium *Vibrio fischeri* revealed elimination of the toxicity of the treated effluent. Bacterial strain *Rhodococcus* sp. ED55 can potentially be applied for bioaugmentation of bioreatores for the enhancement of wastewater treatment.

Acknowledgements

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Bibliography

[1] Zhang, C., Li, Y., Wang, C., Niu, L., Cai, W. (2016). Crit Rev Environ Sci Technol 46, 1-59.

Organic carbon source effect over the performance and the microbial community in a groundwater denitrifying granular sludge bioreactor

OC 04

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Key words: Groundwater, granular sludge, denitrification, microbial ecology, next-generation sequencing.

Abstract
It has been observed that nitrate contamination, disperse into groundwater supplies from large-scale use of agricultural fertilizers, is evidently a significant human health risk. Conventional groundwater treatment technologies, such as anion exchange, reverse osmosis and electrodialysis/electrodialysis reversal, have not proved to be safe environmental technologies because they remove nitrate and other constituents to a concentrated waste stream requiring disposal under high energy requirements [1]. For these reasons, in this project, a novel denitrifying granular sludge bioreactor technology was built, started-up and operated for the treatment of nitrate-contaminated groundwater. The bioreactor was built amended with a nitrate polluted synthetic groundwater composition (100 mg-N L-1 of Nitrate) and a variable concentration of sodium acetate, modified during the operation time to analyze the influence of the influent organic carbon in the denitrification process. Moreover, Molecular biology techniques were done using Illumina MiSeq high throughput sequencing protocols in order to characterize the hypervariable regions V1-V2-V3 of 16S rRNA gene of Bacteria, V3-V4-V5 of 16S rRNA gene of Archaea and ITS region of Fungi [2]. Finally, multivariate redundancy studies linking the microbial community structure with the physicochemical performance were done. The overall conclusion showed that the denitrifying granular sludge amended with low concentrations of sodium acetate is an efficient, cost-friendly alternative for the treatment of groundwater polluted with nitrate.

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Bibliography
[1] NASA (National Aeronautics and Space Administration).(2006) Perchlorate (ClO4 -) Treatment Technologies Literature Review Operable Unit 1 Expanded Treatability Study, EPA ID# CA9800013030.
[2] Gonzalez-Martinez, A., Margareto, A., Rodriguez-Sanchez, A., Pesciaroli, C., Diaz-Cruz, S., Barcelo, D., Vahala, R. (2018) Linking the effect of antibiotics on partial-nitritation biofilters: Performance, microbial communities and microbial activities. Front. Microbiol. 9, 1–16.

Electrokinetic technology for the remediation of contaminants of emergent concern in soil: feasibility assessment

OC 05

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Key words: Electrochemical process, agricultural soil, pharmaceuticals and personal care products, degradation, spatial distribution.

Abstract

In an electrokinetic (EK) remediation process a low-level direct current is applied onto the soil [1]. This technology has been attracting significant research interest for the development of remediation solutions for contaminated soils, especially to remove organic contaminants for which effective and universal solutions are still lacking. The potential of EK remediation to remove from soils one particular group of contaminants -contaminants of emergent concern (CECs), remain largely overlooked. In the present study, the aim was to evaluate the efficiency of the EK process for the remediation of an agricultural clay soils containing CECs [2]. The soil was spiked with four CECs -sulfamethoxazole, ibuprofen, triclosan and caffeine- and their status (i.e. degradation and spatial distribution) evaluated at the seventh day of EK treatment at a defined current intensity, directionality and duration of void period. The CECs degradation in soil after the EK remediation with a current intensity of 10 mA followed a similar trend to that of the natural attenuation: sulfamethoxazole > ibuprofen ≥ triclosan ≥ caffeine. The results support that the application of a unidirectional current favours the CECs remediation (13-85%); coupling an ON/OFF period of 12h resulted in caffeine highest degradation without losing efficiency on the other CECs remediation (36-72%). Our results suggest that the design of an EK assisted remediation process should target the least biodegradable contaminant. Overall this study made evident that EK assisted remediation processes are a promising technology solution to stimulate *in situ* the removal of CECs from agricultural soils [2].

Bibliography

[1] Acar, Y.B., Alshawabkeh, A.N., 1993, Environ. Sci. Technol. 27, 2638–2647
[2] Guedes, P., Lopes, V., Couto, N., Mateus, E.P., Pereira, C.S., Ribeiro, A-B., 2019 (*submitted*).

Application of ultrasounds for membrane cleaning: Study of the microbial biofilm community OC 06

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Key words: MBR, membrane cleaning, biofouling, ultrasounds, biodiversity.

Abstract

Membrane biofouling consists on biofilm growth on membrane surface and results in a flux decline and in a decreasing membrane performance and lifetime, becoming in the main inconvenient in the use of Membrane Bioreactor (MBR) for wastewater treatment. Traditionally physical and chemical methods have been used for membrane cleaning, but have various drawbacks associated with their use, so nowadays the use of ultrasounds has become a promising technology for mitigating this biofouling. In this way, a pilot plant was built consisting on 3 submerged microfiltration membrane modules working in parallel; each module was coupled to a 400W ultrasonic generator which works at 40, 30 and 20 kHz respectively; another module worked without ultrasounds (CWU) [1]. The aim of this work was evaluate the influence of the ultrasonic frequency on the biofilm formation and the biodiversity of the bacterial community formed on membrane surface. Results showed that ultrasonic induce higher evenness in bacterial community, and the species richness decreased mainly al the lowest frequency (20kHz). In addition, the Morisita-Horn index showed that the dominant phylotypes changed strongly and progressively at higher ultrasounds frequency with respect to CWU, while the symmetric index indicated that rare phylotypes showed more similarity under increasing ultrasound frequency, noticeably. On the other hand, the application of ultrasounds resulted in the proliferation of *Gordonia* genus at 20, 30 and 40 kHz, while that *Acinetobacter* genus was dominant in the biofouling of membrane without ultrasound.

Bibliography

[1] Ruiz, L.M., Garralón, G., Pérez, J.I., and Gómez, M.A. (2015). Desalination Water Treat. 56, 3576–3589.

Adaptive wastewater microbiome evolution towards hydrocarbon and lipid enhanced bioremediation OC 07

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Key words: Bioremediation, microbiome, adaptive evolution, PAH, lipids.

Abstract

Wastewater and soil xenobiotics as polyaromatic hydrocarbons (PAH) end-up in landfill for slow bioremediation treatment. Given environmental risk and potential human health hazard, PAH were added to EU Priority Substance List [2]. Since EU regulations prohibit full-scale application of GMOs, evolutionary engineering of microbial strains stands as a natural strategy for improving bioremediation. This work aimed to study microbial community evolution and dynamics during wastewater microbiome adaptive laboratory evolution. Both culture-independent and -dependent approaches were used, to disclose consortium adaptation and isolate novel and enhanced strains for future bioremediation strategies.

Four different adaptive evolution experiments (AEx) were followed for over 100 consecutive cycles. An activated-sludge consortium from industrial wastewater was kept in a mineral media (M9) supplemented with a pollutant as sole carbon source (anthracene, phenanthrene, tristearin and mineral oil). Experiments monitoring included: total cell counts, isolation in selective media, polyphasic genomic PCR-fingerprinting of isolates, phylogenetic identification by 16S-rRNA gene sequencing for selected microorganisms, and microbial communities (bacteria and fungi) characterization by next-generation, NanoPore-based, sequencing approach. AEx with different pollutants allowed to retrieve 455 degradative isolates (30 yeasts and 425 bacteria), some of them with metabolic versatility, ubiquity and persistency, important characteristics for bioremediation applications. Microbial profiling using specific databases as MIDAS [3] for wastewater bacteria and RDP [1] for fungi, showed less diversity over time, in all conditions, suggesting consortia specialization. These experiments allowed to isolate novel strains, belonging to degradative genera, foreseeing improvement of recalcitrant xenobiotic bioremediation in bioremediation applications.

Bibliography

[1] Cole J. R., Wang Q., Fish J. A., Chai B., McGarrell D. M., Sun Y., Brown C. T., Porras-Alfaro A., Kuske C. R., and Tiedje J. M. (2014). Nucl. Acids Res. 42, D633-D642.
[2] European Parliament (2008). OJ L 348, 92.
[3] McIlroy S. J., Kirkegaard R. H., McIlroy B., Nierychlo M., Kristensen J. M., Karst S. M., Albertsen M., Nielsen P. H. (2017). Database, 1-9.

Proposal of a biotechnological alternative to the use of cassava wastewater generated in flour production in Southern Brazil

OC 08

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Key words: Cassava wastewater, environmental damage, elicitor potential, *Haematococcus pluvialis.*

Abstract

The wastewater generated with cassava roots processing for flour production is responsible for serious environmental impacts when disposed of into water bodies, posing serious risks to public health and aquatic communities. In order to minimize these impacts, this study aimed to assess the growth of the microalgae *Haematococcus pluvialis* cultivated with cassava wastewater into its culture medium and to quantify the microalgae astaxanthin biosynthesis. The evaluation of *H. pluvialis* growth *in vitro* was assessed with different wastewater concentrations (2.5%, 5% and 10%) within the culture medium for all three locations of collected samples (Imaruí, Jaguaruna and Paulo Lopes) in the State of Santa Catarina, Brazil. The elicitor potential of the wastewater in astaxanthin production was assessed by extracting and quantifying this carotenoid at the end of the experiment by HPLC. Cell density of *H. pluvialis* showed that the concentration of 2.5% of wastewater from the samples collected in Jaguaruna and Paulo Lopes obtained the best results in growth. Likewise, the astaxanthin content produced by adding 2.5% and 5% of wastewater from Jaguaruna and Paulo Lopes were statistically superior ($p < 0.05$) to the control treatment, which was performed without wastewater addition. These results demonstrate that cassava wastewater has the capacity to stimulate carotenoid astaxanthin accumulation in *H. pluvialis*. Therefore, it is possible to conclude that wastewater usage in *H. pluvialis* cultivation, aiming astaxanthin synthesis, is an important biotechnological alternative to support minimizing damages caused by this agroindustrial wastewater.

Wood pellets as sustainable organic subproducts to enhance denitrification in constructed wetlands treating oligotrophic nitrate-polluted leachates from plant nurseries

OC 09

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Key words: HSSF-CW, nitrates, bacteria, fungi, microbiota, water systems.

Abstract

Constructed wetlands (CW) have been suggested as a sustainable bio-based solution for the removal of organic matter and nutrients. Nonetheless, oligotrophic horticultural leachates need an extra carbon source to enhance the denitrification process. This study aims at gaining deeper insights into denitrification enhancement and microbial dynamics of native CW microbiota, by applying solid organic carbon sources for treating nitrate-rich leachates from plant nurseries.

Initial batch tests by using wood pellets revealed a significant enrichment in denitrifying bacteria ($5 \cdot 10^7$ *nosZ* gene copies g^{-1}) coupled with a 90% nitrate depletion (from initial 150-350 mg NO_3^- L^{-1}) in horticultural leachates after 4-10 days of incubation (0.117 mmol NO_3^- h^{-1} L^{-1}). Interestingly, a 75-99% of nitrate depletion of horticultural leachates (150-350 mg NO_3^- L^{-1}) was also achieved at full-scale HSSF-CW. The highest denitrification rate was observed in the wood pellet tank (82 mmol $N\text{-}NO_3^-$ m^{-2} h^{-1}). Microbial community assessment (wood, gravel and inflow leachates) revealed that the active denitrifying populations were more enriched on the wood pellets than on subsurface gravels. Beta diversity analysis revealed a specialized microbial community of bacteria and fungi on the wood pellets, being different from those attached onto the subsurface gravel and also from the leachates.

The utilization of wood pellets seems to be a sustainable alternative to enhance denitrification in oligotrophic water systems, such as horticultural leachates but also in polluted groundwater.

Surfing in the storm: From biochemical networks of bacteria to deal with pollutants to microbial dynamics during bioremediation processes

OC 10

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Key words: Bioremediation, hydrocarbon, microbial communities, bioaugmentation, biochemical networks.

Abstract

Exploring the microbial diversity in Chile have allowed us to isolate microorganisms for bioremediation in various contaminated terrestrial and marine ecosystems. Bioremediation is an efficient and low-cost treatment for the clean-up of polluted sites. The aims of this study were the analysis of the biochemical networks of bacteria to deal with pollutants and the characterization of the microbial dynamics during bioremediation. The biochemical networks of the model bacteria *Paraburkholderia xenovorans* LB400 and *Cupriavidus metallidurans* CH34 to cope with toxic compounds and heavy metals were elucidated. The influence of pollutants on stress response and biofilm formation by these strains were determined. Bioaugmentation and biostimulation studies of contaminated soils and water were conducted. *Pseudomonas*, *Acinetobacter* and *Rhodococcus* strains were used for bioaugmentation of polluted soils, whereas *Alcaligenes, Dietzia*, *Pseudoarthrobacter* and *Micrococcus* strains were applied for bioaugmentaton of polluted sea water. The microbial dynamics during bioremediation assays were characterized by metagenomic sequence analyses. *Proteobacteria* play an important role in the first weeks of bioremediation, whereas in a second phase an increase in *Actinobacteria* was observed. Relevant aspects in order to carry out efficient bioremediation processes in terrestrial and aquatic systems will be presented. The characterization of bacteria and the study of the role of microbial communities in bioremediation is crucial to improve the clean-up of polluted sites towards a more sustainable development.

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Simultaneous pretreatment and bioremediation with white-rot fungal isolates for improving saccharification of grape stalks

OC 11

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Key words: Biological pretreatment, white-rot fungi, grape stalks, enzymatic hydrolysis.

Abstract

Biological pretreatment of lignocellulosic material represents a low-cost and eco-friendly process. Under the biorefinery concept, delignification of biomass is a simultaneous pretreatment and bioremediation technology whose main objective is to facilitate hydrolysis of structural polysaccharides in order to obtain increased added value products [1].

In this work, grape stalks were pretreated through solid state fermentation (SSF) with six white-rot fungi (*Irpex lacteus*, *Ganoderma resinaceum*, *Bjerkandera adusta*, *Phlebia rufa*, *Trametes versicolor* and *Trametes* sp.) for 21 days. Fungal patterns of ligninolytic enzymes secreted, antioxidant activity (AA) and total polyphenolic compounds (TPP) at the end of pretreatment were investigated. All fungi produced manganese-dependent peroxidase, being the only enzymatic activity detected in *I. lacteus* incubations, and the highest activity expressed by *B. adusta* (≈1 U/mL). Untreated samples presented the highest values for AA and TPP. A positive and significant correlation ($r = 0.987$, $p < 0.05$) between DPPH$^{\bullet}$ scavenging capacity and TPP was observed.

The effect of SSF on saccharification yield increase was evaluated using an enzymatic cocktail. Principal component analysis showed that: *B. adusta*, *P. rufa* and *G. resinaceum* treatment exhibited higher correlation with delignification increase (high content in cellulose and low content in lignin); *I. lacteus* and *Trametes* sp. were correlated with high content in hemicellulose and *T. versicolor* with lower lignin removal. *P. rufa* showed the highest reducing sugars yield (nearly three times increase relatively to controls).

Overall, our results suggest that fungal pretreatment improves enzymatic saccharification by removal of lignin and substrate porosity increase as observed through scanning electron microscopy.

Acknowledgements

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Bibliography

[1] Dias, A.A., Fernandes, J.M.C., Sousa, R.M.O.F., Pinto, P.A., Amaral, C., Sampaio, A., Bezerra, R.M.F. (2018) Fungal Conversion and Valorization of Winery Wastes. In: Prasad R. (eds) Mycoremediation and Environmental Sustainability, 1st edition. Fungal Biology. Springer, Cham, Vol.2, 239-252.

Bioresources from contaminated matrices for the biocontrol of hydrogen sulfide emissions and the recovery of sulphur **OC 12**

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Key words: *Thioalkalivibrio sulfidiphilus* sp., sulfide oxidation, Dissimilatory Sulfite Reductase complex, Hetero Disulfide Reductase complex, Recycling of adsorbing alkaline scrubbers.

Abstract
A new halo-alkaline sulphur oxidising bacterial strain was isolated from brackish estuary sediments contaminated by total petroleum hydrocarbon. The isolate was classified as a new strain of Thioalkalivibrio sulfidiphilus sp., showing a higher capability of adaptation to pH and a higher optimal sodium concentration for growth, when compared to Thioalkalivibrio sulfidiphilus sp. HL-EbGr7, type strain of the species. The strain was capable to growth up to 1.5 M Na+ concentration and up to pH 10. The genome of the new isolate was sequenced and annotated. The comparison with the genome of Thioalkalivibrio sulfidiphilus sp. HL-EbGr7 showed a duplication of an operon encoding for a putative primary sodium extruding pump and the presence of a sodium/proton antiporter with optimal efficiency at halo-alkaline conditions. The new strain was able to oxidise sulphide at halo-alkaline condition at the rate of 1 mmol/mg-N/h, suitable for industrial applications dedicated to the recovery of alkaline scrubber for H2S emission captation and abatement, and the recovery of sulphur.

Full-scale bioremediation of soil and groundwater contaminated with heat transfer fluid   OC 13

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Key words: Microbial consortium, bioremediation, groundwater, heat transfer fluid, soil.

Abstract

Thermo-solar plants use a mixture of 26.5% biphenyl and 73.5% diphenyl ether as heat transfer fluid (HTF). Thermofluids transport energy accumulated on parabolic mirrors to a heat exchanger producing steam, which is finally transformed in electricity by a turbine. Accidental spills can lead to an accumulation of HTF and its degradation by-products in soil and ground water, resulting in environmental and health risks due to its high persistence and toxicity [1]. In this context, bioremediation appears as one of the most sustainable technologies for HTF removal.

KEPLER has recently performed a project in which 1,200 tons of contaminated soil (4.600 mg HTF/Kg) were treated forming dynamic biopilas inoculated with a previously adapted microbial consortium. The activity of microorganisms was stimulated by oxygen, nutrients and humidity supply. The results showed an HTF removal of 99 % after two months. In addition, when spillage occurs under high temperatures and / or rainy seasons, the mobility of HTF increases allowing soil penetration and reaching the aquifer. In this scenario, groundwater can be pumped to be recovered off-site. KEPLER is currently treating 2 m^3/d of groundwater contaminated with an average concentration of 25,000 µg/l. Treatment plant consists in a moving bed biofilm reactor, in which 90% of HTF is removed, followed by a filter unit where remaining product is adsorbed obtaining an effluent concentration under 10 µg/l. The removal ratios obtained on these projects demonstrate the effectiveness of bioremediation as a competitive and sustainable HTF removal technology at full-scale in both soil and groundwater.

Bibliography

[1] Kour, D., Rana, K.L., Kumar, R., Yadav, N., Rastegari, A.A., Yadav, A.N., Singh, K. (2019). New and Future Developments in Microbial Biotechnology and Bioengineering, Elsevier, 1, 1-23.

Integration between mycoaugmentation, bacterial metabarcoding and culturomics: designing bio-based approaches to the decontamination of total petroleum hydrocarbons contaminated soil

OC 14

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Key words: Mycoremediation, hydrocarbons, NGS, generalists, specialists.

Abstract

In Europe soils and sediments are mainly polluted (53%) by hydrocarbons contaminants as TPH (Total Petroleum Hydrocarbons), PAH (Polycyclic Aromatic Hydrocarbons) and BTEX (Benzene, Toluene, Ethylbenzene, Xylenes) [1]. Biological treatments are a reasonable solution to deplete pollutants and to restore the soil features. On the other hand, an optimization is mandatory to make them more economically sustainable. In this study, the optimization of a bio-based treatment was performed using mycoaugmentation approach. A mesocosm scale experiment was carried out using a TPH polluted soil from a dismissed refinery, in Italy. An Ascomycetes, fungal specimen selected for the versatility of the degrading metabolism and isolated from TPH contaminated sediments [2], was inoculated to decontaminate the soil at diverse density, in order to optimise the bioaugmentation protocol. A correlation between the density of the fungal inoculum and the kinetics of TPH degradation was recorded. Besides, the metagenomic analysis of the bacterial community during the process of degradation were performed. Those showed correlations between the kinetics of degradation and specific bacterial phyla and/or group of phyla. Several bacterial strains were isolated by different approaches designed for the recovery of generalists and specialist bacterial candidates, eventually involved in processes such as humification of the organic matter and degradation of the contaminants, respectively. The synergisms between the two bacterial functional groups and the eventually bioaugmented fungal candidate can be engineered to optimise the bio-based process of soil decontamination.

Bibliography

[1] European Environment Agency: http://www.eea.europa.eu, 2012

[2] Becarelli S., Chicca I., Siracusa G., La China S., Gentini A., Lorenzi R. & Di Gregorio S. (2019). Hydrocarbonoclastic Ascomycetes to enhance co-composting of total petroleum hydrocarbon (TPH) contaminated dredged sediments and lignocellulosic matrices. New biotechnol., 50, 27-36.

Bioremediation of oil spills in seawater using lyophilized native hydrocarbon-degrading bacteria

OC 15

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Key words: Oil spills, bioremediation, lyophilized bacteria, native microorganisms, bioaugmentation.

Abstract

Bioremediation with native microorganisms is an ecological and efficient technique to tackle oil spill incidents. A lyophilized product containing oil-degrading bacteria can be applied as a bioremediation tool. In this work, we aimed to study the hydrocarbon degrading performance of a consortium of 3 lyophilized oil-degrading bacterial strains, under simulated natural conditions. The experiment was conducted in flasks containing natural seawater and crude oil, under constant agitation at room temperature under three different experimental conditions: natural attenuation (NA) (seawater+oil); biostimulation (BS) (seawater+oil+nutrients); and bioaugmentation (BA) (seawater+oil+nutrients+lyophilized bacteria). Samples were collected from the flasks throughout the experiment to evaluate the abundance of oil-degrading bacteria by the most probable number (MPN) method, and to recover and identify degrading bacterial strains by cultivation in solid culture medium. After 15 days, the remaining cultures in the flasks were preserved at -20 ^{o}C for hydrocarbon degradation assessment. At this time, both BS and BA treatments showed an emulsion of the oil layer with the seawater, a feature not observed for the NA flasks. The abundance of oil-degrading bacteria increased along time, reaching values higher than 10^{11} MPN/mL for BA treatment after 7 days and for BS after 15 days. The lyophilized strains added to the BA treatment were found along the experimental period (as assessed by colonies morphology comparison). Results indicate that the bacterial strains remained viable after the lyophilization process, without losing their biodegradation potential, and were able to accelerate the natural oil degradation process.

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Bioaugmentation and biostimulation: Learnings from the mining industry OC 16

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Key words: Bioleach, biostimulation, heap Leach, bioremediation.

Abstract

Metallurgists have been using various species of bacteria, knowingly and unknowingly, to leach copper from sulphide ores for centuries and more recently to commercially leach a range of other metals as well as liberate precious metals from sulphide ores, mineral concentrates. These bacteria are primarily sourced from the local environment of the mine and can be readily adapted to produce strains to work more efficiently and commercially at higher temperatures, lower pH and build resistant to high concentrations of toxic metals such as arsenic, antimony and silver. The production of commercial bioleach operations has been achieved either using local naturally occurring bacteria or via bioaugmentation / propriety "designer" bacteria that have been sourced elsewhere and modified via harsh environment selective breeding or genetically to the requirements of the commercial operation. The history of such in commercial bioleaching is discussed with reference to biostimulation and the authors recommendations for efficient bioremediation.

A summary and discussion of the problems and regular failures with scale up from successful laboratory and pilot plant bioleach test work to commercial operations are presented. A discussion and practical advice on percolation and the techniques in construction as well as the binders and additives that result in good percolation and thus ideal consistent chemistry throughout the heap, resulting in much higher conversion rates, be it leaching or bioremediation is included in this paper. The state of the art in design and construction of biologically assisted heap leaching is summarised along with the multi-variable test work required for such and the authors recommendations on how these techniques and analysis of multi-dimensional test work results could best be applied to large scale bioremediation projects in order to scale up successfully and thus improve efficiency of degradation of contaminants as well as reduce the per tonne costs in large scale operations.

Bibliography

[1] John, L.W., 2011, The Art of Heap Leaching – The Fundamentals, Conference Proceedings; SAIMM Percolation Leaching: The status globally and in Southern Africa 2011.

[2] Miller, G., 2003, Ore geotechnical effects on copper heap leach kinetics, Hydrometallurgy 2003 – Fifth International Conference in Honour of Professor Ian Ritchie, Volume 1: Leaching and Solution Purification, Edited by C.A. Young et al., The Minerals, Metals & Materials Society, pp. 329-342.

Innovative strategy for the treatment of landfill leachate: Ascomycetes to deplete contamination and toxicity

OC 17

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Key words: Ascomycetes, leachate, reactor, removal, toxicity.

Abstract

The treatment of landfill leachates (LFLs) is object of study by many authors, mainly by the development of chemical-physical technologies, used to remove the inorganic and organic fraction of the matrices. The last mile of LFLs treatment is represented by the removal and/or reduction of their toxicity.

The most technically feasible solution for the disposal of LFLs is via municipal sewage treatment plants [1] or the dilution with urban wastewater. However, this approach leads to the managing of huge volumes of wastewaters in landfills and the movement of LFLs to external treatment centers.

This work has the aim to develop an innovative biotechnological strategy for the treatment of young, intermediate and old LFLs by myco-based approaches. Batch "hanging biomass" reactors were designed to test the efficacy of the myco-based treatment with Ascomycetes, evaluating the removal of Total Organic Carbon (TOC), changing in biodegradability both by BOD_5 and respirometric tests, and reduction of toxicity using the *Vicia faba* and Microtox assays.

The intermediate leachate treated on continuous batch-scale reactors operated under non-sterile conditions was used as sole carbon source. After 90 days, the Ascomycetes depleted the TOC by 92% and the phyto-, cyto-, and genotoxicity was reduced to a level comparable to the control (deionized water). Laccase activity was measured.

The described approach effected the most recalcitrant fraction of LFLs and depleted the toxicity, allowing a transfer on a pilot-scale reactor plant for the treatment or pre-treatment of landfill leachates with Ascomycetes fungi.

Bibliography

[1] Nghiem, L.D., Hai, F.I., Listowski, A. (2016) Water reclamation and nitrogen extraction from municipal solid waste landfill leachate, Desalin Water Treat 1–8.

Aerobic granular sludge bioreactor performance under different hydrodynamic regimens with or without silver nanoparticles regarding abatement of textile wastewater toxicity

OC 18

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Key words: Textile wastewater treatment, aerobic granular sludge, silver nanoparticles, toxicity, sequencing batch reactors.

Abstract

Aerobic granular sludge (AGS) sequencing batch reactor (SBR) technology was previously validated for the biodecolorization of azo dye laden textile wastewaters (TWW) [1]. The occurrence of silver nanoparticles (AgNPs), a textile finishing additive, in TWW may result in potential ecotoxic effects from both the AgNPs and the released Ag^+ ions [2]. To assess the environmental risks of partially treated TWW discharge, knowledge on toxicity changes during treatment is essential. This work aimed to study the influence of AgNPs on the long-term (113 days) operational performance of anaerobic-aerobic AGS SBRs run under two hydrodynamic regimens, with respect to the potential detoxification of a simulated TWW. Three AGS SBRs were fed with a simulated TWW containing Acid Red 14, with or without AgNPs (<100 nm particle size; 10 mg L-1); SBR 1 and SBR2 operated with fast peripheral feeding, with or without AgNPs respectively; SBR3 operated with slow central feeding, with AgNPs. All SBRs operated in 6-h cycles, including a stirred anaerobic reaction stage followed by an aerobic stage. Samples collected at the end of each of these stages were assessed for potential toxicity using yeast-based bioassays [1]. A microplate susceptibility assay measured inhibitory effects on yeast growth and gene expression assays measured changes in the transcript levels of GRE2 (general stress-induced gene) and RAD54 (specific response to DNA damage) [1]. Overall, only slight differences were found between the three SBRs concerning the evolution of potential toxicity along the treatment cycle.

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Bibliography

[1] N. D. Lourenço, R. D. G. Franca, M. A. Moreira, F. N. Gil, C. A. Viegas, H. M. Pinheiro (2015), Biochem Eng J 104, 57–63.
[2] A. Ivask, I. Kurvet, K. Kasemets, I. Blinova, V. Aruoja, S. Suppi, H. Vija, A. Käkinen, T. Titma, M. Heinlaan, M. Visnapuu, D. Koller, V. Kisand, A. Kahru (2014), PLoS One 9(7), e102108.

SHORT ORAL COMMUNICATIONS
(SOC 01 – SOC 09)

Biodegradation of paroxetine by autochthonous microorganisms in natural media

SCO 01

PC 08

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Key words: Autochthone degrading bacteria, bioremediation, pharmaceuticals.

Abstract

The presence of pharmaceuticals in the environment has been recognized as a growing problem in our society. Degradation of pollutants by native microorganisms is known to be an important detoxification process and thus, bioremediation technologies rise as a promising clean up solution with minimal environmental impact. Bioremediation involves the development of bacterial consortia containing natural degrading bacteria that can be selected through enrichment processes. The aim of this study was to evaluate, in microcosm experiments, the potential of native microorganisms, previously obtained through enrichment processes from an estuarine sediment [1], to biodegrade paroxetine in natural media, either alone or as a consortium.

To assemble the microcosms, water and sediment samples were collected in Douro River Estuary. Half of the microcosms was assembled with 50 mL of estuarine water, whereas the other half was assembled with 40 mL of estuarine water and 10 g of homogenized sediment. Microcosms were inoculated either with a bacterial consortium (10 different bacterial strains obtained from a previous experiment [1]) or with individual bacterial strains (*Pseudomonas* sp. or *Acinetobacter* sp.) selected by their biodegradation potential. The experiment was conducted during 2 weeks, in static and dark conditions. Samples were collected for analysis by HPLC-DAD to evaluate the removal of paroxetine, and for fluoride release analysis, using a fluoride ion-selective electrode, to evaluate the extent of paroxetine defluorination. At the end of the experiment, sediment samples were also collected for community characterization (NGS) and drugs analysis. Data is being analyzed and will be presented.

Acknowledgements

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Bibliography

[1] Duarte *et al*. (2019). *Sci Total Environ*. 655, 796-806.

Enrichment of bacterial consortia capable of biodegrading two persistent fluorinated fungicides

SCO 02
PC 10

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Key words: Biodegradation, defluorination, fungicides, microbial consortia, persistent organic pollutants.

Abstract

Epoxiconazole (EPO) and fludioxonil (FLU) are two fluorinated fungicides that are thriving in the agrochemical industry, characterized by their high recalcitrance, bioaccumulation potential and capacity for endocrine disruption. Both have been detected in the environment with half-life periods of over 1500 days for EPO [1] and 200-300 days for FLU [2]. Biodegradation can be an efficient process to mitigate the negative impacts of xenobiotics in the environment and may be key to reduce the environmental impact of EPO and FLU. Thus, this work aimed to study the potential of environmental bacterial communities to biodegrade EPO and FLU, as well as to identify the involved microorganisms and kinetics of degradation. Degrading bacterial consortia were obtained from an estuarine sediment and an agricultural soil, after an enrichment period of 6 months. Throughout this period, fungicides were supplemented individually to the cultures every 21 days at 5 mgL^{-1}, using sodium acetate as a co-substrate. Biodegradation of EPO and FLU was detected early on the enrichment phase and after ca. 6 months their complete removal and defluorination was observed in periods of 10-15 days in all cultures. Degradation kinetics of the enriched consortia were elucidated and revealed that EPO and FLU were efficiently biodegraded up to 10 mgL^{-1}, with estimated half-live values significantly lower than those reported in literature. 16S rDNA analysis revealed that these consortia harbour bacteria belonging to the Proteobacteria phylum. The optimization of each degrading consortia and the elucidation of the metabolic pathways of these pesticides are currently under study.

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Bibliography

[1] Bromilow R.H., Evans A.A., Nichols P.H., 1999, Pest Sci, 55, 1129-1134.

[2] Marrinozzi M., Coppola L., Monaci E., Karpouzas D.G., Papadopoulou E., Menkissoglu-Spiroudi U., Vischetti C., 2013, Environ Sci Pollut Res, 20, 2546-2555.

Mycoremediation of sulfonamides and tetracyclines from soils with edible fungi

SCO 03

PC 13

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Key words: Sulfamethoxazole, tetracycline, *Pleurotus*, *Agaricus*, soil.

Abstract

Sulfonamides (SAs) and tetracyclines (TC) are some of the most prescribed antibiotics for veterinary use. Only in the European Union, 928 and 2723 tons were respectively consumed in 2017 [1]. Antibiotics cannot be completely metabolized, so a high rate is discharged either to wastewater treatment plants or directly to natural waters or soils [2]. Their presence in the environment constitutes an important threat for global health due to the emergence of multi-resistant bacteria and antibiotics resistant genes (ARGs). The objective of this work is to assess SAs and TCs removal efficiency of two fungi, *Pleurotus ostreatus* and *Agaricus bisporus* growing on two kind of soils (clay and sandy loam). Those fungi have different enzymatic systems (CYP 450, laccase (Lac), Mn peroxidase (MnP)) capable to aerobically co-metabolize those antibiotics to less harmful compounds.

Both soils were spiked with 1 mg/kg of sulfametoxazol (SMX) and TC in glass containers, then two species of ligninolytic fungi (*P. ostreatus* and *A. bisporus*) were inoculated using wheat straw as a carrier. The assay took 6 weeks. SMX and TC were weekly analysed by UPLC-MS, as well as Lac and MnP activity, which were measured according to Garcia-Delgado et al. [3].

Both fungi removed TC at high rates (98%) from the two soils while fungal performance showed difference in SMX removal: 99% in clay by *P. ostreatus* and *A. bisporus* and in sandy loam 90% and 75% by *P. ostreatus* and *A. bisporus* respectively. Extracellular enzymes were poorly expressed although high removal rates were observed.

Bibliography

[1] European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption (2018). Sales of veterinary antimicrobial agents in 30 European countries in 2016, EMA/275982/2018.

[2] Martinez, J.L. (2009). Environ Pollut 157, 2893-2902.

[3] García-Delgado, C., Yunta, F., Eymar, E. (2015). J Hazard Mater 300, 281-288.

Water bio-decontamination with non-release biocidal coated filters

SCO 04

PC 28

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Key words: Biofouling, polymeric coating, tethered biocide, monolith, Multi-resistant *Staphylococcus aureus.*

Abstract
Water bio-contamination with biofouling, an undesirable natural process in which invasive aquatic organisms colonize submerged artificial surfaces, is a social and industrial global concern, particularly, in water supply and treatment circuits. It can lead to subsequent serious penalties, such as bio-corrosion and human infections, becoming a major public health concern. The most effective bio-decontamination strategies comprise the controlled release of toxic and cumulative bioactive agents into the water, which imply limited life-cycle, significant ecotoxicity, and promotion of human pathogens resistance. The aim of this study was to investigate the antimicrobial potential of a recently developed non-biocide-release coating strategy [1], namely against multi-resistant pathogens, to generate bioactive monolithic filters for water bio-decontamination.
Methods: The new non-release-biocide antifouling system comprises a prior Econea® biocide functionalization with a diisocyanate compound, in order to originate isocyanate reactive biocide derivatives (Econea-N=C=O) to be further tethered in polymeric frameworks, such as polydimethylsiloxane (PDMS) and polyurethane based coatings. Antimicrobial susceptibility of the isocyanate reactive agents and developed bioactive coated monolithic filters were evaluated against Gram-positive bacteria, namely Multi-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* (Ef). For the coated monoliths the antimicrobial potential was assessed by a time kill test, which included a study of biocide content effect in the different polymeric matrices.
These new antimicrobial monolithic filters evidenced auspicious antimicrobial and bacteriostatic effects against the tested microorganisms, especially for multi-resistant bacteria. Such promising results can be the key to further exploitation of bioactive eco-coatings for waterborne systems protection against biofouling.

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Bibliography
[1] Silva, E.R.; Ferreira, O.; Ramalho, P.A.; Azevedo, N.F.; Bayón, R.; Igartua, A.; Bordado, J.C.; Calhorda, M.J. (2019). Science of the Total Environment 650(2), 2499-2511.

Specialized degrading granules effective for biaugmentation of Aerobic Granular Sludge reactor treating 2-fluorophenol in wastewater

SCO 05
PC 29

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Key words: aerobic granular sludge, bioaugmentation, bioreactor, 2-fluorophenol.

Abstract

The amount of industrial chemicals being released into the environment has increased. Indigenous microbial communities in wastewater biotreatment processes are not always effective in removing xenobiotics. This work aimed to evaluate the feasibility and efficiency of a promising bioaugmentation strategy in an aerobic granular sludge (AGS) system continuously fed with 2-fluorophenol (2-FP). Bioreactor performance in terms of phosphate and ammonium removal and 2-FP degradation was evaluated.

Granules were produced using extracellular polymeric substances (EPS) extracted from AGS as a carrying matrix and a 2-FP degrading strain, *Rhodococcus* sp. FP1. Afterwards, the produced granules were introduced in the reactor. Shortly after addition, the produced granules broke down into smaller fragments inside the bioreactor, but 2-FP degradation occurred. After 8 days of bioaugmentation, 2-FP concentration inside the reactor started to decrease, and stoichiometric fluorine release was observed 35 days later. 14 Days after the bioaugmentation, phosphate and ammonium removal efficiency improved ca. 36% and 48%, respectively. However, complete phosphorous and ammonium removal was never achieved while the reactor was fed with 2-FP.

The persistency of *Rhodococcus* sp. FP1 in the reactor was followed by qPCR. *Rhodococcus* sp. FP1 was detected 1 day after in the AGS and up to 3 days after bioaugmentation at the effluent. Nevertheless, the degradative ability remained thereafter in the granules. Degrading strain could have persisted even if at lower numbers. Horizontal gene transfer could have happened from the 2-FP degrading strain to indigenous microbiome as some bacteria isolated from the AGS, 3 months after bioaugmentation, degraded 2-FP.

Acknowledgments

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project AGeNT - PTDC/BTA-BTA/31264/2017 (POCI-01-0145-FEDER-031264). We would also like to thank the scientific collaboration under the FCT project UID/Multi/50016/2019.

Efficient Rhizobacterial Consortium to provide bipartite benefit in supporting plant growth in presence of organophosphate pesticide stress

SCO 06

PC 53

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Key words: Rhizobacterial consortium, organophosphate pesticide, bioremediation, degradation enzymes, plant growth promotion (PGP).

Abstract

Modern Indian agricultural practices are shifting towards exploring safer bioinoculants as biological solutions supporting plant growth and protecting host crop and soil health from harmful residual pesticides effect [1]. To accesses the potential of rhizobacterial consortium in supporting host plant growth and protecting host plants when challenged with pesticides Monocrotophos and Dimethoate. Defined objective achieved by employing four native rhizobacterial isolates identified as: *Sphingobacterium* spp., *Brevundimonas* spp., *Pseudomonas* spp. and *Pseudomonas monteilii*, formulated as 15 consortia combinations (singlets, doublets, triplets and quadruplets) with respect to control after cohabitation test. Pesticide degradation properties were assessed by the activity of degradation enzymes: hydrolase, esterase and phosphatase. *In-vitro* plant bioassay (15 days) conducted on lab optimised system ASURE (manuscript under review) to estimate the beneficial impact of different consortia on plant growth promotion. Protective effect of consortia on the host upon pesticide challenge was studied through *in-vitro* plant bioassay after conclusion of a 30 day study. Singlet (T2, T6) and doublet (T3, T5, T8) combinations of *Sphingobacterium* spp., *Brevundimonas* spp. and *Pseudomonas monteilii* were observed to outstand on all grounds compared to triplet and quadruplet combinations. Consortia T3, T6, T8 and T9 showed best PGP properties whereas T2 and T6 showed relatively high degradation potential. Consortia T3, T6 and T5 were observed to protect host plant under 300 ppm of monocrotophos stress whereas T6, T5 and T2 showed better shielding effect to host plant under 50 ppm dimethoate stress. Thus, authors report combinations T6, T3, T5 and T2 as efficient bioinoculant providing bipartite benefits to the host plant and environment [2] [3].

Bibliography

[1] Pattanasupong A., Nagase H., Inoue M., Hirata K., Tani K., Nasu M., and Miyamoto K., (2004). World J Microbiol Biotechno, 20, 517–522.

[2] Abraham J. and Silambarasan S., (2013). Process Biochem, 48, 1559–1564.

[3] Ahemad M. and Khan M.S., (2011). Symbiosis, 54, 17–27.

Possibility of application of *Miscanthus x giganteus* in soil contaminated by the petroleum industry

SCO 07

PC 57

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Key words: *Miscanthus x giganteus*, phytotechnology, petroleum contaminated soil, biomass, degradation.

Abstract

Miscanthus x giganteus (Mxg), C4 perennial grass, is under investigation as one of the most suitable crops for biomass production due to high biomass yields combined with low inputs requirements and other environmental benefits [1]. It is able to grow in moderately heavy metals contaminated soils with slow phytostabilization of metals in root system. Few studies were published also about *Mxg* growing in PAH contaminated soils but generally potential for organic pollution phytoremediation with *Mxg* has not been fully studied yet [2].

Two pot experiments were prepared to investigate possibility of growing *Mxg* in mixtures of highly contaminated soil from petroleum sludge lagoon with uncontaminated soil. At first experiment starting concentrations of C10-C40 aliphatic hydrocarbons were 9 g/kg and 21 g/kg. Although the plants survived, biomass production was very low. That is why second series was prepared with more diluted soil (0 – 4.5 g/kg C10-C40). *Mxg* was again able to grow in all concentrations, but even in the lowest contamination (1.5 g/kg C10-C40) biomass was lower than control. C10-C40 decreased by 31-88% during season. The highest decrease was determined in the lowest C10-C40 concentration. In this concentration, degradation was more intensive in pots with *Mxg* compared to unplanted control while at higher concentrations it was comparable, so no additional phytoremediation effect was revealed.

To conclude, *Mxg* is able to survive even in highly contaminated soils with aliphatic hydrocarbons but the phytotechnology appears to be applicable only in lower concentrations where biomass production is less affected and improvement of degradation was determined.

Bibliography

[1] S. Arnoult, M. Brancourt-Hulmel (2015). BioEnergy Res, 8, 502–526.

[2] V. Pidlisnyuk, T. Stefanovska, E. E. Lewis, L. E. Erickson, L. C. Davis (2014). CRC. Crit. Rev. Plant Sci, 33, 1–19.

Genomic and physiological characterization of *Alcaligenes aquatilis* QD168 reveals a robust adaptive response to polluted marine environments

SCO 08
PC 61

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Key words: *Alcaligenes aquatilis*, abiotic stressor, benzene, salinity, functional genomics.

Abstract
Crude oil-polluted marine sediments are multi-stressor environments were the presence of hydrocarbons, as well as nutrient and salinity fluctuations challenge bacterial fitness. *Alcaligenes aquatilis* QD168 is a hydrocarbonoclastic marine bacterium isolated from crude oil-polluted marine sediment of the Quintero Bay, Valparaíso Region, Central Chile. A complete genome was obtained by PacBio sequencing, achieving a 4.32 Mb circular chromosome [1]. The aims of this study were to identify the genetic determinants involved in *A. aquatilis* QD168 survival to abiotic stressors and to characterize strain QD168 capability to overcome hydrocarbon pollution, nutrient scarcity and salinity fluctuation. Seven central pathways (*e.g.*, *cat* genes) and 16 peripheral pathways/reactions (e.g., *dmp* genes) for the degradation of aromatic compounds were identified in QD168 genome. Strain QD168 is able to grow on 14 aromatic compounds (*e.g.*, benzene, phenol, nicotinate, cinnamate) indicating the functionality of these pathways. QD168 benzene catabolic pathway was further studied by degradation assays and gene expression analysis. Phenol was identified as a metabolic intermediate. An induction by benzene of the transcripts encoding phenol hydroxylase and catechol 1,2-dioxygenase was observed. Genes encoding the short-chain length polyhydroxyalkanoates (scl-PHAs) biosynthetic pathway (*pha* genes) were identified. The synthesis of PHA by strain QD168 was determined. Genes encoding the osmoprotectant ectoine/5-hydroxyectoine (*ect* genes) are present in QD168 genome. Strain QD168 grew in R2A medium with up to 10% NaCl. The physiological adaptation of *A. aquatilis* QD168 to environmental stressors is useful for bioremediation of oil-polluted marine environments.

Bibliography
[1] Durán R. E., Barra-Sanhueza B., Salvà-Serra F., Méndez V., Jaén-Luchoro D., Moore E. R. M., Seeger M. (2019). *Microbiol Resour Announc*. 8:e01664-18.

Flavodoxin FldX1 of *Paraburkholderia xenovorans* LB400 enhances resistance to oxidative stress and improves growth on hydroxyphenylacetates

SCO 09

PC 72

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Key words: *Paraburkholderia xenovorans* LB400; flavodoxins; 3-HPA, 4-HPA.

Abstract

Paraburkholderia xenovorans LB400 is a model bacterium able to degrade 3- and 4-hydroxyphenylacetate (HPA) and other aromatic compounds. This aerobic catabolism of aromatic compounds produce oxidative stress, limiting their degradation [1]. Flavodoxins are small electron transfer proteins, which are induced during oxidative stress and iron starvation [2]. The overexpression of these proteins in bacteria enhanced the resistance to oxidative stress [3]. The aim of this study was to evaluate the protective effect of *P. xenovorans* LB400 flavodoxins during oxidative stress induced by 3- and 4-HPA degradation. Gene encoding flavodoxin FldX1 of *P. xenovorans* LB400 were cloned in a plasmid and overexpressed in this strain. Recombinant strain was exposed to paraquat, a redox-cycling aromatic herbicide. Bacterial sensitivity, survival and biomolecule damage were studied. The growth of *P. xenovorans* recombinant strain on 3- or 4-HPA as sole carbon source were analysed. The recombinant strain showed less growth inhibition than the control strain in 20 mM paraquat and higher survival after exposure to 1 and 20 mM paraquat. Strain p2-FldX1 displayed lower lipid peroxidation after incubation with 1 mM paraquat than control strain. Recombinant *P. xenovorans* strain exhibited faster growth in 3- and 4-HPA than control strain. In conclusion, the flavodoxin FldX1 of *Paraburkholderia xenovorans* LB400 confers protection to oxidative stress and enhances the growth on the aromatic compounds 3- and 4-HPA.

Bibliography

[1] Méndez, V., Agulló, L., González, M., Seeger, M (2011). PLoS ONE. 6, e17583.

[2] Sancho, J. (2006). Cell. Mol. Life Sci. 63, 855–864.

[3] Coba de la Peña, T., Redondo, F.J., Fillat, M.F., Lucas, M.M., Pueyo, J.J. (2013). J Appl Microbiol 115, 236-246.

POSTER COMMUNICATIONS

TOPIC 1 – Degradation of contaminants of emerging concern

(PC 01 – PC 24)

Wastewater disinfection: are Fe-Citrate driven advanced oxidation processes an efficient alternative?

PC 01

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Key words: Wastewater disinfection, *Enterococcus sp.,* peroxymonosulfate, Fe-Citrate like-photo-Fenton.

Abstract

Pathogenic microorganisms present in reclaimed water could have negative impacts on human health if they are not eliminated. Advanced oxidation processes (AOPs) have been proved to be an effective alternative to traditional treatments, as it combines disinfection and oxidation of contaminants. AOPs involve the application of chemical oxidants which do not usually show great disinfectant activity at low doses, but whose action increases when they are activated to produce free radicals [1].

One alternative for radical generation is to use transition metals as catalysts. In previous studies, Fe-citrate has demonstrated to be the most efficient iron specie among the studied in the activation of persulfate and peroxymonosulfate (PMS) for the elimination of *Enterococcus sp.* [2].

The main objective of this study is the evaluation of a hybrid Fe-citrate/UV-A radical generation method applied to several oxidants for the inactivation of wild *Enterococcus* sp. strains. This species can be used as indicator since it is pretty resistant and usually present in wastewater.

The optimization of some photo-assisted treatments has already been carried out using different doses of PMS [0.01 - 1 mM], hydrogen peroxide (H_2O_2) [0.01-1.5 mM] and Fe-citrate [0.2-5 mM]. Preliminary results reveal the increase of bacteria inactivation efficiency by photo-Fenton-like compared to H_2O_2/UV-A. However, high Fe-citrate concentrations might act as scavenger in radical generation since the best performance for H_2O_2 is achieved with a molar ratio 3:1 [H_2O_2/Fe-citrate]. PMS/Fe-citrate processes seem to be highly dependent on the radiation dose, so work is being done on the optimization of this variable.

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Bibliography

[1] Wang, Y.R., Chu, W. (2012). *Applied Catalysis B: Environmental 123-124*, 151-161.

[2] Rodríguez-Chueca, J., Guerra-Rodríguez, S., Raez, J.M., López-Muñoz, M.J., Rodríguez, E. (2019) *Applied Catalysis B: Environmental 248*, 54-61.

Transformation products of ibuprofen in an aqueous solution after a treatment with a microbial extract

PC 02

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Key words: Microbial consortium extract, ibuprofen metabolites, relative bacterial abundance.

Abstract
Ibuprofen (IBP) is a non-steroidal anti-inflammatory drug commonly detected in water resources and wastewaters [1, 2]. Different technologies have been studied for ibuprofen removal but these require large amounts of energy and generate secondary pollution. The objective of this study is to develop a new alternative of bioaugmentation to promote the biodegradation process in water bodies that does not require previous isolation and identification of specific pollutant degraders. We designed a biotreatment using a microbial extract from biomixtures of soil and organic wastes treated previously with a mixture of the pharmaceuticals IBP, diclofenac (DCF) and the biocide triclosan (TCS). The microbial consortium from the biomixture was extracted in aerated liquid solutions following the method described by Arancon et al. [3]. To study the microbial populations in the extracts from contaminated (with IBP, DCF and TCS) or non-contaminated biomixtures, the sequences of 16S rRNA gene amplicons were obtained by Illumina and analysed using QIIME. The removal of ibuprofen in solutions treated with different volumes of extracts containing up to 108 colony forming units mL-1 were incubated and analysed periodically by HPLC. Controls solutions with a sterile extract or obtained from non-contaminated biomixtures were incubated in parallel. Experimental data showed the ability of the extract obtained from contaminated biomixtures to increase the removal of ibuprofen from the liquid solution. Four transformation products identified by GC/MS analysis were also removed by the addition of the extract.

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Bibliography
[1] Delgado Moreno, L., Bazhari, S., Nogales, R., Romero, E. (2019). Sci. Total Environ. 651, 990-997.
[2] Marchlewicz, A., Guzik, U., Smułek, W., Wojcieszyńska, D. (2017). Molecules, 22.
[3] Arancon, N.Q., Edwards, C.A., Dick, R., Dick, L. (2007). BioCycle. 48, 51-52.

Two-stage anaerobic digestion process for the removal of pharmaceutically active compounds from sewage sludge: preliminary studies

PC 03

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Key words: Pharmaceutically active compounds, sewage sludge, two-stage anaerobic digestion, microbial diversity, qPCR.

Abstract

Urban wastewater is the main receiver of pharmaceutically active compounds (PhACs) after their consumption by human population. Some of these substances are efficiently removed by wastewater treatment but most of them are detected both in the treated effluent and in sewage sludge [1]. Two-stage anaerobic digestion (AD) process has been implemented in WWTPs worldwide for the stabilization of sewage sludge [2] although there is still very limited information about the removal efficiencies (REs) of PhACs during the process. The aims of this study were to evaluate the effectiveness of two-stage anaerobic digestion configuration working under different operational and environmental conditions in the removal of 27 PhACs (antibiotics, analgesics/antiinflamatory, β-blockers, antihypertensives/diuretics, lipid regulators and psychiatric drugs) from sewage sludge and to quantify the absolute abundances of total bacteria, archaea and fungi in acidogenic and methanogenic digesters. The sludge subjected to the two-stage AD process came from gravity thickening of primary and secondary sludge of a pilot-scale plant whose biological process consisted of an anaerobic/anoxic/aerobic (A^2O^{TM}) system [3]. Two different sets of hydraulic retention times (HRT) for acidogenic (A) and methanogenic (M) digesters were tested: 2 days (A) and 12 days (M) (phase I) and 5 days (A) and 24 days (M) (phase II), respectively. Ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS) was used for the identification and quantification of PhACs. Moreover, these results were complemented with real time PCR (qPCR) assays to estimate the absolute abundance of total bacteria, archaea and fungi within the sludge microbiota from acidogenic and methanogenic digesters. The absolute abundance of total bacteria and archaea was higher in methanogenic digesters regardless of the phase. Total fungal populations were only detected in phase I. The REs of the tested PhACs were higher in the methanogenic digesters and the operational conditions of phase II resulted in pronounced global REs of several PhACs: diclofenac, ibuprofen, furosemide, propranolol, fenofibrate and lorazepam.

Bibliography

[1] Radjenović et al. (2009). Water Res 43(3), 831-841.
[2] Maspolim et al. (2015). Chemosphere 140, 54-62.
[3] Gallardo-Altamirano et al. (2018). Sci Total Environ 643, 1481-1492.

Tube-in-tube membrane microreactor for heterogeneous TiO_2 photocatalysis with radial addition of persulfate: A Case Study on 17β estradiol and 17α-ethinylestradiol oxidation

PC 04

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Key words: Endocrine disrupting chemicals, nano-engineered membrane, photocatalysis, persulfate, radial permeation.

Abstract

Endocrine Disrupting Chemicals (EDCs) are a group of substances that can disturb the endocrine system in animals or humans, leading to adverse effects in the hormonal control or even cancer [1]. Urban wastewater treatment plants (WWTPs) are one of the main anthropogenic sources of EDCs due to inefficiency of conventional WWTPs to completely remove them. Advanced oxidation processes (AOPs) such as Fenton, photo-Fenton and TiO_2 photocatalysis have been tested for EDCs removal from different water matrices [2]. However, these processes normally use expensive chemicals and catalysts, some of them harmful, which need to be reclaimed. This work proposes a disruptive tube-in-tube membrane microreactor for the intensification of heterogeneous TiO_2 photocatalytic processes in the presence of persulfate. The reactor consists of a ceramic tubular membrane internally fed with persulfate and having on its shell-side surface the deposed catalyst. Polluted water, containing β-estradiol and α-ethinylestradiol, used as model EDCs, is continuously fed into the annular space between the outer quartz tube and the membrane shell side. The oxidant permeates through the membrane pores, being dosed and uniformly delivered to the active catalyst sites, reducing its consumption and improving its contact with the pollutants to be oxidized, also avoiding catalyst deactivation. The helical motion of water around the membrane shell side enhances persulfate radial dispersion, promoting efficiently its UVC photolytic cleavage into sulfate radicals. The unlimited number of persulfate dosing points across the membrane length allows a homogenous distribution of persulfate molecules in the catalyst surface (minimizing electron-hole recombination) and annular reaction zone, boosting the EDCs oxidation rate.

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Bibliography

[1] Becker, D., Rodriguez, S., Insa, S., Schoevaart, R., Barceló, D., Cazes, M., Belleville, M., Sanchez, J., Misovic, A., Oehlmann, J., Wagner, M. (2017). ORG PROCESS RES DEV 21, 480-491.
[2] Hua, M., He, H., Fu, G., Han, F. (2019). Environ. Eng. Sci. 36, 1-9.

Degradation of phenolic contaminants in aqueous mixtures using carbon nitride photocatalysts

PC 05

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Keywords: water treatment, advanced oxidation processes, phenolic compounds, hydrogen peroxide.

Abstract

Water treatment is crucial for a sustainable balance between society and the environment. The industry must treat its wastewaters adequately as they are particularly rich in potentially hazardous compounds, namely phenolic. Heterogeneous photocatalysis with carbon nitride has been proven to be efficient in the degradation of several organic pollutants [1, 2].

Apply carbon nitride photocatalysts in the degradation of different substituted phenolic compounds present in aqueous solutions by using visible light.

The photocatalysts were prepared by thermal decomposition of dicyandiamide as described elsewhere [3]. The photocatalytic experiments were conducted in a batch glass reactor using visible light emitting diodes (LEDs). The experimental conditions were optimised to enhance the degradation and mineralization rates, and improve the simultaneous *in situ* evolution of hydrogen peroxide (H_2O_2). The concentration of the selected compounds was determined by high-performance liquid chromatography, and the amount of produced H_2O_2 followed by a colorimetric method.

The carbon nitride photocatalysts were efficient to oxidise different probe molecules both individually and in simulated mixtures. The removal of the more recalcitrant compounds owns to the relatively high H_2O_2 production rates obtained in less than 180 min. The improvement of the mineralization process was sought with the addition of iron to mimic Fenton-like conditions.

Acknowledgements

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Bibliography

[1] Torres-Pinto, A, Sampaio, M.J., Silva, C.G., Faria, J.L., Silva, A.M.T. (2019) Appl Catal B-Environ 252, 128-137.

[2] Moreira, N.F.F., Sampaio, M.J., Ribeiro, A.R., Silva, C.G., Faria, J.L., Silva, A.M.T. (2019) Appl Catal B-Environ 248, 184-192.

[3] Lima, M.J., Silva, A.M.T., Silva, C.G., Faria, J.L. (2017) J Catal 353, 44-53.

Impact of titanium dioxide nanoparticles on the performance of an aerobic granular sludge-sequencing batch reactor system treating colored textile wastewater

PC 06

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Key words: Titanium dioxide nanoparticles, aerobic granular sludge, sequencing batch reactor, azo dyes, textile wastewater.

Abstract

The textile industry is a major source of water pollution worldwide, producing large wastewater volumes, where azo dyes represent a serious hazard [1], [2]. Lately, engineered nanoparticles, such as titanium dioxide nanoparticles (TiO_2NPs), have been used to improve textiles, while promoting antimicrobial activity [3]. As their presence in biological wastewater treatment plants is expected to increase, concerns are emerging regarding their effect [4]. The aim of this study was to assess the impact of TiO_2NPs on the treatment of azo dye containing textile wastewater, using an aerobic granular sludge sequencing batch reactor (AGS-SBR), considering sludge properties and treatment performance.

Two SBRs were inoculated with conventional activated sludge and subjected to granulation. They were run in 6-h cycles, including 1.5-h of anaerobic mixing and 3.5-h of aeration. Both SBRs were fed simulated textile wastewater containing azo dye Acid Red 14 (40 mg/L), SBR1 also being supplied with TiO_2NPs (5 mg/L), SBR2 being the control. The operation occurred during 336 days, being interrupted between days 62 and 133, biomass being stored at 4°C. The presence of TiO_2NPs initially affected the sludge settling ability in SBR1, reducing biomass accumulation. In the long term, exposure to TiO_2NPs apparently enhanced granule stability, improving biomass settling and accumulation. Color and organic matter degradation yields up to 85-90% were observed before and after storage, irrespective of TiO_2NPs. This efficient treatment in the presence of TiO_2NPs, with fresh and stored biomass, portrays AGS-SBR robustness.

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Bibliography

[1] A. Pandey, P. Singh, L. Iyengar (2007), Int Biodeterior Biodegrad 59(2), 73–84.
[2] R. G. Saratale, G. D. Saratale, J. S. Chang, S. P. Govindwar (2011), J Taiwan Inst Chem Eng 42(1), 138–157.
[3] R. Dastjerdi, M. Montazer (2010). Colloids Surf B: Biointerfaces 79(1), 5–18.
[4] Z. Li, X. Wang, B. Ma, S. Wang, D. Zheng, Z. She, L. Guo, Y. Zhao, Q. Xu, C. Jin (2017), Bioresour Technol 238, 361–368.

Permeable reactive barriers using cork granules for soil remediation containing polycyclic aromatic hydrocarbons

PC 07

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Key words: Soil remediation, PAHs, cork granules, permeable reactive barriers.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are substances with carcinogenic, mutagenic and teratogenic properties commonly found in soils near oil refineries, fuel stations, etc. [1]. Soil washing remediation method using adequate chemicals has been widely used to remove PAHs from soils [2]. This study aims to integrate soil washing with permeable reactive barrier (PRB) techniques, using cork granules as reactive barrier, for ex-*situ* remediation of soils contaminated with PAHs. In this case, the use of sodium dodecyl sulfate (SDS) solutions was

able to efficiently extract the PAHs (phenanthrene and fluorene) from a sandy soil. PAHs concentration was determined by HPLC after a preliminary liquid-liquid extraction with n-hexane for SDS separation. Virgin cork granules (VCG) and thermally treated cork granules at 380 °C with water vapor, called regranulated cork granules (RCG), were used in this work. Preliminary adsorption studies in batch and continuous mode, using a packed-bed column, employing initial PAHs contents below 5 mg/L revealed: (i) ~100% PAHs removal using the two types of cork granules, (ii) RCG presents a higher affinity for phenanthrene and fluorene than VCG, (iii) cork granules shows a higher affinity for phenanthrene than for fluorene, and (iv) SDS has a negative effect on the affinity of PAHs to cork granules. Soil remediation studies were performed in a packed-bed column filled with a bottom layer of contaminated soil and an upper layer of cork granules (PRB). A SDS solution was pumped through the column, washing the soil and the extracted solution containing SDS and PAHs flows through the cork barrier.

Acknowledgements

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Bibliography

[1] Lau, E.V., Gan, S., Ng, H. K., Poh, P.E. (2014). Environ Pollut 184, 640-649.
[2] Santos, E.V., Sáez, C., Cañizares, P., Silva, D.R., Martínez-Huitle, C.A., Rodrigo, M.A. (2017). Chem Eng J 310, 581-588.

Biodegradation of paroxetine by autochthonous microorganisms in natural media

PC 08
SOC 01

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Key words: Autochthone degrading bacteria, bioremediation, pharmaceuticals.

Abstract

The presence of pharmaceuticals in the environment has been recognized as a growing problem in our society. Degradation of pollutants by native microorganisms is known to be an important detoxification process and thus, bioremediation technologies rise as a promising clean up solution with minimal environmental impact. Bioremediation involves the development of bacterial consortia containing natural degrading bacteria that can be selected through enrichment processes. The aim of this study was to evaluate, in microcosm experiments, the potential of native microorganisms, previously obtained through enrichment processes from an estuarine sediment [1], to biodegrade paroxetine in natural media, either alone or as a consortium.

To assemble the microcosms, water and sediment samples were collected in Douro River Estuary. Half of the microcosms was assembled with 50 mL of estuarine water, whereas the other half was assembled with 40 mL of estuarine water and 10 g of homogenized sediment. Microcosms were inoculated either with a bacterial consortium (10 different bacterial strains obtained from a previous experiment [1]) or with individual bacterial strains (*Pseudomonas* sp. or *Acinetobacter* sp.) selected by their biodegradation potential. The experiment was conducted during 2 weeks, in static and dark conditions. Samples were collected for analysis by HPLC-DAD to evaluate the removal of paroxetine, and for fluoride release analysis, using a fluoride ion-selective electrode, to evaluate the extent of paroxetine defluorination. At the end of the experiment, sediment samples were also collected for community characterization (NGS) and drugs analysis. Data is being analyzed and will be presented.

Acknowledgements

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Bibliography

[1] Duarte *et al*. (2019). *Sci Total Environ*. 655, 796-806.

Biodegradation of the fluorinated pharmaceutical atorvastatin by different bacterial consortia **PC 09**

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Key words: Atorvastatin, biodegradation, fluorinated compounds.

Abstract

Atorvastatin (ATO) is a widely prescribed cholesterol-lowering agent, ranking among the most sold drugs in the world [1]. Due to its high use, it has been detected in natural waters and wastewater effluents [2], where it is expected to resist most degradation mechanisms due to the presence of a fluorine atom in its molecular structure [3]. This work aimed to investigate the biodegradation of ATO by bacterial consortia previously enriched with different halogenated compounds. Biodegradation of ATO (3 mgL^{-1}) was in co-metabolism with sodium acetate (500 mgL^{-1}). Microbial cultures were incubated for periods of 21 days, under static conditions, and during this period biodegradation of ATO was followed by weekly monitoring bacterial growth, through spectrophotometry, and the release of fluoride ion (a key indicator of ATO biodegradation) by potentiometry. Tenfold dilutions of the cultures showing complete defluorination of ATO were plated in solid culture media and the obtained isolates were identified through 16S rRNA gene sequence analysis. Preliminary results showed that all the tested consortia had the capability to biodegrade ATO to some extent, but only two consortia were able to completely defluorinate the molecule. Taxonomic identification of the bacterial strains isolated from these consortia revealed that most of the strains present in the original consortia may also be involved in the biodegradation of ATO.

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Bibliography

[1] Gupta, K.K. (2018). Cardiology and Therapy 8, 5-20.
[2] Wilkinson, J. (2017). Environmental Pollution 231, 954-970.
[3] Murphy, C.D. (2016). Applied microbiology and biotechnology 100, 2617-2627.

Enrichment of bacterial consortia capable of biodegrading two persistent fluorinated fungicides

PC 10
SOC 02

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Key words: Biodegradation, defluorination, fungicides, microbial consortia, persistent organic pollutants.

Abstract

Epoxiconazole (EPO) and fludioxonil (FLU) are two fluorinated fungicides that are thriving in the agrochemical industry, characterized by their high recalcitrance, bioaccumulation potential and capacity for endocrine disruption. Both have been detected in the environment with half-life periods of over 1500 days for EPO [1] and 200-300 days for FLU [2]. Biodegradation can be an efficient process to mitigate the negative impacts of xenobiotics in the environment and may be key to reduce the environmental impact of EPO and FLU. Thus, this work aimed to study the potential of environmental bacterial communities to biodegrade EPO and FLU, as well as to identify the involved microorganisms and kinetics of degradation. Degrading bacterial consortia were obtained from an estuarine sediment and an agricultural soil, after an enrichment period of 6 months. Throughout this period, fungicides were supplemented individually to the cultures every 21 days at 5 mgL^{-1}, using sodium acetate as a co-substrate. Biodegradation of EPO and FLU was detected early on the enrichment phase and after ca. 6 months their complete removal and defluorination was observed in periods of 10-15 days in all cultures. Degradation kinetics of the enriched consortia were elucidated and revealed that EPO and FLU were efficiently biodegraded up to 10 mgL^{-1}, with estimated half-live values significantly lower than those reported in literature. 16S rDNA analysis revealed that these consortia harbour bacteria belonging to the Proteobacteria phylum. The optimization of each degrading consortia and the elucidation of the metabolic pathways of these pesticides are currently under study.

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Bibliography

[1] Bromilow R.H., Evans A.A., Nichols P.H., 1999, Pest Sci, 55, 1129-1134.

[2] Marrinozzi M., Coppola L., Monaci E., Karpouzas D.G., Papadopoulou E., Menkissoglu-Spiroudi U., Vischetti C., 2013, Environ Sci Pollut Res, 20, 2546-2555.

Decolorization of textile dyes and effluents by a selected yeast and preliminary characterization of the product metabolites

PC 11

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Key words: Textile effluents, dyes, decolorization, metabolites.

Abstract

Textile industry produces large amounts of effluents that contain a complex mixture of synthetic dyes. These wastewaters can be harmful to the environment, especially if not properly degraded [1,2]. Thus, it is important to complement the traditional treatments, based on chemicals, with the use of alternative biological treatments to potentiate the decolorization of dyes in textile wastewaters. The biological degradation of the synthetic dyes present on the effluents may form various metabolites and their determination is important.

This work aims to assess the decolorization capacity of a selected yeast and characterize, and if possible identify, the metabolites that are formed in the process.

The selected yeast was isolated from textile wastewater and dyes were chosen according to industry representativeness. Simulated effluents with the selected dyes were also tested. Yeast was cultivated in Normal Decolorization Medium (NDM) without yeast extract, supplemented with 25 mg/L of dye or 2.5 g/L of simulated effluent incubated at 25 ºC /100 rpm for 48 h. The product of decolorization was then centrifuged for 15 minutes at 5000 rpm and the supernatant was used to characterize the metabolites by HPLC, according to a published method [3].

The selected yeast was able to decolorize both the dyes and effluents efficiently in the conditions tested, with reduction of the peak corresponding to the dyes and formation of metabolites detected by HPLC.

Bibliography

[1] Kristanti, R.A., Zubir, M.M.F.A., Hadibarata, T. (2016). Journal Environ Manage 172, 107-111.
[2] Raghukumar, C., D'Souza-Ticlo, D., Verma, A.K. (2008). Crit Rev Microbiol 34(3-4), 186-206.
[3] Lourenço, N.D., Franca, R.D.G., Moreira, M.A., Gil, F.N., Viegas, C.A., Pinheiro H.M. (2015). Biochem Eng J 104, 57-63.

Biodegradation of Bisphenol A and Bisphenol S by *Labrys portucalensis* strain F11 PC 12

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Key words: Biodegradation, bisphenol A (BPA), bisphenol S (BPS), *Labrys portucalensis* F11, metabolites.

Abstract

Growing concern about the possible human health effects of Bisphenol A (BPA), particularly as an endocrine disruptor, has led to recent attempts to remove it from some consumer products, often labelled as "BPA-free". However, some of the common replacers are also bisphenols (BPs), such as Bisphenol S (BPS) which may have similar physiological effects in organisms [1]. Industrial and municipal wastewaters are ecosystems particularly exposed to BPs pollution and are common sources of BPs contamination. From the different techniques to remove BPs, biodegradation seems to be the more effective [2].

In this study, biodegradation of BPA and BPS was assessed using the bacterial strain *Labrys portucalensis* F11 in mineral medium (MM). Complete degradation of both compounds supplied at 2.0 mg L^{-1} was reached in independent assays by this bacterium, via co-metabolism, over a 15 days period. BPA and BPS biodegradation by strain F11 was evaluated in a real matrix of municipal wastewater and preliminary results showed a complete degradation of the supplied amount of BPs (2.0 mg L^{-1}) in 17 days. Toxicity assays are being carried out on whole samples of biodegradation experiments. Identification of degradation metabolites is ongoing to better understand the degradation pathway BPA and BPS by *L. portucalensis* F11.

To the best of our knowledge, this is the first report that points to complete degradation of BPS by a single bacterial strain isolated from the environment.

Acknowledgements

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Bibliography

[1] Rochester J.R., Bolden A.L. (2015). *Environ Health Perspect* 123, 643-650.

[2] Noszczynska M., Piotrowska-Seget Z. (2018). *Chemosphere* 201, 214-223.

Mycoremediation of sulfonamides and tetracyclines from soils with edible fungi

PC 13
SOC 03

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Key words: Sulfamethoxazole, tetracycline, *Pleurotus*, *Agaricus*, soil.

Abstract

Sulfonamides (SAs) and tetracyclines (TC) are some of the most prescribed antibiotics for veterinary use. Only in the European Union, 928 and 2723 tons were respectively consumed in 2017 [1]. Antibiotics cannot be completely metabolized, so a high rate is discharged either to wastewater treatment plants or directly to natural waters or soils [2]. Their presence in the environment constitutes an important threat for global health due to the emergence of multi-resistant bacteria and antibiotics resistant genes (ARGs). The objective of this work is to assess SAs and TCs removal efficiency of two fungi, *Pleurotus ostreatus* and *Agaricus bisporus* growing on two kind of soils (clay and sandy loam). Those fungi have different enzymatic systems (CYP 450, laccase (Lac), Mn peroxidase (MnP)) capable to aerobically co-metabolize those antibiotics to less harmful compounds.

Both soils were spiked with 1 mg/kg of sulfametoxazol (SMX) and TC in glass containers, then two species of ligninolytic fungi (*P. ostreatus* and *A. bisporus*) were inoculated using wheat straw as a carrier. The assay took 6 weeks. SMX and TC were weekly analysed by UPLC-MS, as well as Lac and MnP activity, which were measured according to Garcia-Delgado et al. [3].

Both fungi removed TC at high rates (98%) from the two soils while fungal performance showed difference in SMX removal: 99% in clay by *P. ostreatus* and *A. bisporus* and in sandy loam 90% and 75% by *P. ostreatus* and *A. bisporus* respectively. Extracellular enzymes were poorly expressed although high removal rates were observed.

Bibliography

[1] European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption (2018). Sales of veterinary antimicrobial agents in 30 European countries in 2016, EMA/275982/2018.

[2] Martinez, J.L. (2009). Environ Pollut 157, 2893-2902.

[3] García-Delgado, C., Yunta, F., Eymar, E. (2015). J Hazard Mater 300, 281-288.

Removal of organic priority substances and contaminants of emerging concern in aquaculture effluents using constructed wetlands

PC 14

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Key words: Micropollutants, priority substances, contaminants of emerging concern, constructed wetlands, bioremediation.

Abstract

The presence of organic micropollutants (MPs) in the aquatic systems (usually between ng L^{-1} and µg L^{-1}) and their potential effects to wildlife and human health are worldwide issues of increasing concern. In general, these compounds are very difficult to be eliminated by conventional water/wastewater treatment processes, being continuously released into watercourses. Presently, there is no regulation regarding the MPs discharge, however Directive 2013/39/EU and Decision 2018/840/EU indicate respectively, 41 organic priority organic substances (PSs) and 17 contaminants of emerging concern (CECs) which must be monitored in surface waters within the EU [1]. Alternative technologies to eliminate this type of contaminants from water/wastewater are still limited, even more for aquaculture effluents [2]. Constructed wetlands (CWs) are complex systems including water, a substrate, native microorganisms and usually plants. The interaction among these components and the contaminants (dependent upon several biotic and abiotic factors) promotes several processes, namely the bioremediation, which are responsible for the elimination of different pollutants, including PSs and CECs [1]. CWs have exceptional advantages as low-cost, simple operation/maintenance and eco-friendless; but there is still a lot to investigate concerning their application for MPs removal [1,2]. In this sense, this work intends to evaluate the potential of these biological systems to remove PSs and CECs from aquaculture effluents.

Bench-scale experiments were performed using triplicates of planted vertical subsurface flow CWs microcosms (VSSF-CWs). Each microcosm was supplemented with 2 L of aquaculture effluents spiked with 100 ng L^{-1} of each target MP and was operated during four weeks with weekly new feedings, also evaluating the CWs performance along time. Removal efficiencies of CWs were weekly estimated by the difference of MPs concentrations in the CWs inlet and outlet, which were determined by SPE-UHPLC-MS/MS. The obtained results revealed excellent removal rates (varying between 85 and 100%) of the target contaminants. Further investigation is needed to better understand the bioremediation processes involved and to optimize the CW systems for the removal of the most recalcitrant MPs.

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Bibliography

[1] Gorito, A.M., Ribeiro, A.R., Almeida, C.M.R., Silva, A.M.T. (2017). Environ Pollut 227, 428-443.
[2] Gorito, A.M., Ribeiro, A.R., Gomes, C.R., Almeida, C.M.R., Silva, A.M.T. (2018). Sci Total Environ 644, 1171-1180.

Degradation of expanded polystyrene by marine fungi, *Zalerion maritimum* and *Nia vibrissa* PC 15

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Key words: Biodegradation, expanded polystyrene, *Zalerion maritimum*, microplastics.

Abstract
Plastic production has drastically increased over the last few years, due to the high demand of plastic products. Plastic pollution, as consequence has also increased, and despite the efforts made in waste management, sustainable and efficient solutions are still needed for dealing adequately with this environmental threat [1]. Expanded polystyrene (EPS) is one of the most demanded plastic commodities nowadays, due to its versatility, durability and lightness, among other attractive properties. Unfortunately, these desirable properties make them also an environmental threat, as they are easily transported by air and likely to fragment into micro and nanoplastics. In the search of new solutions for the EPS management, a biotechnology-based solution was evaluated: *Zalerion maritimum* and *Nia vibrissa* were screened for their potential in the biodegradation of EPS. The evaluation was performed with 28-day assays, where mass differences were quantified in both the fungi and the microplastic. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was also used for assessing the degradation of EPS. In order to improve the removal of EPS, an optimization procedure was also conducted to obtain the optimum values for EPS concentration, microplastic dimension and malt extract concentration. These were, respectively, 0.1458 g/L, 1-1.40 mm, and 20 g/L. The percentages of removal were variable, but with *Z. maritimum* was possible to obtain percentages between 40% and 80%, and with *N. vibrissa*, percentages between 30% and 50% were achieved.

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Bibliography
[1] Avio, C.G., Gorbi, S., Regoli, F. (2017). Mar Environ Res 128, 2-11.

Optimization of microplastics removal by *Zalerion maritimum* PC 16

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Key words: *Zalerion maritimum*, biodegradation, microplastics, polyethylene, optimization.

Abstract
The presence of microplastics in the environment is one of the major threats to the marine environment due to their indiscriminate use and lack of policies for their management [1]. This leads to a demand for new and sustainable solutions for their removal, and biodegradation has been suggested as a promising one.
Different microorganisms have already been studied for the biodegradation of polyethylene (PE) microplastics, and *Zalerion maritimum* is an example of a marine fungus that has shown results with a great potential [2].
This study aims at developing a bioremediation process for the microplastics in the marine environment, using an optimization procedure in order to improve the use of *Z. maritimum*. Two experimental designs, Uniform Design and Central Composite Design, were applied to find the optimal concentration of the three medium components, to improve the percentages of removal.
This work showed that malt extract was the main regulatory factor for the removal of microplastics, and that the medium also needs to be supplemented with glucose and peptone. The optimal concentrations found with both experimental designs allowed to achieve 90% of removal.

Acknowledgements
Thanks are due for the financial support to CESAM (UID/AMB/50017/2019) to FCT/MCTES through national funds, as well as to project IF/00407/2013/CP1162/CT0023, supported by national funds through FCT/MEC (PIDDAC) and to the University of Aveiro and FCT/MCTES for the financial support for the QOPNA research Unit (FCT UID/QUI/00062/2019) through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. This work is also funded by national funds (OE), through FCT in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19th.

Bibliography
[1] Avio, C.G., Gorbi, S., Regoli, F. (2017). Mar Environ Res 128, 2-11.
[2] Paço, A., Duarte, K., da Costa, J.P., Santos, P.S.M., Pereira, R., Pereira, M.E., Freitas, A.C., Duarte, A.C., Rocha-Santos, T.A.P. (2017). Sci Total Environ 586, 10-15.

Tetracyclines and Sulfonamides presence in horticultural crops PC 17

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Key words: Tomato, pepper, carrot, lettuce, antibiotics.

Abstract

Antibiotics reach the crops due to wastewater irrigation or manure application. Several assays had proved that plants absorbed and translocated them to the edible parts [1]. Although they do not exceed the human exposure doses, the problem of consuming these vegetables is the proliferation of antibiotic resistance bacteria (ARB) and the effect on allergic people [2].

During this work, several crops with different edible parts were grown in presence of sulfonamides and tetracyclines to follow their presence in the plant. Crops of lettuce, pepper and carrot were hydroponically grown. Afterwards, they were spiked with sulfamethoxazole (SMX) and tetracycline (TC) with concentrations between 0.1 mg/L and 15 mg/L. In addition, tomatoes of two commercial greenhouses were sampled and analyzed. To quantify the sulfonamides and tetracyclines, it was necessary an extraction with trichloroethylene (TCE) before their analysis by UPLC-MS/MS.

The results of the hydroponic assays showed that antibiotics reached the aerial part of plants and fruits. The translocation factors were influenced by the kind of crop and exposure time. In the commercial tomatoes, several antibiotics were detected, standing out TC with more than 5 µg/Kg and chlortetracycline with concentrations between 0.5 and 1 µg/Kg. Other antibiotics were detected at ng/Kg level such as SMX with 50-100 ng/Kg and oxytetracycline with 50-75 ng/Kg.

Bibliography

[1] Krzeminski, P., Tomei, M.C., Karaolia, P., Langenhoff, A., Almeida, C.M.R., Felis, E., Gritten, F., Andersen, H.R., Fernandes, T., Manaia, C.M., Rizzo, L., Fatta-Kassinos, D. (2019). Sci Total Environ 648, 1052-1081.
[2] Pan, M., Chu, L.M. (2017). Environ Pollut 231, 829-836.

Toxicity of decolourisation products of synthetic textile effluents by a selected yeast

PC 18

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Key words: Textile effluents, dye decolourisation, yeasts, toxicity.

Abstract
The textile sector is a worldwide industry that produces high volumes of harmful effluents that are discharged in the environment. They are resistant to biodegradation and potentially damaging to the ecosystems [1]. Classic treatment methods are not very effective and biological methods are generally considered more environmentally friendly and of major relevance [2, 3]. Nonetheless, due to variability of biological processes, it is important to access the toxicity of the decolourisation products.
The aim of this work was to access the ability of a selected yeast strain to decolourise specific synthetic textile effluents, with a minimum culture medium, and to assess the toxicity of the resulting decolourisation products.
Yeast LIIIS36B was previously selected and the synthetic textile effluents used were representative of major classes of dyes. Yeast was cultivated in a minimum culture medium containing glucose (20 g/L) supplemented with 2.5 g/L of each synthetic textile effluent selected and incubated at 25 ^{o}C/100 rpm during 48 h. After decolourisation, cultures were centrifuged at 5000 rpm for 15 minutes and the supernatants were tested for its toxicity. Toxicity assays are being performed using organisms of different trophic levels.
Decolourisation of the synthetic effluents tested was efficient only with a minimum culture medium. Different trophic levels seem to show different results for toxicity. Different classes of dye effluents showed similar results.

Bibliography
[1] Dellamatrice *et al*. (2017). Braz J Microbiol 48, 25-31.
[2] Mahmoud, M.S. (2016). HBRC Journal 12(1), 88-98.
[3] Ali, H. (2010). Water Air Soil Poll 213(1-4), 251-273.

Tertiary treatment of mature urban leachates by ozone: Coupling reactors and combining processes **PC 19**

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Key words: FluHelik photoreactor, bubble column reactor, ozone-based AOPs, biodegradability.

Abstract

This study investigated the application of ozonation processes (O_3-only and combined with advanced oxidation processes (O_3-based AOPs)) on the tertiary treatment of a mature leachate, focusing on: (i) degradation of recalcitrant organic matter; (ii) efficiency in ozone consumption; and (iii) increase of biodegradability. The experiments were performed using a pre-treated leachate (biologically nitrified and coagulated, according to [1]), with dissolved organic carbon (DOC) = 404 mg/L and chemical oxygen demand (COD) = 1082 mg/L. All experiments were run in semi-batch mode, during 3h, with a constant $[O_3]_{inlet}$ = 18 mg/min and periodic sampling for analytical characterization (pH, dissolved ozone, DOC, COD and UV-Vis absorbance spectra). First, for O_3-only tests different system setups were evaluated: (a) bubble column reactor, with a porous diffuser (BC-alone); and (b) FluHelik photoreactor coupled in series with the BC, using a porous diffuser (FH/BC-D) or a Venturi injector (FH/BC-V). The system FH/BC-V presented the best treatment performance increasing the volume of treated leachate by 50%, when compared to BC-alone, while maintaining similar DOC and COD removal efficiencies. Afterwards, with FH/BC-V system, the O_3-based AOPs tests (O_3/H_2O_2, O_3/UV_C and $O_3/H_2O_2/UV_C$) showed the synergistic effect of combining multiple processes. The best performance was obtained combining O_3/UV_C, with pseudo-first-order kinetic constants (k, min^{-1}) for DOC and COD removal increasing 2.0 and 1.4-fold, respectively, when compared to the O_3-only treatment. Finally, the biodegradability of the leachate after each treatment was assessed by means of Zhan-Wellens test, decreasing as follows: O_3/UV_C (91%) > $O_3/H_2O_2/UV_C$ (69%) > O_3/H_2O_2 (59%) ≈ O_3-alone (58%).

Acknowledgements

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Bibliography

[1] Silva, T.F.C.V., Soares, P.A., Manenti, D.R., Fonseca, A., Saraiva, I., Boaventura, R.A.R., Vilar, V.J.P. (2017). Sci Total Environ 576, 99-117.

Sulfamethoxazole and trimethoprim degradation mediated by the photodynamic action of a cationic porphyrin

PC 20

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Key words: Antibiotics, pollutants, photodegradation, photodynamic action.

Abstract

The presence of chemical contaminants in the effluents is notorious and cannot be neglected. Effluents from hospital as well as domestic and industrial origin show the presence of human consumption chemicals, mainly personal care products, pharmaceuticals, organic solvents, etc. [1,2]. Once in the wastewater treatment plant (WWTP), the effluents are treated according to the legislation of each country/region. However, the current legislation has shown not to be effective enough concerning the growing presence of chemical pollutants in the natural water reservoirs such as rivers, estuarine environments and even in the oceans. Consequently, new water treatment approaches for water decontamination must be considered, tested and implemented.

We established an experimental procedure in order to study the effects of 5,10,15,20-*tetrakis* (1-methylpyridinium-4-yl) porphyrin tetra-iodide (Tetra-Py$^+$-Me) in the photodegradation of two well-known antibiotics: trimethoprim and sulfamethoxazole. The assays were conducted under two distinct light sources with different electromagnetics spectrum composition and intensity – artificial white light and natural solar irradiation. The photodynamic process occurred in the presence of the photocatalyst (Tetra-Py$^+$-Me), the light source and molecular oxygen and it was mediated by the photogeneration of reactive oxygen species [3], which induced the oxidation of the chemical contaminants.

The main resultant products of the antibiotics photodegradation, in homogenous aqueous matrices, were identified by HPLC-MS.

Bibliography

[1] Almeida, J. et al. (2014). Photochem Photobiol Sci 13(4), 626-33.
[2] Bartolomeu, M. et al. (2018). Photochem Photobiol Sci 17, 1573-1598.
[3] Wainwright, M. et al. (2016). Lancet Infect Dis 3099, 1-7.

Removal and transformation of sulfamethoxazole in biofilters packed with manganese-oxide filtering medium PC 21

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Key words: Sulfamethoxazole, biofilter, ecotoxicity, manganese oxides, transformation products.

Abstract

The goal of this study was assess the removal of sulfamethoxazole (SMX) in biofilters supported by the manganese oxide filtering medium under various operational conditions.

The experimental system consisted of eight types of lab-scale biofilters differed based on: the use/lack of aeration, the type of bed medium (sand or sand mixed with commercial manganese oxides material), the presence/lack of readily available organic carbon source (acetate) in the influent. The sand used in the experiment was taken from biofilters exposed to the presence of SMX for more than 1 year. The influent contained, in all cases, 5 mg/L of SMX.

The removal of SMX was complete with the exception of the anoxic columns fed with the influent containing acetate (regardless of the type of bed medium). The apparent slower rate of the SMX degradation in the anoxic columns prevented complete removal of SMX. Overall, the obtained results suggest that bacteria were able to degrade SMX catabolically and the degradation of this compound was subsequent to the depletion of available acetate (if applicable).

The LC-MS/MS analysis of the effluents indicated eight transformation products of SMX. The transformation reactions relevant for the degradation of SMX in the biofilters are: S-N bond cleavage, isoxazole-ring cleavage, nitrosation, deamonification, hydroxylation and dihydroxylation, and acetylation. The acetylation reaction occurred only in the aerated columns.

Based on the Microtox assay, only the effluents from the anoxic columns filled manganese oxides material were classified as non-toxic.

Degradation of iohexol using biological and photochemical processes **PC 22**

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Key words: Pharmaceutical products, wastewater treatment, photocatalysis.

Abstract

Iohexol (IOX) is one of the commonly used iodinated contrast media (ICM), which are detected in aquatic environment. The concentration of ICM in municipal wastewater are at the levels of ng/L and µg/L, in hospital wastewater they can reach tens of µg/L or even mg/L [1]. Iohexol is derivative of 2,4,6-triiodobenzoic acid and is used in medicine for radiological visualization of the human tissues. It is known that ICM are not metabolized in human body. After intake they are excreted in unchanged form. Subsequently, ICM appear in wastewater treatment plants, later can be also detected in the environmental waters with unknown influence on ecosystem.

The aim of the work was to assess elimination of IOX in biological (e.g. membrane bioreactors (MBR)) and physicochemical (e.g. solar photocatalysis) treatment technologies. In order to improve the photocatalytic activity of TiO_2, its surface modification was made by graphene oxide [2]. Such modification should significantly increase the elimination of organic compounds due to: better sorption properties, higher efficiency not only in the UV range, but also in the visible light and higher selectivity of the decomposition process [3].

The results of elimination of IOX using biological treatments processes were not very promising, but showed that the anaerobic MBR was more effective than aerobic MBR. The efficiency of photocatalysis processes was significantly higher than biological processes and, moreover, advantage of IOX elimination using TiO_2/GO in comparison to TiO_2 was noticed.

Bibliography

[1] Verlicchi, P., Galletti, A., Petrovic, M., Barceló, D. (2010). J Hydrol 389, 416-428.

[2] Faraldos, M., Bahamonde, A. (2017). Catal Today 285, 13-28.

[3] Nguyen-Phan, T.-D., Pham, V.H., Shin, E.W., Pham, H.-D., Kim, S., Chung, J.S., Kim, E. J., Hur, S.H. (2011). Chem Eng J 170, 226-232.

Degradation of imidacloprid by means of solar driven processes PC 23

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Key words: Aquatic environment, degradation, imidacloprid, solar photolysis; photocatalysis.

Abstract
Imidacloprid belongs to the group of neonicotinoids - popular pesticides that are detected in various compartments of the environment. These substances are mentioned to be suspected of causing the *Colony Collapse Disorder* (CCD). Due to its properties and potentially harmful effects, imidacloprid was included in the first EU "Watch List", and after its revision, it was included in the second EU "Watch List". The monitoring data showed that this substance was present in almost 18% from 2385 surface water samples taken from 24 European Union countries [1]. The aim of the research was to assess the decomposition efficiency of imidacroprid the various solar-light-driven processes, i.e. photolysis and photocatalysis using various photocatalysts as TiO_2, ZnO and SnO_2. The investigation was performed at various solar irradiance range, i.e. 250 W/m^2, 500 W/m^2 and 1000 W/m^2. The results of the research allowed determining the kinetic parameters of the above-mentioned photochemical reactions. The research showed that: i) imidacloprid was only partially susceptible to degradation in the solar photolysis - its decomposition after 90 min of the process varied from 12% (irradiance 250 W/m^2) to 30% (irradiance 1000 W/m^2); ii) the addition of photocatalysts, i.e. TiO_2, ZnO and SnO_2, at a dose of 100 mg/L caused that removal of imidacloprid after 90 min of the process was equal to 51.8%, 46.3% and 47.1%, respectively; iii) increasing the ZnO dose to 300 mg/L and 500 mg/L caused that after 90 min of the process, the removal of imidacloprid increased to 60.7% and 63.1%, respectively.

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Bibliography
[1] Loos R., Marinov D., Sanseverino I., Napierska D., Lettieri T. (2018): Review of the 1stWatch List under the Water Framework Directive and recommendations for the 2ndWatch List, EUR 29173 EN, Publications Office of the European Union, Luxembourg, 2018, ISBN 978-92-79-81839-4, doi:10.2760/614367, JRC111198.

Toxicity evaluation of wastewater treated by constructed wetlands PC 24

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Key words: Pharmaceuticals, constructed wetlands, biotests, ecotoxicity.

Abstract

Pharmaceuticals and personal hygiene products (PPCPs) are detected in the natural environment and pose a serious threat to organisms. The main source of environmental pollution by PPCPs are landfills, septic tanks and treatment plants that do not eliminate all micro-contaminants present in the wastewater. For these reasons, new, effective and natural and at the same time economical methods of removing impurities are sought. One of such solutions are constructed wetlands, built to mimic the natural processes occurring in swamp systems.

The aim of the study was to evaluate the detoxification process of wastewater from a constructed wetlands removing model pharmaceutical substances such as diclofenac (DKF) and sulfamethoxazole (SMX), as well as to assess the ecotoxicological properties of these compounds and their equilibrium mixture.

The obtained results indicate that only partial wastewater detoxification was possible in the proposed laboratory model of the hydrophyte treatment plant. The toxicity of the leachate was lower than that of raw sewage, but the risk associated with their presence in the natural environment remained high. The frequency of wastewater dosing and the presence of *Miscanthus giganteus* plants, had an impact on the toxicity reduction process. The detoxification process was more effective when dosing wastewater with a frequency of 1 L / day 5 times a week, which was associated with better oxygen diffusion into the bed and promoted the decomposition of impurities. It was also observed that the presence of plants in the hydrophyte treatment plant intensified the detoxification process.

Effluent from the hydrophyte treatment plant showed a negative effect on *V. fischeri* (mean TU = 0.9), *D. magna* (TU = 2.5, after 48 h) or L. minor (TU = 5.5). In addition, inhibition of *E. fetida* reproduction (at 39%) and genotoxicity against *V. faba* cells (decrease in mitotic index and increase in the number of chromosome aberrations) were observed. Studies have shown changes in the activity of antioxidant enzymes in the cells analyzed. In addition, an increase in the activity of antioxidant enzymes was observed in the leaves of *M. giganteus* taken from the construced wetlands.

POSTER COMMUNICATIONS

TOPIC 2 – New technologies in biotreatment

(PC 25 – PC 34)

Nanoparticles influence on the respiratory activity of nitrifying bacteria PC 25

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Key words: Nitrification, anammox, wastewater, nanoparticles.

Abstract

Anaerobic ammonia oxidation (anammox) process combined with partial nitrification is assumed to be the most efficient method of nitrogen removal from wastewater. One of the main limitations is relatively high optimal temperature (30 and 40°C) for most anammox bacterial species. Recently, several studies revealed that their growth rate and activity can be accelerated by a few kinds of nanoparticles, including manganese dioxide (MnO_2), reduced graphene oxide (RGO) and zero valent iron (ZVI) [1, 2, 3]. However, the role of nitrifying bacteria was omitted in the previous works. For these reasons, the influence of the nanoparticles of MnO_2, RGO, activated carbon (AC) and ZVI on the respiratory activity of the nitrifying bacteria was investigated in this study.

Nitrifying bacteria activity was measured based on the oxygen uptake rate (OUR) measurements, at two temperatures (10 and 20°C), at three different concentrations of nanoparticles. Experimental results show that the nanoparticles influence strongly depends on the temperature, indicating greater susceptibility of activated sludge at 10°C. In most cases, bacterial respiration was not affected significantly or was inhibited, especially by the RGO. On the other hand, ZVI demonstrated stimulation properties. 10 mg/L of ZVI nanoparticles enhanced the activity of nitrifying bacteria about 12% and 39%, at 20 and 10°C respectively. These results coupled with previous research [3] suggest, that ZVI could be one of the most promising nanoparticle in the field of low temperature partial nitrification-anammox process.

Acknowledgments

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Bibliography

[1] Qiao S., Bi Z., Zhou J., Cheng Y., Zhang J., Bhatti Z. (2012). Bioresour Technol 124, 520-525.
[2] Tomaszewski M., Cema G., Ziembińska-Buczyńska A. (2019). Sci Total Environ 646, 206-211.
[3] Erdim E., Özkan Z.Y., Kurt H., Kocamemi B.A (2019). Sci Total Environ 651 (2), 3023-3033.

Effect of immobilization and nanomaterials addition on the anammox process performed in low temperature – carriers characterisation and nitrogen removal

PC 26

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Key words: Anammox, immobilization, reduced graphene oxide, sodium alginate.

Abstract

Biological anaerobic ammonium oxidation (anammox) is a promising and cost – effective process for nitrogen removal from wastewater. Despite the advantages, the anammox process application is not without restrictions. The optimal temperature for anammox bacteria is higher (30°C ± 5) than the average temperature of sewage entering the treatment plant (15°C). It is important to find a way to reduce the temperature of the anammox process without decreasing its efficiency. Under unfavourable conditions in operating system, anammox biomass growth is often inhibited, wherein fast-growing heterotrophs start to dominate. Immobilization of anammox cells may lead to higher anammox biomass retention and will give possibility for anammox bacteria to growth inside the carrier. Moreover, another way to support anammox process at low temperature is nanomaterials addition. Taking these factors into account the aim of this experiment was to combine the immobilization techniques and nanomaterials addition to increase efficiency of the anammox process carried out at low temperatures.

Preliminary research allowed to select sodium alginate (SA) as immobilization agent [1] and reduced graphene oxide (RGO) as a nanomaterial for anammox stimulation [2]. Experiment was carried during temperature change (from 30°C to 18°C) in 4 sequencing batch reactors: control, biomass with RGO addition, biomass immobilized in SA, biomass immobilized in SA with RGO. The morphology and structure of the immobilized samples were characterized via a scanning electron microscope (SEM, QUANTA FEG 250, Thermo Fisher). The physicochemical parameters of the reactors were monitored regularly during the temperature change.

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Bibliography

[1] Banach A., Pudlo A., Ziembińska-Buczyńska A. (2018). E3S Web Conf. Volume 44. In: 10th Conference on Interdisciplinary Problems in Environmental Protection and Engineering EKO-DOK. EDP Sciences.

[2] Tomaszewski M., Cema G., Ziembińska-Buczyńska A. (2019). Sci Total Environ 646, 206-211.

Physico-chemical performance and microbial characterization of granular sludge system for groundwater denitrification PC 27

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Key words: Real time qPCR, aerobic granular sludge, nitrate pollution, groundwater, biological denitrification.

Abstract

The intensive agriculture applications over land have caused damages to the quality water resources. Thus, the fertilizers applied to the land release nitrogen to the groundwater as nitrate ion. The groundwater is the first drinking water resource in human settlements, by this way the nitrogen in drinking water is a precursor of human diseases. Usually, biological denitrification was used for nitrate removal and carried out by heterotrophic-denitrifying bacteria under anaerobic conditions. Thus, the novel aerobic granular sludge technology was implemented for the removal of nitrate from groundwater, due to the understanding that granular biomass favours anaerobic conditions in the core.

The aerobic granular sludge treatment was performed in a lift sequential batch reactor aerated with air through fine bubble at the bottom. The hydraulic retention time was 6 hours; the pH and oxygen were controlled at 7.4±0.3 and 7.8 ± 0.2, respectively. The influent was a synthetic medium simulating contaminated groundwater, and organic matter at decreasing concentrations was added to estimate the lowest concentration for an optimal denitrification process. The nitrogen, organic matter and granules properties were monitored. Biological samples were taken, which were subject to DNA extraction. The DNA pools were used for absolute quantification of the target genes (archaeal and bacterial 16S rRNA, fungal 18S rRNA, *norB*, *nosZ* I and *nosZ* II genes) involved in formation and stabilization of granular sludge and denitrification process. These results suggest that the implementation of aerobic granular sludge system to nitrate removal from groundwater is a successful and innovative technology, which saves costs and is biologically safe.

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Water bio-decontamination with non-release biocidal coated filters

PC 28

SOC 04

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Key words: Biofouling, polymeric coating, tethered biocide, monolith, Multi-resistant *Staphylococcus aureus.*

Abstract
Water bio-contamination with biofouling, an undesirable natural process in which invasive aquatic organisms colonize submerged artificial surfaces, is a social and industrial global concern, particularly, in water supply and treatment circuits. It can lead to subsequent serious penalties, such as bio-corrosion and human infections, becoming a major public health concern. The most effective bio-decontamination strategies comprise the controlled release of toxic and cumulative bioactive agents into the water, which imply limited life-cycle, significant ecotoxicity, and promotion of human pathogens resistance. The aim of this study was to investigate the antimicrobial potential of a recently developed non-biocide-release coating strategy [1], namely against multi-resistant pathogens, to generate bioactive monolithic filters for water bio-decontamination.
Methods: The new non-release-biocide antifouling system comprises a prior Econea® biocide functionalization with a diisocyanate compound, in order to originate isocyanate reactive biocide derivatives (Econea-N=C=O) to be further tethered in polymeric frameworks, such as polydimethylsiloxane (PDMS) and polyurethane based coatings. Antimicrobial susceptibility of the isocyanate reactive agents and developed bioactive coated monolithic filters were evaluated against Gram-positive bacteria, namely Multi-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* (Ef). For the coated monoliths the antimicrobial potential was assessed by a time kill test, which included a study of biocide content effect in the different polymeric matrices.
These new antimicrobial monolithic filters evidenced auspicious antimicrobial and bacteriostatic effects against the tested microorganisms, especially for multi-resistant bacteria. Such promising results can be the key to further exploitation of bioactive eco-coatings for waterborne systems protection against biofouling.

Acknowledgments
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Bibliography
[1] Silva, E.R.; Ferreira, O.; Ramalho, P.A.; Azevedo, N.F.; Bayón, R.; Igartua, A.; Bordado, J.C.; Calhorda, M.J. (2019). Science of the Total Environment 650(2), 2499-2511.

Specialized degrading granules effective for biaugmentation of Aerobic Granular Sludge reactor treating 2-fluorophenol in wastewater

PC 29
SOC 05

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Key words: aerobic granular sludge, bioaugmentation, bioreactor, 2-fluorophenol.

Abstract

The amount of industrial chemicals being released into the environment has increased. Indigenous microbial communities in wastewater biotreatment processes are not always effective in removing xenobiotics. This work aimed to evaluate the feasibility and efficiency of a promising bioaugmentation strategy in an aerobic granular sludge (AGS) system continuously fed with 2-fluorophenol (2-FP). Bioreactor performance in terms of phosphate and ammonium removal and 2-FP degradation was evaluated.

Granules were produced using extracellular polymeric substances (EPS) extracted from AGS as a carrying matrix and a 2-FP degrading strain, *Rhodococcus* sp. FP1. Afterwards, the produced granules were introduced in the reactor. Shortly after addition, the produced granules broke down into smaller fragments inside the bioreactor, but 2-FP degradation occurred. After 8 days of bioaugmentation, 2-FP concentration inside the reactor started to decrease, and stoichiometric fluorine release was observed 35 days later. 14 Days after the bioaugmentation, phosphate and ammonium removal efficiency improved ca. 36% and 48%, respectively. However, complete phosphorous and ammonium removal was never achieved while the reactor was fed with 2-FP.

The persistency of *Rhodococcus* sp. FP1 in the reactor was followed by qPCR. *Rhodococcus* sp. FP1 was detected 1 day after in the AGS and up to 3 days after bioaugmentation at the effluent. Nevertheless, the degradative ability remained thereafter in the granules. Degrading strain could have persisted even if at lower numbers. Horizontal gene transfer could have happened from the 2-FP degrading strain to indigenous microbiome as some bacteria isolated from the AGS, 3 months after bioaugmentation, degraded 2-FP.

Acknowledgments

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project AGeNT - PTDC/BTA-BTA/31264/2017 (POCI-01-0145-FEDER-031264). We would also like to thank the scientific collaboration under the FCT project UID/Multi/50016/2019.

Remediation of petroleum hydrocarbons in polar soil: feasibility of hybrid technologies

PC 30

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Key words: Polar soil, Petroleum hydrocarbons, Natural attenuation, Hybrid technologies.

Abstract

The Arctic environment is very fragile to anthropogenic disturbances. The aim of this work was to develop a mild remediation strategy: bioremediation and electrokinetic process (EK). In the EK process different current strategies were assessed: reversed electrode polarization (REP) and switching the current On/Off. The work was carried out with soil collected in Sisimiut, Greenland from a dump site after an oil spill. The influence of cold temperature (6 ^{o}C, representative temperature of summer at Arctic) in oil bioremediation was assessed by comparing with reference experiments made at room temperature (22 ^{o}C) with and without current. Possibility of enhanced bioremediation using electrokinetics was assessed in experiments lasting 14 d and 5 mA of applied current intensity.

The polar soil had a hydrocarbons concentration of 70 g/kg, which decreased up to 78% after 14 days of treatment. The effect of the current was not observed in the tested conditions as the control showed similar removals to natural attenuation. Among the electrokinetics experiments, the effect of the current showed to be lower (~30%) in the anode at room temperature compared to cold temperature. Naturally present cold-adapted microorganisms showed to be the most important parameter influencing petroleum hydrocarbons degradation in the here tested conditions.

Biofilm interactions in a recirculating aquaculture unit for fish welfare and environmental sustainability

PC 31

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Key words: RAS, microbial community, physicochemical parameters.

Abstract

Recirculating aquaculture systems (RAS) have been developed in order to intensify fish production. They allow water reuse by managing waste and nutrient recycling. A key aspect of these systems is the water treatment performed by the beneficial bacterial community of the biofilter. Water recirculation can, however, promote fish husbandry challenges such as the occurrence of opportunistic pathogen outbreaks [1]. In this study we aim to investigate the dynamic of a RAS microbial community in relation to the variability of water physico-chemical parameters as well as the network interrelationships between key groups. Samples were collected in two RAS, working at two different salinities, covering their inlet and outlet water, as well as fish tank biofilm and biofilter. The same sampling program was applied after three weeks to account for possible temporal changes. Total DNA was isolated from the water samples of several compartments of the RAS system for microbial diversity. Samples were sequenced by Illumina MiSeq® platform and the output analyzed in SilvaNGS pipeline. Preliminary results show that shifts in salinity were translated into distinct microbial communities between the two systems. The genera *Leucothrix* and *Pseudoalteromonas* are the most abundant in the system at salinity 15 and *Tenacibaculum* and *Ardenticatenaceae* in the system at salinity 35. The definition of the core, healthy and established, microbiome community, will help to elucidate the optimum conditions for fish welfare and environmental sustainability of the production. Interactions between groups of interest may enlighten future frameworks for possible modulation formulas.

Acknowledgements

This research was partially supported by the Strategic Funding UID/Multi/04423/2019 through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (POCI-01-0145-FEDER-007621), in the framework of the programme PT2020. Authors also acknowledge the Ph.D. grant with the reference PD/BDE/135542/2018, and Safiestela Sustainable Aquafarming Investments, Lda.

Bibliography

[1] Martins, C. I. M. (2010). Aquacult Eng 43(3), 83-93.

Impact of industrial wastewater on aerobic granules morphology and nitrification process in bioreactors

PC 32

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Key words: Aerobic granules, industrial wastewater treatment, nitrification, quantitative image analysis.

Abstract
Aerobic Granular Sludge (AGS) is an innovative wastewater treatment process used for carbon and nutrients removal from wastewater. Aerobic granules present a compact structure resistant to variable wastewater composition. Process disturbances might affect bacteria, especially those present in the granules outer layers, such as nitrifiers. In this study, fish canning wastewater with variable composition was treated for 107 days using an AGS sequential batch reactor. The operation was divided in 3 phases, according to different periods of organic loading rate (OLR): Phase I: 0.74 to 1.32 kg m^{-3} day^{-1}; Phase II: 1.33 to 1.70 kg m^{-3} day^{-1}; Phase III: 0.12 to 0.78 kg m^{-3} day^{-1}. Carbon removal and nitrification performance were evaluated. Morphological and structural changes within granules were followed by quantitative image analysis (QIA). Principal component analysis (PCA) was performed using QIA data alone and relating QIA with reactor performance. Along the operation, carbon removal was stable, reaching less than 100 mg O_2 L^{-1} at the outlet. Nitrification was inhibited during Phase II but recovered in Phase III. According to QIA data, biomass samples from Phase III clustered together, indicating higher granule stability. PCA analysis also revealed that a higher OLR might have led to a transitory loss of robustness during Phase II, recovered during Phase III.
This study shows that OLR, nitrification process and biomass morphological and structural changes are possibly correlated during the treatment of industrial wastewater by AGS process.

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Granulation and stability: long-term study of aerobic granules in a reactor operated with fill/draw mode treating domestic wastewater PC 33

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Key words: Aerobic granular sludge, low-strength sewage, fill/draw mode, long-term operation.

Abstract

Wastewater treatment using aerobic granular sludge technology has attracted attention in the last years. However, there are challenging issues and limitations relevant for wider applications of this technology, especially regarding the treatment of low-strength sewage [1]. Besides, the majority of the studies follow few months (approximately 100 days) of the reactor operation, and they use synthetic effluents as feeding. The present work aimed a long-term evaluation (>300 days) of the granulation process and stability of aerobic granular sludge (AGS) in a big-scale reactor (1 m³) operated with fill/draw mode treating real domestic wastewater.

Two volumetric exchanged ratios (VERs) were tested (S-1=50% and S-2=75%) in order to compare the loading rates and conversion capacity. The system was operated during 330 days in each strategy. During S-2 (VER=75%) the system was able to withstand this variation by keeping the sludge volume index (SVI) lower than S-1, and consequently keeping greater particles and losing less sludge. Therefore, the outcomes suggest that the aerobic granules in S-2 were robust enough to stand starvation condition and were able to keep stability during over all operation time. Domestic wastewater applied in both strategies presented similar characteristics in terms of influent concentrations. The system treatment performance was a slightly lower in S-2 in term of 5-day biochemical oxygen demand (BOD_5). However, the higher volumetric exchange ratio (75%) was favorable to granulation process, which improved the particles size, settling ability and system stability. In this way, these results indicate the possibility of granulation without inoculation with fill/draw mode in full-scale SBR to increase their volumetric conversion capacity.

Acknowledgements

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Bibliography

[1] Franca, R.D.G., Pinheiro, H.M., van Loosdrecht, M.C.M., Lourenço, N.D. (2018). Biotechnol Adv 36(1), 228-246.

Tube-in-tube membrane microreactor for photochemical UVC/H_2O_2 processes

PC 34

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Key words: Tube-in-tube membrane microreactor, UVC/H_2O_2, H_2O_2 dosing method, process intensification.

Abstract

The occurrence of organic microcontaminants in aquatic ecosystems has become an emerging concern due to the inability of current treatment methods to remove such compounds [1]. Consequently, more efficient wastewater treatment methods are required to reduce the discharge of such compounds into the environment, mainly from urban wastewater treatment plants (WWTPs). With the purpose of increasing the efficiency of UVC/H_2O_2 coupled systems for tertiary treatment of urban wastewaters, an innovative setup is proposed. The main novelty of the new tube-in-tube membrane microreactor for photochemical UVC/H_2O_2 processes relies on the radial addition of H_2O_2 through the membrane porous into the annular reaction zone. This procedure maximises the use of H_2O_2 by maintaining a homogeneous distribution and constant concentration of the injected chemical across the whole length of the reactor. The proposed novel reactor consists of a ceramic ultrafiltration membrane inner tubing and a concentric quartz outer tubing that compose the annulus of the reactor (pathlength of 3.85 mm). The ultrafiltration membrane is used as a dosing system to deliver small amounts of H_2O_2 into the annulus of the reactor. The number of H_2O_2 dosing points available across the membrane length is “virtually” unlimited. In the annulus, where a 2 mg/L of oxytetracycline (OTC) solution flows, UVC light is provided via four mercury lamps located externally to the outer tube. The efficiency of the photochemical UVC/H_2O_2 process was evaluated as a function of the OTC flowrate, H_2O_2 dosage (H_2O_2 stock solution concentration vs H_2O_2 dosing rate), H_2O_2 dosage method and water matrix. An OTC removal of ~36% with a residual H_2O_2 of ~ 14 mg/L was obtained with a synthetic wastewater, while ~7% OTC oxidation and ~12 mg/L of residual H_2O_2 was measured when treating an urban wastewater fortified with the same OTC concentration, considering a low UVC fluence (34 mJ/cm^2) and reactor residence time (4.6 s).

Acknowledgements

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Bibliography

[1] Becker, D., Rodriguez, S., Insa, S., Schoevaart, R., Barceló, D., Cazes, M., Belleville, M., Sanchez, J., Misovic, A., Oehlmann, J., Wagner, M. (2017). Org Process Res Dev 21, 480-491.

POSTER COMMUNICATIONS

TOPIC 3 – Anthropogenic impacts on the microbial communities

(PC 35 – PC 41)

Reuse of treated municipal wastewater: phytotoxicity assessment **PC 35**

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Key words: Municipal wastewater, phytotoxicity, germination index, water reuse.

Abstract

Advances in wastewater treatment have significantly improved the quality of municipal effluents in many parts of the world. Nowadays, this effluent reuse provides valuable solutions to solve the societal challenges of decreasing availability and limiting access to water resources [1]. One way to reuse this effluent is in the irrigation of urban and agricultural areas. However, it is important to evaluate the risk associated with this utilization through ecotoxicological tests. The objective of this study was to evaluate the feasibility of using the municipal WWT effluent for irrigation. Two different bioassays were used: the *Lemna minor* growth; and the germination index (GI) with *Lactuce sativa* and *Nasturtium officinale* [2]. Additionally, the Chlorophyll *a* content in the *Lemna minor* was analyzed. Three effluent samples were collected from municipal wastewater treatment plants (WWTP) in Viseu region, Portugal. Five effluent dilutions were tested (0%, 25%, 50%, 75% and 100%) for each sample collected. The *Lemna minor* growth enhanced in the presence of effluent comparing with control, with an increase in the Chlorophyll *a* content. For all samples analyzed, the GI obtained for both seeds tested were higher than 60%, which indicated that the effluent was not phytotoxic [3].

It was verified that the effluent from the municipal WWTP may be reused for irrigation, as it did not show evidence of toxicity in plant development. However, it is important that complementary studies be carry out in order to have full confidence in the re-use of this effluent.

Bibliography

[1] Moretti, M., Van Passel, S., Camposeo, S., Pedrero, F., Dogot, T., Lebailly, P., Vivaldi, G.A. (2019). Science of the Total Environment 660, 1513–1521.

[2] Zucconi, F., Pera, A., Forte, M., de Bertoldi, M. (1981). Biocycle 22, 54–57.

[3] Pera, A., Vallini, G., Frassinetti, S., Cecchi, F. (1991). Environmental Technology 12, 1137–1145.

Impact of phytotechnologies with cultivation of *Miscanthus x giganteus* to nematode community in metals' contaminated sites

PC 36

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Key words: Metals' contaminated soil, nematode trophic structure, phytotechnology*, Miscanthus x giganteus.*

Abstract

Due to phytostabilisation process, cultivation of *Miscanthus x giganteus (M.x giganteus)* in anthropogenically contaminated soil is widely used approach with many advantages, including soil quality improvement.

The aim of the study was to test nematodes as indicator for monitoring soil quality improvement while growing *M. x giganteus* at metals' contaminated soils.

Nematode isolation/assiging to trophic groups, calulation of relative abundance was done in accordance to methodology [2] at the several metals' contaminated sites with exceeding MPL: Kyiv modelling field, and Kurakhove military site, both-Ukraine and military site in Fort Raily, USA with/without growing *M.x giganteus* and incorporated soil's amendments.

Results indicated that soil nematodes isolated from contaminated soils are assigned to four tropic groups: Plant parasitic, Fungivores, Bacterivores, Omnivores/Predators. Nematodes in the contaminated soils were characterized by low taxonomic richness and species diversity; a shift of community structure; dominance of certain nematode taxa in trophic-groups composition, maturity index decreasing. Growing of *M. x giganteus* did not impacted significantly structure of nematode community. The impact of organic amendments was different depending of the sites and requested further investigation.

Bibliography

[1] Pidlisnyuk V., Erickson L., Stefanovska T. et al (2019), Environmental Pollution, 249, 330.

[2] Skwiercz A., Stefanovska T., Pidlisnyuk V. et al (2017). Comm. Appl. Biol. Sci, Ghent University, 82(3), 281.

Are ozonation by-products the main regrowth drivers after wastewater treatment? PC 37

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Key words: Microbial communities, antibiotic resistance genes, ozonation, urban wastewater, mineral water.

Abstract

Water quality plays an essential role in the wellbeing of populations, demanding a proper wastewater treatment to prevent environmental contamination of the receiving water bodies. Advanced oxidation technologies (AOTs), such as ozonation, have recently emerged as effective tertiary treatments. However, less attention has been given to the effect of these AOTs on the dynamics of the bacterial communities [1]. Previous studies demonstrated that cells surviving the AOTs are capable of regrow in stored treated water, possibly due to the high content of assimilable organic carbon [1].

To investigate if ozonation by-products are the main regrowth drivers in stored treated wastewater. A bubble column reactor operating under continuous mode was used to perform ozonation experiments. All experiments were carried out using secondary wastewater effluent. After ozonation, the surviving cells of one sample aliquot were re-suspended through backwashing with filter-sterilized mineral water, simulating a pristine oligotrophic environment, whereas the cells of another aliquot were maintained in the treated wastewater. Samples were analysed before and after ozonation and after storage at room temperature for 7 days to monitor the total number of cultivable heterotrophs and pseudomonads, as well as the dissolved organic carbon. Antibiotic resistance genes were quantified by real-time PCR, and the bacterial community composition was analysed by Illumina sequencing.

Given the incapability of ozonation to eliminate the whole water microbiota at the tested conditions, regrowth of bacteria and genes occurred in both mineral water (i.e. free of ozonation by-products) and treated wastewater, with the selection of some bacterial groups which may have relevant ecological implications.

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Bibliography

[1] Becerra, C., Macedo, G., Silva, A. M. T., Manaia, C. M., Nunes, O. C. (2016). Sci. Total Environ. 573, 313-323.

Adaptation of soil bacterial communities to long-term Pb contamination PC 38

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Key words: Lead, shooting range, pollution-induced community tolerance, microbial indicators.

Abstract

Soil contamination with toxic heavy metals is a very worrying environmental problem worldwide. In particular, contamination of soil with lead (Pb) has been reported to cause adverse effects to human health and the environment. For instance, soil contamination with high levels of Pb is very common at shooting ranges where bullets have been fired for years at moving clay targets. In this study, we took soil samples (0-10 cm soil depth), along a long-term Pb-contamination gradient (from approximately 300 to 3,000 mg Pb kg^{-1} dry weight soil) in a shooting range where shooting activity has been going on for decades. Initially, we determined total and bioavailable Pb concentrations in soil, observing no clear correlations between both types of concentrations. The relative abundance of the clinical class 1 integron-integrase gene, *intI1*, was quantified to check its usefulness as proxy for anthropogenic metal (Pb) pollution. Pollution-induced community tolerance of soil bacterial communities was studied using Biolog EcoPlates™ supplemented with different concentrations of Pb, reflecting an evolutionary adaptation of the soil cultivable bacterial communities along the shooting range Pb-contamination gradient. In addition, the structure (biodiversity) and composition of soil prokaryotic communities was studied by means of next-generation sequencing with Illumina MiSeq (16S rRNA amplicon sequencing). Both the diversity (α-diversity parameters) and composition of soil prokaryotic communities showed statistically significant differences along the Pb-contamination gradient.

Effect of nanoscale zerovalent iron on microbial communities in an abandoned arsenic-mercury mine

PC 39

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Key words: Field experiment, nanoremediation, nZVI, microbial communities, PCR-DGGE.

Abstract

Nano-scale zero-valent iron (nZVI) is one of the most intensively studied nanomaterials for environmental cleanup uses over the past 20 years. However, information regarding the effectiveness of engineered nanoparticles at field scale and its impacts on soil microbial communities is still scarce. The present study aimed to evaluate the effect of nZVI on soil microbial communities in a long-term experiment at field conditions, in a polluted former mine "El Terronal" (Asturias, Spain).

According to As and Hg concentration, two plots (A, B) of 5 m^2 were established and treated with nZVI (Nanofer 25S, NANO IRON, Czech Republic) at a dose of 2.5%. Composite soil samples were collected at 1, 2, 8 and 14 months. Microbial community was analyzed by PCR-DGGE targeting the 16S rRNA, 18S rRNA and amoA genes, and soil enzymatic arylsulphatase activity was determined.

After 14 months of nZVI application a reduction on the availability of As (A-76%, B-71%), and Hg (A-86%, B-82%) was observed. Diversity and richness data showed that microbial communities were more sensitive to nanoparticles treatment at the initial sampling period (1-2 months). At the end of sampling, the UPGMA dendrograms demonstrated that nZVI application leads to a shift in the communities structure. With regard to arylsulfatase enzyme, an enhanced activity was found along the sampling period. Canonical correspondence analysis indicated that pH and SO_4^{2-} might influence the community structure of bacterial, fungal and ammonia-oxidizing bacteria. These results suggest that nZVI treatment causes significant changes in the available pool of sulfur in these polluted soils.

Soil Microbiome - The indicator of ecosystem stability after biological regeneration of deposols

PC 40

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Key words: Soil microbiome, ecosystem stability, biological regeneration.

Abstract

Kostolac coal basin is next to Kolubara lignite basin, one of the largest open lignite mines in Serbia. In the surface exploitation of this lignite, soil is formed whose depth surface consists of different, non-systematic deposited layers in which microbiological processes have not started [1].

The absence of microorganisms significant in ecological processes represents a significant disadvantage of such substrates. Biological regeneration, under which it is necessary to achieve stability of the ecosystem, is carried out after the technical (mining) recultivation. One of the indicators of such condition is soil microbiome.

Research on the number and diversity of microorganisms was done on samples of substrates from the inner landfill of the "Drmno" open-pit mine in Kostolac coal basin. Biological regeneration was done by the planting of black locust (*Robinia pseudoacacia*) and imperial Paulownia (*Paulownia tomentosa).* By analyzing the samples from these surfaces and surfaces with spontaneously formed vegetation, the low humus and phosphorus amount was detected, and the measured pH values at these sites are extremely high (> 8). Unfavorable ecological conditions have affected the reduction of soil microbiome [2]. Within the soil microbiome most resistant to these unfavorable conditions were representatives of the gram positive bacteria, *Actinomycetes* and *Bacillus.*

The limiting factor for the formation of vegetation includes low levels of nutrients and reduced microbial activity. To achieve the stability of disturbed ecosystems it is necessary to increase the soil microbiome that will interact with the root of the plants. Establishing a complex plant-microbial interaction will contribute to the formation of vegetation.

Bibliography

[1] Drazic, D., Veselinovic, M., Katic, M., Golubovic-Curguz, V., Mihajlovic, D. (2006). Proceedings from the Scientific Conference "Implementation of Remediation in Environmental Quality Improvement" 7-22, Belgrade, 27 November.

[2] Golić, Z., Raičević, V., Jovanović, Lj., Antić-Mladenović, S., Kiković, D. (2006). Soil and plant 55, 203-208.

Sensitivity of soil dehydrogenase and FDA activities to the ionic liquid ethylammonium nitrate and its lithium salt

PC 41

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Key words: Ionic liquids, soil enzyme activities, ethylammonium nitrate, forest soils, agricultural soil.

Abstract

Ionic liquids (ILs) are salts formed by one organic cation and one organic or inorganic anion with melting point below 100 ºC. Because of their negligible volatility, ILs are usually considered as "non-toxic" and frequently are denominated as "green fluids". However, the fact that are not toxic for the atmosphere does not mean they are also innocuous for aquatic and terrestrial environments. Despite this, the number of studies about the toxic effects of ILs on soils is very limited. Due to their thermophysical properties, these compounds have potential use in numerous applications. Ethylammonium nitrate (EAN) and its lithium salt (EAN-Li) are two ILs being investigated for their use as electrolytes for batteries. Soil enzyme activities are highly sensitive parameters to detect any perturbation in soil functioning and therefore could potentially be used as biochemical indicators of ILs toxicity.

In this study, the effect EAN and EAN-Li on soil dehydrogenase and fluorescein diacetate (FDA) activities was investigated. With this purpose, two acidic soils with similar pH and different organic matter content (agricultural and forest) were spiked with increasing amounts of the two compounds, and after three days the activities of both enzymes were analysed. Dehydrogenase activity tended to decrease and FDA to increase with increasing amounts of both ILs, but the extent of the decrease depended on the activity, the IL and the soil. In all cases, dehydrogenase decreased and FDA increased. Generally, dehydrogenase activity and the forest soil were more strongly affected than FDA and the agricultural soil.

Acknowledgements

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POSTER COMMUNICATIONS

TOPIC 4 – New biodegraders and consortium-based strategies

(PC 42 – PC 61)

Isolation and characterization of plant growth promoting microorganisms from heavy metals contaminated soil and their potential role in phytotechnology with *Miscanthus x giganteus*

PC 42

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Key words: Heavy metals contaminated soil, Plant growth promoting bacteria, Phosphate solubilizers

Abstract

Due to rapid industrial developments including various mining activities, heavy metals are accumulated in the environment. This has emerged as a grave problem throughout the world [1].

The aim of this study was the isolation of plant growth promoting (PGP) microorganisms from heavy metals contaminated soil and exploring their metal tolerance and PGP potential.

Isolation of microorganisms was carried out from the heavy metals *viz*. Pb, Cr, Ni, Cu and Zn contaminated soil, collected from the former mining site at Všebořice, Usti region, Northern Czech Republic. The heavy metal tolerant isolates were examined for various PGP characteristics *viz*. Phosphate and Zn solubilization, Indole acetic acid (IAA), Ammonia, HCN, and Siderophore production using the standard assay protocols. The isolates showed higher Pb tolerance and PGP potential were identified using 16S rDNA sequencing. Related field trial studies with *Miscanthus x giganteus* crop as a model plant in artificially contaminated soil with Pb are ongoing.

In this study, a total of 17 bacterial and three actinomycete strains were isolated from the heavy metals contaminated soil, with 13 strains tolerant against $[Pb]_{max}$ = 500 ppm. Based on the Pb tolerance and PGP characteristics (Phosphate solubilizing, Siderophore, Ammonia, HCN and IAA i.e., 22.3 µg/mL production), strain KP-14 was found to be the most promising strain. The selected strain can be used as a potent plant growth promoter and in combination with the second generation energy crop *Miscanthus x giganteus* [2] can enhance the efficiency of phytotechnology applied to the heavy metals contaminated sites.

Bibliography:

[1] Khanna, K., Jamwal, V. L., Gandhi, S. G., Ohri, P., & Bhardwaj, R., (2019). Scientific Reports 9, 5855.
[2] Pidlisnyuk V., Erockson L., Stefanovska T. et al, (2019), Enviornmental Pollution, 249, 330.

Application of nucleic acid mimics and spectral imaging to fluorescence *in situ* hybridization for the characterization of microbial communities

PC 43

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Key words: Microbial communities, fluorescence *in situ* hybridization, spectral imaging, nucleic acid mimics.

Abstract

The application of nucleic acid mimics (NAMs) has improved the performance of FISH methods for the localization, detection and enumeration of microorganisms. However, an important limitation of FISH techniques is the low number of distinguishable targets. Recent advances in combinatorial labelling and fluorescence spectral imaging (CLASI-FISH) have allowed the simultaneous identification of several microorganisms in a single sample. In this work, we aimed to combine NAM-FISH and spectral imaging to develop/validate a new color-coded FISH methodology that allows a multiplexed and robust detection of microorganisms in complex communities. In a first stage, to implement/validate the method, we have selected 8 fluorophores with distinct spectral properties and 7 bacterial species. As a strong variation on the fluorescence intensities is found between species and between fluorophores, 8 versions of EUB LNA/2'OMe probe, each conjugated to one of 8 fluorophores were used to rank species/fluorophores by FISH. Then, mixed populations were used to evaluate the potential of the method for separating/quantifying the different species. Samples were analyzed by confocal laser scanning microscopy coupled with a spectral imaging detector and a linear unmixing algorithm was applied to identify the fluorophores present in each pixel of the images. Validation tests with different proportions of bacteria labelled with the respective fluorophore have shown the ability of the method to correctly distinguish the species. The method shows as a powerful tool for the characterization of environmental microbial consortia that might assist on the monitoring of bioremediation processes.

Acknowledgements

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Identification of the biocatalysts involved in molinate breakdown by *Gulosibacter molinativorax* ON4^T

PC 44

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Key words: Pesticide, genome, transcriptome, enzyme, recombinant expression.

Abstract

Enzymatic bioremediation is a promising approach, in particular because it avoids the introduction of allochthonous microorganisms in the polluted site, and consequently the potential disturbance of the native microbial communities. This technology relies upon the identification of the enzymes involved in the pollutant degradation pathway. *Gulosibacter molinativorax* ON4^T is known to degrade the thiocarbamate herbicide molinate [1]. Although the putative degradation products have been identified [1], only the enzyme responsible for the initial molinate breakdown (molinate hydrolase, MolA) is identified [1]. This study aimed at identifying the enzymes involved in the transformation of the MolA products, namely azepane-1-carboxylate (ACA), by strain ON4^T.

A combined genomic and transcriptomic strategy was used to identify the potential genes coding for the enzymes involved in the degrading pathway. Confirmation of activity has been carried out through recombinant protein expression in *Escherichia coli* BL21(DE3) using a pET system. The comparison of the transcriptomic data from strain ON4^T growing with and without molinate indicated that the genes encoding for Cytochrome P450 (*bioI*), hydantoinases A/B (*hyuA/B*), caprolactone hydrolase (*chnC*) and 6-oxohexanoate dehydrogenase (*chnE*) were over expressed in the presence of molinate. The ongoing work will determine their role in ACA degradation into adipate, which can then enter the β-oxidation pathway, and further enable the kinetic characterization of these novel biocatalysts.

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Bibliography

[1] Nunes, OC. (2013). Appl Microbiol Biotechnol, 97(24), 10275-10291.

Mycoremediation of environmental pollutants using white rot fungi and their enzymes **PC 45**

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Key words: Basidiomycetes, lignin-modifying enzymes, production, application, bioremediation.

Abstract
Contamination of soils and waters with toxic organic pollutants cause detrimental effects on the health of humans, animals, plants, and microbes. Recent fundamental work has revealed the potential application of white rot basidiomycetes (WRB) and their lignin-modifying enzymes (LME) for treatment of soils and effluents contaminated with organic pollutants. The ability of fungi to form extended mycelia networks, the low specificity of their enzymes and their ability of using pollutants as a growth substrate make WRB well suited for bioremediation processes. However, large scale applications of LME for bioremediation are so far limited due to the high cost of enzymes. This presentation summarizes fundamental knowledge on physiological mechanisms regulating LME synthesis by WRB focusing on the common characteristics and unique properties of individual fungi as well as on several approaches providing enhanced secretion of these enzymes by WRB. Firstly, lignocellulosic substrates, some of which containing significant concentrations of soluble carbohydrates and inducers, play a crucial role in enzyme production. Secondly, some microelements and aromatic compounds enhance the LME synthesis although their effect depends on individual fungi physiological peculiarities. Thirdly, expression of basidiomycetes biosynthetic potential depends on the cultivation method. Fourthly, co-culture of compatible fungi may be an appropriate approach to increase laccase and MnP yields. Moreover, the potential of several WRB and their LME in the decolorization of synthetic dyes, removal of oil, trinitrotoluene and individual micropollutants will be analysed.

Acknowledgements
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Comparative genomics of the *Leucobacter* genus reveal a novel sulfonamide degradation gene cluster in strain GP

PC 46

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Key words: Sulfonamides, bacterial consortium, phylogenetic analysis, metagenome-assembled genome.

Abstract

Environmental microbial communities recurrently establish metabolic associations resulting in increased fitness and in the ability to perform complex tasks, such as xenobiotic degradation. In a previous study [1], we have described a sulfonamide-degrading consortium consisting of a novel low-abundant and slow-growing actinobacterium, named strain GP, and *Achromobacter denitrificans* PR1. However, we found that strain GP was unable to grow independently and, thus, could not be further purified. To understand the role of this low abundance bacterium in the degradation of sulfonamides we performed shotgun sequencing of the consortium using two complementary technologies: Miseq paired-end (Illumina) and MinION long-read (Oxford Nanopore). The draft genome of strain GP was then compared to other type strains of the *Leucobacter* genus and to other sulfonamide degraders. The culture-independent approach allowed the recovery of the near complete genome of strain GP (completeness 95.91% and contamination 0.58%). Comparative genomic analysis indicates that strain GP may have lost genes related to tetrapyrrole biosynthesis and thiol transporters, both crucial for the correct assembly of cytochromes and aerobic growth. However, supplying exogenous heme and catalase was insufficient to abolish the dependent phenotype. The actinobacterium harbors at least two copies of a transposable element containing a sulfonamide monooxygenase (*sad*A) flanked by a single IS1380 family transposase. Additionally, two homologs of *sad*B (4-aminophenol monooxygenase) were identified in the draft genome of strain GP, but these were not located in the vicinity of *sad*A nor of mobile or integrative elements.

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Bibliography

[1] Reis, A.C., Čvančarová, M., Liu, Y., Lenz, M., Hettich, T., Kolvenbach, B.A., Corvini, P.F.-X., Nunes, O.C. (2018). Appl Microbiol Biotechnol 102, 10299–10314.

Microalgal biomass production and nutrient recovery using innovative photobioreactors designs based on compound parabolic collectors

PC 47

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Key words: Biomass production, light distribution, microalgae, nutrient recovery, photobioreactor design.

Abstract

Microalgal biomass has high economic potential due to its broad-spectrum biotechnological applications. Several studies have referred the use of microalgae for nutrients recovery from wastewater, CO_2 capture, biofuels production and extraction of high added-value products [1]. Despite all advantages, average biomass productivity of the most common industrial strains is far lower than maximal theoretical estimations. Light distribution inside the photobioreactor strongly influence biomass productivity, nutrient recovery and biochemical composition [2]. In this study, innovative tubular photobioreactors configurations based on compound parabolic collectors were used to enhance biomass (microalga *Chlorella vulgaris*) productivity and nutrient recovery. The photobioreactors are composed of an absorber tube, made of borosilicate glass, and a reflective surface. Photobioreactors performance was evaluated as a function of the reflective surface material (anodized aluminium with (MS) and without (R85) protective coating, and stainless steel (SS)) and geometry (flat (F), simple double-parabola (SP) and traditional double-parabola (DP)). The amount of light reaching the absorber tube, its distribution inside the photobioreactor, the long-term durability and efficiency of the solar collectors are directly related to the reflective surface material and geometry [3]. Improved biomass productivity (110 mg L^{-1} d^{-1}) and nutrient recovery (90% and 45% for nitrates and phosphates, respectively) was obtained for the traditional solar collector with a DP optics made of SS. This configuration allows the illumination of the full perimeter of the absorber tube, resulting in a more homogeneous light distribution inside the reaction medium. Beyond that, SS reflectors present a much lower specular reflectance in the UV region than R85, decreasing the amount of UV light reaching the reaction medium and consequently reducing the algal UV stress.

Acknowledgements

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Bibliography

[1] Gonçalves A.L., Pires J.C.M., Simões M. (2016). Bioresour. Technol. 200, 279-286.
[2] Moreno-Garcia L., Adjallé K., Barnabé S., Raghavan G.S.V. (2017). Renew. Sust. Energ. Rev. 76, 493-506.
[3] Gomes A.I., Silva T.F.C.V., Duarte M.A., Boaventura R.A.R., Vilar V.J.P. (2018). J. Clean. Prod. 199, 369-382.

Potential of green microalgae in the bioremediation of wastewaters from different sources

PC 48

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Key words: Biomass production, bioremediation, microalgae, wastewaters.

Abstract

Microalgae have been proposed as a viable alternative for wastewater remediation from different sources, due to its ability to uptake several organic and inorganic pollutants [1]. As nitrogen and phosphorus are important macronutrients for microalgal growth, microalgae can play an important role in the tertiary treatment of wastewaters containing high amounts of these inorganic species [2]. Accordingly, microalgal growth in wastewaters has the dual effect of promoting wastewater treatment and further reducing biomass production costs (associated with nutrients supply) and environmental impact [1]. Thus, the main goal of this study was to evaluate the application of *Chlorella vulgaris* and *Tetradesmus obliquus* for nutrients removal from landfill leachate and paper industry wastewaters. The experiments were performed in batch mode using different wastewater dilutions, being able to determine the relationship between effluent load and biomass production/nutrients recovery. The results showed that the studied microalgae are able: (i) to grow in these effluents (for example, average biomass productivities determined for *C. vulgaris* grown in landfill leachate ranged between 18 to 51 mg L^{-1} d^{-1}); (ii) to effectively remove nitrates (52%) and phosphates (27%). Another important finding was the formation of microalgal flakes after the first days of cultivation in the landfill leachate. Since separation of microalgal biomass from the culture medium represents an important fraction of the production/treatment costs [3], microalgal growth in the form of flakes without compromising the remediation efficiency can be an important advantage in microalgal remediation systems.

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Bibliography

[1] Gonçalves A.L., Pires, J.C.M., Simões M. (2017). Algal Res. 24, 403-415.
[2] Gonçalves A.L., Pires, J.C.M., Simões M. (2016). J. Clean. Prod. 133, 348-357.
[3] Barros A.I., Gonçalves A.L., Simões M., Pires J.C.M. (2015). Renew. Sust. Energ. Rev. 41, 1489-1500.

Planktonic marine bacterial communities degrading high molecular weight aliphatic hydrocarbons PC 49

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Key words: Marine bacterial community, hydrocarbon degraders, paraffins, nitrogen, high salinity.

Abstract

The degradation of long-chain alkanes (C18–C30) in marine environments is typically less efficient because of their lower water-solubility and low bioavailability for microorganisms [1,2]. Exploring marine microbial communities capable of degrading long-chain alkanes will help to find the best bioremediation strategy for biodegradation of these substrates. This work aimed at assessing the diversity and community structure changes of marine hydrocarbon-degrading bacterial communities growing on octadecane as the sole carbon source under varying ammonium concentrations and salinities. Enrichments were set up on paraffin (C18-alkane) using the surface seawater samples of Menai Strait (Wales, UK) as an inoculum and supplemented with ammonium chloride and sodium chloride. Incubation of enrichments was done at 20ºC for two months. Composition and diversity of bacterial communities in the enrichments were analysed by 16S rRNA gene sequencing using MiSeq Illumina platform. Analysis of sequencing data revealed that the C18 alkane, a nitrogen source and elevated salinity resulted in the increase of *Gamma*- and *Alphaproteobacteria* and the selection of the different types of bacterial communities. Enrichments of seawater on paraffin showed the predominance of genera *Oleibacter, Alteromonas* and *Maricaulis*, while the addition of ammonium chloride and sodium chloride at moderate concentrations favoured the rise in densities of genera *Alcanivorax, Roseivirga* and *Owenweeksia*. The presentation will provide further details on community composition shifts as a response to elevated salinities and simulated eutrophication.

Bibliography

[1] Brzeszcz, J and Kaszycki, P. (2018). J Biodegradation 29:359-407.
[2] Liu et all (2016). Frontiers in Microbiology 7:2131.

Enhanced lead phytoextraction in *Brassica juncea* by endophytes from indigenous plants PC 50

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Key words: Lead, phytoextraction, endophytes, *Brassica juncea.*

Abstract
Lead (Pb) is one of the most common metal pollutant in soil and phytoextraction is a sustainable and cost-effective way to remove it. To increase bioavailability and thus the uptake and translocation of metals into plants, the addition of chelating agents has been extensively used in phytoextraction, and some organic acids such as EDTA is particularly effective in increasing lead solubility [1]. Besides, Plant Growth Promoting Rhizobacteria (PGPR) may be active in supporting the uptake of metals through the root system [2].
Purpose of this work was to develop a phytoextraction strategy able to remove Pb from the soil of a decommissioned fuel depot located in Sardinia (Italy) by the combined use of EDTA and endophytes bacteria isolated from indigenous plants. The contaminated area shows a high spontaneous vegetation and we isolated endophytic microorganisms from the roots of the most represented species: *Lotus cornicolatus, Sonchus tenerrimus, Bromus sterilis, Plantago lagopus, Chrysanthemum coronarium, Phragmites australis, Oxalys pes-caprae*. A total of 46 endophytic strains from the 7 native species were isolated and selected. Genomic DNA from the pure culture of the isolates was subjected to 16S rRNA gene sequencing, leading to identify bacteria belonging to *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus* and *Micrococcus* genera. The isolated microbial strains were then analyzed for the presence of plant growth-promoting properties such as the production of auxins (3-indol acetic acid, IAA), ammonia, siderophores, exopolysaccharides, the formation of biofilm (*in vitro* pellicle), the nitrogen fixation capacity and the inorganic phosphate solubilization ability. A microbial consortium prepared with the twelve PGPR showing the best *in vitro* potential features was used to inoculate microcosms of *Brassica juncea* and *Helianthus annuus.*
Experimental data show that the effect of the addition of endophytes on lead phytoextraction is significantly higher in *B. juncea*, either in shoots (0.21 mg kg^{-1} in control sample, 4.82 mg kg^{-1} after inoculum) or in roots (1.32 mg kg^{-1} in control sample, 55.75 mg kg^{-1} after inoculum). On the other hand, always in *B. juncea*, it appears that the combined addition of EDTA and PGPR have a negative effect on lead uptake: 2.04 mg kg^{-1}. The behavior of *H. annuus* agrees instead to results obtained in previous experiments and the greatest positive effect comes from the combination of EDTA and microbial consortium either in shoots (1.34 mg kg^{-1} in control sample, 1.14 mg kg^{-1} with PGPR, 3.46 mg kg^{-1} with EDTA + PGPR) or in roots (2.14 mg kg^{-1} in control sample, 19.01 mg kg^{-1} with PGPR, 41.98 mg kg^{-1} with EDTA + PGPR). Metagenomics analysis of microbial community of microcosm trials by next generation sequencing (NGS) approach will be shown.

Bibliography
[1] Doumett S, et al., 2008, Chemosphere, 72:1481–1490.
[2] Franchi, E, et al., 2019, Sci Total Environ 655: 328-336.

Influence of rhizosphere bacterial strains on bioavailability of strategic elements in mine-affected soils and implications in phytomining **PC 51**

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Key words: Mine-soils, rare earth elements, restoration, tin, tungsten.

Abstract

The European Commission (EC), within the framework of the Raw Materials Initiative, encourages the development of new means of metal recovery from secondary sources [1]. These initiatives follow the concept of circular economy to ensure the secure supply of raw materials while minimising waste production. Phytomining cultivates hyperaccumulator plant species which can accumulate extreme amounts of metal(s) in their aboveground tissues. It has been shown to be an eco-friendly strategy for the recovery of nickel from ultramafic soils but could be developed for the recovery of other high-value and strategic elements [2]. Implementing phytomining options in mine-affected areas could provide an additional metal source to primary ores and at the same time assist site restoration. Plant-associated bacteria play a central role in the plant's ability to phytoextract and accumulate metals.

This study was carried out using soil from two old mines dedicated to the extraction of either, tungsten (W) and copper (Cu), or to gold (Au) and molybdenum (Mo), in NW Spain. Batch culture assays were performed to evaluate the ability of bacterial strains, originally isolated from plants growing in the same mine areas, to mobilise strategic elements, such as Mo, W and REE, from both mine-soils. Weathering capacity varied amongst isolates, but the activity of several strains induced an acidification of the culture media and significant mobilisation of different critical elements, including Be, Ce, La and Y. Best-performing strains will be used as bioinoculants in future experiments to enhance plant bioaccumulation and extraction of these elements.

Bibliography

[1] EC, 2018. https://ec.europa.eu/commission/publications/report-critical-raw-materials-and-circulareconomy_es (Accessed 27th Apr 2018).
[2] Kidd et al., 2018. Front. Environ. Sci. 6: 44. DOI=10.3389/fenvs.2018.00044.

Bioprospecting microalgae for treatment of marine aquaculture wastewater PC 52

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Keywords: Microalgae, wastewater, marine aquaculture.

Abstract

The high demand for water resources and inefficient wastewater treatment strategies are major drawbacks to the aquaculture industry sustainability [1]. Accordingly, the EU's Blue Growth strategy fosters the development of technologies for proper treatment of aquaculture wastewaters enabling water reuse. Biofilm systems are promising for wastewater treatment, especially those using granules due to their high microbial biomass and cost-effective separation from treated water [2]. Microalgae and bacteria have the ability to grow using nutrients and other organics present in wastewater. The combination of both microorganisms within the same structure could prove beneficial as the synergy established could make the treatment process more efficient.

This study aimed to obtain microalgae strains from a marine environment that will be further used to develop algal-bacterial granular sludge to treat marine aquaculture wastewater. Water samples were collected from a marine aquaculture facility in Murtosa, Portugal. Samples were spread plated in different culture media for microalgae growth. Axenic cultures were obtained after successive streaking and DNA barcoding of the nuclear gene 18S rDNA was used for phylogenetic affiliation. Different algae genera were identified e.g. *Chloroidium*, *Interfilum*, *Pseudochloris*. Simultaneously, enrichment cultures were established from water samples using F/2 culture medium and kept at constant agitation to favour microalgal growth. The dynamics of the microbial communities throughout the enrichment process were followed.

Overall, this study will allow the identification of suitable microalgae for the development of algal-bacterial granules overcoming the challenge regarding biomass separation in wastewater treatment using microalgae.

Acknowledgments

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project GReAT - PTDC/BTA-BTA/29970/2017 (POCI-01-0145-FEDER-029970). We would also like to thank the scientific collaboration under the FCT project UID/Multi/50016/2019.

Bibliography

[1] Chávez-Crooker, P. and Obreque-Contreras, J. (2010) Curr. Opin. Biotechnol. 21, 313–317

[2] Amorim, C.L. et al. (2017) In Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater published by IGI Global, pp. 231–263.

Efficient Rhizobacterial Consortium to provide bipartite benefit in supporting plant growth in presence of organophosphate pesticide stress

PC 53
SOC 06

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Key words: Rhizobacterial consortium, organophosphate pesticide, bioremediation, degradation enzymes, plant growth promotion (PGP).

Abstract

Modern Indian agricultural practices are shifting towards exploring safer bioinoculants as biological solutions supporting plant growth and protecting host crop and soil health from harmful residual pesticides effect [1]. To accesses the potential of rhizobacterial consortium in supporting host plant growth and protecting host plants when challenged with pesticides Monocrotophos and Dimethoate. Defined objective achieved by employing four native rhizobacterial isolates identified as: *Sphingobacterium* spp., *Brevundimonas* spp., *Pseudomonas* spp. and *Pseudomonas monteilii*, formulated as15 consortia combinations (singlets, doublets, triplets and quadruplets) with respect to control after cohabitation test. Pesticide degradation properties were assessed by the activity of degradation enzymes: Hydrolase, esterase and phosphatase. *In-vitro* plant bioassay (15 days) conducted on lab optimised system ASURE (manuscript under review) to estimate the beneficial impact of different consortia on plant growth promotion. Protective effect of consortia on the host upon pesticide challenge was studied through *in-vitro* plant bioassay after conclusion of a 30 day study. Singlet (T2, T6) and doublet (T3, T5, T8) combinations of *Sphingobacterium* spp., *Brevundimonas spp.* and *Pseudomonas monteilii* were observed to outstand on all grounds compared to triplet and quadruplet combinations. Consortia T3, T6, T8 and T9 showed best PGP properties whereas T2 and T6 showed relatively high degradation potential. Consortia T3, T6 and T5 were observed to protect host plant under 300 ppm of monocrotophos stress whereas T6, T5 and T2 showed better shielding effect to host plant under 50 ppm dimethoate stress. Thus, authors report combinations T6, T3, T5 and T2 as efficient bioinoculant providing bipartite benefits to the host plant and environment [2] [3].

Bibliography

[1] Pattanasupong A., Nagase H., Inoue M., Hirata K., Tani K., Nasu M., and Miyamoto K., (2004). World J Microbiol Biotechno, 20, 517–522.

[2] Abraham J. and Silambarasan S., (2013). Process Biochem, 48, 1559–1564.

[3] Ahemad M. and Khan M.S., (2011). Symbiosis, 54, 17–27.

Combination of biological strategies for the remediation of soils simultaneously polluted with heavy metals and organic compounds

PC 54

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Key words: Bioremediation, bioaugmentation, phytoremediation, earthworms, microbial indicators.

Abstract

In this field experiment, a combination of biological strategies was used for the remediation of a soil simultaneously contaminated with heavy metals and organic compounds as a result of the application of sewage sludge. Two different plant species (alfalfa - *Medicago sativa* and rapeseed – *Brassica napus*) were grown on the polluted site for both metal phytostabilization and metal phytoextraction purposes. In addition, a consortium of bacteria was inoculated from a bioaugmentation point of view. Finally, *Eisenia fetida* earthworms were also added to the polluted soil in order to stimulate the biodegradation of the organic contaminants. At the end of the experiment, apart from the quantification of the metal phytoremediation effectiveness and the rate of degradation of the organic contaminants, changes in soil ecotoxicity were determined using a variety of plant and earthworm biomarkers (e.g., seed germination, root elongation, neutral red, etc.). An Ecological Risk Assessment was performed based on our chemical and ecotoxicological results. Interestingly, the effectiveness of our biological remediation combined strategy was assessed using a wide variety of soil microbial parameters which provide information on the biomass, activity and diversity of soil microbial communities. These microbial parameters, together with the chemical analysis, were used as monitoring tools to evaluate the recovery of soil health derived from the application of the biological remediation methods. We observed an improvement in soil health mainly as a consequence of an enhancement in soil microbial activity.

Acknowledgements

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Use of metagenomics for studying fungal and bacterial community dynamics of sewage sludge enrichment experiment with pharmaceutical compounds PC 55

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Key words: Metagenomics, emerging pollutants, sewage sludge, pharmaceutical active compounds, bioremediation.

Abstract

One of the most challenging environmental threats of the last two decades is the impact of emerging pollutants such as pharmaceutical compounds. These synthetic or natural chemicals can be found in prescription medicines, over-the-counter therapeutic drugs [1]. Most generic pharmacons like carbamacepin, ketoprofen and diclofenac had been regularly found in waste water treatment plants (WWTP) as a consequence of its extensive usage and its recalcitrant nature [2]. These emerging and unregulated contaminants end up in soils and water bodies, causing an imbalance of the microbial communities that culminates in a possible development of resistance to antimicrobial agents and finally endangering human health and environmental stability. Native fungal and bacterial communities from sewage sludge play an important role being able to degrade a myriad of pollutants [3]. In this work we performed an enrichment experient using sewage sludge from a WWTP of Granada, Spain, to study both the cultivable and non-culturable microorganisms during the selective preassure with carbamacepine, ketoprofen and diclofenac. DNA extraction and massive sequencing Illumina MiSeq techniques has been used. The results showed a predominance of *Ascomycota* fungi belonging to *Dothiodemycetes*, and *Aspergillaceae* at the end of the experiment. The most abundat bacterial taxa after the enrichment were *Alcaligenes* and *Corynebacterium*. Species like *Cladosporium cladosporoides, Alternaria alternata* and *Penicillium raistrikii* had remarkable degradation rates (25, 90 and 99% of carbamacepin, ketoprofen and diclofenac in 21 days flask experiments, respectively). These results highlight the possibility of selecting native microbial consortiums to carry out composting processes using bioaugmentation techniques.

Aknowdlegments

Proyect CTM2017-84332 (MINECO/AEI/FEDER/UE). CONACyt (A. L. Fellow CVU No. 377965).

References

[1] Verlicchi, P., Zambello, E. (2015). Sci. Total Environ. 538, 750-767.

[2] Thomaidi, V. S., Stasinakis, A. S., Borova, V. L., & Thomaidis, N. S. (2016). Sci Total Environ 548: 280-288.

[3] World Health Organization. (2011). (WHO/HSE/WSH/11.05).

Bacterial roles of a diesel-degrading consortium for the rhizoremediation of diesel-polluted soil: Metagenomic insights PC 56

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Key words: Rhizoremediation, diesel, bacteria, consortium, metagenomics.

Abstract

Diesel is a complex pollutant composed by a mixture of aliphatic and aromatic hydrocarbons. Because of this complexity, diesel bioremediation requires multiple microorganisms, which harbor the catabolic pathways to degrade the mixture. By enrichment cultivation of rhizospheric soil from a diesel-polluted site, we have isolated a bacterial consortium that is able to grow aerobically with diesel and different alkanes and polycyclic aromatic hydrocarbons (PAHs) as the sole carbon and energy source. Microbiome diversity analyses based on 16S rRNA gene showed that the diesel-degrading consortium consists of 49 operational taxonomic units (OTUs) and it is dominated by *Pseudomonas*, *Aquamicrobium*, *Chryseobacterium* and *Sphingomonadaceae.* Changes in microbiome composition was observed when growing on specific hydrocarbons, reflecting that different populations degrade different hydrocarbons. Whole-metagenome sequence analyses have identified redundant genes encoding enzymes implicated in the initial oxidation of alkanes (AlkB, LadA, CYP450) and a variety of hydroxylating and cleavage dioxygenases involved in aromatic and polyaromatic hydrocarbon degradation. The phylogenetic assignment of these enzymes to specific genera allowed us to model the role of specific populations in the diesel-degrading consortium. Rhizoremediation of diesel-polluted soil microcosms using the consortium, resulted in a major reduction of total petroleum hydrocarbons (TPHs), making it suited for rhizoremediation applications. The functional redundancy observed in the metagenome might be related to the plasticity that allows the population to adapt to changes in the environment, and therefore conferring robustness to the degrading hydrocarbon system. Finally, this work shows that an effective autochthonous bacterial consortium can be constructed by successive enrichment cultivation of soils from contaminated sites.

Possibility of application of *Miscanthus x giganteus* in soil contaminated by the petroleum industry

PC 57
SOC 07

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Key words: *Miscanthus x giganteus*, phytotechnology, petroleum contaminated soil, biomass, degradation.

Abstract

Miscanthus x giganteus (Mxg), C4 perennial grass, is under investigation as one of the most suitable crops for biomass production due to high biomass yields combined with low inputs requirements and other environmental benefits [1]. It is able to grow in moderately heavy metals contaminated soils with slow phytostabilization of metals in root system. Few studies were published also about *Mxg* growing in PAH contaminated soils but generally potential for organic pollution phytoremediation with *Mxg* has not been fully studied yet [2].

Two pot experiments were prepared to investigate possibility of growing *Mxg* in mixtures of highly contaminated soil from petroleum sludge lagoon with uncontaminated soil. At first experiment starting concentrations of C10-C40 aliphatic hydrocarbons were 9 g/kg and 21 g/kg. Although the plants survived, biomass production was very low. That is why second series was prepared with more diluted soil (0 – 4.5 g/kg C10-C40). *Mxg* was again able to grow in all concentrations, but even in the lowest contamination (1.5 g/kg C10-C40) biomass was lower than control. C10-C40 decreased by 31-88% during season. The highest decrease was determined in the lowest C10-C40 concentration. In this concentration, degradation was more intensive in pots with *Mxg* compared to unplanted control while at higher concentrations it was comparable, so no additional phytoremediation effect was revealed.

To conclude, *Mxg* is able to survive even in highly contaminated soils with aliphatic hydrocarbons but the phytotechnology appears to be applicable only in lower concentrations where biomass production is less affected and improvement of degradation was determined.

Bibliography

[1] S. Arnoult, M. Brancourt-Hulmel (2015). BioEnergy Res, 8, 502–526.

[2] V. Pidlisnyuk, T. Stefanovska, E. E. Lewis, L. E. Erickson, L. C. Davis (2014). CRC. Crit. Rev. Plant Sci, 33, 1–19

Implementation of different bioremediation treatments on recent and long-term diesel-contaminated soil

PC 58

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Key words: Soil remediation, diesel, bioaugmentation, bio-stimulation, biochar.

Abstract

Soil pollution by diesel fuel is produced by spills and leakages and is a global problem that needs innovative and environmentally friendly solutions. In this framework, the objective of this work is to study the effectiveness of the application of different remediation treatments in a soil that was subjected to two contamination scenarios: (i) long term contamination (aged diesel) and (ii) recent contamination (intentionally contaminated with the same type of diesel). Those bioremediation treatments were: (i) bioaugmentation [1] with a microbial consortium of isolated bacteria from the long term contaminated soil, (ii) bio-stimulation, (iii) addition of biochar (produced from local organic waste from olive and pine pruning) and (iv) different combinations between treatments. Preliminary results showed different effects of treatments in the degradation of aliphatic and aromatic chains between both contamination scenarios. Additionally, our results suggested the existence of a synergy between the bioaugmentation and the biochar treatments.

Bibliography

[1] Mrozik, A., Piotrowska-Seget, Z., (2010). Microbiol. Res. 165, 363-375.

Effect of different light wavelengths on growth, nutrient removal and biomass production of green microalgae

PC 59

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Key words: Biomass production, light supply, microalgae, nutrient removal, wavelengths.

Abstract

Microalgae have several advantageous features, such as high photosynthetic efficiency, fast growth even under unfavorable conditions and the possibility to obtain a diversity of products, due to the biochemically-rich composition of their biomass. Considering the nutritional needs of microalgae in terms of nitrogen and phosphorus, these microorganisms can also be used in the tertiary treatment of wastewater where these macronutrients can have a high concentration [1]. Despite the fast growth and high photosynthetic efficiency, microalgal biomass production and nutrient removal should be further improved. Among the parameters that influence microalgal growth and hence nutrient removal (e.g. nitrogen and phosphorus), light supply is the most important, as it is the energy source used in photosynthetic reactions. Therefore, microalgal growth can be enhanced by changing light conditions, that is, light intensity, wavelength and photoperiod [2]. In this study, the effect of different light wavelengths on nutrient removal from a synthetic effluent and biomass production of *Chlorella vulgaris*, *Tetradesmus obliquus* and *Neochloris oleoabundans* was studied. The experiments were conducted with LEDs with different colors/wavelengths: white (380-750 nm), red (620-750 nm) and blue (450-495 nm). The results indicate that the white LED was the best condition for growth for all the species with a growth rate of 0.179, 0.205 and 0.404 d^{-1} for *C. vulgaris*, *T. obliquus* and *N. oleoabundans*, respectively. Regarding nutrient removal, for the same light conditions, nitrate removal rates obtained for all species were between 8.51 and 8.96 mg L^{-1} d^{-1}; for phosphate, the highest removal rate (2.06 mg L^{-1} d^{-1}) was obtained for *N. oleoabundans*.

Acknowledgements

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Bibliography

[1] Gonçalves, A.L., Pires, J.C.M., Simões, M. (2017). Algal Res 24, 403-415.

[2] Singh, S., Singh, P. (2015). Renew Sust Energ. Rev. 50, 431-444.

Use of vinasse from winery by-products for nutrient removal and production of pigments by *Chlorella protothecoides* PC 60

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Key words: *Chlorella protothecoides*, vinasse, nutrient removal, pigment accumulation.

Abstract

Winery-distillation wastewaters (vinasses) are a major waste stream, which volume and pollution load vary due to the seasonal nature of wine production [1]. Wastewaters can be used as cheap nutrient sources for microalgae biomass production that could be used as a biofertilizer and/or as a source for biofuels and high added value products such as pigments [2]. It was evaluated the total organic carbon (TOC) and total nitrogen (TN) removal efficiencies of the green microalga *Chlorella protothecoides* cultivated in vinasse, as well as the accumulation of pigments in the biomass. *C. protothecoides* was cultivated in media enriched with vinasse at three concentrations (3.12, 6.25 and 12.5%) for 12 days. Pigments (chlorophylls and carotenoids) were quantified along the experiment by spectrophotometry, after extraction with acetone. After filtration of the culture samples, the filtrate was collected and characterized in terms of TOC and TN. The presence of vinasse, rich in organic compounds, in the medium changed the metabolism of the microalgae from auto to heterotrophy, which can be observed by the decrease of TOC and TN. *C. protothecoides* was very efficient in removing nutrients, showing rates around 90 % for TOC and TN removal. Vinasse causes stress on *C. protothecoides* cells which stimulated the production of the alternative chlorophyll c and carotenoids (with antioxidant activity) instead of chlorophylls a and b. Cultivation in 6.25% vinasse seems the best option to produce carotenoids, pigments with high commercial value, achieving a maximum productivity of 0.0096 mg carotenoids/(L.day).

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Bibliography

[1] Ioannou, L. A., Puma, G. L., Fatta-Kassinos, D. (2015). J Hazard Mater 286, 343-368.

[2] Trivedi, J. et al. (2015) Renew Sust Energ Rev 47, 295-307.

Genomic and physiological characterization of *Alcaligenes aquatilis* QD168 reveals a robust adaptive response to polluted marine environments

PC 61
SOC 08

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Key words: *Alcaligenes aquatilis*, abiotic stressor, benzene, salinity, functional genomics.

Abstract

Crude oil-polluted marine sediments are multi-stressor environments were the presence of hydrocarbons, as well as nutrient and salinity fluctuations challenge bacterial fitness. *Alcaligenes aquatilis* QD168 is a hydrocarbonoclastic marine bacterium isolated from crude oil-polluted marine sediment of the Quintero Bay, Valparaíso Region, Central Chile. A complete genome was obtained by PacBio sequencing, achieving a 4.32 Mb circular chromosome [1]. The aims of this study were to identify the genetic determinants involved in *A. aquatilis* QD168 survival to abiotic stressors and to characterize strain QD168 capability to overcome hydrocarbon pollution, nutrient scarcity and salinity fluctuation. Seven central pathways (*e.g.*, *cat* genes) and 16 peripheral pathways/reactions (e.g., *dmp* genes) for the degradation of aromatic compounds were identified in QD168 genome. Strain QD168 is able to grow on 14 aromatic compounds (*e.g.*, benzene, phenol, nicotinate, cinnamate) indicating the functionality of these pathways. QD168 benzene catabolic pathway was further studied by degradation assays and gene expression analysis. Phenol was identified as a metabolic intermediate. An induction by benzene of the transcripts encoding phenol hydroxylase and catechol 1,2-dioxygenase was observed. Genes encoding the short-chain length polyhydroxyalkanoates (scl-PHAs) biosynthetic pathway (*pha* genes) were identified. The synthesis of PHA by strain QD168 was determined. Genes encoding the osmoprotectant ectoine/5-hydroxyectoine (*ect* genes) are present in QD168 genome. Strain QD168 grew in R2A medium with up to 10% NaCl. The physiological adaptation of *A. aquatilis* QD168 to environmental stressors is useful for bioremediation of oil-polluted marine environments.

Bibliography

[1] Durán R. E., Barra-Sanhueza B., Salvà-Serra F., Méndez V., Jaén-Luchoro D., Moore E. R. M., Seeger M. (2019). *Microbiol Resour Announc*. 8:e01664-18.

POSTER COMMUNICATIONS

TOPIC 5 – Waste recovery

(PC 62 – PC 66)

Abundance of denitrification genes in four full-scale wastewater treatment plants — PC 62

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Key words: Wastewater treatment, nitrous oxide, nitrate, denitrification, qPCR.

Abstract
Wastewater treatment plants (WWTPs) are significant sources of greenhouse gases, mainly nitrous oxide (N_2O) [1]. Denitrification is a biochemical pathway of the N-cycle involved in the production of N_2O in WWTPs [2], which requires O_2-limited conditions, and consists of the sequential reduction of nitrate (NO_3^-) to dinitrogen (N_2) via the formation of nitrite (NO_2^-), nitric oxide (NO) and N_2O carried out by the enzymes nitrate (NapA/NarG)-, nitrite (NirK/NirS)-, nitric oxide (cNor/qNor)- and nitrous oxide (NosZI/NosZII)- reductases, encoded by the *napA/narG*, *nirK/nirS*, *norB* and *nosZ*I/*nosZ*II genes, respectively. The aim of this study was to quantify the abundance of denitrifiers in the aerated and anoxic bioreactors (BRs) of 4 full-scale WWTPs located in Granada (Spain) operated under real conditions throughout 5 months, by means of a quantitative PCR (qPCR) approach. Overall, it was observed that samples taken from the anoxic BRs displayed higher abundances of denitrification genes compared to the aerated BRs, regardless of the WWTP analysed. Generally considered, the ratio between genes involved in N_2O production (*nirK* + *nirS* + *norB*) and reduction (*nosZ*I + *nosZ*II) was higher in the anoxic BRs in each WWTP throughout the sampling period. The ratio between genes involved in nitrate (*napA* + *narG*) and nitrite (*nirK* + *nirS*) reductions was higher in Baza WWTP samples, concurring with the highest NO_3^- and NO_2^- concentrations. Our results indicate that monitoring the size of the denitrifying community in WWTPs could be an important key to design mitigation strategies, such as optimising the aeration regime, in order to avoid increases in the abundance of genes involved in N_2O production.

Bibliography
[1] Massara, T.M., Malamis, S., Guisasola, A., Baeza, J.A., Noutsopoulos, C., Katsou, E. (2017). Sci Total Environ 596-597, 106–123.
[2] Castellano-Hinojosa, A., Maza-Márquez, P., Melero-Rubio, Y., González-López, J., Rodelas, B. (2018). Chemosphere 200, 57-66.

Enzymatic transesterification of *Crambe abyssinica* oil for biodiesel production

PC 63

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Key words: Crambe abyssinica, non-edible oil, heterogeneous catalyst, enzymatic transesterification, response surface methodology.

Abstract

Fossil fuels are the most used fuels worldwide, being responsible for 81% of the total primary energy production [1], with high relevance in the transport sector (29%) which results in significant air emission levels (24% of CO_2 global emissions) [2]. Biodiesel help to decrease the emissions associated to fossil diesel consumption, being produced mostly from edible oils. The study of alternative feedstocks is important to increase the economic and environmental viability of biodiesel production. Crambe abyssinica is a promising non-food oilseed crop [3]. The enzymatic production of biodiesel is a relevant process route to be explored aiming at more integrated and environmental friendly production. In this study, biodiesel production from crambe oil by enzymatic transesterification was assessed with the objective of maximizing fatty acid methyl esters yield (EN 14103). Response Surface Methodology was applied with central composite design. The variables chosen were catalyst concentration (x_1), (2−8 wt.%) and methanol:oil molar ratio (x_2), (3:1−9:1). For each assay, the oil was added to a batch reactor in an orbital shaking incubator (200 rpm) during 24 h at 35 °C.

Enzymatic production allowed high yields (84 ± 5 wt.%) after 120 min, reaching 97.5 wt.% at 24 h (6:1 methanol:oil molar ratio, 8 wt.% of enzyme). A predictive model was obtained which estimated a maximum yield of 99 wt.% under such conditions. The study showed that biodiesel with high purity might be obtained by enzymatic transesterification of crambe oil. Further studies are relevant to ensure the viability of its use at higher scale.

Acknowledgements

This work was financially supported by project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC) and Project "LEPABE-2-ECO-INNOVATION" – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). The authors also acknowledge Foundation for Science and Technology for funding Emanuel Costa´s (SFRH/B PD/73809/2010) PhD fellowship.

Bibliography

[1] International Energy Agency, World energy balances: overview. 2018.
[2] Solaymani, S. (2019). Energy, 168, 989-1001.
[3] Costa, E., et al. (2019). Industrial Crops and Products 129, 51-58.

Evaluation of glycerides and free fatty acids conversion through enzymatic hydroesterification of soapstock acid oil aiming biodiesel production

PC 64

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Key words: Acid oil, soapstock, lipase, biodiesel, enzymatic hydroesterification.

Abstract

Currently, feedstock cost is still a significant limitation aiming sustainable biodiesel production [1]. Soapstock is a major by-product from vegetable oil refining with a market price of about one tenth of crude vegetable oil [2], being conventionally treated by acidification aiming lipids recovery [3]. The goal of this study is to characterize glycerides and free fatty acids (FFA) conversion by hydroesterification, comparing it to esterification, in order to support an improved process development aiming at biodiesel production from such by-product. Acidification with HCl was performed, the resultant acid oil containing about 60 wt.% of FFA, 22.6 wt.% of triglycerides, 2.2 wt.% of diglycerides, 1.2 wt.% of monoglycerides, 3.5 wt.% of water and 7.2 wt.% of phospholipids and other minor compounds. The enzymatic hydrolysis was carried out under 35 °C, 200 rpm, 3 wt.% of lipase from *Thermomyces lanuginosus* and 1:0.5 water:oil ratio (w:w); following, enzymatic esterification was performed under 35 ˚C, 200 rpm, 2 wt.% of lipase from *Thermomyces lanuginosus* and 2:1 molar ratio of methanol to acid, during 7 h (when only esterification was performed the same conditions were used for comparison). The reaction was monitored through the quantification of the FFA and glycerides, comparing the results of both processes. The conversions were evaluated to support the application of such processes aiming the development of biodiesel production.

Acknowledgements

This work was financially supported by: project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC). Project "LEPABE-2-ECO-INNOVATION" – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Bibliography

[1] Manaf, I.S.A., et al. (2019). Energ Convers Manage 185 508-517.

[2] Wang, Z.-M., et al. (2007). Korean J. Chem. Eng 24 (6), 1027-1030.

[3] Laoretani, D.S., C.D. Fischer, and O.A. Iribarren. (2017). Food Bioprod Process 101 177-183.

Influence of microbial community on the composting processes using winery wastes PC 65

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Key words: Composting, grape stalks, winery activated sludge, waste recycle, microbial diversity.

Abstract

Composting is a very interesting process from both economic and ecological point of view. In this process, solid organic wastes are decomposed under semi-controlled conditions into a stabilized product. Although generally regarded as being capable of achieving substantial reductions in volume, eutrophication potential and pathogen level, its overall performance is influenced by factors such as composition, aeration rate and ambient temperature [1]. Despite the occurrence of thermophilic range temperatures during the composting process is considered important to assure good sanitization, some mixtures of wastes present technical challenges even when the carbon-to-nitrogen ratio is optimal [2]. A good example of these limitations is the co-composting of winery waste activated sludge and certain solid residues resulting from the wine industry.

The purpose of this study was to obtain useful information from an industrial point of view about the co-composting of winery waste activated sludge and grape stalks.

Different amounts of a mixture containing both materials were co-composted outdoors at pilot-scale and at lab-scale under different temperatures and aeration rates for 2 months. The potential negative effects of reduced microbial diversity on the composts maturity were determined via germination tests and physicochemical analysis.

None of the experiments showed the occurrence of a thermophilic phase, which might suggest biological suppression by the mesophilic microorganisms acclimated to grape phenolic compounds. Even in the absence of a thermophilic stage, the horticultural quality of some substrates indicated that this process could be a versatile tool for the recycle of these wastes.

Bibliography

[1] Anand, D., Veerakumar, V., Gabhane, J., Prince William, S. P. M., Bhange, V. P., Vaidya, A. N., Patil, M. P., Bhattacharyya, J. K., Wate, S. R. (2012). Intern J Recent Trends Science Techn, 5 (1), 09-15.

[2] Roman, P, Martinez, M. M., Pantoja, A. (2015). Santiago: FAO UN.

Biogeochemical behavior of strategic elements in soil–plant systems in an old gold mine in NW Spain **PC 66**

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Key words: Mine-waste, rare earth elements, phytomining, biogeochemistry.

Abstract
Mining activities produce large amounts of waste, consisting of low grade ores, mine tailings, sediments or abandoned sites, which can become secondary sources of metals and other elements of strategic interest. The biogeochemical study of these systems can contribute to the development of plant-based technologies for the recovery of metals from secondary resources. In this context, our aim is to identify sites with a high content of strategic elements and plant species with the capacity to accumulate them in their aboveground biomass. For this purpose, we carried out a prospecting study in an old gold mine in NW Spain. Samples of rocks, soils and plants were collected throughout the mining area and analyzed for the total content of 20 strategic elements. Additionally, geochemical fractions were evaluated in soils using a sequential extraction procedure. The mine wastes showed significant levels of Au and Mo (up to 2 and 100 mg / kg, respectively), moderately high levels of Sb (up to 100 mg / kg) and very high levels of As (up to 1000 mg / kg), with the residual fraction being the most important. These levels were consistent with the sulphide assemblage of the deposit, formed by arsenopyrite and pyrite, with stibnite and molybdenite. Relatively high concentrations of Mo were found in grassy plant species, of As in *Lonicera* sp. and of La in *Dryopteris* sp. The results help our understanding of the environmental behavior of these elements and are useful in the development of green technologies for metal recovery.

POSTER COMMUNICATIONS

TOPIC 6 – Bioremediation of priority pollutants

(PC 67 – PC 87)

Effect of exposure time on cesium uptake by *Ceratophyllum demersum* L. PC 67

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Key words: Cs, phytoremediation, accumulation, hydrophyte, contamination.

Abstract
Cesium is an element naturally occurring in the environment. However, its radioactive isotopes ($^{134,137}Cs$) produced by nuclear fission are a threat to the biosphere. Phytoremediation is a commonly tested method to remove cesium from the environment. While application of terrestrial plants is well studied, utilization of aquatic plants for phytoremediation did not receive so much attention [1]. Therefore, the aim of this study was to determine whether *Ceratophyllum demersum* L. (hornwort) is a good candidate for phytoremediation of aquatic habitats. Phytoremediation ability of *C. demersum* was tested under greenhouse condition for 8, 16 and 24 days. The plants were exposed in randomized block experiment to different concentrations of stable CsCl (0, 0.008, 0.033, 0.133, 0.267, 0.533, 0.800, 1.067 and 1.333 mM). The results revealed a significant effect ($p < 0.001$) of exposure time on the Cs^+ uptake from the solution. Average Cs^+ removal rates were 7.59%, 14.22% and 17.76% for 8, 16 and 24 days respectively. Furthermore, the accumulated amounts of cesium by plants were significantly dependent ($p < 0.001$) on the level of contamination. Hornwort was also able to resist phytotoxic effects of cesium for 16 days without significant effects ($p > 0.05$) on health. Even after 24 days of exposure plants resisted with no significant issues ($p > 0.05$) until 0.533 mM concentration was reached, where health started to deteriorate significantly ($p < 0.001$). These results indicate that Hornwort have potential for remediating aquatic habitats, especially in case of acute events, where short duration of the phytoremediation may take place.

Bibliography
[1] Burger, A., et Lichtscheidl, I. (2018). Science of the Total Environment, 618, 1459-1485.

Effect of copper co-application on the effectiveness of a biobed to remove pesticides

PC 68

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Key words: Bioremediation system, degradation, microbial activity, pesticides, copper.

Abstract

Biobed bioremediation systems have been proved to effectively remove high pesticide doses from agricultural wastewater [1]. Inorganic compounds, such as copper-based fungicides, are frequently co-applied with organic pesticides in agriculture and represent the main fraction in agricultural wastewater. Copper might alter the microbial and enzyme properties of the biomixture in the biobed and, therefore, might reduce their capacity to biodegrade pesticides [2]. The current study explored, for the first time, the effect of copper on the effectiveness of a biomixture composed with soil, vermicompost of olive mill cake and olive tree pruning (1:1:2, v:v:v) for removing eight pesticides with different physicochemical properties. Pesticides were applied to the biomixture at 10 µg g^{-1} each and copper oxychloride (50% w/w) was applied at two dosages: 100 µg g^{-1} (concentration typically found in agricultural wastewater from olive orchards after one copper application) and 400 µg g^{-1} (to simulate several copper applications). Pesticide degradation and dehydrogenase activity were measured in the biomixture at different incubation times. After 60 days of incubation, pesticide degradation ranged from 41 to 68%, depending on the pesticide, except for quizalofop-P (97%). The relatively low degradation observed might be explained by competitive phenomena between pesticides. Dehydrogenase activity decreased with copper concentration. However, the lower enzymatic activity observed in the treatments with copper did not affect pesticide degradation, as indicated by non-significant differences ($p > 0.05$) between pesticides degradation curves in the biomixture with or without copper. Our results suggest that copper can be applied to a biobed system without compromising its effectiveness.

Acknowledgements

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Bibliography

[1] Delgado-Moreno, L.; Nogales, R.; Romero, E. (2017). J. Environ. Manage. 204(1), 160-169.

[2] Wang, Q.Y.; Zhou, D.M.; Cang, L. (2009). Soil Biol. Biochem. 41(7), 1504-1509.

Exploring the biodiesel and toluene anaerobic bioremediation potential in soils

PC 69

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Key words: Biodiesel, toluene, microbial community structure, anaerobic bioremediation, soil.

Abstract

Biodiesel is a renewable fuel which can be mixed with toluene (present in fossil fuels) and accidentally released into anoxic ecosystems, impacting soil microbial communities. The aim of this study was to examine, under nitrate-reduction conditions, the biodegradation of toluene in the presence of two different types of biodiesel (sunflower and rapeseed); and, the biodiesel impact on the bacterial community structure. Sediment samples were spiked individually with toluene, biodiesel, and their blends in laboratory designed microcosms. Degradation of biodiesel alone and blends was monitored by directly measuring the substrate or indirectly by determining nitrate removal during the course of the experiments. Denaturing gradient gel electrophoresis was used to assess impacts on the bacterial community structure exposed to biodiesel and toluene alone as well as to the blends. The results of this study showed that toluene and biodiesel were completely degraded within 10 days. Biodiesel significantly affected the bacterial community structure at a similar magnitude, independently of its origin. Additionally, toluene impacted the bacterial community and denitrification process to a lower extent than biodiesel and a clear decrease in the relative bacterial richness and diversity was shown in samples with biodiesel and blends. To the best of our knowledge, this is one of the first reports describing degradation of biodiesel alone and blends under nitrate-reducing conditions, and also the effects of these compounds on the denitrification process. Additional studies are required to understand the effect of sediment properties on biodiesel biodegradation processes and if the identified bacterial community shift impacts ecosystem functions. Overall, results might be important in implementing bioremediation strategies of renewable and fossil fuel blends in anoxic environments.

Bibliography

Ribeiro H., da Silva J.G., Jesus J., Magalhães M., Dias J.M., Danko A.S. (2019). J Soils Sediments 19(1), 439–450.

Enrichment of soil mixed microbial cultures onto biochar as a proxy for landfarming techniques **PC 70**

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Key words: Contaminated soils, mixed microbial cultures, biodegradation, biochar.

Abstract

Estarreja soils are sinks of long-term contamination due to a Chemical Industrial Complex. We hypothesized that: (i) contaminated soils present microbial communities with better biodegradative performance compared to non-contaminated soils; (ii) such microbial communities can be cultivated *ex situ* to boost their effectiveness in environmental remediation; and (iii) biochar (pyrolysed biomass) can act as a biofilm carrier, thus allowing further field-scale spreading of the biocatalysts.

Soils were sampled from two representative sites (contaminated and non-contaminated) across Estarreja and their microbial communities cultivated inside aerobic bioreactors fed-batch operated with glucose, thereafter progressively replaced with toluene. A third bioreactor was added with biochar to assess biofilm formation. A landfarming assay was performed with the bioactivated biochar in the contaminated soil spiked with glucose and toluene, and the respirometric response quantified.

Both microbial C-biomass and respiration rate were systematically smaller in the contaminated soil (0.9 vs. 1.2 g-C kg^{-1} and 1.9 vs. 4.4 mg-C $kg^{-1}h^{-1}$ respectively), resulting in a lower metabolic quotient yet anticipating a better carbon use efficiency. Indeed, the contaminated soil culture showed a higher COD removal efficiency (48-64% vs. 30-63%) when subjected to toxic conditions (>50% toluene feeding). Although biofilm development was not directly quantified, soil application of the bioactivated biochar resulted in 150% increase in both soil respiration and microbial C-biomass when compared to positive controls (abiotic biochar). These results anticipate the ability of contaminated soil microbial communities to perform effective environmental remediation as well as their capacity to form biofilms onto biochar, which is useful for landfarming techniques.

Implementing a nickel phytomining system in a serpentine quarry as a post-mining land management strategy: field results after two growth seasons

PC 71

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Key words: Agromining, organic amendments, hyperaccumulator, plant-growth promoting bacteria, mine-soil restoration.

Abstract

Nickel phytomining is a green strategy for metal recovery which could potentially be applied to mine affected areas, providing an additional metal source to primary ores and, at the same time, reducing hazardous waste and assisting site restoration. Here we present the results of a two-year phytomining field trial in a serpentine quarry in NW Spain. Specific objectives were to optimise the conditions for plant growth through the selection of the most adequate hyperaccumulating plant species, soil amendments or crop patterns, and the use of bacterial strains with plant growth promoting (PGP) traits and/or which influence plant Ni uptake.

Hyperaccumulators included native populations of *Noccaea caerulescens* and *Odontarrhena serpyllifolia* and the Mediterranean species *Bornmuellera emarginata* and *Odontarrhena muralis*. Two fertilisation regimes were tested (inorganic NPK or composted sewage sludges (CSS)). Plant survival, biomass and Ni yields, soil physico-chemical properties, as well as soil microbial activity and functional diversity were analysed. During the second cropping period, different addition rates of CSS (2.5, 5 and 10%) or distilled grape bagasse (at 2.5% w/w), as well as the Mediterranean spp. *Bornmuellera tymphaea*, were assessed. Also, the benefits of inoculating plants with the PGP bacterial strain *Paenarthrobacter nitroguajacolicus* LA44 were evaluated. All plant species were able to establish and growth in the mine-soil and to generate moderate Ni yields, especially *B. emarginata* and *O. muralis*. Bacterial inoculation stimulated plant growth, which resulted in some cases in higher Ni yields. Finally, soil physico-chemical and biological properties were improved after plant growth, particularly in compost soils.

Flavodoxin FldX1 of *Paraburkholderia xenovorans* LB400 enhances resistance to oxidative stress and improves growth on hydroxyphenylacetates

PC 72
SOC 09

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Key words: *Paraburkholderia xenovorans* LB400; flavodoxins; 3-HPA, 4-HPA.

Abstract

Paraburkholderia xenovorans LB400 is a model bacterium able to degrade 3- and 4-hydroxyphenylacetate (HPA) and other aromatic compounds. This aerobic catabolism of aromatic compounds produce oxidative stress, limiting their degradation [1]. Flavodoxins are small electron transfer proteins, which are induced during oxidative stress and iron starvation [2]. The overexpression of these proteins in bacteria enhanced the resistance to oxidative stress [3]. The aim of this study was to evaluate the protective effect of *P. xenovorans* LB400 flavodoxins during oxidative stress induced by 3- and 4-HPA degradation. Gene encoding flavodoxin FldX1 of *P. xenovorans* LB400 were cloned in a plasmid and overexpressed in this strain. Recombinant strain were exposed to paraquat, a redox-cycling aromatic herbicide. Bacterial sensitivity, survival and biomolecule damage were studied. The growth of *P. xenovorans* recombinant strain on 3- or 4-HPA as sole carbon source were analysed. The recombinant strain showed less growth inhibition than the control strain in 20 mM paraquat and higher survival after exposure to 1 and 20 mM paraquat. Strain p2-FldX1 displayed lower lipid peroxidation after incubation with 1 mM paraquat than control strain. Recombinant *P. xenovorans* strain exhibited faster growth in 3- and 4-HPA than control strain. In conclusion, the flavodoxin FldX1 of *Paraburkholderia xenovorans* LB400 confers protection to oxidative stress and enhances the growth on the aromatic compounds 3- and 4-HPA.

Bibliography

[1] Méndez, V., Agulló, L., González, M., Seeger, M (2011). PLoS ONE. 6, e17583.
[2] Sancho, J. (2006). Cell. Mol. Life Sci. 63, 855–864.
[3] Coba de la Peña, T., Redondo, F.J., Fillat, M.F., Lucas, M.M., Pueyo, J.J. (2013). J Appl Microbiol 115, 236-246.

Genomic analysis of *Acinetobacter radioresistens* DD78: A novel approach to prospecting native hydrocarbon-degrading bacteria PC 73

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Key words: Petroleum, bioremediation limitation, genomic, biosurfactant, salinity-stress.

Abstract

Bioremediation is an efficient and ecological technology for the clean-up of polluted saline sites. Bioremediation by hydrocarbon-degrading bacteria can be limited by salinity and low hydrocarbon bioavailability. The aims of this study were the genomic characterization of hydrocarbons-degrading pathways, and proteins involved in the stress response in *Acinetobacter radioresistens* DD78. DNA extraction was performed used CCUG-Marmur protocol. Genome sequencing was performed using PacBio RSII platform. Reads were assembled with HGAP, v3.0. Annotation was performed using Prokka. Genes were identified by BLAST, using Uniprot-KB/Swiss-Prot database. For phylogenetic identification, MLSA were performed using MrBayes v.2.3.6. *A. radioresistens* was grown on Bushnell Haas (BHB) medium with *n*-hexadecane and diesel 0.1% (w/v) as sole carbon source at 30ºC. Genome of *A. radioresistens* is distributed in four replicons: one chromosome (3 Mb) and three plasmids (90, 81 and 70 kb). Catabolic genes, encoding an alkane monooxygenase and rubredoxin NAD(H) reductase/rubredoxin system, benzoate and anthranilate pathways were identified. *A. radioresistens* DD78 was able to grow in presence of up to 4% (w/v) NaCl. Osmoprotectant betaine operon and osmoprotectant transporters: osmo-dependent choline transporter, sodium/proline symporter and aspartate/alanine antiporter genes were identified. Three biosynthetic genes associated to the biosurfactant alasan were also identified. *Acinetobacter radioresistens* DD78 is an attractive halotolerant hydrocarbonoclastic strain for bioremediation of hydrocarbon-polluted saline soils.

Novosphingobium **sp. HR1a as an excellent biotechnological agent for polycyclic aromatic hydrocarbons degradation and detection** PC 74

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Key words: Rhizoremediation, polycyclic aromatic hydrocarbons (PAHs), biosensor.

Abstract

Novosphingobium sp. HR1a grows using 2-, 3-, or 4-rings aromatic hydrocarbons as the sole carbon source. This strain is able to persist in high numbers in soils (vegetated or not) contaminated with polycyclic aromatic hydrocarbons (PAHs) reducing significantly the concentration and the deleterious effects on the plant growth of these contaminants. Therefore, this microorganism constitutes an excellent candidate to be used in rhizoremediation strategies. To decipher the molecular basis of these biotechnological capabilities, *Novosphingobium* sp. HR1a genome was sequenced. Two transposons containing genes involved in the degradation of PAHs and in its regulation were identified. We have demonstrated the crucial role of the PahAB dioxygenase and the PahR regulator, in the degradation of different PAHs. Mutants in these genes were unable to grow in minimal medium with PAHs as sole carbon source. Using constructions of *gfp* gene (encoding the green fluorescent protein) fused with the dioxygenase or the regulator promoter, we determined that the expression of these genes were specifically induced in the presence of some PAHs and intermediates of their metabolic pathway. Furthermore, we have identified a second regulator, encoded by *orf1998*, which modulates the expression of the *pahAB* and *pahR* genes. To our knowledge, this is the first time that a so complex regulatory circuit controlling the degradation pathway of high-molecular weight PAHs has been investigated. The knowledge on the regulatory elements of PAH degradation has allowed us the designing of biosensors for monitoring environments contaminated with oil-derived mixtures.

Bibliography

[1] Segura A, Hernández-Sánchez V, Marqués S, Molina L. (2017). Sci Total Environ 15, 590-591:381-393.

The potential of *Sarcocornia perennis* applied to a floating wetland island in port marina environment **PC 75**

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Key words: Floating wetland islands, phytoremediation, halophytes, water quality, marina.

Abstract
Floating Wetland Islands (FWI) are a relatively recent nature-based solution, presenting promising remediation potential for the removal of nutrients and other types of pollutants. However, knowledge associated to their ability for contaminant removal combined with the creation of habitats is still scarce and more research is needed to support this ecotechnology.
The present study aims to investigate the application of FWI to promote water quality enhancement and habitat establishment in port marina environments, a harsh environment due to water salinity. To our knowledge this has not yet been explored being an important issue to address for a broaden application of this technology. Having that in consideration, a pilot FWI was implemented in the marina of the Porto Cruise Terminal in Matosinhos. The pilot comprised an agglomerated cork platform (Cork Floating Island®, Bluemater, Lda) where 7 halophyte species were tested. The first months of this study showed that *Sarcocornia perennis* had the best resilience and performance.
Mapping of the micro and macro biotic communities associated to this FWI has been carried out, through microscopy and molecular biology tools, to understand their dynamics along time and possible relation to water depuration processes. Several physical-chemical parameters (e.g. pH, salinity, temperature), nutrients, Total Petroleum Hydrocarbons and Polycyclic Aromatic Hydrocarbons were analyzed to evaluated water quality. Obtained results will be presented.

Acknowledgements
This research was partially supported by the Strategic Funding UID/Multi/04423/2019 through national funds provided by FCT–Foundation for Science and Technology and European Regional Development Fund (POCI-01-0145-FEDER-007621), in the framework of the programme PT2020.

Cesium phytoremediation by three species of aquatic plants PC 76

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Key words: Cesium, phytoremediation, *Calla palustris, Ceratophyllum demersum, Elodea Canadensis.*

Abstract

Radiocaesium started to enter the environment as a result of the aboveground testing of nuclear weapons in 1945 [1]. Later, other anthropogenic activities, such as nuclear accidents, operational drains from nuclear installations, the application of Cs in medicine and other sources, have contributed to increase concentrations of radiocaesium in all parts of the environment. The aim of this paper is to compare phytoremediation ability of three aquatic plants and their uptake of Cs from contaminated solution.

The efficiency of *Calla palustris, Ceratophyllum demersum* and *Elodea canadensis* to remove Cs from contaminated solution was tested during 8 days exposition under greenhouse conditions. The experiment was conducted with stable Cs, provided in the form of CsCl. The plants were exposed to the range of Cs^+ concentrations (0 to 265 mg). The phytoremediation ability of the plant was assessed using removal (%) and bioaccumulation factor (BCF) [2].

The experiment exhibited that the efficiency of Cs removal from water by all tested species is affected by the concentration of Cs^+ ions in water, with the highest efficiency for the medium concentrations, while with the increasing concentration of Cs in solution, the efficiency decreased. The average removal rate was 10% for *C. palustris*, 5% for *C. demersum* and 2.3% for *E. canadensis.* The experiment showed that *C. palustris* is among tested plants most efficient to remove Cs from contaminated solution, while *E. canadensis* showed phytotoxicity effects resulting in significant biomass loss already in low Cs^+.

Bibliography

[1] Burger, A., Lichtscheidl, I. (2018). Sci Total Environ 618, 1459-1485.

[2] Wang, X., Chen, C., Wang, J. (2017). Int J Phytoremediation 19, 402-412.

Enhanced oil spill bioremediation with Corksorb PC 77

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Key words: Corksorb, alkanes, bioremediation, *Alcanivorax borkumensis* SK2, *Rhodococcus opacus* B4.

Abstract

Regranulated cork particles are by-products of cork stopper production, which present very high hydrophobicity and oil sorption capacity after thermal treatment [1]. These thermally treated granules have been used as absorbents in the remediation of oil spills under the commercial brand Corksorb (Corticeira Amorim, S.G.P.S.). Once saturated with oil, cork should be regenerated for cyclic reuse. For that, hydrocarbonoclastic bacteria can be applied to degrade the oil components. Here, we hypothesize that Corksorb granules can potentially stimulate the activity of these bacteria, due to their unique chemical composition, structure and properties [2], thus improving *in situ* bioremediation of oil spills. To test this hypothesis, bacterial growth and hydrocarbons biodegradation were assessed in pure cultures of *Alcanivorax borkumensis* SK2 or *Rhodoccocus opacus* B4 incubated with a mixture of alkanes, and compared with incubations in which the alkanes were sorbed in corksorb. Growth of *Alcanivorax borkumensis* SK2 in alkanes was 1.5 times higher in the assays with corksorb, relatively to the assays without corksorb. Moreover, 72% of the added alkanes were biodegraded in the presence of corksorb, while in its absence only 47% were removed. For *Rhodococcus opacus* B4, hydrocarbons consumption reached 96% and 88% in the presence and absence of corksorb, respectively, although no significant effect could be detected on growth. These results show that corksorb stimulates the activity of hydrocarbonoclastic bacteria, therefore presenting a high potential for improving *in situ* bioremediation of hydrocarbon-contaminated environments by combining absorption with stimulated biodegradation. The mechanisms underlying this stimulatory effect are currently under study.

Bibliography

[1] Pintor, A.M.A., Ferreira, C.I.A., Pereira, J.C., Correia, P., Silva, S.P., Vilar, V.J.P., Botelho, C.M.S., Boaventura, R.A.R. (2012). Water Res 46(10), 3152-3166.

[2] Silva, S.P., Sabino, M.A., Fernandes, E.M., Correlo, V.M., Boesel, L.F., Reis, R.L. (2005). Int Mater Rev 50(6), 345-365.

Georeferenced library of native microbial consortia: a starting point to bioremediate oil spills

PC 78

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Key words: Bioremediation, oil spills, georeferenced library, native microbial communities, next-generation sequencing.

Abstract

During the clean-up of oil spills, conventional treatments are important to control the diffusion and drift of the oil, but they are not suitable for ecological restoration. In this sense, bioremediation can arise as a more sustainable and cost-efficient tool to accelerate the recovery of oil-contaminated environments, by using native microorganisms capable of degrading petroleum compounds. This work aimed to develop a georeferenced library of native microbial consortia with the capability to degrade petroleum hydrocarbons. Seawater samples were collected along the North-West coast of the Iberian Peninsula. These samples were used for microcosms enrichment experiments exposed to crude oil and spiked with nutrients, for two weeks. Afterwards, the initial seawater samples and the final microcosm samples were filtered through Sterivex™ filters for 16S rRNA next-generation sequencing. Taxonomic characterization was made with QIIME2 and the predicted functional profile was obtained by using PICRUSt. Results showed a decrease in microbial richness and diversity after the enrichment with petroleum. In addition, it was also observed an increase in abundance of microorganisms capable of degrading petroleum hydrocarbons, where oil-enriched communities were mainly composed by the genera *Alcanivorax* spp., *Pseudomonas* spp., *Thalassospira* spp. and members of the family *Flavobacteriaceae*. The predicted functional profile showed that the oil-enriched communities had potential to degrade aromatic hydrocarbons such as naphthalene, polycyclic aromatic hydrocarbons, ethylbenzene, toluene and xylene. This work is a starting point for future implementation of new and environmental-friendly approaches to mitigate oil spills in marine environments.

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Mycoremediation of trichloroethene polluted soils by ligninolytic fungi PC 79

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Key words: Chlorinated organic pollutants, *Pleurotus*, *Agaricus*, soil.

Abstract

Trichloroethylene (TCE), a toxic chlorinated organic compound which has been widely used as a solvent in industrial cleaning solutions, is a hazardous environmental pollutant since it is carcinogenic. It is present in soil, air and water. Most studies have been performed to remove TCE from air and water by using different anaerobic bacteria species in addition to a few ones using white-rot fungi, meanwhile there are hardly any in soil [1]. The objective of the present work is to assess TCE removal efficiency of two fungi of genus *Pleurotus* (*Pleurotus ostreatus* and *Pleurotus eryngii*) and *Agaricus bisporus* growing on two different soils (clay and sandy loam). Those fungi have different enzymatic systems (CYP 450, laccase (Lac), Mn peroxidase (MnP)) capable to aerobically co-metabolize TCE to less harmful compounds [2].

Two soils were spiked with 70 and 140 mg/kg of TCE in glass containers, which were inoculated with the fungi using wheat straw as a carrier, then closed and sealed with parafilm. The assay took 4 weeks. TCE was weekly analysed by headspace GC coupled with an electron capture detector. Lac and MnP activities were measured according to Garcia-Delgado et al. [3].

TCE was removed from the two soils reaching dissipation rates of 100% by the three fungi in sandy loam and higher than 92% by *P. eryngii* and *A. bisporus* in clay. Extracellular enzymes were poorly expressed which agreed with the hypothesis of aerobic co-metabolization of TCE with the involvement of CYP 450 [2].

Bibliography

[1] Upadhyay, S.N., Dubey, S.K. (2014). Crit Rev Biotechnol 34, 101-114.

[2] Marco-Urrea, E., Parella, T., Gabarrell, X., Caminal, G., Vicent, T., Reddy, C.A. (2008). Chemosphere 70, 404-410.

[3] García-Delgado, C., Yunta, F., Eymar, E. (2015). J Hazard Mater 300, 281-288.

Study of the efficacy of a bioremediation system treating a contaminated sediment by microalgae ecotoxicity evaluation PC 80

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Key words: Bioremediation, sediment, fungi, ecotoxicity, microalgae.

Abstract

A bioremediation batch test was performed on a sediment sample collected in Bento Rodrigues, after the mining dam failure in Mariana, Brazil. The experiment was carried out in a patented prototype [1] with the application of microbial consortium that includes the fungus *Aspergillus flavus* and *Penicillium spp*. This work intends to evaluate the efficacy of bioremediation by microalgae ecotoxicity evaluation.

Bioremediation experiment was a batch closed system without draining. Besides sediment and inocula, it included the same formulation from a previous study where mangrove oil spill bioremediation was achieved with success: oil, water (sea water) and castor cake (biodiesel production residue). From bioremediation experiment initial sediment and all resulting identifiable products were analyzed corresponding to 4 samples: initial sediment, bioremediate sediment (end of experiment), superficial layer of biorremediate sediment (end of experiment) denominated "crystal" due its characteristics.

For ecotoxicity evaluation microalgae *Pseudokirchneriella subcapitata* was used, and method was adapted from OECD TG 201.

Growth inhibition was determined for each sample and results indicate a significant reduction in the ecotoxicity potential of the sediment. An ecotoxicity abatement of 66%, 41% and 45% was found for each assay, comparing initial sediment with bioremediate sediment, which represents an ecotoxicity average reduction of 51% (STD of 11%). Sample denominated "crystal" on the other end showed an increase in ecotoxicity, which can be due to inhibition to high NaCl content (X-ray results) coming from added sea water, since no metals were found. From ecotoxicity tests, it can be concluded that bioremediation was effective on lowering the ecotoxicity potential of the sediment.

Bibliography

[1] Quintella, C. M., Gonçalves, O. (2012). Brazilian Patent no. PI 10 2012 033531 0.

LMWOA in root exudates as interfaces against toxic metals pollution in soil-plant interaction

PC 81

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Key words: Root exudates, citric acid, oxalic acid, carbonate soil, toxic metals.

Abstract

Root and leaf exudates of epidermal cells are interfaces where plants perform crucial interaction with environment, significant for protection of primary plant metabolism from environmental pollutants. Terrestrial plants during evolution developed strategies to cope with toxic metal pollution in rhizosphere and apoplast of root epidermis. In root epidermal cells, toxic metals exclusion is based on interaction of root exudates (phytosiderophores, carboxylate anions of low molecular weight (LMW) organic acids (oxalic, citric, malic), uronic acids etc.) with metal ions and complexation in rhizosphere environment [1]. In this study, we will present our results about influence of oxalic and citric acids in concentration of 0.01 M, 0.05 M and 0.1 M on concentration of Pb, Cd, Fe, Zn, Mn and Cu in carbonate soil solution. Citric acid (all applied concentrations) increased Cd concentration in soil solution while oxalic had no significant effect. At a concentration of 0.01 M, oxalic acid had no effect on Zn concentration but citric acid efficiently extract Zn from soil. Effect of oxalic and citric acid on Pb in soil solution was significantly affected by extraction time and applied concentration.

The data showed that tested organic acids can increase concentration of metals or induced formation of metal-carboxylate complexes in soil solution, which importance is difficult to assess since it depends on the physiological status of the plant and the pH value of the rhizosphere, which can be significantly altered under stress conditions.

Bibliography

[1] Adeleke, R., Nwangburuka, C., Oboirien, B. (2017). S Afr J Bot 108, 393-406.

Phytostabilization of a contaminated military site using biofuel crop and soil amendments: A field study

PC 82

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Key words: *Miscanthus*, phytostabilization, bioaccessibility, substances, amendments.

Abstract

Extensive areas of productive land can be contaminated by potentially toxic substances due to military activities. Field experiment was initiated in 2016 at Fort Riley, KS military base, in an area with elevated soil lead (Pb). The main objectives of the study were to determine feasibility of using miscanthus for phytostabilization of this contaminated military site and to evaluate the effect of soil amendments on miscanthus growth, soil-plant Pb transfer, bioaccessibility of soil Pb, and soil health. Five treatments were: (i) control plots without tillage and left with natural vegetation, (ii) no tillage, no additional amendments and planted with miscanthus, (iii) tilled soil, no additional amendments and planted with miscanthus, (iv) tilled soil amended with inorganic P (triple superphosphate) and planted with miscanthus, and (v) tilled soil amended with organic P source and planted with miscanthus. Soil and plant samples were analyzed each year. Results from the first year (2016) showed that tilling and soil amendments increased the dry matter yield, and that effects of soil amendments on plant Pb concentration and bioaccessibility of soil Pb were promising. Although there were no differences in the biomass (2017 and 2018), Pb concentrations in plant tissues were continuously lower in biosolids amended plots compared to plots with no added P. Similarly, soils from biosolids amended plots continued to show a low concentration of bioaccessible Pb in 2017 and 2018. Enzyme activities and microbial biomass using phospholipid fatty acids (PLFA) and some soil health parameters were also determined; the results will be discussed.

Intensification of heterogeneous photocatalytic processes using an innovative mili-photoreactor towards indoor air treatment

PC 83

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Key words: Heterogeneous photocatalysis, mili-Photoreactor, indoor air treatment, n-decane, illumination mechanism.

Abstract

Indoor air quality (IAQ) has become an important concern due to the increased amount of personal time spent in indoor environment, having a significant impact on human health, comfort and productivity [1]. Intensification of heterogeneous photocatalytic processes using an innovative mili-photoreactor (NETmix) towards indoor air treatment is been considered a promising strategy, considering the abatement of volatile organic compounds (VOCs), nitrogen oxides (NO_X) and sulphur dioxide (SO_2). The mili-photoreactor consists of a series of cylindrical chambers interconnected by prismatic transport channels mechanically engraved in a stainless steel slab irradiated by UVA LEDs [2]. The network of chambers and channels and/or the borosilicate slab will be coated with TiO_2 thin films using a simple spray pyrolysis method.

The illumination efficiency is one of the biggest limitations of photocatalytic processes in large-scale applications. Due to the specific internal geometry of the NETmix, some shadow zones can occur inside the reactor, reducing significantly the real illuminated catalyst coated surface [3]. Taking this into account, a reactor with a lower depth was designed and constructed, going from 3 mm to 1 mm, resulting in a higher efficiency on *n*-decane removal, used as model VOC.

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Bibliography

[1] Zhong, L., Haghighat, F. (2015). Build Environ 91, 191-203.

[2] Laranjeira, P.E., Martins, A.A., Lopes, J.C.B, Dias, M.M. (2009). AIChE J 55, 2226-2243.

[3] da Costa Filho, B.M., Araujo, A.L.P., Padrão, S.P., Boaventura, R.A.R., Dias, M.M., Lopes, J.C.B., Vilar, V.J.P. (2019). Chem Eng J 366, 560-568.

Cork-based permeable reactive barriers coupled to electrokinetic for interrupting pollutants to reach groundwater: A case study on hexavalent chromium-contaminated soil

PC 84

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Key words: Hexavalent chromium, soil remediation, electrokinetic technology, permeable reactive barriers, cork granules.

Abstract
Soils and water are frequently contaminated by hexavalent chromium (Cr(VI)) due to stormwater run-off, accidental spills and uncontrolled leaching from contaminated dumps or storage ponds [1,2]. During stoppers production, a large amount of cork by-products is generated and, usually, is used as low-value material. Cork presents a macroporous honeycomb-like structure composed by 40% of suberin, which can act as (i) electron donor for the reduction of Cr(VI) to trivalent chromium (Cr(III)), and as (ii) a binder for the reduced Cr(III) in aqueous solutions. Therefore, this work proposes a novel and green technology for the remediation of soils contaminated with Cr(VI), avoiding it to reach groundwater. The treatment strategy combines electrokinetic (EK) with permeable reactive barriers (PRBs) composed by cork granulates (CG). Soil remediation tests were performed in a lab-scale prototype composed by an acrylic EK cell divided into 5 compartments, a power supply set at 20 V and two graphite electrodes. The central compartment was filled with 2.5 kg of kaolinite-based clay soil (low permeability) spiked with 50 mg/kg of Cr(VI). The graphite electrodes were assembled in the compartments (anodic and cathodic) adjacent to the central compartment, where the electrolyte solutions were added. The remediation of the Cr(VI)-spiked soil was evaluated as a function of the CG-PRB (10 cm × 10 cm × 1 cm) position, electrolyte type, current intensity and polarity reverse. CGs barrier was more effective when located near the anode compartment, mainly associated with the acidic pH, which favors the Cr(VI) reduction process.

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Bibliography
[1] Fonseca, B., Pazos, M., Tavares, T., Sanromán, M. (2013). Environ Sci Pollut Res 19, 1800-1808.
[2] dos Santos, E.V., Ferro, S., Vocciante, M. (2019). The Handbook of Environmental Remediation: Classic and Modern Techniques, Electrokinetic remediation, Royal Society of Chemistry.

Bioremediation of petroleum hydrocarbons by fungi of the genus *Aspergillus* in different compartments PC 85

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Key words: *Aspergillus*, biodegradation, petroleum hydrocarbon, pollution, salinity.

Abstract
The fungi of the genus *Aspergillus* are found in the most diverse terrestrial compartments. These microorganisms are often found in sites contaminated with petroleum hydrocarbons [1] and can achieve removals of these pollutants up to 86% [2] or even higher, which depends on factors such as compound and concentration involved, species and conditions such as pH, temperature, and salinity, among others [3]. In a search conducted in the sciencedirect.com database using the keywords "fungi, degradation, hydrocarbon and petroleum", "fungi, degradation and PHA" and "fungi, degradation and BTEX", 33, 4 and 1 publications were found. Of these, in 55% (21) was referred species of the genus *Aspergillus* involved in the degradation of compounds present in petroleum and its derivatives. There has been a substantial increase in these studies in the last 4 years, with Mexico, Brazil and Nigeria as the countries with the highest number of publications. Most of the *Aspergillus* species were directly isolated from the soil (50%), possibly due to the greater ease of access to this compartment, followed by wastewater (13.6%) and less from mangrove isolation (9.1%) and in seas and marine sediment (9.1%). However, species from micro-collections are often used (9.1%). Although the main origin of the strains employed came from the soil, most of the degradation studies occurred in aqueous medium (76.2%). Less emphasis has been placed on the study of bioremediation of these compounds in the marine environment, so that only 19% of the articles sought to evaluate the potential degradation of petroleum hydrocarbons under the effect of salinity, a factor of great relevance considering the high incidence of oil spillage in the sea, which indicates a promising bioremediation research area.

Bibliography
[1] Ghizelini, A.M., Martins, K.G., GieBelmann, U.C., Santoro, E., Pasquallete, L., Mendonça-Hagler, L.C.S., Rosado, A.S., Macrae, A. (2019). Mar Pollut Bull 139, 181-188.
[2] Al-Hawash, A.B., Zhang, J., Liu, S., Ghalib, H.B., Zhang, X., Ma, F. (2018). Ecotoxicol Environ Saf 164, 398-408.
[3] González-Abrandelo, D., Pérez-Llaño, Y., Folch-Mallol, J.L., Aranda, E., Vaidyanathan, V.K., Cabana, H., Gunde-Cimerman, N., Batísta García, R.A. (2019). Bioresour Thecnol 189, 287-296.

Utilization of non-exhaustive extraction techniques for estimation of bioavailability of aliphatic hydrocarbons in soil

PC 86

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Key words: Non-exhaustive extraction techniques, 1-butanol extraction, biodegradation, bioavailability.

Abstract

Petroleum hydrocarbons are the most common soil contaminants worldwide and petroleum contamination of soil and groundwater is of concern in many parts of the world. Biological treatment, or bioremediation, is a desirable soil remediation option due to the low costs associated with its application. Many factors may affect the bioremediation process. Among them is the bioavailaility or bioaccessibility of contaminants, which can determine achieved end-points, while there is no single set of contaminant/soil characteristics able to effectively predict the biodegradable proportion of contaminant. Non-exhaustive extraction techniques (NEETs) utilise low-molecular weight primary alcohols, such as 1-butanol and 1-propanol, in simple shake extractions, which provide a mild extraction of hydrophobic compounds from soil.

In this study, the bioavailability of aliphatic hydrocarbons in different soils was predicted using three NEETs. We have tried to predict the biodegradable amount of aliphatic hydrocarbons C_{10} – C_{40} in soil of park, forest, meadow, field and soil from industrial zone. Three NEETs were used, i.e. 1-butanol, 1-propanol with water (1:1 v/v) and hydroxypropyl-β-cyclodextrine (HPCD). Extracted amounts were compared to residual hydrocarbon concentrations after 1-year biodegradation with bioaugmented *Comamonas acidovorans*.

The best prediction of biodegradable portion of aliphatic hydrocarbons was achieved using 1-butanol (predominantly ±10% difference from non-biodegraded residuum), followed by mixture of 1-propanol with water (1:1 v/v) which tended to underestimate the biodegradation by approx. 20%. Contrary the HPCD, known for good prediction of aromatic hydrocarbon biodegradation, underestimated the aliphatics biodegradation by two orders of magnitude. The results show that single-step NEETs can be used for effective prediction of biodegradation, but used solvent has to be properly selected.

Mycoremediation of environmental pollutants using white rot fungi and their enzymes — PC 87

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Key words: *Basidiomycetes,* lignin-modifying enzymes, production, application, bioremediation.

Abstract

*C*ontamination of soils and waters with toxic organic pollutants cause detrimental effects on the health of humans, animals, plants, and microbes. Recent fundamental work has revealed the potential application of white rot basidiomycetes (WRB) and their lignin-modifying enzymes (LME) for treatment of soils and effluents contaminated with organic pollutants. Ability of fungi to form extended mycelia networks, the low specificity of their enzymes and their ability of using pollutants as a growth substrate make WRB well suited for bioremediation processes. However, large scale applications of LME for bioremediation is so far limited due to enzyme high cost. This presentation summarizes fundamental knowledge on physiological mechanisms regulating LME synthesis by WRB focusing on the common characteristics and unique properties of individual fungi as well as on several approaches providing enhanced secretion of these enzymes by WRB. Firstly, lignocellulosic substrates, some of which containing significant concentrations of soluble carbohydrates and inducers, play a crucial role in enzyme production. Secondly, some microelements and aromatic compounds enhance the LME synthesis although their effect depends on individual fungi physiological peculiarities. Thirdly, expression of basidiomycetes biosynthetic potential depends on the cultivation method. Fourthly, co-culture of compatible fungi may be an appropriate approach to increase laccase and MnP yields. Moreover, potential of several WRB and their LME in the decolorization of synthetic dyes, removal of oil, trinitrotoluene and individual micropollutants will be analysed.

Acknowledgements

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POSTER COMMUNICATIONS
TOPIC 7 – Identification and monitoring of pollutants
(PC 88 – PC 91)

Monitoring of organic micropollutants in environmental matrices **PC 88**

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Key words: Environment, monitoring, micropollutants, EU-Directive 2013/39, EU-Decision 2018/840.

Abstract

Environmental contamination due to organic micropollutants continuously released into the environment at trace concentrations (ng L^{-1} to µg L^{-1}) is a worldwide issue of concern [1]. These pollutants are generally not completely removed during conventional wastewater treatment, reaching other water matrices such as ground (GW) and surface waters (SW), which might be sources for drinking water (DW) production, thus jeopardizing the human health [2]. The European Union (EU) has recommended the monitoring of specific priority substances (PSs, Directive 2013/39) and some contaminants of emerging concern (CECs, Decision 2018/840) in surface waterbodies. Therefore, their determination at trace levels in this matrix is an important issue that demands fast and sensitive analytical procedures.

In this sense, the present study aimed to develop analytical methodologies based on offline solid phase extraction (SPE) followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) to assess the occurrence of EU-relevant micropollutants in DW and SW samples. The optimized SPE-UHPLC-MS/MS methods were successfully applied in DW samples from different sources (such as tap, fountain and well water) and in stressed Portuguese rivers (Ave and Leça) [3,4]. A widespread occurrence of PSs and CECs was verified at ng L^{-1} levels in both matrices, highlighting the need of more monitoring programs for further prioritization and risk assessment of PSs and CECs in watercourses.

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Bibliography

[1] A.R. Ribeiro, M. Pedrosa, N.F.F. Moreira, M.F.R. Pereira, A.M.T. Silva, *J Chromatogr A*, 1418 (2015) 140-149;
[2] M.O. Barbosa, N.F.F. Moreira, A.R. Ribeiro, M.F.R. Pereira, A.M.T. Silva, *Water Res*., 94 (2016) 257-279;
[3] M.O Barbosa, A.R. Ribeiro, M.F.R. Pereira, A.M.T. Silva, *Anal. Bioanal. Chem*, 408 (2016) 8355-8367;
[4] M.O. Barbosa, A.R. Ribeiro, N. Ratola, E. Hain, V. Homem, M.F.R. Pereira, L. Blaney, A.M.T. Silva, *Sci. Total Environ.*, 644 (2018) 1128-1140.

Occurrence of anticancer drugs in influents and effluents from a Portuguese Wastewater Treatment Plant PC 89

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Key words: Anticancer drugs, monitoring, liquid-liquid extraction, wastewaters, pollution.

Abstract

Anticancer drugs, also known as cytostatics, are compounds used in chemotherapy. Although they are employed to treat cancer, these chemicals are not specific for damaged cells, meaning that they may interfere with healthy tissues, representing a potential risk for aquatic species and humans [1]. When administered, anticancer drugs are partially excreted through urine and feces, reaching sewage waters. Currently, there are no effective treatments for these hazardous compounds at wastewater treatment facilities and, therefore, they end up in surface waters and possibly in potable water [2].

The objectives of this work are: (i) to evaluate the contamination of influents and effluents of one wastewater treatment plant, located at northern Portugal, regarding specific anticancer drugs of concern (mycophenolic acid, mycophenolate mofetil, bicalutamide and cyclophosphamide) [3]; (ii) to assess the temporal variation of anticancer drugs concentration in wastewaters; and (iii) to get information about the efficiency of secondary and tertiary treatments in the overall cytotoxic removal. For such a purpose, the influent and the effluents of the secondary and tertiary treatments were collected every day during a week (24-h composite samples). Anticancer drugs were extracted from the samples by liquid-liquid extraction and then analyzed by liquid chromatography-tandem mass spectrometry. The results show that some anticancer drugs may reach surface waters, even when a tertiary treatment step is applied.

Acknowledgements

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Bibliography

[1] Olalla, A., et al. (2017) Chemosphere, 190, 417-430.
[2] Franquet-Griell et al. (2016) Environ Pollut, 208, 532-536.
[3] Santos et al. (2017) Chemosphere, 184, 1250-1260.

Toxic metals in the ecosystems of 22 urban reservoirs of Prague metropolitan area

PC 90

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Key words: Toxic metal, urban reservoir, chronic toxicity, sediment, fish.

Abstract

Urban areas are major sources of toxic metals (TM), originating from different anthropogenic activities [1]. Large quantity of metals ends in aquatic ecosystems mainly due direct discharge or surface runoff from impervious surfaces. The increasing concentration of TM is a serious treat for the environment and human population. The aim of the study was to investigate the level of water, sediment and fish pollution by TM in 22 urban reservoirs of Prague metropolitan area.

The survey was conducted during 2 year period, when water, sediment and fish were collected and analysed for metals content (Cd, Cu, Cr, Ni, Pb, Zn, Mn, Fe, Al) [2]. Complementary, basic water quality parameters were measured and chronic toxicity test of sediments was conducted with *Heterocypris incongruens*.

More than 50% of the reservoirs exceeded the Czech Environmental Quality Standards (EQS) for total organic carbon, chemical oxygen demand and phosphate, indicating high level of eutrophication. Copper was identified as the most hazardous TM in water. Nickel, copper and zinc exceeded EQS in sediment of most reservoirs. In a few cases, increased concentrations of chromium, cadmium and lead were monitored. A high variability of metals levels was detected in fish species, according to their age and food habits. In the case of most reservoirs, the chronic toxicity test showed negative effect of the sediments on mortality and growth of *Heterocypris incongruens*.

The type of urban drainage is an important factor affecting not only level of pollution, but also remobilization of metals from sediment.

Bibliography

[1] Sutherland, R.A. (2000). Environ Geol 39(6), 611-627.

[2] Komínková, D., Nábělková, J., Vitvar, T. (2016). J Soil Sediment 16(5), 1569-1583.

Thermodynamic properties of chemical fragrances: benchmark tools for environmental risk assessment

PC 91

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Key words: Thermodynamic properties, molecular energetics, group contribution models, reactivity assessment, emerging pollutants.

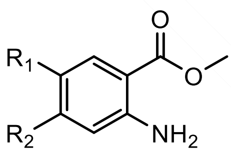
Abstract

What does happen to the residues of all the highly-fragranced products used every day?

Due to ineffective wastewater treatment, these chemical fragrances are continually being released with wastewater effluent. The persistence of these chemicals can origin long-term hazards whose effects for aquatic life and the food chain are unknown. In this context, environmental risk monitoring of fragrances for the protection of ecosystems should be done with rigor prior to their commercial use. This assessment is basically made by different approaches, bringing into question the reliability of the estimate made.

The objective of this work is the development of experimental and computational studies to provide accurate structural, thermodynamic and electronic properties of fragrances, filling data gaps for important classes of fragrances, in order to build a solid foundation of data. Their knowledge leads to the design of robust and accurate group contribution models, allowing to estimate properties of other related compounds with reliability. This procedure will enable to do a correct prediction of fragrances environmental partitioning (air, water and biota) and, consequently, to take preventive actions and solve problems.

The current study addresses a thermodynamic study regarding three fragrance compounds, methyl anthranilate derivatives, based on both experimental and computational research, with the main goal of evaluating the thermodynamic effects inherent to the presence of different substituents (methyl, hydroxy, and dimethoxy - Figure 1) in the ring.



R_1=OH, R_2=H, Methyl 5-hydroxyanthranilate
R_1=CH_3, R_2=H, Methyl 3-methylanthranilate
R_1=OCH_3, R_2=OCH_3, Methyl 4,5-dimethoxyanthranilate

Figure 1. General molecular formulae of the methyl anthranalite derivatives presented in this work.

Acknowledgements

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POSTER COMMUNICATIONS

TOPIC 8 – Mathematical models for bioremediation processes

(PC 92)

Bioremediation of soils contaminated with VOC's – fugacity based kinetics modelling PC 92

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Key words: Enhanced bioremediation; xylene, ethylbenzene; calcareous, granitic, and schist soils, fugacity; modeling.

Abstract

Assisted bioremediation of soils has been gaining relevance in areas with widespread contamination requiring on-site intervention since it is a cost-effective technology with zero-to-negative environmental impact.

A consortium of heterotrophic microorganisms extracted from a soil collected in a crude oil contaminated site, and subsequently adapted and developed in a xylene enriched liquid mineral medium promoted the biodegradation tests. The resulting microbial community from the second transfer culture was characterized, revealing a predominance of *Chitinophagaceae* (46.17%) and *Pseudomonadaceae* (32.72%) families. The microbial culture was augmented and used in biodegradation and bioremediation tests at different lab scales. The soil contaminants tested were two volatile organic compounds (ethylbenzene and xylene). Assisted bioremediation assays used three types of natural soils (calcareous, granitic, and schist). Contamination levels were monitored in discontinuous mode by daily measurements of ethylbenzene and xylene concentration in the gas phase using gas chromatography. Bioventing was promoted by controlled ventilation through a respirometric system which in addition to air supply, also monitored oxygen and carbon dioxide within each column [1].

Moreover the kinetic models of biodegradation, the Mackay's concept of fugacity was adopted to develop mathematical models able to describe and reproduce enhanced bioremediation of natural soils contaminated with ethylbenzene and xylene. Calibration of the developed models was based on experimental data obtained in laboratory tests. Fugacity-based models were successfully applied to lab scale microcosms, opening an advantageous path to access and predict the behavior of contaminants (mainly their distribution by soil phases) in the aptitude tests required before field application of any remediation technology. This methodology proved to be robust, describing, and predicting the behavior and time evolution of the main variables involved in the processes.

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Bibliography

[1] Carvalho, M. M., Vila, M. C., Delerue-Matos, C., Oliva-Teles, T., & Fiúza, A. (2015). Assisted bioremediation tests on three natural soils contaminated with benzene. Eurasian Journal of Soil Science. 4 (3), 153-160. doi:10.18393/ejss.2015.3.153-160.

POSTER COMMUNICATIONS

TOPIC 9 – Other

(PC 93)

The catabolic potential of hydrocarbons of the hydrocarbonoclastic bacterium *Achromobacter* sp. strain B7

PC 93

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Key words: *Achromobacter*, hydrocarbon catabolism, aliphatic hydrocarbon.

Abstract

Achromobacter species are Gram-negative bacilli commonly found in soil and water but also are associated with human clinical samples. *Achromobacter* sp. B7 is a bacterium isolated during bioremediation trials by bioaugmentation of a diesel-spiked soil from Valparaiso Region, Chile [1]. The aim of this study is the reconstruction of hydrocarbon catabolic pathways in *Achromobacter sp.* strain B7. Strain B7 was grown on several hydrocarbons (aliphatic and aromatic hydrocarbons) as sole carbon source. The genome was sequenced on an Illumina HiSeq 4000 system and Nanopore MinION instrument, assembled with SPAdes v.3.11.1 and annotated using PROKKA software. The identification of catabolic genes and their genomic context was carried out through homology-based analysis using Blast tool. Strain B7 is able to grow in BHB minimal medium on hexane, octane, hexadecane and diesel as sole carbon sources at 30°C. The highest growth was observed on octane. The hybrid assembly resulted in a closed and complete chromosome sequence of 6,236,552 bp with a G+C content of 64.8%. 5526 coding sequences and 65 tRNA were identified. Strain B7 possessed complete catabolic pathways of aliphatic hydrocarbons (*alk* genes). Genes involved in the catabolism of aromatic hydrocarbons were annotated in strain B7 genome. A high number of peripheral (*e.g.*, benzoate) and central pathways (*e.g.*, gentisate, catechol) were identified. This study indicate that strain B7 is able to grow in aliphatic hydrocarbons as sole carbon sources and harbours a wide range of genes involved in hydrocarbon degradation.

Acknowledgements

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Bibliography

[1] Méndez, V., Hernández, L., Salvà-Serra, F., Jaén-Luchoro, D., Durán, R. E., Barra, B., Beatriz Piñeiro-Iglesias, Edward R. B. Moore & Seeger, M. (2018). *Microbiol Resour Announc, 7*(19), e01326-1.

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