Student: Ana Sofia Teixeira Oliveira

Supervisor(s): Paula M.L. Castro, Catarina L. Amorim, Mark van Loosdrecht

Thesis Tittle: EPS_AuGmentS - Aerobic Granular Sludge technology combined with bioaugmentation with immobilized bacteria as a tool to degrade micropollutants from wastewater

Abstract (max. 3000 characters, including spaces)

The variety and amount of industrial chemicals being released into the environment has raised. Indigenous microbial communities in wastewater biotreatment processes may not be effective in removing xenobiotic contaminants. A major aim for the 3 year of scholarship was to design new bioaugmentations strategies and to evaluate its efficiency in an aerobic granular sludge sequencing batch reactor (AGS-SBR) system continuously fed with 2-fluorophenol (2-FP). Bioreactor performance in terms of phosphate and ammonium removal and 2-FP degradation and microbial communities shifts were evaluated.

Granules were produced using extracellular polymeric substances (EPS) obtained from excess biomass as a carrying matrix and a 2-FP degrading strain, *Rhodococcus* sp. FP1. Afterwards, the produced granules were introduced in the SBR. The produced granules broke down into smaller fragments inside the bioreactor shortly after addition, but 2-FP degradation was maintained. After 8 days of the bioaugmentation process, 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 during the feeding with 2-FP.

The persistency of *Rhodococcus* sp. FP1 in the SBR was followed by qPCR. *Rhodococcus* sp. FP1 was detected up to 3 days after bioaugmentation in the SBR effluent. Horizontal gene transfer could have happened between the 2-FP degrading strain and AGS indigenous microbial strains. Some bacteria isolated from the AGS were able to degrade 2-FP after bioaugmentation and the occurrence of HGT among the degrading strains is under evaluation.

Student: Helena Alexandra Gonçalves Ferreira

Supervisor(s): Elisabete Pinto, Marta W. Vasconcelos, Ana M. Gil

Thesis Tittle: IMPULSE - IMpact of a PULSE-based partial replacement diet on metabolome and health

Abstract (max. 3000 characters, including spaces)

The IMPULSE project generally intends to assess human health adaptations of daily replacing one typical omnivorous lunch meal with a vegetarian meal using protein-rich grain legumes, such as beans, chickpeas, lentils or peas, also known as pulses, as animal-based foods, like meat, alternatives. For this purpose, we will make use of a combination of food and nutritional research classical tools with cutting edge "omics approaches", namely, Metabolomics through body fluid analysis by Nuclear Magnetic Resonance (NMR) spectroscopy. The first year of this PhD was dedicated to the planification of the quasi-experimental dietary intervention and data collection protocols. In this context, a systematic review on the topic has been performed and the manuscript has been accepted for revision by the journal *Critical Reviews in Food Science and Nutrition*. This review was also accepted for a poster presentation at the *XVII Congress of Food and Nutrition of the Portuguese Association of Nutrition*. From the end of the first year and through the second, two dietary interventions have been carried out: from March to May 2018 (n=19) and from October to December 2018 (n=8), respectively. In order to achieve a more representative number of participants we are planning a third intervention, possibly from October to December 2019. Notwithstanding, until the present date we have gathered data from 27 volunteers, and all has been assembled in databases. NMR sample acquisition has been initiated and about 45% of samples available were analysed so far (171 urine samples out of 378 samples). No data processing has been performed yet, hence no results regarding metabolomics can be presented at this workshop. Nevertheless, preliminary analysis from other data sources like food preference tests, as well as, physical measures and blood biochemistry assessments, was performed. In short, results point out to general good acceptance by participants to the proposed diet, to an overall maintenance of anthropometric parameters and to a maintenance (e.g. iron) or slight improvement in few health indicators (e.g. blood lipid profile). Some of these findings were presented as posters at the *XVIII Congress of Food and Nutrition of the Portuguese Association of Nutrition* and as an oral communication at the *2nd Mediterranean Legume Innovation Network Workshop* in frame of the European TRUE project. So far, one of the main limitations has been the recruitment of enough volunteers. Also, common data assessment delays, such limitations on NMR equipment availability, have not allowed the start of the analysis of all types of biological samples. To sum up, the majority of tasks proposed for the first and second years of this PhD have been accomplished successfully. As future work we intend to 1) plan the next dietary intervention; 2) advance NMR metabolomics analysis of all biological samples; 3) advance food and nutritional intake data analysis and 4) begin fecal microbiota analysis.

Student: Inês Gonçalves de Azevedo Moreira

Supervisor(s): Prof. Doutora Paula Teixeira; Doutora Helena Albano; Doutora Joana Barbosa

Thesis Tittle: Tradition, science and innovation: bioactive substances to mitigate microbiological risks of (innovative) *alheiras*

Abstract (max. 3000 characters, including spaces)

In response to nutritional and health concerns, the food industry has begun to offer a wider variety of products that reflect changing consumer preferences. In addition to traditional *alheiras*, made with pork and/or poultry meats, other varieties of *alheiras* ("innovative") made from codfish, mushrooms, tofu, soy and vegetables were launched on the Portuguese market.

The objective of this study involves the chemical characterization of 22 *alheiras*, including fourteen "innovative" products and eight corresponding traditional *alheira (*produced by the same company). In addition, three selected *alheiras* were contaminated with four different pathogens to evaluate their behaviour in these matrices along storage period.

Results

Water activity and pH values of "innovative" *alheiras* ranged from 0.961 to 0.991 and from 4.0 to 5.5, respectively, and nitrites, nitrates and biogenic amines were found to be within accepted limits for this kind of products.

At the end of 2 months storage (shelf life period reached) we were able to see that, unlike traditional *alheiras*, most of the tested pathogens did not survive in some of the "innovative" *alheira* matrix tested.

Future work

Studies concerning the potential of bacteriocinogenic lactic acid bacteria and other bioactive substances to mitigate possible microbiological risks will be conducted and also metagenomic approaches to evaluate the microbial diversity on "innovative" a*lheira* matrix will be next investigated.

Student: Jaqueline Rocha

Supervisor(s): Célia Manaia

Thesis Tittle: Novel approaches on the characterization of the wastewater resistome: possible implications on human health and water quality management

Abstract (max. 3000 characters, including spaces)

Klebsiella pneumoniae is able to colonize humans and is a major infection cause by Gram-negative bacteria in hospitals. Besides its clinical importance, this species can also be found in the environment, in particular in soil, plants and waterways. Since the relationship between environmental and human bacteria is still unclear, although crucial for understanding the paths of transmission to humans, we aimed to assess whether clinical isolates of *K. pneumoniae* maintain clinically relevant traits once in the environment. A group of 59 wastewater (n=25) and clinical (n=34) isolates of third-generation cephalosporin-resistant *K. pneumoniae* was characterized based on their antibiotic resistance phenotype and genotype, horizontal gene transfer capacity and biofilm production. The infection capacity potential of a selected group of 47 isolates (23 environmental and 24 clinical) was assayed using the model organism *Galleria mellonella*. Part of these isolates and others, whose genomes were available in public databases, in a total of 73 environmental and 78 clinical, were subjected to comparative genomic analyses. Most wastewater (76%, 19/25) and clinical isolates (94%, 32/34) were multidrug resistant. ESBL genes were more prevalent in clinical (>65%, >22/34) than in wastewater isolates (>56%, >14/25) and the bla_{KPC} gene was only found in clinical isolates (5/34). Conjugative capacity was more frequent in clinical (76%, 26/34) than in wastewater isolates (40%, 10/25). *G. mellonella* health index was lower after infection with clinical than with wastewater isolates. The whole genome sequence analysis targeting 6 groups of genes related to antibiotic and heavy metal resistance, virulence, efflux systems, oxidative stress and quorum sensing, revealed 1406 gene variants. The 11 genes found related to oxidative stress or quorum sensing were common to all isolates, while 485 and 460 genes related to resistome or virulence were found exclusively in clinical and environmental isolates, respectively. These results reveal that a certain degree of specialization may occur in environmental or in clinical niches. However, it is suggested that putative clinically relevant traits may persist in environmental bacteria.

Student: Joana Ribeiro Costa

Supervisor(s): Prof. Doutora Manuela Pintado, Lorenzo Pastrana & Lourdes Cabral

Thesis Tittle: Valorization of grape pomace through extraction of xylooligossacharides for potential application in functional ingredients

Abstract (max. 3000 characters, including spaces)

Grapes are one of the most cultivated fruit crops worldwide, from which more than 70% is generated in wine industry in the form of grape skin, seeds, stems and residual pulp, known as grape pomace (GP)[1,2]. Grape pomace extract was obtained through enzymatic extraction, characterized and tested for its antimicrobial and antioxidant capacities. The extract seemed to be promisor until significantly decreased after gastrointestinal digestion, suggesting the need for an alternative system to protect it from the harsh gastrointestinal conditions.

The encapsulation of the extract into alginate or chitosan through ionic gelation was optimized through central composite design, and allowed the formation of nanoparticles with sizes between 400 and 1000 nm, suitable for oral delivery, and high association efficiency of phenolic compounds (> 50%). Both systems were successful, since the phenolic compounds present in the extract were protected from the harsh gastrointestinal conditions, and were delivered into the intestine, where they were able to exert antioxidant and antimicrobial activity.

References:

1. Spanghero, M. *et al.*, 2009. Anim Feed Sci Technol. 152 (3-4), 243 – 255.
2. Corbin, K. R. *et al.*, 2015. Bioresour Technol. 193, 76 – 83.

Student: Joana Lopes Abreu Miranda

Supervisor(s): António Osmaro Santos Silva Rangel and Raquel Beatriz Ribeiro Mesquita

Thesis Tittle: Development and application of automatic and miniaturized methods for iodine, thyroid peroxidase and iron quantification for thyroid-related disorders

Abstract

The main objective of this PhD project is to contribute to the development of cost-effective, robust, automatic, reliable and miniaturized methods for the determination of iodine and iron in biological samples, as there is evidence of a strong interaction between iron, iodine and thyroid metabolism. The developed methodologies will hopefully provide an effective tool to study the relationship between the assessed parameters in thyroid-related disorders. To achieve the above-mentioned purpose, we are developing flow techniques, to automate chemical and biochemical assays.

The micro sequential injection lab-on-valve (μSI-LOV) concept, associated to the bead injection technique and to solid phase spectrometry was the appropriate choice for biochemical assays due to the possibility of handling small amounts of fluids (i.e. microliters). During the second year of the PhD, the objective was to accomplish both iron(III) retention and spectrophotometric measurement, by functionalizing beads with the bidentate 3,4–hydroxypyridinone (3,4-HPO) ligand. This way, by using the sorbent/colour reagent in consecutive cycles, the ligand consumption could be minimized. The spectrophotometric reaction was carried out at the beads surface, packed in a LOV flow cell, in a solid phase spectrometry (SPS) approach. The functionalized beads proved to be an efficient solid sorbent to retain and therefore to quantify iron(III) at pH~7. This is an important factor to a direct application to biological samples, namely blood serum. The determinations were carried out with the minimal consumption of reagents, standards, and synthetic biological samples.

During the end of the second year of the PhD, in the context of the NORTE2020 scholarship, a work, in partnership with a University of the Balearic Islands (Spain), was developed for the quantification of iodine. The determination of iodine status is based on the concentration of iodine excreted in urine. So, a method consisting in a spectrofluorimetric determination of iodine in urine, using a miniaturized analyzer chip in a multi-syringe flow system, was developed. The detection was based on the catalytic effect of iodide on the redox reaction between Ce(IV) and As(III), using the Sandell-Kolthoff reaction. During the third year of the PhD, the method was improved for the determination of iodine in urine samples; to eliminate interferences (namely from thiocyanate) and release iodine from organo-iodine compounds, an on-line oxidation process aided by UV radiation was implemented in the developed system. The developed method is simpler and faster than the classic approach of the Sandell-Kolthoff reaction.

Student: José Carvalho Soares

Supervisor(s): Marta Vasconcelos

Thesis Tittle: Effects of induced iron deficiency under high CO2 concentration in bean and soybean plants

Abstract (max. 3000 characters, including spaces)

The increases in atmospheric carbon dioxide concentrations can enhance plant growth and change their nutrient demands. The identification of cultivars or the characteristics of cultivars which have superior performance at eCO_2 might support their adaptation to this global environmental change by facilitating the development of varieties that can exploit the increase in CO_2. This performance must encompass not only productivity traits but, as demonstrated recently, also must include nutritional resilience to eCO_2 conditions.

Therefore, in the current study, we focused on basically three main objectives:

a) Study of intraspecific variation of yield responses under eCO_2 in a controlled environment in two legume species: soybean and common bean, while assessing aspects pertaining to nutritional quality. The results indicate that consistent and significant variation in the response of seed yield to eCO_2 under controlled conditions does exist among varieties of bean and common bean, and that variation in the response of pod and seed number are a suitable phenotype for predicting the responsiveness to future eCO_2. Moreover, it was demonstrated that eCO_2 decreased the nutritional value of both species.

b) Characterization of leaf photosynthesis and yield parameters of bean and soybean genotypes under Free-Air CO_2 Enrichment in a field experiment. From these findings, we observed that plant biomass, seed yield and photosynthetic capacity were increased under CO2 enrichment conditions. In addition, nutrient assimilation match carbon assimilation under eCO_2 and leads to photosynthesis up-regulation to eCO_2.

c) Responses to Fe limitation in soybean as affected by the atmospheric CO_2 concentration. We investigated how eCO_2 affects the Fe status of soybean plants grown in Fe-sufficiency or Fe-deficiency conditions. We demonstrate that elevated CO_2 can improve plant Fe nutrition under Fe-limited conditions by inducing physiological, and molecular changes that enhance Fe uptake.

Student: Miguel António Marcos Ramos

Supervisor(s): Prof. Paula Castro

Thesis Tittle: Selection of strains of edible mycorrhizal fungi for improved field persistence and mycelial expansion

Abstract (max. 3000 characters, including spaces)

Trees are key elements in mitigating the common environmental problems in urban areas, provisioning crucial ecosystem services such as air quality improvement, decrease of water runoff and microclimate mitigation. Ectomycorrhizal Fungi (ECM) may play an important role in urban tree management, improving tree vigor, and thus the extent of ecosystem services delivered by urban trees under stress. An in-depth exploitation of the potential of dedicated EcM inocula as the active factor in management of trees in urban ecosystems through an analysis of tree physiology, growth response remains to be done.

The major aims of this work were to assess the growth performance of *Tilia tomentosa* inoculated with ECM species and study plant response to water stress. An 9 months in-vivo experiment was established with seedlings exposed to different substrate pH (Acid and Alkaline), 5 inoculation treatments and different water stress conditions (no stress and stress).
Inoculation of *T. tomentosa* seedlings with selected inocula significantly promoted height growth, diameter, foliar dry weight of seedlings in acid substrate. SPAD levels were measured at different time points in a stress dynamic experiment (before stress, water stress, recover). Under the stress period a protection/mitigation effect was observed from ECM to the plants in acid and alkaline substrate. Plant that were exposed to water stress have normalized their SPAD values under recovery and at the end stage. These results represent an important contribution for development and application of ECM inocula in urban context.

Student: Nazareno Scaccia

Supervisor(s): Prof. Célia Manaia and Dr. Ivone Vaz-Moreira

Thesis Tittle: Evaluation of possible risks of antibiotic resistance transmission to humans by treated WW-irrigated crops.

Abstract (max. 3000 characters, including spaces)

The use of treated wastewater to irrigate crops may be a source of antibiotic resistant bacteria and antibiotic resistance genes (ARB&ARGs) capable of contaminating the human food chain, and therefore threat human health. The major objective of this research project is to assess the risks of wastewater ARB transmission to humans via gastrointestinal tract. The research question of this work regards the capability of environmental ARB&ARGs to survive in the presence of the autochthonous human gut microbiome. In order to address this question, we have been carried out fecal microcosm assays (FMAs) aiming to assess the fitness of environmental ARB in the presence of the complex human gut microbiome. For that, feces-based microcosm assays were inoculated with wastewater isolates of *Escherichia coli* (strain A2FCC14) and *Enterococcus faecalis* (strain H1EV10), harboring the ARGs bla_{TEM}, bla_{CTX}, bla_{OXA-A} and *van*A, respectively. The effect of variables such as the presence or absence of oxygen, cefotaxime or vancomycin on the bacterial community composition, ARB survival and ARGs persistence were studied.

The fecal bacterial community was characterized by the predominance of members of the phyla *Firmicutes, Bacteroidetes, Actinobacteria*, and *Verrucomicrobia*. The spiked ARB were able to survive in fecal microbiota for at least a week and their ARGs could be quantified for at least one month, under both aerobic and anaerobic conditions. The presence of sub-inhibitory concentrations of antibiotics did not induce significant differences on the survival of the tested ARB or ARGs when compared with antibiotic-free microcosms. This study demonstrated the potential of wastewater ARB to survive the human gut microbiome and of their ARGs to persist for longer periods supporting the hypothesis of a potential successful transmission to humans, a topic that deserves further investigation.

Student: Ricardo Gómez García

Supervisor(s): Dr. Cristóbal N. Aguilar, Dra. Ana Raquel Madureira and Dra. Manuela Pintado

Thesis Tittle: Functional ingredients from valorization of melon (*Cucumis melo L.)* by-products: production, bioactivity and potential application

Abstract (max. 3000 characters, including spaces)

Inodorous melon peels are considered as wastes causing environmental pollution, requiring an integral valorization explored in this study. So, the first step started by a chemical characterization and antioxidant capacity evaluation in order to identify the potential industrial applications. Melon peels were fractioned using simple mechanism and separation methods as milling, centrifugation and decantation. The fractions were characterized to investigate their chemical and bioactive compounds composition and its antioxidant activity. Melon peels fractions revealed great content of carbohydrates in the liquid fraction (82.80%), dietary fiber in the solid fraction (44.42%) and protein content in the pellet fraction (34.90%). The analysis of structural carbohydrates in the solid fraction (SF) showed a composition of cellulose (27.68%), hemicellulose (8.2%) and lignin (26.46%). Also, *inodorus* peels presented significant amounts of phenolic compounds (PC) (798.43 mg gallic acid equivalents/ 100 g dry matter = DM), total carotenoids (98.59 mg b-carotene equivalents/ 100 g DM) and total chlorophylls (1681.71 mg/ 100 g DM). Through LC-MS methodology, sixteen PC were identified, where luteolin-7-glycoside (316.37 mg/ 100 g DM) and 4-hydroxybenzoic acid (123. 60 mg/ 100 g DM) were the two most prominent compounds. The carotenoids HPLC profile showed the presence of b-carotene, lutein, b-cryptoxanthin and violaxanthin (91.52, 0.70, 4.92 and 1.63 mg/100 g DM, respectively). The results of DPPH, ABTS and ORAC radicals scavenging, revealed a high antioxidant activity equivalents to 924.77 μM TE/ 100 g DM, 344.46 mg AAE/ 100 g DM and 52.49 μM TE/ mL of extract. The three fractions proved to be prototypes of functional ingredients due to the high content of relevant nutrients namely fiber, pectin and protein as well as different bioactive compounds which are related to the antioxidant activity against free radicals and their association in the prevention of certain types of diseases. The results open new perspectives on integral valorization *Inodorous* melon peels toward added value ingredients with zero waste.

Student: Tatiana Paula Vilela

Supervisor(s): Prof. João Paulo Ferreira and Prof. Ana Maria Gomes

Thesis Tittle: Development of New Added-Value Dairy Products from Cheese Surpluses - Biochemical, Structural and Sensorial Characterization

Abstract (max. 3000 characters, including spaces)

The study of interactions between carbohydrates and milk proteins were studied, in order to understand the function of starch in the dispersion of cheese.

Cheese can be described as a bi-continuous gel structure consisting of a porous protein matrix (casein) interspaced with localized domains of fat. The way this matrix is formed is key, since it plays a role in the final microstructure of cheese and, consequently, its texture, flavour and overall quality. There is not a consensus in literature regarding the interactions that are responsible for maintaining the protein structure in cheese, with some authors pointing to electrostatic and ionic interactions as the main ones, others pointing to hydrophobic interactions and others to a combination between various interactions

Emmental cheese was used, which is a Swiss-type, semi-hard curd cheese, where the matrix acidification occurs after pressing of the curd. Hence the concentration of colloidal calcium increases proportionally with the concentration of casein at drainage, leading to the formation of a highly cohesive and mineralised matrix.

Urea, SDS, EDTA, NaCl and NaOH solutions are commonly used as protein denaturants. In this work, we aimed to determine the contribution that each type of interaction had in cheese structure. For that, we used the different dissociating agents referred above, at different concentrations and combinations.

In this work, in order to determine total protein in cheese, we developed a modification of an UV absorbance method, preceded by the dissolution of the samples in NaOH. The method was also adjusted to quantify protein in solutions where cheese was dispersed.

Sample solutions of dissociating agent and Emmental cheese were heated to 70 °C with constant agitation. This protocol, involving heating the cheese, pretends to approximate the conditions cheese is subjected when preparing cheese-containing food products, such as sauces or processed cheeses, among others. Afterwards, the samples were centrifuged, and the supernatant separated. Aliquots of this were diluted in NaOH 0.1 M, before measurement by UV absorbance at 280 nm. The solubilized protein by each dissociating solution was then evaluated and compared to the total protein in cheese.

Results showed that a cooperation between hydrophobic interactions, hydrogen bonds and ionic interactions are involved in the protein structure of Emmental cheese, since combinations of urea, SDS and EDTA show a high solubility for the cheese proteins. Urea, alone, at the concentration of 6 M, was able to solubilize up to 85% of cheese proteins, while urea (6 M), SDS (2.5%) and EDTA (4 mM) combined were able to solubilize up to 92% of the cheese proteins.

In the future, other dissociating agents will be tested, as well as a particular type of interaction in the formation of MCBs, namely carbohydrate-π interactions, which have not been looked into in studies of dairy proteins – hydrocolloid interactions.