

POLIS_MSCA-IF-661429_DATASET-2

Project: POLIS

Marie Skłodowska-Curie Individual Fellowships

MSCA-IF-2014-EF

GA No. 661429



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Dataset name	POLIS_MSCA-IF-661429_DATASET-2
Grant number	661429 - POLIS - H2020-MSCA-IF-2014
Project number	661429
Project acronym	POLIS
Project title	Studying the bricks of microbial cities: characterization and structural properties of exopolysaccharides and their interaction with proteins and cations in anammox granular sludge
Call (part) identifier	H2020-MSCA-IF-2014
Topic	MSCA-IF-2014-EF Marie Skłodowska-Curie Individual Fellowships (IF-EF)
Fixed EC Keywords	Environmental biotechnology, bioremediation, biodegradation
Free keywords	anammox, granular sludge, biofilm, exopolymeric substances, exopolysaccharides, EPS, rheology, exopolymeric proteins, mono-divalent cations
Beneficiary	Polytechnic University of Milan (PIC: 999879881)
Department	Department of Civil and Environmental Engineering (DICA)
Supervisor	Prof. Francesca Malpei
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Project description	This research project is in the context of environmental engineering, in particular in the field of wastewater treatment, more specifically focused on biofilm-based innovative technologies for nitrogen removal. The anaerobic ammonium oxidation (anammox) bacteria are recently discovered players of the biogeochemical nitrogen cycle. The bioprocess based on anammox metabolism is an innovative technology for the removal of nitrogen from municipal and industrial wastewaters



allowing important savings on operational costs due to the requirement of 60% less oxygen (aeration), no need for organic carbon and the production of 90% less excess sludge, compared with conventional nitrogen removal technologies. Its potential application to municipal wastewater (sewage) would allow a complete redesign of the present energy-consuming into an energy-yielding sewage treatment plant. Due to the slow growth rate of anammox bacteria, their retention in the system is one of the main concern for process stability. This is the reason why in most of the different anammox-based technologies currently applied, anammox bacteria are cultivated in the form of biofilm and in particular in the form of self-aggregating biofilm (i.e. granular sludge). Biofilm stability is closely related to the properties of the extracellular polymeric substances (EPS) constituting the matrix in which microorganisms live and grow. EPS are high-molecular weight compounds secreted by microorganisms establishing the functional and structural integrity of biofilms, and are considered the fundamental component that determines the physiochemical properties of a biofilm. EPS are mostly composed of polysaccharides and proteins, but also include other macro-molecules such as DNA, lipids and humic substances. The aim of the present research was to investigate the structural components of anammox EPS matrix unveiling the mechanisms involved in anammox biofilm (specifically granular sludge) formation and stability. Several extraction methods were tested to evaluate the extraction yield and the total carbohydrates/protein content. Mass spectrometry (e.g. MALDI MS) was used to investigate functional EPS and their fine structures, with special focus on hydrogel and film forming components. Rheometric analysis were used to evaluate the viscoelastic characteristics of anammox granular sludge in comparison with the hydrogel (potentially) formed by extracted EPS and their interaction with mono/divalent-cations. Microscopy techniques (mainly AFM, SEM) were used to image the morphology of the extracted biopolymer and the film structure.

Considering that excess sludge is currently one of the main waste products of wastewater treatment facilities, the recovery of a bio-based polymer and its application in other industrial sectors would contribute to the transition towards a circular economy fostering sustainable economic growth. The results of the present research could identify the potential applications of a bio-based polymer recovered from excess biofilm sludge based on its properties.

Open access to scientific publications and underlying data

Scientific peer-reviewed publications such as journal articles will be deposited upon publication in Zenodo repository (<https://zenodo.org/>), an OpenAIRE/CERN repository. The deposition of the research data needed to validate the results presented in the deposited scientific publications



('underlying data') will be evaluated from time to time by the Researcher (Ing.Tommaso Lotti, PhD) and the Supervisor (Prof.Francesca Malpei) along the project development according to the approach of the European Commission “as open as possible, as closed as necessary”. The research data decided to be open up will be organized in separate data sets. For the description of each data set and the relative depositing procedure, the reader is referred to the “DATA SETS” section below. The list of openly shared data sets will be updated and/or modified whether necessary during the time course of the project.

DATA SETS

Data set name POLIS_MSCA-IF-661429_DATASET-2

Data set description Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules such as biopolymers. MALDI methodology is a three-step process. First, the sample is solubilized in a solvent, mixed with a suitable matrix material and applied to a metal plate. Second, a pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material. Finally, the analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases, and can then be accelerated into the mass spectrometer.

The present dataset comprises the mass spectra of the extracellular polymeric substances (EPS) samples extracted from anammox granular sludge. EPS were extracted from freeze-dried anammox granules originating from the full-scale anammox reactor of the wastewater treatment plant of Rotterdam, Sluisjesdijk-Dokhaven (van der Star et al., 2007).

EPS was dissolved in ammonium bicarbonate (5 mM) and run in a conventional SDS-PAGE gel and stained with Coomassie Blue. Stained gel bands were cut and trypsin digestion was performed following the Protease-Max digestion protocol (Promega). α -Cyano-4-hydroxycinnamic acid (HCCA) was used as matrix and dissolved to a final concentration of 10 mg/mL in a solution of pure acetonitrile and 0.1% trifluoroacetic acid (70/30% v/v). The digested EPS solution was mixed with the matrix (50/50% v/v), spotted on the target plate and then analyzed by means of a MALDI TOF/TOF Ultraflex III (Bruker). After complete drying, the samples were submitted to *mass* spectrometry analysis on a MALDI TOF TOF Ultraflex III (Bruker Daltonics, Germany), which was operated in *positive reflectron* for peptide analyses. The peptidic fraction of the



venom was acquired in the range m/z 900-4500.

Standards and metadata Metadata describing the nomenclature used for the storage of research data are given in a separate text file named "DATASET-2_metadata". The file "DATASET-2_metadata" contains the list of acronyms and symbols used for data presentation as well as the matrix and solvent used for sample preparation. The mass spectrometer output files is presented in the standard format "mzML" (Mass Spectrometry Markup Language) in order to facilitate data sharing and reuse. The format "mzML" is a unified open file format containing the peaks list and information about the precursor MS signals and about the associated metadata (i.e., instrument settings and description, acquisition mode, etc).

Data sharing A selection of research data are deposited, upon publication of the scientific publication reporting the elaborated research data, in Zenodo repository (<https://zenodo.org/>), an OpenAIRE/CERN repository.

Peer-reviewed scientific publications will be deposited in self archiving/green "OA" repositories such as Tommaso Lotti's page on ResearchGate (https://www.researchgate.net/profile/Tommaso_Lotti) and Polytechnic University of Milan institutional self-archiving system (<https://re.public.polimi.it/>).

Research data as well as scientific publications and conference papers are available upon request via e-mail.

References van der Star, W.R.L., Abma, W.R., Blommers, D., Mulder, J.-W., Tokutomi, T., Strous, M., Picioreanu, C., van Loosdrecht, M.C.M. (2007). Startup of reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam. *Water Res.* 41, 4149-63.

