SSM
Scuola Superiore Meridionale

MERC PhD Project Proposal 2023/2024

Title of the research project:

Modelling and control of complex biological systems for wastewater treatment for agricultural and industrial processes

Keywords (up to five)

Complex systems, Synthetic biology, Nonlinear dynamical systems, Ecology

Supervisors (at least two from two different areas):

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Nonlinear systems and control, Synthetic biology, Switched and hybrid systems, Multi-agent systems

Supervisor 2 (name, contact details, homepage, area of expertise)
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Applied Mathematics, Ecology, System Biology, System Dynamics

Supervisor 3 (name, contact details, homepage, area of expertise)
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Complex systems, Nonlinear dynamics, Synthetic biology

Project description (max 5000 characters)

Wastewater treatment involves the elimination of contaminants from various sources such as households, industries, and agriculture, transforming them into harmless effluent suitable for reintroduction into the water cycle or for reuse in irrigation or manufacturing. Biodegradable organic substances like fats, food residues, detergents, and human waste are degraded by microorganisms such as bacteria, protozoa, and yeast through natural processes within controlled environments like trickling filters, bio-towers, and other types of bioreactors. Depending on the complexity of the degradation required, different microbial species may be employed simultaneously, each contributing to a specific phase of the process. Alternatively, a more appealing approach is to employ a microbial community, i.e., an ensemble of different species of microorganisms cooperating and sharing resources, which can carry out more than a biodegradation process at a time with higher efficiency. These benefits come at the cost of engineering more complex systems in which each actor involved, i.e., a microbial species, cooperates with the others, sharing resources for their growth and communicates to maintain the community in health and the rate of bioreactions at the desired level. Microbial communities are delicate and complex systems, requiring careful design of internal cellular mechanisms to regulate growth rates and ensure coordination among species through biochemical signaling. These mechanisms are integrated into the cells by introducing new genetic circuits into their genomes.

The goal of this project is to develop new methodologies to accelerate the engineering, deployment, and control of synthetic microbial communities for more efficient and reliable treatment of wastewaters. The specific objectives of the research project are summarized below.

[O1] Self-regulation control of populations in microbial communities.
This objective is focused on the design of embedded biomolecular feedback controllers allowing cells to autonomously regulate their composition in a microbial community comprising two or more cellular populations.

[O2] Balancing labor division and cooperation through communication.
This objective is aimed to design reliable molecular communication mechanisms to regulate the activities in the microbial community and coordinate the effort to efficiently produce target molecules/compounds of interest for biodegradation and treatment of wastewaters.

[O3] Scalability and environmental risks.
The aim of this objective is to study the scalability and reliability of the designed microbial communities for their deployment in large-scale applications, ranging from industrial reactors to water tables. Moreover, the potential impact and risks of their deployment in natural ecosystems and their interaction with natural resident species will be investigated, such as invasion and/or symbiosis and use of common resources.

To achieve the above objectives, we will extend growth control and communication mechanisms presented in the literature, originally designed for simpler scenarios involving one or two cellular populations to encompass situations involving more than two populations. These mechanisms rely on diffusing molecules, such as quorum sensing molecules, which are produced by each population. This allows cells to sense their own density within the environment as well as that of other populations, or to communicate among themselves. However, as the number of cellular populations within the microbial community increases, the use of independent communication channels becomes impractical due to limited availability of specific diffusing molecules and the necessity of incorporating numerous genetic "sensor circuits" into each cell, one for every population within the microbial community. Instead of using dedicated quorum sensing molecules for the intercellular communication as in the current approach, we plan to exploit the same target molecules produced by the cells as sensing channels. This requires the development of a new biochemical mechanism all inside each cell that interacts with the diffusing target molecules and inform the cells about their density (and hence the density of the corresponding population that produced it) – without consuming the target molecules – and regulate accordingly the growth rate of the cell. This strategy allows to free up biochemical channels that will be used to coordinate in the consortium the production of the final bioproduct or compound of interest for the biodegradation process. While this novel communication architecture is inherently scalable, it requires a rethinking of the overall multicellular feedback scheme to ensure that all sensing and growth control loops involved do not interfere with one another, thereby enabling the microbial community to achieve the desired level of production of target molecules or compounds of interest.

Relevance to the MERC PhD Program (max 2000 characters)

The project is strongly interdisciplinary, comprising elements from synthetic biology, molecular biology, dynamical systems, complex network, control theory, and ecology. Complexity in these biological systems arises at three different levels; first, genes, promoters and transcription factors form a network that allows the cells to respond to external stimuli and to keep memory of past events; second, signalling molecules chemically react together to realize communication between the same or different cellular species; third, by sharing common resources and by producing signalling molecules, different cellular species form a network that can regulate itself to balance labour and production in the microbial community. These types of interactions can be mathematically described by graphs and their dynamics by complex networks and compartmental models. Moreover, numerical simulation of these systems can be challenging due to different timescale and layers of interactions, stochasticity and spatial effects.

The research outputs of this project have the potential to be applied to challenging problems (such as wastewater treatment, bioremediation of soil from pollutant, and industrial bioprocessing) and to improve the sustainability of human development by decreasing its impact on natural ecosystems and environments and thus enhance their resilience.

Key references

[1] Dangi et al. "Bioremediation through microbes: systems biology and metabolic engineering approach." Critical reviews in biotechnology 39.1 (2019): 79-98.
[2] Zhou et al. "Distributing a metabolic pathway among a microbial consortium enhances production of natural products." Nature Biotechnology 33.4 (2015): 377-383.
[3] Shong et al. "Towards synthetic microbial consortia for bioprocessing." Current Opinion in Biotechnology 23.5 (2012): 798-802.
[4] Shou et al. "Synthetic cooperation in engineered yeast populations." Proceedings of the National Academy of Sciences 104.6 (2007): 1877-1882.
[5] Gonze et al. "Microbial communities as dynamical systems." Current opinion in microbiology 44 (2018): 41-49.
[6] Ren, et al. "Population regulation in microbial consortia using dual feedback control." In Proc. of the IEEE 56th Conference on Decision and Control, (2017).
[7] Fusco, et al. "Embedded control of cell growth using tunable genetic systems." International Journal of Robust and Nonlinear Control (2021): 1- 15.
[8] Miller et al. "Quorum sensing in bacteria." Annual review of microbiology 55.1 (2001): 165-199.
[9] Kylilis et al. "Tools for engineering coordinated system behaviour in synthetic microbial consortia." Nature communications 9.1 (2018): 1-9.
[10] Brune et al. "Engineering microbial consortia to enhance biomining and bioremediation." Frontiers in microbiology 3 (2012): 203.
[11] Fiore et al. "Multicellular feedback control of a genetic toggle-switch in microbial consortia." IEEE Control Systems Letters 5.1 (2020): 151-156.
[12] Martinelli et al. "Multicellular PI control for gene regulation in microbial consortia." IEEE Control Systems Letters 6.1 (2022): 3373 - 3378.
[13] Salzano et al. "Ratiometric control of cell phenotypes in monostrain microbial consortia." Journal of the Royal Society Interface 19.192 (2022): 20220335.
[14] Salzano et al. "Ratiometric control for differentiation of cell populations endowed with synthetic toggle switches." In Proc. of the IEEE 58th Conference on Decision and Control, (2019).

Joint supervision arrangements

Meetings will be scheduled every two weeks with the supervisors to ensure the advancement of the project and provide the required support. Davide Fiore, with the co-supervision of Mario di Bernardo, will support the activities during all the duration of the project, while Francesco Giannino will support the activities of the student for the achievement of objective [O3]. The student will also be expected to participate in weekly meetings with the research group, exchanging ideas and attending to seminars of colleagues.

Location and length of the study period abroad (min 12 months)

The student will spend 6 months at the Universitat Politècnica de València, hosted by Prof. Alejandro Vignoni, who already agreed to host the student at the Synthetic Biology and Biosystems Control Lab. The student is expected to visit the Universitat Politècnica de València after the second year of the PhD course (tentative schedule: 01/02/2026-31/07/2026).

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Moreover, in agreement to the requirement reported in the DM n. 118/2023 for the PhDs programs in digital and Ambiental transitions (PNRR-TDA), the student will spend 6 months (tentative schedule: 01/02/2025-31/07/2025) at the Telethon Institute of Genetics and Medicine (TIGEM) in Pozzuoli, Italy, hosted by Prof. Diego di Bernardo.

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Any other useful information

The activities of this project are related to the PRIN 2022 PNRR project “Control of smart microbial communities for wastewater treatment” funded by MUR, of which Davide Fiore is Principal Investigator. The PhD student will have the opportunity to participate to related research activities and collaborate with other partners.