

Microbial applications and tools for environmental management and assessment

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Abstract

Different microbial applications and tools were developed for environmental management and assessment. We lead applied research working at different scales, beginning with projects at a laboratory scale, but as well experimenting with demonstrative projects (pilot plants). We work to discern about mechanisms related to **pathogen removal, water treatment and reuse, and biomaterials production**, both combining traditional and molecular microbiology techniques. We also work in the production, characterization and processing of biopolymers, as well as in the analysis of their degradation by microorganisms.

Introduction

Global water crisis is improving research and development of wastewater reuse and many developed countries are increasing reuse of treated wastewater for irrigation, domestic and industrial applications. In the context of sustainable development, natural systems such as constructed wetlands, sand filters and other decentralized solutions appear as an increasing popular alternative to conventional wastewater systems when treating wastewater from small communities due to its efficiency, low cost and low management requirements.

The presence of pathogens, many of which are capable of long-term survival in the environment, pose health risks to farmers and their families, consumers, and nearby communities. Pathogenic organisms should be removed before discharge, especially if the reuse of effluent is to be considered. Although many studies have been focused on the removal of pathogens in constructed wetlands, some questions still remain unknown about the nature and pathogen removal mechanisms in these systems. We work to discern about these mechanisms both combining traditional and molecular techniques.

Plastics are synthetic polymeric materials that serve as raw material for the production of a large variety of commodities and are utilized in almost every manufacturing industry ranging from automotive and packaging to medicine. The total global capacity of commodity plastic production dramatically increased in the last decades until 250 Mtons/year, fact that leads to environmental and economic problems. The use of biomaterials independent of the oil industry can partially solve this problem, but nowadays still they are not competitive due to its high cost. On the other part, it may indicate that the microorganisms produce a high degree of degradation of the materials that it is important to quantify it in order to avoid or to induce it.

Multi-network Projects

The complexity of what we are now facing in a rapidly climate changing world suggests that no one individual, group or organisation has all the necessary skills or competencies either to comprehensively understand the challenges involved or to design appropriate solutions. Therefore, and considering the environmental challenges today, the majority of the R&D projects developed by our group are carried out by powerful research networks and thanks to collaborative projects. To foster these dynamics work, we try to promote the establishment of internal and external networks. The use of networks that complement each other, in a multi-network scheme, strengthens the capacities of individual groups and especially, it extends the capabilities of the groups involved in the different planned activities, whether education and training, R & D, innovation, diffusion and transfer.

The core activity of the MSMLab and AQUASOST groups is research on microbial applications and tools for environmental management and assessment. But in this sense, we try to make an approach considering the different scales, from micro to macro scale. In the micro scale we have a direct collaboration in the GAIA building with GENIUL, a molecular biology company. In the macro scale we have a collaboration with TYPESA-TECNOMA, the first civil engineering company in Spain today.

Finally, all of our efforts will be directed to make more resilient social and ecological urban systems. In this sense, the concept "Recycling cities" is developed in a collaborative project launched by the UNESCO Chair on Sustainability of the Polytechnical University of Catalonia (UPC) and a network of Universities and researchers from Europe and Latin America.

Results

Several applied studies at different scales were developed and results were shown in this presentation. As an example of micro-scale experiment, a study was carried out to determine the effects of some pollutants (three pesticides, diuron, alachlor and endosulfan), on biofilm bacterial viability in pilot-scale constructed wetlands. Undisturbed mature biofilms (grown in Siporax® rings) from two wetlands, pre-treated and non-pre-treated with pesticides, were collected and exposed individually to the three pesticides. The aim of the study was to determine and compare the pesticides impact on these mature biofilms. To estimate bacterial viability in Siporax® rings a new colorimetric approach based on the reduction of tetrazolium sodium salts such as XTT was used. The results of this study indicate that pesticides impact on biofilm bacterial viability, and inhibition of bacterial activity on non-pre-treated biofilms were higher than on previously treated biofilms. However, a tolerance and even resistance to pesticides was present on both treatments.

In the field of biomaterials, the studies are related with the isolation of new bacterial strains from environmental samples that accumulate a high concentrations of PHA's, its characterization, transformation and formation of antibiotic-loaded nano and micro spheres to reduce the incidence of bacterial infections associated with the implantation of biomaterials in the body. The last results show the higher production of PHB homopolymer cited in the literature from an environmental bacterium of the genus *Bacillus* in industrial conditions. It is a new strain deposited in the Spanish Type Culture Collection. The fact that the strain can properly develop and synthesise PHB in presence and absence of salt suggests that the strain is a great candidate for biotechnological applications in different areas. It is possible for the first time, to degrade high molecular mass PHB polymer and copolymer into low molecular mass by means of commercial lipases. In these conditions, different techniques and methods have allowed

to form for the first time doxycycline-loaded PHB nano- and micro-spheres, for odontological applications.

As an example of more macro-scale and demonstrative experiment, the LIFE project REAGRITTECH will aim to demonstrate a method for recycling water resources at parcel scale, in order to optimize the resources for its best use in the ecosystem and therefore achieve a sustainable and integrated river basin, by improving the chemical characteristics of the reused water and, therefore improve the natural environment surrounding them. The main advantages of this demonstration project will be the optimization of water resources within the supply system, decreasing the contributions of pollutant loads on the hydrological system, the recovery of habitats and riparian and aquatic ecosystems degraded by human pressure, the improvement of the ecological status of surface water bodies, the development of a remedy for potential changes in the environment as a result of its transformation into irrigated agricultural areas, defined by different watershed plans and programs, and reducing investments in treatment facilities of river water for consumption within the plans purification of agencies.

Finally, an integration of different appropriate technologies for water treatment using constructed wetlands and buffer strips were applied in the socio-environmental restoration of the "Morro de Moravia" in Medellín (Figure 1), Colombia. Management of wastewater and rainwater in Morro de Moravia was inadequate, as the area does not have a sewerage system. Pollutants from the dumped waste combined with rainwater to produce a noxious leachate containing high concentrations of organic matter, nutrients, pathogens and heavy metals. The authorities therefore decided to apply low-cost alternatives such as constructed wetlands and buffer strips to remove pollutants from the run-off. The buffer strip at Morro de Moravia consists of a strip of trees, shrubs and grasses planted along a hillside, between the contaminant source and the constructed wetlands. This buffer acts like a living filter, improving water quality by biodegrading, trapping and filtering nutrients, pesticides and other pollutants, and by slowing down run-off. A documentary film of this transformation process has been produced (<http://www.youtube.com/watch?v=-bFyRYNp4Uk&feature=youtu.be>).

Conclusions

Water shortages in arid and semi-arid areas, such as the Mediterranean, have prompted projects for wastewater reuse. Reclamation can be achieved through conventional intensive systems or through naturally- engineered treatments such as reed beds (horizontal subsurface-flow constructed wetlands). In recent years, the natural option using treatment systems working with attached microorganisms has garnered worldwide interest because it is easy to operate and requires less energy than conventional systems, although it does require more surface area than conventional systems. The utilisation of different appropriate technologies is essential to progress towards a more ecologically sound water supply, from water collection through to its treatment and reuse.

Different microbial applications and tools are essential today for environmental management and assessment. We work to discern about mechanisms related to **pathogen removal, water treatment and reuse, and biopolymers production**, both combining traditional and molecular microbiology techniques.



Figure 1. Different images from the environmental restoration of “Morro de Moravia” garbage dump in Medellin, using buffer strips.

References

- Agudelo, R.; Peñuela, G.; Jaime, N.; Morató, J.; Jaramillo, M. (2010) Simultaneous removal of chlorpyrifos and dissolved organic carbon using horizontal sub-surface flow pilot wetlands. *Ecological Engineering*. *Ecological Engineering* 36 (2010) 1401–1408.
- Agudelo, R.M., Machado, C., Aguirre, N.J., Morató, J. and Peñuela, G. (2011) 'Optimal conditions for chlorpyrifos and dissolved organic carbon removal in subsurface flow constructed wetlands', *International Journal of Environmental Analytical Chemistry*, 91: 7, 668 — 679
- Agustí, G., Fittipaldi, G., Morató, J., Codony, F. 2013. Viable quantitative PCR for assessing the response of *Candida albicans* to antifungal treatment. *Appl Microbiol Biotechnol* (2013) 97:341–349.
- Agustí, G., Fittipaldi, M., Morató, J., Codony, F. 2012. Viable quantitative PCR for assessing the response of *Candida albicans* to antifungal treatment. *Applied microbiology and biotechnology*. 11/2012. DOI:10.1007/s00253-012-4524-z
- Casas-Zapata, J.C., Rios, K., Florville-Alejandre, T.R.m Morató, J., Peñuela, G. 2013. Influence of chlorothalonil on the removal of organic matter in horizontal subsurface flow constructed wetlands. *Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes*. 01/2013; 48(2):122-32.
- LLop, J.Ma, Salas, H. & Morató, J. (Eds). 2013. *Reciclar Ciudad: Repensar la Transformación de las Ciudades*. Cátedra UNESCO de Sostenibilidad, Universitat Politècnica de Catalunya – BarcelonaTech.
- Codony, F., Fittipaldi, M., Lopez, E., Morató, J., Agustí, G. 2012. Well Water as a Possible Source of *Waddlia chondrophila* Infections. *Microbes and environments / JSME*. 10/2012
- Codony, F., Martin Perez, L., Adrados, B., Agustí, G., Fittipaldi, M. & Morató, J. 2012. Amoeba-related health risk in drinking water systems: could monitoring of amoebae be a complementary approach to current quality control strategies? *Future microbiology*. 01/2012; 7(1):25-31.
- Codony, F.; Fittipaldi, M.; Adrados, B.; Morató, J.; Pérez, L.; Álvarez, B.; Peñuela, G.A. 2010. A new microtiter-plate screening method for evaluating the viability of aerobic respiring bacteria in high surface biofilms. *Letters in Applied Microbiology*, 51(3): 331-337.
- Fittipaldi, M., Codony, F., Adrados, B., Camper, A.K. and Morató, J. 2011. Viable Real-Time PCR in Environmental Samples: Can All Data Be Interpreted Directly? *Microbial ecology*. *Microb Ecol* (2011) 61:7–12.
- Fittipaldi, M., Pino, N.J., Adrados, B., Agustí, G., Peñuela, G., Morató, J. & F. Codony. 2011. Discrimination of Viable *Acanthamoeba castellanii* Trophozoites and Cysts by Propidium Monoazide Real-Time Polymerase Chain Reaction. *J. Eukaryot. Microbiol.* 58(4):359-64. doi: 10.1111/j.1550-7408.2011.00557
- Fittipaldi, M., Rodriguez, N.J.P., Codony, F., Adrados, B., Peñuela, G.A., Morató, J. 2010. Discrimination of infectious bacteriophage T4 virus by propidium monoazide real-time PCR, *Journal of Virological Methods* (2010), 168 -1-2, pp. 228 – 232; doi:10.1016/j.jviromet.2010.06.011
- Garcia, J., Rousseau, D.P.L., Morató, J., Usage, E., Matamoros, V. & Bayona, J.M. (2010). 'Contaminant Removal Processes in Subsurface-Flow Constructed Wetlands: A Review. *Critical Reviews In Environmental Science and Technology*, 40: 7, 561 – 66

Martín Perez, L., Codony, F., Ríos, K., Peñuela, G., Agradados, B., Fittipaldi, M., de Dios, G., J. Morató. 2011. Searching *Simkania negevensis* in environmental waters. *Folia Microbiol.* (2012) 57(1):11-14.

Montoya, J., Cuesta, O., Flecha, O., Viade, D., Gallegos, A. & J. Morato. 2011. Moravia como ejemplo de transformación de áreas urbanas degradadas: tecnologías apropiadas para la restauración integral de cuencas hidrográficas, *NOVA* 9-15, 38-49; iSSN:1794-2470.

Rodriguez-Contreras, A., Margarita Calafell-Monfort, María Soledad Marqués-Calvo. Enzymatic degradation of P(3HB-co-4HB) by commercial lipases. *Polymer Degradation and Stability*, 2012, 97: 597-604.

Rodriguez-Contreras, A., Margarita Calafell-Monfort, María Soledad Marqués-Calvo. Enzymatic degradation of poly(3-hydroxybutirate) by a commercial lipase. *Polymer Degradation and Stability*, 2012, 97: 2473-2476.

Rodriguez-Contreras, A., Martin Koller, Miguel Miranda-de Sousa Diaz, Margarita Calafell, Gehart Braunegg, María Soledad Marqués-Calvo. Novel Poly[(R)-3-hydroxybutyrate]-producing bacterium isolated from a Bolivian hypersaline lake. *Food Technology and Biotechnology*, 2013, 51: 123-130.

Rodriguez-Contreras, A., Martin Koller, Miguel Miranda-de Sousa Diaz, Margarita Calafell, Gehart Braunegg, María Soledad Marqués-Calvo. High production of poly(3-hydroxybutyrate) from a wild *Bacillus megaterium* Bolivian strain. *Journal of Applied Microbiology*, 2013, 114: 1378-1387.