



INTERACTIONS BETWEEN EUKARYOTIC AND PROKARYOTIC MICROORGANISMS IN ACTIVATED SLUDGE: A MOLECULAR APPROACH TO IMPROVE WASTEWATER TREATMENT

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ABSTRACT

Wastewater treatment is one of the most important biotechnological processes which are used worldwide to treat municipal and industrial sewage. Activated-sludge processes aim to achieve a maximal reduction of the Biological Oxygen Demand (BOD) of wastewater with a minimal production of biological solids. Even though many microbiological methods are available to study the activated sludge communities, many recent questions on the role of certain microorganisms are kept unanswered. A crucial addressed by the present work is to know if the presence of different groups of bacteria and protozoa lead to significant consequences on the process performance. Focusing on the importance of the interactions between the prokaryotic and the eukaryotic populations, the work will contribute to the knowledge of how microbial communities are established in a complex environment as the activated sludge processes.

WASTEWATER AND MICROORGANISMS: A WORLD TO DISCOVER

The improvement of global health and sanitation and consequent reduction in the spread of disease depends largely on good hygiene practices, availability of health facilities, and reliable collection and treatment of waste water. The World Health Organization (WHO) estimates that 2.4 billion people lack access to any type of sanitation equipment. The overall sustainability of a wastewater treatment technology is a function of economic, environmental and social dimensions (Muga and Mihelcic, 2008). The European Union across its 27 Member States, has a population of more than 492 million people. Wastewater generated by these people, as well as by industry, is a major source of pollution of natural waters (COM, 2007). In Portugal, wastewater treated summed up 392.8 millions of m³ and 8.35

million was the number of people covered with wastewater treatment (Águas de Portugal, 2010).

Bacteria clearly play a vital role in the conversion of the wide diversity of organic compounds and in the removal of nitrogen and phosphorus in aquatic ecosystems, and particularly in activated-sludge. The efficiency and robustness of a wastewater treatment plant (WWTP) mainly depend on the composition and activity of its microbial community (Wagner *et al.*, 2002). Ciliate protozoa not only reduce the density of dispersed bacteria in the environment by feeding, but also lead to a reduction in the concentration of soluble BOD, being the causes not determined so far.

The role of the grazers on the prokaryotic populations clearly influence the performance of the treatment plant and also lead to a change of the kinetic parameters of the microorganisms from one WWTP to another. Several studies have demonstrated the potential of protozoa as bio-indicators of the process performance and effluent quality (Nicolau *et al.*, 2007). Regardless the advance in aquatic ecology, the study of the interactions between eukaryotic and prokaryotic microorganisms in activated sludge remains a relatively new field of research.

Molecular Approach

Examples of the benefits of population dynamics research in improving the performance and in assisting the design and operation of biological wastewater treatment processes are known. On the other hand, the study of the mechanisms supporting population dynamics in activated sludge are still built on bacteria-substrate or bacteria-oxygen dependences, hardly considering other components, even if the protozoa are nowadays considered to play fundamental roles in this complex environment. Molecular techniques have only recently been applied to determine the phylogenetic diversity and relationships of free-living protists in aquatic ecosystems, and to identify changes in abundance of particular species of protists (Sherr and Sherr, 2002). A lot of work has been conducted in both



mathematical modelling and in the study of the microbial ecology of activated sludge systems, but the role of protozoa in the determination of the whole community of activated sludge has been largely neglected. Misleading and difficult identification of bacteria by traditional microscopic techniques directed research towards molecular methods, namely DGGE and FISH techniques. Nevertheless, studies integrating both the prokaryotic and the eukaryotic populations in activated-sludge are non-existent. In many cell biological studies, gene expression is the starting point for elucidation of mechanism at the microscopic and molecular levels. Describing the coordination of gene expression is therefore a central step towards understanding cellular systems, (Larson *et al.*, 2009).

The highly complex microbial communities in WWTP are barely understood, remaining, most of the time, as a “black box”. The application of molecular tools in wastewater microbiology has revolutionized our view on the microbial ecology of these systems. In wastewater microbiology, more detailed knowledge on the biology of the non-cultured bacteria needs to be gained. Regarding application, the most obvious benefit of the progress described is that it provides a basis for a more knowledge-driven treatment of WWTP failures (Wagner *et al.*, 2002).

Then... Questions take place

How do the biotic factors, along with the physical-chemical and the operational traits, determine the occurrence of particular groups of the prokaryotic component? Can the prevalence of the different groups of protozoa be associated with any or some of these prokaryotic groups? Can the occurrence or dominance of some groups of protozoa be the direct result of abiotic factors, or are they determined by the composition of the prokaryotic populations as well? And to what extend?

Discovery Plan

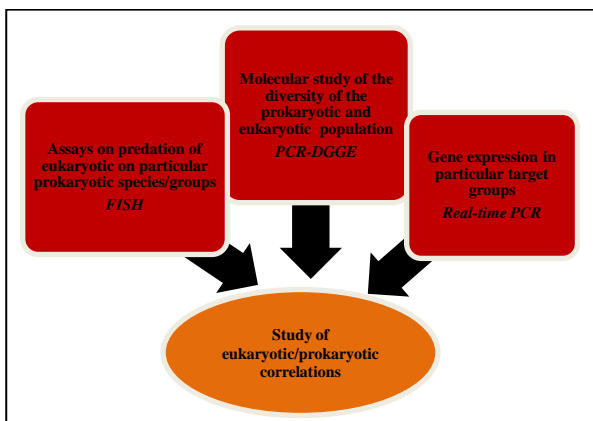


Figure 1: Outline of the work plan

At the end, important results concerning the microbial ecology of activated sludge will have been attained. The occurrence and the activity of some relevant prokaryotic and eukaryotic groups will be investigated in real activated sludge systems, and the abiotic and biotic factors contributing for their prevalence in such environments will be determined.

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MARTA M. NETO was born in Almeida, Portugal and went to the Universidade Nova de Lisboa where she studied environmental engineering and obtained her master degree in 2008. She worked with environmental microbiology and molecular ecology, her research focus being on how the network of microorganisms degrades contaminants in natural environment, maximizing their potential and leading to the biological decontamination. At the moment, she is starting her PhD (Programa Doutoral em Engenharia Química e Biológica) which aims to contribute to the improvement of wastewater treatment by knowing the impact of microorganisms' interactions in the performance of activated sludge processes and the reason why they happen to occur in these specific man-made ecosystems.