The Biotransformation of Hydrophobic and Hydrophilic Pharmaceuticals and Their Metabolites by Nitrifying and Heterotrophic Cultures

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ABSTRACT: Residues of human and veterinary pharmaceuticals are introduced into the environment via a number of pathways, but primarily from discharges of wastewater treatment plants or land application of sewage sludge and animal manure. Presently, it is difficult to accurately assess the overall ecological impacts of pharmaceutical residues in the environment because information on the identities and quantities of their degradation products is very limited. This lack of information is largely due to analytical challenges encountered in detecting and quantifying trace levels of unknown compounds in complex environmental matrices. The objective of this research was to examine the removal efficiencies of selected pharmaceuticals in a pilot scale membrane bioreactor (MBR) and a full-scale conventional wastewater treatment plant in Barcelona, Spain, and compare the results with the data from our current studies in Buffalo, New York (USA). The research was performed at the Chemical & Environmental Research Institute of Barcelona (Institut d'Investigacions Quimiques i Ambientals de Barcelona or IIQAB), Spain. To investigate the pharmaceutical removal efficiencies of various full-scale wastewater treatment systems in Barcelona, Spain and compare their efficiencies with the common treatment systems in the United States. We exchanged graduate student between our institutions and utilized state-of-the art instrumentation for trace analysis of pharmaceutical contaminant is environmental samples. These include a new ultra-high pressure liquid chromatograph with quadrupole time-of-flight mass spectrometer (UPLC Q-ToF-MS), a high resolution mass spectrometer, in the identification of trace levels of pharmaceutical metabolites formed during their treatment and degradation in the environment.

INTRODUCTION

The name of awardee institution for current NSF award is University at Buffalo. The names of travelers were Dr. Diana S. Aga (PI) and Mary Dawn Celiz (graduate student). The dates of travel were June 15, 2007 – September 14, 2007.

In the current NSF award, one of our objectives is to determine the rate and extent of model pharmaceutical biotransformation and byproduct formation by *Nitrosomonas europaea*, and by an enriched, non-nitrifying heterotrophic culture derived from activated sludge. However, it is important to compare the biodegradation of pharmaceuticals and the identities of their by-products formed in laboratory-scale bioreactors with those with full-scale wastewater treatment plants (WWTPs). The fate of certain pharmaceuticals in sewage during treatment has been studied to some extent, but because almost every treatment facility is unique in terms of its catchment area and treatment processes, a careful investigation of the sample matrices is useful in constructing predictive environmental risk assessment models.

In this research supported by IREE, a major treatment facility in Barcelona [receiving various proportions of domestic, hospital, industrial and agricultural wastes] was investigated in terms of its efficiency in pharmaceutical removal. This facility has a conventional treatment and a pilot-scale membrane bioreactor (MBR) technology. Due to the increasing importance of water reuse in highly populated cities worldwide, advanced treatment technologies are becoming more common in pursuit of providing better water quality to the public. The MBR technology is considered as the most promising development in microbiological wastewater treatment due to two important features: (1) the low sludge load in terms of biological oxygen demand which enables bacteria to mineralize poorly degradable organic compounds, and (2) the high sludge age that gives the bacteria time to adapt to these substances.

The research was conducted at the Institut d'Investigacions Quimiques I Ambientals de Barcelona (IIQAB), a research institute of the Spanish National Research Council (Consejo Superior de Investigaciones Científicas, CSIC). The research focus of IIQAB-CSIC includes basic and technologic areas of Chemistry and the institute aims to contribute to the knowledge of the geosphere, the biosphere and to the impact of the human activity on the ecosystem. IIQAB is well equipped with state-of-the-art analytical instrumentation and specializes in LC/MS and LC/MS/MS integrated systems. Many researchers from other European institutions collaborate intensively with IIQAB because funding typically comes from the European Union. Hence, there are several scientists, postdoctoral fellows, and Ph.D. students from other European countries conducting research at IIQAB. The foreign collaborator, **Professor Damia Barceló**, is a full Research Professor at IIQAB-CSIC and Head of the Environmental Chemistry Department. His research interests include analysis and fate of organic pollutants, surface and ground water, coastal water and wastewater characterization, endocrine disrupting compounds and biosensors for toxicity assessment.

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

During the IREE trip, we had three major activities: (1) attended an international meeting in Frankfurt, Germany, where we presented results from our current NSF project, (2) collected and analyzed samples from a major WWTP in Barcelona, with MBR and conventional activated sludge (CAS), and (3) joined the departmental activities including visit to a typical Catalan village and the Barcelona drinking water tower. These activities are described below.

1. Diana Aga (PI) and Mary Dawn Celiz (graduate student) both gave an oral presentation at the *5th IWA Specialized Conference on Assessment and Control of Micropollutants / Hazardous Substances in Water* , 17 - 20 June 2007, held at Frankfurt, Germany. The IWA conference provided an international platform for drinking water and wastewater engineers, environmental chemists, water managers, hydrogeologists, ecotoxicologists and toxicologists to discuss the removal and the effects of micropollutants and hazardous substances in water systems.

2. Research at the IIQAB-CSIC involved sample collection and analysis from an MBR and a CAS treatment systems at the Rubi WWTP, which receives municipal, hospital, and industrial wastewater. Composite samples were collected from the effluent of the primary sedimentation tank which serves as the influent to the CAS and MBR (hence labeled influent in figure 1), and from CAS and MBR effluents 4 times during July 2007.

Table 1.0 compares the removal efficiency of selected pharmaceuticals included in this study in CAS and MBR. In general, the MBR has a higher %removal compared to CAS, which may be attributed to the infinite SRT and a higher biomass concentration in MBR. Adsorption on the biomass may also play a role, but most of these pharmaceuticals are highly water soluble, hence we suspect that removal by sorption is not the major mechanism of removal. The compounds found to be resistant to biodegradation are carbamazepine, diatrizoate, and iopamidol.

Table 1. Comparison of the removal efficiency of MBR and CAS

Compound Analyzed	% removed	
	CAS	MBR
Carbamazepine	10	2
Diclofenac	8	78
Diatrizoate	18	32
Enalapril	79	95
Enalaprilat	80	93
Trimethoprim	29	97
Iopromide	19	83
Iopamidol	13	33
Iohexol	39	84
Aceclofenac	49	51

The concentrations of two of the compounds we are studying under our current NSF funding (iopromide and trimethoprim) in the Barcelona WWTP are presented in

Figure 1.0. These pharmaceuticals are present in pg/L to ng/L range. It is interesting to note that while we observe 0 to less than 15% biodegradation of iopromide and trimethoprim in the lab-scale bioreactors using *Nitrosomonas europea*, we observed high removal of iopromide and trimethoprim in the MBR. We are currently investigating the important mechanisms governing this high removal rates of pharmaceuticals in MBR relative to CAS.

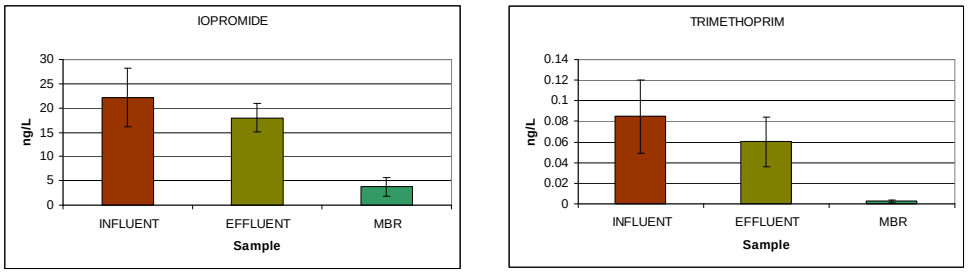

Figure 1. Concentrations of iopromide (x-ray contrast agent) and trimethoprim (antibacterial agent) in the influent and effluents of a conventional activated sludge and membrane bioreactor (MBR).

3. During our stay at the IIQAB-CSIC, we learned how to use all the new mass spectrometry instruments, we interacted (both in the lab and in social settings) and established relationships with the students and scientists in the environmental chemistry department. The group was composed of students and postdocs from different areas in Spain, and from other countries such as Brazil, Denmark, France, Portugal, Slovenia, Serbia, and Switzerland, which provided an international atmosphere. We joined a field trip to one of the wineries in the Catalunya region and an educational tour of the Torre Agbar, an architechtural wonder in Barcelona which offices the Agbar Group, a world leader in activities related to the water cycle and in the services of health and certification. Spain, being one of the oldest civilizations provided the student and faculty participants a diverse experience in culture, architecture, and food.

BROADER IMPACTS OF THE INTERNATIONAL TRAVEL

The supplement award supported the international travel of a female junior faculty and a female graduate student. All activities described above has helped the recipients to establish a long-term collaboration with several European scientists, including those whom we have met at the Mocropol meeting in Germany. To date, the host institution has invited other graduate students from our laboratory to conduct summer research in Barcelona, in exchange with Spanish graduate students who will conduct research at the Universit at Buffalo. We are currently preparing a collaborative proposal with the host institution, to be submitted to the European Union Work Program for 2008, which will enhance the career of a female faculty. Our cultural activities in Barcelona have enhanced our understanding of the Spanish government, work habits, traditions and educational system. Our visit to the Torre Agbar gave us very good perspectives of new architechtural designs that are highly energy efficient while maintaining a conducive working environment.

DISCUSSION AND SUMMARY

Results from our experiments at the IIQAB-CSIC provided new data which suggest that our batch reactors using pure nitrifying cultures may give us different biological degradation pathways of pharmaceuticals, and these may not necessarily explain what is observed in actual WWTPs. The observed enhancement in removal of many pharmaceutical compounds by MBR is promising in terms of improving our current technologies for treating micropollutants, such as antibiotics and endocrine disrupting chemicals. Our results gave us new directions on how to proceed with our on-going laboratory experiments to investigate the role of heterotrophic cultures in continuous flow systems. Our international experience gave us a unique opportunity to meet and discuss research ideas with the leading European experts in this area of research. We were able to use modern analytical instruments at the host institution that allowed us to analyze very low concentrations of pharmaceuticals and their metabolites in WWTPs.

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BRIEF BIOGRAPHIES OF RESEARCHERS

Diana S. Aga is an Associate Professor of Chemistry at the University at Buffalo, The State University of New York, Buffalo, NY. Diana Aga received her B.S. degree in Agricultural Chemistry at the University of the Philippines at Los Baños (1988), and her Ph.D. in Environmental and Analytical Chemistry at the University of Kansas (1995). She was a research assistant at the United States Geological Survey, Lawrence, Kansas (1993-1996), and a postdoctoral fellow at the Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Switzerland (1996-1998). Diana Aga is recipient of various research awards such as the National Science Foundation CAREER Award, the North Atlantic Treaty Organization Scientific and Environmental Affairs Fellowship, and the Alexander von Humboldt Research Fellowship.

Mary Dawn Celiz is a Ph.D. student in Analytical Chemistry at the University at Buffalo, The State University of New York, Buffalo, NY. She received her B.S. degree in Agricultural Chemistry at the University of the Philippines at Los Baños. She is currently supported by the NSF grant no. BES 0504359.