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**IMPACTO DE HIDRELÉTRICAS BRASILEIRAS NAS  
MUDANÇAS CLIMÁTICAS: MICRO-ORGANISMOS  
ASSOCIADOS À EMISSÃO DE METANO EM RESERVATÓRIOS**

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**IMPACT OF BRAZILIAN HYDROPOWER ON CLIMATE  
CHANGE: MICROORGANISMS ASSOCIATED WITH METHANE  
EMISSIONS IN RESERVOIRS**

Thesis presented to the Graduate Program in  
Microbiology from the Institute of Biomedical  
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## ABSTRACT

Torres-Ballesteros AM. Impact of Brazilian hydropower on climate change: microorganisms associated with methane emissions in reservoirs [Ph. D. thesis (Microbiology)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2016.

Brazil generates most of their electricity from Hydropower, however carbon footprint energy from this source has been questioned in the last years. One of the main critics is that methane emission is still not included in assessments of environmental impact. We studied the microbial ecology related with this greenhouse gas, measuring abundance of methanogenic and methanotrophic microorganisms in hydropower reservoirs. We found a strong correlation between relative abundance of these microbial groups and methane fluxes in the air–water interface. Was demonstrated that relations in gene abundances correspond with methane cycle in the six ecosystems studied. As reported in previous works, we confirmed that geographical location influences methane emissions and microbiology related to carbon fixation in aquatic environments. According to multivariate analysis, oxygen, geophysical and climatological parameters shape the balance of microbial communities related with methane cycle. Also we collected samples of sediment in São Marcos River, area chosen for a new hydroelectric power plant, and tested the methanogenic potential incubating the sample under anoxic gas different carbon source conditions. Methane production was measured during three months and analysed by gas chromatography. After incubations were completed, an aliquot was taken for nucleic acid. Our results showed a significant difference in methane production rates and lag times among the microcosms. The earlier production of methane from acetate microcosm over time confirms the importance of this process in this environment. Methanol is a substrate used exclusively by methanogenics, and thus, might explain the shortest time for the production of methane in the cultures with this carbon source. The potential methane emission in the sample was confirmed with the increasing of gas production during incubation and the amount of methane produced depends of carbon source type. These conditions also have a significant effect in the community structure of methanogenic Archaea. Findings from this work would provide scientific evidence to mitigate methane emission and manage hydroelectric development in tropical countries, one of the central sources of energy. Information acquired in this work also will contribute to achieve the challenge for future hydropower projects: settles the reduction of emissions greenhouse gases and guarantee the supply of energy demands.

**Keywords:** Microbial ecology. Climate change. Renewal energy. Carbon cycle. Greenhouse gases. Methanogenesis. Methane oxidation.

## RESUMO

Torres-Ballesteros AM. Impacto de hidrelétricas brasileiras nas mudanças climáticas: micro-organismos associados à emissão de metano em reservatórios [tese (Doutorado em Microbiologia)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2016.

Brasil gera a maior parte de sua eletricidade a partir de hidrelétricas, no entanto energia a partir desta fonte tem sido questionada nos últimos anos pela pegada de carbono. Uma das principais críticas é que a emissão de metano que ainda não está incluído nas avaliações de impacto ambiental. Nós estudamos a ecologia microbiana relacionadas com este gás de efeito estufa, medindo abundância de bactérias metanogênicas e metanotróficas em reservatórios hidrelétricos. Descobrimos uma forte correlação entre a abundância relativa destes grupos microbianos e fluxos de metano na interface ar-água. Foi demonstrado que as relações em abundâncias de genes correspondem com a regulação de metano nos seis ecossistemas estudados. Também confirmamos que a localização geográfica influencia as emissões de metano e a dinâmica de micro-organismos relacionadas com a fixação de carbono em ambientes aquáticos. De acordo com a análise multivariada, oxigênio, parâmetros geofísicos e climatológicas moldam o equilíbrio das comunidades microbianas relacionadas com o ciclo de metano. Também foram coletadas amostras de sedimentos no rio São Marcos, área escolhida para uma nova usina hidrelétrica, e testamos o potencial metanogênico incubando a amostra sob anóxia e em diferentes condições da fonte de carbono. A produção de metano foi medida durante três meses. Após as incubações, uma alíquota foi feita para o extração de ácidos nucleicos. Nossos resultados mostraram uma diferença significativa nas taxas de e tempos de produção de metano entre os microcosmos. A quantidade de metano produzido a partir de Acetato confirma a importância deste substrato neste ambiente. O metanol é um substrato utilizado exclusivamente para metanogênese, e, portanto, pode explicar o menor tempo para a produção de metano em culturas com essa fonte de carbono. O potencial de emissão de metano na amostra foi confirmada com o aumento da produção de gás durante a incubação e a quantidade de metano produzido depende do tipo de fonte de carbono. Esta condição também tem um efeito significativo na estrutura da comunidade de micro-organismos metanogênicas. Os resultados deste trabalho fornece evidências científicas para mitigar a emissão de metano e promover o desenvolvimento hidrelétrico, uma das fontes centrais de energia em países tropicais. As informações obtidas neste trabalho também contribuem para alcançar o desafio de projetos hidrelétricos futuros: redução das emissões de gases de efeito estufa e garantir o suprimento de demandas energéticas.

**Palavras-chave:** Ecologia microbiana. Mudanças climáticas. Energia renovável. Ciclo do carbono. Gases de efeito de estufa. Metanogênese. Oxidação de metano.

# **CHAPTER 1 - MICROORGANISMS RELATED TO METHANE CYCLE IN HYDROPOWER RESERVOIRS**

## **1.1 INTRODUCTION**

Research and development in energy efficiency could help in adaptation and mitigation strategies of climate change while enhancing the prospect of achieving sustainable growth. Climate change affects the function and operation energy infrastructure (as hydroelectricity) due to increasing of water demand in the coming decades, primarily because of population growth (Bates et al., 2008).

Hydropower is one of the main energy sources in tropical areas. In Brazil, 67% of the power demand is supplied by hydroelectric plants (The Brazilian Electricity Regulatory Agency, ANEEL, 2016). This renewal energy has the potential to mitigate climate change, but carbon footprint of hydropower reservoirs has been questioned in the last years (Fearnside, Pueyo, 2012; Fearnside, 2015; Hu, Cheng, 2013). Although methane (CH<sub>4</sub>) is known as one of main greenhouse gases (GHD), the emission of this gas is still not included in assessments of environmental impact of hydroelectricity (Li, Lu, 2012). In this regard, recent works have tried to define and quantify methane emission factors in hydropower reservoirs (Barros et al., 2011; Maeck et al., 2013; Roland et al., 2010).

Brazil also developed a project (Balcar study) to obtain data related to greenhouse gas emissions from hydropower reservoirs. Besides monitoring, this information is used to estimate potential emissions (by modeling) and to establish future management practices in hydropower plants. The project was coordinated by the Electric Power Research Center (CEPEL) and headed by The Brazilian Electricity Regulatory Agency (in Portuguese, Agência Nacional de Energia Elétrica, ANEEL) with the Ministry of Mines and Energy (MME). Research teams associated to Balcar study, including Sao Paulo University (USP) and Federal University of Pará (UFPA), collected data from reservoirs located in different biomes. Professor Artur da Costa da Silva from UFPA leads the microbiology group, in which this PhD work is framed.

In freshwater ecosystems, organic carbon sources (carbohydrates, long-chain fatty acids and alcohols) are mineralized and a proportion of final products are channeled in to CH<sub>4</sub> biogenesis (Liu, Whitman, 2008); by anaerobic microorganisms called methanogens (Conrad, 2009). However biological processes related with methane formation (methanogenesis) remains unexplored in reservoirs sediments, mainly in tropical areas, where carbon cycle is more productive (Raddatz et al., 2007) and methane emissions seem to be higher than temperate areas (Yang et al., 2014a).

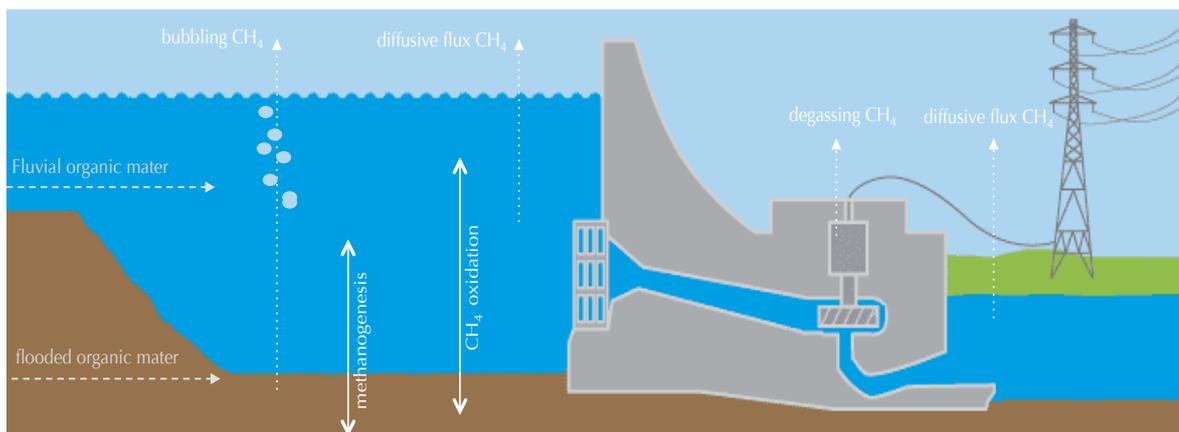
Methanogens diversity and metabolic capabilities are usually associated with substrate chemistry (Conrad, 2007); as well as other microbial communities respond to composition, quantity, and variety of carbon molecules in their environment (Hernández, Hobbie, 2010; Wang et al., 2013). Therefore, organic carbon input management could be a key element in reducing methane emissions from reservoirs (Bergier et al., 2014); to date no study has been performed to verify the effect of carbon in methanogen communities and CH<sub>4</sub> fluxes in hydropower reservoirs.

Carbon input differences in reservoirs can modify methane formation at community and molecular level, resulting in changes of methane emission to the atmosphere (Jugnia et al., 2005). Also metabolic capabilities of methanogens could be different in response to organic matter changes. After flooding soil during hydropower reservoir construction, carbon and oxygen varies considerably (Friedl, Wüest, 2002) and could influence methanogenic communities. To test this hypothesis was designed an experiment with sediment samples from São Marcos River, area chosen for a new hydroelectric power plant. Chapter Two present results of methanogenic potential from those samples incubated in microcosms simulating flooded, anoxic and different carbon source conditions. Additionally, data from microcosm in those conditions can be an indicator of land use changes effects in microbial communities.

Methanogenesis is very well described in freshwater sediments (Borrel et al., 2012), and recently it has also been detected in the water column (Bogard et al., 2014). Bacterial communities (methanotrophs) consume a proportion of methane produced by methanogens (methane oxidation). The final methane flux result from the balance between those two microbial processes linked to a variety of physical factors, as geometry and hydrodynamic of reservoirs (Bambace et al., 2007; Ometto et al., 2013) (see figure 1).

Analyses described in Chapter Three shows how methane fluxes in the air–water interface relates with abundance of methanogens and methanotrophs. Also, from data collected *in situ* was possible to infer which parameters shape the balance of microbial communities associated with methane cycle in reservoirs. Chapter Three presents relative abundance of methanogenic and methanotrophic communities in six Brazilian hydropower projects: Xingó, Balbina, Tucuruí, Três Marias, Segredo and Funil. Findings from this work would provide scientific evidence to mitigate future methane emissions and manage hydroelectric development in tropical countries.

Among innumerable methods to study microbial communities from sediments and soils, incubation in microcosm has the great advantage to enrich organism involved in specific metabolism (as methanogenesis or methane oxidation) (Konopka et al., 2015). Microbial diversity response to environmental changes could be monitored with new generation sequencing of 16S rRNA gene (methodology employed in Chapter Two). Besides 16S rRNA gene, Nunoura et al., (2008) suggested the use of functional genes as alternative molecular marker for methanogens and methanotrophic communities in environmental samples (without enrichments or incubations). In Chapter Three quantitative Polymerase Chain Reaction (qPCR) was used to detect those functional genes associated with methane cycle; since it is rapid, sensitive and a high efficiency technique (Colwell et al., 2008; Nunoura et al., 2008; Sharkey et al., 2004; Yu et al., 2005).



**Figure 1 - Methane cycle in hydropower reservoirs.**

Fluvial and flooded organic mater (soil, plant material, wood) is degraded to simplest compounds that are substrates for methanogenesis. Methane formation occurs mainly in sediments. Bacteria in the superficial sediment and column water oxidize a proportion of methane. The other part of methane is emitted to the atmosphere by bubbling, diffusive flux and in a degassing process at the dam. (Adapted from concepts in Bogard et al., 2014; Borrel et al., 2011; Goldenfum, 2010)

## **1.2 OBJECTIVES**

The objectives of this study were:

- (i)* To measure the potential methane formation in an area designed for a hydropower reservoir and to evaluate changes in the diversity of Archaea and Bacteria communities with different carbon source conditions in sediment samples.
  
- (ii)* To estimate the abundance of methanogens and methanotrophic microorganism in six hydroelectric reservoirs using phylogenetic markers; and to identify relations between microbial communities and methane fluxes in the atmosphere-water interface.

## **1.3 DOCTORAL PROJECT MOTIVATION**

A proportion of research related to climate change is driven by the need to mitigate effects of rising global temperatures. Existing information of greenhouse gases emissions from hydropower is not enough and there are difficulties in making decisions in energy infrastructure investment. The information generated from this project would provide scientific evidence to take actions to mitigate methane emission and stimulate hydroelectric development in tropical regions (one of the main sources of energy).

This research also will bring benefits to the scientific community interested in energy production and climate change. The results may provide some keys to design strategies in which renewable resources are used and emissions of greenhouse gases are minimized. Microbial ecology techniques will be used to test hypothesis; studying microorganisms in these environments is an innovative contribution in this field.

## 4 CONCLUSIONS AND FINAL REMARKS

Differences in carbon entry into new reservoirs modify the microbial community structure resulting in potential emissions of methane. A methane increase was observed after incubating soil samples (collected in areas intended to reservoirs) with different methanogenic carbon sources. However, the amounts of methane were not significant when compared between the carbon sources.

Metabolic pathways associated to acetate seem more productive, since the methane produced was the highest in microcosm with this carbon source. Bacteria and Archaea diversity changes in response to organic matter differences in the flooded samples.

Assessment of stress (anoxic conditions and carbon source changes) changes in identity of the dominant species and the community structure after flooding soil simulations. As expected shifts in microbial community composition following the exposure to a novel environmental regime lead to increased productivity (methane production, in the case of methanogenic archaea) and ecosystem performance.

The strong correlation between relative abundance of microbial groups and methane fluxes in the air–water interface, demonstrated that relations in gene abundances correspond with methane cycle in the six ecosystems studied. Geographical location influences methane emissions and microbiology related to carbon fixation in aquatic environments. According to multivariate analysis, oxygen, geophysical and climatological parameters shape the balance of microbial communities related with methane cycle. Methanotrophic organisms consume a proportion of methane product of organic matter decomposition resulting less CH<sub>4</sub> emitted into the atmosphere. With hydropower operational planning (based in microbial ecology information) will be possible manage quantity of methane emissions.

The balance between methanogenesis and methane oxidation, quantified by *mcrA* and *pmoA* abundances, will result in different values for methane fluxes at the air-water interphase. Also if methanotrophic organisms consume a proportion of methane product of organic matter decomposition; the amount of CH<sub>4</sub> emitted into the atmosphere could be managed with hydropower operational planning (based in microbial ecology information).

Efforts have been invested in order to identify potential mitigation strategies for the CH<sub>4</sub> emission in hydropower reservoirs and prevent higher consequences for the climate change scenario. The challenges for future hydropower projects are settles the reduction of emissions greenhouse gases and guarantee the supply of energy demands, sustainably.

The Energy Research Company estimates that Brazil needs 6.350 megawatts of new electricity generation per year between now and 2022. Today the country obtains 70% of their energy from hydroelectric power plants. Hydropower is an attractive energy option for many reasons. It is cheaper than thermoelectric power and most other renewable forms of electricity, can provide energy at scale more easily and with fewer disruptions than wind or solar, and can potentially provide electrical energy with lower levels of greenhouse gas emissions than thermoelectric energy, although its effect on methane production could counteract this benefit.

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