

Inspired by the finding that bacteria can generate electricity from mud, **Professor Abraham Esteve Núñez** is developing bacterial-driven devices for bioremediation and water treatment

## What are the driving forces guiding your research?

Twelve years ago, the fascinating finding that bacteria could generate electricity from mud emerged. Out of this mechanism, several potential applications appeared. During my time as a postdoctoral researcher at the University of Massachusetts Amherst, I explored the physiology and biochemistry of Geobacter, the first microorganism reported to produce electricity and the most effective at performing such a reaction. As a biochemist, the challenge of elucidating the bacteria-electrode interface and the possibilities that could arise from such a mechanism was exciting. Now, I am a professor in a Chemical Engineering Department - an ideal location for developing applied technologies for environmental purposes.

## How does your work utilise mechanisms found in nature?

Living organisms are in an energetic crisis from the outset: they face a lack of nutrients and resources, and problems in dealing with waste. We are aiming to mimic some of the methods that microorganisms have developed to cope with that stress. For instance, they have developed extracelullar electron transfer (EET) to deal with energy deficiency, and we aim to merge this natural mechanism with electrochemical engineering.

# Can you explain what microbial electrochemical technologies (METs) are, and the role they play in your research?

METs link microbial metabolism to an electrochemical system to, for example, capture electrons from organic waste. Within

this scheme, there are different configurations, such as microbial fuel cells (MFCs) to harvest energy or microbial electrolysis cells (MECs) to invest energy in setting an artificial voltage. METs are strongly associated with applied science, and some start-ups based upon them have recently emerged. I am not only concerned with the practical applications of this process, but also what happens inside the bacteria in terms of metabolism, and the interaction between them and the electrodes.

## What do you hope to achieve through your work with electroactive bacteria?

We aim to provide insights into the mechanisms bacteria use for donating or accepting electrons from conductive materials. Specifically, one of our goals is to stimulate natural electroactive bacteria to enhance bioremediation of polluted environments, or alternatively we can also harvest clean energy from natural environments. In addition, my group is designing sustainable technologies for treating wastewater in order to produce freshwater from saline water at zero energy cost.

## Can you expand on your recent development of a conductive biofilter?

During wastewater treatment, the supply of oxygen comprises a significant portion of operating costs, and generates a large volume of biomass or sewage sludge due to the high growth yield from aerobic microorganisms. We developed the conductive biofilter to solve some of these problems. It works using the capacity that certain conductive materials have to stimulate the exchange of electrons between microorganisms and their environment, and consists of a device filled with biocompatible electrically conductive particles. These particles

act as a support for electroactive biofilm growth. This 'bed' enhances microbial oxidation reactions, increasing the ability of bacteria to transfer electrons generated during these reactions to a final electron acceptor.

## In what ways does your team support you in this study? Do you share similar expertise or take a more multidisciplinary approach?

We are a large, multidisciplinary team comprising biotechnologists, biologists, electrochemists, chemical engineers, civil engineers, environmentalists and technicians. We each bring different approaches and solutions. Everyone conveys their own expertise, which makes our research more fruitful. Scientific debate is an essential and exciting part of our work, not only among the team but also with colleagues in the field.

## What are the biggest challenges you have faced in this investigation?

Actually, setting up such a multidisciplinary group was one of the main challenges we faced when we started the Bioelectrogenesis group in 2009, as successfully performing MET requires a number of professionals with very diverse backgrounds. Another big challenge was testing our ideas and devices at large scales. This was made feasible thanks to national programmes from the Spanish government devoted to improving cooperation between researchers and private companies.



# Bioelectrogenesis: sustainable biotechnology

Researchers at the University of Alcalá and IMDEA-Water, Spain, are investigating how electron transfer in bacteria can be used to generate green energy. In doing so, they aim to develop next-generation technologies for wastewater treatment



METland unit at CENTA facilities in Seville, Spain.

MUD, A SEEMINGLY lifeless and uninteresting material, can in fact be employed to generate electricity by using bacteria. When bacteria consume an organic substance under conditions lacking oxygen, like those found in mud, they produce CO<sub>2</sub>, protons and electrons. Using electrochemically active proteins on their outer membranes, certain types of bacteria can directly transfer these electrons to electrical conductive materials – producing electricity that can be harvested. This process, called microbial electrogenesis, is a novel source of green energy that could revolutionise the way we deal with bugs.

Electrogenic bacteria are found in many anaerobic environments, including the sediments of rivers, lakes and seas. Of these, the *Geobacter* genus is one of the best characterised. These bacteria attach to iron and mineral particles from sediments and breathe them, just as humans do it with oxygen. Professor Abraham Esteve Núñez is an expert on the *Geobacter* genus and has published several papers on the mechanisms underlying its electricity harvesting. Today, as leader of the Bioelectrogenesis (Bioe) group at the University of Alcalá, Esteve Núñez continues to investigate microbial electrogenesis.

#### MICROBIAL FUEL CELLS

Bacterial extracellular electron transfer can be utilised to convert chemical energy into

electricity through electrochemical devices called fuel cells, which combine hydrogen and oxygen to produce electricity. This clean technology only produces water and heat as by-products and is already being employed in some forms of public transport.

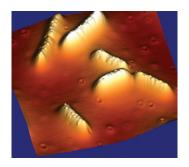
A microbial fuel cell (MFC) is a variation on the typical fuel cell. In an MFC, bacteria create electrical power by oxidising the organic matter found in, for instance, wastewater - cleaning this wastewater at the same time. MFCs are made of two compartments, an anode and a cathode, separated by a membrane that is selective for positively charged ions. In the anode, fuel is oxidised by microorganisms, generating electrons and protons. The electrons are transferred into the cathode by an external electronic circuit, while the protons can pass through the membrane owing to their positive charge. Next, the electrons and protons are consumed, combining with oxygen to form water.

MFCs can be placed in natural habitats, allowing carbon cycles from soil and plants to provide a sustainable and continuous fuel source, and form the basis of sedimentary MFCs.

#### **BACTERIAL MADE CURRENT**

In order to investigate the industrial potential of MET, Esteve Núñez leads the Bioe research

**Left**: *Geobacter* cells on HOPG graphite. Image produced using atomic force microscopy (AFM). Photo credit: Celia Rogero & Abraham Esteve. **Right**: sMFC charging an iPod using soil bacteria through an Universal Bioelectric Storage designed and constructed by the start-up Nanoelectra.





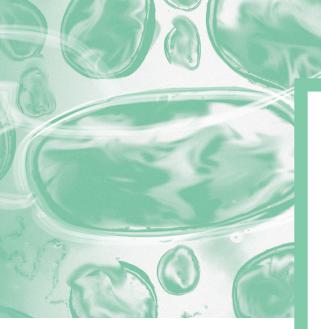
group, which is funded by Spanish and European research programmes, as well as private companies. The Bioe group aims to explore three interconnected research areas: the physiology and biochemistry of microbial electrogenesis, environmental microbial electrogenesis, and microbial electrogenesis and bioengineering.

To access the equipment necessary for such research, the team works across three different sites. Most of the research is carried out in The University of Alcalá (UAH)'s Department of Chemical Engineering, which has experience in bioengineering and houses MFC facilities as well as dedicated equipment for studying anaerobic bacteria. The group is also partnered with IMDEA-Water and Center of New Water Technologies Foundation (CENTA), both institutes devoted to the investigation of water technologies, which provides access to cutting-edge analytical techniques. Outside of the laboratory, the Bioe group develops field applications using the facilities at the Royal Botanic Garden Juan Carlos I, including greenhouses and wetland areas.

#### **IMPROVING WASTEWATER TREATMENT**

The group has recently been developing MET for water treatment – an industry in dire need of improvement. Indeed, the way wastewater is treated at present has several problems, many of which are energy-related. "One hundred years ago we began treating wastewater using aerobic microorganisms, but this has two problems: the generation of biomass (sludge) and the supply of oxygen to the biological system, which represents a high-energy cost," Esteve Núñez explains.

By contrast, anaerobic bacteria have a low biomass yield and can use excess electrons to produce methane, which can be used as fuel to generate electrical and thermal energy. On the downside, methane is a greenhouse gas, and anaerobic microbial metabolism can become slow due to the limited number of electron acceptors. Fortunately, MET overcomes both of these limitations by channelling electrons



into electrodes and offering an unlimited sink of electrons. To translate this potential to real-world application, Esteve Núñez is adapting MET for use in existing water treatment plants. This work forms part of Aquaelectra, a project funded by the Spanish Ministry of Economy and Innovation's INNPACTO programme, which aims to encourage academic-industry collaboration.

Already, the initiative has developed three patented technologies for wastewater treatment based on the principles of microbial electrogenesis, one of which involves using electrogenic properties in a biofilter. This electrically conductive biofilter, based on the capacity of certain materials to stimulate the exchange of electrons between bacteria and their environment, solves the two major problems of wastewater treatment: cost and biomass generation.

#### **COMBINING TECHNOLOGIES**

One of the most promising applications of this biofilter is in artificial wetlands, to treat wastewater in small communities worldwide. When applied to wetlands, it induces the oxidation (and thus removal) of organic matter present in wastewater. These promising results led to performing a full scale assay where thousands of litters of wastewater were daily treated by electrogenic bacteria, in which constructed wetlands are combined with MET to create a hybrid technology, which Esteve Núñez calls METland.

Using the METland concepts developed in Aquaelectra, the multidisciplinary team has already improved the efficiency of constructed wetlands. Their ultimate vision of such innovative technology incorporates three key elements – self-control, renewable energy and telecommunications through a project called Smart Wetland (www.smartwetland.es). This low energy, low cost, bacteria-driven wetland would be able to self-manage its electrogenic performance in response to the characteristics of the wastewater

### THE BIOELECTROGENESIS GROUP: IMPACT AREAS

#### **Basic science**

- Showed the involvement of proteins called cytochromes in transferring electrons from bacteria to electrodes
- Demonstrated that bacteria do not need to form a biofilm covering a solid electrode to produce electricity

#### **Natural environments**

- Developed novel strategies for harvesting electric energy from soil, which can be used to power USB devices including mobile phones
- Improved the bioremediation of polluted soils by stimulating electroactive bacteria

#### Wastewater

- Merged microbial electrochemical technologies with artificial wetlands to develop METlands, a new system to treat urban wastewater
- Currently using microbial desalination cells to produce freshwater from saltwater
- Carried out wastewater treatment using fluid-like anodes in bioelectrochemical fluidised bed reactors

after considering the microbial electricity as a signal.

#### LOOKING AHEAD

Based on the promising outcomes of the Smart Wetland project, Esteve Núñez plans to implement his METlands at full scale in four locations: the Mediterranean, Northern Europe, South America and North America. This will form part of an international consortium supported by Horizon 2020. Alongside his continuing research efforts, Esteve Núñez is already taking his innovations to the market. "We have founded two start-up companies to commercialise our bioelectrochemical tools in the clean technologies and wastewater treatment fields," he concludes.

#### **BIOELECTROGENESIS**

#### **OBJECTIVES**

- To investigate and develop devices operated by electroactive bacteria able to perform extracellular electron transfer (EET)
- To provide insights into the interface bacteriaelectrode, with also a strong applied component

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ABRAHAM ESTEVE NÚÑEZ is a professor at University of Alcalá, Spain, and leader of Bioe Group. His research is focused on the microbial electrogenesis field to

further understand bacteria-electrode interactions but also has a strong applied component, with the aim of researching and developing bioelectrogenic devices. He is currently the Secretary of the International Society for Microbial Electrochemistry and Technology (ISMET). He coordinates the Action group MEET-ME4WATER at EIP water. He is also the founder of two start-ups – nanoelectra and METfilter.





