PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

EVALUATION OF MICROBIAL ELECTROCHEMICAL TECHNOLOGIES AS A SUSTAINABLE TREATMENT STRATEGY FOR NITROGEN REMOVAL IN POLLUTED COASTAL MARINE SYSTEMS.

MARÍA JOSÉ DE LA FUENTE TRAVERSO

Thesis submitted to the Office of Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

IGNACIO VARGAS C.
RODRIGO DE LA IGLESIA C.

Santiago de Chile, Marzo, 2020

© 2020, María José De La Fuente Traverso

DocuSign Envelope ID: 5EA1FA6E-E6B1-4C4C-81BA-4A6C835C3923

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
SCHOOL OF ENGINEERING

EVALUATION OF MICROBIAL ELECTROCHEMICAL TECHNOLOGIES AS A SUSTAINABLE TREATMENT STRATEGY FOR NITROGEN REMOVAL IN POLLUTED COASTAL MARINE SYSTEMS

MARÍA JOSÉ DE LA FUENTE TRAVERSO

Members of the Committee:

IGNACIO VARGAS

RODRIGO DE LA IGLESIA

JUAN FRANCISCO ARMIJO

GONZALO PIZARRO

JUAN PABLO PAVISSICH

ROBERT NERENBERG

JUAN DE DIOS ORTÚZAR

Thesis submitted to the Office of Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

Santiago de Chile, March 2021

"Si buscas algo distinto, no hagas siempre lo mismo". Albert Einstein

ACKNOWLEDGEMENTS

First, I want to sincerely thank my advisors and mentors, Dr. Ignacio Vargas and Dr. Rodrigo De la Iglesia, for their support and guidance throughout my Ph.D studies. I am grateful for their help to overcome difficulties during research, willingness to help with everything I needed, and for all they taught me. Both are great humans and researchers; I feel fortunate to have been part of their research groups. Especially, I want to thank Dr. Rodrigo De la Iglesia for forming me as a scientist from when I was an undergraduate to this point, starting as a mentor and ending as a great friend. For that and much more, thank you!

I would also like to thank committee members Dr. Gonzalo Pizarro, Dr. Juan Francisco Armijo, Dr. Juan Pablo Pavissich, and Dr. Juan de Dios Ortuzar for their valuable comments and suggestions to improve this work and for their willingness to help me. Additionally, I would like to thank Dr. Holger Daims for receiving me as an intern in his laboratory in Vienna and contributing to the review of my thesis results. I thank Dr. Laura Farias for always warmly welcoming me in her laboratory to perform my nutrient analysis, and I am thankful for her contributions during the writing of my articles.

Thanks to my research group at PUC for their suggestions and disposition to hear about my research; they always provide feedback that helped me improve every day. Thanks to Leslie Daille for always being open to answering my questions and supporting me emotionally during my Ph.D research. Thanks to Francisca Rubio and Mauricio Medel for always helping me with my troublesome and tangled experiments; he always had the patience and peace of mind to help me solve any unforeseen event. Thank you to Evelyn Cruz from the marine microbiology laboratory, who was always there to help me with

whatever I needed, in addition to the supporting me as a friend throughout my doctorate. Thanks to Karen Yanira Sanzana for helping me do my hundreds of nutrient tests with so much patience and many good vibes. Thank you to all the administrative staff at DIHA, Fernanda, Carmen Gloria, and María Inés. I also want to thank all the ECIM staff for helping me every time I needed it.

This research would not have been possible without the financial support of FONDECYT 11201134, FONDECYT 1160917 and FONDECYT 1181326, the Office of Naval Research (Grant NICOP-N62909-17-1-2012, ONR N62909-17-1-2012), the Energy Research & Innovation Center (MERIC) (Project CORFO 14CEI2-28228), the CEDEUS Center CONICYT/FONDAP/15110020 and the Vicerrectoría de Investigación of Pontificia Universidad Católica de Chile.

To my PUC friends, Diana, Luis and Felipe. I am very happy to have met such great friends. Thank you for all your help and for always cheering me up. Furthermore, I want to thank the most important people in my life, my mom, brothers, and sister. This would not have been possible without their unconditional love, endless support, and encouragement.

Finally, I want to thank Martin, my life partner. Thank you for your infinite love and unwavering support with this and absolutely everything in life. I would never have achieved this without your support; infinite thanks.

GENERAL INDEX

Page

TABLE INDEX

Page

FIGURE INDEX

Page

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

ESCUELA DE INGENIERÍA

EVALUATION OF MICROBIAL ELECTROCHEMICAL TECHNOLOGIES AS A SUSTAINABLE TREATMENT STRATEGY FOR NITROGEN REMOVAL IN POLLUTED COASTAL MARINE SYSTEMS

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences by

MARÍA JOSÉ DE LA FUENTE TRAVERSO.

ABSTRACT

For many years, the world's coastal marine ecosystems have received industrial waste with high concentrations of nitrogen, generating the eutrophication of these ecosystems. In this context, microbial electrochemical technologies (METs) have begun to gain attention due to their cost efficiency in removing nitrogen and organic matter using the metabolic capacity of microorganisms. However, they still have limitations, such as the enrichment strategy for specific microbial communities on the electrodes under natural conditions. In this sense, applying an overpotential to an electrode has become a sustainable and efficient microbial enrichment technique. This thesis was focused on two main themes. First, the use of overpotentials as a microbial enrichment technique from natural seawater is consolidated and denitrifying microorganisms are enriched. Second, a MET capable of removing nitrogen from natural seawater was developed using microbial enrichment by overpotentials.

The results obtained in this thesis show that the application of overpotentials to an electrode was a useful technique for enriching microbial metabolism. Specifically, it was demonstrated that by applying -260 mV (vs. Ag/AgCl) to the working electrode, it is possible to significantly enrich denitrifying microorganisms. Based on these results, it was possible to develop a MET capable of significantly removing nitrogen and organic matter. In this case, and different from similar reactors reported in the literature, microbial enrichment in the electrodes was carried out by applying overpotentials without use of a culture medium from natural seawater. Specifically, significant enrichment of ammonium-oxidizing and nitrite-oxidizing microorganisms was observed at the anode, while at the cathode, denitrifying microorganisms and planctomycete enrichment was observed. Finally, it was determined that overpotentials could accelerate the start-up process of microbial electrochemical technologies and help accelerate their implementation in real environments.

Members of the Doctoral Thesis Committee:
Ignacio Vargas
Rodrigo De la Iglesia
Juan Francisco Armijo
Gonzalo Pizarro
Juan Pablo Pavissich
Robert Nerenberg
Juan de Dios Ortuzar

Santiago December 2020.

Keywords: Nitrogen removal, microbial electrochemical technologies, bioelectrochemical systems, nitrification, denitrification, seawater, overpotential, microbial enrichment.

EVALUACIÓN DE TECNOLOGÍAS ELECTROQUÍMICAS MICROBIANAS COMO ESTRATEGIA DE TRATAMIENTO SOSTENIBLE PARA LA ELIMINACIÓN DE NITRÓGENO EN SISTEMAS MARINOS COSTEROS CONTAMINADOS

Tesis enviada a la Dirección de Investigación y Postgrado en cumplimiento parcial de los requisitos para el grado de Doctor en Ciencias de la Ingeniería.

MARÍA JOSÉ DE LA FUENTE TRAVERSO.

RESUMEN

Los ecosistemas marinos costeros del mundo han sido utilizados durante muchos años como receptores de residuos industriales con altas concentraciones de nitrógeno, generando la eutrofización de estos ecosistemas. En este contexto las tecnologías electroquímicas microbianas (MET) han comenzado a ganar atención por su costo y eficiencia en la remoción de nitrógeno y materia orgánica,, utilizando la capacidad metabólica de microorganismos. Sin embargo, todavía tienen limitaciones, como la estrategia de enriquecimiento para comunidades microbianas específicas en los electrodos en condiciones naturales. En esta línea, la aplicación de un sobrepotencial a un electrodo se ha convertido en una técnica de enriquecimiento microbiano sostenible y eficaz. Considerando estos antecedentes, esta tesis estuvo enfocada en dos temas principales. Primero, consolidar la utilización de sobrepotenciales como técnica de enriquecimiento microbiano desde agua de mar natural y lograr enriquecer microorganismos desnitrificantes de manera específica, y (2) desarrollar una MET capaz de remover nitrógeno desde agua de mar natural, utilizando la técnica de enriquecimiento microbiano por sobrepotenciales.

Los resultados obtenidos en esta tesis muestran que la aplicación de sobrepotenciales a un electrodo es una técnica efectiva de enriquecimiento de metabolismos microbianos desde un inoculo de agua de mar natural. Específicamente, se demostró que aplicando al electrodo de trabajo -260 mV (vs Ag / AgCl), es posible enriquecer significativamente el establecimiento de microorganismos desnitrificantes. Tomando estos resultados, se logró desarrollar una MET capaz de remover nitrógeno y materia orgánica de manera significativa. En este caso, y diferente a reactores similares reportados en la literatura, el enriquecimiento microbiano en los electrodos se llevó a cabo mediante la aplicación de sobrepotenciales en agua de mar natural, sin la necesidad de uso de medio de cultivo. Específicamente, en el ánodo se observó un enriquecimiento significativo de microorganismos amonio-oxidantes y nitrito-oxidantes, mientras que en el cátodo, se observó un enriquecimiento de microorganismos desnitrificantes y *planctomycetes*. Finalmente, se logró determinar que el uso de sobrepotenciales podría acelerar el proceso de puesta en marcha de las tecnológicas electroquímicas microbiana y, por ende, ayudar a acelerar su implementación en ambientes reales.

Miembros de la comisión de tesis doctoral:
Ignacio Vargas
Rodrigo De la Iglesia
Juan Francisco Armijo
Gonzalo Pizarro
Juan Pablo Pavissich
Robert Nerenberg
Juan de Dios Ortuzar

Santiago, Diciembre de 2020.

Palabras clave: Remoción de nitrógeno, tecnologías electroquímicas microbianas, sistemas bioelectroquímicos, nitrificación, desnitrificación, agua de mar, sobrepotencial, enriquecimiento microbiano.

1. INTRODUCTION

1.1 Nitrogen cycle disturbance: a big silent problem

Today, the world is experiencing one of the most massive human health crises of the last 100 years. However, while the world focuses on this health crisis, other silent problems can cause a more significant global effect than the current pandemic. The most commonly discussed problem is climate change, but there is an even more silent substantial problem than climate change: nitrogen cycle alteration. Rockström et al. (2009) proposed nine processes that regulate the Earth system's stability and resistance (Figure 1-1). Within these processes, limits were determined on which humanity can continue to develop sustainably over time. It is postulated that exceeding these limits increases the probabilities of abrupt and even irreversible changes (Rockström et al., 2009).

One significant impact that population growth has generated globally is accelerating nitrogen entry rates into the biosphere (Rockström et al., 2009; Sutton et al., 2013) (Figure 1-1). The annual nitrogen deposition limit has been calculated at 35 million tons of nitrogen, and today, the yearly nitrogen deposition is close to 150 million tons of nitrogen (Rockström et al., 2009; Steffen et al., 2015). In this context, projections have been made, and the results only show that this alteration in the nitrogen deposition rate, which is altering the nitrogen cycle, will continue to increase (Galloway et al. 2004; J Galloway et al. 2013). Thus, alteration of the nitrogen cycle is a problem that should be addressed as a priority worldwide.

Figure 1-1: Current status of the control variables for seven of the planetary boundaries. The green zone is the safe operating space, the yellow zone represents the uncertainty zone (increasing risk), and the red zone is a high-risk zone (Steffen et al., 2015).

1.2 Sources and effects of excess nitrogen on coastal marine ecosystems

It has been determined that more than 40% of coastal marine ecosystems are impacted by anthropogenic activity, especially pollution by nutrients such as nitrogen (Smith, 2003; Halpern et al., 2007, 2008). Nitrogen can come from different anthropogenic sources, such as agriculture, livestock, industrial waste, and wastewater (Ansari et al., 2010). However, it has been identified that the anthropogenic sources with the most significant oceanic

nitrogen discharge are submarine sewage outfalls and fish farming (Soto and Norambuena, 2004; Abessa et al., 2005; Powley et al., 2016).

Submarine sewage outfalls (SSOs) in Chile are widely used as a treatment system, in which approximately 29% of wastewater is transported directly to the sea through SSO, corresponding to 254 MM m^3 yr^{-1} (SISS, 2016). All this wastewater volume goes to the sea without nutrient removal treatment. Only the largest solids and the bacteria present in the water are previously treated by cloration (Abessa et al., 2005; Powley et al., 2016). The significant marine dilution and the pressure applied to the discharged water allow submarine outfalls to reduce pollutant concentrations that were not eliminated and comply with environmental standards (Feitosa et al., 2013; De-la-Ossa-Carretero et al., 2016). Consequently, SSO technology does not remove dissolved carbon, nitrogen, or phosphorus, which are the primary pollutants in wastewater.

The fish farming industry has sustained growth since 1980 (Buschmann et al., 2009). Marine aquaculture production of fish, shellfish, and seaweed in Chile reached 1.4 million tons in 2019, making it the leading mariculture producer in the western world (SERNAPESCA, 2019). This industry's primary wastes are uneaten food, fish faeces, and urea, contributing significantly to nutrient pollution in adjacent marine ecosystems (Buschmann et al., 2009). These wastes are particularly rich in nitrogen (ammonia and nitrite), which generates an excess of the system's load capacity, leading to a deterioration in water quality and even eutrophication of the ecosystem (Naylor et al., 2000; Buschmann et al., 2006, 2009). Today, nitrogen treatment options in Chilean fish farming are only based on controlling the intensity of fish production (Ervik et al., 1997; Hansen et al.,

2001; Stigebrandt et al., 2004). However, due to technological advances, the use of recirculating aquaculture systems (RASs) for massive marine farming has begun.

Among those sources, fish farming and urban wastewater discharge zones have shown concentrations of 12.48 mg L^{-1} to 40.67 mg L^{-1} for nitrate and 5.29 mg L^{-1} mg L^{-1} to 54.90 mg L^{-1} for ammonium. (Orhon et al., 1997; Abessa et al., 2005; Lananan et al., 2014; Bahroun et al., 2016; Guldhe et al., 2017). These measurements are 20 to 70 times and 40 to 3,000 times higher than the nitrate and ammonium averages in the coastal sea, respectively (Quinn et al., 1988; Libes, 2009). These high concentrations of nitrogen can generate eutrophication of marine systems adjacent to fish farming or SSO areas. The main environmental effects of eutrophication are (Figure 1-2); i) the increase in suspended particles due to macroalgae blooms, leading to a decrease in light penetration, and ii) the change in biomass of primary producers (Glibert, 2017). Additionally, another significant effect is hypoxia caused by the decrease in dissolved oxygen. The production of CO_2 associated with the decomposition of organic matter produced by the increase in primary productivity and suspended algae generates an increase in anoxic areas (Smith, 2003). Eutrophication is usually followed by the proliferation of microalgae that are generally harmful and cause adverse effects on aquatic animals, such as obstruction of fish gills and localized anoxia (Anderson et al., 2002; Granéli and Turner, 2006).

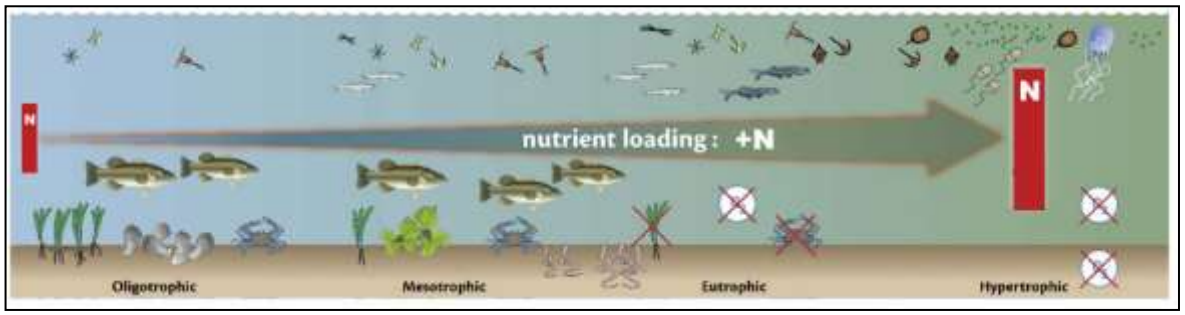

Figure 1-2: Conceptual schematic showing a system's progression towards eutrophication with the increased loading of nitrogen (Modified from Gilbert, 2017).

1.3 Microorganisms responsible for the natural metabolization of nitrogen

The nitrogen cycle is carried out by a wide diversity of microorganisms responsible for maintaining the balance in nitrogen concentrations in coastal (and other) marine ecosystems.

Nitrogen naturally enters marine systems through the nitrogen fixation process, where molecular nitrogen is transformed into ammonium (Figure 1-3). This process is carried out by N_2-fixing microorganisms such as *Prochlorococcus, Synechococcus,* and *Trichodesmium* (Zehr, 2011; Zehr and Kudela, 2011). Then, the oxidation of ammonium to nitrite is carried out by ammonia-oxidizing bacteria (AOB), such as *Nitrosomonas* and *Nitrosospira*. Then, the oxidation of nitrite to nitrate is carried out by nitrite-oxidizing bacteria (NOB), such as *Nitrobacter*, *Nitrococcus, Nitrospina,* and *Nitrospira* (Bock and Wagner, 2006). However, a few *Nitrospira* species can carry out the complete nitrification process, transforming ammonium to nitrate. This last metabolism is called complete ammonia oxidation (COMAMMOX) (Daims et al., 2016; Hu and He, 2017). In the next stage of microbial nitrogen metabolism, nitrate is reduced to molecular nitrogen by a broad

group of microorganisms, denitrifying bacteria (Zehr and Kudela, 2011). In addition to denitrification, another type of microbial metabolism is responsible for removing nitrogen from the system by anaerobic ammonium oxidation (Anammox) (Kuenen, 2008). These bacteria, belonging to the *Planctomycetes* group, can metabolize nitrite and ammonium to transform it into nitrogen gas. This metabolism, like denitrification, is carried out under anaerobic conditions (Zehr and Kudela, 2011).

These bacterial metabolic processes are called dissimilative because microorganisms perform cellular oxidation-reduction processes to remove or provide electrons for cellular energetics. On the other hand, nitrogen assimilation processes are those where nitrate is reduced to ammonium for cellular biosynthesis (Zehr and Kudela, 2011; Daims et al., 2016). Under undisturbed conditions, these microorganisms can maintain the balance of nitrogen concentrations in the medium. However, with nitrogen discharges to the ocean at concentrations 20 to 3,000 times higher than the average nitrogen in the coastal sea, the normal cycle of microbial nitrogen metabolization is not sufficient to remove the excess of this compound in the medium. For this reason, it is necessary to find a way to remove this excess nitrogen to avoid eutrophication problems in coastal marine systems.

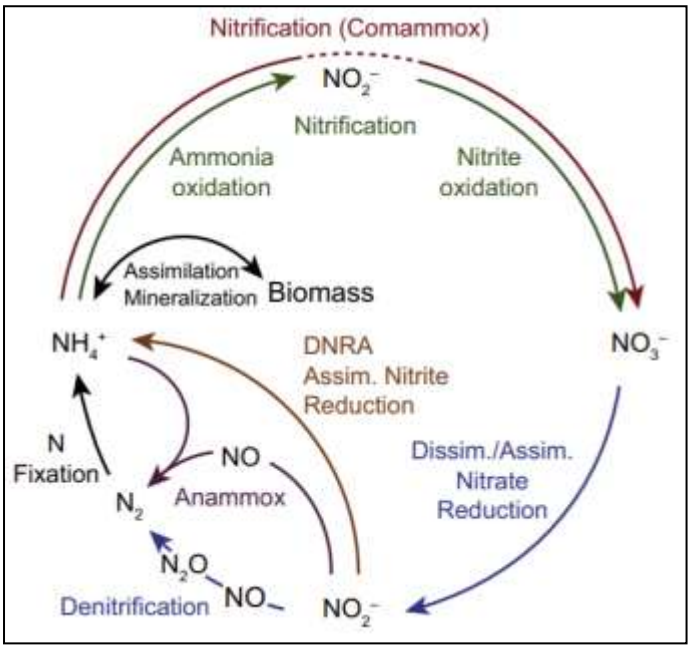

Figure 1-3: Biogeochemical nitrogen cycle. Schematic illustration of the key processes of the nitrogen cycle (obtained from Daims, Lücker, and Wagner 2016).

1.4 Conventional technologies for nitrogen removal.

To remove the nitrogen present in wastewater and RAS, a variety of technologies have been developed. Today, physicochemical, chemical and biological technologies are used to remove nitrogen from wastewater (Rezvani et al., 2019). Table 1-1 shows all the physicochemical technologies conventionally used to remove nitrogen, including ion exchange (IE), reverse osmosis (RO), electrodialysis (ED), and adsorption by activated carbon (Mook et al., 2012; Rezvani et al., 2019). Although all of these technologies have proven to be efficient in nitrogen removal, they present unsolved problems such as high chemical use, high brine production, high energy consumption, and high material and

regeneration cost (Table 1-1) (Nataraj et al., 2006; Bellona et al., 2008; Mook et al., 2012; Kalaruban et al., 2016).

On the other hand, biological treatments are carried out by bacteria, which convert ammonium and nitrate to nitrogen gas. These methods do not present problems such as byproduct formation or brine production, and in general, the operational cost is lower than that of physicochemical and chemical techniques (Soares, 2000; Aslan and Türkman, 2003; Mook et al., 2012; Rezvani et al., 2019).

Bacteria of the nitrogen cycle carry out biological treatments (see Section 1.3). Under aerobic conditions, the microbial oxidation of ammonium to nitrate occurs, and then, under anaerobic conditions, the microbial reduction of nitrate to molecular nitrogen takes place (Davis, 2010; Van Hulle et al., 2010). The first limitation of biological treatments is the differences in the oxygen level in which each process takes place. These differences make it challenging to carry out both operations simultaneously. For the same reason, today, they are carried out separately. In the nitrification or comammox process, aeration is used to maintain an optimum oxygen concentration to ensure the sufficient presence of oxygen for the oxidation of ammonium to nitrite and then nitrite to nitrate. In the denitrification process, aeration is not needed, but an electron donor (organic or inorganic) is necessary to ensure nitrate reduction (Van Hulle et al., 2010). Adding an inorganic electron source is significantly less expensive than adding an organic electron source for denitrification. However, it is still considered a significant expense during the nitrogen removal process (Park and Yoo 2009). Similarly, the biological treatment of nitrogen generates an

excessive production of sludge that, in the long term, ends up being a problem (Pérez-Elvira et al., 2006).

Based on the disadvantages of conventional nitrogen treatment technologies, other strategies, such as microbial electrochemical technologies (METs), have begun to receive more attention.

Although these technologies were initially conceived to produce energy, taking advantage of the microbial metabolism has proven to be a sustainable option for removing pollutants such as nitrogen compounds (Sun et al., 2016). The difference between a MET and a traditional nutrient removal system basically resides in the opportunity to use the same chemical energy present in the waste for its treatment, reducing the operating energy expenses (e.g., aeration systems) (Mook et al., 2012).

Table 1-1: Comparison of different nitrogen removal techniques (modified from Rezvani, Sarrafzadeh, and Ebrahimi 2019)

TECHNOLOGY	ADVANTAGES	DISADVANTAGES	REFERENCES
Physicochemical			
Ion Exchange	Selective resins for different pollutants, common application, low production cost.	It requires regeneration of the resin, production of brine, high use of chemicals (salt).	Samatya, Kabay, and Yu 2006; Kalaruban et al. 2016
Reverse Osmosis	Remove multiple contaminants, low production cost, environmentally friendly.	Need for post-treatment to remove accumulated contaminants in brine, membrane fouling, high operating cost.	Darbi et al. 2006; Schoeman and Steyn 2003; Bellona et al. 2008
Electrodialysis	Multiple removals of pollutants, higher water recovery (less waste).	High energy consumption, complex construction, and operation skipping brine production as final waste.	Hell et al. 1998; Nataraj, Hosamani, and Aminabhavi 2006
Activated Carbon Absorption	It does not generate residues of brine or concentrates, high adsorption capacity, elimination of multiple contaminants.	High cost of material and high price of regeneration.	Mook et al. 2012; Monsalvo, Mohedano, and Rodriguez 2011
Chemical			
Chemical denitrification.	Does not generate residues of brine or concentrates, nitrate reduction instead of accumulation in residues, elimination of multiple pollutants.	Inconsistency in nitrate reduction, pH, and temperature dependence. Risk of ammonia or nitrite production in the nitrate removal process.	Chaplin et al. 2012; Huang and Zhang 2004
Biological			
Biological nitrification and denitrification.	No dangerous by-products are generated, no additional treatment is required, removal of multiple pollutants, lower cost of operation than physicochemical treatments in general.	Constant oxygenation of the medium is necessary (nitrification), and the addition of organic or inorganic electron donor (denitrification) post-treatments is also required for turbidity and sludge removal.	Soares 2000; Aslan and Türkman 2003

1.5 Principles of Microbial Electrochemical Technologies (METs)

METs are bioelectrochemical devices formed by an anode and a cathode connected through an external circuit, which allows the flow of electrons between the electrodes. A microbial biofilm grows on each electrode surface, which catalyses electron transfer in the system (Franks & Nevin, 2010). The operation of a MET is based on three fundamental stages (Figure 1-4). The first stage consists of the microbial oxidation of organic and inorganic compounds, thus managing to transfer electrons to the electrode to fulfill the role of the anode in the system (Lovley, 2011). This process is carried out by electrochemically active bacteria (EAB) capable of transferring electrons to the electrode (Franks and Nevin, 2010; Lovley, 2011). During the second stage, a proton gradient occurs as a result of microbial metabolic processes. This is a critical point in the operation of a MET since if the proton concentrations increase excessively, the pH of the system will drop abruptly and prevent the normal growth and development of the microbial communities in the system (Lovley, 2011). This problem can be solved using seawater as the medium since, due to its high buffering capacity, and it is possible to counteract the increased production of protons (Whitfield, 1974). Finally, the electrons transferred to the anode by the bacteria are transferred to the cathode, generating an electric current. In the cathode, the EAB can use the electrons transferred from the anode to a terminal electron acceptor such as nitrate. This process can also be chemically catalysed (e.g., platinum), for example, for oxygen reduction (Lovley, 2011).

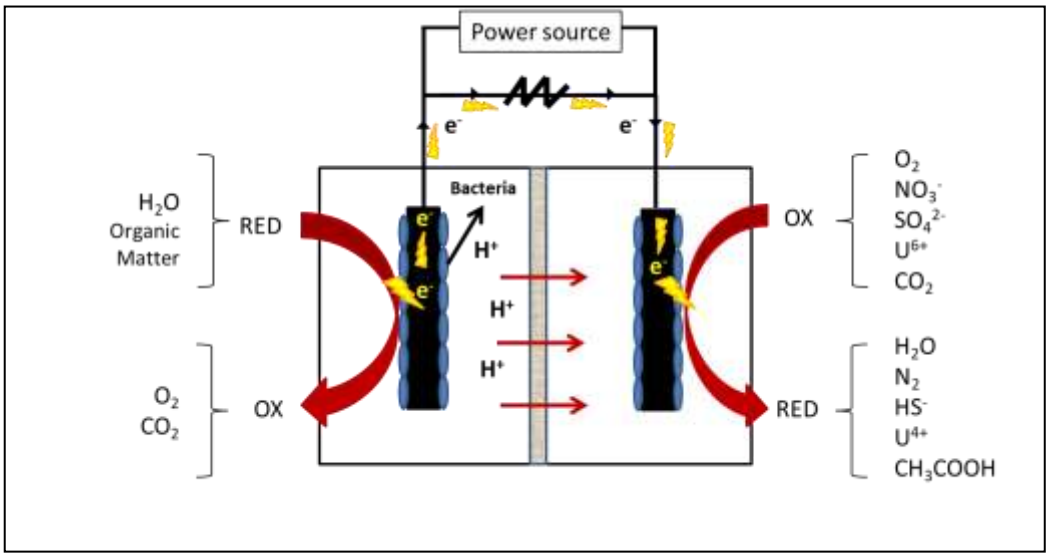

Figure 1-4: Illustration of the operation of a MET. OX: oxidized compounds, RED: compounds reduced (Modified from Lovley, 2011).

EABs have specific mechanisms that allow them to capture or transfer electrons from or to the electrode. There are three types of extracellular electron transfer (EET) processes in bacteria: c-type cytochromes, shuttles, and nanowires (Yang et al., 2012). C-type cytochromes works transfering electrons directly between the electrode and the bacteria (Figure 1-5). Therefore, for this EET mechanism to work, the bacteria must contact the electrode. The shuttles are molecules secreted by bacteria to transfer electrons to the electrode. Through studies carried out with *Shewanella* species, it has been possible to determine that mainly riboflavin and flavin mononucleotides can act as shuttles (Marsili et al., 2008; von Canstein et al., 2008). Other redox compounds widely distributed in environments or in the extracellular polymeric substances (EPS) present in biofilm (e.g., humic, manganese species, or polysulfide) could also be used as electron shuttles (Torres et al., 2010). Therefore, in this case, the bacterium does not need to be in direct contact

with the electrode for electron transfer. The third EET mechanism is through bacterial nanowires, which were found for the first time as electrically conductive pili in *Geobacter sulfurreducens* (Reguera et al., 2005). Bacterial nanowires can be achieved by extending to tens of microns to contact electron acceptors or other bacteria. Electrical conductivity has been demonstrated along nanowires (El-naggar et al., 2010), which means that bacteria, such as shuttles, may not be in direct contact with an electrode to capture or transfer electrons using bacterial nanowires.

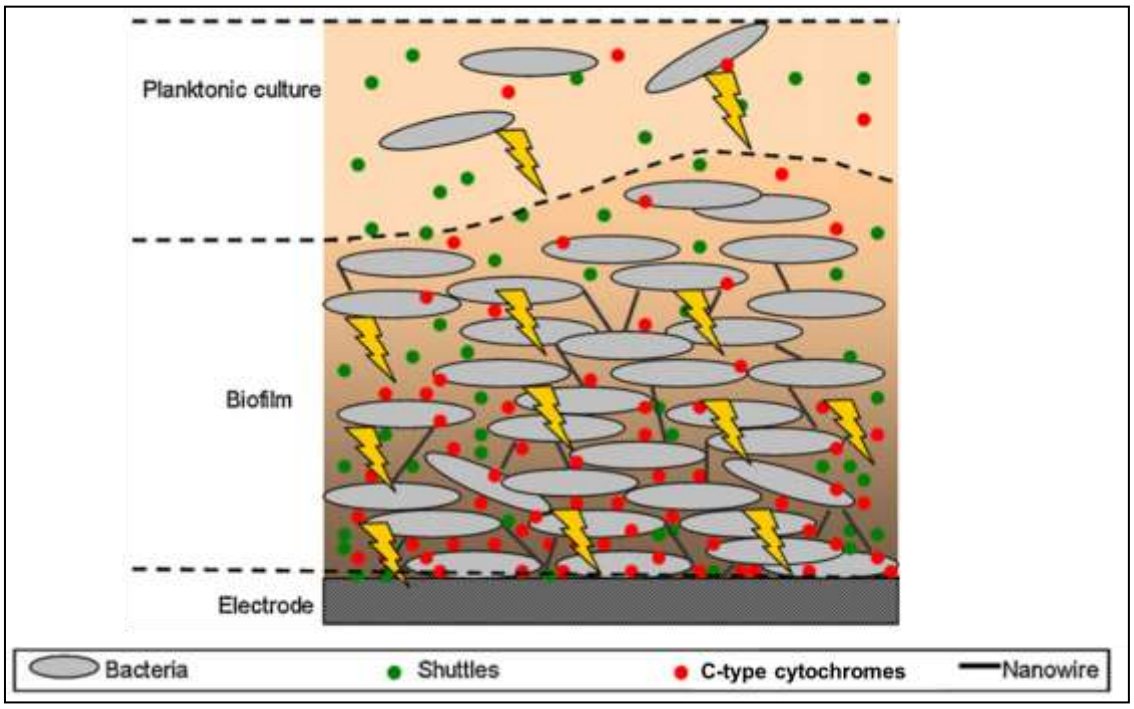

Figure 1-5: Representation of the 3 microbial electron transport mechanisms in a biofilm: c-type cytochromes, shuttles and nanowires. (Modified from Yang et al. 2012)

Although MET was initially conceived to produce electrical energy taking advantage of the metabolism of EAB (i.e., microbial fuel cells), today, this family of technologies represents an energy-efficient and sustainable alternative for transforming different types of

compounds. In this context, it has been possible to develop MET capable of removing carbon and nitrogen simultaneously (Sun et al., 2016). Achieving the removal of carbon and nitrogen is a challenge mainly due to the microbial metabolism associated with the complete metabolism of nitrogen. As indicated in Section 1.4, microbial nitrification only occurs under strict aerobic conditions, while microbial denitrification occurs under anaerobic settings. This is why some of the first developed bioelectrochemical reactors (BERs) capable of removing carbon and nitrogen simultaneously separated nitrification from denitrification processes (Virdis et al., 2008; Xie et al., 2011a). Since those early experiments, it was then discovered that denitrification could occur even with concentrations of up to 5 mg L^{-1} oxygen in the medium (Virdis et al., 2011). This was possible due to the microbial stratification and the oxygen gradients generated in a biofilm, which would allow complete metabolization of nitrogen in the same biofilm (Virdis et al., 2010; Ryu et al., 2013; Zhang et al., 2013, 2014; Zhu et al., 2019). All these factors are due to the microbial stratification generated in the biofilm developed in the cathode (Figure 1-6). Although these reactors can simultaneously remove carbon and nitrogen, they still present challenges when applied in a complex system such as the sea (Sun et al., 2016; Pous et al., 2020). These reactors have been developed using an inoculum from activated sludge from a reactor operating for several months or from previously enriched microbial consortia (Zhu et al., 2019; Pous et al., 2020). Using such inoculum ensures a significant abundance of nitrifying and denitrifying microorganisms in the reactor and its efficiency. The high physicochemical and biological complexity that characterizes the marine environment makes it impossible to ensure a high abundance of nitrifying and denitrifying

microorganisms in a small volume (Sunagawa et al., 2015). This represents a considerable challenge to apply a MET capable of removing nitrogen and carbon in marine environments.

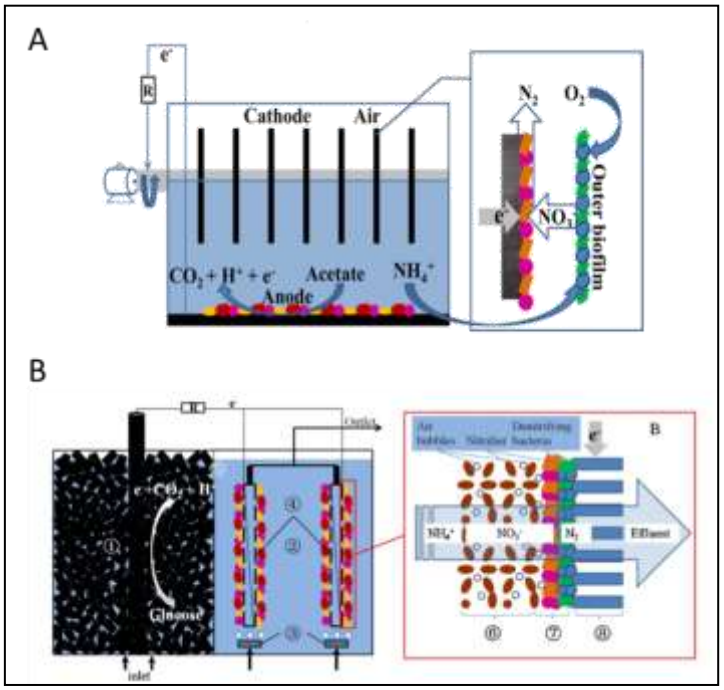

Figure 1-6: scheme of two reactors capable of removing carbon and nitrogen simultaneously, (A) Zhang et al. (2013) and (B) Zhang et al. (2014). In both cases, it was reported the complete metabolization of nitrogen in the cathode, thanks to microbial stratification. As can be seen, in both cases the nitrifying bacteria are found in the outer layers and the denitrifying bacteria in deeper layers directly associated with the cathode.

1.7 Electrochemical overpotentials as a microbial enrichment technique

In nature, there are microorganisms capable of using inorganic compounds as an electron source or final electron acceptors, such as iron-oxidizing bacteria and sulphate-reducing bacteria, respectively (Castro et al., 2000; Emerson et al., 2010). Each of these cellular oxidation-reduction processes occurs at specific electrochemical potentials (redox tower). Taking this information as a basis, investigations have been carried out to determine the effect of the use of electrode polarization on microbial colonization of these electrodes (Torres et al., 2009; Miceli et al., 2012; Zhan et al., 2014; Rowe et al., 2015; Z. Wang et al., 2015). Several authors have proven this strategy to modulate and enrich the presence of a particular EAB. An example of this is the research conducted by Torres et al. (2009), where different overpotentials were applied to different electrodes. In this work, wastewater was used as an inoculum. As a result, different biofilms can be observed, depending on the overpotential applied to each electrode (Figure 1-7). When analysing each electrode's community composition, it was also possible to observe significant differences depending on the applied overpotential. (Torres et al., 2009). Various authors have used this technique to enrich or promote specific microbial metabolism in an electrode (Miceli et al., 2012; Rowe et al., 2015; Z. Wang et al., 2015; Pous et al., 2020). Then, the electrochemical enrichment of specific metabolisms emerges as a novel and efficient strategy for biofilm modulation and control. This feature could be of particular interest in bioreactors operated under open environmental conditions by accelerating the startup process and improving their performance.

To determine if this tool could contribute to the scalability and applicability of MET in real environments, it is necessary to develop efforts for moving research from controlled laboratory conditions to complex natural settings, such as coastal marine environments.

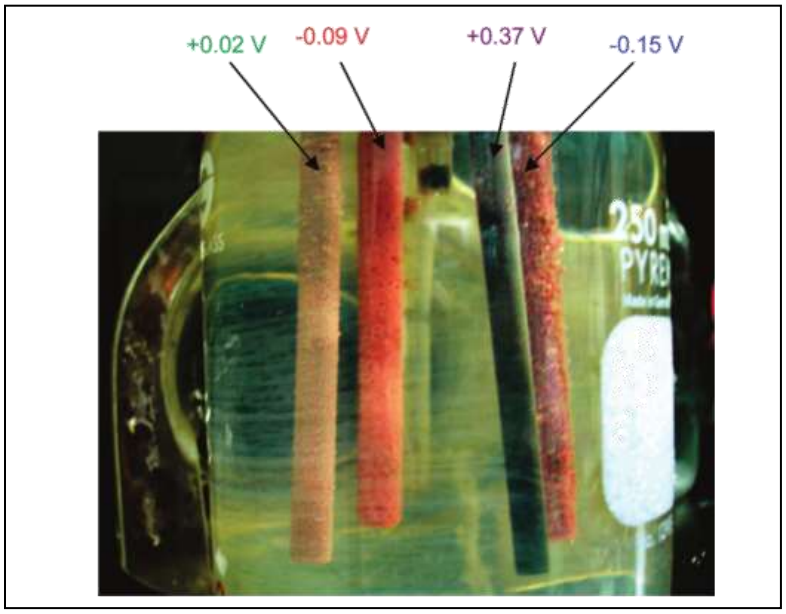

Figure 1-6: Representative image of the biofilms developed in each electrode and their difference in colors according to the applied potential during the research carried out by Torres et al. (2009)

1.8 Hypothesis and objectives

Coastal systems have long been used as receptors for nitrogenous wastes, and there are currently many eutrophic coastal areas globally. Although efficient METs have been developed to remove nitrogen and organic matter, there are still challenges to be solved to accomplish the use of METs in real and complex systems such as the sea.

Under this conceptual framework, this research's hypothesis is that the use of specific electrochemical overpotentials in a MET allows the enrichment of a marine microbial biofilm specialized in the removal of nitrogen compounds.

In this context, three specific objectives were established:

1. To develop a microbial enrichment tool from marine inoculum through the use of electrochemical overpotentials.
2. To enrich a microbial community specialized in the denitrification process by applying electrochemical overpotentials associated with this metabolism.
3. To develop a laboratory-scale bioelectrochemical reactor with biofilms specialized in the metabolization of nitrogen compounds in seawater.

In general, this work seeks to develop a laboratory-scale reactor capable of significantly removing nitrogen and carbon in seawater, representing a sustainable alternative to solve the eutrophication problem in coastal and marine environments. Hence, a transdisciplinary approach was needed to achieve the objectives of this work. Chapter I of this thesis provides a general introduction to the problem of nitrogen pollution in the sea, identifying the primary anthropogenic sources responsible for excess nitrogen, the environmental consequences, and conventional nitrogen treatment technologies. Then, at the end of the chapter, METs are described and exposed as technologies that have proven to be efficient in removing nitrogen and carbon but still present challenges that have not yet proven to be applied in natural systems. Chapter II was developed to diagnose efficient overpotentials as

a microbial enrichment technique for an inoculum of natural seawater without using a culture medium. Chapter III was conducted to verify that the enrichment of denitrifying microorganisms was possible through reported overpotentials for this metabolism. Finally, in Chapter IV, the objective was to develop a laboratory-scale reactor enriched with microorganisms of interest through the use of overpotentials identified in the previous chapters capable of significantly removing nitrogen and carbon from seawater.

This dissertation is based on a compilation of three papers: two Q1 journal papers as the principal author and one journal paper submissions. The papers constitute the core of this thesis and are presented in “List of Papers”.

List of Papers

I. De La Fuente, M. J., Daille, L. K., De la Iglesia, R., Walczak, M., Armijo, F., Pizarro, G. E., & Vargas, I. T. (2020). Electrochemical Bacterial Enrichment from Natural Seawater and Its Implications in Biocorrosion of Stainless-Steel Electrodes. Materials, 13(10), 2327. https://doi.org/10.3390/ma13102327

II. De La Fuente, M. J., De la Iglesia, R., Farias, L., Daims, H., Lukumbuzya, M., & Vargas, I. (2021). Electrochemical Enrichment of Marine Denitrifying Bacteria to Enhance Nitrate Metabolization in Seawater. Journal of Environmental Chemical Engineering, 9(4), 105604. https://doi.org/10.1016/j.jece.2021.105604

III. De La Fuente, M. J., De la Iglesia, R., Farias, L., Glasner, B., Torres-Rojas, F., Muñoz, D., Daims, H., Lukumbuzya, M., & Vargas, I. Enhanced Nitrogen and Carbon

Removal in Natural Seawater by Electrochemical Enrichment in a Bioelectrochemical Reactor. (Submitted to Journal of Environmental Chemical Engineering)

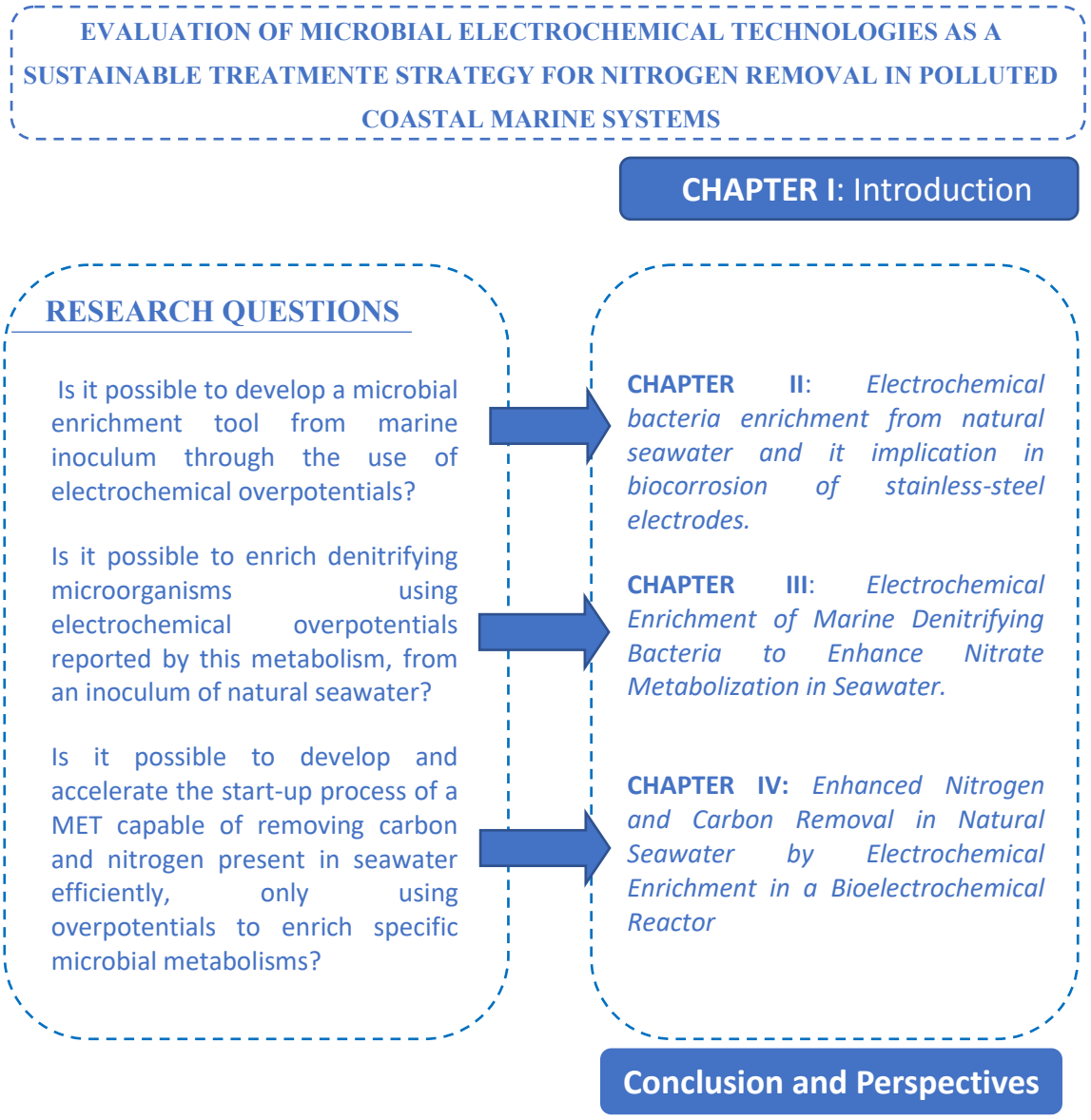

Figure 1-7: Outline summary of the thesis

2. ELECTROCHEMICAL BACTERIAL ENRICHMENT FROM NATURAL SEAWATER AND ITS IMPLICATIONS IN BIOCORROSION OF STAINLESS-STEEL ELECTRODES.

Published in *Materials* (2020) Vol. 13(10), p. 2327.

2.1. Introduction

Microbial electrochemical technologies (METs) take advantage of microbial communities to catalyze redox reactions over electrodes (Rao et al., 1976; Roller et al., 1984; Berks et al., 1995; He et al., 2009). Even though the first generation of METs were designed to produce electricity, their efficiency as wastewater treatment systems has also been proven (He et al., 2009; Virdis et al., 2010; Li and Yu, 2015; Jiang et al., 2017). The next generation of METs were then conceived to accomplish selective and energy-efficient treatment, remediation, and recovery of nutrients, metals, and by-products with industrial value (Wang and Ren, 2013). However, many of these novel and disruptive METs have only been tested at the laboratory scale and under controlled conditions. Therefore, some of the main ongoing challenges for METs are to overcome the practical limitations associated with (i) the performance requirements of cost-efficient materials used as electrodes, and (ii) the enrichment of specific microbial communities which can catalyze reactions of interest in a MET under natural, uncontrolled environmental conditions (Logan, 2010; Rowe et al., 2015), which is related to the use of METs in the implementation of bioremediation strategies.

The durability of electrode materials under exposure to aggressive natural environments where complex microbial communities can develop (e.g., coastal areas) is a major concern for the scaling-up of METs. Due to their biocompatibility and corrosion

resistance, carbon-based materials (e.g., graphite and carbon felt) are commonly used as electrode materials in laboratory scale METs. However, in pilot systems, they exhibit limitations in terms of resistivity, mechanical strength, and cost (Kim et al., 2007; Logan, 2010; Baudler et al., 2015). Stainless steel (SS) is a common industrial material characterized by its good mechanical properties, corrosion resistance, conductivity, cost efficiency, and easy scalability (Dumas et al., 2007; Call et al., 2009; Selembo et al., 2009). Pocaznoi et al. (2012) (Pocaznoi et al., 2012) compared the abilities of carbon cloth, graphite plate, and SS as bioanodes in bioelectrochemical reactors (BERs), concluding that SS electrodes presented the highest current density among the tested materials. Furthermore, SS electrodes have been successfully proven to be effective as both anodes and cathodes in marine METs (Dumas et al., 2007), producing power levels similar to those produced with graphite electrodes (0.2 W m^{-2}) (Tender et al., 2002). Thus, although the material evaluation of METs under real service conditions is in its infancy, SS emerges as a good candidate as an electrode material for the scaling-up of METs in marine environments.

Poised potential experiments have been used to study the effect of electrode potential on microbial colonization of the electrode in vitro (Torres et al., 2009; Miceli et al., 2012; Zhan et al., 2014; Rowe et al., 2015; Z. Wang et al., 2015). This strategy has been proven by several authors to be able to modulate and enrich the presence of particular electrochemically active bacteria (EAB) which can transform contaminants in a BER. Indeed, while negative potentials have been shown to enrich heterotrophic exoelectrogenic bacteria (Torres et al., 2009; Miceli et al., 2012), the use of a positive potential tends to

enrich autotrophic microorganisms that are able to directly or indirectly receive electrons from an electrode (Z. Wang et al., 2015). In general, these types of experiments have been developed in culture media under controlled conditions using an inoculum obtained from wastewater treatment plants or anaerobic soil, where the microorganisms of interest are found in high abundance (Juretschko et al., 2002). In consequence, it is necessary to evaluate the effectiveness of the application of an overpotential to an electrode as a tool to enrich the presence of EAB in METs. To determine if this tool could help with the scalability and applicability of MET in real environments, this evaluation should be accomplished under exposure to complex natural environments such as coastal marine environments.

In this work, we investigated whether the durability of SS electrodes exposed to a marine environment changes according to the applied overpotential and the enrichment of specific bacteria, as well as the application of an overpotential as a microbial enrichment tool to improve the applicability and scalability of MET. To address the proposed objective, a complete and multi-technique approach was used to evaluate the effect of different overpotentials on both the corrosion and the biological colonization of SS as an electrode material for METs operating in a coastal marine environment. Our results demonstrated differential electrochemical bacterial enrichment under the different overpotentials tested. In particular, the highest positive potential led to clear enrichment in members of the *Roseobacter/Rhodobacter* clade, and exerted a strong effect on deterioration of the SS electrodes.

2.2. Materials and Methods

2.2.1. Reactor Configuration

In this work, two sets of experiments were conducted. The first experiment was carried out to determine the effect of overpotential application on microbial enrichment from natural seawater samples, and the second experiment was conducted to isolate the effects of both the applied potential (abiotic) and the enriched microorganisms (biotic) on the metal corrosion.

The first experiment was run using four 100 mL glass bottles as three-electrode electrochemical cells, which were modified to allow a continuous closed flow of natural seawater during the experiment (Figure 2-1). Each bottle contained four AISI 316L (X2CrNiMo17-12-2, material number 1.4404) SS electrodes as the working electrode (1.5 cm^3 each), one graphite rod as the counter electrode (2 cm^3), and one Ag/AgCl electrode as the reference electrode (3 M KCl) (Figure 2-1). This experimental design was based on the reactor designed by (Torres et al., 2009). Before seawater exposure, each SS plate was polished using emery paper (grit 240), rinsed with distilled water, degreased with acetone, and finally dried by blowing hot air. An Interface 1000TM potentiostat (GAMRY, Warminster, PA, USA) connected to an ECM8™ electrochemical multiplexer (GAMRY, Warminster, PA, USA) was used to poise each cell at one of three specific potentials over the ten-day course of the experiments. The potentials were: (i) −150 mV (vs. Ag/AgCl), representing the reported optimal applied potential for enriching marine EAB (Rowe et al., 2015); (ii) +100 mV (vs. Ag/AgCl), corresponding to the open circuit potential (OCP) reported for 316L SS under marine conditions after 10 days (Fischer et al., 2016) and

allowing for the enrichment of autotrophic exoelectrotrophic microorganisms on the electrodes (Z. Wang et al., 2015); and (iii) +310 mV (vs. Ag/AgCl), representing a near potential of one of most-positive potentials reported for the oxygen reduction reaction (Ter Heijne et al., 2010). As a control, a fourth electrochemical cell was run under OCP conditions.

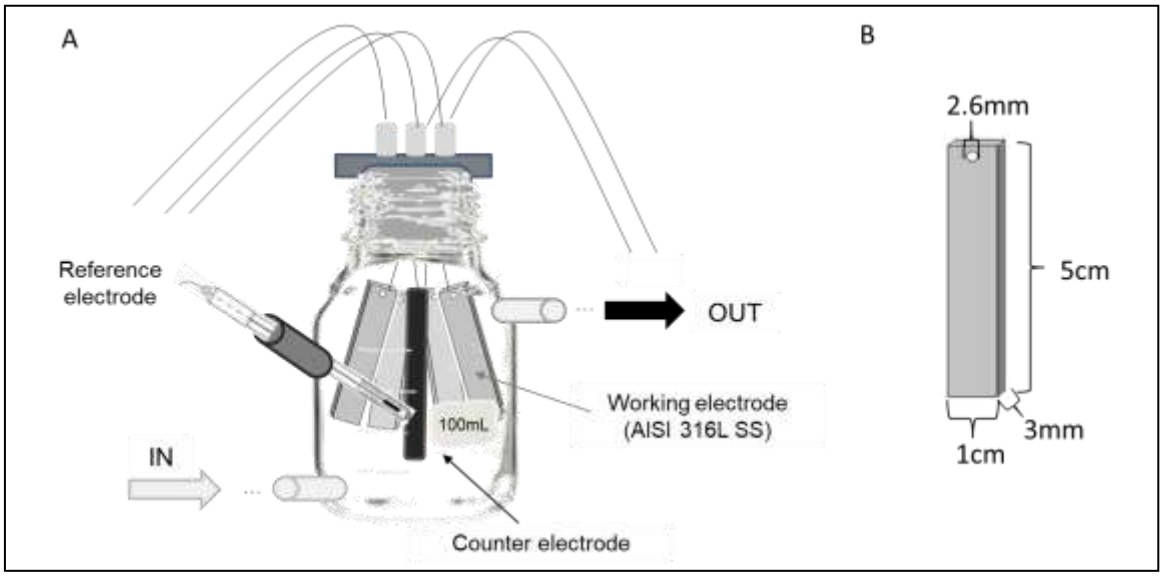

Figure 2-1: . Experimental design. (A) Design of the bottles, indicating seawater inlet and outlet and the arrangement of the AISI 316L Stainless Steel (SS) electrodes, reference electrode, and counter electrode. (B) Dimensions of SS electrodes.

The second experiment involved the same experimental setup as that used in the first, but two cells were set under abiotic conditions, with 0.2 µm-filtered natural seawater. Inside of one of these two cells the working electrode was polarized at +310 mV (vs. Ag/AgCl) and the other was run as the control under OCP conditions. A third electrochemical cell was

tested under biotic conditions (using unfiltered seawater) and the working electrode was poised at +310 mV (vs. Ag/AgCl).

For both experiments, fresh, natural coastal seawater was collected from the Estación Costera de Investigaciones Marinas (ECIM) of the Pontificia Universidad Católica de Chile, located in the Eastern Pacific Ocean at 33°30'16"S, 71°38'23"W. Water was stored in a temperature-controlled room at 20 °C. Air pumps and a light schedule (12 h/12 h) were used to maintain saturated conditions of phototrophic activity and dissolved oxygen (8 mg L^{-1}) during the experiment. Electrochemical cells were connected to a tank in a closed-loop system. A peristaltic pump was used to maintain a constant flow of seawater (0.5 mL s^{-1}) throughout the 10 days of the experiment.

2.2.2. Surface Analysis for Corrosion Evaluation

2.2.2.1. Weight Loss

To assess weight loss, each coupon was weighed before the assembly of the experiments with an analytical balance with a precision of 0.1 mg (Shimadzu ATY224, Kyoto, Japan). The samples analyzed were the three SS electrodes after they were first sonicated for community analysis (described in Section 2.3). After sonication, the SS electrodes were washed, degreased with acetone, and dried with hot air to be reweighed. A one-way analysis of variance (ANOVA), followed by an a posteriori Tukey test, were performed using GraphPad Prism version 6 to check for significant differences between treatments (Boivin et al., 2006).

2.2.2.2 Atomic Force Microscopy (AFM).

For the second experiment, after the cleaning of coupons to remove the biofilm, the topographies of the surfaces of the SS electrodes were observed using atomic force microscopy (AFM). The measurements were performed with an Innova® Atomic Force Microscope (Bruker, Billerica, MA, USA). Each coupon was measured in triplicate at different points to obtain a representative analysis. The topography was measured in air, using the tapping mode with a silicon nitride probe. Roughness analysis was carried out on the images obtained, over an area of 35 mm × 35 mm.

2.2.3. Microbial Community Analysis

2.2.3.1. DNA Extraction

After the exposure time, three of the four SS electrodes in each bottle were removed and sonicated in 50 mL of sterile seawater for 5 min (Elmasonic S 30H, Elma Schmidbauer GmbH, Frechen, Germany). The sonicated products were filtered through a 0.2-µm membrane filter to collect the biomass and perform the subsequent DNA extraction with phenol:chloroform based on a previously reported protocol (Henríquez-castillo et al., 2015). Additionally, at the end of the experiment, 2 L of water from the tank was filtered through a 0.2-µm membrane filter to assess changes in the microbial community of the water column present in the tank. DNA quantification was performed with a Qubit® 2.0 fluorometer (InvitrogenTM, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol.

2.2.3.2. Fragment Analysis (FA) of 16S rRNA Genes

To identify the microbial community composition developed on the tested electrodes, amplification of 16S rRNA genes was performed in all DNA samples. The 16S rRNA genes were amplified by polymerase chain reaction (PCR), using the 5'-NED-labeled primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and the primer 1492R (5'-GGYTACCTTGTTACGACTT-3'), without fluorescent labels (Weisburg et al., 1991). The final concentrations in the PCR mix were 1.2 mM $MgCl_2$, 1X KAPA PCR buffer B (KB1002), 0.3 mM dNTPs, 0.3 μM for each primer, and 1 U KAPA Taq polymerase (KAPABiosystems, Cape Town, South Africa). PCR conditions were as follows: 95 °C for 5 min, 25 cycles of 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min, and a final elongation step at 72 °C for 7 min. 16S rRNA gene PCR amplicons (1492 bp in length) were checked by electrophoresis on a 1% agarose gel. PCR products (17.5 μL) were digested independently with 2.5 U of *Hae*III restriction endonuclease and incubated overnight at 37 °C. Terminal restriction fragments (T-RFs) were detected using capillary electrophoresis (Macrogen, Seúl, Korea), and data were obtained using Peak Scanner software (v1.0, AB Applied Biosystems, Foster City, California, USA). The sizes of the T-RFs were determined using the internal standard LIZ1200 (Applied Biosystems, Rotkreuz, Switzerland). Data analysis included the T-RFs between 50 and 900 nucleotides and excluded those representing less than 0.5% of the total area/abundance (Osborne et al., 2006), thus recalculating the area of each selected T-RF as a proportion of the total area.

2.2.3.3. 16S rRNA Gene Clone Library Construction

To identify the different operational taxonomic units (OTUs) observed in the Terminal Restriction Fragment Length Polymorphism (T-RFLP) profiles, a clone library was created

for the 16S rRNA gene. For this purpose, amplification and subsequent cloning of the 16S rRNA gene were performed from the DNA of each sample using primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Weisburg et al., 1991). The PCR products obtained were cloned into the vector TOPO-TA using a mixture containing 4 µL of PCR product, 1 µL of salt solution, and 1 µL of cloning vector (Cloning Kit R for Sequencing, Invitrogen). This mixture was incubated at room temperature for 20 min, and then 45 µL of competent cells of *Escherichia coli* (DH5α) were added and incubated in ice for 20 min. The mixture was then placed in the thermoregulated bath at 42 °C for 50 s and finally placed in ice for 2 min. After transformation, the mixture was incubated in 950 µL of Super Optimal Broth (SOB) medium (20 mM glucose, 20 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 0.5 g L^{-1} NaCl, 2.5 mM KCl, 5 mM $MgCl_2$, and 5 mM $MgSO_4$, pH 7.0) for 1.5 h at 37 °C with constant agitation. Afterward, 100 µL of the incubated solution was collected and seeded on LB agar plates supplemented with ampicillin (50 µg mL^{-1}), 10 µL of X-gal (80 mg mL^{-1}), and 40 µL of IPTG (500 mM). The seeded plates were incubated at 37 °C overnight.

To corroborate the presence of the fragment of interest in the cloning vector, cloned sequences were amplified by PCR using the M13 forward (M13F) (5'-GTAAAACGACGGCCAG-3') and M13 reverse (M13R) (5'-CAGGAAACAGCTATGAC-3') primers. The final concentrations in the PCR mix (final volume 25 µL) were: 1.2 mM $MgCl_2$; 1X buffer without Mg^{2+}; 0.25 mM dNTPs; 0.5 µM each of M13F and M13R; and 1.25 U KAPA Taq polymerase. PCR conditions were: 94 °C for 10 min; 25 cycles at 94 °C for 45 s; 50 °C for 1 min; 72 °C for 1 min; and a final

elongation step at 72 °C for 7 min. PCR amplicons were checked by electrophoresis on a 1.5% agarose gel. Positive clones were confirmed based on their length of about 780 bp. Positive clones were sequenced at Macrogen Inc., Seoul, Korea. The obtained clone sequences are available in the Sequence Read Archive database (Table S2-1).

2.2.3.4. Analysis of Clone Sequences

Sequence Scanner Software v1.0 (AB Applied Biosystems, Foster City, CA, USA) was used to check the quality of the sequences. Sequences were then analyzed with VECTOR NTI software Version 11.5.4 (Thermo Fisher Scientific, Waltham, MA, USA) to eliminate the remaining vector sequence and leave the sequences in the 5'–3' direction, considering the primer 27F as the beginning. Through this software, an in-silico analysis was also performed with the restriction enzyme *Hae*III for the subsequent taxonomic assignment of the observed T-RFs. The T-RFs of each condition were assigned with the in-silico analysis of the sequenced clones for each condition, except in the + 100mV (vs. Ag/AgCl) condition. In this condition no clones were obtained, and only taxonomic assignment was made using the T-RFs. All edited sequences were compared to the published database (nr/nt) using BLASTn (Altschul et al., 1990) from the National Center for Biotechnology and Information (NCBI, December 2018).

2.2.3.5. Amplicon Analysis of 16 rRNA Gene Sequences

To obtain a deeper taxonomic analysis of the microbial community enriched for under each set of conditions, amplicon analysis (AA) using next-generation sequencing (NGS) of the V4 hypervariable region of the 16S rRNA gene was performed with the Illumina MiSeq platform (Integrated Microbiome Resource (IMR), Toronto, Canada). The

sequences obtained in the present study are publicly available in the Sequence Read Archive database under the accession number PRJNA603906. The 16S rRNA gene amplicon sequences were processed using Mothur (Schloss et al., 2009). Sequences were de-multiplexed, assembled, and assigned to samples by matching them to barcode sequences using the *make.contigs* script and primers were removed using *cutadapt* (Martin, 2011). Sequences with undesired lengths (200–300 bp), ambiguous nucleotides, and homopolymers longer than 8 bp were removed before further analysis. Afterward, sequences were aligned using the recreated Silva SEED v119 (Quast et al., 2013) as the reference. Chloroplast, eukaryotic, archaeal, and mitochondrial sequences were discarded. Sequences were also checked for PCR chimeras using UCHIME version 4.2.40 (mybiosoftware) (Edgar et al., 2011). High-quality sequences were clustered into operational taxonomic units (OTUs) with the furthest-neighbor algorithm, with a minimum sequence identity cut-off of 97%. Taxonomic assignments were performed against Silva v119 (Quast et al., 2013). OTUs formed by 20 or fewer reads were not considered in subsequent analyses. For the OTUs that could not be identified at the gender level, a manual BLASTn was performed, wherein each sequence was compared to the published database (December 2018) (nr/nt) using BLASTn (Altschul et al., 1990) from NCBI.

2.2.3.6. Statistical Analysis of Fragment and Amplicon Analysis Profiles

To determine the similarity between the samples and the robustness of the results obtained by FA and AA, fragment and amplicon profiles were statistically analyzed via multivariate analysis using Primer-E software version 6 (Primer-E, Plymouth, UK). For each dataset (FA and AA), the average of the relative abundance profile of each replicate

of the OTU was transformed to its square root, and then a similarity matrix was obtained using the Bray–Curtis coefficient (Beals, 1984). With the similarity matrix thus generated, a cluster was performed for visual interpretation of the grouping, where the proximity between samples corresponded to their similarity. To evaluate the statistical significance of the differences between and within communities, a one-way ANOSIM and a SIMPROF analysis were performed (R.M.Warwick, 2007).

2.2.3.7. Similarity Analysis between Cloning and Amplicon Sequences

The sequences obtained via cloning and amplicon sequences were compared to determine the similarity between sequences and therefore the robustness of the community composition results. For this, the sequences of the most abundant OTUs, associated with the same bacterial genera in cloning and amplicon sequences, were used to perform a BLAST between sequences. With these results, a literature search was carried out to identify the metabolism and natural environment reported for each detected genus.

To determine the difference and/or similarity in bacterial identification and metabolic inference between samples, main bacterial taxa were identified using 16S rRNA gene sequences of clone library and AA results. Each sequence was taxonomically assigned by manual BLASTn of the sequence against the NCBI nt/nr database. For this analysis, only the families with a relative abundance higher than 1% of the total abundance in each sample were used.

2.2.3.8. Microscopy Analysis

The SS coupons used for microscopy were cut into two parts. One part was used for epifluorescence microscopy and the other for scanning electron microscopy (SEM). For

epifluorescence microscopy, each coupon was immersed in 0.1% w/v acridine orange (Sigma Chemical Co., St. Louis, MO, USA) for 5 min at room temperature. Then, each coupon was washed with Phosphate Buffered Saline (PBS). Each coupon was examined using an Olympus CX31 microscope (Olympus, Tokyo, Japan). The other halves of the coupons were used for SEM analysis. For SEM analysis, samples were fixed with 2% glutaraldehyde, treated by critical-point drying, and coated with a thin gold film. Each coupon was examined using a LEO 1420VP microscope (LEO Electron Microscopy Inc., New York, NY, USA) (Fischer et al., 2016). For each coupon, three pictures were taken at randomly selected areas.

2.3. Results and Discussion

2.3.1. Electrode Deterioration

Three different potentials were applied to the 316L SS electrodes to determine the effect of overpotential application on electrode deterioration and bacterial enrichment. After 10 days of exposure, the maximum weight loss (as an indicator of deterioration) was observed for the + 310 mV (vs. Ag/AgCl) treatment, with significant differences ($P < 0.05$) from that which occurred under the other conditions (Figure 2-2A).

To determine whether the significant weight loss observed under the +310 mV (vs. Ag/AgCl) potential was due to the effect of the electrochemically enriched bacteria or the potential itself, an abiotic control experiment was conducted. Figure 2-2B shows that the maximum weight loss, which occurred in the SS electrodes poised at +310 mV (vs. Ag/AgCl) under biotic conditions, was significantly different from the corrosion produced

under abiotic conditions both with and without the overpotential *(P* < 0.05). This result suggests that the observed deterioration was associated more with the enrichment of specific bacteria than with abiotic corrosion. Besides, AFM analysis of the electrodes showed that the applied potential of +310 mV (vs Ag/AgCl) significantly increased the surface roughness, probably due to electrode corrosion (Figure 2-3).

Indeed, biocorrosion of SS has been linked with the action of autotrophic metabolism (Olesen et al., 2000; Chamritski et al., 2004; Trigodet et al., 2019). This type of corrosion is frequently associated with the ennoblement of the OCP involving iron-oxidizing bacteria, manganese-oxidizing bacteria (Olesen et al., 2000; Chamritski et al., 2004), and, more recently, electro-autotrophic bacteria able to use an electrode (i.e., a SS coupon) as the electron donor to reduce oxygen (Eddie et al., 2016, 2017; Trigodet et al., 2019). Furthermore, the ennoblement of SS plates exposed to natural seawater typically shifts the OCP by +200 to +300 mV (vs. Ag/AgCl), bringing it closer to the pitting corrosion potential (Trigodet et al., 2019). This microbially driven phenomenon results in the initiation of SS pitting and crevice corrosion. Pitting corrosion of 316L SS coupons exposed to seawater has been reported under an applied potential ranging from +500 to +600 mV (vs. Ag/AgCl) after a few days of immersion (Compère et al., 1996). Therefore, the significant differences in weight loss (Figure 2-2) and roughness (Figure 2-3) exhibited by the coupons exposed to the +310 mV (vs. Ag/AgCl) potential, in comparison to those produced the other tested conditions, may be linked with the enrichment for particular bacterial groups that could catalyze electron transfer, rather than with the applied potential itself.

Under the tested conditions, no significative damage was observed on SS electrodes polarized at +100 mV (vs. Ag/AgCl) that correspond to OCP reported for 316L SS under marine conditions after 10 days (Fischer et al., 2016). These results suggest that SS is a good candidate to be used as electrode material for the scaling-up of METs in marine environments. Hence, this finding opens the opportunity to explore long-term evaluation of this material in marine METs involving biocathodic reactions.

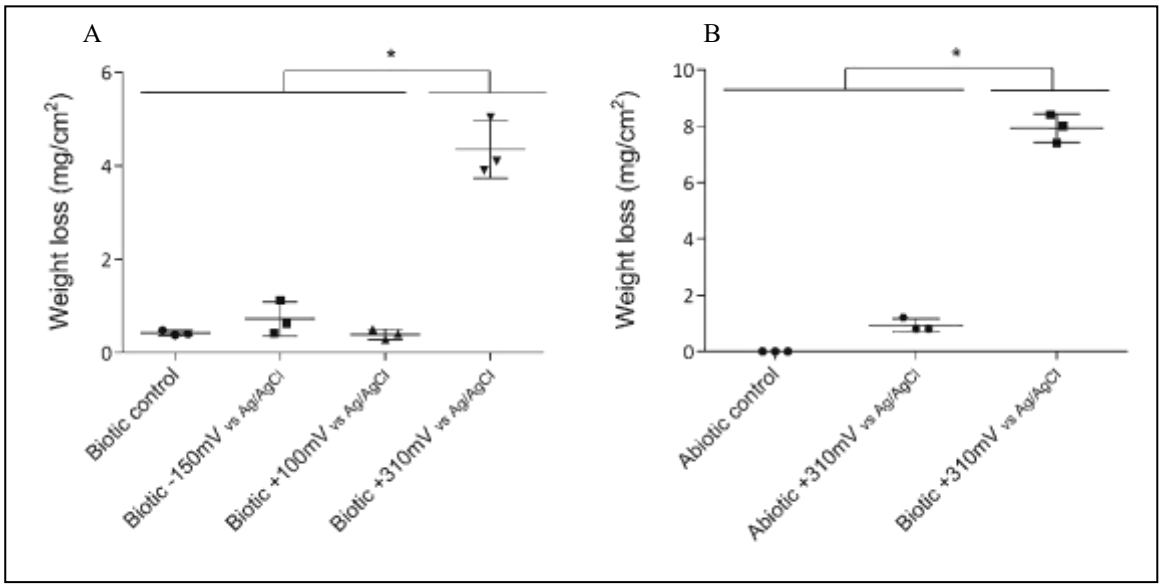

Figure 2-2: Weight loss of SS electrodes under each treatment after 10 days. To determine the significant differences between the treatments, a posteriori Tukey test was performed. Significant differences between treatments are indicated with asterisks, where each asterisk group treatments according to similarity. (A) Weight losses in the initial experiment under biotic conditions. (B) Weight losses under the abiotic and biotic conditions with the +310 mV (vs. Ag/AgCl) potential applied.

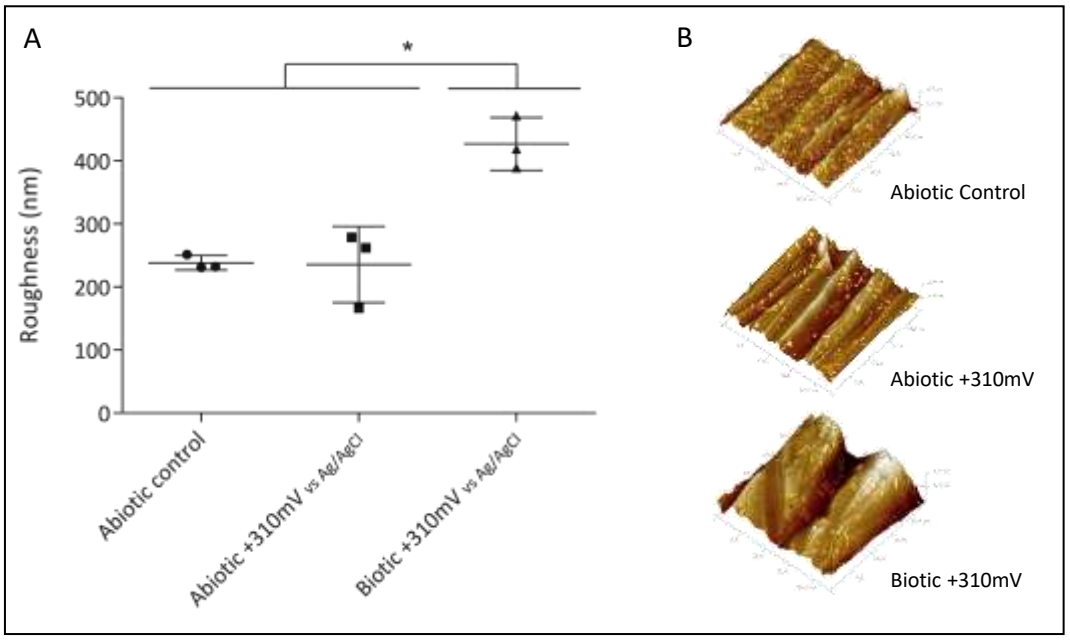

Figure 2-3: Roughness of SS electrodes (second experiment), measured by atomic force microscopy (AFM). Asterisks represent significant differences between treatments ($P < 0.05$). (A) Average roughness of SS electrodes with standard deviation and (B) 3D Atomic Force Microscope (AFM) images of SS electrodes obtained over an area of 35 µm × 35 µm.

2.3.2. Bacterial Enrichment Using Overpotentials

To corroborate the microbial community development on the tested electrodes, epifluorescence microscopy and SEM were performed (supplementary materials Figure S2-1 and S2-2). Microscopic analysis revealed the presence of microorganisms attached to the surface of the electrode, with the presence of extracellular polymeric-like substances (Figure S2-2). These results confirm early biofilm development on all the coupons.

During the experiment, three different enrichment levels were expected because of the experimental design, which would be influenced by (i) the effect of water storage (bottle effect), (ii) the effect of the SS surface (i.e., material composition and roughness), and (iii)

the effect of the overpotential application. The storage conditions and the surface were transversal in all the conditions studied, allowing the isolation of the specific effect of microbial enrichment upon application of the overpotential.

To evaluate the effect of the applied potentials on specific microbial enrichment, the bacterial community developed under each treatment was characterized at the end of the experiment using its 16S rRNA gene profiles. Cluster analysis based on the Bray–Curtis similarity coefficients obtained from both FA and AA datasets indicated a similar and consistent pattern, with the samples exposed at +310 mV (vs. Ag/AgCl) being the most different in each dataset (Figure 2-4), with 65% and 80% similarity, respectively. Differences were supported by SIMPROF (Pi 4,114, $P < 0.05$) and ANOSIM (R: 0.718, $P < 0.05$) analyses.

Cluster analyses were complemented with taxonomic assignation to the family level of the main OTUs detected by cloning (i.e., FA) and AA. In the case of the samples exposed at +310 mV (vs. Ag/AgCl), the Rhodobacteraceae bacterial family was highly enriched from a natural inoculum of seawater without any nutrients added. Both techniques, FA and AA, gave similar results in terms of relative abundance of Rhodobacteraceae in the +310 mV (vs. Ag/AgCl) samples, with an average relative abundance of 81.80% by FA and 69.31% (±2.81) by AA.

On the other hand, the +100 mV, −150 mV (vs. Ag/AgCl) polarization, and control treatments resulted in similar bacterial compositions (Figure 2-4), suggesting that the effect of applying 100 mV, −150 mV (vs. Ag/AgCl) to an electrode, on the enrichment of specific marine bacteria, was not greater than the effect generated by the material itself.

Sulfitobacter and *Glaciecola*, two of the most abundant microbial genera identified in the +100 mV (vs. Ag/AgCl) and −150 mV (vs. Ag/AgCl) samples (Table 2-2), were previously identified as the most abundant genera in microbial communities developed over SS surfaces (Erable et al., 2017). In the case of the control, wherein no potential was applied, typical microbial genera previously associated with electroactive microbial communities were identified. When exposed to seawater, the 316L SS surface potential naturally reached +100 mV (vs. Ag/AgCl) after 10 days (Fischer et al., 2016), and as is shown in Figure 2-4, the control and +100 mV (vs. Ag/AgCl) treatments resulted in similar microbial community compositions. These results suggest that microbial settling in natural complex environments, as well as in reactors run under controlled laboratory conditions (Torres et al., 2009; Miceli et al., 2012; Zhan et al., 2014; Z. Wang et al., 2015), depends on the value of the SS electrode potential.

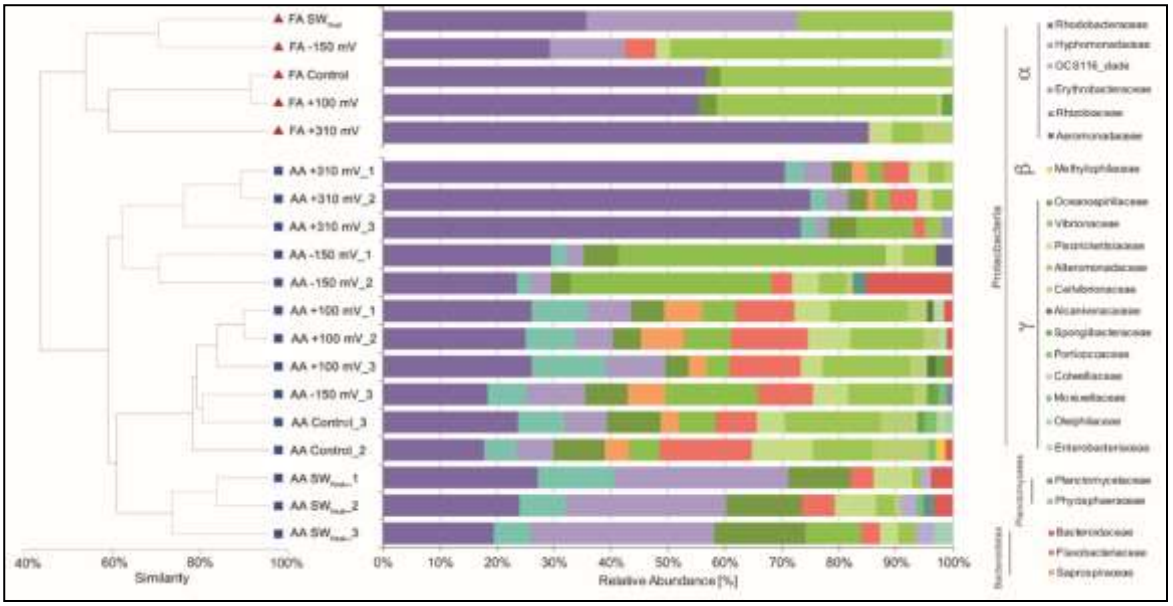

Figure 2-4: Cluster and relative abundance family-level taxonomic analysis of the bacterial communities associated with each treatment.

2.3.3. Metabolic Inference of Electrochemically Enriched Bacterial Communities

To gain more information about the microbial community identified in each sample, the most abundant genera were characterized, identifying their metabolisms and the environments in which they have been found, according to previous reports in the literature. Table 2-1 lists, in decreasing order of abundance, the most abundant genera of each family that were identified for each treatment. Furthermore, the similarity between the sequences obtained by FA and AA, associated with each gender, was analyzed. All pairs of sequences had similarities of >80% and were related to the same bacterial genera, confirming the correspondence between a fingerprinting technique (such as FA) and AA analysis (Gobet et al., 2014; van Dorst et al., 2014).

For the discussion of the relative abundance of the most abundant microbial genera identified in each condition, only the AA analyzes were used. The reason for this is due to the low number of clones obtained by condition, which generates an oversize of the relative abundance values of the genera identified by the clones in each condition (Table S2-2). The genera with higher relative abundances were *Roseobacter, Pheobacter*, and *Sulfitobacter*, with relative abundances of 16.8%, 10.5%, and 9.9%, respectively (Table 2-2). These genera showed the highest relative abundances in the +310 mV (vs. Ag/AgCl) samples. All of these genera have been related to autotrophic microorganisms and reported in electrochemically active microbial communities (Allgaier et al., 2003; Vandecandelaere et al., 2009; Parot et al., 2011; Erable et al., 2017). Other genera that have not been previously implicated in biocorrosion of SS, including *Aestuariicella, Lewinella*, and *Spongiibacter,* were also detected, with relative abundances of 2.43%, 3.18%, and 0.5%,

respectively. The majority of the genera shown in Table 2-1 have been previously associated with electroactive microbial communities developed on SS plates or electrodes [42,45,48–58].

As described in Materials and Methods, the potential of −150 mV (vs. Ag/AgCl) was selected as a representative midpoint potential capable to enrich for reported marine EAB from marine sediments (Rowe et al., 2015). The groups of microorganisms reported by Rowe et al. (2015) at −150 mV (vs. Ag/AgCl) were not found in our experiments. Nevertheless, *Sulfitobacter* and *Glaciecola* have been reported as the predominant bacteria in electroactive microbial communities on SS electrodes (Erable et al., 2017), and these were identified as two of the most abundant microbial genera enriched on the electrodes polarized at -150 mV (vs. Ag/AgCl) in our experiments (Table 2-2).

Poised potentials around +100 mV (vs. Ag/AgCl) have been identified as selective pressures for specific microbial communities mostly composed by *Marinobacter*, *Chromatiaceae*, and *Labrenzia* (MCL). This particular community, identified as a MCL-biocathode, has been reported to be capable of using the electrons supplied by the electrochemical system to drive CO_2 fixation and O_2 reduction (Strycharz-glaven et al., 2013; Leary et al., 2015; Z. Wang et al., 2015). Although *Marinobacter* and *Labrenzia* were not the most abundant genera identified in our electrodes tested over +100 mV (vs. Ag/AgCl), the abundances of both genera were significantly higher in comparison with their abundances in the other samples (Figure S2-3). It is important to highlight that the aforementioned MCL-biocathode cluster was obtained using marine sediment as the inoculum (Malik et al., 2009). In this study, electrochemical enrichment was accomplished

directly from natural seawater. In consequence, differences in bacterial composition and abundance between these experiments were expected.

Roseobacter and *Sulfitobacter* were two of the most abundant genera identified in the +310 mV (vs. Ag/AgCl) samples (Table 2-2). Both groups of microorganisms have been identified as EAB with high efficiency in the catalysis of the oxygen reduction reaction (Parot et al., 2011). As indicated in Materials and Methods, this overpotential has been related to the oxygen reduction reaction (Ter Heijne et al., 2010; Rimboud et al., 2016). From a bioenergetic perspective, with higher potentials closer to the theoretical reduction potential of oxygen (i.e., +600 mV vs. Ag/AgCl at pH = 7 and pO_2 = 0.2 bar), aerobic microorganisms gain less energy (Ter Heijne et al., 2010). Using an inoculum collected from a wastewater treatment plant, Ter Heijne et al. (2010) studied the effect of positive overpotentials (+50 mV, +150 mV, and +250 mV vs. Ag/AgCl) on the development of oxygen-reducing biocathodes. While the biocathodes polarized at +50 mV and +150 mV (vs. Ag/AgCl) produced current during the first day after inoculation, that polarized at +250 mV (vs. Ag/AgCl) produced no current until day 15, probably due to slow bacterial growth and a longer start-up time (Ter Heijne et al., 2010). On the other hand, in a bioelectrochemical study of *Candidatus* Tenderia electrophaga (belonging to the Chromatiaceae family), Eddie et al. (2017) showed that by applying a potential as positive as +470 mV (vs. SHE) or +250 mV (vs. Ag/AgCl), there was an increase in the expression of genes related with nitrate and oxygen reduction (Eddie et al., 2017). Luo and Moran (2014) (Luo and Moran, 2014) reported that about half of the marine *Roseobacter* clade present genes related to the nitrate reduction process, and as indicated above, *Sulfitobacter*

and *Roseobacter* can reduce oxygen using electrons from a polarized electrode (Parot et al., 2011). Our results showed that by applying a positive potential as high as +310 mV (vs. Ag/AgCl) to SS electrodes exposed to natural seawater, it was possible to establish a high enrichment pressure for enriching autotrophic oxygen-reducing EAB such as *Sulfitobacter* and *Roseobacter*.

The presence of autotrophic oxygen-reducing EAB has been related to the acceleration of biocorrosion processes (Eddie et al., 2016, 2017; Trigodet et al., 2019). The highest abundances of *Sulfitobacter* and *Roseobacter* were found in the +310 mV (vs. Ag/AgCl) treatment, and at the same time, this condition resulted in significant differences in weight loss (Figure 2-2) and roughness (Figure 2-3) in comparison with those produced under the other tested conditions. Therefore, the results of this research suggest that high abundances of *Sulfitobacter* and *Roseobacter* are directly related to electrode deterioration, specifically the biocorrosion of 316L SS.

Table 2-1: Similarity analysis between sequences obtained by fragment analysis (FA) and amplicon analysis (AA), and their metabolisms and the environments wherein have been reported. The similarity between sequences obtained by AA and FA is expressed as % similarity.

Family	Most Abundant Genus Identified by AA	% Similarity	Most Abundant Genus Identified by FA	Metabolism and Environments Wherein They Have Been Reported
Rhodobacteraceae	*Roseobacter*	90	*Roseobacter*	1. Aerobic anoxygenic photosynthesis (Allgaier et al., 2003). 2. Identified as primary colonizers on surfaces exposed to seawater (Dang and Lovell, 2000). 3. Reported as EAB with high efficiency in the catalysis of the oxygen reduction reaction (Parot et al., 2011).
	Pheobacter	93	*Pheobacter*	1. Reported in polarized stainless steel cathode (Vandecandelaere et al., 2009). 2. Association with *Roseobacter* during primary colonization (Vandecandelaere et al., 2009). 3. Aerobic anoxygenic photosynthesis (Vandecandelaere et al., 2009).
	Sulfitobacter	80	*Sulfitobacter*	1. Reported on the bacterial communities associated with the early stages of marine corrosion of carbon steel (Dang et al., 2008). 2. Reported as EAB with high efficiency in the catalysis of the oxygen reduction reaction (Parot et al., 2011). 3. Has been found in both anodic and cathodic biofilms (Erable et al., 2017).
Vibrionaceae	*Vibrio*	-	-	1. Reported in graphite bioanodes present in bioelectrochemical systems [54].
Hyphomonadaceae	*Hyphomonas*	99	*Hyphomonas*	1. Identified in graphite biocathodes present in an MFC (Chen et al., 2010).
	Maricaulis	90	*Maricaulis*	1. Identified as a typical bacterioplankton in marine ecosystems (Abraham et al., 1999). 2. Reported as the primary colonizer in biofilm developed on stainless steel (Moura et al., 2018).
Flavobacteriaceae	*Muricauda*	87	*Muricauda*	1. Reported in a biocathode microbial community (Malanoski et al., 2018).
Alteromonadaceae	*Alteromonas*	85	*Alteromonas*	1. Reported on electrochemically active biofilms [51]. 2. Chemo-heterotrophic halophytes [51].
	Glaciecola	87	*Glaciecola*	1. Aerobic chemo-heterotrophic bacteria (Yuan et al., 2011). 2. Reported as predominant bacteria in electroactive biofilms on stainless steel electrodes (Erable et al., 2017).
Phycisphaeraceae	Planctomycetes	-	-	1. Reported in marine phototrophic consortia that can transfer electrons to electrodes in response to reductive stress (Darus et al., 2016).
Oceanospirillaceae	*Neptuniibacter*	-	-	1. Reported in microbial community associated with stainless steel coupons [56].
Piscirickettsiaceae	Methylophaga	-	-	1. Reported in stainless steel and carbon steel cathodes [57]. 2. Has been reported to reduce nitrate to nitrite [71]. 3. Halophilic methylotrophic metabolism [72].
Cellvibrionaceae	Aestuariicella	-	-	1. Aliphatic hydrocarbon-degrading bacterium [73]. 2. Not yet reported in biofilms associated with stainless steel [73].
Saprospiraceae	Lewinella	-	-	1. Isolated from marine sediment [74]. 2. Not yet reported in biofilms associated with stainless steel [74].
Bacteroidaceae	Bacteroides	-	-	1. Reported on stainless steel electrodes [58].
Colwelliaceae	Colwellia	89	Colwellia	1. Reported on carbon steel cathodes [71]. 2. Identified in a marine biofilm exposed to high concentration of nitrate [71].
Spongiibacteraceae	Spongiibacter	92	Spongiibacter	1. Halophilic marine bacterium [75]. 2. Not yet reported in biofilms associated with stainless steel [75].

Table 2-2: Relative abundance (%) of most-abundant genre identified by amplicon analysis (AA) in each sample (+ 100mV, −150mV, + 310mV vs. Ag/AgCl and Control).

Family	Genus	Relative Abundance (%)			
		AA			
		+100 mV	−150 mV	+310 mV	Control
Rhodobacteraceae	Roseobacter	4.3	3.9	34.0	6.4
	Phaeobacter	6.0	4.8	18.8	5.9
	Sulfitobacter	7.2	7.9	15.2	3.5
	Ruegeria	1.3	1.0	0.5	1.2
	Labrenzia	1.1	0.0	0.1	0.1
Vibrionaceae	Vibrio	4.0	3.3	3.8	5.0
	Photobacterium	0.1	0.0	0.0	0.0
	Aliivibrio	0.0	0.0	0.0	0.0
Hyphomonadaceae	Hyphomonas	4.3	3.4	3.6	5.1
	Maricaulis	1.0	0.3	0.7	0.8
Flavobacteriaceae	Muricauda	5.3	2.1	1.7	3.7
	Maribacter	0.0	0.0	0.0	0.0
	Cellulophaga	0.0	0.0	0.0	0.0
Alteromonadaceae	Alteromonas	0.6	0.1	2.5	11.5
	Glaciecola	7.3	5.9	0.0	0.6
	Marinobacter	2.9	0.2	0.0	0.2
Phycisphaeraceae	Plantomycete	10.3	4.2	2.2	6.6
Oceanospirillaceae	Neptuniibacter	3.7	4.3	3.3	6.5
	Amphritea	0.1	0.0	0.0	0.0
	Oleibacter	0.7	0.1	0.0	1.1
Piscirickettsiaceae	Methylophaga	5.1	3.3	2.1	6.9
Cellvibrionaceae	Aestuariicella	2.3	0.8	0.5	4.5
Saprospiraceae	Lewinella	5.5	1.7	1.3	1.7
Bacteroidaceae	Bacteroides	1.1	4.6	0.0	0.5
Colwelliaceae	Colwellia	0.9	0.0	0.0	0.7
Spongiibacteraceae	Spongiibacter	0.5	0.5	0.0	0.6

2.4. Conclusions

Electrochemical bacterial enrichment from natural seawater without nutrients amended was successfully achieved for the first time to the best of our knowledge. Under the tested conditions, no significant damage was observed on SS electrodes poised at up to +100 mV

(vs. Ag/AgCl). This finding opens the opportunity for long-term evaluation of SS as electrode material in marine METs. On the other hand, a significant selection for putative EAB within the *Roseobacter* and *Sulfitobacter* genera present in a marine inoculum was observed upon applying an overpotential of +310 mV (vs. Ag/AgCl), and the resultant microbial community accelerated corrosion, compromising the lifetimes of the SS electrodes. Finally, further attention should be given to the aforementioned bacterial groups as potential biological drivers of pitting corrosion in 316L SS in marine environments.

2.5. Acknowledgements

This work was supported by the Vicerrectoría de Investigación de la Pontificia Universidad Católica de Chile (Project VRI-PUC Nº01/2013), the Office of Naval Research (Grant NICOP-N62909-17-1-2012), FONDECYT 1160917, and FONDECYT 1181326. This study was conducted at the Marine Energy Research and Innovation Center (MERIC) (Project CORFO 14CEI2-28228).

We give special thanks to Benjamin Lagos, Mauricio Medel, and Evelyn Cruz for their assistance in the laboratory and help with the experimental setup.

3. ELECTROCHEMICAL ENRICHMENT OF MARINE DENITRIFIYING BACTERIA TO ENHANCE NITRATE METABOLIZATION IN SEAWATER.

Published in *Journal of Environmental Chemical*, Volume 9(4), p.105604

3.1. Introduction

Discharge of municipal wastewater and of sewage from fish aquaculture industry into coastal and marine environments is of significant concern owing to the high bioavailable nitrogen concentration. The nitrate concentrations in fish farming and urban wastewater discharge zones have been measured, obtaining concentrations of 40.67 mg L^{-1} oand 12.48 mg L^{-1}, respectively (Bahroun et al., 2016; Guldhe et al., 2017). These concentrations of nitrate are 20 to 70 times higher than the average nitrate concentration in the sea (Libes, 2009). The final consequence of this nitrogen excess in the sea is eutrophication (Howarth and Marino, 2006). The main environmental effects of eutrophication are (i) increase in suspended particles due to macroalgae blooms, which leads to a decrease in water clarity, and (ii) increase in the precipitation rate of organic matter, which causes the destruction of benthic habitats and promotes the development of anoxic conditions, thereby affecting benthic communities and the biogeochemical balance of aquatic systems (Smith, 2003; Ansari et al., 2010; Kennish, 2015; Wurtsbaugh et al., 2019).

Several strategies and technologies have been developed in the last decade, including physical, chemical, and biological methods, to remove nitrate present in wastewater. Physical separation techniques such as activated carbon adsorption, ion exchange

processes, reverse osmosis, electrochemical reduction, and electrodialysis have shown efficiency in nitrate removal. However, these technologies have high operating costs and an excessive production of brines, which compromise the sustainability of the process (Bhatnagar and Sillanpää, 2011; Huno et al., 2018; Rezvani et al., 2019). Inconsistency of nitrate reduction, risk of nitrite formation (potential incomplete denitrification) and the reduction of it to ammonia, are important limitations for scaling up chemical denitrification processes (Rezvani et al., 2019). Biological denitrification has been proven to be the most cost-efficient technique in nitrate removal, but the addition of organic matter is still necessary to achieve effective removal. This heterotrophic growth leads to excessive sludge production that requires additional operation units (Ahn, 2006; Bhatnagar and Sillanpää, 2011; Huno et al., 2018; Rezvani et al., 2019). In this context, bioelectrochemical methods for dissimilatory nitrate reduction, such as Bioelectrochemical Denitrification Systems (BEDS), have emerged as a promising nitrate removal technology because no organic electron donor is needed, thereby increasing the cost-efficiency of the system and preventing the subsequent generation of sludge (Park et al., 2005; Kelly and He, 2014; Kondaveeti et al., 2014).

In general, BEDS have been tested under controlled laboratory conditions using a culture medium, a matrix of wastewater, and activated sludge as inoculum (Virdis et al., 2008, 2010; Xie et al., 2011b; Zhang et al., 2013, 2014). In a real environment, such as the sea, physicochemical conditions and nutrient fluctuation cannot be controlled. In addition, a low relative abundance of denitrifying bacteria (compared to activated sludge) in the system could compromise the efficacy of the treatment (Juretschko et al., 2002). Thus, the

development of BEDS for treating nitrate-impacted coastal and marine environments has emerged as a key challenge to expand the range of application of this technology.

The application of overpotentials to an electrode has been demonstrated to be useful in the in vitro enrichment of Electrochemically Active Bacteria (EAB) (Torres et al., 2009; Miceli et al., 2012; Rowe et al., 2015; Wang et al., 2015). In the case of Rowe et al. (2015), marine EAB from sediments were enriched by applying overpotentials, and were then characterized electrochemically. Most of the EAB isolated in this research were capable of taking electrons from an inorganic electron donor to reduce nitrate. Furthermore, Kondaveeti et al. (2013) reported that the bioelectrochemical treatment of nitrate shows a microbial reduction peak at -130 mV (vs. Ag/AgCl) if the medium contains nitrate and at -260 mV (vs. Ag/AgCl) if the medium contains nitrite (NO_2^-). In the case of nitrite, a reduction peak appears at -570 mV (vs. Ag/AgCl) if the medium contains nitrate.

Therefore, these three potentials previously associated with the microbial denitrification process (-130, -260, and -570 mV vs. Ag/AgCl) could be used as a microbial enrichment tool for denitrifying bacteria without the need for the use of culture medium. To the best of our knowledge, this mechanism has not been previously evaluated. For this reason, in this study we evaluated (i) the enrichment of denitrifying marine bacteria from seawater in a working electrode using specific overpotentials and (ii) the effect of this enrichment of denitrifying marine bacteria on nitrate removal in a bioelectrochemical reactor. To accomplish these objectives, three-electrode electrochemical cells were used and three proposed potentials (-130, -260, and -570 mV vs. Ag/AgCl) were applied to the working

electrodes. To determine the nitrate removal, its concentration was measured during the experiment. At the end of the experiment, to identify the presence and abundance of potentially denitrifying bacteria, bacterial community analysis using Next Generation Sequencing (NGS) and Fluorescence In Situ Hybridization (FISH) was performed. Furthermore, Cyclic Voltammetry (CV) was used to confirm that the formed biofilm used the electrode as an external electron donor.

3.2. Material and Methods

3.2.1. Reactor Configuration

The experiment was run using twelve 250 mL autoclavable plastic flasks. Each flask had three carbon felt (AvCarb C100 Soft Carbon Battery Felt, Fuel Cell Store) electrodes as one working electrode (1.5 cm^3), one graphite rod as a counter electrode (2.0 cm^3), and one Ag/AgCl electrode (3 M KCl; 0.21 V vs. SHE) as a reference electrode (CHI111, CH Instruments Inc.) (Figure 3-1). Three different overpotentials were applied to the carbon felt working electrodes, namely (i) -130 mV (vs. Ag/AgCl) representing the nitrate reduction potential in a biocathode where only nitrate was added and (ii) -260 mV (vs. Ag/AgCl) and (iii) -570 mV (vs. Ag/AgCl) corresponding to the nitrate and nitrite reduction potential, respectively, in a biocathode where both compounds were added (Kondaveeti et al., 2014). Each condition had three replicates, and three of them worked as a control without any overpotential (i.e. open circuit condition) (Figure 3-1). All the reactors were kept under constant agitation (100 rpm) during the 30 d of the experiment in a temperature-controlled room at 19 °C. To maintain the cathode at the three different

potentials for 30 d, an Interface 1000TM potentiostat (GAMRY, PA, USA) connected to an ECM8™ Electrochemical Multiplexer (GAMRY, PA, USA) was used.

The inoculum for all the reactors was fresh, natural coastal seawater collected from the Estación Costera de Investigaciones Marinas of the Pontificia Universidad Católica de Chile located at the eastern Pacific Ocean (33°30'16"S, 71°38'23"W). To ensure a significant number of microorganisms in each reactor, 60 L of seawater was concentrated to a volume of 3 L by tangential flow filtration using a Vivaflow 200 cartridge equipped with a 100 000 MWCO RC membrane (Sartorius Biotechnologie SAS, France). Subsequently, 0.909 g of KNO_3 was added to the 3 L to be later distributed equally in each flask. Thus, each reactor started the experiment with 3 mM of nitrate.

Based on the buffer capacity of the natural seawater and the lack of any compound competing with the cathode as electron source for electroactive denitrifying microorganisms, the use of a membrane was considered not necessary.

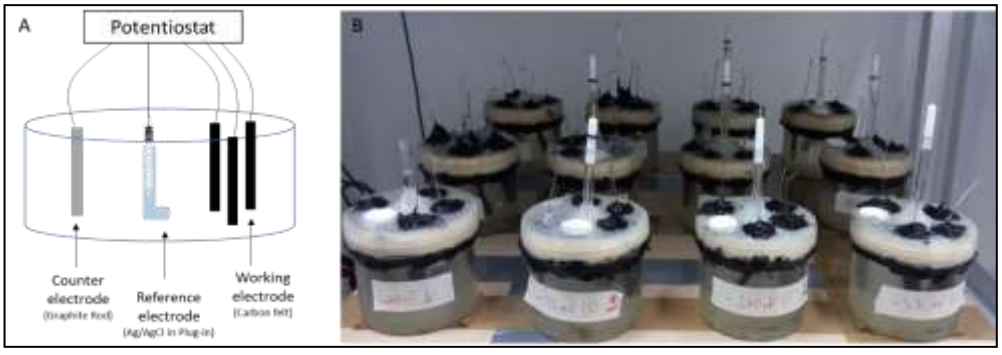

Figure 3-1: Experimental design. (A) Design of the flasks: each flask had three carbon felt electrodes as one working electrode, one graphite rod as a counter electrode, and one Ag/AgCl electrode as a reference. (B) Photograph of the used reactors and their replicates.

3.2.2. Microbial community analysis

After the exposure time and electrochemical analysis, the three working electrodes (i.e. cathodes) of each reactor were cut in half. One piece was used to analyze the microbial community present in each electrode by FISH, and the other piece was analyzed by NGS.

3.2.2.1. Fluorescence in situ hybridization

To determine the microbial community composition present in the electrodes, each electrode was analyzed by rRNA-targeted FISH. The three electrode pieces from each condition were fixed in a 2% (v/v) formaldehyde solution for one week at 4 °C. Fixed samples were washed in 1 × PBS (phosphate-buffered saline), resuspended in a 1:1 mixture of 1 × PBS and 96% (v/v) ethanol (Daims et al., 2005), and stored at -20 °C. Before performing the hybridization process, the samples were sonicated in 50 mL of Milli-Q water for 3 min (Elmasonic S 30H, Elma Schmidbauer GmbH, Germany). Sonicated samples were filtered through a 0.2 μm membrane filter to collect the cells. FISH was performed directly on the filter. Each reactor had three working electrodes, and the total number of reactors was 12 (3 for each treatment/fixed potential); therefore, in total 36 filters were analyzed.

The rRNA-targeted oligonucleotide probes used in this study are listed in Table 3-1. Probes were mono-labelled at their 5’ ends with fluorophores (Table 3-1). The probes used to identify Rhodobacteria were selected because in the sequencing results a high abundance of microorganisms associated with the denitrification capacity belonged to the group of Rhodobacteria. On the other hand, the probes used to identify *Planctomycetes*

were selected because annamox belongs to this group of microorganisms. FISH was performed for 2 h at 46 °C according to a standard protocol (Manz et al., 1992; Daims et al., 2005). After the hybridizations, each sample was also stained with DAPI (4′,6-diamidino-2-phenylindole).

Table 3-1: Ribosomal RNA-targeted oligonucleotide probes used in this study.

Probe	Fluorophore	Target organism	Probe sequence (5'-3')	FA[c] (%)	Reference
DEN 124	Atto 494	Denitrifiers*	CGA CAT GGG CGC GTT CCG AT	35	Ginige et al., 2005
RHOB 1002	Atto 633	Rhodobacteria	ACC ATC TCT GGA ACC GCG	35	Figuerola & Erijman, 2007
PARA 739	Atto 532	Rhodobacteria	GCG TCA GTA TCG AGC CAG	35	Thayanukul et al., 2010
PLA 46	Cy 3	*Planctomycetes*	GAC TTG CAT GCC TAA TCC	35	Neef et al., 1998
PLA 886	Cy 3	*Planctomycetes*	GCC TTG CGA CCA TAC TCC C	35	Neef et al., 1998

* Acetate-utilizing denitrifiers

Fluorescence micrographs were acquired with an inverted Leica TCS SP8X confocal laser scanning microscope equipped with a 405 nm UV diode, a Leica supercontinuum white-light laser, two photomultiplier detectors, three hybrid detectors, and the Leica Application Suite AF 3.2.1.9702. For each filter three images were taken at random positions, and a total of 108 images was obtained for the analyzed 36 filters. The relative biovolumen of the probe-target populations (compared to all DAPI-stained microbes) were quantified by using the software *daime* (version 2.1) (Daims et al., 2006). These results were statistically analyzed through a multivariate analysis using GraphPad Prism 6 (GraphPad Software, San Diego, CA) to determine the similarity between the samples.

3.2.2.2. DNA Extraction

The remaining three working electrode pieces from each bottle were sonicated in 50 mL of sterile seawater for 5 min (Elmasonic S 30H, Elma Schmidbauer GmbH, Germany). Sonicated samples were filtered through a 0.2 µm membrane filter to collect the biomass and perform the subsequent DNA extraction with a phenol/chloroform-based protocol (Fuhrman et al., 1988). Additionally, 2 L of water from the tank was filtered through a 0.2 µm membrane filter at the end of the experiment to analyze the changes in the microbial community of the water column present in the tank. DNA quantification was performed with a Qubit® 2.0 Fluorometer (InvitrogenTM, Life Technologies, CA, USA) according to the manufacturer's protocol.

3.2.2.3. Amplicon analysis of 16 rRNA gene sequences

In order to obtain a taxonomic overview of the microbial community enriched in each condition, Amplicon Analysis (AA) using NGS of V4 hypervariable region of the 16S rRNA gene was performed using the Illumina MiSeq platform (Integrated Microbiome Resource, Canada). The sequences obtained in the present study are publicly available in the Sequence Read Archive database under the accession number PRJNA666464. The 16S rRNA gene amplicon sequences were processed using Mothur29. Sequences were demultiplexed, assembled, and assigned to samples by matching to barcode sequences using *make.contigs* script, and primers were removed using *cutadapt30*. Sequences with undesired lengths (300–200 bp), ambiguous nucleotides, and homopolymers longer than eight base pairs were removed before further analysis. Sequences were then aligned using

the recreated Silva SEED v11931 as the reference. Chloroplast, eukaryotic, archaeal, and mitochondrial sequences were discarded. Sequences were also checked for PCR chimeras using UCHIME version 4.2.4032. High-quality sequences were clustered into operational Taxonomic Units (OTUs) using the furthest neighbour algorithm with a minimum sequence identity cut-off of 97%. Taxonomic assignments were performed against the Silva v11931 database. OTUs formed by 20 or fewer reads were not considered in subsequent analyses. For the OTUs that could not be identified at the genus level, manual BLASTn searches were performed against the NCBI nr/nt database (December, 2018).

3.2.2.4. Statistical analysis of AA profiles

To determine the similarity between the samples, multivariate statistical analyses were conducted using Primer 6 software (Primer-E, Plymouth, UK). The average of the OTU relative abundance profiles of each replicate was transformed to its square root, and then a similarity matrix was obtained using the Bray-Curtis coefficient (Beals, 1984). Thus, with the similarity matrix generated, a cluster was performed for visual interpretation of the grouping where the proximity between samples corresponds to their similarity. To evaluate the statistical significance of the difference between communities and within communities, a one-way ANOSIM and a SIMPROF analysis were performed (R.M.Warwick, 2007). Furthermore, a shade plot was created to visualize the diversity patterns of microbial genera present in each sample, which was responsible for the clustering. For this analysis, only bacterial genera with an abundance greater than 100 reads were used.

3.2.3. Electrochemical analysis

To confirm that the bacteria used the electrode as an electron donor and to measure the effectiveness of the catalyst in the nitrate reduction process, a CV technique was used in each reactor at the final stage of the experiment. Each CV analysis was performed using a potentiostat (Interface 1000TM potentiostat, GAMRY, PA, USA), where the tested carbon felt electrodes, a graphite rod electrode (99.9% purity), and an Ag/AgCl electrode served as the working, counter, and reference electrodes, respectively. The CV analyses were conducted by sweeping the cell in a potential range from -700 mV to +500 mV (vs Ag/AgCl) with a scan rate of 0.5 mV/s.

3.2.3. Nitrogen removal analysis

Nitrate removal was obtained by measuring nitrate consumed by microorganisms during the experiment, following the definition reported by; Yu et al., 2010; Yan et al., 2012 and Kondaveeti et al., 2014. To measure nitrate concentrations, samples of 1.5 mL were collected every 72 h. Each sample was filtered (pore size 0.2 μm) to remove microorganisms and particles, and was then kept at -20 °C until further processing. The nitrate concentrations were measured by standard methodology (Koroleff, 1999), using a Seal AutoAnalyzer (Seal Analytical AA3, Wisconsin, USA). Owing to the nitrate detection range of the equipment (<0.1 μM to 45.0 μM), the samples were diluted 1:50 prior to the measurements. The similarity between the samples was assessed by multivariate statistical analyses using GraphPad Prism 6 (GraphPad Software, San Diego,

CA).Using the same sampling protocol and equipment, nitrite was measured over time. Its concentration was under the used machine detection limit (<0.015 μM).

3.2.3. Coulombic efficiency (CE)

Coulombic efficiency (CE) was calculated according to Equation (1)(Torres-rojas et al., 2020)

$$CE_{(NO3)^-} = (Q_{output}/Q_{input}) \times 100\% \quad (1)$$

where $CE_{(NO3)-}$ is the CE based on nitrate removal, Q_{output}, is the total coulombs calculated by integrating the current (Ampere) over time (seconds) and Q_{input}, is the total charge available in the substrate. Q_{input} is defined as the multiplication of the Faraday's constant (F= 96,485 C/mol ē), the volume solution in the cathode (v=0.25 L), the number of electron involved (n=10) and the amount of nitrate removed (mol/L) within time t.

3.3 Results

3.3.1 Microbial community composition

To compare the bacterial composition among samples, a cluster analysis was conducted based on the Bray-Curtis coefficient. As can be observed in Figure 3-2, the three control replicates grouped separately from the other conditions, and the experiment treatment most similar to the control was -130 mV (vs. Ag/AgCl). The difference between groups was also supported by SIMPROF analysis. In addition, a shade plot based on the relative abundance was created to visualize the difference in genus abundance. The most abundant genera

were *Marinobacter*, *Neptunibacter*, *Alteromonas*, *Kordiimonas*, *Roseovarius*, and *Planctomyces*, with total relative abundances of 8.75%, 7.58%, 6.95%, 5.13%, 4.68%, and 4.13%, respectively.

From the group of bacterial genera represented in Figure 3-2, a deeper analysis was conducted to identify all the bacterial genera with facultative potential to carry out denitrification process. Figure 3-3 shows the total relative abundance of genera that were previously associated with the denitrification process per sample. Only the case of -260 mV (vs. Ag/AgCl) showed a significantly higher abundance of denitrifying bacteria compared with those of the other conditions ($P < 0.05$). Table 3-2 shows the relative abundance of each microbial genus reported with the denitrification capacity in each condition with a total abundance of ≥ 1%. In the case of the control and -260 mV (vs. Ag/AgCl) samples, the most abundant genus associated with the denitrification process was *Marinobacter* (9.2% and 15.4%, respectively), and that in the -130 mV and -570 mV (vs. Ag/AgCl) samples was *Planctomyces* (5.2% and 10.8%, respectively).

The relative abundances of selected microbial groups were also determined by quantitative FISH using the probes DEN 124 for denitrifiers, RHOB 1002 and PARA 739 for Rhodobacteria, and PLA 46 and PLA 886 for *Planctomycetes*. The relative biovolumen per condition was similar, and showed no significant differences (Figure S3-1). The FISH images shown in Figure 3-4 are from samples that best represent the results obtained after analyzing each replicate and condition with each probe. The FISH analysis only showed a significantly higher abundance of DEN 124 (denitrifiers) in the case of -260 mV (vs.

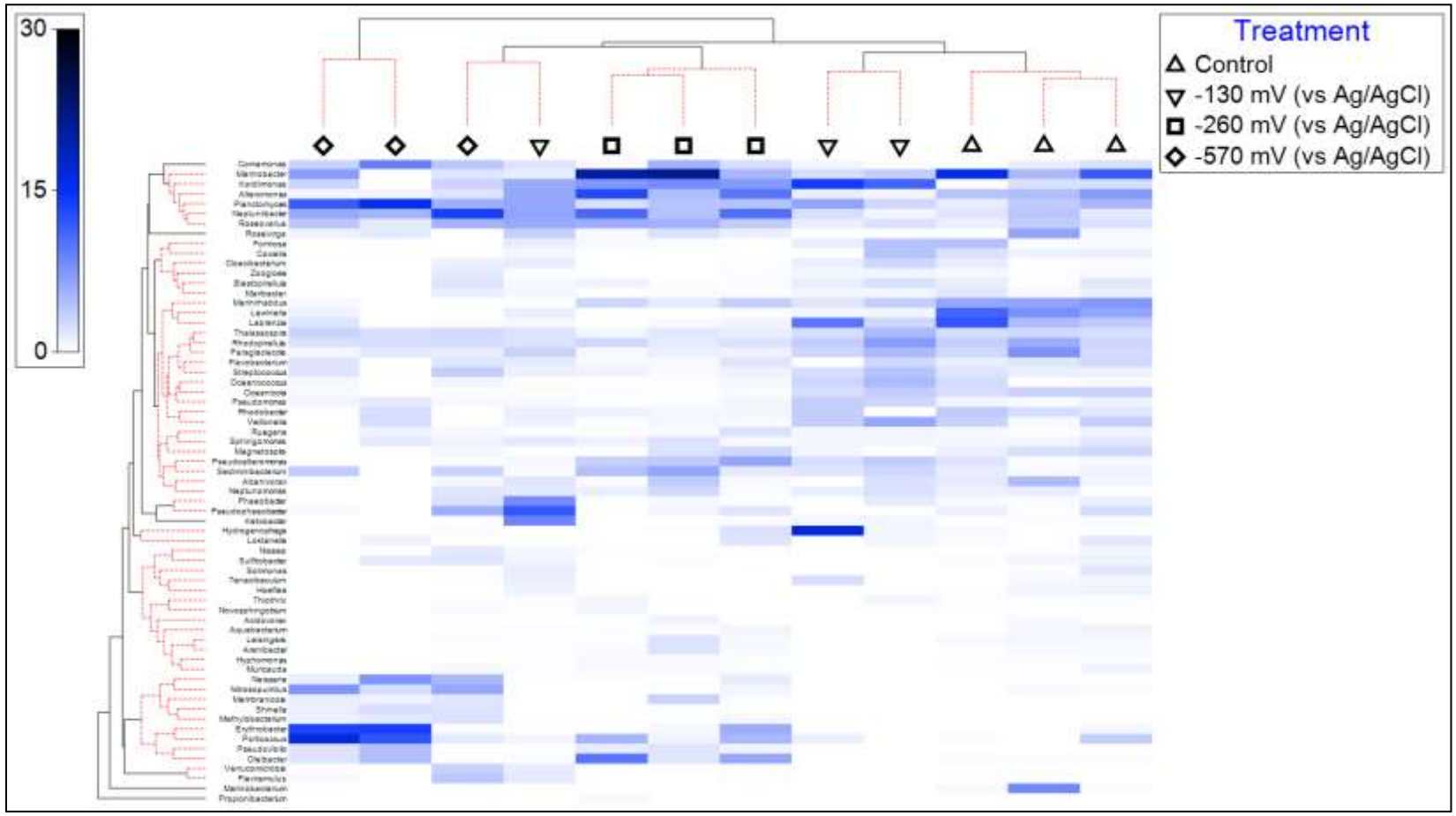

Figure 3-2: Shade plot illustrating the relative abundance of genera in each treatment. The upper dendrogram clusters the samples based on the microbial community composition. On the left, the most abundant genera with an abundance greater than 100 reads were used. Darker shades in each cell of the array represent higher relative densities. White space denotes the absence of the given species.

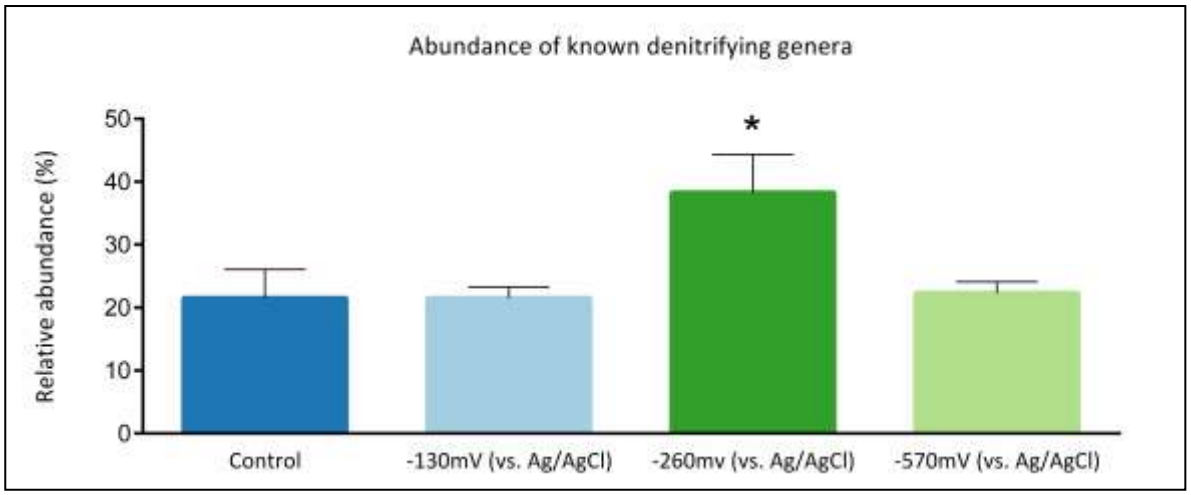

Figure 3-3: Relative abundance (%) of the sum of all reported microbial genera with denitrification capacity in each condition. To determine the significant differences between the treatments, a Tukey *a posteriori* test was conducted. Significant differences between the treatments are indicated with asterisks.

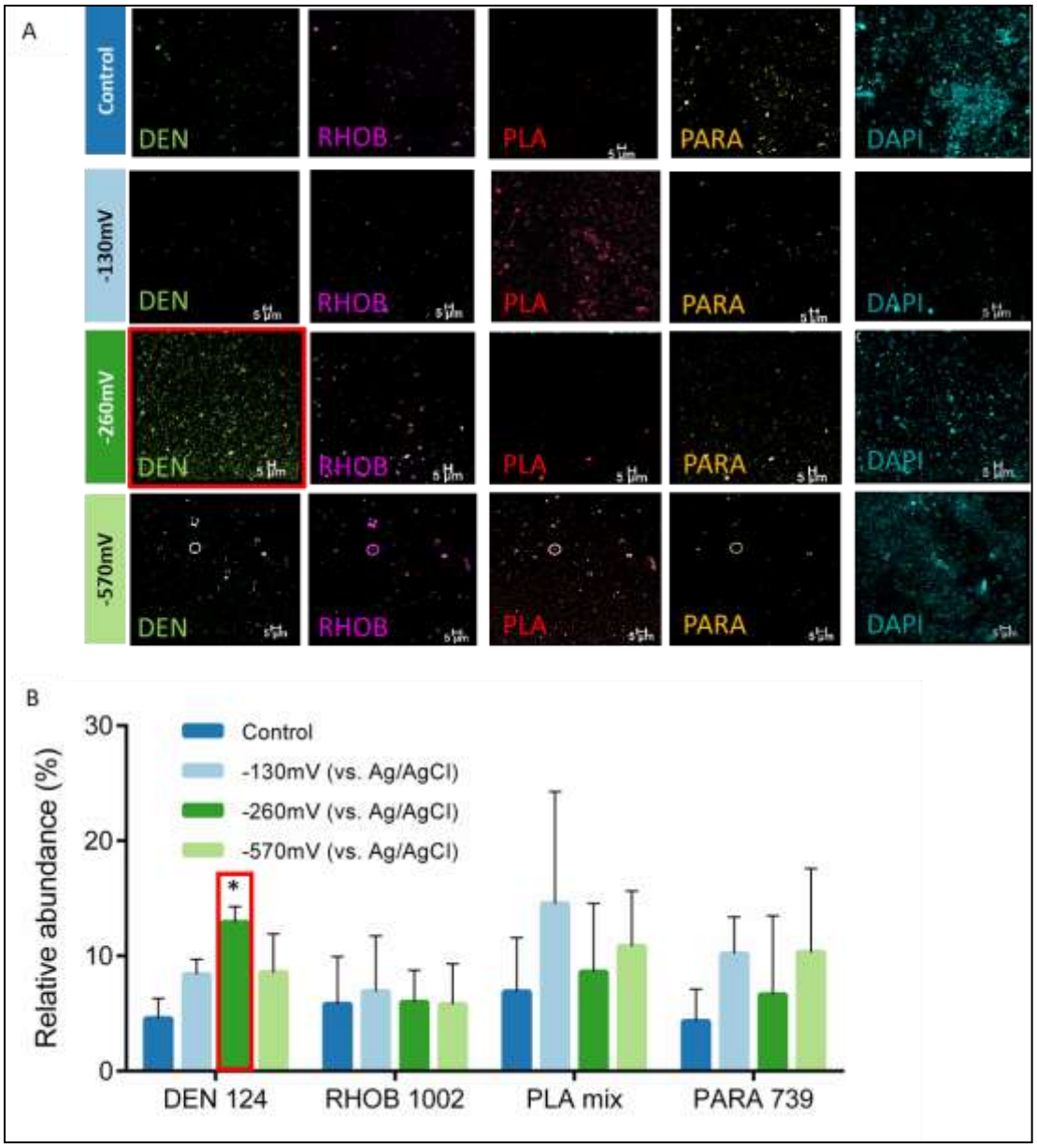

Figure 3-4: Abundance of the different microbial groups determined by quantitative fluorescence in situ hybridization (FISH). The probes used for assignment to the various groups were DEN 124 for denitrifiers, RHOB 1002 and PARA 739 for Rhodobacteria, and PLA 46 and PLA 886 for *Planctomycetes*. (A) Representative FISH images for each condition. (B) Relative abundance (%) of each probe in each treatment. Significant differences between treatments are indicated with a red square.

3.3.2 Electrochemical characterization of the microbial community

To verify that the microbial community present in the working electrodes (acting as biocathodes) catalyzed electron transport from the cathode electrode owing to nitrate bioreduction, CV analyzes were performed. In general, all the peaks observed in the CV analyzes under biotic conditions were reductive, and the CV performed under abiotic conditions showed no peak (Figure 3-5). The registered peaks by CV for all conditions were between -210 mV and -420 mV (vs. Ag/AgCl). The average peaks for each condition were -460 mV (± 98 mV), -125 mV (± 31 mV), -340 mV (± 45 mV), and -263 mV (± 40 mV) (vs. Ag/AgCl) for the control and the -130 mV, -260 mV, and -570 mV (vs. Ag/AgCl) reactors, respectively. Therefore, the presence of reductive peaks (and the absence of oxidative ones) revealed by the CV results suggested that EAB within the formed biofilm, used the poised electrode as external electron donor.

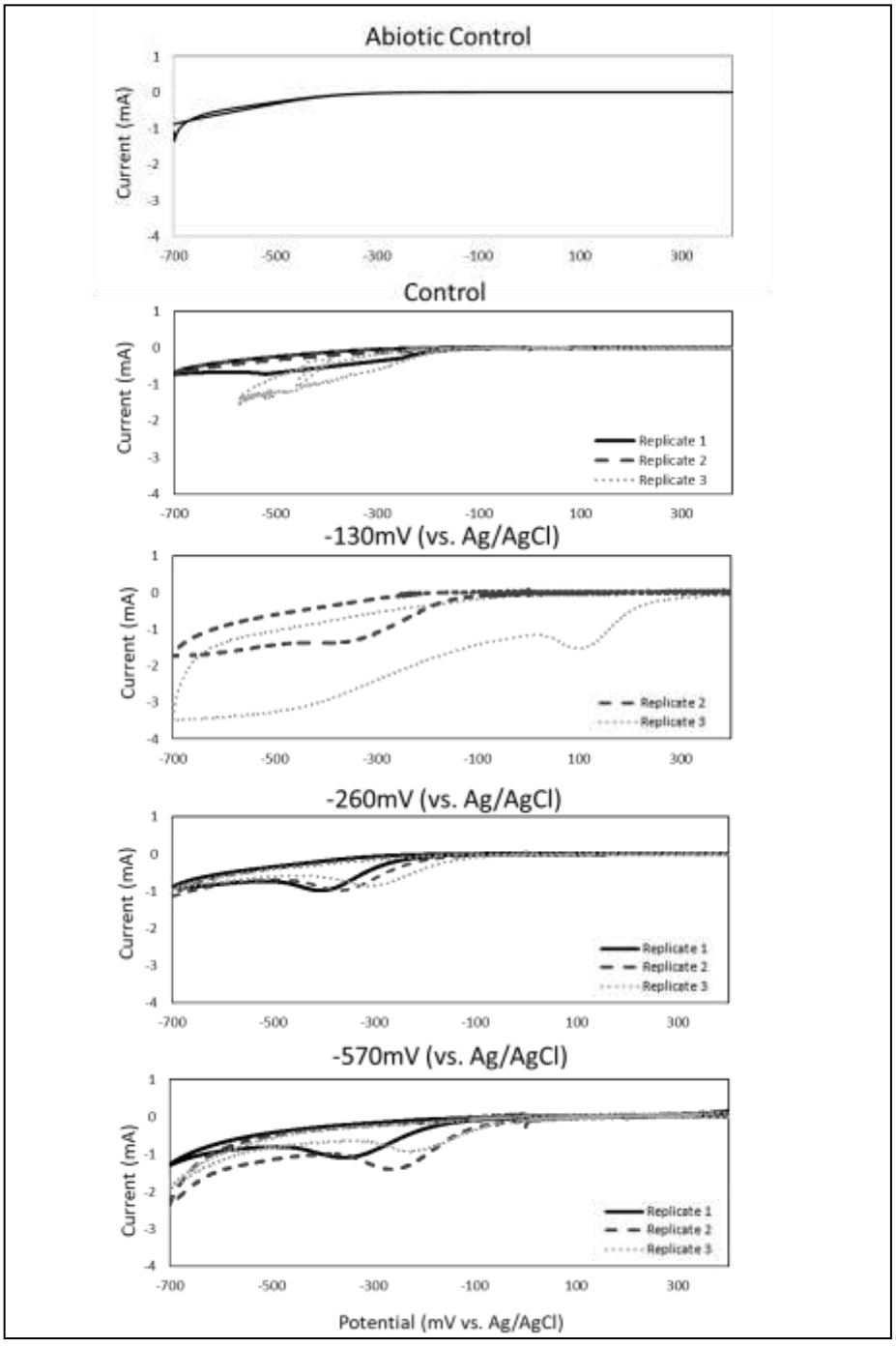

Figure 3-5: Cyclic voltammetry (CV) for working electrodes after four weeks in each condition. Each CV analysis was run with a scan rate of 0.5 mV/s over a voltage range from -700 mV to +500 mV (vs. Ag/AgCl). The different lines in each figure represent three replicates of each treatment.

3.3.3 Nutrient analysis

To determine the nitrate removal, its concentration in each condition was measured throughout the experiment (Figure 3-6A and 3-6B). All the conditions demonstrated a decrease in nitrate concentration in the medium over time from ~2500 µM to ~2000 µM, but in the -260 mV (vs. Ag/AgCl) treatment, this decrease was significantly greater ($P < 0.05$) than that in the other conditions (Figure 3-6A). The difference between the -260 mV (vs. Ag/AgCl) treatment and the other conditions after 30 d of operation is presented in Figure 3-6B, where the percentage of total removal of the -260 mV (vs. Ag/AgCl) treatment was significantly higher ($P < 0.05$) compared with that of the other treatments and the control.

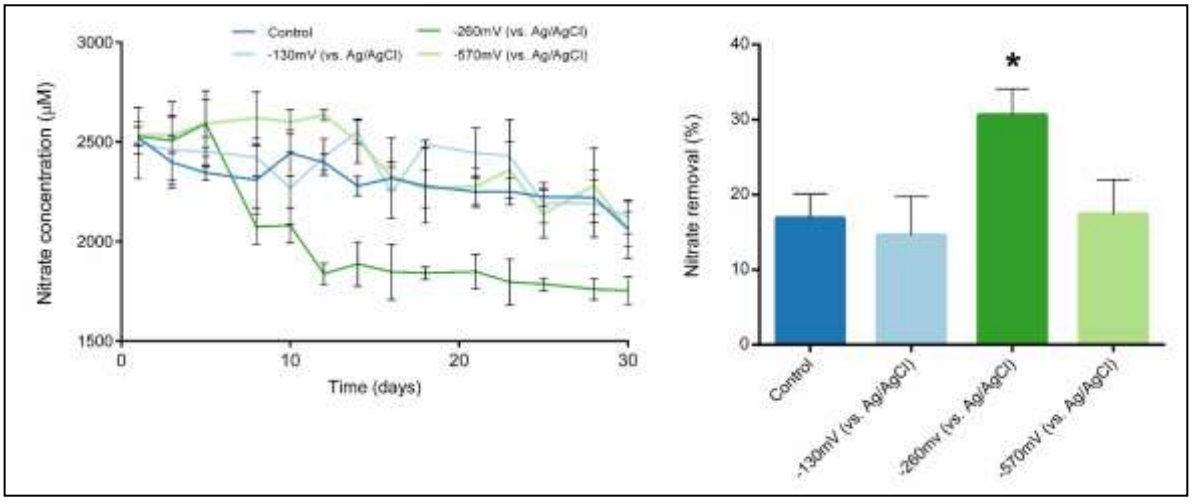

Figure 3-6: (A) Nitrate concentration of each treatment after 30 d. (B) Total nitrate removal (%) after 30 d of operation. To determine the significant differences between the treatments, a Tukey a posteriori test was conducted. Significant differences ($P < 0.05$) between treatments are indicated with asterisks.

3.4 Discussion

The results of this research show for the first time that it is possible to enrich denitrifying microorganisms using electrochemical overpotentials previously reported for the reduction of nitrate and nitrite (Kondaveeti et al., 2014). This finding demonstrates that applying electrochemical overpotentials on electrodes could be used as a bacterial enrichment tool, in this specific case, of denitrifying microorganisms. Even more, the resulted community of denitrifies was enriched from a natural seawater inoculum, avoiding the use of a culture medium or any other enrichment strategy. The results of this research demonstrate that the application of an overpotential produces an effect on the microbial community composition, thereby allowing enrichment of marine denitrifying bacteria from seawater. In general, the six most abundant genera found in the electrodes were associated with denitrifying metabolisms (Enger et al., 1987; Li et al., 2013; H. Wang et al., 2015; Rowe et al., 2015), and approximately 25% of the obtained sequences were associated with bacterial genera with denitrification capacity. However, even though the three electrochemical potentials used in this research were associated with biological nitrate reduction peaks (Kondaveeti et al., 2014), a significantly ($P < 0.05$) greater denitrifying bacterial enrichment was only observed in the case of -260 mV (vs. Ag/AgCl) (Figure 3-3). The same observation was made by FISH analysis, where the highest relative abundance of DEN 124 (denitrifiers) was found in the -260 mV (vs. Ag/AgCl) condition (Figure 3-4).

Regarding the nitrate removal capacity, nitrate analyses over time showed that when -260 mV (vs. Ag/AgCl) was applied to an electrode for one month, nitrate removal was

significantly higher than that in the other conditions (Figure 3-6A and 3-6B). The nitrate removal rate for -260 mV (vs. Ag/AgCl) was calculated 1.5 mg L^{-1} d^{-1} (±0.12). This nitrate removal rate was lower than the values obtained by previous BEDS designed for nutrient removal (from 5 to 500 mg L-1 d-1)(Kelly and He, 2014; Molognoni et al., 2017; Kondaveeti et al., 2019). However, these authors tested bioelectrochemical reactors inoculated with activated sludge and pre-acclimatized for several months using a culture medium. In general, this approach has been commonly used to ensure a high abundance of microorganisms of interest, thereby improving the reactor performance (Rozendal et al., 2010; Fang et al., 2011; Jiang et al., 2016; Ding et al., 2018). Therefore, although the removal rate in the present research was lower, seawater was used for both the inoculum and culture medium. Consequently, our results suggest that denitrifying bacteria can be enriched by selecting an appropriate overpotential, even in environments with low abundance of these microorganisms (Figs. 3 and 6B).

The *Marinobacter* strain isolated from marine sediments has been shown to have denitrification capacity and be electrochemically active with a reductive nitrate peak around -350 mV (vs. Ag/AgCl) (Rowe et al., 2015). In the present research, the electrodes poised with -260 mV (vs. Ag/AgCl) presented a significantly high relative abundance of *Marinobacter (*15.4%) compared with that of other conditions (Table 3-2). Simultaneously, the CV analysis for the -260 mV (vs. Ag/AgCl) treatment showed an average reductive peak equal to -350 mV ± 45 mV (vs. Ag/AgCl) (Figure 3-5). This could indicate that *Marinobacter* is the principal bacterium responsible for the nitrate reduction efficiency when -260 mV (vs. Ag/AgCl) is applied to an electrode inoculated with seawater. The

overpotential application to an electrode could have a crucial effect on the nitrate removal efficiency because in the control reactors, which also presented a high abundance of *Marinobacter* (9.2%), nitrate removal was significantly lower than that in the -260 mV (vs. Ag/AgCl) condition.

Table 3-2: Average relative abundance (%) of all microbial genera with denitrification capacity in each condition with a total abundance of ≥ 1%.

	Relative abundance (%)			
Genus	Control	-130 mV (vs. Ag/Ag/Cl)	-260 mV (vs. Ag/Ag/Cl)	-570 mV (vs. Ag/Ag/Cl)
Marinobacter	9.2 ± 3.60	2.4 ± 1.0	15.4 ± 3.90	3.6 ± 1.6
Alteromonas	3.3 ± 0.20	2.7 ± 3.0	9.1 ± 3.70	1.0 ± 0.9
Planctomyces	2.7 ± 1.60	5.2 ± 0.9	3.6 ± 1.30	10.8 ± 1.9
Pseudoalteromonas	0.9 ± 1.10	2.1 ± 1.5	4.4 ± 1.60	0.0
Thalassospira	1.6 ± 0.10	3.8 ± 0.9	1.1 ± 0.50	2.5 ± 0.5
Rhodobacter	2.0 ± 0.80	1.1 ± 1.1	0.7 ± 0.10	0.7 ± 0.4
Neptunomonas	0.6 ± 0.50	1.9 ± 0.7	1.4 ± 1.30	0.5 ± 0.4
Pseudovibrio	0.00	0.0	1.3 ± 0.60	1.4 ± 1.2
Pseudomonas	0.5 ± 0.30	1.0 ± 1.0	0.2 ± 0.20	0.8 ± 0.5
Ketobacter	0.1 ± 0.09	0.8 ± 0.5	0.00	0.0
Nisaea	0.2 ± 0.10	0.2 ± 0.1	0.1 ± 0.02	0.5 ± 0.3
Ruegeria	0.4 ± 0.20	0.4 ± 0.1	0.9 ± 0.80	0.4 ± 0.3

Planctomyces has been reported in farm aquaculture environments where nitrate concentrations reaches values over 40 mg L^{-1}, which is 70 times higher than the average nitrate concentration in the sea (Sugita et al., 2005; Bissett et al., 2006; Kawahara et al., 2009; Libes, 2009; Lage and Joana, 2012). In this research, we used nutrient concentrations to simulate these environments (farm aquaculture). Therefore, this could explain the high abundance of *Planctomyces* in the reactors. In general, *Planctomycetes* has been associated with the ability to conduct dissimilatory nitrate/nitrite reduction to ammonium (DNRA) (Mohan et al., 2004). Furthermore, it has been reported that DNRA is conducted efficiently in BES when a potential of -500 mV (vs. Ag/AgCl) is applied (Su et al., 2012). This could explain the high abundance of *Planctomyces* (10.8%) and the significant microbial enrichment of this genus in the samples of -570 mV (vs. Ag/AgCl) (Table 3-2). To determine whether applying -570 mV (vs. Ag/AgCl) to an electrode can significantly enhance DNRA, it will be necessary to conduct ammonium measurements to determine the efficiency of nitrate reduction to ammonium.

Since the objective of this study was the electrochemical selection of marine denitrifying microorganisms, the reactor configuration, removal and CE could be improved. Indeed, the CE calculated for all the tested overpotentials was lower than 1%. The low CE values could be associated to the high internal resistance of the system. Using similar three-electrode reactors and a volumetric biocathode configuration, Torres-Rojas et al. obtained similar low values of CE for chlorate bioelectrochemical reduction (Torres-rojas et al., 2020). To obtain higher CEs, several improvements in the reactor design and operation should be accomplished.

Finally, the results of this study show enrichment of electroactive denitrifying microorganisms when -260 mV vs Ag/AgCl was applied. In recent studies by Jia et al. (2021) and Ramos et al. (2017), the authors identified a potential range between -500 mV and -170 mV (vs. Ag/AgCl) where electron shuttles (e.g., Riboflavin) and outer membrane C-type cytochromes are activated in denitrifying microorganisms. Similar conceptual models have been proposed for other electron acceptors like chlorate and perchlorate (Torres-rojas et al., 2020). Therefore, the applied potential of -260 mV (vs. Ag/AgCl) could activate the electron transfer mechanisms of denitrifying microorganisms, favouring their enrichment in an electrode. Further research is required to find the optimal electrochemical overpotential, probably in the vicinity of the tested -260 mV (vs Ag/AgCl). This will lead us to optimize the enrichment of denitrifying microorganisms and the subsequent enhance of nitrate metabolization in a BEDS.

3.5 Conclusions

In this research, enrichment of denitrifying bacteria from seawater was conducted by applying -260 mV (vs. Ag/AgCl) to an electrode without the need for the addition of organic matter as an external electron donor. Since only reductive peaks were observed by the CV analysis, the polarized electrodes at different overpotentials could had work as external electron donors for nitrate reduction. These results are the starting point to begin testing this enrichment technique for other types of microbial metabolisms of interest.

Finally, this work not only represents the first successful attempt to electrochemically enrich marine denitrifying microorganisms, but also presents a technique to accelerate the

start-up process of BEDS, thereby avoiding the use of culture media and an exogenous inoculum. This strategy could help to improve the scaling up of BEDS designed to operate in complex environments such as the sea.

3.6. Acknowledgements

This work was supported by FONDECYT projects 11201134 and 1181326, the CEDEUS center CONICYT/FONDAP/15110020 and the Vicerrectoría de Investigación of Pontificia Universidad Católica de Chile. This study was conducted at the Marine Energy Research & Innovation Center (MERIC) (Project CORFO 14CEI2-28228). We give special thanks to Leslie K. Daille, Mauricio Medel, Francisca Rubio, Karen Yanira Sanzana and Evelyn Cruz for their assistance in the laboratory and help with the experimental setup.

4. ENHANCE OF NITROGEN AND CARBÓN REMOVAL IN NATURAL SEAWATER BY ELECTROCHEMICAL ENRICHMENT IN A BIOELECTROCHEMICAL REACTOR

To be submitted to *Journal of Environmental Chemical Engineering*

4.1. Introduction

Coastal systems have long been used for waste disposal, such as industrial and agricultural by-products, tourism, submarine tailings, placements and pipes, and salmon farming (Nixon, 1995). These constant nutrient inputs into coastal systems have led to a nutrient unbalance , resulting in eutrophication (Ansari et al., 2010; Smith, 2003). Nitrogen is one of the main factors in water body eutrophication and reaches systems from different anthropogenic sources (Smith, 2003). Among those sources, fish farming and urban wastewater discharge zones have shown concentrations from 12.5 to 40.7 mg/L nitrate and from 5.3 to 30 mg/L ammonium (Bahroun et al., 2016; Guldhe et al., 2017; Lananan et al., 2014; Orhon et al., 1997). These concentrations are 20 to 70 times and 40 to 3000 times higher than the average nitrate and ammonium concentrations observed in the coastal sea, respectively (Libes, 2009; Quinn et al., 1988).

Nitrogen removal in recirculating aquaculture systems is conventionally performed by physicochemical and biological treatment technologies (Mook et al., 2012). However, these technologies still have problems to be solved, such as toxic by-product generation and excessive sludge production (Bhatnagar and Sillanpää, 2011; Kapoor and Viraraghavan, 1997; Park and Yoo, 2009). In this context, microbial electrochemical

technologies (METs) have gained attention due to their cost/efficiency and capability of removing different pollutants of interest (Mook et al., 2012).

Several METs capable of removing dissolved nitrogen and carbon simultaneously have been developed (Ryu et al., 2013; Sun et al., 2016; Virdis et al., 2010; Xie et al., 2011; Zhang et al., 2013; Zhu et al., 2013). In general, these reactors have been inoculated with samples taken from an activated sludge system, reactors operating for several months, or from previously enriched microbial consortia. The use of these inocula ensures a high enough abundance of nitrifying and denitrifying microorganisms for acceptable reactor efficiency. However, the marine environment is characterized by high physicochemical and biological complexity, which makes it impossible to ensure a high abundance of nitrifying and denitrifying microorganisms (Sunagawa et al., 2015). This represents a considerable challenge to develop METs capable of the simultaneous removal of nitrogen and carbon from polluted marine environments.

Techniques involving the application of overpotentials to an electrode are useful in modelling the microbial community composition (Miceli et al., 2012; Rowe et al., 2015; Torres et al., 2009; Wang et al., 2015). From this, repowered electrochemical potentials were identified for the microbial denitrification process. Through this analysis, denitrifying microbial enrichments were achieved by applying -260 mV (vs. Ag/AgCl) to the electrode (Kondaveeti et al., 2014; Pous et al., 2020). Under this framework, in this study, we were able to (1) develop a methodology to generate an enrichment of nitrifying and denitrifying microorganisms without the need for culture media, (2) develop a bioelectrochemical reactor (BER) capable of significant dissolved inorganic nitrogen and organic matter

removal from natural seawater influent, and (3) propose a strategy for accelerating the reactor start-up process. To ensure the presence of electrochemically active microorganisms (EAMs) in the anode and denitrifying microorganisms in the cathode, the reactor electrodes were polarized. The current, nitrogen, and carbon were measured throughout the experiment to determine the reactor's performance. At the end of the experiment, the microbial communities present on the electrodes of each reactor were characterized by fluorescence in situ hybridization (FISH), microscopy, and next-generation sequencing (NGS).

4.2 Materials and Methods

4.2.1 Reactor configuration

The experiment was conducted using two-chamber BERs (Figure 4-1) with anodic and cathodic compartments with volumes of 252 and 504 cm^3, respectively. Each compartment had four graphite plates (Impervious Bipolar Graphite Plate, Fuel Cell Store) that form a sinusoidal path in the flow of water to increase the BER hydraulic retention time (6.5 h) (Figure 4-1B). The anode and cathode graphite plate volumes were 4.5 and 9 cm^3, respectively. Each graphite plate was united to a platinum wire and used to connect the electrode to the 1000TM multichannel potentiostat (GAMRY, PA, USA) (Figure 4-1B and 4-1C). Both compartments were filled with irregular sieved graphite granules (2–12 mm diameter) (Asbury Carbons, Sunbury, Pennsylvania), leaving effective volumes of 95.76 cm^3 and 191.52 cm^3 for the anodic and cathodic chambers, respectively. An ion-exchange membrane (Nafion™ 212, FuelCell Store, USA) was used to separate the compartments.

The reference electrode, an Ag/AgCl electrode (3 M KCl) (CHI111, CH Instruments Inc.), was installed in both compartments. Poised potential BERs and control (open circuit) reactors were operated in triplicate.

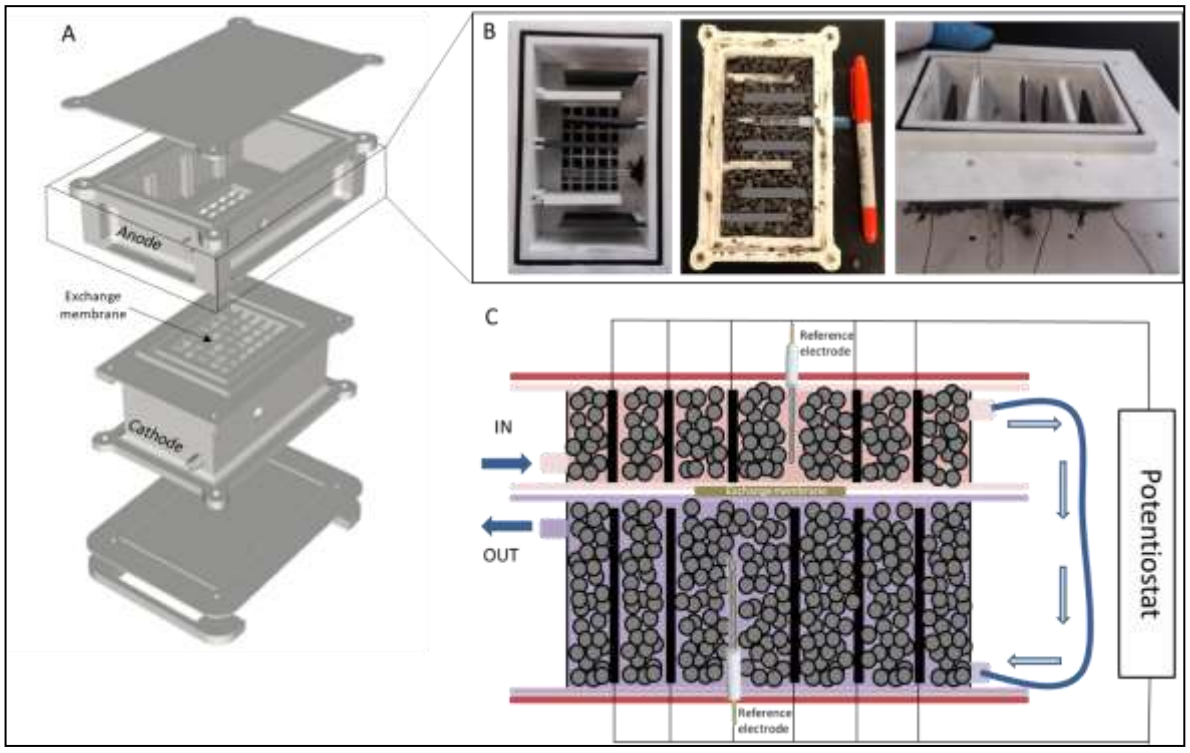

Figure 4-1. Experimental design. (A) 3D image of the complete reactor, indicating the cathode, anodic compartment, and place where the exchange membrane was installed. (B) Illustration of the internal conformation of each compartment, the arrangement of the graphite plates, the reference electrode, and the graphite granules. (C) Illustration of the operating reactor connected to the potentiostat through the graphite plates. The water flow through the reactor is also visualized.

4.2.2. System operation, inoculation, and enrichment

The six BERs were operated for 37 days under flow-recirculation mode, and throughout the experiment, the same flow was maintained (2 mL min^{-1}) using a peristaltic pump. The inoculum and medium for all reactors were natural coastal seawater collected from the Estación Costera de Investigaciones Marinas (ECIM) of the Pontificia Universidad Católica de Chile, located in the eastern South Pacific Ocean, 33° 30’16 “S; 71° 38’23 "W. The seawater was supplied with ammonium and acetate in the form of sodium acetate and ammonium chloride at concentrations of 160 mg/L NH_4Cl and 744 mg/L $NaC_2H_3O_2$, simulating the concentrations that have been reported for wastewater (Abessa et al., 2005). The six reactors were operated in four phases. Phase I had a cycle of seven days, phase II consisted of four cycles of five days (5 days/cycle), phase III and IV had just one cycle of five days. Between each phase and cycle (in the case of phase II), fresh natural seawater and nutrients were renewed. The control reactors were kept under the same conditions as the treatment reactors but remained in the open circuit throughout the experiment without applying any potential.

In phase I, to ensure the presence of EAM in the anode, a negative overpotential was applied to the anode of the BERs (Miceli et al., 2012). This potential had to be more negative than that applied to the cathode to ensure the operation of the electrode as an anode. Therefore, -500 mV (Ag/AgCl) was applied to the anode. To ensure significant colonization of microorganisms on the electrodes, the six reactors were operated with continuous closed flow connected to a 60-L tank of fresh natural seawater. Only in phase I was the anode polarized; subsequently, the polarization was focused on the cathode.

In phase II, to ensure a significant abundance of denitrifying microorganisms in the cathode, -260 mV (vs. Ag/AgCl) was applied to the cathodes of the three treatment reactors. Only the natural-fresh seawater volume necessary to keep the reactor full (1 L) with a continuous closed flow was utilized in this and the next phases. This phase lasted four cycles of five days (20 days).

In phase III, to determine the reactor's performance when it was connected to a resistance, the treatment reactors were connected to a 1 kΩ resistor for one cycle of five days. Finally, phase IV consisted of repeating phase II, but this time for five days. The control reactors were kept under the same conditions as the treatment reactors but remained in the open circuit throughout the experiment without applying any potential.

To maintain the overpotentials applied to the treatment reactors in each phase, an Interface 1000TM Multichannel potentiostat (GAMRY, PA, USA) was used. In phase three, the current generated by the treatment reactors was measured using a Multimeter 2700 (Model 2700, Keithley Instruments, USA). The maximum current density reported in each phase was normalized by the effective volumes of the anode and cathode electrode using the following equation (modified from Oon et al., 2016):

$$J = I / vV \qquad (1)$$

where J is the normalized current density (A m^{-3}), I is the maximum current measured in each phase, v is the porosity (0.38 for graphite) (Doherty et al., 2015), and V is the total reactor volume (m^3) (i.e., the empty bed volume).

4.2.3 Nutrient analysis

The variation in nutrients and their removal rate were obtained by measuring ammonium, nitrate, nitrite, and total organic carbon (TOC) during the experiment, following the definition reported by (Kondaveeti et al., 2014; Yan et al., 2012; Yu et al., 2010). These measurements were performed before and after each phase and cycle (in phase II, four cycles of 5 days) by taking 15 mL from each reactor and filtering through a 0.2-µm membrane filter to eliminate microorganisms. Samples were kept in in 15 ml falcon tubes at -20°C, until measurement.

The ammonium concentration was measured using the protocol described by Holmes et al., (1999). Nitrate and nitrite concentrations were measured using standard methods (Koroleff, 1999) with a Seal AutoAnalyser (Seal Analytical AA3, Wisconsin, USA). The calibration curve was performed between 0.5 to 50 µM. Total organic carbon (TOC) was measured using a TOC-L analyser (Shimadzu, Kyoto, Japan) with a non-purgeable organic carbon (NPOC) approach.

For each cycle, the removal rates for NH_4^+-N and TOC were calculated by the difference of the initial and final concentration of each cycle, divided by its days of duration. The removal efficiency of each reactor was obtained by transforming the total NH_4^+-N and TOC removed by each reactor into a percentage. Similarities between samples were statistically analysed through Sidak's multi comparisons test using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

4.2.4 Microbial community analysis

At the end of the experiment, graphite granules were used for DNA extraction, and then the microbial community was characterized by tag sequencing. Graphite plates were used to characterize the microbial community using the FISH microscopy technique.

4.2.4.1 DNA extraction

At the end of the experiment, graphite granules from each reactor were removed and sonicated in 150 mL of sterile seawater for 5 min (Elmasonic S 30H, Elma Schmidbauer GmbH, Germany). Sonicated products were filtered through a 0.2 μm membrane filter to collect the biomass and to perform the subsequent DNA extraction with a phenol:chloroform-based protocol (Fuhrman et al., 1988). DNA quantification was performed with a Qubit® 2.0 Fluorometer (InvitrogenTM, Life Technologies, CA, USA) according to the manufacturer's protocol. To corroborate the DNA integrity, the 260/280 and 260/230 ratios were measured using NanoDrop® ND-1000 spectrophotometers (Thermo Fisher Scientific, Massachusetts, USA).

4.2.4.2 Amplicon analysis of 16 rRNA gene sequences

To obtain a taxonomic analysis of the microbial community established in each reactor, amplicon analysis (AA) using next-generation sequencing (NGS) of the V4 hypervariable region of the 16S rRNA gene was performed with the Illumina MiSeq platform (Integrated Microbiome Resource (IMR), Canada). The sequences obtained in the present study are

publicly available in the Sequence Read Archive database under the accession number PRJNA666469.

Sequences obtained were analysed by the DADA2 tool and compiled into amplicon sequence variants (ASVs), allowing maximal lengths of 240 and 200 for forward and reverse reads, respectively. Additionally, non-ambiguities were permitted, and the maximal number of expected errors was 2. The resulting sequences were taxonomically assigned with the SILVA 16S database version 132. The biological matrix was filtered with a minimum amount of reads per sample of 5000. ASVs were filtered by abundance, fixing a minimum of 20 reads in at least one sample, leading to a final biological matrix with 14 samples with 1727 ASVs in the total dataset. The biological matrix was transformed into a binary matrix representing the presence/absence categories of all ASVs in the dataset. Taxa that showed a differential preference for each experimental condition were selected, and the most abundant ASV per taxon was used for dendrograms and sequence comparisons. Venn diagrams and ASV computations were performed in R environment version 3.6.1 (https://www.r-project.org/), and dendrograms, alignments, and sequence analysis were performed in MEGA-X software (https://www.megasoftware.net/) using the MUSCLE alignment algorithm with 1000 iterations. The neighbour-joining algorithm was run with 1000 bootstrapping iterations with uniform rates among sites.

4.2.4.3 FISH microscopy analysis

To determine the microbial community composition present in the graphite plate electrode, each cathode and anode per condition was analysed by rRNA-targeted FISH and

fluorescence microscopy. Three graphite plate electrodes of each condition, from the anode and cathode, were fixed in a 2% (v/v) formaldehyde solution for one week at 4°C. Fixed samples were then washed in 1x PBS, resuspended in a 1:1 mixture of 1x PBS and 96% (v/v) ethanol (Daims et al., 2005), and stored at -20°C. Before performing the hybridization, the samples were sonicated in 50 mL of MQ water for 3 min (Elmasonic S 30H, Elma Schmidbauer GmbH, Alemania). Sonicated samples were filtered through a 0.2 µm membrane filter to collect the cells, and FISH was performed directly on the filters. With this procedure, a total of 36 filters were analyzed.

The rRNA-targeted DNA oligonucleotide probes used in this study are listed in Table 4-1. Probes were monolabelled at their 5'-ends with fluorophores as listed in Table 4-1. FISH was performed at 46°C according to a standard protocol for rRNA-targeted FISH (Manz et al., 1992; Daims et al.,2005), where the hybridizations were performed for two hours. After the hybridizations, each sample was stained with DAPI to visualize all microbial cells. The samples were hybridized to a mixture of different probes. Nsm156, Nsm443, and NEU (all labelled with FLUOS) to detect ammonia-oxidizing bacteria (AOB). Ntspa662, Ntspa712, and Ntspa1151 (all labelled with Cy3) to detect nitrite oxidizing bacteria. Pla46 and Pla886 to detect *Planctomycetes* (both labelled with Cy3). DEN124 to identify Acetate-utilizing denitrifiers (labelled with Atto 594). RHOB 1002 to detect Rhobacteria (labelled with Atto 633) (Table 4-1).

Table 4-1: Ribosomal RNA-targeted oligonucleotide probes used in this study.

Probe	Fluorophore	Target Organisms	Probe sequence (5'-3')	FA[c] (%)	Reference
DEN 124	Atto 594	Acetate-utilizing denitrifiers	CGA CAT GGG CGC GTT CCG AT	35	Ginige et al., 2005
RHOB 1002	Atto 633	Rhodobacteria	ACC ATC TCT GGA ACC GCG	35	Figuerola & Erijman, 2007
S-G-Ntspa-662-a-A-18 (Ntspa 662)	Cy 5	Genus *Nitrospira*	GGA ATT CCG CGC TCC TCT	35	Daims et al., 2001
S-*-Ntspa-712-a-A-21 (Ntspa 712)	Cy 5	Most members of the phylum Nitrospirae	CGC CTT CGC CAC CGG CCT TCC	35	Daims et al., 2001
S-*-Ntspa-1151-a-A-20 (Ntspa 1151)	Cy 5	Sublineage II of the genus *Nitrospira*	TTC TCC TGG GCA GTC TCT CC	35	Maixner et al., 2006
S-*-Nsm-0651-a-A-18 (NEU)	FLUOS.	Most halophilic and halotolerant *Nitrosomonas* spp.	CCC CTC TGC TGC ACT CTA	35	Wagner et al., 1994
S-G-Nsm-0155-a-A-19 (Nsm 156)	FLUOS	*Nitrosomonas* spp., *Nitrosococcus mobilis*	TAT TAG CAC ATC TTT CGA T	35	Mobarry et al., 1996
pB-250 (Nsv 443)	FLUOS	*Nitrosospira* spp.	CCG TGA CCG TTT CGT TCC G	35	Mobarry et al., 1996
PLA 46	Cy 3	*Planctomycetes*	GAC TTG CAT GCC TAA TCC	35	Neef et al., 1998

FISH images were acquired with an inverted Leica TCS SP8X confocal laser scanning microscope (CLSM) equipped with a 405-nm UV diode, a Leica supercontinuum white-light laser, two photomultiplier (PMT) detectors, three hybrid (HyD) detectors, and the Leica Application Suite AF 3.2.1.9702. Three photos were taken at random positions over each filter (36 filters x 3 pictures; 108 photos in total) for further analysis. To obtain the abundance data of the different microbial groups hybridized with each probe in each sample, FISH images were analysed using the Leica Application Suite X and DAIME version 2.1 (Daims et al., 2006).

The abundance of microorganisms hybridized with each probe was standardized by the total counts of DAPI-stained microbial cells. These results were subjected to multivariate analysis using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

4.3 Results

4.3.1 Reactor performance

To determine nitrogen as ammonium and TOC removal throughout the experiment, their removal rates in each cycle were measured (Figure 4-2 and Figure 4-S2). In the case of NH_4^+-N, the removal rate of the treatment condition proved to be significantly higher than that of the control in all phases of the experiment ($P < 0.05$). The highest removal rate was 7.5 mg NH_4^+-N L^{-1} day^{-1} and was observed in cycle 7 (Phase IV) of the experiment when -260 mV (vs. Ag/AgCl) was applied again to the cathode (Figure 4-2A). In the case of TOC, a significantly ($P < 0.05$) higher removal rate of polarized reactors in comparison with control reactors was observed in cycles 5, 6, and 7 (Figure 4-2B). The highest TOC removal rate was 99.52 mg L^{-1} day^{-1}, which was found in cycle 7, when -260 mV (vs. Ag/AgCl) was reapplied to the cathode. The percentages of total removal of NH_4^+-N and TOC were 96.1% and 68.7%, respectively (Table S4-2). The specific removal rate value in each phase and cycle for NH_4^+-N and TOC and the affluent and effluent concentrations of each phase and stage are provided in Tables S4-1 and S4-2, respectively.

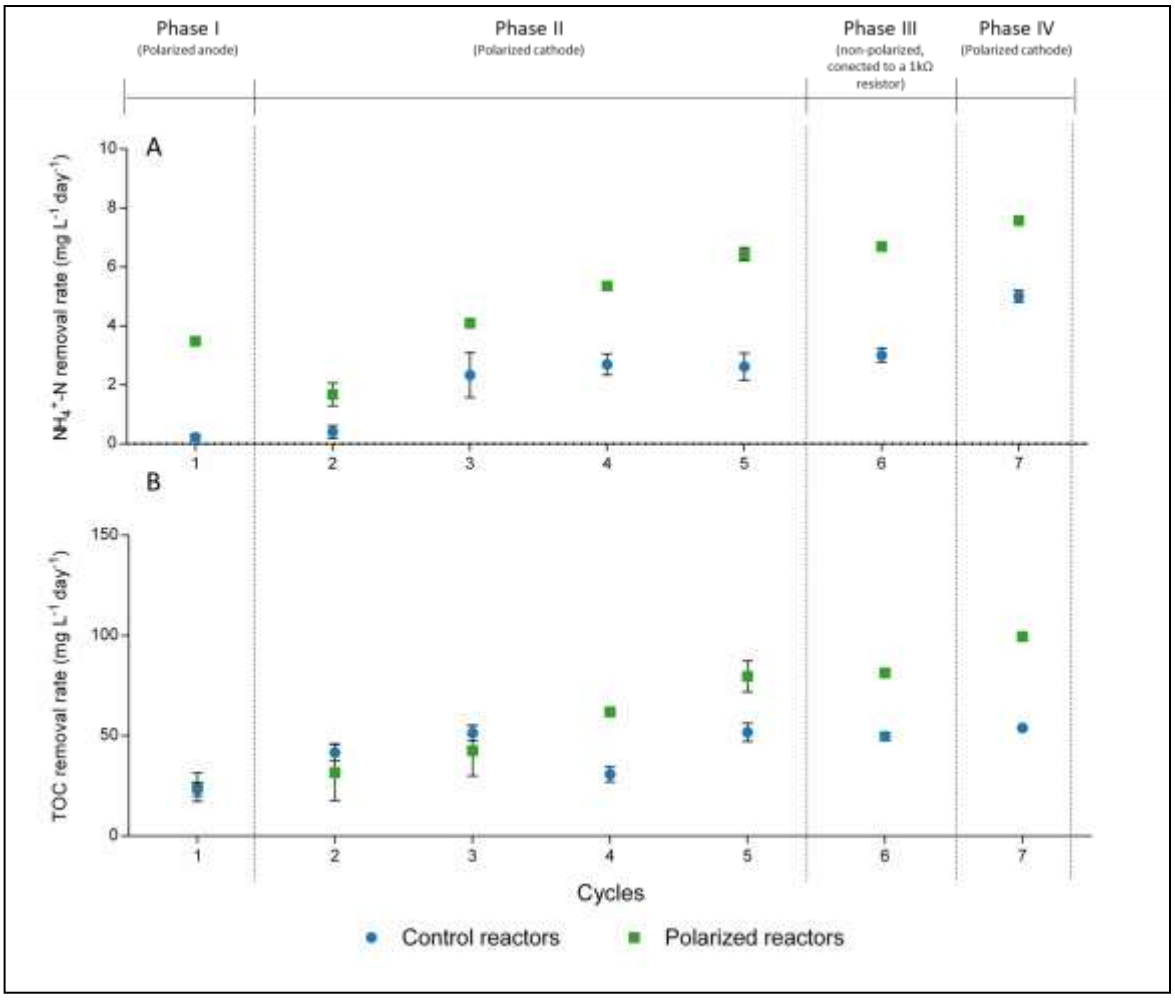

Figure 4-2: Removal rate (mg L^{-1} day^{-1}) of ammonium (A) and total organic carbon, TOC (B), recorded for control reactors (dark circles) and polarized reactors (grey circles) during each phase of the experiment. The error bars represent the standard deviation of the average of three replicates of each condition.

Throughout the experiment, a difference in NH_4^+-N and TOC removal between each condition was observed. The removal differences between the control and polarized reactors stabilized after the third cycle. At this point, the polarized reactors removed more than 1.5 times the nitrogen and carbon than the controls (Figure 4-2). The variations in the concentrations of NO_3^--N and NO_2^--N concentration at the beginning and end of each cycle

and phase were also measured (Table S4-2). As shown in Table S4-2, these concentrations in the polarized reactors' effluents were significantly lower than those in the control reactors.

In the case of current generation, the maximum current density during each phase was determined (Table 4-2). As shown in Table 2, reactor 4 (R4) had the highest current during the experimental stages. On average, among the three reactors, maximum current densities of 5.8 A m^{-3} (± 1.1), 9.3 A m^{-3} (± 2.8), 0.4 A m^{-3} (± 0.1) and 7.0 A m^{-3} (± 3.0) were achieved for phases I, II, III and IV, respectively.

Table 4-2: Maximum current density (A m^{-3}) reported for each polarized reactor during each phase of the experiment.

Phases	Duration (Days)	Maximum current density (A m^{-3}) in polarized reactor		
		Replicate 1	Replicate 2	Replicate 3
I (polarized anode at -500 mV vs. Ag/AgCl)	7	7.0	5.5	4.9
II (polarized cathode at -260 mV vs. Ag/AgCl)	20	11.6	10.2	6.2
III (cell connected to a 1kΩ resistor)	5	0.9	0.2	0.1
IV (polarized cathode at -260 mV vs. Ag/AgCl)	5	10.1	8.5	2.5

4.3.2 Microbial community and functional characterization

To determine differences in the composition of the microbial community present in each condition, an observed frequency analysis of microbial taxa was performed (Figure 4-3A). In the case of unique ASV detection in polarized reactors, identification frequencies of 100% and 83% were observed for the genera Halomonas and Desulfovibrio, respectively.

In the control condition, identification frequencies of 100% and 90% were found for the genera Thiomicrospira and Desulfoconvexum, respectively (Figure 4-3A). Furthermore, for shared ASVs among experimental conditions, the genus Halomonas showed a higher detection rate in the treatment condition compared to the control. For the control condition, the microbial genera Thiomicrospira, Anaerofustis, Dethiosulfatibacter, Desulfoconvexum, and Desulfobacter were detected at frequencies of 78.9%, 81.8%, 81.8%, 81.8% and 87.1%, respectively.

To evaluate whether the detected ASVs under these experimental conditions were similar with previous data, a phylogenetic tree based on ASV sequences was constructed (Figure 4-3B) (Kondaveeti et al., 2014; Rowe et al., 2015). Previously reported sequences were included from Rowe et al., 2015 (Figure 4-3B, identified with an *) and Kondaveeti et al., 2014 (Figure 4-3B, identified with an Ł), and dominant genera were used. Similar dendrogram topology was found in this comparison, and genera such as Halomonas showed high bootstrapping values in nodes related to referential sequences.

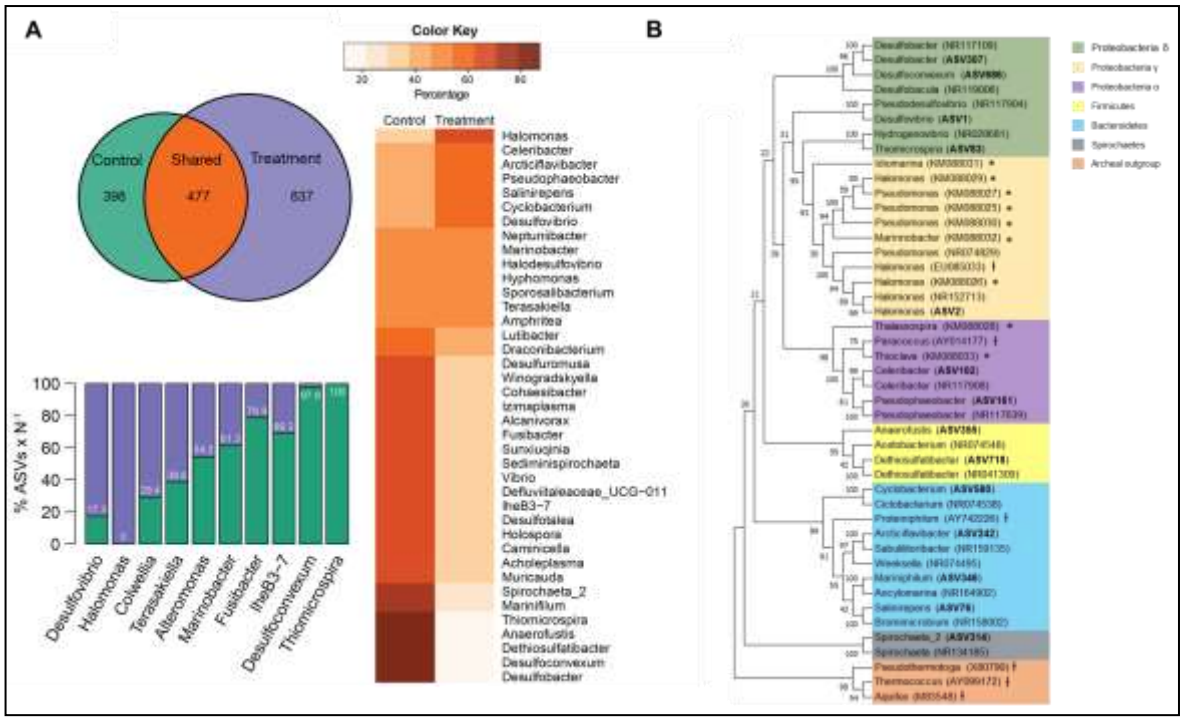

Figure 4-3: Bacterial Community Analysis. (A) Analysis of the frequency of bacterial taxa detection within the experimental setup. Upper left, Venn diagram with total ASVs detected that were exclusive or shared in absolute numbers. Bottom left, the number of ASVs detected per taxonomic category normalized by sampling size in each experimental condition. The top 10 taxa were plotted and sorted by frequencies in the R4-6 condition. Right panel, shared ASVs detected and taxonomically classified by the SILVA database among experimental conditions, with percentages with respect to all detections. (B) Neighbour-joining computed phylogenetic tree that includes taxa identified in this study and in similar studies. Sequence IDs in GenBank are shown as well as genera as taxonomic classifications, and major taxonomic categories are shown in colours. Previously described taxa by Rowe et al., 2015 (*) and Kondaveeti et al., 2014 (Ⱨ) were included.

To determine the microbial enrichment of functional groups associated with the nitrogen cycle in the anodes and cathodes of each reactor, FISH microscopy analysis was performed. Representative FISH images of the obtained results are shown in Figure 4-4. Nonsignificant differences ($P > 0.05$) were detected in the total number of cells per condition (Figure S1). In the case of the community identified by FISH in the anodes of each reactor, the poised reactors showed a significantly higher ($P < 0.05$) NOB abundance than the control reactors (unpoised) (Figure 4-4A). Conversely, the cathode FISH image analysis of each condition showed significant differences in all probe abundances except for RHOB (AOB, DEN, PLA, and NOB; $P < 0.05$; Figure 4-4B). Polarized reactors showed a significantly higher abundance of microorganisms belonging to the denitrifiers and *Planctomycetes* (DEN and PLA, respectively; Figure 4-4B). In the nonpolarized control reactors, a significantly higher abundance of AOB and NOB probes was observed in their cathodes (Figure 4-4B).

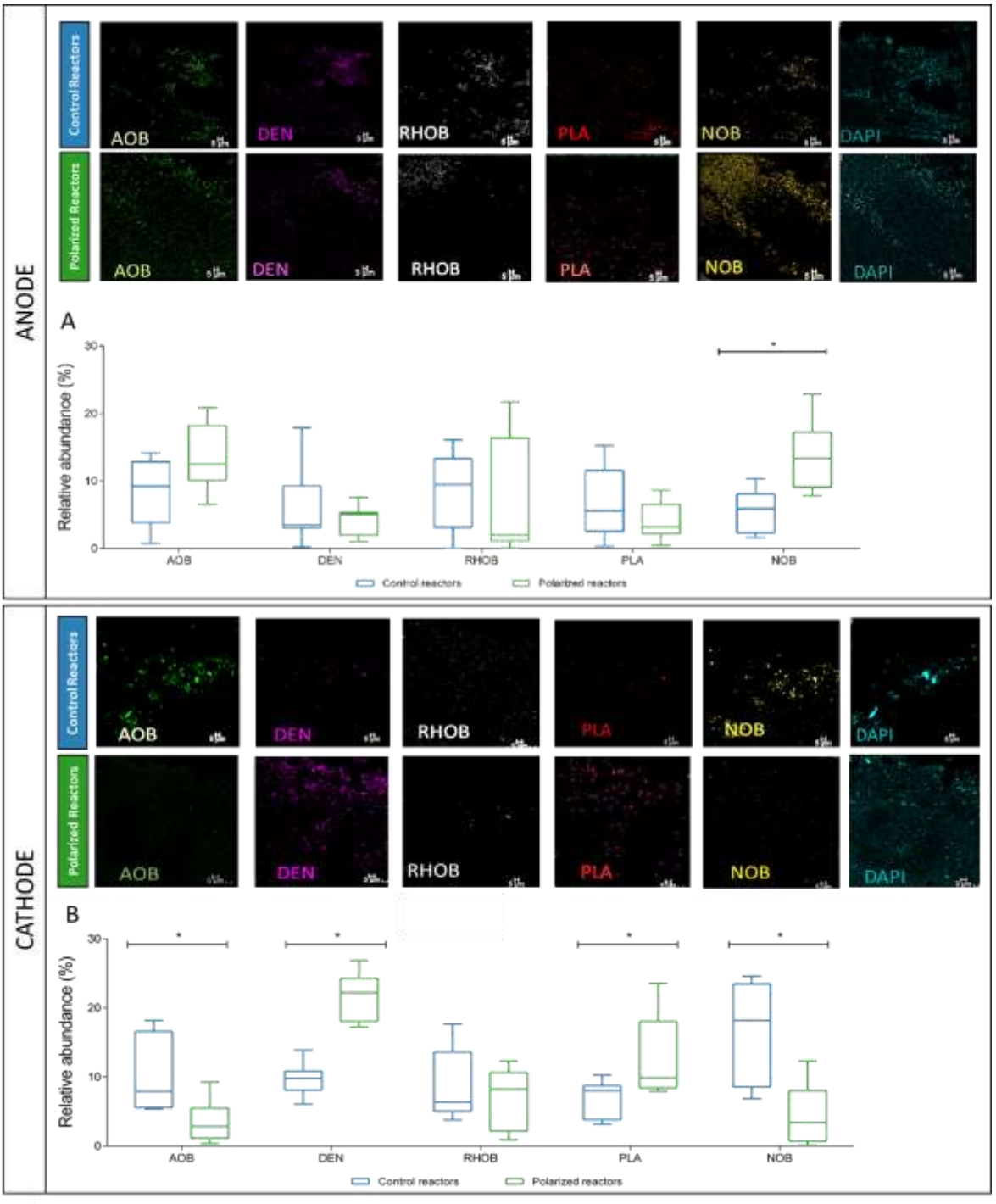

Figure 4-4: Relative abundance (%) of the groups of microorganisms hybridized with each probe in the anodes (A) and cathodes (B) of the control (blue boxes) and polarized reactors (green boxes). The applied FISH probes and their target organisms are listed in Table 4-1.

Significant differences between treatments, according to a Tukey *a posteriori* test, are indicated with asterisks. The displayed FISH images are representative for the FISH results in each condition.

4.4 Discussion

In this work, it was possible to combine the existing knowledge of microbial enrichment by applying overpotential and the development of reactors for the simultaneous removal of dissolved inorganic nitrogen and total organic carbon. The results reported in this study show that nitrification can be effectively coupled with denitrification, and at the same time, both processes could be accelerated by applying an overpotential

4.4.1 Reactor performance.

In the poised reactor case, significant increases in nitrogen and TOC removal were observed when -260 mV (vs. Ag/AgCl) potential was applied to the cathode. In fact, during the cycles where the cathode was maintained polarized, the highest removal rates were reported for ammonium and TOC. In the case of ammonium, the maximum removal rate (7.5 mg NH_4^+-N L^{-1} day^{-1}) proved to be lower than that reported by similar experimental conditions (Pous et al., 2020; Zhang et al., 2013; Zhu et al., 2019). In these studies, it took four and a half months (Pous et al., 2020), three months (Zhang et al., 2013), and six months (Zhu et al., 2019) to reach removal values of 39 mg NH_4^+-N L^{-1} day^{-1}, 19.4 mg NH_4^+-N L^{-1} day^{-1} and 13 mg NH_4^+-N L^{-1} day^{-1}, respectively. In comparison, in this study, it only took one month to reach a removal rate of 7.5 mg NH_4^+-N L^{-1} day^{-1}. However, in the case of total NH_4^+-N removal, our results show the removal efficiency of 96.1% ± 1.7% of NH_4^+-N, which is even higher (85.8% to 91.5% of NH_4^+-N) than previous work (Pous et al., 2020; Zhang et al., 2013; Zhu et al., 2019). It is important to

highlight that in the case of Pous et al. (2020), the inoculum contained effluent from different reactors performing nitrification, denitrification, anammox and activated sludge. In the case of Zhang et al. (2013) and Zhu et al. (2019), activated sludge was used as the inoculum. Therefore, all of these reactors either already had a microbial community associated with the nitrogen cycle or activated sludge was used as inoculum, which is known to have a high abundance of nitrifying and denitrifying microorganisms (Juretschko et al., 2002).

In the sea, a high abundance of microorganisms associated with the nitrogen cycle cannot be ensured due to the fluctuating nature of the ecosystem (Sunagawa et al., 2015). Therefore, the applied seawater inoculum did not ensure a high abundance of nitrogen-cycling microorganisms or removal rates close to those reported by Zhang et al. (2013) and Zhu et al. (2019). However, the TOC maximum removal rate (99.5 mg L^{-1} day^{-1}) was higher (30-90 mg L^{-1} day^{-1}) than the rates in previous work (Zhang et al., 2013; Zhu et al., 2019). Thus, our results suggest that the start-up process of MET can be accelerated by applying an overpotential to the electrode.

With regard to current density, the maximum for each polarized reactor was recorded in phase II of the experiment when -260 mV (vs. Ag/AgCl) was applied to the cathode (Table 4-2). In general, MET reported capable of removing nitrogen, and organic matter have been developed connected to an external resistance, which maximum current density reported range was from from 0.84 to 1.8 A m^{-3} (Ryu et al., 2013; Zhang and He, 2012; Zhang et al., 2013; Zhu et al., 2013). These current densities were higher than the maximum current density (0.9 A m^{-3}) reported by our setup when the reactors were

connected to a resistance of 1 kΩ. It is important to emphasize that, in general, the reported MET need months of start-up to register significant current densities (Zhang and He, 2012; Ryu et al., 2013; Zhang et al., 2013; Zhu et al., 2013) and in this study only took 20 days to reach a current density of 0.9 (Table 4-2). The application of overpotentials to an electrode to accelerate EAM enrichment, and therefore current production, has been previously reported by several authors (Miceli et al., 2012; Rowe et al., 2015; Torres et al., 2009; Wang et al., 2015). Therefore, the results obtained in our research reinforce the idea of MET polarization to accelerate the process of generating a significant current density.

To consider the complete removal of nitrogen species from seawater, gas species forms of nitrogen (i.e., N_2, N_xO) should also be assured. Nevertheless, during the polarization period (phase II), in particular since cycle 4, the concentration of NO_3^--N in the effluent reach values significantly lower than the affluent. In addition, the amount of NO_2^--N measured in both, effluent and affluent, was four order of magnitude lower than the nitrogen concentration added as ammonium (Table S4-2). On the other hand, the polarized reactors showed significantly lower NO_3^--N and NO_2^--N concentrations in their effluents than the concentrations observed in the control reactors at the end of each cycle (Table S4-2). Then, the results showed that a significant amount of nitrogen was removed from the liquid face. This removal is suggested due to the significant enrichment of denitrifying microorganisms in the cathode generated by applying -260 mV (vs. Ag/AgCl), which has been associated with the denitrification process (Kondaveeti et al., 2014). In addition, the maximum assimilation rate of ammonium and nitrate by bacteria in the seawater has been previously determined, reaching a value of 1.96 x 10-3 mg L^{-1} day^{-1} (Allen et al., 2002).

This value is at least three orders of magnitude lower than the removal rates obtained in this study (Figure 4-2). However, it cannot be ruled out that a fraction of the nitrogen compounds was assimilated by bacteria. Yet, nitrate removal by electrochemical reduction is discarded since it occurs at a significantly higher potential (-2.9 V vs. Ag/AgCl) (Katsounaros et al., 2006) than those applied in this research.

4.4.2 Effects of the overpotential on the enrichment of bacteria associated with the nitrogen cycle

From the community analysis carried out, AA detected a higher rate of specific taxonomic groups in the polarized reactors. From taxa within those categories, ASVs associated with the genus Halomonas were highlighted (Figure 4-3A). Within the genus Halomonas, denitrification capacity is an important taxonomic marker (González-Domenech et al., 2010). Simultaneously, Halomonas strains isolated from marine sediments are EAM, with a reductive nitrate peak at approximately -220 mV (vs. Ag/AgCl; (Rowe et al., 2015). Therefore, because the reductive peak reported for Halomonas is similar to the applied overpotential in this investigation (-260 mV vs. Ag/AgCl), the enrichment of this genus in the community present in polarized reactors was expected. Moreover, similar associations have been observed in previous works (Kondaveeti et al., 2014; Rowe et al., 2015), suggesting a direct relationship between Halomonas-related bacteria and the removal of nitrogen compounds (Figure 4-3B).

Community sequencing methods were complemented with FISH for the specific identification of enriched functional groups. The FISH analysis (Figure 4-4) corroborated the effect of electrode polarization on the enrichment of nitrifiers and bacteria related to

denitrifiers and to *planctomycetes* in the anode and cathode, respectively. However, in nonpolarized cathodes, an enrichment of nitrifying microorganisms was observed. A different response pattern has been reported in previous designs (Virdis et al., 2011; Zhang et al., 2014, 2013) in which total nitrogen metabolization is carried out in the cathode. While nitrifying bacteria have been associated with shallow layers in cathodic biofilms, denitrifying bacteria have been identified in deeper layers interacting with the cathode (Virdis et al., 2011; Zhang et al., 2013; Zhang et al., 2014). Indeed, in Figure 4-4, it can be seen that the control condition is more similar to the microbial distribution of the reactors developed by Virdis et al., (2011) and Zhang et al., (2013 and 2014), where both nitrifying and denitrifying microorganisms were identified in the cathode. Therefore, these results suggest that it is possible to use overpotential to enrich microorganisms with a specific metabolism, in this case, associated with the nitrogen cycle, without the need for the use of a culture medium.

The -260 mV (vs. Ag/AgCl) condition has been associated with the denitrification process (Kondaveeti et al., 2014), so a significantly higher abundance of denitrifying bacteria in the cathode was expected. However, the high quantity of *planctomycetes* identified in polarized cathodes is striking (Figure 4B). Within the *planctomycetes* clade, anaerobic oxidation of ammonium (ANAMMOX) is a characteristic that has also been reported as EAM (Kuenen, 2008; Shaw et al., 2020). Moreover, the enzyme hydrazine hydrolase (involved in the anammox process) and the enzyme nitrite reductase (involved in the denitrification process) have been reported to have the same standard reduction potential (Jetten et al., 2009). Overall, it is postulated that the application of -260 mV (vs. Ag/AgCl)

could activate both enzymes associated with denitrification and anammox metabolism, and therefore, the enrichment of annamox bacteria could also be promoted. But to verify this hypothesis, it is necessary to perform FISH analysis using specific probes for anammox, since the probe used in this study is for the group of *planctomycetes* that is composed of a great variety of bacteria.

4.5 Conclusion

The results of this research suggest that the effect of microbial enrichment by applying overpotentials to the electrodes could accelerate the start-up process of the reactor in terms of its nutrient removal rates and current production. The use of overpotential is proposed as an enrichment technique for microorganisms with specific metabolisms in environments where it is difficult to ensure a significant abundance of microorganisms of interest. Specifically, the application of -260 mV (vs. Ag/AgCl) to the cathode demonstrated significant enrichment of denitrifying microorganisms and *planctomycetes* from an inoculum of natural seawater. These results demonstrate that it is unnecessary to use a culture medium to enrich and maintain a microbial community in a reactor, which could also help in the successful application of MET in real environments.

The development of the reactor developed in this research, capable of removing carbon and nitrogen from polluted seawater, could represent a solution to solve the eutrophication problem in marine environments. However, before applying this technology in a real marine system and achieving the desired impact, improvements are needed to maximize the nutrient removal efficiency.

4.6 Acknowledgements

This work was supported by the FONDECYT 11201134 and FONDECYT 1181326, the CEDEUS center CONICYT/FONDAP/15110020 and the Vicerrectoría de Investigación of Pontificia Universidad Católica de Chile. This study was conducted at the Marine Energy Research & Innovation Center (MERIC) (Project CORFO 14CEI2-28228). We give special thanks to Leslie K. Daille, Mauricio Medel, Francisca Rubio, Karen Yanira Sanzana and Evelyn Cruz for their assistance in the laboratory and help with the experimental setup.

5. CONCLUSIONS AND PERSPECTIVES.

The results obtained in this thesis provide new information regarding the development and construction of MET-type reactors capable of removing nitrogen and organic matter for removal in marine environments. The contribution of this thesis was achieved by combining different disciplines (i.e., electrochemistry, marine microbiology, reactor design, and environmental science and engineering). It was possible to verify the hypothesis thanks to the verification of (1) the analysis of overpotentials as a microbial enrichment tool and (2) the identification of an efficient overpotential in enriching denitrifying microorganisms. The results allowed for the development of a laboratory-scale bioelectrochemical reactor with marine biofilms specialized in removing nitrogen compounds (i.e., nitrification and denitrification).

5.1 Conclusions.

The results obtained in Chapter II verified that (1) SS 316 L is a promising material to be used as an electrode, but corrosion problems were presented when polarized at + 310 mV and (2) the overpotentials were confirmed as a tool for microbial enrichment without the need of an enriched culture medium. Specifically, it was observed that by applying +310 mV (vs. Ag/AgCl), it was possible to select EAB of the genera *Sulfitobacter, Roseobacter,* and *Phaeobacter*. These genera have been related to autotrophic oxygen-reducing EABs, which have been reported as biocorrosion accelerators. For this reason, the electrodes polarized at +310 mV (vs. Ag/AgCl) were the most affected by the phenomenon of biocorrosion.

From the results obtained in Chapter III, it was possible to determine that the overpotential of -260 mV (vs. Ag/AgCl) was the most effective in enriching denitrifying bacteria. This was achieved using natural seawater as inoculum and without the need to use an external electron donor. In this case, the electrode functioned as an electron donor. The use of the electrode as a source of electrons could be verified from the results obtained by cyclic voltammetry, which showed only negative peaks, which indicates that microorganisms are only reducing compounds. In addition, the -260 mV (vs. Ag/AgCl) condition demonstrated significant nitrate removal compared to the other tested overpotentials during the 30 days of operation of the reactors in batch mode. This result was directly related to the abundance of denitrifying microorganisms in the electrodes polarized at -260 mV (vs. Ag/AgCl).

Finally, from the results obtained in Chapter IV, the construction of a MET-type reactor capable of removing nitrogen from natural seawater was achieved. A reactor composed of an anode compartment and a cathode compartment separated by an ion-exchange membrane was used. The six BERs were operated for 37 days under flow-recirculation mode. The inoculum was natural seawater supplied with ammonia and acetate, simulating concentrations reported in wastewater. Considering the results obtained in Chapters II and III, a potential of -260 mV (vs. Ag/AgCl) was used to enrich denitrifying microorganisms in the cathode. From this, it was possible to determine that overpotentials can accelerate the BER start-up process, which were directly related to the EAB enrichment effect produced by applying overpotentials to the electrodes. Overpotential application generated cathode enrichment of denitrifying microorganisms and *planctomycetes*, the latter related to anammox metabolism. In the anode, an enrichment of microorganisms AOB and NOB

was observed. The significant enrichment of these metabolisms in the anode and cathode was directly related to the reactors' removal capacity. The polarized reactors showed significantly greater removal of TOC and ammonia than the controls. Therefore, based on the information gathered in Chapters II and III, it was possible to successfully develop a BER that efficiently removes TOC and nitrogen from marine environments.

5.2 Perspectives

The reactor developed in this research, capable of removing carbon and nitrogen from marine environments, could represent an alternative to prevent eutrophication problems in marine environments. Before applying this technology in a real marine system and achieving the desired impact, improvements and previous analyses are needed.

The following previous analysis and research are suggested to apply the designed reactor in a real environment. First, it is essential to find the optimal overpotential to enrich microorganisms associated with the nitrogen cycle. The reason for this is that only three reported overpotentials for the denitrification metabolism were tested. Simultaneously, the necessary time needs to be determined for reactor electrode polarization to reach an optimal level of enrichment of microorganisms associated with the nitrogen cycle. After this, it is important to explore each electrode's ideal area to achieve maximum nitrogen removal efficiency. With this, it will be possible to decide on the number of reactors in series that will be needed to achieve effective nitrogen removal. Having performed all this analysis, the first field tests could be carried out and thus achieve a MET that can help reverse the damage generated in marine environments.

On the other hand, to better understand the microbial metabolic processes that occur in the developed MET, it would be necessary to isolate these microorganisms. By isolating them, each microorganism's role in MET could be expressly understood, and this could also allow controlling more variables of this technology. Furthermore, enrichment of nitrifying microorganisms was observed in the anode of the reactors that were polarized. This type of microorganism has not been reported as EAB. Therefore, it would be essential to isolate these nitrifying microorganisms and assess whether they are EAB. This analysis provides new knowledge regarding the microorganisms that carry out processes associated with nitrification.

Finally, decreasing excessive nitrogen discharges to the sea could help reduce the system's overload and aid the regeneration of impacted ecosystems. Therefore, achieving a successful implementation of the technology developed in this thesis could avoid reaching a point of no return in altering the nitrogen cycle and helping sustainable development.

6. BIBLIOGRAFY

Abessa, D.M.S., Hortelani, M.A., Sarkis, J.E., 2005. Influence of a Brazilian sewage outfall on the toxicity and contamination of adjacent sediments. Mar. Pollut. Bull. 50, 875–885. https://doi.org/10.1016/j.marpolbul.2005.02.034

Abraham, W., Strompl, C., Meyer, H., Lindholst, S., Moore, E.R.B., Christ, R., Vancanneyt, M., Tindali, B.J., Bennasar, A., Smit, J., Tesar, M., 1999. Phylogeny and polyphasic taxonomy of Caulobacter species . Proposal of Maricaulis gen . nov . with Maricaulis maris (Poindexter) comb . nov . as the type species , and emended description of the genera Brevundirnonas and Caulobacter. Int. J. Syst. Bacteriol. 1053–1073.

Ahn, Y., 2006. Sustainable nitrogen elimination biotechnologies : A review. Process Biochem. 41, 1709–1721. https://doi.org/10.1016/j.procbio.2006.03.033

Allgaier, M., Uphoff, H., Felske, A., Wagner-do, I., 2003. Aerobic Anoxygenic Photosynthesis in Roseobacter Clade Bacteria from Diverse Marine Habitats. Appl. Environ. Microbiol. 69, 5051–5059. https://doi.org/10.1128/AEM.69.9.5051

Altschul, S.F., Gish, W., Miller, W., Myers, E.E.W.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. https://doi.org/10.1016/S0022-2836(05)80360-2

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful Algal Blooms and Eutrophication Nutrient Sources , Composition , and Consequences. Estuaries 25, 704–726.

Ansari, A.A., Singh, S., Lanza, G.R., Rast, W., 2010. Eutrophication: Causes, Consequences and Control, 2nd ed. Springer Science & Business Media.

Aslan, S., Türkman, A., 2003. Biological denitrification of drinking water using various natural organic solid substrates. Water Sci. Technol. 48, 58140.

Bahroun, S., Bousnoubra, H., Drouiche, N., Kherici, N., 2016. Analysis of wastewater discharges to the Wadi Kebir East River by the environmental discharge objectives (EDO) method. Desalin. Water Treat. 57, 24750–24754. https://doi.org/10.1080/19443994.2016.1146924

Barco, R. a., Emerson, D., Sylvan, J.B., Orcutt, B.N., Jacobson Meyers, M.E., Ramírez, G. a., Zhong, J.D., Edwards, K.J., 2015. New Insight into Microbial Iron Oxidation as Revealed by the Proteomic Profile of an Obligate Iron-Oxidizing Chemolithoautotroph. Appl. Environ. Microbiol. 81, 5927–5937. https://doi.org/10.1128/AEM.01374-15

Barco, R.A., Emerson, D., Sylvan, J.B., Orcutt, B.N., Meyers, E.J., Ramírez, G.A., Zhong, J.D., Edwards, J., 2015. New Insight into Microbial Iron Oxidation as Revealed by the Proteomic Profile of an Obligate Iron-Oxidizing Chemolithoautotroph. Appl. Environ. Microbiol. 81, 5927–5937. https://doi.org/10.1128/AEM.01374-15

Baudler, A., Schmidt, I., Langner, M., Greiner, A., Schröder, U., 2015. Does it have to be carbon? Metal anodes in microbial fuel cells and related bioelectrochemical systems. Energy Environ. Sci. 8, 2048–2055. https://doi.org/10.1039/c5ee00866b

Beals, E.W., 1984. Bray-curtis ordination: An effective strategy for analysis of multivariate ecological data. Adv. Ecol. Res. 14, 1–55. https://doi.org/10.1016/S0065-2504(08)60168-3

Bellona, C., Drewes, J.E., Oelker, G., Luna, J., Filteau, G., Amy, G., 2008. Comparing nanofiltration and reverse osmosis for drinking water augmentation. J. / Am. Water Work. Assoc. 100. https://doi.org/10.1002/j.1551-8833.2008.tb09724.x

Berks, B.C., Ferguson, S.J., Moir, J.W.B., Richardson, D.J., 1995. Enzymes and associated electron transport systems that catalyse the respiratory reduction of nitrogen oxides and oxyanions. Biochim. Biophys. Acta 2728, 97–173.

Bhatnagar, A., Sillanpää, M., 2011. A review of emerging adsorbents for nitrate removal from water. Chem. Eng. J. 168, 493–504. https://doi.org/10.1016/j.cej.2011.01.103

Bissett, A., Bowman, J., Burke, C., 2006. Bacterial diversity in organically-enriched ¢ sh farm sediments. FEMS Microbiol. Ecol. 55, 48–56. https://doi.org/10.1111/j.1574-6941.2005.00012.x

Bock, E., Wagner, M., 2006. Oxidation of Inorganic Nitrogen Compounds as an Energy Source, in: The Prokaryotes. pp. 457–495.

Boivin, M.E.Y., Greve, G.D., Kools, S.A.E., van der Wurff, A.W.G., Leeflang, P., Smit, E., Breure, A.M., Rutgers, M., van Straalen, N.M., 2006. Discriminating between effects of metals and natural variables in terrestrial bacterial communities. Appl. Soil Ecol. 34, 103–113. https://doi.org/10.1016/j.apsoil.2006.03.009

Buschmann, A.H., Cabello, F., Young, K., Carvajal, J., Varela, D.A., Henr??quez, L., 2009. Salmon aquaculture and coastal ecosystem health in Chile: Analysis of regulations, environmental impacts and bioremediation systems. Ocean Coast. Manag. 52, 243–249. https://doi.org/10.1016/j.ocecoaman.2009.03.002

Buschmann, A.H., Riquelme, V.A., Hern??ndez-Gonz??lez, M.C., Varela, D., Jim??nez, J.E., Henr??quez, L.A., Vergara, P.A., Gu????ez, R., Fil??n, L., 2006. A review of the impacts of salmonid farming on marine coastal ecosystems in the southeast Pacific. ICES J. Mar. Sci. 63, 1338–1345. https://doi.org/10.1016/j.icesjms.2006.04.021

Call, D.F., Merrill, M.D., Logan, B.E., 2009. High Surface Area Stainless Steel Brushes as Cathodes in Microbial Electrolysis Cells. Environ. Sci. Technol. 43, 2179–2183.

Castro, H.F., Williams, N.H., Ogram, A., 2000. Phylogeny of sulfate-reducing bacteria 1. FEMS Microbiol. Ecol. 31, 1–9.

Chamritski, I.G., Burns, G.R., Webster, B.J., Laycock, N.J., 2004. Effect of iron-oxidizing bacteria on pitting of stainless steel. Corrosion 60, 658–669.

https://doi.org/10.5006/1.3287842

Chaplin, B.P., Reinhard, M., Schneider, W.F., Schu, C., Shapley, J.R., Strathmann, T.J., Werth, C.J., 2012. Critical Review of Pd-Based Catalytic Treatment of Priority Contaminants in Water. Environ. Sci. Technol. 46, 3655–3670.

Chen, G.W., Cha, J.H., Choi, S.J., Lee, T.H., Kim, C.W., 2010. Characterization of an open biocathode microbial fuel cell for electricity generation and effluent polish. Korean J. Chem. Eng. 27, 828–835. https://doi.org/10.1007/s11814-010-0142-6

Compère, C., Jaffré, P., Festy, D., 1996. Aging of Type 316L Stainless Steel in Seawater : Relationship Between Open-Circuit Potential , Exposure Time , and Pitting Potential. Corros. Sci. 52, 496–501.

Daims, H., Lücker, S., Wagner, M., 2016. A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria. Trends Microbiol. 24, 699–712. https://doi.org/10.1016/j.tim.2016.05.004

Daims, H., Nielsen, J.L., Nielsen, P.E.R.H., Schleifer, K., Wagner, M., 2001. In Situ Characterization of Nitrospira -Like Nitrite-Oxidizing Bacteria Active in Wastewater Treatment Plants. Appl. Environ. Microbiol. 67, 5273–5284. https://doi.org/10.1128/AEM.67.11.5273

Dang, H., Li, T., Chen, M., Huang, G., 2008. Cross-Ocean Distribution of Rhodobacterales Bacteria as Primary Surface Colonizers in Temperate Coastal Marine Waters □ †. Appl. Environ. Microbiol. 74, 52–60. https://doi.org/10.1128/AEM.01400-07

Dang, H., Lovell, C.R., 2000. Bacterial Primary Colonization and Early Succession on Surfaces in Marine Waters as Determined by Amplified rRNA Gene Restriction Analysis and Sequence Analysis of 16S rRNA Genes. Appl. Environ. Microbiol. 66, 467–475.

Darbi, A., Viraraghavan, T., Butler, R., Corkal, D., 2006. Pilot-Scale Evaluation of Select

Nitrate Removal Technologies. J. Environ. Sci. Heal. 4529. https://doi.org/10.1081/ESE-120022873

Darus, L., Ledezma, P., Keller, J., Freguia, S., 2016. Marine phototrophic consortia transfer electrons to electrodes in response to reductive stress. Photosynth Res 347–354. https://doi.org/10.1007/s11120-015-0193-y

Davis, M.L., 2010. Secondary treatment by suspended growth biological processes., in: Water and Wastewater Engineering: Design Principles and Practice. pp. 1–115.

De-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Gimenez-Casalduero, F., Sánchez-Lizaso, J.L., 2016. Monitoring the effects of wastewater treatment strategies. Environ. Monit. Assess. 188, 110. https://doi.org/10.1007/s10661-016-5092-y

Ding, A., Zhao, D., Ding, F., Du, S., Lu, H., Zhang, M., Zheng, P., 2018. Effect of inocula on performance of bio-cathode denitrification and its microbial mechanism. Chem. Eng. J. 343, 399–407. https://doi.org/10.1016/j.cej.2018.02.119

Dumas, C., Mollica, A., Féron, D., Basséguy, R., Etcheverry, L., Bergel, A., 2007. Marine microbial fuel cell : Use of stainless steel electrodes as anode and cathode materials. Electrochim. Acta 53, 468–473. https://doi.org/10.1016/j.electacta.2007.06.069

Eddie, B.J., Wang, Z., Iv, W.J.H., Leary, D.H., Malanoski, A.P., Tender, L.M., Lin, B., Strycharz-glaven, S.M., 2017. Metatranscriptomics Supports the Mechanism for Biocathode Electroautotrophy by “Candidatus Tenderia electrophaga.” Mol. Biol. Physiol. 2, 1–13.

Eddie, B.J., Wang, Z., Malanoski, A.P., Hall, R.J., Oh, S.D., Heiner, C., Lin, B., Strycharz-glaven, S.M., Eddie, B.J., 2016. ‘ Candidatus Tenderia electrophaga ’ , an uncultivated electroautotroph from a biocathode enrichment 2178–2185. https://doi.org/10.1099/ijsem.0.001006

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves

sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

El-naggar, M.Y., Wanger, G., Man, K., Yuzvinsky, T.D., Southam, G., Yang, J., 2010. Electrical transport along bacterial nanowires from. PNAS 107, 18127–18131. https://doi.org/10.1073/pnas.1004880107

Emerson, D., Fleming, E.J., Mcbeth, J.M., 2010. Iron-Oxidizing Bacteria : An Environmental and Genomic Perspective. Annu. Rev. Microbiol. 64, 561–583. https://doi.org/10.1146/annurev.micro.112408.134208

Enger, O., Nygaard, H., Solberg, M., Schei, G., Nielsen, J., Dundas, I., 1987. Characterization of Alteromonas denitrificans sp. nov. Int. J. Syst. Bacteriol. 37, 416-421.

Erable, B., Byrne, N., Etcheverry, L., Achouak, W., Bergel, A., 2017. Single medium microbial fuel cell : Stainless steel and graphite electrode materials select bacterial communities resulting in opposite electrocatalytic activities. Int. J. Hydrogen Energy 42, 26059–26067. https://doi.org/10.1016/j.ijhydene.2017.08.178

Erable, B., Roncato, M.-A., Achouak, W., Bergel, A., 2009. Sampling Natural Biofilms : A New Route to Build Efficient Microbial Anodes. Environ. Sci. Technol. 43, 3194–3199.

Ervik, A., Hansen, P.K., Aure, J., Stigebrandt, A., Johannessen, P., Jahnsen, T., 1997. Regulating the local environmental impact of intensive marine fish farming I . The concept of the MOM system. Aquac. Eng. 158, 85–94.

Fang, C., Min, B., Angelidaki, I., 2011. Nitrate as an Oxidant in the Cathode Chamber of a Microbial Fuel Cell for Both Power Generation and Nutrient Removal Purposes 464–474. https://doi.org/10.1007/s12010-010-9148-0

Feitosa, R.C., Rosman, P.C.C., Carvalho, J.L.B., Côrtes, M.B. V, Wasserman, J.C., 2013.

Comparative study of fecal bacterial decay models for the simulation of plumes of submarine sewage outfalls. Water Sci. Technol. 68, 622–631. https://doi.org/10.2166/wst.2013.286

Figuerola, E.L.M., Erijman, L., 2007. Bacterial taxa abundance pattern in an industrial wastewater treatment system determined by the full rRNA cycle approach. Environ. Microbiol. 9, 1780–1789. https://doi.org/10.1111/j.1462-2920.2007.01298.x

Fischer, D.A., Daille, L., Aguirre, J., Galarce, C., Armijo, F., Iglesia, R.D. la, Pizarro, G., Vargas, I., Walczak, M., 2016. Corrosion of stainless steel in simulated tide of fresh natural seawater of South East Pacific. Int. J. Electrochem. Sci. 11, 6873–6885. https://doi.org/10.20964/2016.08.50

Franks, A.E., Nevin, K.P., 2010. Microbial Fuel Cells, A Current Review. Energies 3, 899–919. https://doi.org/10.3390/en3050899

Fuhrman, J.E.D.A., Comeau, D.E., Hagstrom, A.K.E., Chan, A.M.Y.M., 1988. Extraction from Natural Planktonic Microorganisms of DNA Suitable for Molecular Biological Studies. Appl. Environ. Microbiol. 54, 1426–1429.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vörösmarty, C.J., 2004. Nitrogen cycles : past , present , and future. Biogeochemistry 70, 153–226.

Galloway, J.N., Trends, R., Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2013. Transformation of the Nitrogen Cycle : Potential Solutions 889. https://doi.org/10.1126/science.1136674

Ginige, M.P., Keller, J., Blackall, L.L., 2005. Investigation of an Acetate-Fed Denitrifying Microbial Community by Stable Isotope Probing , Full-Cycle rRNA Analysis , and

Fluorescent In Situ Hybridization-Microautoradiography. Appl. Environ. Microbiol. 71, 8683–8691. https://doi.org/10.1128/AEM.71.12.8683

Glibert, P.M., 2017. Eutrophication , harmful algae and biodiversity — Challenging paradigms in a world of complex nutrient changes. Mar. Pollut. Bull. 0–1. https://doi.org/10.1016/j.marpolbul.2017.04.027

Gobet, A., Boetius, A., Ramette, A., 2014. Ecological coherence of diversity patterns derived from classical fingerprinting and Next Generation Sequencing techniques. Environ. Microbiol. 16, 2672–2681. https://doi.org/10.1111/1462-2920.12308

Graeber, I., Kaesler, I., Borchert, M.S., Dieckmann, R., Pape, T., Lurz, R., Nielsen, P., von Döhren, H., Michaelis, W., Szewzyk, U., 2008. Spongiibacter marinus gen. nov., sp. nov., a halophilic marine bacterium isolated from the boreal sponge Haliclona sp. 1. Int. J. Syst. Evol. Microbiol. 58, 585–590. https://doi.org/10.1099/ijs.0.65438-0

Granéli, E., Turner, J.T., 2006. Ecology of Harmful Algae. Springer Berlin Heidelberg.

Guldhe, A., Ansari, F.A., Singh, P., Bux, F., 2017. Heterotrophic cultivation of microalgae using aquaculture wastewater: A biorefinery concept for biomass production and nutrient remediation. Ecol. Eng. 99, 47–53. https://doi.org/10.1016/j.ecoleng.2016.11.013

Halpern, B.S., Selkoe, K.A., Micheli, F., Kappel, C. V, 2007. Evaluating and Ranking the Vulnerability of Global Marine Ecosystems to Anthropogenic Threats. Conserv. Biol. 21, 1301–1315. https://doi.org/10.1111/j.1523-1739.2007.00752.x

Halpern, B.S., Walbridge, S., A. Selkoe, K., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., Signaling, 2008. global human impact on marine ecosystems. Science (80-.). 319, 948–953.

Hansen, P.K., Ervik, A., Schaanning, M., Johannessen, P., Aure, J., Jahnsen, T., Stigebrandt, A., 2001. Regulating the local environmental impact of intensive , marine fish farming II . The monitoring programme of the MOM system ž Modelling – Ongrowing fish farms – Monitoring /. Aquaculture 194, 75–92.

He, Z., Kan, J., Wang, Y., Huang, Y., Mansfeld, F., Nealson, K.H., 2009. Electricity Production Coupled to Ammonium in a Microbial Fuel Cell. Environ. Sci. Technol. 43, 3391–3397. https://doi.org/10.1021/es803492c

Hell, F., Lahnsteiner, J., Frischherz, H., Baumgartner, G., 1998. Experience with full-scale electrodialysis for nitrate and hardness removal. Desalination 7, 173–180.

Henríquez-castillo, C., Rodríguez-marconi, S., Rubio, F., Trefault, N., Andrade, S., Iglesia, R. De, 2015. Eukaryotic picophytoplankton community response to copper enrichment in a metal-perturbed coastal environment. Phycol. Res. 189–196. https://doi.org/10.1111/pre.12087

Holland, R.L., Cooper, B.H., Helgeson, N.G., Mccracken, A.W., 1980. Automated detection of microbial growth in blood Automated Detection of Microbial Growth in Blood Cultures by Using Stainless-Steel Electrodes. J. Clin. Microbiol. 12, 180–184.

Howarth, R.W., Marino, R., 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems : Evolving views over three decades. Limnol. Oceanogr. 51, 364–376.

Hu, H.W., He, J.Z., 2017. Comammox—a newly discovered nitrification process in the terrestrial nitrogen cycle. J. Soils Sediments 17, 2709–2717. https://doi.org/10.1007/s11368-017-1851-9

Huang, Y.H., Zhang, T.C., 2004. Effects of low pH on nitrate reduction by iron powder. Water Res. 38, 2631–2642. https://doi.org/10.1016/j.watres.2004.03.015

Huno, S.K.M., Rene, E.R., van Hullebuschm, E.D., Annachhatre, A.P., 2018. Nitrate

removal from groundwater : a review of natural and engineered processes Solomon. J. Water Supply Res. Techonology-Aqua 8, 885–902. https://doi.org/10.2166/aqua.2018.194

Janvier, M., Grimont, P.A.D., 1995. The genus Methylophaga, a new line of descent within phylogenetic branch γ of proteobacteria. Res. Microbiol. 146, 543–550. https://doi.org/10.1016/0923-2508(96)80560-2

Jia, Y., Qian, D., Chen, Y., Hu, Y., 2021. Intra / extracellular electron transfer for aerobic denitrification me diate d by in-situ biosynthesis palladium nanoparticles. Water Res 189. https://doi.org/10.1016/j.watres.2020.116612

Jiang, C., Yang, Q., Wang, D., Zhong, Y., Chen, F., Li, Xin, Zeng, G., Li, Xiaoming, 2017. Simultaneous perchlorate and nitrate removal coupled with electricity generation in autotrophic denitrifying biocathode microbial fuel cell. Chem. Eng. J. 308, 783–790. https://doi.org/10.1016/j.cej.2016.09.121

Jiang, C., Yang, Q., Wang, D., Zhong, Y., Chen, F., Li, Xin, Zeng, G., Li, Xiaoming, Shang, M., 2016. Simultaneous perchlorate and nitrate removal coupled with electricity generation in autotrophic denitrifying biocathode microbial fuel cell. Chem. Eng. J. 308, 783–790. https://doi.org/10.1016/j.cej.2016.09.121

Juretschko, S., Loy, A., Lehner, A., Wagner, M., 2002. The Microbial Community Composition of a Nitrifying-Denitrifying Activated Sludge from an Industrial Sewage Treatment Plant Analyzed by the Full-Cycle rRNA Approach. Syst. Appl. Microbiol. 25, 84–99.

Kalaruban, M., Loganathan, P., Shim, W.G., Kandasamy, J., Naidu, G., Nguyen, T.V., Vigneswaran, S., 2016. Removing nitrate from water using iron-modified Dowex 21K XLT ion exchange resin: Batch and fluidised-bed adsorption studies. Sep. Purif. Technol. 158, 62–70. https://doi.org/10.1016/j.seppur.2015.12.022

Kawahara, N., Shigematsu, K., Miyadai, T., Kondo, R., 2009. Comparison of bacterial communities in fi sh farm sediments along an organic enrichment gradient. Aquaculture 287, 107–113. https://doi.org/10.1016/j.aquaculture.2008.10.003

Kelly, P.T., He, Z., 2014. Nutrients removal and recovery in bioelectrochemical systems : A review. Bioresour. Technol. 153, 351–360. https://doi.org/10.1016/j.biortech.2013.12.046

Kennish, M.J., 2015. Eutrophication of mid-Atlantic coastal bays Eutrophication of Mid-Atlantic Coastal Bays. Bull. New Jersey Acad. Sci. 54, 5–13.

Kim, J.R., Jung, S.H., Regan, J.M., Logan, B.E., 2007. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresour. Technol. 98, 2568–2577. https://doi.org/10.1016/j.biortech.2006.09.036

Kondaveeti, S., Kang, E., Liu, H., Min, B., 2019. Continuous autotrophic denitrification process for treating ammonium-rich leachate wastewater in bioelectrochemical denitrification system (BEDS). Bioelectrochemistry 130, 107340. https://doi.org/10.1016/j.bioelechem.2019.107340

Kondaveeti, S., Lee, S., Park, H., Min, B., 2014. Bacterial communities in a bioelectrochemical denitrification system : The effects of supplemental electron acceptors. Water Res. 51, 25–36. https://doi.org/10.1016/j.watres.2013.12.023

Koroleff, F., 1999. Determination of nutrients., in: Grasshoff, K., Kremling, K., Ehrhardt, M. (Eds.), Methods of Seawater Analysis. pp. 159–226.

Kuenen, J.G., 2008. Anammox bacteria : from discovery to application. Nat. Rev. Microbiol. 6, 320–326.

Lage, O.M., Joana, L., 2012. Isolation and characterization of Planctomycetes from the sediments of a fish farm wastewater treatment tank. Arch. Microbiol. 194, 879–885. https://doi.org/10.1007/s00203-012-0821-2

Lananan, F., Hajar, S., Hamid, A., Nur, W., Din, S., Khatoon, H., Jusoh, A., Endut, A., 2014. International Biodeterioration & Biodegradation Symbiotic bioremediation of aquaculture wastewater in reducing ammonia and phosphorus utilizing Effective Microorganism (EM-1) and microalgae (Chlorella sp .). Int. Biodeterior. Biodegradation 95, 127–134. https://doi.org/10.1016/j.ibiod.2014.06.013

Leary, D.H., Hervey, J.W., Malanoski, A.P., Wang, Z., Eddie, B.J., Tender, G.S., Vora, G.J., Tender, L.M., Lin, B., Strycharz-Glaven, S.M., 2015. Metaproteomic evidence of changes in protein expression following a change in electrode potential in a robust biocathode microbiome. Proteomics n/a-n/a. https://doi.org/10.1002/pmic.201400585

Lee, S.D., 2007. Lewinella agarilytica sp. nov., a novel marine bacterium of the phylum Bacteroidetes, isolated from beach sediment. Int. J. Syst. Evol. Microbiol. 57, 2814–2818. https://doi.org/10.1099/ijs.0.65254-0

Li, H., Zhou, E., Zhang, D., Xu, D., Xia, J., Yang, C., Feng, H., Jiang, Z., Li, X., Gu, T., Yang, K., 2016. Microbiologically Influenced Corrosion of 2707 Hyper-Duplex Stainless Steel by Marine Pseudomonas aeruginosa Biofilm. Sci. Rep. 6, 1–12. https://doi.org/10.1038/srep20190

Li, R., Zi, X., Wang, X., Zhang, X., Gao, H., Hu, N., 2013. Marinobacter hydrocarbonoclasticus NY-4 , a novel denitrifying , moderately halophilic marine bacterium. Springerplus 2, 346–355.

Li, W.-W., Yu, H.-Q., 2015. Stimulating sediment bioremediation with benthic microbial fuel cells. Biotechnol. Adv. 33, 1–12. https://doi.org/10.1016/j.biotechadv.2014.12.011

Libes, S.M., 2009. Introduction to marine biogeochemistry.

Lo, N., Kim, K.H., Baek, K., Jia, B., Jeon, C.O., 2015. Aestuariicella hydrocarbonica gen. nov., sp. Nov., an aliphatic hydrocarbon-degrading bacterium isolated from a sea tidal

flat. Int. J. Syst. Evol. Microbiol. 65, 1935–1940. https://doi.org/10.1099/ijs.0.000199

Logan, B.E., 2010. Scaling up microbial fuel cells and other bioelectrochemical systems. Appl. Microbiol. Biotechnol. 85, 1665–1671. https://doi.org/10.1007/s00253-009-2378-9

Lovley, D.R., 2011. Powering microbes with electricity: direct electron transfer from electrodes to microbes. Environ. Microbiol. Rep. 3, 27–35. https://doi.org/10.1111/j.1758-2229.2010.00211.x

Luo, H., Moran, M.A., 2014. Evolutionary Ecology of the Marine Roseobacter Clade. Microbiol. Mol. Biol. Rev. 78, 573–587. https://doi.org/10.1128/MMBR.00020-14

Maixner, F., Noguera, D.R., Anneser, B., Stoecker, K., Wegl, G., Wagner, M., Daims, H., 2006. Nitrite concentration influences the population structure of Nitrospira -like bacteria. Environ. Microbiol. 8, 1487–1495. https://doi.org/10.1111/j.1462-2920.2006.01033.x

Malanoski, A.P., Lin, B., Brian, J., Wang, Z., Iv, W.J.H., Glaven, S.M., 2018. Relative abundance of ‘ Candidatus Tenderia electrophaga ’ is linked to cathodic current in an aerobic biocathode community. Microb. Biotechnol. https://doi.org/10.1111/1751-7915.12757

Malik, S., Drott, E., Grisdela, P., Lee, J., Lee, C., Lowy, D.A., Tender, L.M., 2009. A self-assembling self-repairing microbial photoelectrochemical solar cell. Energy Environ. Sci. 2, 292–298. https://doi.org/10.1039/b816417g

Marsili, E., Baron, D.B., Shikhare, I.D., Coursolle, D., Gralnick, J.A., Bond, D.R., 2008. Shewanella secretes flavins that mediate extracellular electron transfer. PNAS 105, 6–11.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10. https://doi.org/10.14806/ej.17.1.200

Martins, G., Peixoto, L., Ribeiro, D.C., Parpot, P., Brito, A.G., Nogueira, R., 2010. Bioelectrochemistry Towards implementation of a benthic microbial fuel cell in lake Furnas (Azores): Phylogenetic af fi liation and electrochemical activity of sediment bacteria. Bioelectrochemistry 78, 67–71. https://doi.org/10.1016/j.bioelechem.2009.07.003

Miceli, J.F., Parameswaran, P., Kang, D., Krajmalnik-brown, R., 2012. Enrichment and Analysis of Anode-Respiring Bacteria from Diverse Anaerobic Inocula. Environ. Sci. Technol. 46, 10349–10355. https://doi.org/10.1021/es301902h

Mobarry, B.K., Wagner, M., Urbain, V., Rittmann, B.E., Stahl, D.A., 1996. Phylogenetic Probes for Analyzing Abundance and Spatial Organization of Nitrifying Bacteria. Appl. Environ. Microbiol. 62, 2156–2162.

Mohan, S.B., Schmid, M., Jetten, M., Cole, J., 2004. Detection and widespread distribution of the nrfA gene encoding nitrite reduction to ammonia , a short circuit in the biological nitrogen cycle that competes with denitrification. FEMS Microbiol. Ecol. 49, 433–443. https://doi.org/10.1016/j.femsec.2004.04.012

Molognoni, D., Devecseri, M., Cecconet, D., Capodaglio, A.G., 2017. Cathodic groundwater denitrification with a bioelectrochemical system. J. Water Process Eng. 19, 67–73. https://doi.org/10.1016/j.jwpe.2017.07.013

Monsalvo, V.M., Mohedano, A.F., Rodriguez, J.J., 2011. Activated carbons from sewage sludge Application to aqueous-phase adsorption of 4-chlorophenol. Desalination 277, 377–382. https://doi.org/10.1016/j.desal.2011.04.059

Mook, W.T., Chakrabarti, M.H., Aroua, M.K., Khan, G.M. a., Ali, B.S., Islam, M.S., Abu Hassan, M. a., 2012. Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. Desalination 285, 1–13. https://doi.org/10.1016/j.desal.2011.09.029

Moura, V., Ribeiro, I., Moriggi, P., Capão, A., Salles, C., Bitati, S., Procópio, L., 2018. The influence of surface microbial diversity and succession on microbiologically influenced corrosion of steel in a simulated marine environment. Arch. Microbiol. 200, 1447–1456. https://doi.org/10.1007/s00203-018-1559-2

Nataraj, S.K., Hosamani, K.M., Aminabhavi, T.M., 2006. Electrodialytic removal of nitrates and hardness from simulated mixtures using ion-exchange membranes. J. Appl. Polym. Sci. 99, 1788–1794. https://doi.org/10.1002/app.22710

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., I, J.L., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017–1024.

Neef, A., Arnann, R., Schlesner, H., Schleiferl, K., 1998. Monitoring a widespread bacterial group : in situ detection of planctomycetes with 16s rRNA-targeted probes. Microbiology 144, 3257–3266.

Oldham, A.L., Steinberg, M.K., Duncan, K.E., Makama, Z., Duncan, K.E., 2017. Molecular methods resolve the bacterial composition of natural marine biofilms on galvanically coupled stainless steel cathodes. J. Ind. Microbiol. Biotechnol. 44, 167–180. https://doi.org/10.1007/s10295-016-1887-7

Olesen, B.H., Avci, R., Lewandowski, Z., 2000. Manganese dioxide as a potential cathodic reactant in corrosion of stainless steels 42, 211–227.

Orhon, D., Ates, E., Sözen, S., Çokgör, E.U., 1997. CHARACTERIZATION A N D COD FRACTIONATION OF DOMESTIC WASTEWATERS. Environ. Pollut. 95, 191–204.

Osborne, C.A., Rees, G.N., Bernstein, Y., Janssen, P.H., 2006. New Threshold and Confidence Estimates for Terminal Restriction Fragment Length Polymorphism Analysis of Complex Bacterial Communities †. Appl. Environ. Microbiol. 72, 1270–

1278. https://doi.org/10.1128/AEM.72.2.1270

Park, H. Il, Kim, D. kun, Choi, Y., Pak, D., 2005. Nitrate reduction using an electrode as direct electron donor in a biofilm-electrode reactor. Process Biochem. 40, 3383–3388. https://doi.org/10.1016/j.procbio.2005.03.017

Park, J.Y., Yoo, Y.J., 2009. Biological nitrate removal in industrial wastewater treatment : which electron donor we can choose. Appl. Microbiol. Biotechnol. 82, 415–429. https://doi.org/10.1007/s00253-008-1799-1

Parot, S., Vandecandelaere, I., Cournet, A., Délia, M., Vandamme, P., Bergé, M., Roques, C., Bergel, A., 2011. Catalysis of the electrochemical reduction of oxygen by bacteria isolated from electro-active biofilms formed in seawater. Bioresour. Technol. 102, 304–311. https://doi.org/10.1016/j.biortech.2010.06.157

Pérez-Elvira, S.I., Diez, P.N., Fdz-Polanco, F., 2006. Sludge minimisation technologies. Rev. Environ. Sci. Bio/Tecnology 5, 375–398. https://doi.org/10.1007/s11157-005-5728-9

Pocaznoi, D., Calmet, A., Etcheverry, L., Erable, B., Bergel, A., 2012. Stainless steel is a promising electrode material for anodes of microbial fuel cells. Energy Environ. Sci. 5, 9645–9652. https://doi.org/10.1039/c2ee22429a

Pous, N., Korth, B., Alvarez, M.O.-, Dolors, M., Harnisch, F., 2020. Electrifying biotrickling filters for the treatment of aquaponics wastewater. Bioresour. Technol. 319, 124221. https://doi.org/10.1016/j.biortech.2020.124221

Powley, H.R., Du, H.H., Lima, A.T., Krom, M.D., Cappellen, P. Van, 2016. Direct Discharges of Domestic Wastewater are a Major Source of Phosphorus and Nitrogen to the Mediterranean Sea. Environ. Sci. Technol. 50, 8722–8730. https://doi.org/10.1021/acs.est.6b01742

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner,

F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. https://doi.org/10.1093/nar/gks1219

Quinn, P.K., Charlson, R.J., Bates, T.S., 1988. Simultaneous observations of ammonia in the atmosphere and ocean. Nature 335, 336–338.

R.M.Warwick, K.R.C. and, 2007. Change in marine communities: An Approach to Statistical Analysis and Interpretation. Am. J. Clin. Nutr. https://doi.org/1

Rajasekar, A., Ting, Y.P., 2011. Role of inorganic and organic medium in the corrosion behavior of bacillus megaterium and pseudomonas sp. in stainless steel SS 304. Ind. Eng. Chem. Res. 50, 12534–12541. https://doi.org/10.1021/ie200602a

Ramos, S., Almeida, R.M., Cordas, C.M., Moura, J.J.G., Pauleta, R., Moura, I., 2017. Insights into the recognition and electron transfer steps in nitric oxide reductase from Marinobacter hydrocarbonoclasticus. J. Inorg. Biochem. 177, 402–411. https://doi.org/10.1016/j.jinorgbio.2017.09.001

Rao, J.R., Richter, G.J., Von Sturm, F., Weidlich, E., 1976. The Performance of Glucose Electrodes and the Characteristics of Different . Biofuel Cell Constructions. Bioelectrochemistry Bioenerg. 3, 139–150.

Reguera, G., Mccarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., Lovley, D.R., 2005. Extracellular electron transfer via microbial nanowires. Nature 435, 1098–1101. https://doi.org/10.1038/nature03661

Rezvani, F., Sarrafzadeh, M., Ebrahimi, S., 2019. Nitrate removal from drinking water with a focus on biological methods : a review. Environ. Sci. Pollut. Res. 26, 1124–1141. https://doi.org/10.1007/s11356-017-9185-0

Rimboud, M., Bergel, A., Erable, B., 2016. Multiple electron transfer systems in oxygen reducing biocathodes revealed by different conditions of aeration / agitation.

Bioelectrochemistry 110, 46–51. https://doi.org/10.1016/j.bioelechem.2016.03.002

Ringelberg, D.B., Foley, K.L., Reynolds, C.M., 2011. Electrogenic capacity and community composition of anodic biofilms in soil-based bioelectrochemical systems. Appl. Microbiol. Biotechnol. 1805–1815. https://doi.org/10.1007/s00253-011-3264-9

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F.S., Lambin, E., Lenton, T.M., Scheffer, M., Folke, C., Joachim, H., Nykvist, B., Wit, C.A. De, Hughes, T., Leeuw, S. Van Der, Rodhe, H., Sörlin, S., Snyder, P.K., Costanza, R., Svedin, U., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Liver, D., 2009. Planetary Boundaries : Exploring the Safe Operating Space for Humanity. Ecol. Soc. 14.

Roller, S.D., Bennetto, H.P., Delaney, G.M., Mason, J.R., Stirling, J.L., Thurston, C.F., 1984. Electron-transfer Coupling in Microbial Fuel Cells: 1. Comparison of Redox-mediator Reduction Rates and Respiratory Rates of Bacteria. J. Chem. Technol. Biotechnol. 34, 3–12.

Rowe, A.R., Chellamuthu, P., Lam, B., Okamoto, A., Nealson, K.H., 2015. Marine sediments microbes capable of electrode oxidation as a surrogate for lithotrophic insoluble substrate metabolism. Front. Microbiol. 6, 1–15. https://doi.org/10.3389/fmicb.2014.00784

Rozendal, A., Yuan, Z., Virdis, B., Rabaey, K., 2010. Simultaneous nitrification , denitrification and carbon removal in microbial fuel cells. Water Res. 44, 2970–2980. https://doi.org/10.1016/j.watres.2010.02.022

Ryu, J.H., Lee, H.L., Lee, Y.P., Kim, T.S., Kim, M.K., Anh, D.T.N., Tran, H.T., Ahn, D.H., 2013. Simultaneous carbon and nitrogen removal from piggery wastewater using loop configuration microbial fuel cell. Process Biochem. 48, 1080–1085. https://doi.org/10.1016/j.procbio.2013.05.016

Samatya, S., Kabay, N., Yu, M., 2006. Removal of nitrate from aqueous solution by nitrate

selective ion exchange resins. React. Funct. Polym. 66, 1206–1214. https://doi.org/10.1016/j.reactfunctpolym.2006.03.009

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09

Schoeman, J.J., Steyn, A., 2003. Nitrate removal with reverse osmosis in a rural area in South Africa. Desalination 5, 15–26.

Schwermer, C.U., Lavik, G., Abed, R.M.M., Dunsmore, B., Ferdelman, T.G., Stoodley, P., Gieseke, A., De Beer, D., 2008. Impact of nitrate on the structure and function of bacterial biofilm communities in pipelines used for injection of seawater into oil fields. Appl. Environ. Microbiol. 74, 2841–2851. https://doi.org/10.1128/AEM.02027-07

Selembo, P.A., Merrill, M.D., Logan, B.E., 2009. The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells. J. Power Sources 190, 271–278. https://doi.org/10.1016/j.jpowsour.2008.12.144

Smith, V.H., 2003. Eutrophication of Freshwater and Coastal Marine Ecosystems A Global Problem. Environ. Sci. Pollut. Res. 10, 126–139.

Soares, M.I.M., 2000. Biological denitrification of groundwater. Water. Air. Soil Pollut. 123, 183–193.

Soto, B.D., Norambuena, F., 2004. Evaluation of salmon farming effects on marine systems in the inner seas of southern Chile : a large-scale mensurative experiment. J. Appl. Ichthyol. 20, 493–501.

Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., De Vries, W., De Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sörlin, S., 2015. Planetary boundaries: Guiding human development on a changing planet. Science (80-.). 347. https://doi.org/10.1126/science.1259855

Stigebrandt, A., Aure, J., Ervik, A., Kupka, P., 2004. Regulating the local environmental impact of intensive marine fish farming III . A model for estimation of the holding capacity in the Modelling – Ongrowing fish farm – Monitoring system. Aquaculture 234, 239–261. https://doi.org/10.1016/j.aquaculture.2003.11.029

Strycharz-glaven, S.M., Glaven, R.H., Wang, Z., Zhou, J., Vora, G.J., Tender, L.M., 2013. Electrochemical Investigation of a Microbial Solar Cell Reveals a Nonphotosynthetic Biocathode Catalyst. Appl. Environ. Microbiol. 79, 3933–3942. https://doi.org/10.1128/AEM.00431-13

Su, W., Zhang, L., Li, D., Zhan, G., 2012. Dissimilatory Nitrate Reduction by Pseudomonas alcaliphila With an Electrode as the Sole Electron Donor. Biotechnol. Bioeng. 109, 2904–2910. https://doi.org/10.1002/bit.24554

Sugita, H., Nakamura, H., Shimada, T., 2005. Microbial communities associated with filter materials in recirculating aquaculture systems of freshwater fish. Aquaculture 243, 403–409. https://doi.org/10.1016/j.aquaculture.2004.09.028

Sun, H., Xu, S., Zhuang, G., Zhuang, X., 2016. Performance and recent improvement in microbial fuel cells for simultaneous carbon and nitrogen removal : A review. J. Environ. Sci. 39, 242–248. https://doi.org/10.1016/j.jes.2015.12.006

Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., Djahanschiri, B., Zeller, G., Mende, D.R., Alberti, A., Cornejo-castillo, F.M., Costea, P.I., Cruaud, C., Ovidio, F., Engelen, S., Ferrera, I., Gasol, J.M., Guidi, L., Hildebrand, F., Kokoszka, F., Lepoivre, C., 2015. Structure and function of the global

ocean microbiome. Science (80-.). 348, 1–10.

Sutton, M.A., Bleeker, A., Howard, C.M., Erisman, J.W., Abrol, Y.P., Bekunda, M., Datta, A., Davidson, E., Vries, W. de, Oenema, O., Zhang, F.S., 2013. Our nutrient world. The challenge to produce more food & energy with less pollution. Edinburgh, Scotland.

Tender, L.M., Reimers, C.E., Iii, H.A.S., Holmes, D.E., Bond, D.R., Lowy, D.A., Pilobello, K., Fertig, S.J., Lovley, D.R., 2002. Harnessing microbially generated power on the seafloor. Nat. Biotechnol. 20, 821–825. https://doi.org/10.1038/nbt716

Ter Heijne, A., Strik, D.P.B.T.B., Hamelers, H. V., Buisman, C.J., 2010. Cathode Potential and Mass Transfer Determine Performance of Oxygen Reducing Biocathodes in Microbial Fuel Cells. Environ. Sci. Technol. 44, 7151–7156.

Thayanukul, P., Zang, K., Janhom, T., Kurisu, F., 2010. Concentration-dependent response of estrone-degrading bacterial community in activated sludge analyzed by microautoradiography-fluorescence in situ hybridization. Water Res. 44, 4878–4887. https://doi.org/10.1016/j.watres.2010.07.031

Torres-rojas, F., Muñoz, D., Tapia, N., Canales, C., Vargas, I.T., 2020. Bioresource Technology Bioelectrochemical chlorate reduction by Dechloromonas agitata CKB. Bioresour. Technol. 315, 123818. https://doi.org/10.1016/j.biortech.2020.123818

Torres, C.I., Krajmalnik-Brown, R., Parameswaran, P., Marcus, A.K., Wanger, G., Gorby, Y.A., Rittmann, B.E., 2009. Selecting anode-respiring bacteria based on anode potential: Phylogenetic, electrochemical, and microscopic characterization. Environ. Sci. Technol. https://doi.org/10.1021/es902165y

Torres, C.I., Marcus, A.K., Lee, H., Parameswaran, P., Krajmalnik-brown, R., Rittmann, B.E., 2010. A kinetic perspective on extracellular electron transfer by anode-respiring bacteria ′. FEMS Microbiol. Rev. 34, 3–17. https://doi.org/10.1111/j.1574-

6976.2009.00191.x

Trigodet, F., Larché, N., Morrison, H.G., Jebbar, M., Thierry, D., Maignien, L., 2019. Electroactive Bacteria Associated With Stainless Steel Ennoblement in Seawater. Front. Microbiol. 10, 1–9. https://doi.org/10.3389/fmicb.2019.00170

van Dorst, J., Bissett, A., Palmer, A.S., Brown, M., Snape, I., Stark, J.S., Raymond, B., Mckinlay, J., Ji, M., Winsley, T., Ferrari, B.C., 2014. Community fingerprinting in a sequencing world. FEMS Microbiol. Ecol. 89, 316–330. https://doi.org/10.1111/1574-6941.12308

Van Hulle, S.W.H., Vandeweyer, H.J.P., Meesschaert, B.D., Vanrolleghem, P.A., Dejans, P., Dumoulin, A., 2010. Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. Chem. Eng. J. 162, 1–20. https://doi.org/10.1016/j.cej.2010.05.037

Vandecandelaere, I., Nercessian, O., Faimali, M., Segaert, E., Mollica, A., Achouak, W., De Vos, P., Vandamme, P., 2010. Bacterial diversity of the cultivable fraction of a marine electroactive biofilm. Bioelectrochemistry 78, 62–66. https://doi.org/10.1016/j.bioelechem.2009.07.004

Vandecandelaere, I., Segaert, E., Mollica, A., Faimali, M., Vandamme, P., 2009. Phaeobacter caeruleus sp. nov., a blue-coloured, colony-forming bacterium isolated from a marine electroactive biofilm. Int. J. Syst. Evol. Microbiol. 59, 1209–1214. https://doi.org/10.1099/ijs.0.002642-0

Virdis, B., Rabaey, K., Rozendal, R.A., Yuan, Z., Keller, J., 2010. Simultaneous nitrification , denitrification and carbon removal in microbial fuel cells. Water Res. 44, 2970–2980. https://doi.org/10.1016/j.watres.2010.02.022

Virdis, B., Rabaey, K., Yuan, Z., 2008. Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Res. 42, 3013–3024.

https://doi.org/10.1016/j.watres.2008.03.017

Virdis, B., Read, S.T., Rabaey, K., Rozendal, R.A., Yuan, Z., Keller, J., 2011. Biofilm stratification during simultaneous nitrification and denitrification (SND) at a biocathode. Bioresour. Technol. 102, 334–341. https://doi.org/10.1016/j.biortech.2010.06.155

von Canstein, H., Ogawa, J., Shimizu, S., Lloyd, J.R., 2008. Secretion of Flavins by Shewanella Species and Their Role in Extracellular Electron Transfer □. Appl. Environ. Microbiol. 74, 615–623. https://doi.org/10.1128/AEM.01387-07

Wagner, M., Anmus, B., Hartmannt, A., Hutzlers, P., Amann, R., 1994. In situ analysis of microbial consortia in activated sludge using fluorescently labelled , rRNA-targeted oligonucleotide probes and confocal scanning laser microscopy. J. Microsc. 1, 181–187.

Wang, H., He, Q., Chen, D., Wei, L., Zou, Z., Zhou, J., Yang, K., Zhang, H., 2015. Microbial community in a hydrogenotrophic denitrification reactor based on pyrosequencing. Appl. Microbiol. Biotechnol. 99, 10829–10837. https://doi.org/10.1007/s00253-015-6929-y

Wang, H., Ren, Z.J., 2013. A comprehensive review of microbial electrochemical systems as a platform technology. Biotechnol. Adv. 31, 1796–1807. https://doi.org/10.1016/j.biotechadv.2013.10.001

Wang, Z., Leary, D.H., Malanoski, A.H.P., Li, R.W., Judson Hervey, W., Eddie, B.J., Tender, G.S., Yanosky, S.G., Vora, G.J., Tender, L.M., Lin, B., Strycharz-Glaven, S.M., 2015. A previously uncharacterized, nonphotosynthetic member of the Chromatiaceae is the primary CO2-fixing constituent in a self-regenerating biocathode. Appl. Environ. Microbiol. 81, 699–712. https://doi.org/10.1128/AEM.02947-14

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. Weisburg, WG 173, 697–703. https://doi.org/n.a.

Wurtsbaugh, W.A., Paerl, H.W., Dodds, W.K., 2019. Nutrients , eutrophication and harmful algal blooms along the freshwater to marine continuum. Wiley Interdiscip. Rev. Water 6, 1–27. https://doi.org/10.1002/wat2.1373

Xia, J., Yang, C., Xu, D., Sun, D., Nan, L., Sun, Z., Li, Q., Gu, T., Yang, K., 2015. Laboratory investigation of the microbiologically influenced corrosion (MIC) resistance of a novel Cu-bearing 2205 duplex stainless steel in the presence of an aerobic marine Pseudomonas aeruginosa biofilm. Biofouling 31, 481–492. https://doi.org/10.1080/08927014.2015.1062089

Xie, S., Liang, P., Chen, Y., Xia, X., Huang, X., 2011a. Simultaneous carbon and nitrogen removal using an oxic / anoxic-biocathode microbial fuel cells coupled system. Bioresour. Technol. 102, 348–354. https://doi.org/10.1016/j.biortech.2010.07.046

Xie, S., Liang, P., Chen, Y., Xia, X., Huang, X., 2011b. Bioresource Technology Simultaneous carbon and nitrogen removal using an oxic / anoxic-biocathode microbial fuel cells coupled system. Bioresour. Technol. 102, 348–354. https://doi.org/10.1016/j.biortech.2010.07.046

Yan, H., Saito, T., Regan, J.M., 2012. Nitrogen removal in a single-chamber microbial fuel cell with nitrifying biofilm enriched at the air cathode. Water Res. 46, 2215–2224. https://doi.org/10.1016/j.watres.2012.01.050

Yang, Y., Xu, M., Guo, J., Sun, G., 2012. Bacterial extracellular electron transfer in bioelectrochemical systems. Process Biochem. 47, 1707–1714. https://doi.org/10.1016/j.procbio.2012.07.032

Yu, C., Liang, Z., Das, A., Hu, Z., 2010. Nitrogen removal from wastewater using

membrane aerated microbial fuel cell techniques. Water Res. 45, 1157–1164. https://doi.org/10.1016/j.watres.2010.11.002

Yuan, L.N., Ren, L.F., Li, Y.T., Han, W.J., Yu, Yong, Chu, Y.N., Liu, G.M., Yu, D., Teng, M.J., Wang, L., Wang, X.M., Zhou, X.G., Yu, Yu De, Yu, J., 2011. A complete genome assembly of Glaciecola mesophila sp. nov. sequenced by using BIGIS-4 sequencer system. Sci. China Life Sci. 54, 835–840. https://doi.org/10.1007/s11427-011-4211-9

Zehr, J.P., 2011. Nitrogen fixation by marine cyanobacteria. Trends Microbiol. 19, 162–173. https://doi.org/10.1016/j.tim.2010.12.004

Zehr, J.P., Kudela, R.M., 2011. Nitrogen Cycle of the Open Ocean : From Genes to Ecosystems. Ann. Rev. Mar. Sci. 3, 197–225. https://doi.org/10.1146/annurev-marine-120709-142819

Zhan, G., Zhang, L., Tao, Y., Wang, Y., Zhu, X., Li, D., 2014. Anodic ammonia oxidation to nitrogen gas catalyzed by mixed biofilms in bioelectrochemical systems. Electrochim. Acta 135, 345–350. https://doi.org/10.1016/j.electacta.2014.05.037

Zhang, G., Zhang, H., Ma, Y., Yuan, G., 2014. Membrane filtration biocathode microbial fuel cell for nitrogen removal and electricity generation. Enzyme Microb. Technol. 60, 56–63. https://doi.org/10.1016/j.enzmictec.2014.04.005

Zhang, Guangyi, Zhang, H., Zhang, C., Zhang, Guoquan, Yang, F., Yuan, G., Gao, F., 2013. Simultaneous nitrogen and carbon removal in a single chamber microbial fuel cell with a rotating biocathode. Process Biochem. 48, 893–900. https://doi.org/10.1016/j.procbio.2013.03.008

Zhu, G., Huang, S., Lu, Y., Gu, X., 2019. Simultaneous nitrification and denitrification in the bio-cathode of a multi-anode microbial fuel cell. Environ. Technol. 0, 1–11. https://doi.org/10.1080/09593330.2019.1663938

7. SUPPLEMENTARY MATERIAL

7.1 Chapter II

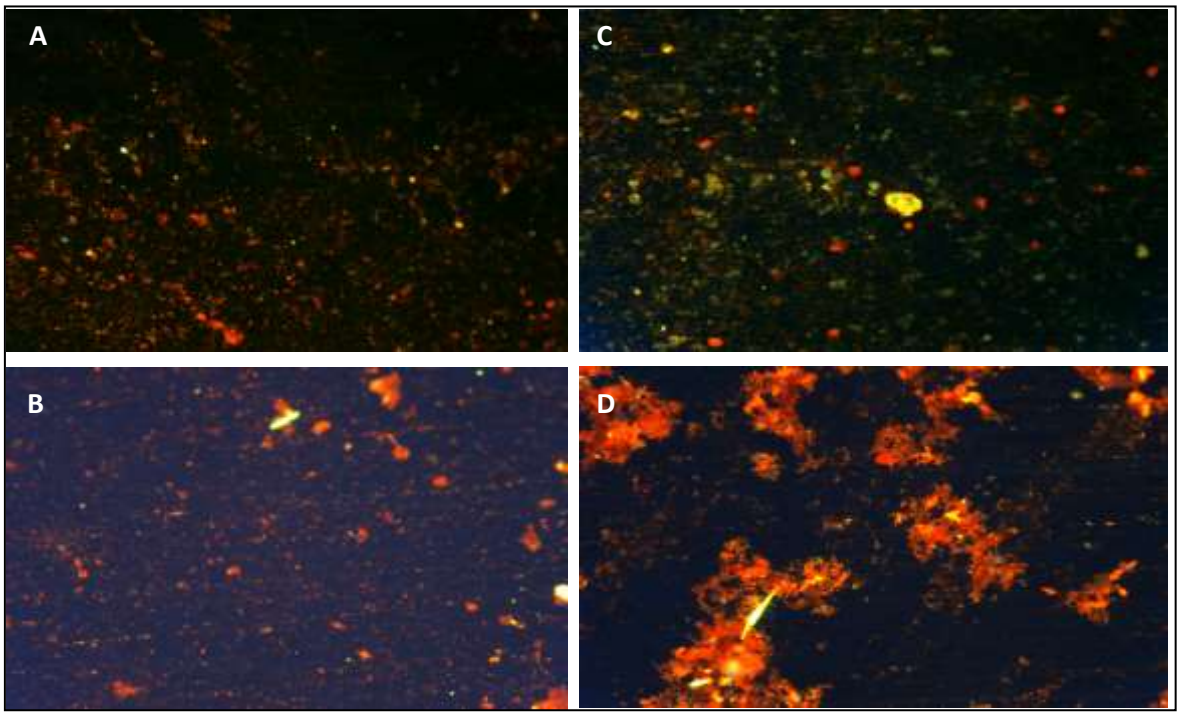

Figure S2-1: Eplifluorescense microscopy of each treatment, using acridine orange 0.1% w/v. When an acridine orange binds with DNA, it will exhibit a green color, and when it binds with RNA, it will exhibit a red color. A: +310 mV vs Ag/AgCl, B: -150 mV vs Ag/AgCl, C: +100 mV vs Ag/AgCl, and D: Control. All images are in 10X.

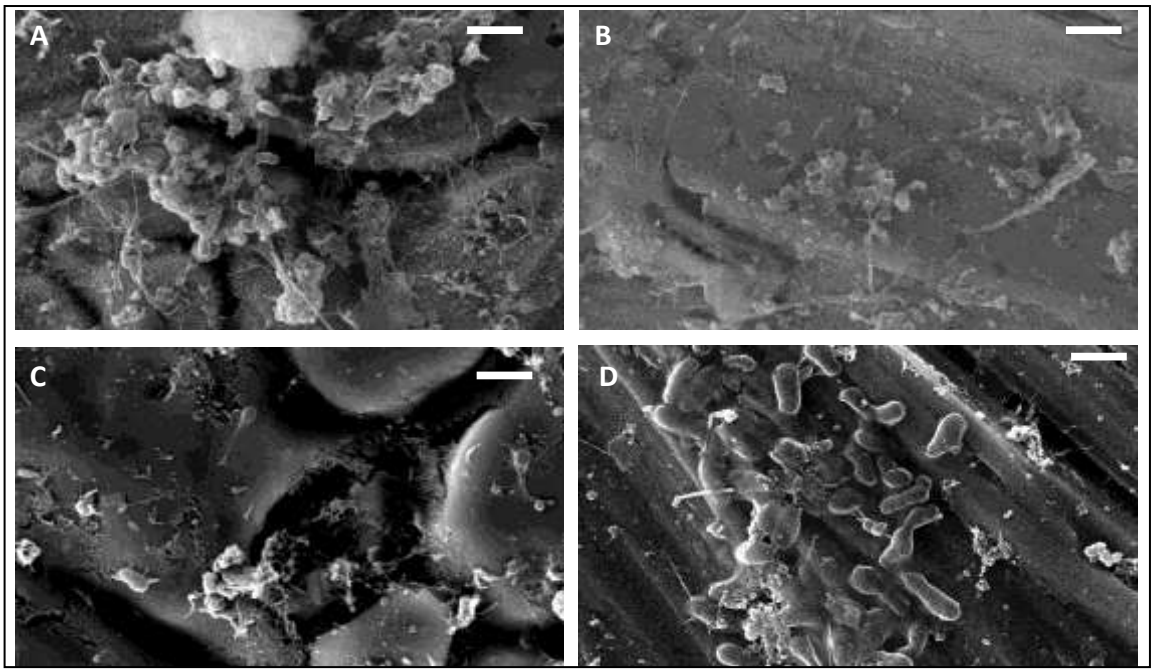

Figure S2-2: Scanning Electron Microscopy of each treatment. A: +310 mV vs Ag/AgCl, B: -150 mV vs Ag/AgCl, C: +100 mV vs Ag/AgCl, and D: Control. Bar scale represents a size of 2 µm. The magnifications of these images are 13,900 X.

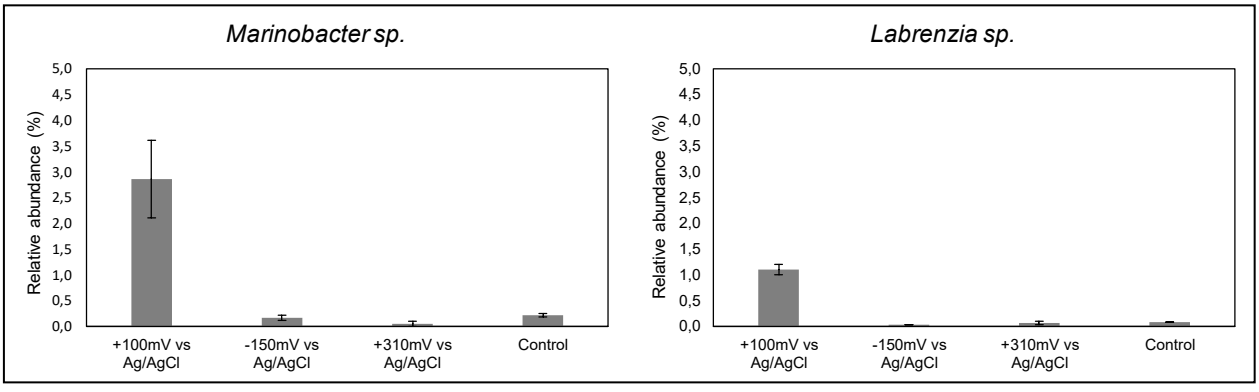

Figure S2-3: Relative abundance (%) of *Marinobacter* sp. and *Labrenzia* sp. in each sample.

Table S2-1: Accession number of the clone sequences reported in this thesis, and the most closely related organisms, with its accession number to the NCBI nucleotide database.

Condition	Accession N° (for this research)	Most closely related organisms	Accession N°
+310mV (vs Ag/AgCl)	MT026239	*Phaeobacter* sp.	MN099587.1
+310mV (vs Ag/AgCl)	MT026240	*Methylophaga sp.*	KC295387.1
+310mV (vs Ag/AgCl)	MT026241	*Roseobacter sp.*	AF098493.1
+310mV (vs Ag/AgCl)	MT026242	*Alteromonas sp.*	JX022750.1
+310mV (vs Ag/AgCl)	MT026243	*Halioxenophilus aromaticivorans*	AB809162.1
+310mV (vs Ag/AgCl)	MT026244	*Pseudophaeobacter sp.*	MK737663.1
+310mV (vs Ag/AgCl)	MT026245	*Phaeobacter caeruleus*	HM031996.1
+310mV (vs Ag/AgCl)	MT026246	*Leisingera sp.*	CP038234.1
+310mV (vs Ag/AgCl)	MT026247	Uncultured bacterium clone methane	GU584300.1
+310mV (vs Ag/AgCl)	MT026248	*Roseobacter sp.*	JQ661253.1
+310mV (vs Ag/AgCl)	MT026249	*Phaeobacter sp.*	HE818248.1
-150mV (vs Ag/AgCl)	MT026250	Uncultured Piscirickettsiaceae bacterium	DQ234105.2
-150mV (vs Ag/AgCl)	MT026251	*Roseobacter sp.*	AY576690.1
-150mV (vs Ag/AgCl)	MT026252	*Ruegeria sp.*	MN099589.1
-150mV (vs Ag/AgCl)	MT026253	Uncultured *Colwellia sp.*	JN860307.1
-150mV (vs Ag/AgCl)	MT026254	*Hyphomonas sp.*	CP017718.1
-150mV (vs Ag/AgCl)	MT026255	*Marinobacterium stanieri*	NR_024699.1
Control	MT026256	*Alteromonas stellipolaris*	LR218097.1
Control	MT026257	*Glaciecola sp.*	JX310209.1
Control	MT026258	Uncultured bacterium clone	KX177808.1
Control	MT026259	*Spongiibacter marinus*	NR_118015.1
Control	MT026260	Uncultured *Alteromonas sp.*	KC917978.1
Control	MT026261	*Phaeobacter sp.*	FJ436728.1
Control	MT026262	*Phaeobacter gallaeciensis*	CP015124.1
Control	MT026263	*Aestuariicella hydrocarbonica*	NR_135890.1
Control	MT026264	*Phaeobacter sp.*	FJ436729.1
Control	MT026265	*Pseudoalteromonas sp.*	AM162590.1
Counter	MT026266	*Alteromonas sp.*	JX022750.1
Counter	MT026267	*Alteromonas macleodii*	CP018321.1
Counter	MT026268	*Vibrio sp.*	LC506146.1
Counter	MT026269	*Hyphomonas sp.*	KC295391.1
Counter	MT026270	*Phaeobacter sp.*	FJ436728.1
Counter	MT026271	*Roseobacter sp.*	AY576690.1
Counter	MT026272	*Phaeobacter sp.*	FJ014980.1
Counter	MT026273	*Sulfitobacter sp.*	EU864265.1

Initial Sea water	MT026274	Uncultured Flavobacteria	AM279180.1
Initial Sea water	MT026275	*Planktomarina temperata*	NR_125550.1
Initial Sea water	MT026276	Uncultured Flavobacteria	AM279180.1
Initial Sea water	MT026277	*Winogradskyella sp.*	CP019332.1
Initial Sea water	MT026278	Uncultured Flavobacteria	EF202334.1
Initial Sea water	MT026279	*Loktanella sp.*	MK737661.1
Initial Sea water	MT026280	Uncultured bacterium clone	MK176136.1
Initial Sea water	MT026281	*Planktomarina temperata*	NR_125550.1
Initial Sea water	MT026282	Uncultured Pseudomonas sp.	KP453918.1
Initial Sea water	MT026283	Uncultured bacterium clone	JQ198943.1
Final Sea water	MT026284	*Sulfitobacter sp.*	MF600215.1
Final Sea water	MT026285	*Hyphomonas sp.*	KC295391.1
Final Sea water	MT026286	Uncultured bacterium clone	JQ200192.1
Final Sea water	MT026287	*Maricaulis maris*	CP000449.1
Final Sea water	MT026288	Uncultured *Hyphomonas sp.*	FJ425626.1
Final Sea water	MT026289	*Pseudoalteromonas sp.*	KF009870.1
Final Sea water	MT026290	*Maricaulis maris*	CP000449.1
Final Sea water	MT026291	*Roseobacter sp.*	AJ534238.1
Final Sea water	MT026292	*Hyphomonas sp.*	KC295391.1

Table S2-1: Relative abundance (%) of the most abundant genre identified by Amplicon Analysis (AA) and Fragment Analysis (FA) in each sample (+100 mV, -150 mV, +310 mV vs Ag/AgCl and Control).

			Relative abundance (%)							
			AA				FA			
	Family	Genus	+100 mV	-150 mV	+310 mV	Control	+100 mV	-150 mV	+310 mV	Control
	Rhodobacteraceae	Roseobacter	4.3	3.9	34.0	6.4	0.0	18.2	37.0	0.0
		Phaeobacter	6.0	4.8	18.8	5.9	55.2	0.0	44.8	56.0
		Sulfitobacter	7.2	7.9	15.2	3.5	0.0	0.0	0.0	0.0
		Ruegeria	1.3	1.0	0.5	1.2	0.0	4.3	0.0	0.0
		Labrenzia	1.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0
	Vibrionaceae	Vibrio	4.0	30.3	3.8	5.0	0.0	0.0	0.0	0.0
		Photobacterium	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Aliivibrio	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Hyphomonadaceae	Hyphomonas	4.3	3.4	3.6	5.1	0.0	13.3	0.0	0.0
		Maricaulis	1.0	0.3	0.7	0.8	0.0	0.0	0.0	0.0
	Flavobacteriaceae	Muricauda	5.3	2.1	1.7	3.7	0.0	0.0	0.0	0.0
		Maribacter	0.0	0.0	0.0	0.0	0.0	5.3	0.0	0.0
		Cellulophaga	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Alteromonadaceae	Alteromonas	0.6	0.1	2.5	11.5	32.0	47.6	5.2	37.0
		Glaciecola	7.3	5.9	0.0	0.6	6.3	0.0	0.0	2.6
		Marinobacter	2.9	0.2	0.0	0.2	0.0	0.0	0.0	0.0
	Phycisphaeraceae	Plantomycete	10.3	4.2	2.2	6.6	0.0	0.0	0.0	0.0
	Oceanospirillaceae	Neptuniibacter	3.7	4.3	3.3	6.5	3.2	0.0	0.0	2.3
		Amphritea	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Oleibacter	0.7	0.1	0.0	1.1	0.0	0.0	0.0	0.4
	Piscirickettsiaceae	Methylophaga	5.1	3.3	2.1	6.9	0.0	2.6	3.8	0.0
	Cellvibrionaceae	Aestuariicella	2.3	0.8	0.5	4.5	0.7	0.0	5.2	0.3
	Saprospiraceae	Lewinella	5.5	1.7	1.3	1.7	0.0	0.0	0.0	0.0
	Bacteroidaceae	Bacteroides	1.1	4.6	0.0	0.5	0.0	0.0	0.0	0.0
	Colwelliaceae	Colwellia	0.9	0.0	0.0	0.7	0.0	1.9	0.0	0.0
	Spongiibacteraceae	Spongiibacter	0.5	0.5	0.0	0.6	2.1	0.0	0.0	0.4

7.2 Chapter III

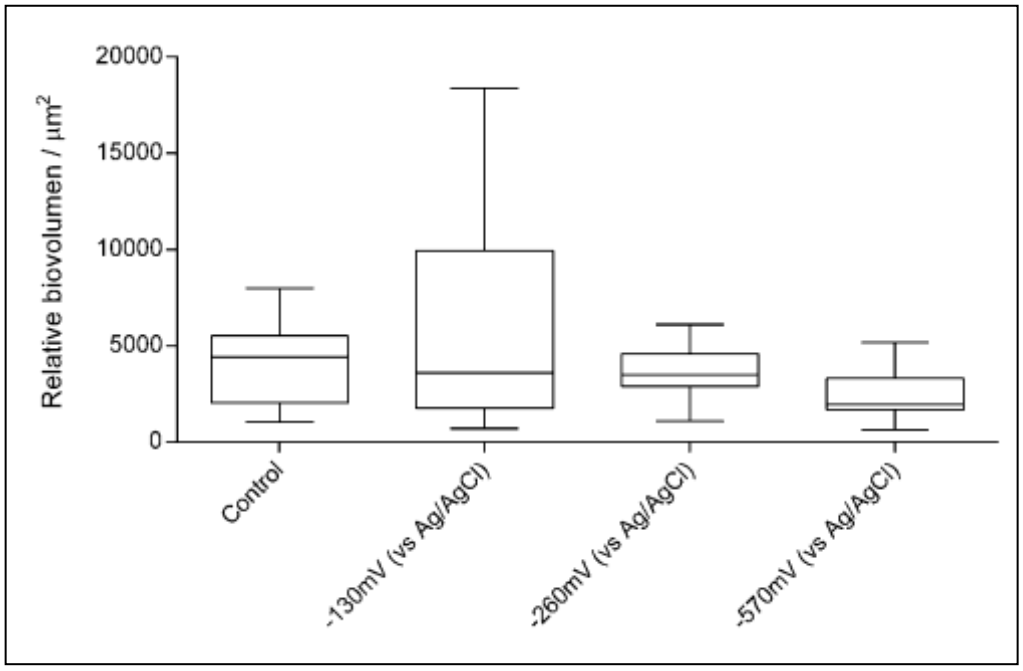

Figure S3-1. Relative biovolumen per μm^2, count by DAPI in each sample.

7.3 Chapter IV

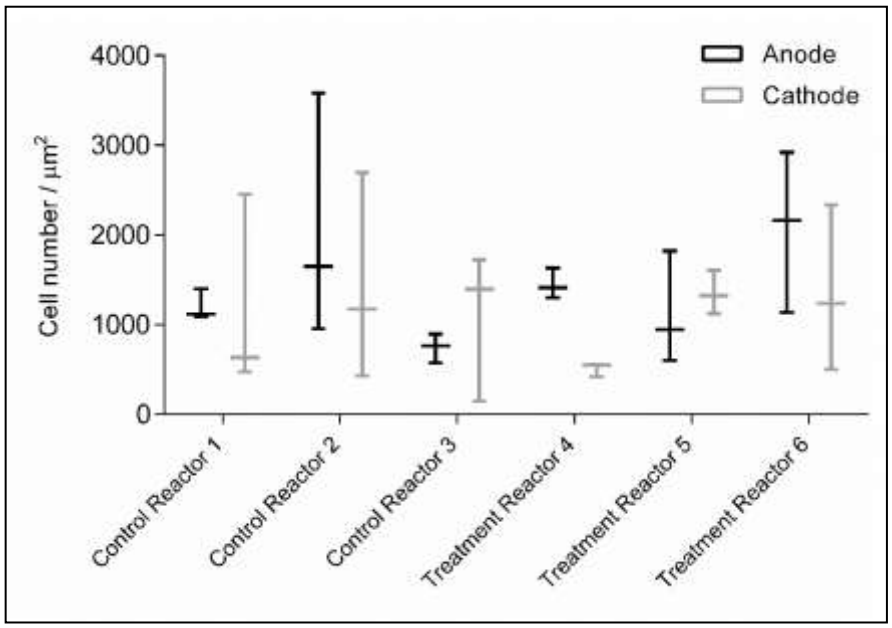

Figure S4- 1. Cell number per μm^2, count by DAPI in each electrode of each reactor.

Table S4-1: Removal rate (mg L^{-1} day^{-1}) of each ammonium and TOC matter during each phase along the experiment, as averages ± standard error.

		Removal rate (mg L^{-1} day^{-1})			
		NH_4^+-N		**TOC**	
Phases	**Cycles**	Control reactors	Polarized reactors	Control reactors	Polarized Reactors
I (anode at -500 mV vs Ag/AgCl)	1	0.22 ± 0.11	3.48 ± 0.12	23.38 ± 3.3	24.30 ± 7.1
II (cathode at -260 mV vs Ag/AgCl)	2	0.40 ± 0.21	1.67 ± 0.38	41.68 ± 4.3	31.68 ± 8.1
	3	2.33 ± 0.75	4.09 ± 0.11	51.34 ± 3.8	42.50 ± 8.9
	4	2.69 ± 0.35	5.35 ± 0.11	30.79 ± 3.8	61.97 ± 1.3
	5	2.61 ± 0.46	6.43 ± 0.21	51.69 ± 4.5	79.63 ± 7.7
III (cell connected to a 1kΩ)	6	3.00 ± 0.23	6.69 ± 0.15	49.67 ± 2.0	81.31 ± 1.4
IV (cathode at -260 mV vs Ag/AgCl)	7	5.00 ± 0.19	7.57 ± 0.08	53.87 ± 0.5	99.51 ± 1.5

Table S4-2: Summary of nitrogenous and carbon concentrations measured at the beginning and end of each phase and cycle, as averages ± standard error.

	Phases						
	I (polarized anode at -500 mV vs Ag/AgCl)	II (polarized cathode at -260 mV vs Ag/AgCl)				III (cell connected to a 1kΩ resistor)	IV (polarized cathode at -260 mV vs Ag/AgCl)
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7
NH_4^+-N (mg/L)							
Affluent	41.9 ± 0.8	39.8 ± 0.3	39.8 ± 0.2	37.8 ± 0.2	36.9 ± 0.5	37.7 ± 0.7	39.4 ± 0.8
Control Reactors effluent	40.5 ± 2.3	37.8 ± 1.2	28.1 ± 4.3	24.4 ± 1.3	23.9 ± 2.7	22.6 ± 2.4	14.4 ± 1.2
Polarized reactors effluent	17.6 ± 1.3	31.4 ± 2.6	19.3 ± 0.6	11.1 ± 1.2	4.8 ± 1.3	4.2 ± 1.1	1.5 ± 0.3
NO_3^--N (mg/L)							
Affluent	0.31 ± 0.012	0.26 ± 0.0015	0.17 ± 0.005	0.20 ± 0.002	0.15 ± 0.004	0.19 ± 0.001	0.21 ± 0.003
Control Reactors effluent	0.29 ± 0.03	0.47 ± 0.01	0.16 ± 0.012	0.09 ± 0.003	0.06 ± 0.0008	0.05 ± 0.009	0.05 ± 0.007
Polarized reactors effluent	0.35 ± 0.04	0.33 ± 0.04	0.05 ± 0.003	0.002 ± 0.0001	0.0003 ± 0.0001	0.0003 ± 0.0002	0.0003 ± 0.0002
NO_2^--N (mg/L)							
Affluent	0.0007 ± 0.0004	0.007 ± 0.0001	0.006 ± 0.0002	0.006 ± 0.0001	0.007 ± 0.0005	0.012 ± 0.0001	0.007 ± 0.0004
Control Reactors effluent	0.005 ±0.0003	0.001 ± 0.0004	0.003 ± 0.00004	0.015 ± 0.00006	0.018 ± 0.0001	0.008 ± 0.00004	0.008 ± 0.0002
Polarized reactors effluent	0.005 ± 0.0005	0.0001 ± 0.0004	0.003 ± 0.00006	0.004 ± 0.00002	0.002 ±0.0001	0.002 ± 0.0008	0.004 ± 0.0001
TOC (mg/L)							
Affluent	729.7 ± 2.1	717.3 ± 4.5	739 ± 2.1	689.4 ± 5.6	731.3 ± 3.5	717.4 ± 2.1	724.3 ± 2.5
Control Reactors effluent	563.5 ± 18.8	508.9 ± 21.7	482.3 ± 19.3	535.4 ± 19.1	472.8 ± 22.6	469.1 ± 10.2	454.9 ± 2.9
Polarized reactors effluent	557.6 ± 42.1	558.9 ± 70.1	526.5 ± 62.4	379.5 ± 6.7	333.2 ± 38.7	310.8 ± 7.5	226.7 ± 7.4