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A NEW BACTERIUM INTRIGUING THE SCIENTIFIC WORLD BY ITS PARTICULARITIES: HALOMONAS TITANICAE

From its properties to the potential applications to solve some environmental issues

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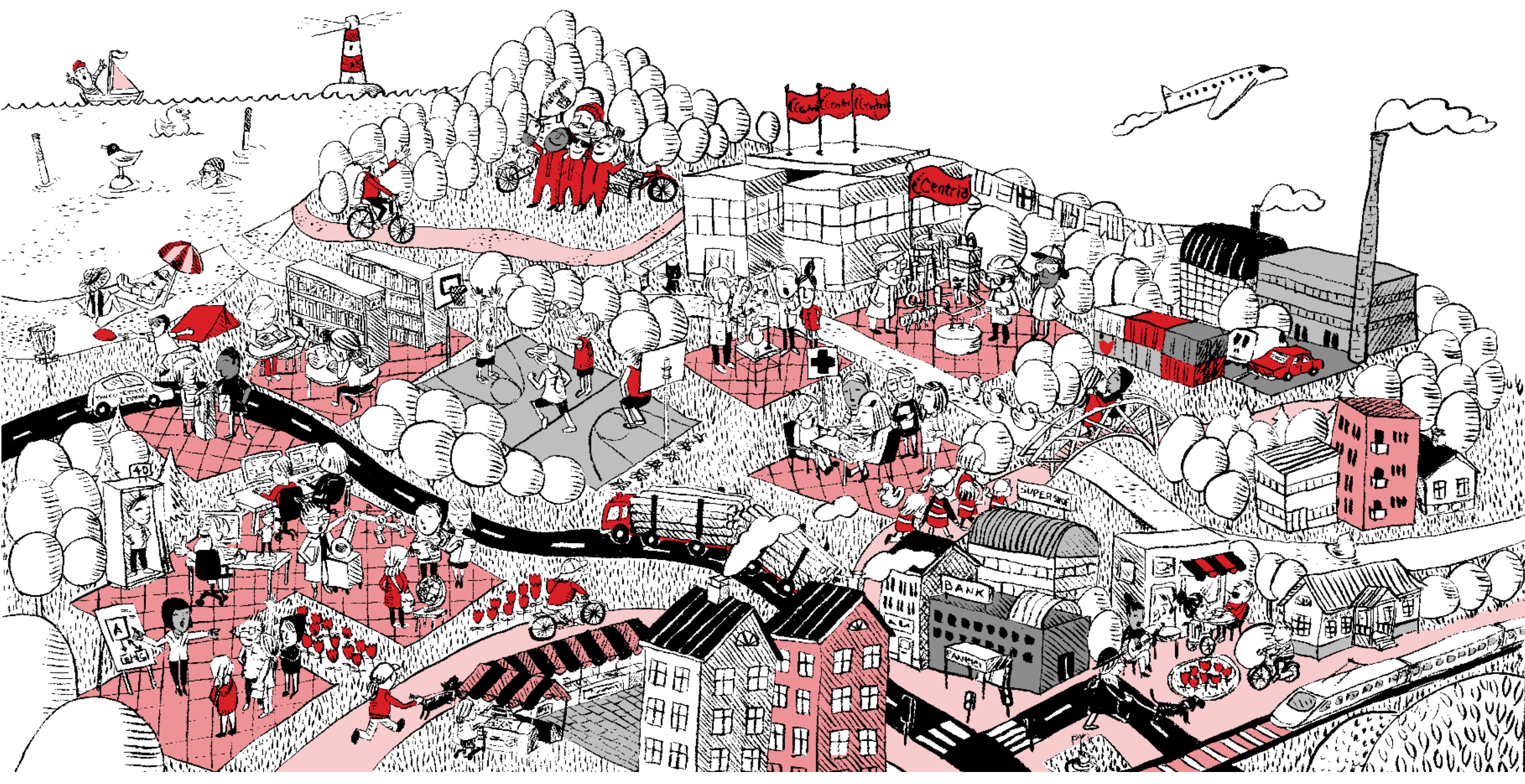

ABSTRACT

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Name of thesis A NEW BACTEIRUM INTRIGUING THE SCIENTIFIC WORLD BY ITS PARTICULARITIES: HALOMONAS TITANICAE. From its properties to the potential applications to solve some environmental issues.		
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The aim of this thesis was to present a new discovery in the scientific world which is the bacterium Halomonas Titanicae. Beyond being an incredible microorganism resisting to extremes conditions, it represents a hope for the science regarding its properties of "chewing" the iron and the steel. However, due to its irreversible activity, it represents a danger for the conservation of the UNESCO heritage which wanted to keep the Titanic wreckage intact. Finally, considering the actions of the bacterium upon the iron and the steel, it would be interesting to study the eventual applications concerning the cleaning of the seas and oceans.

This report gives different points of views of this new bacterium, causing so many debates, to understand what it is so special, and how it represents a challenge for the scientific and the cultural world.

The thesis is divided in six main sections including the introduction giving general information about the subject of this thesis to have some basis before getting into the report. The second chapter gives information about the bacterium to understand where it comes from, to detail its structure and biological activity. The third chapter is about the reaction occurring at the bacteria – iron interface. The corrosion is a natural phenomenon which can damage structures, but it exists different forms of corrosion. In this report, the aqueous corrosions and the microbiologically influenced corrosion will be studied more deeply. The next section deals with the experiments that could have been done. It was to show the influence of different factors (environmental conditions, microorganisms) on the corrosion rate to overcome how the Titanic could disappear by 2030. The fifth chapter is about all the different applications possible concerning the bacterium, the issue it caused regarding the Titanic future, and all the unanswered interrogations concerning Halomonas Titanicae.

It is important to study the nature and the mysteries of this bacterium because in some cases, it is possible to apply the properties for good purposes such as the cleaning of the Earth which is polluted by humans: it is called biomimetic. Understanding the biological process of organisms is a clever way to cope with the environmental issues and impacts around the world.

Key words
Halomonas Titanicae, Corrosion, Bacterium, Cleaning of the oceans and seas

CONCEPT DEFINITIONS

Bp: Base pair

DO: Dissolved oxygen

IRB: Iron Reducing Bacteria

MIC: Microbiologically Influenced corrosion

MPa: Mega Pascal

NaCl: Sodium Chloride

O_2: Oxygen

PET: Polyethylene terephthalate

RNAr: Ribosomal RNA

SRB: Sulphate Reducing Bacteria

UNESCO: United Nations Educational, Scientific and Cultural Organization

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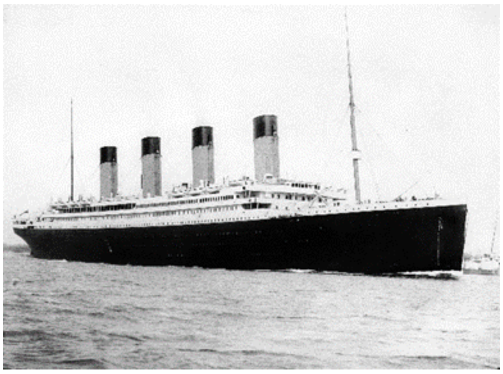
1 INTRODUCTION

Hidden deep into the Atlantic Ocean, 3.8 km below the surface, is living a fascinating microorganism which could solve some current world issues due to its remarkable properties. This thesis will be about the bacterium Halomonas Titanicae discovered on the Titanic wreckage (National Geographic 2010). It has the ability of eating, or chewing matters composed of metals or irons. The topic has been chosen because it belonged to the marine biology sector, which presents many challenges explaining why the report focused on this incredible microorganism. Halomonas Titanicae is a discovery because it belongs to a new type of bacterium. It has never been identified precisely, except grouping into other wide categories (bacteria or fungi). The ship (PICTURE 1) sank 98 years ago and has been discovered again in 1985.



PICTURE 1. RMS Titanic leaving the Southampton harbour the 10th of April 1912 (BBC 2021)

From this event, there were studies made on the organisms living on the wreckage to analyse them. It was this way that Halomonas Titanicae has been discovered by scientists (National Geographic 2010).

The main question about this bacterium was how it was possible for a microorganism to consume metals or irons, but then the study has been carried out on a bigger scale: How does the Halomonas Titanicae bacterium represent a challenge for the scientific community regarding its remarkable properties to exploit but also a challenge for the cultural world since the Titanic wreckage is under the protection of the United Nations Educational, Scientific and Cultural Organization? (UNESCO)

To answer this question, the starting point of the study will be to go through the history of the bacterium and its characteristics to understand how it works. Then, the reactions occurring on the wreckage will be developed to explain in which way the bacterium degrades the metal or iron. In the third chapter of this thesis, the goal was to develop my own knowledge by doing different experiments to illustrate the actions of the bacterium but because of some complications, only the protocols and experiments conjectures will be developed to explain the reasoning. To make the thesis more concrete and scientific, data generated by other scientists will be added to the description of the experiments. In the final chapter, there will be a discussion about all the data gathered about the bacterium to determine if the properties of the bacterium could not be used for different environmental purposes. The action of the bacterium on the wreckage causing ethical debates will be also discussed.

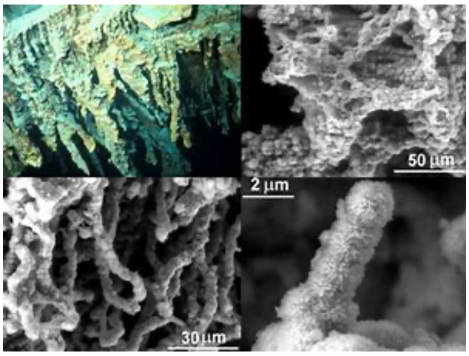
The expectations form this thesis would be to develop the reasoning by having a concrete and real study case, to improve the scientific way of thinking, to develop the ability of analysing and criticizing data extracted from scientific documents and experiments' protocols. Finally, associating the scientific knowledge to the study realized in this report, the reasoning will be extended the idea to real applications to overcome world issues such as pollution. It will also develop an opinion regarding cultural debates existing upon the disappearance of the Titanic.

2 THE METAL – MUCHING BACTERIA

This new bacterium discovered on the Titanic is particular because of its activity and the potential it represents. It has created a real interest in the scientific community because its properties representing a challenge for scientists to understand. Besides, as far as it considers the shipwreck of the Titanic, some ethical aspects can be addressed regarding the conservation of the UNESCO heritage wreck.

2.1 History: from the catastrophe of the Titanic in 1912 to the appearance of the bacterium threating the survival of the wreckage

It started on the 14th and 15th of April 1912, when the Titanic catastrophe occurred. It has caused more than 1500 victims and the wreck sank 3800 m below the surface of the water (Loumé 2018). It has stayed there many years before being discovered Robert Ballard, oceanographer at the university of Rhode Island in Narragansett in 1985 (BBC 2018). The first identification of the bacteria had been made by scientist at Dalhousie University in Halifax in Canada. They collect samples of the surface of the wreckage to analyse them. The remaining question was to know if the bacteria existed before the sinking of the Titanic or not. In 1991, these samples were compared to rust formations called "rusticles" (PICTURE 2). It is a porous structure of corrosive by-products (National Geographic 2010).



PICTURE 2. "Rusticles" on the Titanic wreckage (BBC, 2020)

At this stage, the bacterium was not officially identified because it had not been isolated from the mix composed of other types of microorganisms. Because even though the Titanic is a wreck, it is home to thousands of micro-organisms. They colonized the wreck from the moment it has touched the bottom of the seas/oceans and they make quickly what is called biofilms over all the surfaces of the wreck (Loumé 2018). But at this place, where there is no light, high pressure (38 MPa), and low temperature (4 ºC), life would not be able to survive or even to appear. Under these conditions, if they had not had any life forms, the corrosion should not have appeared, and the wreck should have been preserved (BBC 2018). Some years later, it has been finally the scientist Henrietta Mann who isolated and identified one category of bacteria from the mix which was Halomonas Titanicae (National Geographic 2010). Twenty-seven other species have been discovered in the rusticles but research is still ongoing to find other bacteria with the same function as Halomonas Titanicae. However, the bacterium has been classified due to the variability of the 16S rRNA gene (Sanchez-Porro 2013). From analysis made on the microorganism, it was possible to estimate the year of the Titanic disappearance based on the bacteria activity which would accelerate the speed of the metal corrosion. By 2030, the Titanic should no longer exist in the depths of the Atlantic Ocean. (BBC 2018).

2.2 Halomonas Bacteria

First, the family to which belongs the bacterium studied should be introduced. Due to its name Halomonas, the bacteria belonging to this family are halophiles which is the principal characteristic. It means that their growth is based on the high NaCl concentrations in the medium. Beisdes, Halomonas bacteria are adaptable to different conditions (temperature and pH parameters), so it can be used for other industrial purposes (Sanchez-Porro 2013). Besides, the halophiles microorganisms have a tolerance towards acidic pH such as sodium acetate, succinic acid, or itaconic acid (Zhang 2020).

The general structure characteristics of the Halomonas bacteria are that they are Gram negative cells in the shape of sticks (PICTURE 3). The cells are often not coloured or yellowish.

PICTURE 3. Bacterium, Halomonas Titanicae (BBC 2010)

Furthermore, as most microorganisms live in extreme conditions, an equilibrium with their environment must be achieved to survive. They are composed of fatty acids such as C (18:1) omega7c, or C (16:0) for example. They are not immobile microorganisms and often move by means of a flagellum or by using polar forces. (Sanchez-Porro 2013).

Halomonas bacteria consume essentially micro and macro nutrients present in their surroundings. However, it is possible for certain type of bacteria to survive without some of the nutrients required. For example, if there is no nitrate in the environment, the bacteria can survive in anaerobic conditions using glucose as nutrients. Others denitrify nitrate into nitrogen to gain energy for their metabolism (Sanchez-Porro 2013).

As they live in extremes conditions, there are specific places around the world where they will preferentially grow. The main characteristic of the medium is the high salinity such as the Dead Sea and the frigid waters of Antarctica. However, Halomonas Titanicae belongs to the ones living in deep-sea waters (Sanchez-Porro 2013).

Due to the scientific and technological progress, the sequencing of the genome has been developed. Indeed, from 2010 to 2013, about sixteen strains have been sequenced entirely or partially (Daoud 2020). In TABLE1, there are some of the strains which have been completely sequenced by scientists.

TABLE 1. Halophilic strains having their genomes completely sequenced (Daoud 2020)

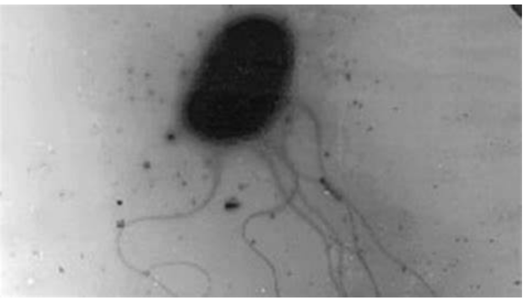
Strain	Lenght (pb)	Proteins	Submission date	Last modification	Genome ID in NCBI
Halomonas elongata DSM 2581	4.061.300	3628	17/9/2010	30/07/2015	3125
Halobacterium hubeiense	3.130.350	3102	17/12/2015	7/1/2016	42779
Halobacterium salinarum ATCC 700922	2.571.010	2540	14/07/2000	3/8/2015	1051(1)a
Halobacterium salinarum DSM 671	2.668.780	2592	12/2/2008	3/8/2015	1051(2)b
Haloaroula narismortai ATCC 43049	4.274.640	4100	2/11/2004	2/8/2015	1084
Natrinema pellirubrum DSM 15624	4.309.270	3981	27/12/2012	2/12/2015	11383
Haloferax volcanii DS2	3.939.820	3734	23/03/2010	15/09/2016	1149
Halobacillus halophilus DSM 2266	4.170.010	3942	11/4/2012	1/8/2016	11352
Chromohalobacter salexi-gens	3.696.650	3233	11/4/2006	3/8/2015	828

2.3 From Rusticles to New Type of Bacteria: Halomonas Titanicae

The given name of the bacteria comes from the place where it has been discovered: on the RMS Titanic. However, it is not only the bacteria activity which causes the Titanic destruction but a combination of different factors involving the current, the salt and seas/oceans reactions (NBC New York 2019).

2.3.1 Characteristics and Structure of The Bacterium

Halomonas Titanicae is not a pigmented bacterium, or it can be only yellow tinted. Even if the bacteria have been discovered by extraction on rusticles' samples, its identification has been done only in 2010 (Sanchez-Porro 2013). In fact, according to scientist Henrietta Mann, the bacteria would "chew" the steel/iron and transform it into iron crystals which will be released into the environment (Cite Scolaire Internationale NK). They use the rust to gain energy in the form of electrons from the iron degradation. Besides, it seems that the reactions have occurred inside and outside the microorganism bodies. It is for this reason that scientists have described the bacteria as an organism "eating" the wreck by aqueous corrosion. But it is still a chemo-organotrophic organism with a respiratory system. It means that they oxidize organic matters to produce energy which is the case of many bacteria (Sanchez-Porro 2013). The reactions are possible because the organism produces itself the oxidase or the catalase required. However, the growth can also happen in anaerobic conditions, but glucose is needed. Furthermore, the metal remains the main source energy of the bacteria. Besides, Halomonas Titanicae is a bacterium which is halophile, gram negative, of the genus Halomonas as explained. As the bacterium is halophilic, this means that it can survive in an environment with a high concentration of salt, such as oceans or salt marshes, for example. These concentrations scale can vary from 0.5% to 25% but as every organism the best conditions for the growth of the bacteria are from 2% to 8% of NaCl and between 30 to 37 ºC (Cite Scolaire Internationale NK). However, the bacterium is psychrotolerant mesophilic, which means that it can grow at low temperatures, such as 4 ºC, which can be found in the depths of the oceans/seas where the pressure is high. The rate of development of the organism is just slowed down. In 2010, Sanchez-Porro wrote an article about bacterium's cell structure, size, metabolism, and genome (Sanchez-Porro 2013). Halomonas Titanicae is not a big bacterium, it has a bacilli shape, and it measures approximately 0.5-0.8 x1.5-6.0 µm. Its genome is composed of 1453 bp and it is linear. Moreover, this microorganism is not immobile and can move thanks to peritrichous flagellum (PICTURE 4).


PICTURE 4. Halomonas Titanicae, a moving bacterium (Bhavieen Kaur & Henrietta Mann NK)

In its genome composition, there is a high Guanine/Cytosine content giving to the bacteria this ability to grow in extreme conditions. Halomonas Titanicae is a bacterium that can survive in conditions which are often not suitable to most lifeforms: deep water where the light is absent at crushing pressures (Sanchez-Porro 2013).

The pathology degree of Halomonas Titanicae has not been clearly established but scientists know they could infect humans. Indeed, there is no evidence of actual impacts. The bacteria still a hazardous organism to metals specially with the ones having high levels of rust. However, regarding the environmental conditions of the bacteria, there is an interrogation about the ability of Halomonas Titanicae to survive in such hostile environment (BBC 2018).

2.3.2 Extremophile Bacteria: Ectoin

Because of the location of the wreck of the Titanic, the conditions of living for microorganisms are extreme, and make it difficult for them to survive (National Geographic 2010). However, some organisms have succeeded to grow in these environments: they are called extremophile organisms (BBC 2018). Different factors make the life difficult to settle such as the salt concentration in the water which often causes cell death. Water of oceans and seas are composed of many components such as nutrients and other bio elements which play a role in the osmosis phenomenon (BBC 2018). Due to this reaction, the salt concentrations are important regarding the stability of the microorganisms. To cope with this issue, they produce organic matters like sugars and amino acids to keep the balance of the cell. But in the environment studied, the salt concentrations are more important than other places in the world making the process even more difficult (BBC 2018). As consequence the osmosis is increased, and it can affect the cell integrity.

However, Joe Zaccai's team from the Laue-Langevin Institute in Grenoble discovered in 2016 that the bacteria Halomonas Titanicae produced a molecule capable of making the cell resistant to the osmotic pressure. This molecule is the extremolytes called ectoin. Ectoin has been identified by Galinski in 1985 in another species of bacteria named Ecthothiorhodospira Halochloris. They belong to the same type of bacteria which are extremophile and need to resist to the extreme conditions present in their environment. But this ability has been discovered in a wider range of bacteria Gram +/- like Halomonas Titanicae (CEA 2016). So, ectoin is a small molecule, composed of a cyclic amino acid derivative (FIGURE 1).

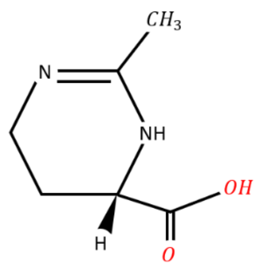


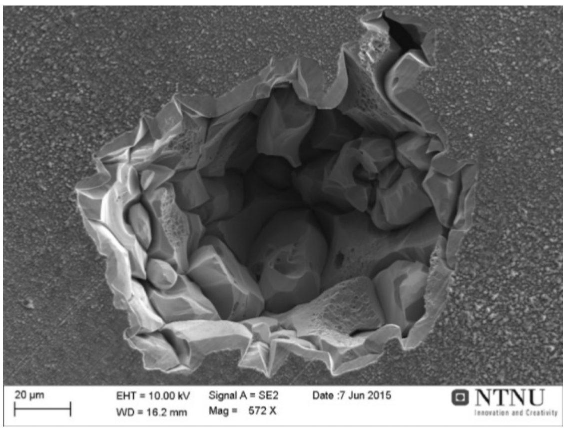
FIGURE 1. Ectoin (CEA 2016)

The concentration of the extremolyte depends on the salt concentrations and other factors such the temperature of the environment (Park 2020). Ectoin minimizes the water effect on the cell when it is in high concentration (Scientific Information NK). The molecule helps resist osmotic pressure, which can be lethal by increasing the molecular composition of the cell. The process is based on preferential exclusion in addition to the effect of water which will remove osmolytes from the surface of the protein leaving the protein to hydrate (Scientific Information NK).

Furthermore, the process is composed of several reactions. The steric exclusion, which is based on the size of the molecule, is needed when the protective molecules on the surface are larger than water. There are also the pressure changes which cause the exclusion of ectoin from the water-molecule interface. The third step is the preferential hydration which is the action of the formation of water molecules in clusters due to the osmolytes effect (Scientific Information NK). As the salt concentrations in the environment where Halomonas Titanicae live are high, this increases the phenomenon of osmosis. Besides, the NaCl has a chaotropic effect on water molecules by decreasing the interaction between them. Whereas if ectoin is in the solution, it will increase the number of water molecules, so ectoin increases the water molecules interaction (Scientific Information, NK). It demonstrates the organizing and complexing properties of the molecules. By keeping the water molecules close to the surface of the cell, it regulates the osmosis and does not destroy the cell. It protects the lipid membranes composed of different systems responsible of the viability of the organism. Even though Halomonas Titanicae can survive in a such extreme environment, the mechanism of corrosion operating on the wreckage will be studied more in detail to determine if the bacterium has a real impact on the rate of the corrosion.

2.3.3 A Symbiotic Organism

A lot of microorganisms have found a way to survive by living in symbiose with other organisms when the environment is not sufficient. Due to the location of the Titanic wreckage, the nutrients, and the oxygen that the bacteria require can be rare, so the symbiosis of the organism with another one is not impossible. Furthermore, Halomonas Titanicae has been extracted from an algae culture, Spirulina Patensis. In the marine environment, the pioneer bacterium first creates a biofilm over a surface. The biofilm created will provide a suitable and attractive environment for organisms to develop like algae. So, in the biofilm the algae produce oxygen and organic matters which can be used after by the bacteria to grow. Because it is a symbiotic relation, the bacterium also provides resources for algae which is often vitamins or carbon dioxide. This association represents a danger for the marine materials which is the biofouling effect damaging the boats' structure. Indeed, the formation of the biofilms over the surface of the machines can change the physical-chemical properties of the components at the metal/biofilm interface. It can be described as a degradation by localized corrosion, as shown on PICTURE 5 (Dong 2019).



PICTURE 5. Localized corrosion (Erik Haugan)

3 CORROSION

Since the bacteria Halomonas Titanicae has been identified on the wreckage of the Titanic, the reactions occurring on the components of the boat have been studied. Indeed, it appears that the bacteria were "chewing" the boat by forming a layer of rust weakening the structure of the wreck (National Geographic 2010). It has been compared and identified as corrosion due to the formation of "rusticles".

3.1 Definition

The corrosion can be defined as an action of corrosive substances or due to the corrosive environment. It changes the internal structure and composition of materials by chemical or physical transformations. The reaction is irreversible. It can be described as the ability of metals and their alloys to return to their original condition under atmospheric agents. The efficiency of the reaction depends on the corrosive substances and the environment considered (Balan 2018). For example, metals which are the components considered in this study case, are chemically active under air and moisture, shown on FIGURE 2. Some of them are most reactive than other like magnesium, iron, or aluminium. This chemical change can happen in natural condition (preferred) or caused by human activities (Balan 2018).

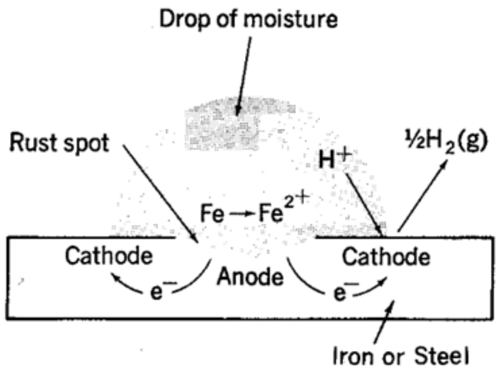


FIGURE 2. Corrosion process under air and moisture conditions (M. Di Benedetti)

Corrosion results from the degradation of components due to the oxidation of two main oxidants which are air and water. It weakens the structure of the object until it deteriorates. Besides, not all materials react in the same way to corrosion, it depends on the fluid in contact with the materials and the properties of the material itself. The result of the corrosion is to convert the metal to more chemically stable form like oxide, hydroxide, or sulfide (Balan 2018).

As expected, corrosion only takes place in certain places under certain conditions, otherwise the planet would disappear in a short time. Indeed, there are certain conditions that must be met for the reaction to occur. First, the presence of oxygen or air in the atmosphere is mandatory as well as the presence of moisture or water. They are the required fluids. Then, there are the materials such as metals in the case of the Titanic (Tanzi 2019). Furthermore, the rate of corrosion is based on different factors which can increase or decrease the rate. The structure of metal, the electrical sensing nature of the metal, the presence of gases in the atmosphere, and the presence of salt in the water are four factors to consider when the rate of corrosion is studied (Tanzi 2019). Corrosion is the progressive degradation of the structure due to the oxidation of the component weakening it. Besides, sometimes it is not the only result of one cause but the combination of several reactions such as natural degradation due to the environment, or due to human activity which can have an impact on the environment composition.

3.2 Different Types of Corrosion: Mic and Aqueous Corrosion

There is not only one form of corrosion, depending on the components, but several types of corrosion also exist. However, the corrosion occurring on the wreckage of the titanic belongs to the microbiological one and the aqueous corrosion due to the location of the boat. So, the focus will be done on these two types to explain to what extend Halomonas Titanicae is involved in the degradation of the wreckage. First, the microbiological corrosion because it happens between the bacteria and the wreckage structure, then the aqueous because it occurs in the depth of the Atlantic Ocean which is salty water (Phd Javaherdashti 2020).

Corrosion is based on an interaction between a matter and a fluid. It can be described as the exchange of electrons through a conductive metal or due to ions present in a conductive medium. The microbiologically influenced corrosion is the one involving microorganisms, shown in the PICTURE 6.

PICTURE 6. Microbiologically influenced corrosion (Tim O'Leary 2017)

In the case of the Titanic, it is about algae and the bacterium HT. In fact, observations made on both organisms revealed often that microorganisms live in symbiose with other organisms to survive (Telegdi 2017).

Due to the presence of microorganisms, the notion of biologically influenced corrosion can be introduced. Even if we are considering the reaction, they are not directly involved in the reaction because they can just accelerate the mechanism. The action of the bacteria has been identified as a factor of the rapid corrosion of the metals and its alloys in many conditions such as seawater (AMPP NK). Then, because the wreck is in the ocean, we can speak also about aqueous corrosion, shown on the PICTURE 7. It is an electrical reaction caused by the environmental parameters and it often degrades the iron and steel in the case of the marine engines (Waverley Brownall 2016).

PICTURE 7. Aqueous corrosion (LPD Lab Service)

3.3 Corrosion Upon the Eh40 Steel – Iron by Halomonas Titanicae

By focusing more on the reactions happening on the Titanic wreck, some studies have been made about the corrosion process and the different factors which could influence the rate of the reaction or its efficiency. The corrosion is not only influenced by the organism considered but also by the environment. Regarding Halomonas Titanicae and the steel/iron of the Titanic (EH40), two different conditions have been tested: anaerobic and aerobic systems. As the corrosion is based on the electron acceptors used, the oxygen concentration was studied because oxygen plays a role in the corrosion process (Wang 2021).

They conjectured that depending on the conditions of living of Halomonas Titanicae, the corrosion would be inhibited or favourited. The oxygen concentration in the medium was determined as a factor of the rate of the corrosion because depending if the system was (an)aerobic, it had an influence on the reaction. Indeed, the corrosion is based on the final electron acceptor efficiency, which is the reduction of the oxygen by the reduction of the electron acceptor solid Fe(III) to Fe(II) which is soluble (Wang 2021).

Since microorganisms are involved in the corrosion process, the reaction can be defined as a Microbiologically Influenced Corrosion (MIC). The reaction includes their activity which can be direct or indirect, and it represents about 20% of the loss by corrosion. Although biocorrosion has always existed, scientists do not know the full process. Different categories of bacteria have been identified such as the sulfate-reducing bacteria (SRB), the acid-producing bacteria, and the iron-oxidizing bacteria which correspond to Halomonas Titanicae (Wang 2021).

The category to which Halomonas Titanicae belongs is that of iron-oxidizing also called the iron reducing bacteria (IRB). It induces the metals corrosion by reducing the Fe(III) solid to Fe(II) soluble. The result of this reaction is to destroy the corrosion layer which will expose a new surface. Due to the specific properties of the reaction, the type of IRB depends on the environment and the particularities of the bacteria. For example, a group of scientists has discovered that the ability of bacteria to react with the metal was potentially in relation with another organism (Wang 2021).

So, the study was to describe the mechanism occurring on the Titanic components (EH40) due to Halomonas Titanicae activity. They focused on electron acceptors, a common regulation parameter for bacteria and corrosion. It was determined that under aerobic conditions, the bacteria only used oxygen as the final electron acceptor causing the decrease in DO concentration and inhibition of corrosion. Whereas in anaerobic conditions, the bacteria used the Fe(III) as final electrons acceptors which stimulated the rate of the corrosion (Wang 2021).

3.4 Association of Algae

Halomonas Titanicae, identified from a rusticle, is involved in symbiosis with other organisms for survival reasons which made scientists wonder if they were also involved in the corrosion phenomenon. (Dong 2019). Algae are known to be corrosive organism but few of them have been reported to be involved in the corrosion reaction. The bacterium increases the rate of corrosion upon the material of the wreck, and it seems that the corrosion is influenced depending on the medium. From this statement, the question was to clear the relation between the algae Spirulina Patensis and Halomonas Titanicae beyond the symbiotic exchange (Dong 2019).

Zhang, et al. (2020) studied the corrosion upon the boat component which is often 304L by using the bacteria and electrochemical techniques. They first purified a sample collected from the RMS Titanic to collect only the bacteria and the algae as shown on the FIGURE 3.

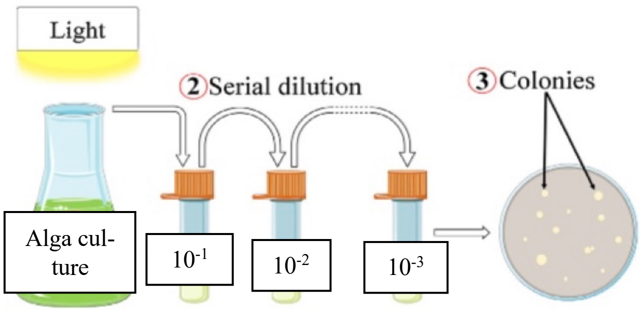
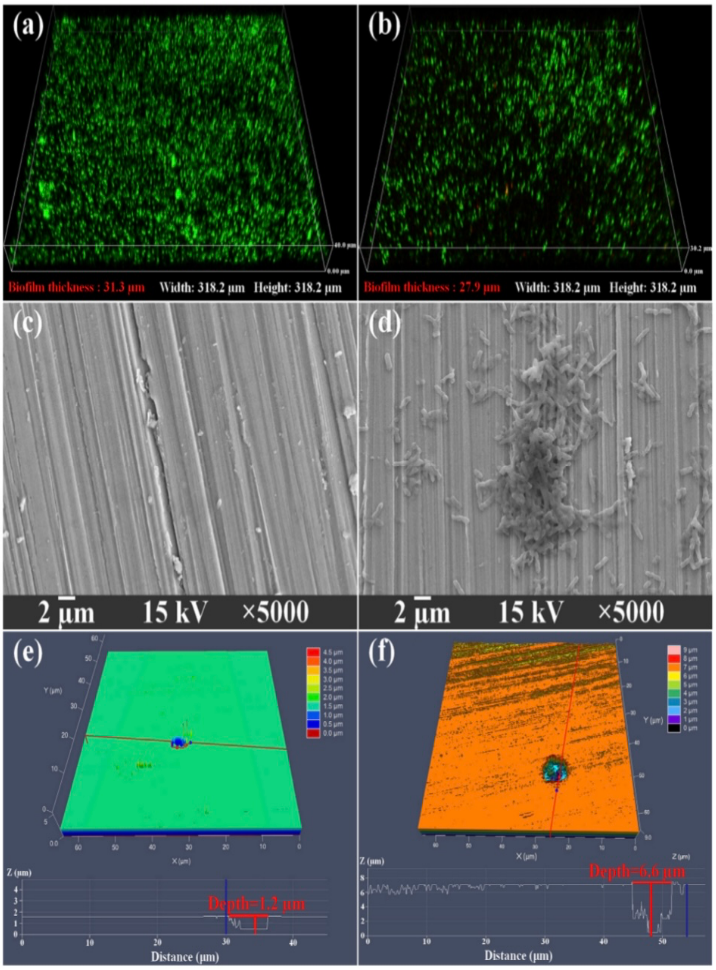


FIGURE 3. Schematic illustration of isolation and identification of the algae associated bacteria from S. patensis culture (Dong 2019)

Then, they were put into cultured cells to be purified again to increase their number. The sample is treated by RNAr 16S sequencing. To study the bacteria development and activity, different mediums were used, one is sterile and another one with artificial sea water (Dong 2019).

The results of the experiments showed that Halomonas Titanicae induced a Microbiologically Influenced Corrosion. Besides, the two mediums used, have illustrated the fact that the bacteria had a higher activity in biotic medium, where algae were (PICTURE 8). Through this study, the role of algae has not been determined because scientists do not know if the algae are involved in the biocorrosion (Dong 2019).



PICTURE 8. CLSM 3-D images of H. Titanicae biofilm on the 304L SS samples surfaces after (a) 7 d, (b) 12 d; SEM images of the 304L SS coupon surfaces after exposure to (c) sterile medium and (d) biotic medium for 14 d. The largest pit depth on 304L SS coupon surfaces after 14 d of exposure to (e) sterile medium and (f) biotic medium

4 EXPERIMENTS: TO UNDERSTAND THE CORROSION MECHANISM AND HOW THE BACTERIA INFLUENCES CORROSION

Initially, different series of experiments were planned to explain the overall functioning of corrosion but also the corrosion that the bacteria operated on the wreckage. The first purpose was to show the impact of environmental conditions on the corrosion process. Then, the idea was to understand the effect of the bacteria and the role it would play on the corrosion rate of the metal. However, due to some issues (difficulty of having the bacteria in laboratory, time management to realize the experiments), the realization of them was not possible, but a comparison with other similar experiments will be developed in the report to keep on the general reasoning of the thesis. Besides, it appears that the bacteria were pathogenic for humans' health, and it was complicated to make it grow in a laboratory due to the specific conditions.

4.1 Protocols

Due to the fact that my subject was about a living organism, it would be interesting to try to realize different experiments to observe and analyse the behaviour of the microorganism in different conditions. The purpose of the experiments would be to understand the organism, its mechanisms and the effects it has on its environment.

4.1.1 Influence Of Environmental Conditions on The Corrosion

The objective of this experiment was to determine if the environmental parameters influenced the corrosion of the metal. The idea was to treat different steel/metal sticks into different medium and to observe how the corrosion would act on it. Therefore, the experiments must be repeated many times for the statistics to be meaningful. As reactant of the corrosion, we know that the oxygen present in the atmosphere and the water under the diatomic form O_2 is the final electron acceptors of the corrosion. By changing the composition of the medium, the effects on the corrosion is observed. Besides, trying to be as realistic as possible, every other parameter such as the temperature, the stream will be reproduced by laboratory techniques. Knowing the dioxygen properties, the corrosion products must be in the medium where the oxygen concentration would be the higher. To do the experiment, thirty-two beakers with a stick of metal immerged in each of them will be prepared. They had different compositions as shown in TABLE 2.

TABLE 2. Compositions of the different medium where steel and metal sticks are submitted for corrosion observation

<table>
<tr><th colspan="2"></th><th>Water (ml)</th><th>Water + oil layer (ml)</th><th>Water (ml) + NaCl (g)</th><th>Water + NaCl + Oil Layer</th></tr>
<tr><td>High [O2]</td><td rowspan="2">Cold T</td><td rowspan="8">40 w</td><td rowspan="8">20 w / 20 o</td><td rowspan="8">40 w / 1.6 g</td><td rowspan="8">20 w / 20 o / 1.6g</td></tr>
<tr><td>Low [O2]</td></tr>
<tr><td>High [O2]</td><td rowspan="2">Hot T</td></tr>
<tr><td>Low [O2]</td></tr>
<tr><td>High [O2]</td><td rowspan="2">Cold T + agitation</td></tr>
<tr><td>Low [O2]</td></tr>
<tr><td>High [O2]</td><td rowspan="2">Hot T + agitation</td></tr>
<tr><td>Low [O2]</td></tr>
</table>

The first experiment was based on visual observations, so the beakers must be left for several days. With the results, it would be possible to determine which medium favourited the most the corrosion. The mediums with low oxygen concentrations are supposed to be the ones with the high corrosion products because the steel would be the principal source for the electrons' transfer of the reaction. Then, the medium with the agitator will also have a higher percentage of the corrosion because it would increase the contact of the water with the stick of steel by frictional forces. Besides, the temperature is supposed to accelerate the time of reaction, so when the temperature is cold the corrosion would be more present. Finally, for the composition of the medium, as the oxygen is present into the water and the atmosphere, the beakers with the oil layer would have less corrosion than the other because one source of oxygen (air) will be removed.

4.1.2 Influence Of Bacteria on The Corrosion Rate of The Iron

This experiment was to determine the degree of implication of the bacteria upon the corrosion reaction. Two models are required, one which is the control sample and another one which is the test with the microorganisms. The medium composition will be the same as the place where the Titanic wreck is. It means that the conditions of the depths of the Atlantic will have to be recreated, as shown in the FIGURE 4. The pressure must be at 38 MPa, the temperature at 4 ºC, the streams of the ocean are reproduced using an agitator and the absence of light by doing the experiment in a dark room.

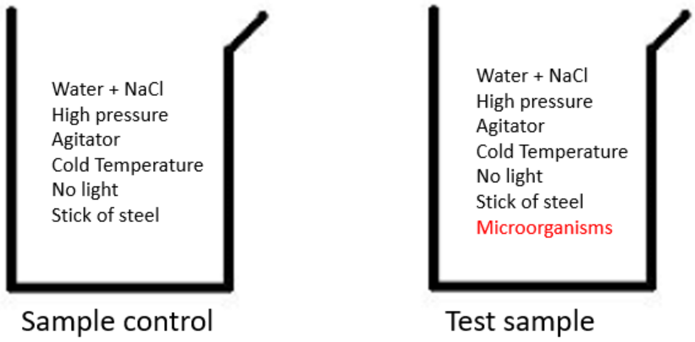


FIGURE 4. Schemas of the experiment: Testing the microorganisms' influence on the corrosion

To be able to compare the rate of corrosion, different factors will be studied and measured. The pH, due to the reaction the ions concentration will change (Fe^{3+}). The potential can also be measured because the corrosion is basically an oxidation and a reduction of a chemical element. As the potential cannot be measured directly, another electrode will be used: a saturated camel electrode which is the reference. Then, by comparing the different measures over a certain period, it would be easy to determine if the bacteria have a real influence on the reaction.

4.2 Bibliographic Data About Experiments

Because the experiments were not possible, this part of the thesis is based on theoretical work. The aim is to get as close as possible to the expected results by referring to experiments done by other scientists. However, due to the recent discovery of this bacterium, there was very little scientific literature that could provide information on the bacterium.

4.2.1 Influence Of Environmental Conditions on The Corrosion

It has been determined that oxygen had a role in the process. Molecular oxygen is a soluble gas which is present in many environments such as the water or the atmosphere. Some studies have determined that the oxygen had a real influence in aqueous solutions regarding the oxidation yields of the solutes (Lapuerta 2005). This is what is called the water chemistry involving the oxidation-reduction couple O_2/H_2O.

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \qquad (1)$$

Besides, it is important to notice that the dissolved oxygen is a good hydrate electron collector because it creates radicals which can react with the iron for example.

$$e^-_{aq} + O_2 \rightarrow O_2^{\cdot-} \qquad (2)$$

$$H^{\cdot} + O_2 \rightarrow HO_2^{\cdot} \qquad (3)$$

The final radical ($HO_2^{\cdot}$) will have the role of oxidant at the iron/water or iron/air interface. Moreover, due to its composition, the air is naturally oxidant (Lapuerta 2005). So, based on this information it would be logical that the samples with the oil layer would have less corrosion than the one in contact with the air because it has removed a source of oxidant (Lapuerta 2005). Besides, depending on the oxygen concentration the corrosion could have a different impact on the structure because if the concentration is high, the oxygen would be the main oxidant source whereas if the concentration is low, the oxygen will be replaced by another source such as the iron.

Concerning the agitation induced to reproduce the streams of the seas and oceans, it appears that the movement of the fluid (water) is not directly responsible of the corrosion but of the erosion. Indeed, it participates to the weakening if the iron structure (Insa Lyon NK). So, the samples having the agitation would have the highest corrosion products because it "favourited" the reaction by destroying the protection of the iron with the erosion.

Regarding the temperature, as every chemical reaction when the temperature rises the rate of the reaction increases too protection of the iron with the erosion (Konovalova 2021). The samples where the temperature is hot, there would have the more corrosion products. Finally, the salt in the water accelerates the degradation of the component five to ten times more than normal air or water. Moreover, the bacteria can cause damages too, which will be studied later (Rodriguez 2018).

4.2.2 Influence Of Bacteria on The Corrosion Rate of The Iron

The Halomonas Titanicae bacteria is unique for its activity on the shipwreck. It attacks the structure to degrade it. By analysing the process occurring on the different component of the ship, it appears that depending on the final acceptors used, the rate of corrosion will change. The oxygen and the iron are the two electron acceptors in competition depending on the environmental conditions (Wang 2021). When the oxygen is in large quantity (aerobic conditions), Halomonas Titanicae will tend to use the oxygen for its metabolism more than for the corrosion. However, if it is in anaerobic environment the bacteria will not have enough oxygen for its metabolism, so to create enough energy through reaction it will use the solid Fer(III) to turn it into soluble Fe(II) into the water weakening the structure of the wreck. In the depth of the Atlantic Ocean, there are less streams than at the surface, so the oxygen level is not often renewed explaining the lack of oxygen (Wang 2021).

Depending on the oxygen concentration, Halomonas Titanicae will not have the same behaviour. Indeed, the corrosion appears more frequently when the oxygen is in low concentration because it will tend to use the iron as source of electron acceptor to generate energy for its metabolism (Wang 2021).

4.3 Interpretations

Based on the results of two experiments, an assumption can be formulated which is that the corrosion is a natural phenomenon which is influenced by the surrounding organisms. The environment plays a role in the reaction regarding its composition (organic matters, salt concentrations, streams, microorganism) because it can affect the rate of the corrosion. Besides, in this present case, the microorganism considered is Halomonas Titanicae, and it has been demonstrated that its activity is maximal when the oxygen concentration is low. Consequently, the bacterium's activity is added to the natural corrosion rate, the degradation will be accelerated.

5 DISCUSSION

Within more data about the bacterium and the effects it has on the metals, it is interesting to focus on a bigger study scale. Indeed, Halomonas Titanicae has a real potential in environmental industry. Furthermore, some debates appear due to its hostile activity to the wreckages. Besides, as it is a new microorganism, many questions about it still unanswered.

5.1 Titanic Wreckage Facing Its Own Degradation Due to The Bacteria

Through the studies made on the Halomonas Titanicae bacteria and its activity on the Titanic wreck, it appears that it is responsible for its progressive deterioration over the years. Indeed, it does not only degrade the steel and metal structure of the wreck by corrosion, but it also accelerates its mechanism. Considering the properties of the bacteria and the studies realized on its activity, it is quite probable that the Titanic would disappear in a few years, 2030 according to the scientists (Loume 2018). Wrecks are not naturally occurring elements in the natural environment and are therefore contaminants in the marine environment, posing a threat to wildlife. However, beyond the source of pollution that the wreck of the Titanic represents, it has found its place in the marine world by serving as a refuge for animals. Moreover, the Titanic is not only a simple ship, but also the largest ocean liner ever built and is part of the UNESCO world heritage since April 15th of 2012, in accordance with the 2001 convention relating to the protection of any site having a cultural or historical character (UNESCO 2017). However, this legal protection is jeopardized by the Halomonas Titanicae bacterium that keeps deteriorating it. This puts the conservation of the wreck at the heart of many debates in the scientific community. While some perceive Halomonas Titanicae as a solution to eliminate metal from the oceans, others would like to see it controlled from a scientific point of view to ensure the conservation of the ship.

5.2 Questioning About Halomonas Titanicae

Several questions remain unanswered about the Halomonas Titanicae bacteria. One of the questions revolves around the origin of the bacteria. Because of the extreme living conditions to which the bacteria have adapted to survive (high pressure, very low temperature, no light source), the appearance of the microorganism remains a mystery. Although it was identified on the Titanic, scientists wonder if Halomonas Titanicae was already present before the sinking of the ship or if it is the event that causes the appearance of the bacterial strain. Moreover, Halomonas Titanicae needs specific parameters to live and have a stable activity as studies have previously shown.

On the other hand, as the main activity of the bacteria is corrosion attacking the shipwreck, iron is defined as the major element in its functioning. However, there is an interrogation of the utility of the iron in the survival of the bacteria. The parameters specific to the bacteria can be a criterium of survival of the bacterium and caused it disappearance if not present in the environment.

Another aspect of the bacterium that can be raised is its properties. Indeed, they corrode the wrecks in the seabed so they can represent a solution for cleaning the oceans, but do they have other properties that could be useful for other applications.

Therefore, further studies should be conducted to better understand the functioning of the bacterium and the potential or danger it may represent for the environment.

5.3 Applications (Industrial and Environmental)

Due to the incredible properties of the bacterium, humans can be inspired by the organism, which is called biomimetic, and use them as a tool to manage the water pollution, or to treat the water used in industries for instance.

5.3.1 Industrial Potential of the Metal Degradation caused by the Bacterium

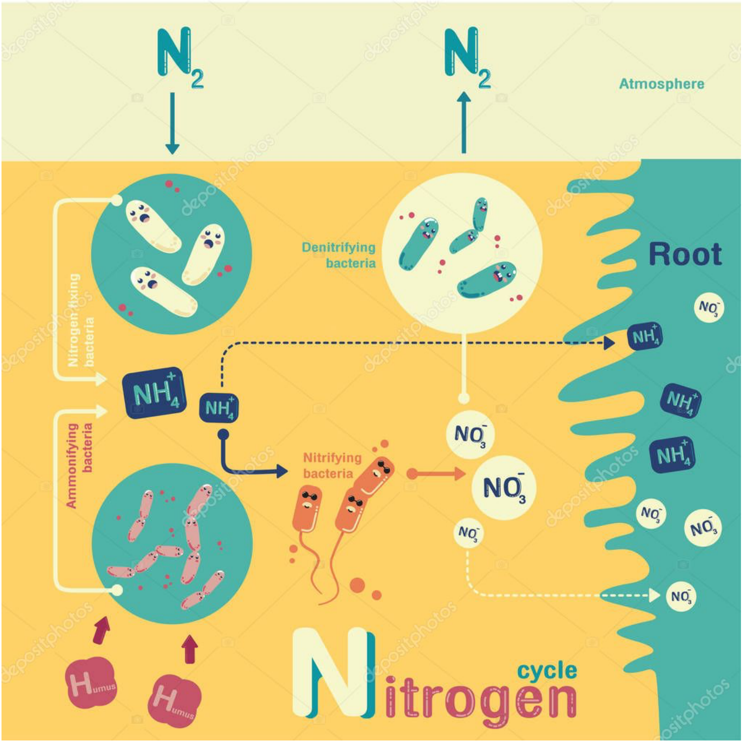
The specific properties of the bacteria are interesting for different sectors of the industry because it can be a source of development for biocatalysts. Indeed, the discovery of new bacteria means the discovery of new proteins which could be used for different purposes. The different microorganisms also live in many different environments so it could be advantageous to study them to learn how they survive in such biotopes. Metagenomic studies have shown the diversity of organisms living within certain organisms that may or may not be cultivated. It allows to identify and to isolate new enzymes having a potential in the biotechnology sector. For example, the halophilic cellulase is one the enzymes which can be produced presenting some survival properties like thermostable, halo-stable, and alkali-stable. The final products can be used in different fields like in the textile, detergent, and the food industries (Daoud 2020).

5.3.2 Removal Of Metals from The Oceans and Seas

From the Titanic catastrophe to today, there were several ships, planes, or other machines which have sunk into the oceans. Made from different metals and steels, they represent a source of pollution for the marine life all around the life. UNESCO (2003) reported about three million shipwrecks in the depth of the oceans (Little 2016). The bacteria could be used for cleaning the ocean by controlling its property of destroying metal/iron. It could be a biological way to clean up the oceans. However, Halomonas Titanicae cannot survive in other environmental conditions, so it could be interesting the biomimetic techniques to copy the bacteria's activity. But the interesting point of the bacteria was its ability to resist to extreme conditions corresponding often to the places where wrecks could be found.

5.3.3 Denitrification And Nitrification Due to Human's and Natural Activities

Pollution due to humans' activity has become one of biggest challenge to fix. Regarding the marine world and environmental problems, it is the nitrogen pollution caused in aquaculture sector. The pollution is generated due to the culture feeding which can release nitrogen into the environment. These elements are considered as pollutant for the ecosystem and need to be removed because they represent an obstacle to the ecosystem balance. However, it is not the only source of pollution, it can come from any sort of organic waste such as sewage released from the cities and other organic streams due to the humans' activity. Based on the nitrogen cycle, shown on PICTURE 9, it appears that some bacteria can manage the transformation of the nitrogen under its different forms. The reactions considered are the nitrification and the denitrification (Huang 2020). But the studies have mainly focused on bacteria found in fresh water, and scientists do not have enough information about the ones acting in salty water (oceans and seas).



PICTURE 9. Nitrogen cycle (Dr. Ray L. Winstead)

Studies are processed on the bacteria which can be found in mariculture. The goal is to develop a pool of organisms having the ability to remove or eliminate the nitrogen efficiently. Through all the different species existing and reported, only few of them have been isolated due to their high efficiency in the removal of nitrogen in the environment: *Marinomonas, Marinobactetium, Halomonas, and Cobetia*. The particularity of these organisms is that they work by association which means that they reach their full potential when they are associated to another one. That is the case of Halomonas Titanicae which is more efficient and stable when it coexists with another bacteria. The high ratio of nitrogen removal has been reported for the association of Halomonas Titanicae and Marinomonas Communis with 0.76 mg NO_2—N/L/h (Huang 2020).

The interesting property of the bacteria is that it transforms the nitrogen into biomass by assimilation or dissimilation. The main purpose of scientists is to design the best association between bacteria to clean water but the different biological techniques combining nitrification (autotrophic) and denitrification (heterotrophic) are complicated. The type of consortium used would depend on the type of pollution and the type of water considered because some of bacteria would be more efficient in some cases. However, it appears that the strains present in mariculture were using different ways of nitrogen degradation belonging to the nitrogen cycle (Huang 2020).

5.3.4 Combination With Other Biological Techniques of Cleaning

The progress of the biotechnology sector allows scientists to use organisms' properties to fix some world issue like the pollution caused by humans. As presented in this thesis, the bacteria Halomonas Titanicae has been identified and analyzed to understand its operation. Beyond the corrosion it does on the shipwreck of the Titanic, it has been determined that the bacterium has other properties such as the removal of the nitrogen in the environment. Based on this information, the bacterium can clean the ocean from impurities such as the metal/steel and the nitrogen. But the nature has other secrets because Halomonas is not the only bacteria able to purify its environment.

By focusing on the ocean pollution, metals are not the only pollutants but the plastic microparticles too (Zewe 2016). There are about five trillion of plastic items across the world which represent a threat for the ecosystem. A research team of Harvard University designed a project called Plastiback based on the bacteria E. Coli. By modifying its genome, it would be possible to destroy one of the main plastic pollutants present in water, namely polyethylene terephthalate (PET). The bacteria are in bioreactor acting on the polluted flows which are treated then released in another bioreactor for further processes, as shown on the FIGURE 5 (Zewe 2016).

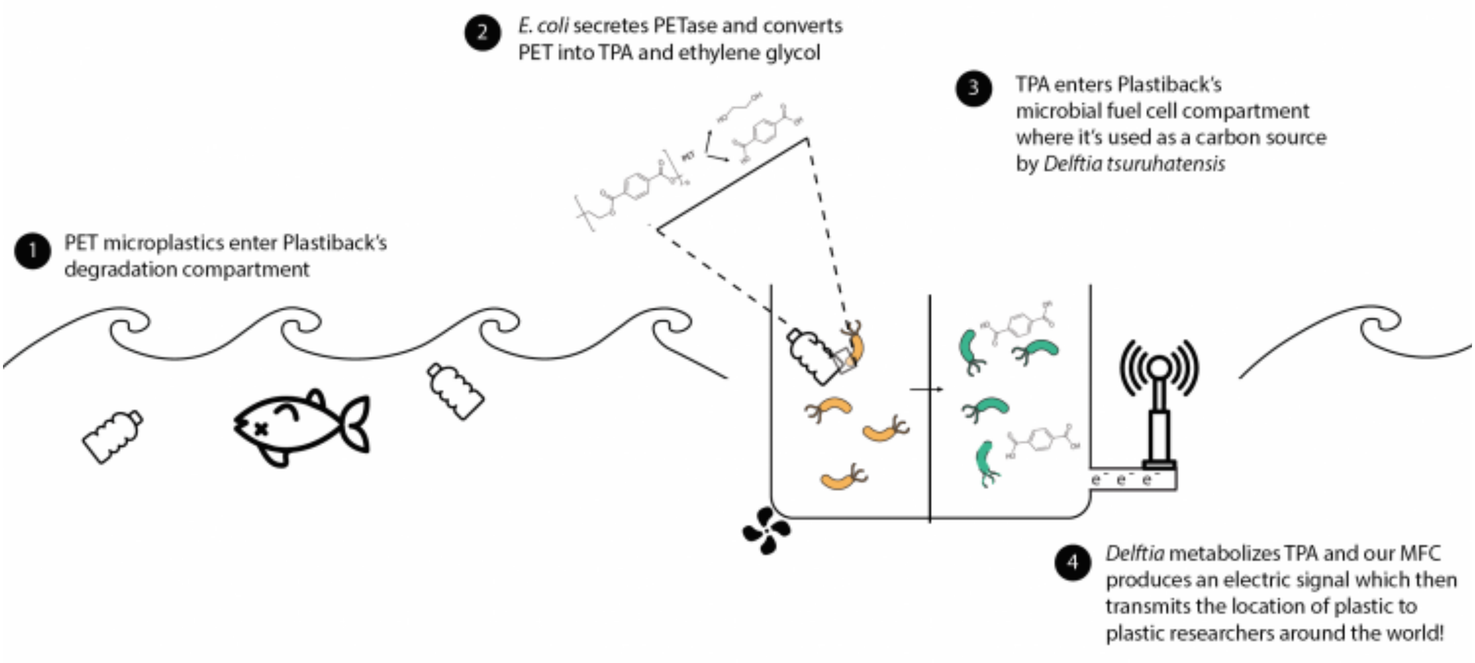


FIGURE 5. Cleaning water process through the project Plastiback (Harvard BioDesign 2016)

By combining the action of different organisms, it would be possible to clean the oceans and seas from the pollution caused by humans' activity through the years. It would represent a greener way of processing because no harmful or toxic substances would be released due to the biological properties of the organisms.

6 CONCLUSION

The world is still full of mysteries and knowledge that humans still underestimate. However, with new technologies and scientific progress, researchers are constantly making new discoveries. This was the case with the Halomonas Titanicae bacterium, which was discovered near the wreck of the Titanic. It aroused curiosity in the scientific community because the fate of the Titanic was at stake. Due to microbiological and aqueous corrosion mechanisms, less than ten years would remain before the Titanic disappeared. After studies on these microorganisms, the structure as well as the mode of operation of the bacteria could be described, but many points remain to be clarified. For example, the existing symbiosis between the bacteria and the algae raises questions about its consequences. Indeed, beyond the survival that the association of the two organisms ensured for each other, one wonders if it did not intervene in the corrosion mechanism. Despite the experiments carried out to establish any relationship between this symbiosis and corrosion, scientists have not come to a clear conclusion as to the participation of the alga in the process, even though the corrosive properties of alga are known.

Furthermore, while this bacterium seemed unique, there is an interrogation about its uniqueness and whether it could be found anywhere else on Earth. One might wonder if by gathering the same conditions in another place the bacteria could grow. The origin of the bacterium has also not been defined, as it is still not known whether it appeared before or at the time of the sinking of the Titanic. However, Halomonas Titanicae also represents a real potential for the biotechnology sector. By learning from its abilities, it may be possible to design solutions for the biological cleaning of oceans and seas by removing iron and ammonia. Extending this mode of operation by using the natural properties of organisms for environmental purposes, E. Coli which has been modified to clean the oceans of polyethylene terephthalate. It is therefore conceivable that by combining different living organisms, green cleaning systems could be developed.

On the other hand, leaving aside the biological aspect of the bacteria, the topic covers an ethical aspect about the Titanic. While it is part of the world's heritage of human activity, its disappearance is a matter of debate. Some would like to see the wreck remain on the ocean floor by developing a treatment for the bacteria, while others would simply like to see the Titanic disappear and extend this action to all wrecks found on the sea floor. There are two possible directions, one would be to find a treatment for the bacteria so that it can be controlled, and the Titanic can be preserved, while the other would be to simply exploit the corrosion capacity to purify the waters. However, there are other issues at stake, such as the maritime market where ships' hulls can be damaged, so the bacteria represent a possible economic market for the maritime sector. To conclude, this bacterium is a source of innovation and knowledge in the scientific and biotechnological world that required to be explored.

Through this thesis, many skills have been developed such as the skills in research and interpretation of scientific data. In addition, this thesis has treated of a fascinating sector of activity which the marine biology. It has a real potential of development for the biotechnologies. A lot of knowledge has been collected about this field and the function of the organisms living in the depths, especially the extremophilic living beings. However, the only disappointment was about the experiments which have not been realized, so the thesis was more theorical than expected. Despite this, this thesis has increased the skills into practice to carry out a protocol and an experimental study.

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