Chapter 4

Recent Trend on Bioremediation of Polluted Salty Soils and Waters Using Haloarchaea

Sonia Aracil-Gisbert, Javier Torregrosa-Crespo and Rosa María Martínez-Espinosa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70802

Abstract

Pollution of soils, sediments, and groundwater is a matter of concern at global level. Industrial waste effluents have damaged several environments; thus, pollutant removal has become a priority worldwide. Currently, bioremediation has emerged as an effective solution for these problems, and, indeed, the use of haloarchaea in bioremediation has been tested successfully. A bibliographic review is here presented to show the recent advances in bioremediation of polluted soil and wastewater using haloarchaea. Several aspects related to the publications in the context of bioremediation and the innovative proposal of using haloarchaea are also analyzed. The results obtained claim that most of the countries show an alarming contamination issue, which focuses the finance into research about environmental friendly remediation approaches to solve this problem. Concerning bioremediation, strategies to treat soils and polluted waters have been much more studied than bioremediation processes addressed to industrial residues. Publications about bioremediation mainly comes from USA, China, and India at the time of writing this work. However, works using haloarchaea in bioremediation came from Kuwait and Spain. Haloarchaea have been investigated as a potential tool for industrial and environmental purposes. Further research is needed to elucidate the optimal growth conditions and environmental parameters for this proposal.

Keywords: bioremediation, haloarchaea, *Haloferax mediterranei*, heavy metals, green biotechnology, nitrogen, (per)chlorates, phosphorous

1. Introduction

Last decades highlight the importance of the increasing water demand, the environmental pollution or the presence of excess nitrogen in the environment, causing serious alterations in soil, water, and atmosphere [1, 2]. The presence of heavy metals in the environment as well as the C:N ratio has been a major problem in soils and waters due to their toxicity; therefore, their elimination before being released into the media is relevant from environmental, economic, and social points of view [3]. There are many methods to remove pollutants, involving physical, chemical, or biological approaches. During recent decades, the latter has received an increasing attention as a way of removing or neutralizing pollutants from a contaminated site using native or introduced microorganisms [4].

One of those biological strategies recently applied is the bioremediation, which is defined as the application of the metabolic capabilities of bacteria, fungi, yeast, algae, plants, and microbial mats to degrade harmful contaminants to living organisms or transform them into less toxic compounds by naturally enhancing degradation processes [3, 5]. Two types of bioremediation might be found according to the way of use: (i) intrinsic *in situ* bioremediation: natural degradation of remaining contaminants in the environment, without any external intervention; (ii) engineered *in situ* bioremediation: requires an external supply to increase the biodegradation rate by accelerating the growth of further organisms through the secretion of essential nutrients. In most of the situations, engineered bioremediation prevails over the intrinsic one except for those cases in which the natural occurrence of biodegradation is faster than the migration [6].

Engineered bioremediation includes two approaches: biostimulation and bioaugmentation. The addition of limiting nutrients to a contaminated environment where all the necessary natural microorganisms are assumed to be present refers to biostimulation. Bioaugmentation controls the quantity of microorganisms with the desired catalytic capability needed for biodegradation of specific compounds [7, 8]. A combination between these disciplines is suggested to be a promising strategy to speedup bioremediation [5]. Nowadays, biological remediation is considered one of the best options for the treatment of contaminated environments [3] due to their numerous advantages such as *in situ* treatment of soils or waters, high efficiency, and no secondary pollution [5, 9].

1.1. Microorganisms and bioremediation: the case of haloarchaea

Microorganisms are the ones which oversee the bioremediation process, thanks to their ability of using environmental contaminants as source of carbon and energy and their small contact surface [6]. Prokaryotes are the main biotechnological agents used for biodegradation of organic matter, although fungi, algae, and protozoa can be used for the same purpose but affecting differently the nature of the compound for the degradation [10]. In addition, phytoremediation has been for a long time as a promising technique to degrade contaminants from soil sediment, surface, and groundwater [11, 12]. It was proposed the use of microbial consortium, such as phosphate solubilizing microbes, in association with phytoremediation to achieve a most trustworthy approach for the enhancement of the remediation of metal-contaminated soils [13].

More recently, some research has shown that archaea can be of high interest for biotechnological purposes including bioremediation. Archaea were first characterized as a group of single-celled prokaryotic microorganisms living in extremophile environments with elevated temperatures (thermophiles), low or high pH (acidophiles, alkalophiles), high salinity (halophiles),

http://dx.doi.org/10.5772/intechopen.70802

or strict anoxia [14]. The ability of those species to grow under a wide range of extreme conditions equally to their mechanisms supporting genetic plasticity [15] make them good candidates for research in different fields, including biotechnology and, consequently, bioremediation [9, 14, 16].

Nowadays, there is an increasing interest in the optimization of bioremediation approaches in high salt environments, which are mostly influenced by the discharge of industrial effluents [2, 17]. The increase of salinity and nitrogen compounds in soils and ground waters during the last years is focusing attention in the physiological and molecular mechanisms involved in salt-stress tolerance and nitrate metabolism [18]. Conventional microbiological processes are not capable of being executed at high salt concentrations, indicating that microbial bioremediation of hypersaline produced water requires halophiles [19, 20].

In this sense, haloarchaea have been successfully tested for biotechnological applications throughout the last decade [16, 21, 22]. This is because of the extreme properties of their enzymes: high thermostability and resistance to denaturing agents such as detergents, organic solvents, and extreme pH [23]. Moreover, their proteins are rich in acidic amino acids, which allow the maintenance of the stable conformation and activity at high salt concentrations [24].

Most of the species from *Halobacteriaceae* and *Haloferacaceae* families can grow under anaerobic conditions, and they are more abundant and diverse than other microbes in environments containing increasing salt concentrations (more than 20–25% salts) [25, 26]. They are found in natural salty lakes and saltmarshes created by human beings, which are extremely alkalinized places, likewise in submarine brine pools and brine pockets within sea ice [23, 27].

General biochemical pathways related to nitrogen cycle, heavy metals, hydrocarbons, or aromatic compounds have been described in haloarchaea as efficient pathways. Consequently, they might be applied for bioremediation proposals in saline and hypersaline wastewater treatments, thanks to their high tolerance to salt, metals, and organic pollutants [3, 14, 25].

1.2. *Haloferax mediterranei*: a good model for viable bioremediation applications

Hfx. mediterranei is a haloarchaeon from the family *Haloferacaceae* [28], first isolated from seawater evaporation ponds near Alicante, Spain [29]. It can grow in a broad range of NaCl concentrations (1.0–5.2 M) [14], thanks to its remarkable metabolic efficiency and genome stability at high salt concentrations [25, 30].

Hfx. mediterranei is also a denitrifying haloarchaeon based on its ability to reduce nitrate and nitrite under oxic and anoxic conditions [31, 32]. In the presence of oxygen, this haloarchaeon takes profit of nitrate and nitrite as nitrogen sources for its own proliferation [33] through assimilation pathway. Under oxygen depletion, it uses both ions as terminal electron acceptors in the respiratory chain via denitrification [32, 34]. Once nitrogenous compounds are consumed, brines become free of nitrogenous compounds such as nitrate, nitrite, or ammonium.

Owing to this specific metabolism, it was demonstrated that *Hfx. mediterranei* was capable of removing most of the nitrogen compounds present in a treated medium, specifically in anoxic conditions after the induction of the denitrification pathway [14]. Its resistance to very high nitrate (up to 2 M) and nitrite (up to 50 mM) concentrations makes them the highest described from a prokaryotic microorganism [3, 35]. Furthermore, the respiratory nitrate reductase of this organism can reduce efficiently bromate and (per)chlorate thanks to the use of these compounds as terminal electron acceptors [36].

Because of its capability of growing faster than most of other members of the same family and their described characteristics, *Hfx. mediterranei* has served as a good model for haloarchaeal physiology and metabolic studies during several decades [30, 37]. Therefore, it represents a highly interesting model for research in potential development of bioremediation approaches in polluted soils, sediments, and waters.

We had reviewed on bioremediation of polluted salty soils and water using haloarchaea in our previous manuscript [3, 22, 25, 34, 36] and had introduced the importance of haloarchaea as a bioremediation tool. Thus, in this manuscript, we statistically investigated the recent trend of the study and estimated the characteristics.

2. Research method

The starting point for this work was the bibliographic-bibliometric review about the most interesting keywords related to the topic. To optimize the search of information, it was used "PubMed" (https://www.ncbi.nlm.nih.gov/pubmed/) as a free search engine, as well as two well-known subscription-based scientific databases: "Scopus" (https://www.scopus.com/) and "Web of Science (WoS)" (https://www.webofknowledge.com/). The main keywords that have been used include "archaea," "bioremediation," "contaminated soil," "haloarchaea," "industrial waste," "salty soil," and "wastewater." In addition, some keywords were employed for the sole purpose to corroborate the total number of publications considering synonyms or similar words as the previous ones, such as "halophilic archaea," "industrial raw," and "salted soil."

The combination between these keywords was used to optimize the search for publications. All the keywords were grouped into two subclasses: the first one includes the terms "archaea," "bioremediation," "haloarchaea," and "halophilic archaea"; the second class involves "contaminated soil," "industrial raw," "industrial waste," "salted soil," "salty soil," and "wastewater." These classes of keywords and their synonyms were crossed, obtaining a total pool of 27 concepts: "archaea and contaminated soil," "archaea and bioremediation," "archaea and industrial raw," "archaea and industrial waste," "archaea and salted soil," "archaea and salty soil," "archaea and wastewater," "bioremediation and contaminated soil," "bioremediation and haloarchaea," "bioremediation and halophilic archaea," "bioremediation and industrial raw," "bioremediation and industrial waste," "bioremediation and salted soil," "bioremediation and salty soil," "bioremediation and wastewater," "haloarchaea and contaminated soil," "haloarchaea and industrial raw," "haloarchaea and industrial waste," "haloarchaea and salted soil," "haloarchaea and salty soil," "haloarchaea and wastewater," "halophilic archaea and contaminated soil," "halophilic archaea and industrial raw," "halophilic archaea and industrial waste," "halophilic archaea and salted soil," "halophilic archaea and salty soil," and "halophilic archaea and wastewater." The following fields were selected in the databases to delimit the results: "article title," "abstract," and "keywords."

In April 2017, the search about number of publications per year was finished, using a range of 27 years (1990–2016). They were obtained regarding the following items: country, type of document, language, and publication date. The language and type of document were not indispensable for analyzing purposes. The research question was: what is the impact of haloarchaeal applications in bioremediation in research/science?

To assess the differences in the search of publications between the three mentioned databases, the analysis of variance (ANOVA) was performed using R-project statistics. ANOVA statistics was chosen, since it is usually employed for this kind of studies. The significant threshold for these analyses had a p value of 0.05 except for those cases where the transformation of the data was not efficient, and it was needed to change it ($p = 0.01$). In those cases where at least one of the levels was statistically different, it was performed a test to delimit the class which is different from the rest or differences between classes. The test used was Tukey's HSD. For the average calculations and graphical representations, Microsoft Excel 2016 was employed.

3. Results

3.1. Analysis of databases results regarding publications per year

First, a contrast between PubMed, Scopus, and Web of Science was performed regarding the number of publications per year. The purpose of this approach was to study if there were significant differences in the search concerning the database based on the keywords selected. To summarize the data, only publications from a range of years (1990–2016) were used in the statistical test of the total 10 keywords, including synonyms. The year 2017 was not considered in this interval due to the inaccuracy of the results and the unpredictable number of total publications at the end of the year.

An ANOVA test was performed to analyze the differences between the three databases (PubMed, Scopus, and Web of Science) and to compare them looking for statistical significance in the number of publications. The requirements for ANOVA test were corroborated as follows: independence of the data, normality of the results, and homogeneity of the variances. ANOVA statistics was done obtaining that at least one of the databases was different ($p < 0.05$), so Tukey's test was performed to distinguish the variable class by comparing each level with the rest (**Figure 1**). Concerning the p value of the results, there are only significant differences between Web of Science and PubMed as it is displayed in **Figure 1** by means of lines referring to similarities. The differences between the databases are remarked in the way PubMed is significantly different from Web of Science ($p = 0.0018$) and not for Scopus ($p = 0.39$). No significant differences were documented between Scopus and Web of Science regarding the number of total publications per year ($p = 0.07$). PubMed, as a service from the US National Library of Medicine (NLM), carries the highest number of publications. However, Web of Science is one of the most used search database, containing quality publications from editorials with high impact factor.

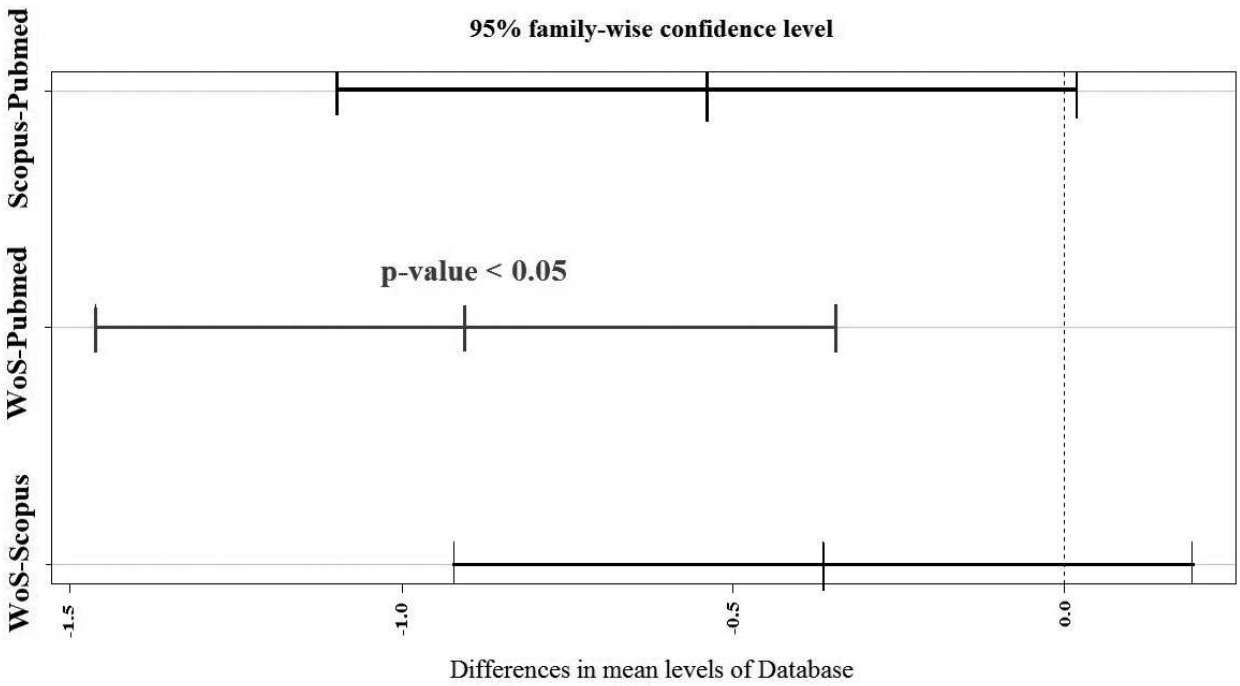

Figure 1. Plot based on Tukey's test showing the differences between the databases. There are significant differences between Web of Science and PubMed ($p = 0.0018$).

3.2. Study of the number of publications according to continents and countries

The location of the research related to the topic keywords according to continents and countries was also analyzed. Most of the countries in the world possess almost one publication along time. However, United States of America (USA) and China present the highest number of publications (111,392 and 89,849, respectively). The whole subset of countries with representativeness in the research about bioremediation using haloarchaea in soils, waters, and industrial raw is shown in **Figure 2**, with different intensities according to the abundance of publications. The language was not a representative deal since almost all the publications were written in English (97.4%). The diversity of the language in the different countries is remarkable despite of this, where other writing languages are German (0.5%), Spanish (0.4%), and Portuguese (0.4%).

As a further matter, all the data from the number of publications per country were grouped into continents for a more general view of the representativeness. The following results were obtained concerning total publications in the search: Asia (210,850), Europe (210,086), America (165,946), Oceania (19,038), Eurasia (18,752), and Africa (18,591). Africa has the lowest value of publications although the abundance of countries with positive results is large. Eurasia includes Turkey, Russia, Kazakhstan, and Georgia, which can be considered from Europe and Asia due to their geographical localization. Instead of including these countries on a single continent, a different class of continent is considered due to their high values of publications. The final values of Europe and Asia are so close with a difference of 800 publications, which implies a possible change of dominance in the next few years.

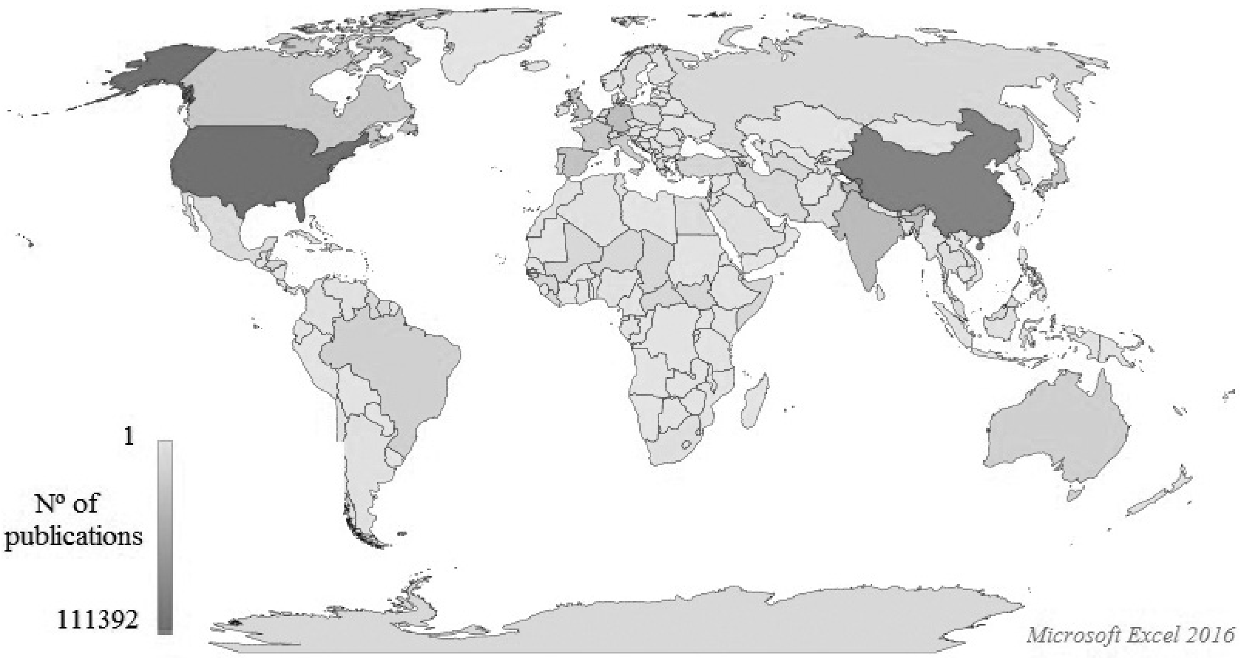

Figure 2. Global map representing the total publications per country by increasing intensity of color. Total number of publications is referred to the 10 keywords summation of results.

3.3. Impact of bioremediation using haloarchaea in literature

Haloarchaea were not directly related to bioremediation in literature until year 2007, and, even after this year, the number of publications has been low as can be noted in the percentage of occurrence of haloarchaea in bioremediation (0.06%). This value was calculated by analyzing the total publications per year regarding "bioremediation" as keyword in comparison to the combination of "bioremediation and haloarchaea or halophilic archaea."

The main lines of investigation regarding bioremediation using haloarchaea are developed by Bonete MJ (University of Alicante), Al-Mailem DM (University of Kuwait), and Martínez-Espinosa RM (University of Alicante). Furthermore, University of Alicante, University of Kuwait, and University of East Anglia are the most important institutions in terms of developing research lines about haloarchaeal species applied in bioremediation. These affiliations may be duplicated due to the fact some publications can contain two or more institutions in collaboration. Many of the authors with active experimental approaches in this way belong to the University of Alicante in collaboration with University of East Anglia (approximately, 50% of publications from the University of Alicante are in collaboration; 100% of publications from University of East Anglia are made in collaboration with University of Alicante).

Besides, the influence of the keywords "bioremediation" and "haloarchaea or halophilic archaea" was analyzed to delimit the total number of publications per year in the chosen databases. These results are plotted in **Figure 3**. There is an increasing tendency of the publications, with higher quantities of results during last years both in bioremediation (**Figure 3A**) and haloarchaea (**Figure 3B**). The keyword "halophilic archaea" shows a higher representativeness than "haloarchaea" in the search due to the presence of the word "halophilic" which can be

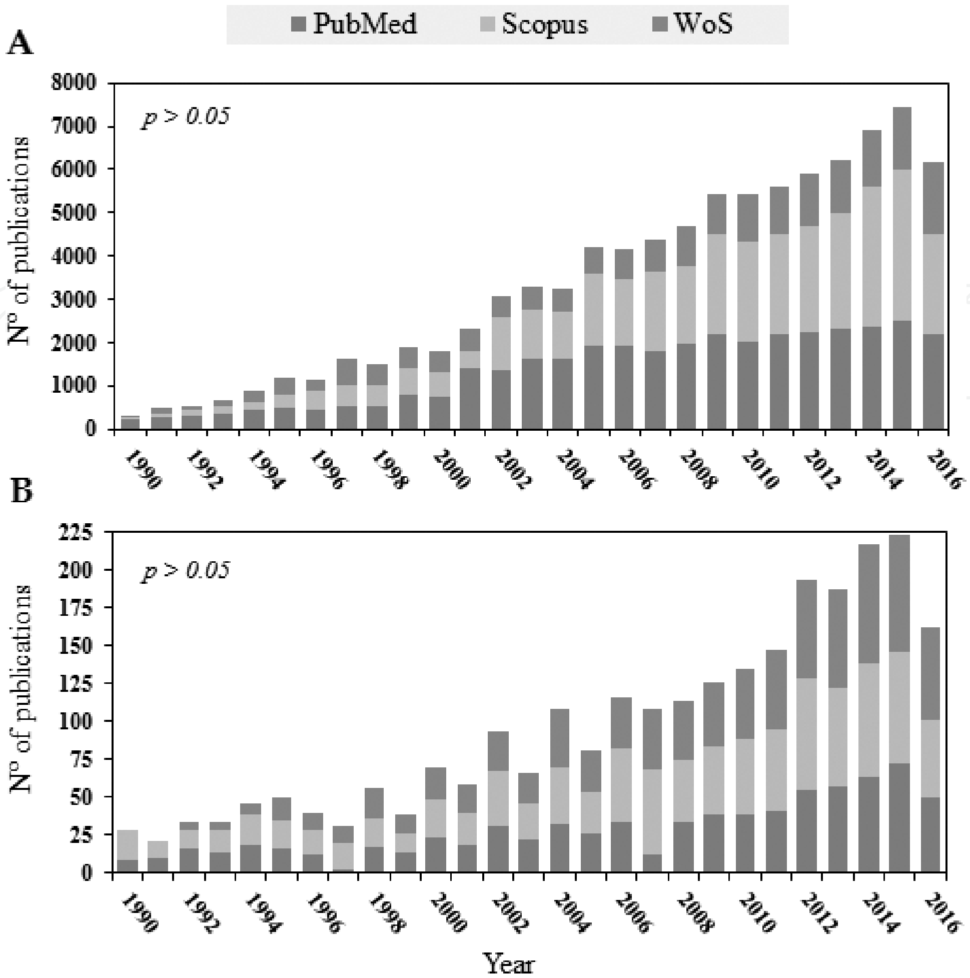

Figure 3. Graphical representation of the total number of publications per year from three databases (PubMed, Scopus, and Web of Science) concerning the keywords (A) "bioremediation"; and (B) "haloarchaea" and "halophilic archaea."

used for referring other species different from archaea (bacteria, microorganism, and plant; **Figure 3B**).

Both "bioremediation and haloarchaea" and "halophilic archaea" publications present a lower value of results (**Figure 4**). Since "haloarchaea" have a lower representativeness in literature than "bioremediation," the results show a limitation in the number of publications per year. Increasing results appear since year 2007, although some publications have been documented before in the way of potential studies using haloarchaea. Below year 2005, no guaranteed results concerning the topic are shown due to the lower interest in haloarchaea and the probable appearance of other microorganisms because of the use of "halophilic" in the search.

The impact of bioremediation research by countries is shown in **Figure 5**. This representation remarks the abundance of publications in the top 10 countries, where the USA is the dominant followed by China and India. This is not surprising owing to the presence of those countries in the top three of publications regarding all the keywords. In **Figure 6**, the top 10 countries that investigate in bioremediation using haloarchaea are shown, with Kuwait and Spain dominating the volume of total publications. Although Kuwait is not on the top 10 countries in

http://dx.doi.org/10.5772/intechopen.70802

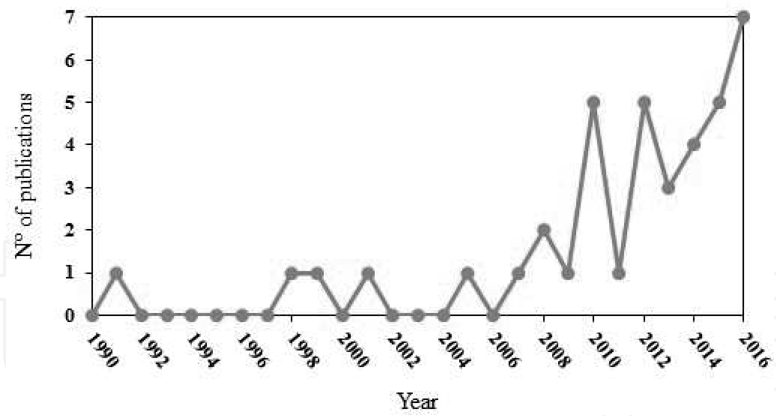

Figure 4. Graphical representation of the total publications per year PubMed, Scopus and Web of Science (WoS) databases using as keywords the average of "bioremediation and haloarchaea" and "bioremediation and halophilic archaea." Both combinations are represented as average due to their synonym relevance.

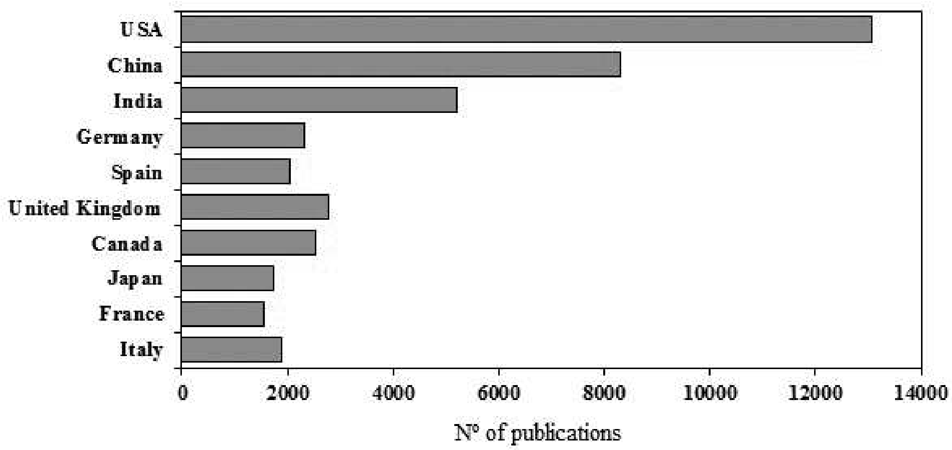

Figure 5. Main countries with the highest rate of publications in regard to bioremediation. Both Scopus and Web of Science results have been considered.

bioremediation publications, it is important to highlight its predominance in the study of haloarchaea as bioremediation tool.

Several areas of Kuwait are mainly characterized by hypersaline environments. Due to this fact, desalination is sometimes the only way of reliable water supply in most of the Arabian Gulf states. In South Kuwait, a desalination plant has showed a continued increase of salt, which can affect the local marine biodiversity. In 2011, it was suggested a distinct water quality approach to detect those issues without affecting the marine environment surrounding the water plant [38]. Desalination plants manage a huge quantity of salty water, although they imply several negative impacts. The main cause of desalination plant pollution in the environment includes noise pollution, visual pollution, reduction in recreational fishing and swimming areas, emission of materials into the atmosphere, and brine discharge [39]. Likewise, the climate is very like the one in Spain.

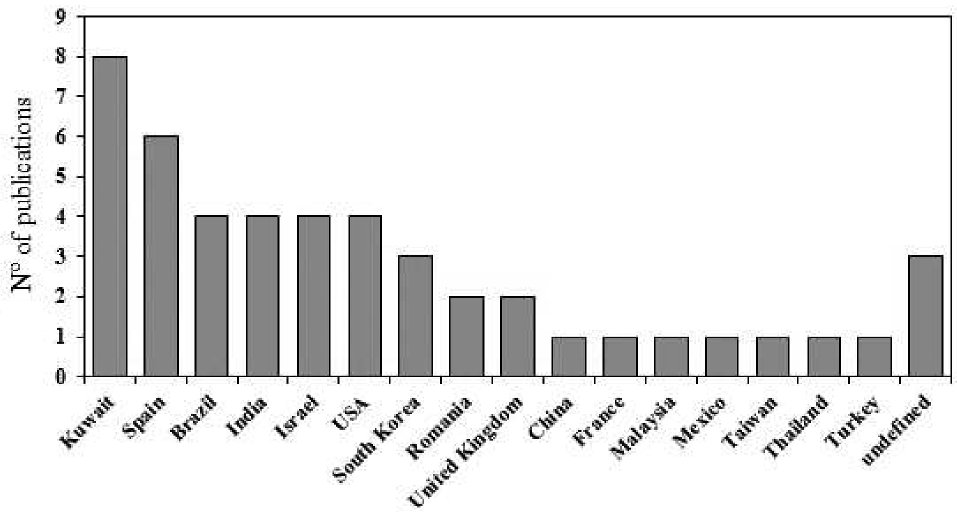

Figure 6. Mean total number of publications concerning the combination of keywords "bioremediation and haloarchaea" from Scopus and Web of Science search databases.

3.4. Bibliographic review of *Haloferax mediterranei*

Between years 2013 and 2016, there was an increase of interest for the study of this haloarchaeon and their possible applications. The highest number of publications concerning *Hfx. mediterranei* comes from Spain, with descriptions of different type of applications (industrial, environmental or medical) concerning this haloarchaeon. Authors like Bonete and Martínez-Espinosa (University of Alicante) or Pfeifer (Technische Universitat Darmstadt) appear as the first representative authors in this field, developing independent research lines in their institutions. In fact, the University of Alicante (Spain) is the first research institution in the ranking of number of publications about this haloarchaeon (**Figure 7**). Besides, those

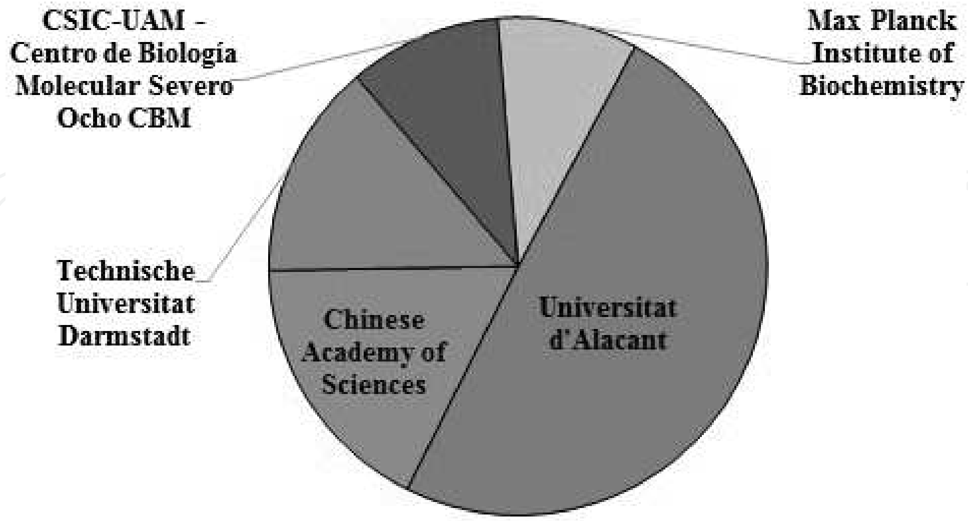

Figure 7. Top five of institutions/affiliations which are involved in research about *Hfx. mediterranei*: Universitat d'Alacant-University of Alicante (50%), Chinese Academy of Science (17%) Technische Universitat Darmstadt (14%), CSIC-UAM—Centro de Biología Molecular Severo Ochoa CBM (10%), Max Planck Institute of Biochemistry (9%). The publications may be duplicated in the way one article may be published by two affiliations.

http://dx.doi.org/10.5772/intechopen.70802

authors are studying the biochemistry, molecular biology, and potential applications of *Hfx. mediterranei* since its discovery. This work is a contribution to that research group, the most involved in the understanding of *Hfx. mediterranei* in natural contaminated water and further applications [3, 25, 31].

4. Discussion

Globally, alternative remediation processes are gaining importance instead of chemical or solid methods in polluted compounds treatment. There is a global concern about wastewater and residue management due to the presence of toxic compounds [40]; thus, environmental friendly approaches are being financed by those countries with the higher rate of contamination, such as China or India [41]. Bioremediation, biostimulation, and bioaugmentation are suggested to be suitable alternatives and, due to this fact, they have received a great deal of attention in recent years [7].

Extremophiles may represent a good option for bioremediation of contaminated sites due to their biochemical and molecular properties. Halophilic microorganisms, especially haloarchaea, represent a highly promising way for removing polluted compounds thanks to their effective metabolism under extreme conditions or even in the presence of highly toxic compounds [2, 42]. Previous studies show the potential use of haloarchaea in bioremediation [3, 22]. Thus, a bibliographic review was developed along this work for the sole purpose to show the recent advances in bioremediation using these microorganisms. The recent gain of interest in potential haloarchaeal innovations is also shown. The source of information was based on three databases, some of which present high-quality impact in publications: PubMed, Scopus, and Web of Science. It was surprising the volume of publications from PubMed and Scopus in contrast to Web of Science, a scientific database containing high-quality publications and worldwide used. In fact, Web of Science is considered the most important database in terms of research evaluation of standards in some institutions such as universities, research institutes, or other institutions involved in research management.

Regarding to the topic, several countries are increasing their efforts in developing environmental friendly methods for removing contaminants; thus, the number of publications concerning bioremediation is highly increasing since recent years. The severe contamination documented in China and India may explain the high number of publications in benefit of bioremediation. The need to find solutions to this problem has increased the public and private finance for such projects. Bioremediation might be the solution for soils and water problems of pollution. The concerning about natural water contamination has always been present, but there is an increasing attention of creating soil quality remediation plans in China. Serious pollution risks were confirmed by the Chinese authorities in April 2014. About 16% of soils in China and 34.9% of brownfields exceed national standards for pollution with heavy metals and pesticides [43]. In India, in addition to the drinking water demand, there are evidences of groundwater contamination due to wastes of local industries. People experience symptoms of disease when drinking water from rivers, which reinforce the actual problem of water contamination [44].

Despite this, the diversity of metabolic pathways of *Hfx. mediterranei*, and in general haloarchaea, is greater than earlier assumed. The research about biochemical and molecular characteristics of haloarchaea will be a potential issue in biotechnological applications, especially on bioremediation of natural contaminated water. Furthermore, more investigation about the potential use of those microorganisms is needed to elucidate the optimum range of conditions for suitable bioremediation applications.

Author details

Sonia Aracil-Gisbert, Javier Torregrosa-Crespo and Rosa María Martínez-Espinosa*

*Address all correspondence to: rosa.martinez@ua.es

Departamento de Agroquímica y Bioquímica, Facultad de Ciencias, Universidad de Alicante, Alicante, Spain

References

[1] Breisha GZ, Winter J. Bio-removal of nitrogen from wastewaters–a review. Journal of American Science. 2010;**6**(12):508-528

[2] Le S, Paniagua D, Vazquez-Duhalt R. Biodegradation of organic pollutants by halophilic bacteria and archaea. Journal of Molecular Microbiology and Biotechnology. 2008;**15**:74-92. DOI: 10.1159/000121323

[3] Bonete M, Bautista V, Esclapez J, García-Bonete M, Pire C, Camacho M, Torregrosa-Crespo J, Martínez-Espinosa R. New uses of haloarchaeal species in bioremediation processes. In: Shiomi M, editor. Advances in Bioremediation of Wastewater and Polluted Soil. 1st ed. InTech; 2015. p. 23-49. DOI: 10.5772/60667

[4] Azubuike C, Chikere C, Okpokwasili G. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World Journal of Microbiology and Biotechnology. 2016;**32**:180. DOI: 10.1007/s11274-016-2137-x

[5] El S, Agathos S. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Current Opinion in Microbiology. 2005;**8**(3):268-275. DOI: 10.1016/j.mib.2005.04.011

[6] USA National Research Council. In Situ Bioremediation: When Does it Work? Washington, D.C.: National Academy Press; 1993. Available from: http://www.nap.edu/catalog/2131.html Accessed: March 8, 2017

[7] Adams G, Fufeyin P, Okoro S, Ehinomen I. Bioremediation, biostimulation and bioaugmentation: A review. International Journal of Environmental Bioremediation & Biodegradation. 2015;**3**(1):28-39. DOI: 10.12691/ijebb-3-1-5

[8] Cycoń M, Mrozik A, Piotrowska-Seget Z. Bioaugmentation as a strategy for the remediation of pesticide-polluted soil: A review. Chemosphere. 2017;**172**:52-71. DOI: 10.1016/j.chemosphere.2016.12.129

[9] Xu Y, Zhou N. Microbial remediation of aromatics-contaminated soil. Frontiers of Environmental Science & Engineering. 2016;**11**(2):1. DOI: 10.1007/s11783-017-0894-x

[10] Ivanov V, Hung Y. Applications of environmental biotechnology. In: Wang LK, Ivanov V, Hung Y, editors. Handbook of Environmental Engineering. Vol. 10: Environmental Biotechnology. CBS Publishers, New Delhi, India: Springer Science+Business Media; 2010. p. 1-27. DOI: 10.1007/978-1-60327-140-0_1

[11] Subhashini V, Swamy A. Phytoremediation of Pb and Ni contaminated soils using *Catharanthus roseus* (L.). Universal Journal of Environmental Research and Technology. 2013;**3**(4):465-472

[12] Zhang H, Tian Y, Wang L, Zhang L, Dai L. Ecophysiological characteristics and biogas production of cadmium-contaminated crops. Bioresource Technology. 2013;**146**:628-636. DOI: 10.1016/j.biortech.2013.07.148

[13] Jia X, Liu C, Song H, Ding M, Du J, Ma Q, Yuan Y. Design, analysis and application of synthetic microbial consortia. Synthetic and Systems Biotechnology. 2016;**1**(2):109-117. DOI: 10.1016/j.synbio.2016.02.001

[14] Nájera-Fernández C, Zafrilla B, Bonete M, Martínez-Espinosa R. Role of the denitrifying haloarchaea in the treatment of nitrite-brines. International Microbiology. 2012;**15**(3):111-119. DOI: 10.2436/20.1501.01.164

[15] Capes M, Coker J, Gessler R, Grinblat-Huse V, DasSarma S, Jacob C, Kim J, DasSarma P, DasSarma S. The information transfer system of halophilic archaea. Plasmid. 2011;**65**(2):77-101. DOI: 10.1016/j.plasmid.2010.11.005

[16] Arora S, Vanza M, Mehta R, Bhuva C, Patel P. Halophilic microbes for bio-remediation of salt affected soils. African Journal of Microbiology Research. 2014;**8**(33):3070-3078. DOI: 10.5897/AJMR2014.6960

[17] Cuadros-Orellana S, Pohlschröder M, Grossman M, Durrant L. Biodegradation of aromatic compounds by a halophilic archaeon isolated from the dead sea. Chemical Engineering Transactions. 2012;**27**:13-18. DOI: 10.3303/CET1227003

[18] Ventosa A, Nieto J, Oren A. Biology of moderately halophilic aerobic bacteria. Microbiology and Molecular Biology Reviews. 1998;**62**(2):504-544. 1092-2172/98/$04.0010

[19] Bonfá M, Grossman M, Mellado E, Durrant L. Biodegradation of aromatic hydrocarbons by haloarchaea and their use for the reduction of the chemical oxygen demand of hypersaline petroleum produced water. Chemosphere. 2011;**84**:1671-1676. DOI: 10.1016/j.chemosphere.2011.05.005

[20] Ghosal D, Ghosh S, Dutta T, Ahn Y. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Frontiers in Microbiology. 2016;**7**:1369. DOI: 10.3389/fmicb.2016.01369

[21] Oren A. Industrial and environmental applications of halophilic microorganisms. Environmental Technology. 2010;**31**(8–9):825-834. DOI: 10.1080/09593330903370026

[22] Bonete M, Martínez-Espinosa R. Enzymes from halophilic archaea: Open questions. In: Ventosa A, Orena A, Ma Y, editors. Halophiles and Hypersaline Environments. 1st ed. Berlin: Springer-Verlag; 2011. p. 359-373. DOI: 10.1007/978-3-642-20198-1_19

[23] Castillo Rodríguez F, Roldán Ruiz M, Blasco Plá R, Huertas Romera M, Caballero Domínguez F, Moreno-Vivián C, Martínez Luque-Romero M. Biotecnología Ambiental. 1st ed. Spain: Editorial Tébar; 2005. p. 377-387

[24] Oren A. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Systems. 2008;**4**:2. DOI: 10.1186/1746-1448-4-2

[25] Torregrosa-Crespo J, Martínez-Espinosa R, Esclapez J, Bautista V, Pire C, Camacho M, Richardson D, Bonete M. Anaerobic metabolism in *Haloferax* genus: Denitrification as case of study. In: Poole RK, editor. Advances in Microbial Physiology. 1st ed. Vol. 68. Oxford Academic Press; 2016. p. 41-85. DOI: 10.1016/bs.ampbs.2016.02.001

[26] Valentine D. Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nature Reviews Microbiology. 2007;**5**:316-323. DOI: 10.1038/nrmicro1619

[27] Munn C. Marine Microbiology: Ecology and Applications. 1st ed. Vol. 2004. Garland Science/BIOS Scientific Publishers; 2004. p. 81-120

[28] Gupta R, Naushad S, Baker S. Phylogenomic analyses and molecular signatures for the class *Halobacteria* and its two major clades: a proposal for division of the class *Halobacteria* into an emended order *Halobacteriales* and two new orders, *Haloferacales* ord. nov. and *Natrialbales* ord. nov., containing the novel families *Haloferacaceae* fam. nov. and *Natrialbaceae* fam. nov. International Journal of Systematic and Evolutionary Microbiology. 2015;**65**(3):1050-1069. DOI: 10.1099/ijs.0.070136-0

[29] Rodríguez-Valera F, Ruiz-Berraquero F, Ramos-Cormenzana A. Isolation of extremely halophilic bacteria able to grow in defined inorganic media with single carbon sources. Journal of General Microbiology. 1980;**119**(2):535-538. DOI: OO22-1287/80/0000-902

[30] Han J, Zhang F, Hou J, Liu X, Li M, Liu H, Cai L, Zhang B, Chen Y, Zhou J, Hu S, Xiang H. Complete genome sequence of the metabolically versatile halophilic archaeon *Haloferax mediterranei*, a poly(3-hydroxybutyrate-co-3-hydroxyvalerate) producer. Journal of Bacteriology. 2012;**194**(16):4463-4464. DOI: 10.1128/JB.00880-12

[31] Bonete M, Martínez-Espinosa R, Pire C, Zafrilla B, Richardson D. Nitrogen metabolism in haloarchaea. Saline Systems. 2008;**4**:9. DOI: 10.1186/1746-1448-4-9

[32] Lledó B, Martínez-Espinosa R, Marhuenda-Egea F, Bonete M. Respiratory nitrate reductase from haloarchaeon *Haloferax mediterranei*: Biochemical and genetic analysis. Biochimica et Biophysica Acta (BBA—General Subjects). 2004;**1674**(1):50-59. DOI: 10.1016/j.bbagen.2004.05.007

[33] Martínez-Espinosa R, Marhuenda-Egea F, Bonete M. Purification and characterization of a possible assimilatory nitrite reductase from the halophile archaeon *Haloferax mediterranei*. FEMS Microbiology Letters. 2001;**196**(2):113-118. DOI: 10.1111/j.1574-6968.2001.tb10550.x

[34] Martínez-Espinosa R, Richardson D, Butt J, Bonete M. Respiratory nitrate and nitrite pathway in the denitrifier haloarchaeon *Haloferax mediterranei*. Biochemical Society Transactions. 2006;**34**(1):115-117. DOI: 10.1042/BST0340115

[35] Martínez-Espinosa R, Lledó B, Marhuenda-Egea F, Díaz S, Bonete M. NO_3^-/NO_2^- assimilation in halophilic archaea: Physiological analysis, nasA and nasD expressions. Extremophiles. 2009;**13**(5):785-792. DOI: 10.1007/s00792-009-0266-y

[36] Martínez-Espinosa R, Richardson D, Bonete M. Characterisation of chlorate reduction in the haloarchaeon *Haloferax mediterranei*. Biochimica et Biophysica Acta (BBA)—General Subjects. 2015;**1850**(4):587-594. DOI: 10.1016/j.bbagen.2014.12.011

[37] Fang C, Ku K, Lee M, Su N. Influence of nutritive factors on C50 carotenoids production by *Haloferax mediterranei* ATCC 33500 with two-stage cultivation. Bioresource Technology. 2010;**101**(16):6487-6493. DOI: 10.1016/j.biortech.2010.03.044

[38] Uddin S, Al Ghadban A, Khabbaz A. Localized hyper saline waters in Arabian Gulf from desalination activity—an example from South Kuwait. Environmental Monitoring and Assessment. 2011;**181**(1–4):587-594. DOI: 10.1007/s10661-010-1853-1

[39] Tularam G, Ilahee M. Environmental concerns of desalinating seawater using reverse osmosis. Journal of Environmental Monitoring. 2007;**9**(8):805-813. DOI: 10.1039/b708455m

[40] Toxics Action Center. The Problems with Waste. [Internet]. 2015. Available from: http://www.toxicsaction.org/problems-and-solutions/waste [Accessed: May 21, 2017]

[41] Numbeo. Pollution Index for Country 2017. [Internet]. 2017. Available from: https://www.numbeo.com/pollution/rankings_by_country.jsp [Accessed: May 21, 2017]

[42] Falb M, Müller K, Königsmaier L, Oberwinkler T, Horn P, von S, González O, Pfeiffer F, Bornberg-Bauer E, Oesterhelt D. Metabolism of halophilic archaea. Extremophiles. 2008;**12**(2):177-196. DOI: 10.1007/s00792-008-0138-x

[43] Yang H. China's soil plan needs strong support. Nature. 2016;**536**:375. DOI: 10.1038/536375a

[44] Harris T. Rains or not, India faces drinking water crisis. Phys.org. [Internet]. 2016. Available from: https://phys.org/news/2016-06-india-crisis.html [Accessed: May 12, 2017]