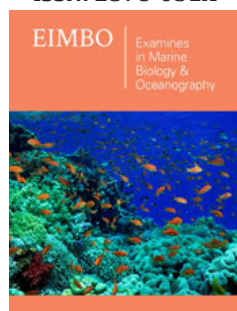


Alcanivorax borkumensis and Bioremediation: The Actual Perspective of a Vintage Bacteria

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Opinion

Approximately twenty-six years after its discovery, *Alcanivorax borkumensis* and the hydrocarbonclastic bacteria present themselves as one of the most efficient microbiological tools offered by nature to be used in the environmental recovery processes (bioremediation) of areas contaminated by oil and its derivatives. However, the careful study of the physiological and metabolic properties of this bacterium and the development of modern technologies for environmental recovery are of primary importance for achieving increasingly better performance (maximum degradation in the shortest possible time).

Twenty-six years ago, in 1998 Yakimov et al. reported the isolation and characterization from a sediment collected in the island of Borkum (Germany), of a new bacterium strain named *Alcanivorax borkumensis*, an unusual marine microorganism able to grow using as only carbon and energy sources a highly restricted spectrum of substrates, predominantly alkanes [1]. This discovery of this bacterium is the beginning of an important advancement in microbiological research and at the same time, in the technology for the development of strategies for bio-recovery of environments polluted by hydrocarbons (bioremediation). The isolation of *Alcanivorax* correspond to the identification a one eco-physiologically unusual group of marine hydrocarbon-degrading bacteria named Obligate Hydrocarbonoclastic Bacteria (OHCB) comprising, overtime, different bacterial genera, such as *Cycloclasticus*, *Oleiphilus*, *Oleispira*, *Thalassolituus* and clearly *Alcanivorax*. Within a short period of time, was massive the isolation of bacteria related to *Alcanivorax*, or detection of its 16S rRNA gene sequences, from samples taken from surface water, shallow and deep sea water bodies, sediments, hydrothermal vents and mud volcanoes, ridge flank crustal fluids and grey whale carcass, in corals, sponges and aquaculture-poisoning dinoflagellates. Sequences of *Alcanivorax* like bacteria have also been detected in a few terrestrial environments that share relevant properties (salinity, presence of hydrocarbons) with marine ecosystems.

This ubiquity of *A. borkumensis* presumably results from its capacity to grow on many saturated petroleum fraction constituents and on biogenic hydrocarbons: Straight-chain and branched alkanes, isoprenoids and long sidechain alkyl compounds [2,3]. At the same time, the presence of *A. borkumensis* associated with marine invertebrates seems to reflect a special ecological niche containing readily accessible hydrocarbons produced by the animal partners. Interestingly, although bacteria and *Alcanivorax*-related 16S rRNA gene sequences has been retrieved from microbial communities inhabiting cold polar areas, the organism itself has so far only been isolated from more temperate lower latitudes [2,4,5]. Normally, this bacterium is found in low numbers in unpolluted environments, but it quickly becomes the dominant microbe in oil-polluted open ocean and coastal waters, where it may comprise up to 80–90% of the oil-degrading microbial community [6-8]. Several field studies on bacterial

community dynamics and hydrocarbon degradation in coastal systems have demonstrated the pivotal role of *A. borkumensis* in oil-spill bioremediation [9]. The type of strain of genus *Alcanivorax* is North Sea isolate *A. borkumensis* SK2 [1]. Since its isolation, different species have thus far been described, including *A. jadensis* and *A. venustensis* [10,11], *A. dieselolei* [12], *A. balearicus* [13], *A. hongdengensis* [14] and *A. pacificus* [15] that were isolated from marine environments. Recently been isolated other species as *A. marinus* [16], *A. xenomutans* [17], *A. gelatiniphagus* [18], *A. mobilis* [19], *A. nanhaiticus* [20], *A. indicus* [21], *A. profundus* [22], *A. sediminis* [23], *A. profundimaris* [24], *A. limicola* [25], *A. quisquiliarum* [26] and *A. xiamenensis* [2].

A. borkumensis [27] is classified within the order *Oceanospirillales* of the class of the Gammaproteobacteria. Recent phylogeny analysis [16S rDNA sequencing, DNA-DNA Hybridization (dDDH), Average Nucleotide Identity (ANI), Average

Amino Acid Identity (AAI), Percentage of Conserved Proteins (POCP) and comparative genomic studies [20,28] showed that *Alcanivorax* species formed three clades named *Isoalcanivorax* gen. nov. and *Alloalcanivorax* gen. nov., respectively, along with the emended description of the genus *Alcanivorax* sensu stricto (Table 1). The peculiar physiological and metabolic characteristics of *Alcanivorax* group are, absolutely recognized, the basis of the application of environmental recovery techniques based on the use of microorganisms. Bioremediation is considered as one of the most important eco-friendly and cost-effective technologies for marine ecological restoration, which leads to a complete decomposition of complex petroleum hydrocarbons of spilled oil into non-toxic compound [29,30]. Bioremediation, including biostimulation and bioaugmentation, has proven to be an effective method for cleaning up residual oil in a variety of environments [31] and has been proposed as the only viable management option that can be implemented on a large scale in marine environments [29,31].

Table 1: Schematic representation of taxonomic distribution of bacteria related to *Alcanivoracaceae* family.

Class	Order	Family	Genus	Species	Ref.	Recl.
Gammaproteobacteria	oceanosoirillales	Alcaniv oracacae	<i>Alcanivorax</i>	<i>Alcanivorax borkumensis</i>	Yakimov et al. [1]	
				<i>Alcanivorax hongdengensis</i>	Wu et al. [14]	
				<i>Alcanivorax jadensis</i>	Bruns & Berthe-Corti [10]	
				<i>Alcanivorax limicola</i>	Zhu et al. [25]	
				<i>Alcanivorax nanhaiticus</i>	Lai et al. [20]	
				<i>Alcanivorax profundus</i>	Liu et al. 2019	
				<i>Alcanivorax quisquiliarum</i>	An et al. [26]	
				<i>Alcanivorax sediminis</i>	Liao et al. [23]	
			<i>Alloalcanivorax</i>	<i>Alloalcanivorax balearicus</i>	Rivas et al. [13]	Rai et al. [27]
				<i>Alloalcanivorax dieselolei</i>	Liu and Shao [12]	Rai et al. [27]
				<i>Alloalcanivorax gelatiniphagus</i>	Kwon et al. [18]	Rai et al. 2023 [27]
				<i>Alloalcanivorax marinus</i>	Lai et al. [15]	Rai et al. [27]
				<i>Alloalcanivorax mobilis</i>	Yang et al. [19]	Rai et al. [27]
				<i>Alloalcanivorax profundimaris</i>	Dong et al. [24]	Rai et al. [27]
				<i>Alloalcanivorax venustensis</i>	Fernández-Martínez et al. 2003	Rai et al. [27]
				<i>Alloalcanivorax xenomutans</i>	Rhau et al. 2014	Rai et al. [27]
			<i>Isoalcanivorax</i>	<i>Isoalcanivorax indicus</i>	Song et al. [21]	Rai et al. [27]
				<i>Isoalcanivorax pacificus</i>	Lai et al. 2010	Rai et al. [27]

In marine environment, hydrocarbons biodegradation can be limited by many factors (e.g. nutrients, pH, temperature, oxygen and contaminant presence, Figure 1). Biostimulation involves the

modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of limiting nutrients and electron acceptors, which

are otherwise available in quantities low enough to constrain microbial activity. As indicated [31] as the addition of nutrients, oxygen or other electron donors and acceptors to the coordinated site in order to increase the population or activity of naturally occurring microorganisms available for bioremediation. The

primary advantage of biostimulation is that bioremediation will be undertaken by already present native microorganisms that are well-suited to the subsurface environment and are well distributed spatially within the subsurface (Figure 2).

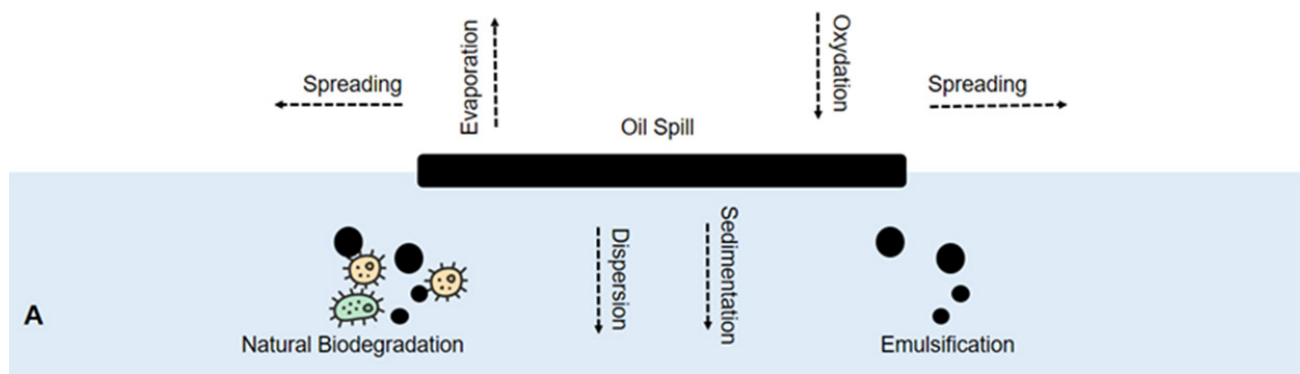


Figure 1: Fate of an oil spill. Oil fate is dependent on many natural processes as the oil begins to reach the surface. These processes include spreading, evaporation, and photo-oxidation on the surface of the water. Emulsification and dispersion take place below the surface, which can assist with microbial degradation of the oil. Oil can also sediment to the sea floor. A, seawater / water column.

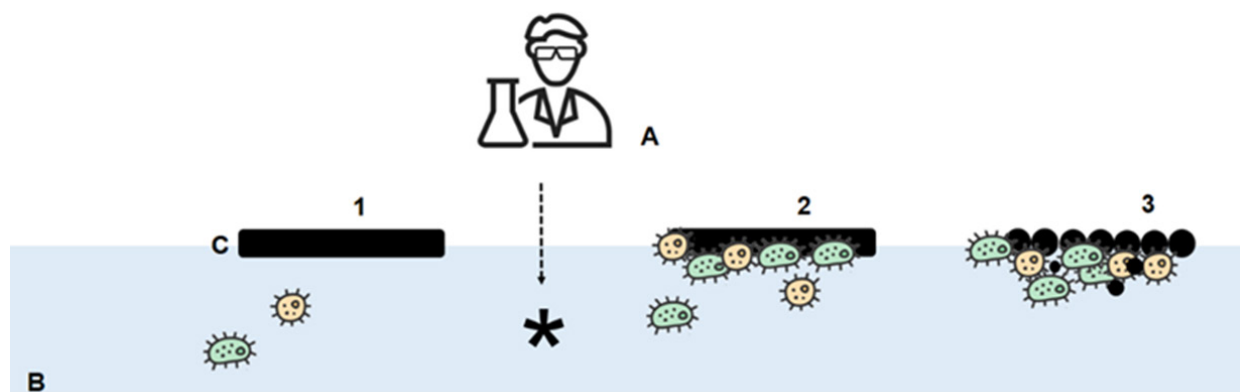


Figure 2: Schematic description of bioremediation (biostimulation) process in marine environment. A: Specialist that according operative protocols alter the natural condition (insertion of nutrients, surfactants, enhancement of microbial growth) to favour the development of natural microbial population with biodegradative capability; B: seawater (water column); C: crude oil. 1: stimulation of natural bacteria; 2: adhesion of bacterial inoculated to crude oil and initial proliferation; 3: growth of bacterial and increment of biodegradation process.

Bioaugmentation involves the introduction of microorganisms isolated from the contaminated site, from a historical site or carefully selected and genetically modified to support the remediation of petroleum hydrocarbon contaminated sites based on the assumption and/or confirmation that indigenous organisms within the impacted site cannot biodegrade petroleum hydrocarbon. Successful bioaugmentation treatments depend on the use of inocula consisting of microbial strains or microbial consortia that have been well adapted to the site to be decontaminated. Foreign

microorganisms (those in inocula) have been applied successfully but their efficiency depends on ability to compete with indigenous microorganisms, predators and various abiotic factors. Factors affecting proliferation of microorganisms used for bioaugmentation including the chemical structure and concentration of pollutants, the availability of the contaminant to the microorganisms, the size and nature of the microbial population and the physical environment should be taken into consideration when screening for microorganisms to be applied (Figure 3).

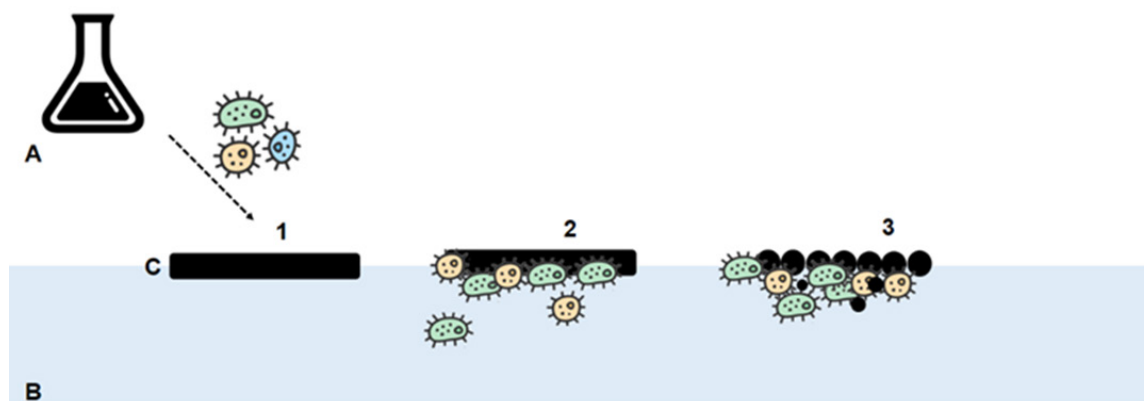


Figure 3: Schematic description of bioremediation (bioaugmentation) in marine environment. A: Flask with microbial culture of hydrocarbon degrading bacteria and/or hydrocarbonoclastic bacteria; B: seawater (water column); C: crude oil. 1: inoculum of bacteria cultivated in laboratory; 2: adhesion of bacterial inoculated to crude oil and initial proliferation; 3: growth of bacterial and increment of biodegradation process.

Furthermore, recent studies [32-34] have proven how *Alcanivorax* bacteria is important polypropylene degraders in mesopelagic environments. *Alcanivorax* strain isolated from plastic marine debris [34] exhibited rapid growth in the presence of weathered low density PE (LDPE) as the sole carbon source; in the same time bacteria related to *Alcanivorax borkumensis* were indicated to be enriched most abundantly on liquid polypropylene and on its structurally similar branched alkane, pristane. Conceptually, the evolution of the application of this bacteria (or bacteria belonging to the same family) can pass through a synergy with different chemical-physical-engineering strategies and technologies. In fact, the application of particular tools (e.g. bioreactor, systems with activated carbon filters...) can favour an increase in biodegradative capability, which therefore allow the normal limits imposed by the natural physiology of these bacteria to be overcome. However, the search for new genera and increasingly performing bacteria, through bi-prospecting that aims at the study of atypical environmental matrices and which could be innovative sources of microorganisms must always be a present point.

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