Research review paper

Bioreactor technology in marine microbiology: From design to future application

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ABSTRACT

Marine micro-organisms have been playing highly diverse roles over evolutionary time: they have defined the chemistry of the oceans and atmosphere. During the last decades, the bioreactors with novel designs have become an important tool to study marine microbiology and ecology in terms of: marine microorganism cultivation and deep-sea bioprocess characterization; unique bio-chemical product formation and intensification; marine waste treatment and clean energy generation. In this review we briefly summarize the current status of the bioreactor technology applied in marine microbiology and the critical parameters to take into account during the reactor design. Furthermore, when we look at the growing population, as well as, the pollution in the coastal areas of the world, it is urgent to find sustainable practices that beneficially stimulate both the economy and the natural environment. Here we outlook a few possibilities where innovative bioreactor technology can be applied to enhance energy generation and food production without harming the local marine ecosystem.

Contents

1. Introduction

The marine environment is the largest habitat on earth, accounting for more than 90% of the biosphere by volume, 71% by area and harbouring micro-organisms responsible for about 50% of the total global primary production (Lauro et al., 2009). Marine micro-organisms have defined the chemistry of the oceans and atmosphere over evolutionary time: they are playing highly diverse roles in terms of ecology and biochemistry. However, mainly because of their extreme living environments compared with terrestrial bacteria, the vast majority of marine microbes have not yet been cultured (Alain and Querellou, 2009; Lang et al., 2005; Vartoukian et al., 2010). Especially, the micro-organisms living in marine sediments usually have a very small niche for survival, under defined physico-chemical conditions. Only under these specific conditions, the thermodynamic energy gained from their metabolic reactions is sufficient to sustain

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themselves. This means that in terms of maintaining or cultivating these organisms one has to take notice of these specific conditions with respect to temperature, pressure and fluid/gas flux (Amend and Shock, 2001). During the last decades, the bioreactors with novel design elements have been applied for studying and even enriching marine microbes (Aertsen et al., 2009; Lang et al., 2005). For this purpose, the bioreactors (as opposed to (fed)-batch incubations) are the only tools to simulate the natural conditions to a certain extent with a good control of the environmental factors.

Besides the fundamental research of the marine micro-organisms, the bioreactor technology has been applied to study water treatment, the production of bioactive compounds and clean energy (Bond et al., 2002; Lang et al., 2005). Firstly, the marine micro-organisms have developed unique cellular properties such as high salt tolerance, hyperthermo-stability, piezophilicity, and cold adaptivity, due to their extreme living conditions (Debashish et al., 2005). Their special

metabolites have been considered as a source of unique bioactive compounds with the potential for industrial development such as pharmaceuticals, cosmetics, nutritional supplements, enzymes, fine chemicals and agrichemicals (Debashish et al., 2005; Leary et al., 2009). A well known example, the biosynthetic capacity to make bryostatin, a powerful PKC-activating cancer cell toxin, was localized to a marine bacterium *Endobugula sertula* associated with *Bugula neritina* (Sudek et al., 2007). The research progress up to year 2005 in the field of bioreactor engineering for the generation of marine bacterial products, has been well reviewed by Lang et al. (2005). Since 2005, along with the application of advanced immobilization and purification technologies, more and more large-scale cultivations of marine bacteria have been reported (Muffler and Ulber, 2008; Sarkar et al.; Sarkar et al., 2008). Also, aquaculture industry is expanding fast: the world aquaculture production from marine waters increased from 19.3 million tons in year 1998 to 34.1 million tons in 2007 (FAO). For each ton of fish production, 7.0 kg

Box 1

Bio Electrochemical Systems.

Here, a brief description of a BES with examples applicable to a marine environment will be provided. Extensive reviews on general BES functioning can be found elsewhere (Logan et al., 2006; Pham et al., 2009; Rabaey and Verstraete, 2005). A BES is a system that is capable of harvesting electrons from microbial respiration. When current is harvested it is called a Microbial Fuel Cell (MFC) and when extra current is supplied to the system to stimulate an electrochemical reaction it is called a Microbial Electrolysis Cell (MEC). Harvesting electrons can be done because some micro organisms are able to respire with solid electron acceptors via direct cell-electrode contact. For instance Geobacter species are able to respire with Fe^{3+} hydroxides. Others are able to respire for instance with reduced Uranium species. Organisms that have this trait can also respire with electrodes made of carbon, platinum or other conductive materials. Another subgroup of micro-organisms is able to use suspended electron shuttles for their respiration with solid materials. These shuttles can be sulphur or manganese or other ionic species but can also be indigenously produced electron mediators such as flavins or phenazines. Once electrons, liberated from the anaerobic degradation of organic matter, are deposited on the anode electrode, these move through an external circuit to the cathode electrode where they are used for a reduction reaction. In the external circuit the electrons are used to perform the work. The most common reduction reaction in the cathode is O_2 reduction to H_2O. The importance is that the anode and cathode reactions are separated in a space to prevent a short circuit. The separation is achieved by adding an ion exchange membrane or sediment. Due to this separation a pH gradient can develop (acidic in the anode environment vs basic in the cathode environment). This gradient is due to the slower movement of the protons compared to the electrons. This movement of protons is needed for the charge balancing of the overall reaction. No research has been presented up till now that investigated the buffering capacity of the marine sediment/water environments for BES functioning. The amount of energy that can potentially be harvested with a marine BES (MFC) can be calculated according to Logan et al. (2006). The true energy produced is also heavily dependent on the mass transfer and electrode kinetics.
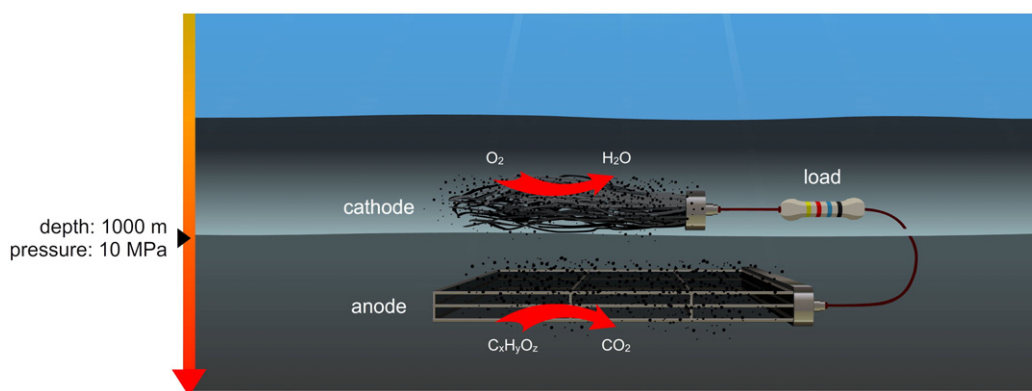
References

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., et al. Microbial fuel cells: methodology and technology. Environ Sci Technol 2006; 40,5181-5192.
Pham, H., Boon, N., Marzorati, M., Verstraete, W. Enhanced removal of 1, 2-dichloroethane by anodophilic microbial consortia. Water Res 2009;43, 2936-2946.
Rabaey, K., Verstraete, W. Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol 2005;23,291-298.

P and 49.3 kg N are released into the water column every year (Cao et al., 2007). This can lead to eutrophication and an alteration of the indigenous aquatic ecosystem. Therefore treatment of aquaculture wastes in marine ecosystems needs to be better managed. Pioneer works have demonstrated that the reactor technology can be used for land based aquaculture wastewater treatment on a small scale (Crab et al., 2007; Lezama-Cervantes and Paniagua-Michel, 2010). However, it remains challenging to transfer those technologies to marine environments. Finally, a certain type of bioreactors, Bio Electrochemical Systems (BES), are capable of harvesting energy from microbial metabolism by separating oxidation and reduction reaction in space and time (Box 1).

Over half of the global human population lives and works in a coastal strip just 200 km wide (Hinrichsen, 1998). Nevertheless the bioreactor technology for marine (eco)systems has not been well explored compared to the terrestrial (eco)systems. The purpose of this review is to give an overview of the possibilities of marine reactor engineering for an improved use of coastal areas for the production of valuables and cleaning of wastes, thereby using the present natural resources in a better and more sustainable fashion for future generations in the highly populated coastal areas.

2. Bioreactor construction and operation

An overview of 6 types of conventional reactor designs suited for marine ecosystem research has been summarized in Table 1. According to the research purpose, modifications and combinations of different configurations in one reactor setup are commonly found. Regarding the specific characteristics of marine microbial processes,

there are special requirements for bioreactor construction and operation.

2.1. In situ incubations and enrichments

The most straightforward option to enrich and cultivate micro-organisms of interest is to use the local environment as a "natural lab" and take the enriched culture for analysis. This approach is mainly suited for marine micro-organisms whose optimal growth conditions are not well defined but whose growth locations are. Moreover, these micro-organisms need to have a natural tendency to attach to surfaces and grow on them, like some groups of microalgae and anammox bacteria (Moreno-Garrido, 2008). For example, in order to enrich marine anammox bacteria, a non-woven fabric was fixed on the seabed and used as a support surface for the anammox bacteria to grow (Nakajima et al., 2008). Another example entails the addition of substrates to an existing sediment-BES for increasing power output and enriching the microbial community (Rezaei et al., 2007). Besides, a precise monitoring of the in situ environmental parameters that are present in the "natural lab" can provide a better understanding of the relation between the marine micro-organisms and their living conditions and thus enhance the chances for ex-situ lab cultivation (de Beer et al., 1997; Wieland et al., 2001).

2.2. Sampling biological resources for marine biotechnology

In the oceanographic literature, different regions of the ocean are divided into distinct provinces based upon their general physical and

Table 1
Bioreactor configurations and their applications.

Bioreactor configuration	Characteristics	Applications
Stirred tank reactor (STR)	Batch and continuous fed mode cultivation	Bioactive compounds production in large scale (Muffler and Ulber, 2008; Sarkar et al., 2008)
Rotating disk bioreactor (RDBR)	Bio-film formation on the disk	Bioactive compound production (Konopka, 2000)
	Collect/remove fast growing/bio-film forming biomass from the disk	Cultivation of oligotrophiles/slow-growing bacteria (Konopka, 2000)
	Mimic the environments with interval of oxic/anoxic	
Gas-lift reactor (ALR)	Gas-form substrate is fed from the bottom to induce mixing	Characterization of processes with gas substrate involved (Meulepas et al., 2009)
Membrane reactor (MR)	Selective membrane is applied to keep biomass	Cultivation of slow growing bacteria (Meulepas et al., 2009)
Continuous high-pressure reactor (CHPR)	High-pressure pump needed to induce high-hydrostatic-pressure	Characterization of processes under high-hydrostatic-pressure (Parkes et al., 2009; Pradillon et al., 2004; Wright et al., 2003; Zeng et al., 2009)
	High-pressure gas or a compressor can be combined to induce high-gas-pressure	Increase gas-form substrate concentration (Deusner et al., 2009; Y. Zhang et al., 2010)
Bio Electrochemical System (BES)	Separation of bioelectrochemical reactions in space and time	Production of direct electricity or energy carriers (Clauwaert and Verstraete, 2009; Logan et al., 2006).
		Isolation of organisms capable of extracellular electron transfer (EET) (Zuo et al., 2008).

Clauwaert, P., Verstraete, W. Methanogenesis in membraneless microbial electrolysis cells. Applied Microbiology and Biotechnology, 2009; 82, 829–836.

Deusner, C., Meyer, V., Ferdelman, T.G. High-pressure systems for gas-phase free continuous incubation of enriched marine microbial communities performing anaerobic oxidation of methane. Biotechnology and Bioengineering, 2009; 105, 524–533.

Konopka, A. Microbial physiological state at low growth rate in natural and engineered ecosystems. Current Opinion in Microbiology, 2000; 3, 244–247.

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J.r., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. Microbial fuel cells: methodology and technology. Environmental Science & Technology, 2006; 40, 5181–5192.

Meulepas, R.J.W., Jagersma, C.G., Gieteling, J., Buisman, C.J.N., Stams, A.J.M., Lens, P.N.L. Enrichment of anaerobic methanotrophs in sulfate-reducing membrane bioreactors. Biotechnology and Bioengineering, 2009; 104, 458–470.

Muffler, K., Ulber, R. Fed-batch cultivation of the marine bacterium *Sulfitobacter pontiacus* using immobilized substrate and purification of sulfite oxidase by application of membrane adsorber technology. Biotechnology and Bioengineering, 2008; 99, 870–875.

Parkes, R.J., Sellek, G., Webster, G., Martin, D., Anders, E., Weightman, A.J., Sass, H. Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). Environmental Microbiology, 2009; 11, 3140–3153.

Pradillon, F., Shillito, B., Chervin, J.C., Hamel, G., Gaill, F. Pressure vessels for in vivo studies of deep-sea fauna. High Pressure Research, 2004; 24, 237–246.

Sarkar, S., Saha, M., Roy, D., Jaisankar, P., Das, S., Roy, L.G., Gachhui, R., Sen, T., Mukherjee, J. Enhanced production of antimicrobial compounds by three salt-tolerant actinobacterial strains isolated from the Sundarbans in a niche-mimic bioreactor. Marine Biotechnology, 2008; 10, 518–526.

Wright, P.C., Westacott, R.E., Burja, A.M. Piezotolerance as a metabolic engineering tool for the biosynthesis of natural products. Biomolecular Engineering, 2003; 20, 325–331.

Zeng, X., Birrien, J.L., Fouquet, Y., Cherkashov, G., Jebbar, M., Querellou, J., Oger, P., Cambon-Bonavita, M.A., Xiao, X., Prieur, D. Pyrococcus CH1, an obligate piezophilic hyperthermophile: extending the upper pressure–temperature limits for life. Isme Journal, 2009; 3, 873–876.

Zhang, Y., Henriet, J.-P., Bursens, J., Boon, N. Stimulation of *in vitro* anaerobic oxidation of methane rate in a continuous high-pressure bioreactor. Bioresource Technology, 2010; 101, 3132–3138.

Zuo, Y., Xing, D.F., Regan, J.M., Logan, B.E. Isolation of the exoelectrogenic bacterium *Ochrobactrum anthropi* YZ-1 by using a U-tube microbial fuel cell. Applied and Environmental Microbiology, 2008; 74, 3130–3137.

chemical characteristics (Schrenk et al., 2010). In each province there are distinct groups of micro-organisms living 1) in free suspension; 2) attached to flocculated material; 3) in the sediment; 4) on animate and inanimate surfaces; and 5) as partners in symbiosis or in commensalism with other marine organisms (DeLong, 1997; Lang et al., 2005). Depending on the origin, specific sampling strategies have to be taken into account to ensure the successful extraction of the mixed microbial cultures. One research line is to develop sampling devices to take the sediment or water out of the ocean without changing the conditions, for example keep the same pressure and temperature as *in situ*. For this purpose, the most challenging task is to take samples from the deep-sea where the pressure is highly elevated and plays an important role on the microbial diversity (Box 2). Parkes and co-workers have developed a system to perform sampling, cultivation, isolation and sub-sampling without depressurization (Parkes et al., 2009). The success of this system opens new possibilities to study the depressurization-sensitive, anaerobic, piezophilic prokaryotic communities. Another research line is to develop techniques to separate different species from each other starting from the original mixed-culture samples. For this purpose, methods based on the "dilution-to-extinction" concept are commonly applied. One example is the so-called high-throughput culturing (HTC) method, which was to dilute natural communities of micro-organisms to a known number, ranging from 1 to 10 cells per well, and then examine the potential microbial growth in each well (Connon and Giovannoni, 2002). This HTC approach has been successfully applied to isolate marine bacterial from SAR11 clade, the marine group I Crenarchaeota and the OM43 clade (Connon and Giovannoni, 2002; Konneke et al., 2005; Stingl et al., 2007).

2.3. Nutrient level

Composing a correct growth medium remains a difficult task when aiming at the maintenance of a high activity and/or at the enrichment of specific species. Special attention has to be given to the fact that for some marine species a high activity is not necessarily linked with a high growth rate because of their low biomass yield. For example, the marine anaerobic methane oxidizing consortia, achieve a biomass yield per mol of the methane oxidized which is 23 times lower compared to the aerobic methane oxidizing consortia from a wastewater treatment plant (Nauhaus et al., 2007; van der Ha et al.,

2010). Nutrient levels in pelagic waters are not uniform. Large expanses of water are relatively nutrient depleted (e.g. open Atlantic Ocean water), whereas other zones are relatively nutrient rich (e.g. Pacific Ocean coastal water) (Lauro et al., 2009). Many marine bacteria have evolved to grow optimally at either high (copiotrophic) or low (oligotrophic) nutrient concentrations, enabling different species to colonize distinct trophic habitats in the ocean (Lauro et al., 2009). Most of the micro-organisms found in the sea are halophiles, which have a specific requirement for the sodium ion (about 3% or even higher) and other minerals as the trace elements to maintain their metabolic activity (Lang et al., 2005). To neutralize the sodium ion, a chloride ion is often used. Minimal salt medium does not always suffice for growing or maintaining certain species. Some marine species also have a requirement for complex carbon sources, including proteins or polysaccharides (Lang et al., 2005). Finally, experimental evidence showed that mixed substrates improve the metabolic flexibility of the microorganisms toward changes in the environment and promote a faster growth in an oligotrophic environment (Egli, 2004). Therefore a complex medium composition, but not necessarily at a high concentration of each substrate (in the order of mg/L), appears to be appealing for slow-growing marine microbes (Law and Button, 1977).

On the other hand, if the harvesting of secondary metabolites is the main purpose, the specific nutrient supply to sustain growth (e.g. carbon or nitrogen source) sometimes has to be limited to keep the cells in the stationary phase, while the substrate needed for the formation of the desired product has to be present in excess. The cells at tropophase (or growth phase) generally have their secondary metabolism, such as antibiotic production, "switched off" until they reach the idiophase (or production phase) (Marwick et al., 1999).

2.4. Temperature

Almost 95% of the seafloor lies in water depths where the temperatures are close to freezing (-1 °C to 4 °C) (Jorgensen and Boetius, 2007). Despite the low global temperature in the deep sea, the hydrothermal vents are hotspots, where the temperature can reach up to 1000 °C, these were discovered some 30 years ago (Jorgensen and Boetius, 2007). Water remains liquid in these regions due to the high hydrostatic pressure (Rothschild and Mancinelli, 2001) (see also Box 2 on thermodynamics). Although the temperature

Box 2
Thermodynamic parameters of deep sea live.

One essential physical parameter determining live in marine ecosystems is the hydrostatic pressure. The hydrostatic pressure correlates with water depth in the sea: there is about 0.1 MPa pressure additional from 10 m water. At the same time, the dissolvability of gas has an almost linear correlation with the hydrostatic pressure. For example, at the water surface and at a water depth of 1000 m, the pressures are atmosphere (0.1 MPa) and 10 MPa; resulting in the saturation concentrations of methane 1.3 mM and 127 mM in seawater at 4 °C (Duan and Mao, 2006). The pressure is also directly influencing the types of bacteria habituating different water depths: non-piezophilic bacteria are living at the surface or in shallow water; piezophilic bacteria are living at the bottom of the sea and in the sediment; while piezo-tolerant bacteria are living in between. Another essential parameter is the light density: depending on water turbidity, sunlight can penetrate a few to hundreds of meters down in the seawater, therefore phototrophic and non-phototrophic zones are also defined. Besides, at different water depths, different compounds are available as electron acceptors in the microbiological reactions to supply energy for microbial growth. The standard Gibb's free energy ($\triangle G^{o\prime}$) is in the first order of the standard redox potential ($\triangle E^{o\prime}$): $\triangle G^{o\prime} = -nF\triangle E^{o\prime}$, (Eq. (1)). Microorganisms prefer to carry out the reaction with the highest possible redox potential difference. Generally oxygen is depleted first; below a few centimetres in the sediment, the anaerobic zone starts. After oxygen, iron, manganese, nitrate and sulfate became the dominant electron acceptors to support the growth of the local ecosystems.

Reference

Duan, Z.H., Mao, S.D. A thermodynamic model for calculating methane solubility, density and gas phase composition of methane-bearing aqueous fluids from 273 to 523 K and from 1 to 2000 bar. Geochim Cosmochim Acta 2006; 70, 3369-3386.

close to the thermal vents decreases fast because of the high thermal conductivity of water (0.6 W/m/K), cell growth over 100 °C can be found close to the vents. The thermophilic vent archaea strain 121 is able to grow at 121 °C at ambient pressure (Kashefi and Lovley, 2003). Recent study has demonstrated that the elevated hydrostatic pressure extends the temperature maximum for possible cell proliferation of a hyperthermophilic methanogen (*Methanopyrus kandleri* strain 116) from 116 °C at 0.4 MPa to 122 °C at 20 MPa (Takai et al., 2008). To maintain the correct incubation temperature of the marine organisms in the lab, either the reactor is placed inside a thermal controlled incubator (Y. Zhang et al., 2010) or surrounded by a layer filled with temperature-controlled water (Meulepas et al., 2009). These methods can normally control the temperature in a range of 1–80 °C. If the incubation temperature has to be even higher, which is not feasible by hot water, a hot air incubator can be applied (Kashefi et al., 2002; Reysenbach et al., 2006).

2.5. Pressure

Pressure is another important thermodynamic parameter, especially at higher depths in the marine environment (Box 2). Indeed, the largest fraction of the ocean is at depths of more than 200 m (Whitman et al., 1998) and 75% of the marine biosphere is located below 1000 m depth (Aertsen et al., 2009). Marine micro-organisms can live up to about 110 MPa hydrostatic pressure which is three orders of magnitude higher than the atmospheric pressure (Aertsen et al., 2009; Lauro and Bartlett, 2008; Simonato et al., 2006; Zeng et al., 2009).

Regarding the design of a high-pressure reactor, both high-hydrostatic-pressure and high-gas-pressure need to be considered. For the piezophilic bacteria whose substrates are easily dissolved at atmospheric conditions, hydrostatic pressurization is sufficient to alter their gene expression to achieve maximum cell growth. High-hydrostatic-pressure can easily be built up with a standard high-pressure liquid chromatography (HPLC) pump, and released by a back-pressure regulator (BPR) (Fig. 1). Depending on the research purpose, different types of high-hydrostatic-pressure reactors have been constructed: the pressurized chemostat (Grossart and Gust, 2009; Jannasch et al., 1996), pressurized thermal gradient block (Kallmeyer et al., 2003), and continuous-mode high-hydrostatic-pressure reactor (Parkes et al., 2009). For certain piezophilic microorganisms, their main substrates are under gaseous form and as a consequence in most cases poorly dissolvable under atmospheric pressure (such as methane or hydrogen). Hence an *in vitro* high-gas-pressure is needed to enhance their metabolic activity and growth rate. For example, anaerobic methanotrophs have an apparent affinity (K_M) of 37 mM for methane, which equals about 3 MPa methane partial pressure (Y. Zhang et al., 2010). To obtain a high dissolved gas concentration, two approaches can be considered: a free gas-phase in the high-pressure incubation vessel with biomass (Nauhaus et al., 2002); or pre-dissolve gas under high-pressure in a conditioning vessel before transferring the saturated medium to the incubation vessel (Y. Zhang et al., 2010) (Fig. 1). In the latter case there is no free gas-phase in the incubation vessel where the biomass is located (Deusner et al., 2009; Y. Zhang et al., 2010).

2.6. Materials

Numerous parameters have to be taken into account when choosing reactor materials, such as chemical composition, surface morphology, and mechanical and chemical stability. As discussed before, some marine micro-organisms specifically require high concentrations of the sodium ion for their growth (Kogure, 1998). High salt levels (in terms of Cl^-, which is commonly the counterpart of the sodium ion) in combination with aeration may cause corrosion problems on austenitic steels. For the cultivation of aerobic marine
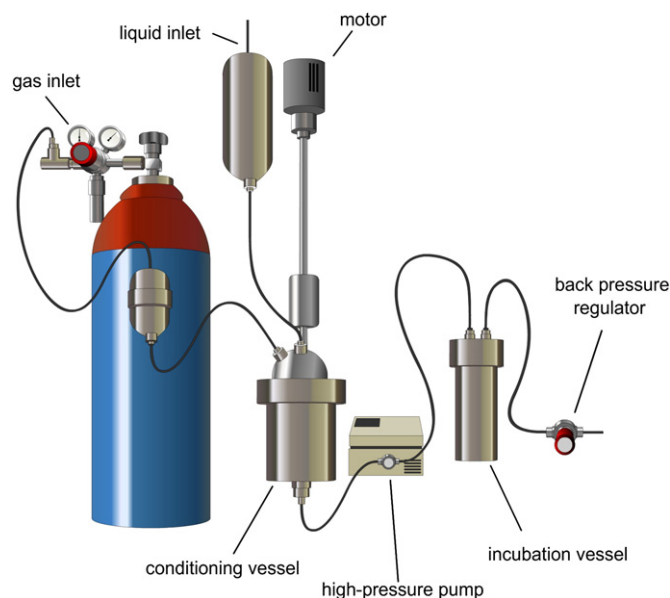

Fig. 1. The scheme of a typical high-pressure bioreactor system (modified from Y. Zhang et al. (2010)).

bacteria, molybdenum is additionally added for pitting resistance (i.e. with grade 316 stainless steel) (Kielemoes et al., 2002). Another type of corrosion is caused by microorganisms, either by their end products or by their enzymatic catalysis (Scotto et al., 1985). For example, denitrification (Kielemoes et al., 2000) and sulfate reducing processes (Muyzer and Stams, 2008) influence the corrosion of iron and stainless steel by using the cathodically produced hydrogen when iron is immersed in anoxic aqueous liquids. This microbiologically influenced corrosion has been well reviewed by Landoulsi et al. (2008) and Kielemoes et al. (2002). For high-pressure incubations, materials like PEEK plastic (Houghton et al., 2007), stainless steel (Deusner et al., 2009; Parkes et al., 2009; Y. Zhang et al., 2010) and titanium (Deusner et al., 2009; Y. Zhang et al., 2010) have been used for the vessels and the tubing. For the corrosion information on these materials, detailed information is available on the website of Parr Instrument Company (Parr). For incubations at atmospheric pressure, glassware has been widely used, as it is inert to any corrosion. Photo-transparency of the reactor (incubation vessel) is sometimes required for photo-synthetically active cells (Meunier et al., 2010). Inside the reactors, carrier materials, for example different polymer materials (Meulepas et al., 2009; Sudarno et al., 2010), have been tested to provide sufficient surface to form a biofilm.

Materials used in a BES in a marine setting are not different from the materials that are used in the BES in other environments. This is due to the intrinsic nature of a BES, which actually promotes 'corrosion' i.e. redox reactions to happen. Therefore materials that are useful as electrodes in a BES are conductive but stabile, as they do not participate in the redox reactions. For the BES housing, the materials need to be isolated to prevent a short circuit from occurring. Examples of conductive materials are graphite granules, felt and rods with different porosities and surface characteristics (Clauwaert et al., 2008; Scott et al., 2008a, 2008b). Stainless steel and other materials have also been used in a BES (Dumas et al., 2007; Thrash and Coates, 2008). A special note is in its place for the cathode, where in lab scale reactors platinum is often used for catalyzing a high rate oxygen reduction reaction (ORR). This is however not needed when a biological oxygen-reducing cathode is used (Clauwaert et al., 2007a, 2007b; Cournet et al., 2010; He and Angenent, 2006).

Table 2
In vitro simulations of marine microbial processes and environments.

Processes and environments	Reactors and references
Intertidal estuarine	Rotating disk bioreactor (Sarkar et al., 2008)
Sinking from surface water to deep ocean	Pressurized microcosm (Grossart and Gust, 2009)
Cold water seeps	Continuous flow-through reactor (Girguis et al., 2003); Continuous high-pressure reactor (Y. Zhang et al., 2010) Membrane bioreactor (Meulepas et al., 2009)
Hydrothermal vents	Continuous high-pressure reactor (Houghton et al., 2007; Imai et al., 1999) Gas-lift bioreactor (Postec et al., 2007) Fermentor (Mukhopadhyay et al., 1999)
Hyperbaric environment	A diamond anvil cell (Aertsen et al., 2009)

Reference:
Aertsen, A., Meersman, F., Hendrickx, M.E.G., Vogel, R.F., Michiels, C.W. Biotechnology under high pressure: applications and implications. Trends in Biotechnology, 2009; 27, 434–441.

Girguis, P.R., Orphan, V.J., Hallam, S.J., DeLong, E.F. Growth and methane oxidation rates of anaerobic methanotrophic archaea in a continuous-flow bioreactor. Applied and Environmental Microbiology, 2003; 69, 5472–5482.

Grossart, H.P., Gust, G. Hydrostatic pressure affects physiology and community structure of marine bacteria during settling to 4000 m: an experimental approach. Marine Ecology-Progress Series, 2009; 390, 97–104.

Houghton, J.L., Seyfried, W.E., Banta, A.B., Reysenbach, A.L. Continuous enrichment culturing of thermophiles under sulfate and nitrate-reducing conditions and at deep-sea hydrostatic pressures. Extremophiles, 2007; 11, 371–382.

Imai, E., Honda, H., Hatori, K., Brack, A., Matsuno, K. Elongation of oligopeptides in a simulated submarine hydrothermal system. Science, 1999; 283, 831–833.

Meulepas, R.J.W., Jagersma, C.G., Gieteling, J., Buisman, C.J.N., Stams, A.J.M., Lens, P.N.L. Enrichment of anaerobic methanotrophs in sulfate-reducing membrane bioreactors. Biotechnology and Bioengineering, 2009; 104, 458–470.

Mukhopadhyay, B., Johnson, E.F., Wolfe, R.S. Reactor-scale cultivation of the hyperthermophilic methanarchaeon *Methanococcus jannaschii* to high cell densities. Applied and Environmental Microbiology, 1999; 65, 5059–5065.

Postec, A., Lesongeur, F., Pignet, P., Ollivier, B., Querellou, J., Godfroy, A. Continuous enrichment cultures: insights into prokaryotic diversity and metabolic interactions in deep-sea vent chimneys. Extremophiles, 2007; 11, 747–757.

Sarkar, S., Saha, M., Roy, D., Jaisankar, P., Das, S., Roy, L.G., Gachhui, R., Sen, T., Mukherjee, J. Enhanced production of antimicrobial compounds by three salt-tolerant actinobacterial strains isolated from the Sundarbans in a niche-mimic bioreactor. Marine Biotechnology, 2008; 10, 518–526.

Zhang, Y., Henriet, J.-P., Bursens, J., Boon, N. Stimulation of *in vitro* anaerobic oxidation of methane rate in a continuous high-pressure bioreactor. Bioresource Technology, 2010; 101, 3132–3138.

3. Current status of reactor technology

The processes that have already been simulated *in vitro* by a reactor system are listed in Table 2. The choice of the most suitable system is situation-dependent. Depending on the research question, three different best practices can be distinguished for 1) mimicking the natural environment, 2) stimulating the uncultured or producing metabolites of interest and 3) controlling redox conditions on the sediment/water interface.

If the research goal is to mimic the natural environmental conditions as precise as possible, the high-pressure bioreactor system should be applied since most of the marine environment is under pressure (Fig. 1; Table 1). However, the cost for materials and operation, together with the safety training for operational personnel are the main difficulties when scaling up. For an accurate simulation, biogeochemical *in situ* measurements need to be as detailed as possible. This is not always easy, since the environmental parameters are highly location-dependent and the sites, such as hydrothermal vents, are difficult to access with sophisticated instruments for precise measurements. Nowadays, video-guided *in situ* analytical instruments, such as sensors for temperature, pH, metals, CO_2 and sulphide, are widely used (de Beer et al., 1997; Lichtschlag et al., 2010; Wieland et al., 2001). The concept of underwater modules that autonomously

measure and record data electronically is already successfully demonstrated (Jorgensen and Boetius, 2007). However, their application for scientific interests and their influence on the ecosystems need to be better explored.

If the research aim is to stimulate, enrich or cultivate previously uncultured micro-organisms and/or produce metabolites of interest, the use of relatively low substrate concentrations, long incubation periods, and even additional signalling compounds are advantageous to increase the cultivability and improve the recovery of prokaryotes from the different natural samples (Alain and Querellou, 2009). The rotating disk bioreactor (RDBR) has successfully been applied for these purposes (Table 1). In order to ensure economically viable metabolite production, the concentration and purity of the product of interest are the two key criteria to take into account for reactor design and operation. New strategies need to be developed to up-concentrate valuable products (involving novel reactor designs) and to increase the immobilization of active cells in the reactor (use of carrier materials). These are the two elements that need better investigation (Meunier et al., 2010).

A third path of marine (sediment) reactor technology can be found in the Bio Electrochemical Systems (BES). This technology has been an unconventional one in marine research but is gaining more and more attention. This technology has mainly been researched for wastewater treatment and other freshwater applications but the high ionic strength and thus high conductivity and low ohmic resistance of marine and brackish waters are favourable attributes for high electron transfer (Box 1). Detailed descriptions regarding the application of BES in marine settings can be found elsewhere (Reimers et al., 2001; Tender et al., 2002). It has already been shown that this technology can power remote sensors (Donovan et al., 2008) and meteorological buoys (Tender et al., 2008) in the field. On the anode side different (polluting) compounds can be bio-electrochemically removed such as organic sediments (i.e. anodic dredging) (Hong et al., 2010), aromatic hydrocarbons (T. Zhang et al., 2010;), azodyes (Mu et al., 2009) and halogenated compounds (Pham et al., 2009). The anode can thus be used to stimulate anaerobic degradation processes by offering an alternative electron acceptor, i.e. the electrode. This electrode can be poised at a set potential, thus selecting for specific reactions (not) to occur or for different microbial consortia to establish themselves on the anode (Aelterman et al., 2008). The same concept goes for the cathode where electrons can be provided from an alternative source, also at a given potential. Other than producing power or anodically oxidizing compounds, a BES operated under electrolysis conditions can also efficiently make several products such as, but not limited to, H_2 (Rozendal et al., 2008), CH_4 (Clauwaert and Verstraete, 2009; Villano et al., 2010) and ethanol (Steinbusch et al., 2010) by choosing the right conditions in the cathode.

4. Outlook and opportunities

Since the development of the bioreactor technology, marine microbiology has not been limited anymore to *in situ* observation and quantification. It has been intensively studied *in vitro* in which the bioreactor has been proven as a good tool to: simulate the various natural environments (Y. Zhang et al., 2010); to support the growth of marine microbes (Meulepas et al., 2009; Nakajima et al., 2008); to industrialize the production of bioactive compounds (Lang et al., 2005) and to control the E_h of marine sediments while harvesting energy or making a product (Bond et al., 2002; Timmes et al., 2010). The research focus has shifted from *in situ* to *in vitro*, from exploration to experimentation. However, bioreactors are not restricted to be placed in labs or on site in factories. To tackle future technological and/or biological challenges a solution can be found in the application of the bioreactor technology *in situ*. Pioneer work has demonstrated the success of underwater modules, which were placed on the seafloor by submersibles, for autonomously measuring and recording

data electronically (Alain and Querellou, 2009; Jorgensen and Boetius, 2007). From our point of view, the following lines of research and development can be of great interest to apply bioreactor technology in natural marine environments:

1) BES and controlling the marine redox environment.

   As discussed above a BES in the MFC mode is able to provide long-term low power outputs to remote sensing systems (Donovan et al., 2008; Tender et al., 2008). It is, however, not able to sustain large power outputs for use in urban or industrialized areas. Therefore we propose to use a BES in a different fashion near human occupied coastal areas. Usually in these areas there is a degree of hypoxia caused by eutrophication (Levin et al., 2009; Paerl, 2006; Rabalais et al., 2010). This eutrophication is due to increased primary production due to the anthropogenic release of nutrients (nitrogen, phosphorus and trace elements) and COD (chemical oxygen demand) into the coastal waters. Subsequent aerobic decay of all this organic matter leads to hypoxia, which in its turn leads to a drop in higher marine life and food (fish, shrimp, and crustaceans) and an increase in jellyfish which can have an even more detrimental effect on the ecosystem functioning and economic services of the marine coastal area (Purcell et al., 2007). With a BES, it is possible to introduce an alternative electron acceptor to the benthic zone and thus decreasing the dependence on oxygen as a terminal electron acceptor for the decay of organic matter in the sediment. This proposal has never been tested in practice or in a lab scale experiment. One can envision that by providing an alternative electron acceptor, possibly seeded with a

suitable active biofilm, metabolic process will take a different route. A comparable process, illustrating the principle with other reactants, has been seen in a lab scale BES where providing an electrode to a methanogenic anaerobic environment, electricity is produced in conjunction with a drop in methane production (Pham et al., 2006). Also the reverse is true, a BES with a high organic load will revert to methanogenesis when the substrate transfer to the anodic biofilm is limited (Freguia et al., 2008; Pham et al., 2006). However for applying such a system of redox control, a suitable cathodic reaction needs to be found. An oxygen reduction reaction is not a solution as this will be counterproductive towards mitigation of the eutrophication. However a long distance cathode (Williams et al., 2010) can be used when it is placed on the water surface where there is a lot of photosynthetic oxygen production. Another possibility is to make use of a nitrate reducing biocathode reaction (Clauwaert et al., 2007a, 2007b). Nitrate is one of the nutrients causing the eutrophication (Rabalais et al., 2010) thus the mitigation of the eutrophication can work two ways. The first is to lessen the dependence on oxygen to break down organic matter; the second is to lower the availability of the nitrate which is a cause of eutrophication.

2) BESs for product formation.

   Another application for a BES is to operate it in an electrolysis mode (microbial electrolysis cell, MEC). In this mode gaseous energy carriers can be produced or even better, when focusing on the mitigation of anthropogenic effects, CO_2 can be captured and sequestered in the form of CH_4 (Cheng et al., 2009; Clauwaert and Verstraete, 2009). Methane is also a greenhouse gas but not directly
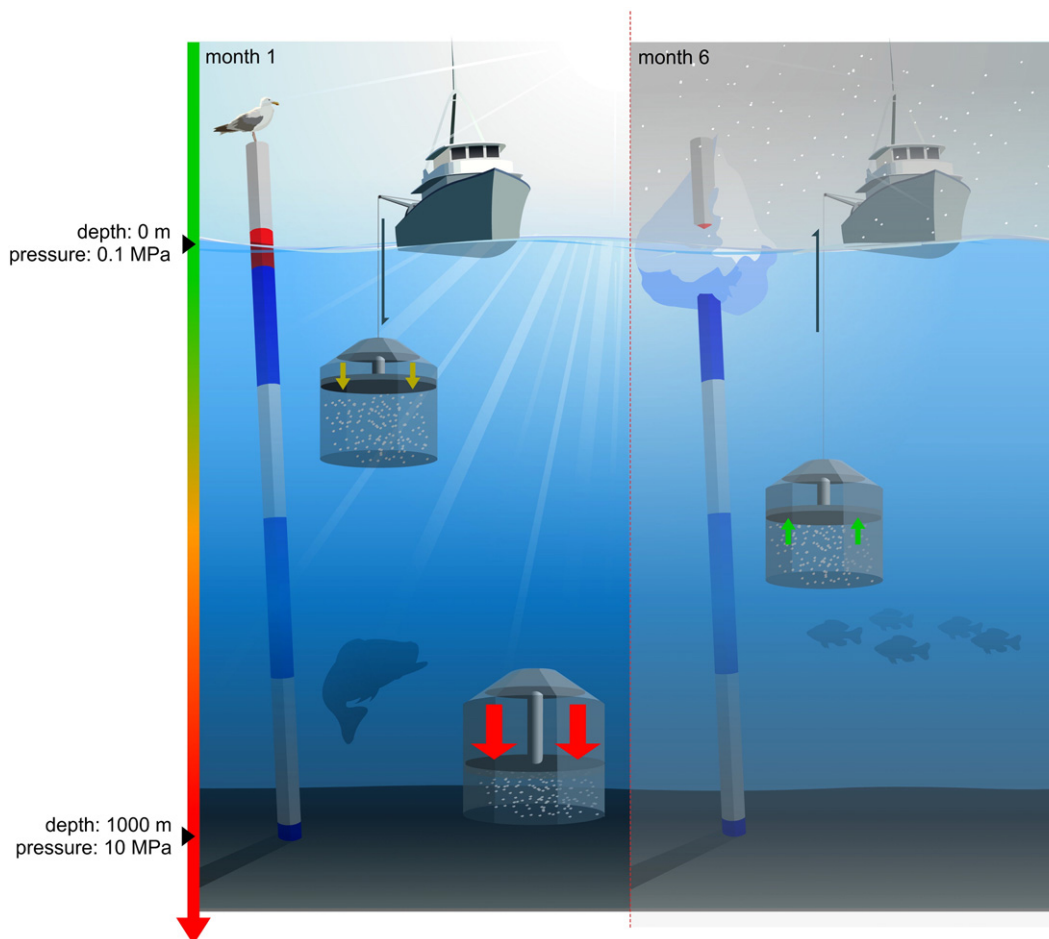

Fig. 2. The use of natural water depth to pressurize a bioreactor.

involved in ocean acidification. When it is produced in a controlled environment, it can be harvested and put to good use as a bio-fuel. Producing and harvesting the CH_4 is enhanced by the lower solubility of CH_4 as compared with CO_2 (approximately $10 \times$ less soluble at all depths (Duan and Mao, 2006; Duan and Sun, 2003)).

3) Using the deep sea as natural pressuring device.
   We also envision the possibility to use the water pressure at increasing depths to grow piezophillic organisms and to speed up the piezophillic reactions. Although high-pressure bioreactors have been shown to be the best tool to simulate deep-sea environments, they are still not in a large-scale application after 20 years of development. Safety reasons put restrictions on operation of a large-scale high-pressure (bio)reactor on land (especially when a pressurized gas phase is included). Cost issues have also played a major role in hampering the use of large scale pressurized (bio)reactors on land. Two major costs occurred in the pressurizing of a large volume and cooling the whole system. A solution is to construct a piston-based bioreactor, which can be sent into the deep water (Fig. 2). The reactor is pressurized by the spontaneous increase of hydrostatic pressure while it is sinking to the required depth. This is especially interesting for the production of bioactive compounds from piezophilic bacteria at low temperature. The challenge is to load the module and collect the module or the products cost efficiently. Selective membranes can be applied to allow natural seawater to penetrate the reactor housing and serve as a substrate for micro-organisms while simultaneously locking the product inside the module. This allows the device to be immobilized for a longer period to reduce the cost of loading and unloading.

4) A self-powered deep sea digester for biochemical production.
   Another possibility to sustainably exploit the natural resources of the ocean, is to develop an on site bioreactor producing valuables from planktonic biomass. The bioreactor would be operated in a similar way as the reactor described in Fig. 2. The biomass can be harvested actively or passively through a microfiltration membrane unit. After filtration this planktonic biomass is further transferred to an anaerobic digester to produce fatty acid and protein, with the same approach as applied in wastewater treatment (Verstraete et al., 2005, 2009). These products can serve as building blocks for the production of bio-products, like polyhydroxyalkanoates (PHA) (Rehm, 2007). In view of the transition from an oil based economy to a green economy (Jones and De Meyere, 2009), these PHA can be used for the production of biopolymers and bioplastics (Morgan-Sagastume et al., 2010). Ecological considerations were made that "green bioplastics" like PHA are gaining popularity as alternatives to common polymeric materials (Martin et al., 2007). Furthermore, to be sustainable the above-described bioreactor needs to be self-powered. To meet the energy requirement for self-powering, the organic carbon not used for PHA production can be reduced to methane. The *in situ* high pressure keeps methane compressed into the liquid phase and is thus easily stored. Once in a while, this bioreactor travels from the deep water to the sea surface, where methane can be combusted with air. The energy derived from the combustion is transferred as electricity and stored in a battery.

5. Conclusions

The application of the bioreactor technology in and/or for marine (eco)systems has been implemented in various research fields: blue biotechnology, such as marine micro-organism cultivation and deep-sea bioprocess characterization; white biotechnology, such as bioactive compound production and green biotechnology, such as marine waste treatment, and energy generation. For future applications, it is promising to combine organic matter removal with food production and even energy generation by using off shore *in situ* bioreactors. The

BES has a great potential to be applied in coastal areas or in sediments for energy harvesting and controlling marine sediment E_h. The application of the bioreactor technology has allowed us to switch our research strategy from *in situ* exploration to *in vitro* experimentation. Now is the time to bring the bioreactor technology back to the sea, to provide the coastal populations with technology for sustainable utilization of the natural resources of the sea.

References

Aelterman P, Freguia S, Keller J, Verstraete W, Rabaey K. The anode potential regulates bacterial activity in microbial fuel cells. Appl Microbiol Biotechnol 2008;78:409–18.

Aertsen A, Meersman F, Hendrickx MEG, Vogel RF, Michiels CW. Biotechnology under high pressure: applications and implications. Trends Biotechnol 2009;27:434–41.

Alain K, Querellou J. Cultivating the uncultured: limits, advances and future challenges. Extremophiles 2009;13:583–94.

Amend JP, Shock EL. Energetics of overall metabolic reactions of thermophilic and hyperthermophilic archaea and bacteria. FEMS Microbiol Rev 2001;25:175–243.

Bond DR, Holmes DE, Tender LM, Lovley DR. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 2002;295:483–5.

Cao L, Wang W, Yang Y, Yang C, Yuan Z, Xiong S, et al. Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. Environ Sci Pollut Res 2007;14:452–62.

Cheng SA, Xing DF, Call DF, Logan BE. Direct biological conversion of electrical current into methane by electromethanogenesis. Environ Sci Technol 2009;43:3953–8.

Clauwaert P, Verstraete W. Methanogenesis in membraneless microbial electrolysis cells. Appl Microbiol Biotechnol 2009;82:829–36.

Clauwaert P, Rabaey K, Aelterman P, De Schamphelaire L, Ham TH, Boeckx P, et al. Biological denitrification in microbial fuel cells. Environ Sci Technol 2007a;41:3354–60.

Clauwaert P, Van der Ha D, Boon N, Verbeken K, Verhaege M, Rabaey K, et al. Open air biocathode enables effective electricity generation with microbial fuel cells. Environ Sci Technol 2007b;41:7564–9.

Clauwaert P, Aelterman P, Pham TH, De Schamphelaire L, Carballa M, Rabaey K, et al. Minimizing losses in bio-electrochemical systems: the road to applications. Appl Microbiol Biotechnol 2008;79:901–13.

Connon SA, Giovannoni SJ. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl Environ Microbiol 2002;68:3878–85.

Cournet A, Délia M-L, Bergel A, Roques C, Bergé M. Electrochemical reduction of oxygen catalyzed by a wide range of bacteria including Gram-positive. Electrochem Commun 2010;12:505–8.

Crab R, Avnimelech Y, Defoirdt T, Bossier P, Verstraete W. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 2007;270:1-14.

de Beer D, Glud A, Epping E, Kuhl M. A fast-responding CO_2 microelectrode for profiling sediments, microbial mats, and biofilms. Limnol Oceanogr 1997;42:1590–600.

Debashish G, Malay S, Barindra S, Joydeep M. Marine enzymes marine biotechnology I. Berlin: Springer-Verlag Berlin; 2005. p. 189–218.

DeLong EF. Marine microbial diversity: the tip of the iceberg. Trends Biotechnol 1997;15:203–7.

Deusner C, Meyer V, Ferdelman TG. High-pressure systems for gas-phase free continuous incubation of enriched marine microbial communities performing anaerobic oxidation of methane. Biotechnol Bioeng 2009;105:524–33.

Donovan C, Dewan A, Heo D, Beyenal H. Batteryless, wireless sensor powered by a sediment microbial fuel cell. Environ Sci Technol 2008;42:8591–6.

Duan ZH, Mao SD. A thermodynamic model for calculating methane solubility, density and gas phase composition of methane-bearing aqueous fluids from 273 to 523 K and from 1 to 2000 bar. Geochim Cosmochim Acta 2006;70:3369–86.

Duan ZH, Sun R. An improved model calculating CO_2 solubility in pure water and aqueous NaCl solutions from 273 to 533 K and from 0 to 2000 bar. Chem Geol 2003;193:257–71.

Dumas C, Mollica A, Feron D, Basseguy R, Etcheverry L, Bergel A. Marine microbial fuel cell: use of stainless steel electrodes as anode and cathode materials. Electrochim Acta 2007;53:468–73.

Egli T. Microbial growth with mixtures of carbon substrates: what are its implications for the degradation of organic pollutants in particular and for microbial ecology in general? Leiden: A a Balkema Publishers; 2004.

FAO. FAO yearbook of fishery statistics summary tables.

Freguia S, Rabaey K, Yuan ZG, Keller J. Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environ Sci Technol 2008;42:7937–43.

Girguis PR, Orphan VJ, Hallam SJ, DeLong EF. Growth and methane oxidation rates of anaerobic methanotrophic archaea in a continuous-flow bioreactor. Applied and Environmental Microbiology 2003;69:5472–82.

Grossart HP, Gust G. Hydrostatic pressure affects physiology and community structure of marine bacteria during settling to 4000 m: an experimental approach. Mar Ecol Prog Ser 2009;390:97-104.

He Z, Angenent LT. Application of bacterial biocathodes in microbial fuel cells. Electroanalysis 2006;18:2009–15.

Hinrichsen D. Coastal waters of the world: trends, threats, and strategies. Washinton D.C. Island Press; 1998.

Hong SW, Kim HS, Chung TH. Alteration of sediment organic matter in sediment microbial fuel cells. Environ Pollut 2010;158:185–91.

Houghton JL, Seyfried WE, Banta AB, Reysenbach AL. Continuous enrichment culturing of thermophiles under sulfate and nitrate-reducing conditions and at deep-sea hydrostatic pressures. Extremophiles 2007;11:371–82.

Imai E, Honda H, Hatori K, Brack A, Matsuno K. Elongation of oligopeptides in a simulated submarine hydrothermal system. Science 1999;283:831–3.

Jannasch HW, Wirsen CO, Doherty KW. A pressurized chemostat for the study of marine barophilic and oligotrophic bacteria. Appl Environ Microbiol 1996;62:1593–6.

Jones PT, De Meyere V. Terra reversa. Uitgeverij ASP. Brussels: Academic & Scientific Publishers; 2009.

Jorgensen BB, Boetius A. Feast and famine — microbial life in the deep-sea bed. Nat Rev Microbiol 2007;5:770–81.

Kallmeyer J, Ferdelman TG, Jansen KH, Jorgensen BB. A high-pressure thermal gradient block for investigating microbial activity in multiple deep-sea samples. J Microbiol Meth 2003;55:165–72.

Kashefi K, Lovley DR. Extending the upper temperature limit for life. Science 2003;301:934.

Kashefi K, Holmes DE, Reysenbach AL, Lovley DR. Use of Fe(III) as an electron acceptor to recover previously uncultured hyperthermophiles: isolation and characterization of *Geothermobacterium ferrireducens* gen. nov., sp nov. Appl Environ Microbiol 2002;68:1735–42.

Kielemoes J, De Boever P, Verstraete W. Influence of denitrification on the corrosion of iron and stainless steel powder. Environ Sci Technol 2000;34:663–71.

Kielemoes J, Bultinck I, Verstraete W. Biocorrosie van austenitisch roestvast staal. Mechelen: Kluwer Techniek; 2002.

Kogure K. Bioenergetics of marine bacteria. Curr Opin Biotechnol 1998;9:278–82.

Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 2005;437:543–6.

Konopka A. Microbial physiological state at low growth rate in natural and engineered ecosystems. Current Opinion in Microbiology 2000;3:244–7.

Landoulsi J, El Kirat K, Richard C, Feron D, Pulvin S. Enzymatic approach in microbial-influenced corrosion: a review based on stainless steels in natural waters. Environ Sci Technol 2008;42:2233–42.

Lang S, Huners M, Lurtz V. Bioprocess engineering data on the cultivation of marine prokaryotes and fungi marine biotechnology II. Berlin: Springer-Verlag Berlin; 2005. p. 29–62.

Lauro FM, Bartlett DH. Prokaryotic lifestyles in deep sea habitats. Extremophiles 2008;12:15–25.

Lauro FM, McDougald D, Thomas T, Williams TJ, Egan S, Rice S, et al. The genomic basis of trophic strategy in marine bacteria. Proc Natl Acad Sci USA 2009;106:15527–33.

Law AT, Button DK. Multiple-carbon-source-limited growth kinetics of a marine coryneform bacterium. J Bacteriol 1977;129:115–23.

Leary D, Vierros M, Hamon G, Arico S, Monagle C. Marine genetic resources: a review of scientific and commercial interest. Mar Policy 2009;33:183–94.

Levin LA, Ekau W, Gooday AJ, Jorissen F, Middelburg JJ, Naqvi SWA, et al. Effects of natural and human-induced hypoxia on coastal benthos. Biogeosciences 2009;6:2063–98.

Lezama-Cervantes C, Paniagua-Michel J. Effects of constructed microbial mats on water quality and performance of *Litopenaeus vannamei* post-larvae. Aquacult Eng 2010;42:75–81.

Lichtschlag A, Felden J, Bruchert V, Boetius A, de Beer D. Geochemical processes and chemosynthetic primary production in different thiotrophic mats of the Hakon Mosby Mud Volcano (Barents Sea). Limnol Oceanogr 2010;55:931–49.

Martin K, Paula H, Rodolfo B, Christoph K, Aid A, Gerhart B. Current advances in cost efficient polyhydroxyalkanoate production. Curr Top Biotechnol 2007;3:1-13.

Marwick JD, Wright PC, Burgess JG. Bioprocess intensification for production of novel marine bacterial antibiotics through bioreactor operation and design. Mar Biotechnol 1999;1:495–507.

Meulepas RJW, Jagersma CG, Gieteling J, Buisman CJN, Stams AJM, Lens PNL. Enrichment of anaerobic methanotrophs in sulfate-reducing membrane bioreactors. Biotechnol Bioeng 2009;104:458–70.

Meunier CF, Dandoy P, Su BL. Encapsulation of cells within silica matrixes: towards a new advance in the conception of living hybrid materials. J Colloid Interface Sci 2010;342:211–24.

Moreno-Garrido I. Microalgae immobilization: current techniques and uses. Bioresour Technol 2008;99:3949–64.

Morgan-Sagastume F, Karlsson A, Johansson P, Pratt S, Boon N, Lant P, et al. Production of polyhydroxyalkanoates in open, mixed cultures from a waste sludge stream containing high levels of soluble organics, nitrogen and phosphorus. Water Res 2010;44:5196–211.

Mu Y, Rabaey K, Rozendal RA, Yuan ZG, Keller J. Decolorization of azo dyes in bioelectrochemical systems. Environ Sci Technol 2009;43:5137–43.

Muffler K, Ulber R. Fed-batch cultivation of the marine bacterium *Sulfitobacter pontiacus* using immobilized substrate and purification of sulfite oxidase by application of membrane adsorber technology. Biotechnol Bioeng 2008;99:870–5.

Mukhopadhyay B, Johnson EF, Wolfe RS. Reactor-scale cultivation of the hyperthermophilic methanarchaeon Methanococcus jannaschii to high cell densities. Applied and Environmental Microbiology 1999;65:5059–65.

Muyzer G, Stams AJM. The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 2008;6:441–54.

Nakajima J, Sakka M, Kimura T, Furukawa K, Sakka K. Enrichment of anammox bacteria from marine environment for the construction of a bioremediation reactor. Appl Microbiol Biotechnol 2008;77:1159–66.

Nauhaus K, Boetius A, Kruger M, Widdel F. In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. Environ Microbiol 2002;4:296–305.

Nauhaus K, Albrecht M, Elvert M, Boetius A, Widdel F. In vitro cell growth of marine archaeal–bacterial consortia during anaerobic oxidation of methane with sulfate. Environ Microbiol 2007;9:187–96.

Paerl HW. Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: interactive effects of human and climatic perturbations. Ecol Eng 2006;26:40–54.

Parkes RJ, Sellek G, Webster G, Martin D, Anders E, Weightman AJ, et al. Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). Environ Microbiol 2009;11:3140–53.

Parr. http://www.parrinst.com/default.cfm?Page_ID=357.

Pham TH, Rabaey K, Aelterman P, Clauwaert P, De Schamphelaire L, Boon N, et al. Microbial fuel cells in relation to conventional anaerobic digestion technology. Eng Life Sci 2006;6:285–92.

Pham H, Boon N, Marzorati M, Verstraete W. Enhanced removal of 1, 2-dichloroethane by anodophilic microbial consortia. Water Res 2009;43:2936–46.

Postec A, Lesongeur F, Pignet P, Ollivier B, Querellou J, Godfroy A. Continuous enrichment cultures: insights into prokaryotic diversity and metabolic interactions in deep-sea vent chimneys. Extremophiles 2007;11:747–57.

Pradillon F, Shillito B, Chervin JC, Hamel G, Gaill F. Pressure vessels for in vivo studies of deep-sea fauna. High Pressure Research 2004;24:237–46.

Purcell JE, Uye S, Lo WT. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Mar Ecol Prog Ser 2007;350:153–74.

Rabalais NN, Diaz RJ, Levin LA, Turner RE, Gilbert D, Zhang J. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 2010;7:585–619.

Rehm BHA. Biogenesis of microbial polyhydroxyalkanoate granules: a platform technology for the production of tailor-made bioparticles. Curr Issues Mol Biol 2007;9:41–62.

Reimers CE, Tender LM, Fertig S, Wang W. Harvesting energy from the marine sediment–water interface. Environ Sci Technol 2001;35:192–5.

Reysenbach AL, Liu YT, Banta AB, Beveridge TJ, Kirshtein JD, Schouten S, et al. A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. Nature 2006;442:444–7.

Rezaei F, Richard TL, Brennan RA, Logan BE. Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems. Environ Sci Technol 2007;41:4053–8.

Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature 2001;409:1092–101.

Rozendal RA, Jeremiasse AW, Hamelers HVM, Buisman CJN. Hydrogen production with a microbial biocathode. Environ Sci Technol 2008;42:629–34.

Sarkar S, Saha M, Roy D, Jaisankar P, Das S, Roy LG, et al. Enhanced production of antimicrobial compounds by three salt-tolerant actinobacterial strains isolated from the Sundarbans in a niche-mimic bioreactor. Mar Biotechnol 2008;10:518–26.

Schrenk MO, Huber JA, Edwards KJ. Microbial provinces in the subseafloor. Annu Rev Mar Sci 2010;2:279–304.

Scott K, Cotlarciuc I, Hall D, Lakeman JB, Browning D. Power from marine sediment fuel cells: the influence of anode material. J Appl Electrochem 2008a;38:1313–9.

Scott K, Cotlarciuc I, Head I, Katuri KP, Hall D, Lakeman JB, et al. Fuel cell power generation from marine sediments: investigation of cathode materials. J Chem Technol Biotechnol 2008b;83:1244–54.

Scotto V, Dicintio R, Marcenaro G. The influence of marine aerobic microbial film on stainless-steel corrosion behavior. Corros Sci 1985;25:185–94.

Simonato F, Campanaro S, Lauro FM, Vezzi A, D'Angelo M, Vitulo N, et al. Piezophilic adaptation: a genomic point of view. J Biotechnol 2006;126:11–25.

Steinbusch KJJ, Hamelers HVM, Schaap JD, Kampman C, Buisman CJN. Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. Environ Sci Technol 2010;44:513–7.

Stingl U, Tripp HJ, Giovannoni SJ. Improvements of high-throughput culturing yielded novel SAR11 strains and other abundant marine bacteria from the Oregon coast and the Bermuda Atlantic Time Series study site. ISME J 2007;1:361–71.

Sudarno U, Bathe S, Winter J, Gallert C. Nitrification in fixed-bed reactors treating saline wastewater. Appl Microbiol Biotechnol 2010;85:2017–30.

Sudek S, Lopanik NB, Waggoner LE, Hildebrand M, Anderson C, Liu HB, et al. Identification of the putative bryostatin polyketide synthase gene cluster from "Candidatus endobugula sertula", the uncultivated microbial symbiont of the marine bryozoan Bugula neritina. J Nat Prod 2007;70:67–74.

Takai K, Nakamura K, Toki T, Tsunogai U, Miyazaki M, Miyazaki J, et al. Cell proliferation at 122 degrees C and isotopically heavy CH4 production by a hyperthermophilic methanogen under high-pressure cultivation. Proc Natl Acad Sci USA 2008;105:10949–54.

Tender LM, Reimers CE, Stecher HA, Holmes DE, Bond DR, Lowy DA, et al. Harnessing microbially generated power on the seafloor. Nat Biotechnol 2002;20:821–5.

Tender LM, Gray SA, Groveman E, Lowy DA, Kauffman P, Melhado J, et al. The first demonstration of a microbial fuel cell as a viable power supply: powering a meteorological buoy. J Power Sources 2008;179:571–5.

Thrash JC, Coates JD. Review: direct and indirect electrical stimulation of microbial metabolism. Environ Sci Technol 2008;42:3921–31.

Timmes TC, Kim HC, Dempsey BA. Electrocoagulation pretreatment of seawater prior to ultrafiltration: pilot-scale applications for military water purification systems. Desalination 2010;250:6-13.

van der Ha D, Hoefman S, Boeckx P, Verstraete W, Boon N. Copper enhances the activity and salt resistance of mixed methane-oxidizing communities. Appl Microbiol Biotechnol 2010;87:2355–63.

Vartoukian SR, Palmer RM, Wade WG. Strategies for culture of 'unculturable' bacteria. FEMS Microbiol Lett 2010;309:1–7.

Verstraete W, Morgan-Sagastume F, Aiyuk S, Waweru M, Rabaey K, Lissens G. Anaerobic digestion as a core technology in sustainable management of organic matter. Water Sci Technol 2005;52:59–66.

Verstraete W, de Caveye PV, Diamantis V. Maximum use of resources present in domestic "used water". Bioresour Technol 2009;100:5537–45.

Villano M, Aulenta F, Ciucci C, Ferri T, Giuliano A, Majone M. Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. Bioresour Technol 2010;101:3085–90.

Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proc Natl Acad Sci USA 1998;95:6578–83.

Wieland A, de Beer D, Damgaard LR, Kuhl M, van Dusschote D, Van As H. Fine-scale measurement of diffusivity in a microbial mat with nuclear magnetic resonance imaging. Limnol Oceanogr 2001;46:248–59.

Williams KH, Nevin KP, Franks A, Englert A, Long PE, Lovley DR. Electrode-based approach for monitoring in situ microbial activity during subsurface bioremediation. Environ Sci Technol 2010;44:47–54.

Wright PC, Westacott RE, Burja AM. Piezotolerance as a metabolic engineering tool for the biosynthesis of natural products. Biomolecular Engineering 2003;20:325–31.

Zeng X, Birrien JL, Fouquet Y, Cherkashov G, Jebbar M, Querellou J, et al. Pyrococcus CH1, an obligate piezophilic hyperthermophile: extending the upper pressure–temperature limits for life. ISME J 2009;3:873–6.

Zhang T, Gannon SM, Nevin KP, Franks AE, Lovley DR. Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. Environ Microbiol 2010a;12:1011–20.

Zhang Y, Henriet J-P, Bursens J, Boon N. Stimulation of in vitro anaerobic oxidation of methane rate in a continuous high-pressure bioreactor. Bioresour Technol 2010b;101:3132–8.

Zuo Y, Xing DF, Regan JM, Logan BE. Isolation of the exoelectrogenic bacterium Ochrobactrum anthropi YZ-1 by using a U-tube microbial fuel cell. Applied and Environmental Microbiology 2008;74:3130–7.