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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, 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 bioreactor technology applied in marine microbiology and the critical parameters to take into account during reactor design. Furthermore, when we look at the growing population, as well as, 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.

Keywords:

Bio electrochemical system; high-pressure bioreactor; marine resources; eutrophication; sustainable green technology

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 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, 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 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, 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, bioreactors (as opposed to (fed)-batch incubations) are the only tools to simulate the natural conditions to a certain extent with good control of the environmental factors.

Besides the fundamental research of marine micro-organisms, 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, 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 know 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 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 ton in year 1998 to 34.1 million ton 2007 (FAO). For each ton of fish production, 7.0 kg 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 reactor technology can be used for land based aquaculture wastewater treatment on 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 bioreactor technology for marine (eco)systems has not been well explored compared to 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 area's for 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, non-woven fabric was fixed on the seabed and used as a support surface for 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 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; 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 successful extraction of mixed microbial cultures. One research line is to develop sampling devices to take 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 maintenance of high activity and/or at 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, marine anaerobic methane oxidizing consortia, achieve a biomass yield per mol of methane oxidized which is 23 times lower compared to 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 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 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, 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 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 marine organisms in the lab, either the reactor is placed inside a thermal controlled incubator *(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 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 (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 (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; 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 cultivation of aerobic marine 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 sulphate 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; Zhang *et al*., 2010) and titanium (Deusner *et al*. 2009; 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 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 need to in the redox reactions. For the BES housing, the materials need to be isolating 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; Scott *et al*., 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*., 2007; Cournet *et al*., 2010; He and Angenent, 2006).

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 needs 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 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 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 (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 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 (Zhang *et al.*, 2010); to support the growth of marine microbes (Meulepas *et al*., 2009; Nakajima *et al*., 2008); to industrialize 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 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 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 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, crustaceans) and an increase in jellyfish which can have an even more detrimental effect on 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 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 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*., 2007). 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 nitrate which is a cause of eutrophication.

2) *BESs for product formation.*

Another application for a BES is to operate it in electrolysis mode (microbial electrolysis cell, MEC). In this mode gaseous energy carriers can be produced or even better, when focusing on 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 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 (approx.10 x 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 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 are occurred in the pressurizing 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; Verstraete *et al*., 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 make 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 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. BES has great potential to be applied in coastal areas or in sediments for energy harvesting and controlling marine sediment E_h. The application of 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.

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References

Aelterman, P., Freguia, S., Keller, J., Verstraete, W., Rabaey, K. The anode potential regulates bacterial activity in microbial fuel cells. Applied Microbiology and Biotechnology, 2008; 78, 409-418.

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.

Alain, K., Querellou, J. Cultivating the uncultured: limits, advances and future challenges. Extremophiles, 2009; 13, 583-594.

Amend, J.P., Shock, E.L. Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria. Fems Microbiology Reviews, 2001; 25, 175-243.

Bond, D.R., Holmes, D.E., Tender, L.M., Lovley, D.R. Electrode-reducing microorganisms that harvest energy from marine sediments. Science, 2002; 295, 483-485.

Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., Xiong, S., Diana, J. Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. Environmental Science and Pollution Research, 2007; 14, 452-462.

Cheng, S.A., Xing, D.F., Call, D.F., Logan, B.E. Direct Biological Conversion of Electrical Current into Methane by Electromethanogenesis. Environmental Science & Technology, 2009; 43, 3953-3958.

Clauwaert, P., Aelterman, P., Pham, T.H., De Schamphelaire, L., Carballa, M., Rabaey, K., Verstraete, W. Minimizing losses in bio-electrochemical systems: the road to applications. Applied Microbiology and Biotechnology, 2008; 79, 901-913.

Clauwaert, P., Rabaey, K., Aelterman, P., De Schamphelaire, L., Ham, T.H., Boeckx, P., Boon, N., Verstraete, W. Biological denitrification in microbial fuel cells. Environmental Science & Technology, 2007; 41, 3354-3360.

Clauwaert, P., Van der Ha, D., Boon, N., Verbeken, K., Verhaege, M., Rabaey, K., Verstraete, W. Open air biocathode enables effective electricity generation with microbial fuel cells. Environmental Science & Technology, 2007; 41, 7564-7569.

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

Connon, S.A., Giovannoni, S.J. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Applied and Environmental Microbiology, 2002; 68, 3878-3885.

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. Electrochemistry Communications, 2010; 12, 505-508.

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 CO2 microelectrode for profiling sediments, microbial mats, and biofilms. Limnology and Oceanography, 1997; 42, 1590-1600.

Debashish, G., Malay, S., Barindra, S., Joydeep, M. Marine enzymes Marine Biotechnology I. Springer-Verlag Berlin, Berlin, 2005.pp. 189-218.

DeLong, E.F. Marine microbial diversity: The tip of the iceberg. Trends in Biotechnology, 1997; 15, 203-207.

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.

Donovan, C., Dewan, A., Heo, D., Beyenal, H. Batteryless, Wireless Sensor Powered by a Sediment Microbial Fuel Cell. Environmental Science & Technology, 2008; 42, 8591-8596.

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. Geochimica Et Cosmochimica Acta, 2006; 70, 3369-3386.

Duan, Z.H., Sun, R. An improved model calculating CO2 solubility in pure water and aqueous NaCl solutions from 273 to 533 K and from 0 to 2000 bar. Chemical Geology, 2003; 193, 257-271.

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. Electrochimica Acta, 2007; 53, 468-473.

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? A a Balkema Publishers, Leiden.2004

FAO. FAO Yearbook of Fishery Statistics Summary tables.

Freguia, S., Rabaey, K., Yuan, Z.G., Keller, J. Syntrophic Processes Drive the Conversion of Glucose in Microbial Fuel Cell Anodes. Environmental Science & Technology, 2008; 42, 7937-7943.

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.

He, Z., Angenent, L.T. Application of Bacterial Biocathodes in Microbial Fuel Cells. Electroanalysis, 2006; 18, 2009-2015.

Hinrichsen, D. Coastal Waters of the World: Trends, Threats, and Strategies. Washinton D.C. Island Press, 1998.

Hong, S.W., Kim, H.S., Chung, T.H. Alteration of sediment organic matter in sediment microbial fuel cells. Environmental Pollution, 2010; 158, 185-191.

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.

Jannasch, H.W., Wirsen, C.O., Doherty, K.W. A pressurized chemostat for the study of marine barophilic and oligotrophic bacteria. Applied and Environmental Microbiology, 1996; 62, 1593-1596.

Jones, P.T., De Meyere, V. Terra Reversa. Uitgeverij ASP (Academic & Scientific Publishers), Brussels.2009

Jorgensen, B.B., Boetius, A. Feast and famine - microbial life in the deep-sea bed. Nature Reviews Microbiology, 2007; 5, 770-781.

Kallmeyer, J., Ferdelman, T.G., Jansen, K.H., Jorgensen, B.B. A high-pressure thermal gradient block for investigating microbial activity in multiple deep-sea samples. Journal of Microbiological Methods, 2003; 55, 165-172.

Kashefi, K., Holmes, D.E., Reysenbach, A.L., Lovley, D.R. Use of Fe(III) as an electron acceptor to recover previously uncultured hyperthermophiles: Isolation and characterization of Geothermobacterium ferrireducens gen. nov., sp nov. Applied and Environmental Microbiology, 2002; 68, 1735-1742.

Kashefi, K., Lovley, D.R. Extending the upper temperature limit for life. Science, 2003; 301, 934-934.

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

Kielemoes, J., De Boever, P., Verstraete, W. Influence of denitrification on the corrosion of iron and stainless steel powder. Environmental Science & Technology, 2000; 34, 663-671.

Kogure, K. Bioenergetics of marine bacteria. Current Opinion in Biotechnology, 1998; 9, 278-282.

Konneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., Stahl, D.A. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature, 2005; 437, 543-546.

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. Environmental Science & Technology, 2008; 42, 2233-2242.

Lang, S., Huners, M., Lurtz, V. Bioprocess engineering data on the cultivation of marine prokaryotes and fungi Marine Biotechnology Ii. Springer-Verlag Berlin, Berlin, 2005.pp. 29-62.

Lauro, F.M., Bartlett, D.H. Prokaryotic lifestyles in deep sea habitats. Extremophiles, 2008; 12, 15-25.

Lauro, F.M., McDougald, D., Thomas, T., Williams, T.J., Egan, S., Rice, S., DeMaere, M.Z., Ting, L., Ertan, H., Johnson, J., Ferriera, S., Lapidus, A., Anderson, I., Kyrpides, N., Munk, A.C., Detter, C., Han, C.S., Brown, M.V., Robb, F.T., Kjelleberg, S., Cavicchioli, R. The genomic basis of trophic strategy in marine bacteria. Proceedings of the National Academy of Sciences of the United States of America, 2009; 106, 15527-15533.

Law, A.T., Button, D.K. MULTIPLE-CARBON-SOURCE-LIMITED GROWTH KINETICS OF A MARINE CORYNEFORM BACTERIUM. Journal of Bacteriology, 1977; 129, 115-123.

Leary, D., Vierros, M., Hamon, G., Arico, S., Monagle, C. Marine genetic resources: A review of scientific and commercial interest. Marine Policy, 2009; 33, 183-194.

Levin, L.A., Ekau, W., Gooday, A.J., Jorissen, F., Middelburg, J.J., Naqvi, S.W.A., Neira, C., Rabalais, N.N., Zhang, J. Effects of natural and human-induced hypoxia on coastal benthos. Biogeosciences, 2009; 6, 2063-2098.

Lezama-Cervantes, C., Paniagua-Michel, J. Effects of constructed microbial mats on water quality and performance of Litopenaeus vannamei post-larvae. Aquacultural Engineering, 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). Limnology and Oceanography, 2010; 55, 931-949.

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

Marwick, J.D., Wright, P.C., Burgess, J.G. Bioprocess intensification for production of novel marine bacterial antibiotics through bioreactor operation and design. Marine Biotechnology, 1999; 1, 495-507.

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.

Meunier, C.F., Dandoy, P., Su, B.L. Encapsulation of cells within silica matrixes: Towards a new advance in the conception of living hybrid materials. Journal of Colloid and Interface Science, 2010; 342, 211-224.

Moreno-Garrido, I. Microalgae immobilization: Current techniques and uses. Bioresource Technology, 2008; 99, 3949-3964.

Morgan-Sagastume, F., Karlsson, A., Johansson, P., Pratt, S., Boon, N., Lant, P., Werker, A. Production of polyhydroxyalkanoates in open, mixed cultures from a waste sludge stream containing high levels of soluble organics, nitrogen and phosphorus. Water Research, 2010; 44, 5196-5211.

Mu, Y., Rabaey, K., Rozendal, R.A., Yuan, Z.G., Keller, J. Decolorization of Azo Dyes in Bioelectrochemical Systems. Environmental Science & Technology, 2009; 43, 5137-5143.

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.

Muyzer, G., Stams, A.J.M. The ecology and biotechnology of sulphate-reducing bacteria. Nature Reviews Microbiology, 2008; 6, 441-454.

Nakajima, J., Sakka, M., Kimura, T., Furukawa, K., Sakka, K. Enrichment of anammox bacteria from marine environment for the construction of a bioremediation reactor. Applied Microbiology and Biotechnology, 2008; 77, 1159-1166.

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. Environmental Microbiology, 2007; 9, 187-196.

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. Environmental Microbiology, 2002; 4, 296-305.

Paerl, H.W. Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: Interactive effects of human and climatic perturbations. Ecological Engineering, 2006; 26, 40-54.

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.

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

Pham, H., Boon, N., Marzorati, M., Verstraete, W. Enhanced removal of 1,2-dichloroethane by anodophilic microbial consortia. Water Research, 2009; 43, 2936-2946.

Pham, T.H., Rabaey, K., Aelterman, P., Clauwaert, P., De Schamphelaire, L., Boon, N., Verstraete, W. Microbial fuel cells in relation to conventional anaerobic digestion technology. Engineering in Life Sciences, 2006; 6, 285-292.

Purcell, J.E., Uye, S., Lo, W.T. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Marine Ecology-Progress Series, 2007; 350, 153-174.

Rabalais, N.N., Diaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., Zhang, J. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences, 2010; 7, 585-619.

Rehm, B.H.A. Biogenesis of microbial polyhydroxyalkanoate granules: a platform technology for the production of tailor-made bioparticles. Current Issues in Molecular Biology, 2007; 9, 41-62.

Reimers, C.E., Tender, L.M., Fertig, S., Wang, W. Harvesting energy from the marine sediment-water interface. Environmental Science & Technology, 2001; 35, 192-195.

Reysenbach, A.L., Liu, Y.T., Banta, A.B., Beveridge, T.J., Kirshtein, J.D., Schouten, S., Tivey, M.K., Von Damm, K.L., Voytek, M.A. A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. Nature, 2006; 442, 444-447.

Rezaei, F., Richard, T.L., Brennan, R.A., Logan, B.E. Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems. Environmental Science & Technology, 2007; 41, 4053-4058.

Rothschild, L.J., Mancinelli, R.L. Life in extreme environments. Nature, 2001; 409, 1092-1101.

Rozendal, R.A., Jeremiasse, A.W., Hamelers, H.V.M., Buisman, C.J.N. Hydrogen production with a microbial biocathode. Environmental Science & Technology, 2008; 42, 629-634.

Sarkar, S., Roy, D., Mukherjee, J. Production of a potentially novel antimicrobial compound by a biofilm-forming marine Streptomyces sp in a niche-mimic rotating disk bioreactor. Bioprocess and Biosystems Engineering, 2009; 33, 207-217.

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.

Schrenk, M.O., Huber, J.A., Edwards, K.J. Microbial Provinces in the Subseafloor. Annual Review of Marine Science, 2010; 2, 279-304.

Scott, K., Cotlarciuc, I., Hall, D., Lakeman, J.B., Browning, D. Power from marine sediment fuel cells: the influence of anode material. Journal of Applied Electrochemistry, 2008a; 38, 1313-1319.

Scott, K., Cotlarciuc, I., Head, I., Katuri, K.P., Hall, D., Lakeman, J.B., Browning, D. Fuel cell power generation from marine sediments: Investigation of cathode materials. Journal of Chemical Technology and Biotechnology, 2008b; 83, 1244-1254.

Scotto, V., Dicintio, R., Marcenaro, G. The influence of marine aerobic microbial film on stainless-steel corrosion behavior. Corrosion Science, 1985; 25, 185-194.

Simonato, F., Campanaro, S., Lauro, F.M., Vezzi, A., D'Angelo, M., Vitulo, N., Valle, G., Bartlett, D.H. Piezophilic adaptation: a genomic point of view. Journal of Biotechnology, 2006; 126, 11-25.

Steinbusch, K.J.J., Hamelers, H.V.M., Schaap, J.D., Kampman, C., Buisman, C.J.N. Bioelectrochemical Ethanol Production through Mediated Acetate Reduction by Mixed Cultures. Environmental Science & Technology, 2010; 44, 513-517.

Stingl, U., Tripp, H.J., Giovannoni, S.J. 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 Journal, 2007; 1, 361-371.

Sudarno, U., Bathe, S., Winter, J., Gallert, C. Nitrification in fixed-bed reactors treating saline wastewater. Applied Microbiology and Biotechnology, 2010; 85, 2017-2030.

Sudek, S., Lopanik, N.B., Waggoner, L.E., Hildebrand, M., Anderson, C., Liu, H.B., Patel, A., Sherman, D.H., Haygood, M.G. Identification of the putative bryostatin polyketide synthase gene cluster from "Candidatus endobugula sertula", the uncultivated microbial symbiont of the marine bryozoan Bugula neritina. Journal of Natural Products, 2007; 70, 67-74.

Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H., Nakagawa, S., Nunoura, T., Horikoshi, K. Cell proliferation at 122 degrees C and isotopically heavy CH4 production by a hyperthermophilic methanogen under high-pressure cultivation. Proceedings of the National Academy of Sciences of the United States of America, 2008; 105, 10949-10954.

Tender, L.M., Gray, S.A., Groveman, E., Lowy, D.A., Kauffman, P., Melhado, J., Tyce, R.C., Flynn, D., Petrecca, R., Dobarro, J. The first demonstration of a microbial fuel cell as a viable power supply: Powering a meteorological buoy. Journal of Power Sources, 2008; 179, 571-575.

Tender, L.M., Reimers, C.E., Stecher, H.A., Holmes, D.E., Bond, D.R., Lowy, D.A., Pilobello, K., Fertig, S.J., Lovley, D.R. Harnessing microbially generated power on the seafloor. Nature Biotechnology, 2002; 20, 821-825.

Thrash, J.C., Coates, J.D. Review: Direct and indirect electrical stimulation of microbial metabolism. Environmental Science & Technology, 2008; 42, 3921-3931.

Timmes, T.C., Kim, H.C., Dempsey, B.A. 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. Applied Microbiology and Biotechnology, 2010.

Vartoukian, S.R., Palmer, R.M., Wade, W.G. Strategies for culture of 'unculturable' bacteria. Fems Microbiology Letters, 2010; 309, 1-7.

Verstraete, W., de Caveye, P.V., Diamantis, V. Maximum use of resources present in domestic "used water". Bioresource Technology, 2009; 100, 5537-5545.

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 Science and Technology, 2005; 52, 59-66.

Villano, M., Aulenta, F., Ciucci, C., Ferri, T., Giuliano, A., Majone, M. Bioelectrochemical reduction of CO2 to CH4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. Bioresource Technology, 2010; 101, 3085-3090.

Whitman, W.B., Coleman, D.C., Wiebe, W.J. Prokaryotes: The unseen majority. Proceedings of the National Academy of Sciences of the United States of America, 1998; 95, 6578-6583.

Wieland, A., de Beer, D., Damgaard, L.R., Kuhl, M., van Dusschote, D., Van As, H. Fine-scale measurement of diffusivity in a microbial mat with nuclear magnetic resonance imaging. Limnology and Oceanography, 2001; 46, 248-259.

Williams, K.H., Nevin, K.P., Franks, A., Englert, A., Long, P.E., Lovley, D.R. Electrode-Based Approach for Monitoring In Situ Microbial Activity During Subsurface Bioremediation. Environmental Science & Technology, 2010; 44, 47-54.

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, T., Gannon, S.M., Nevin, K.P., Franks, A.E., Lovley, D.R. Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. Environmental Microbiology, 2010; 12, 1011-1020.

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.

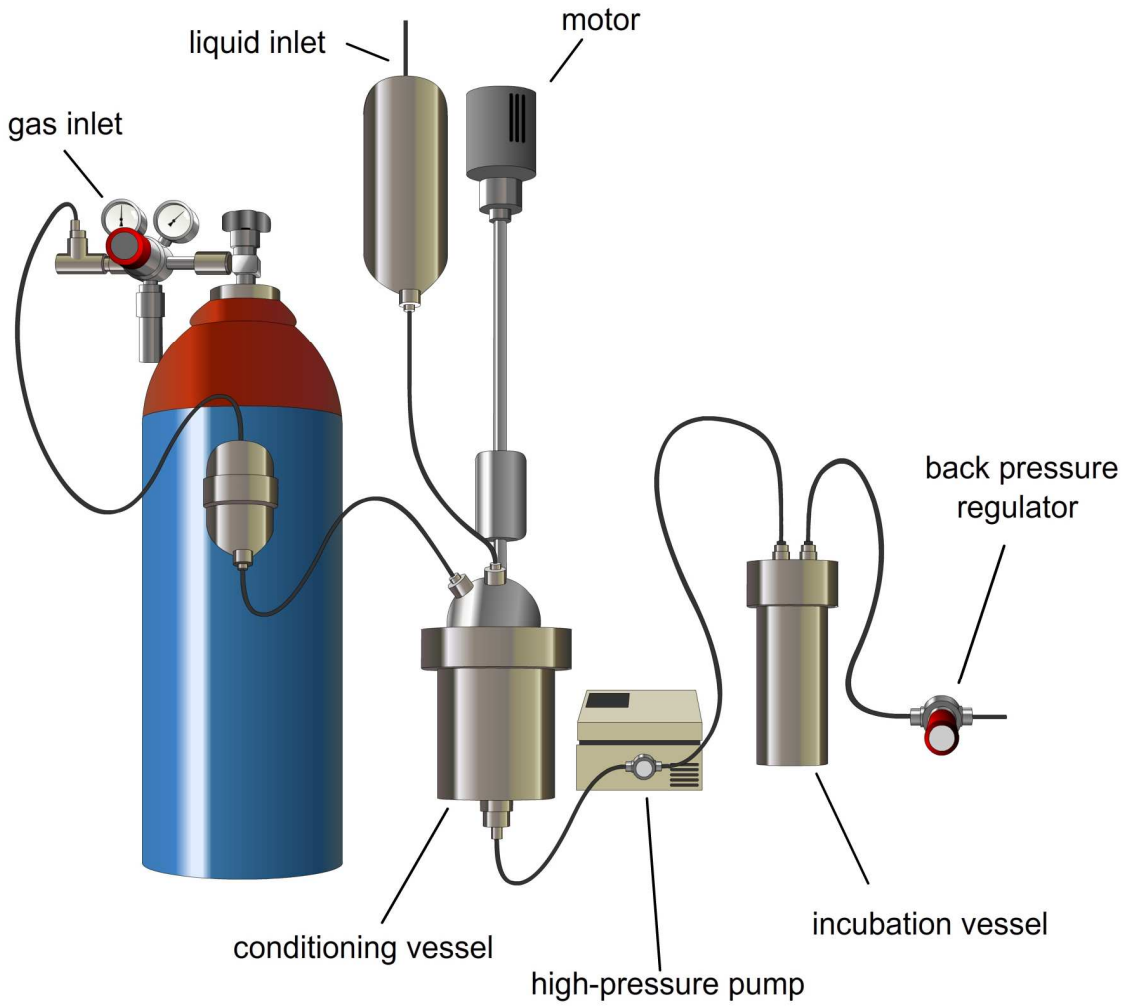


Fig 1. The scheme of a typical high-pressure bioreactor system (modified from Zhang, *et. al.* (2010)).

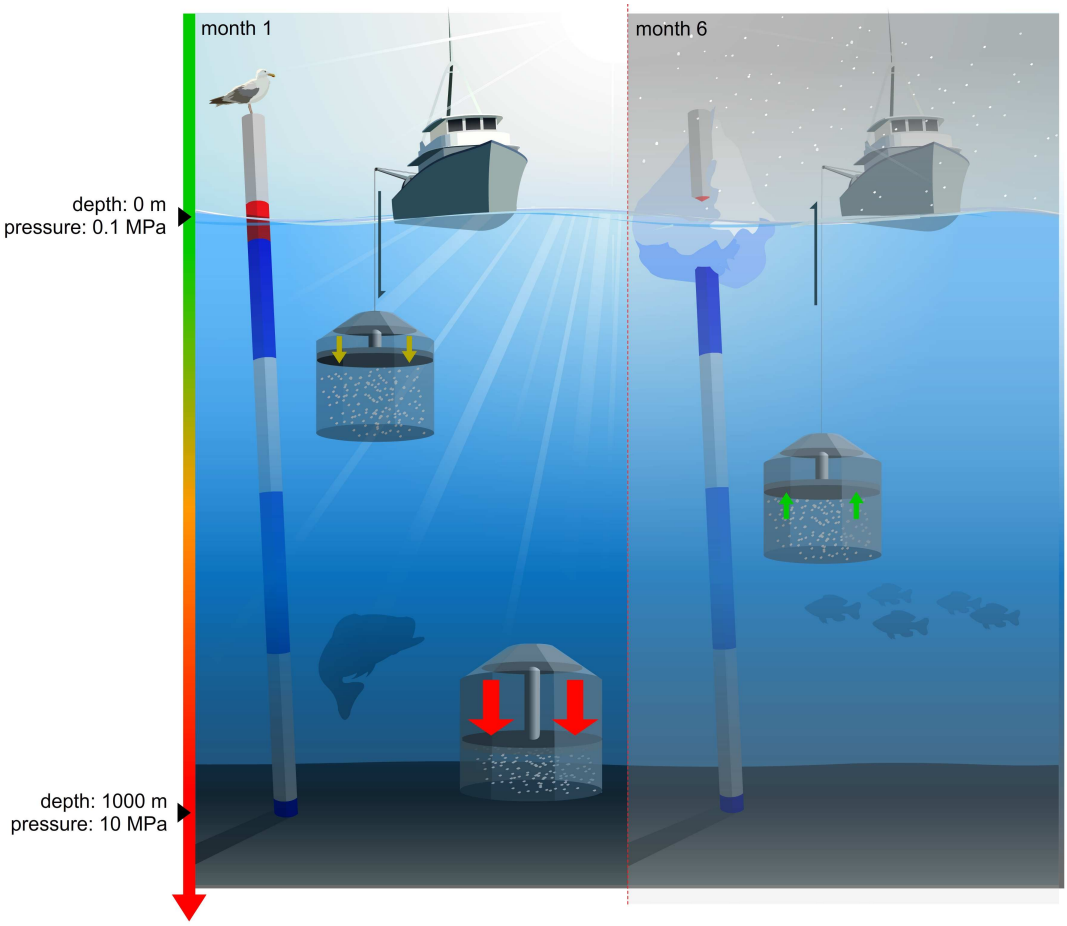


Fig. 2. The use of natural water depth to pressurize a bioreactor.

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; Collect/remove fast growing/bio-film forming biomass from the disk; Mimic the environments with interval of oxic/anoxic	Bioactive compound production (Konopka, 2000); Cultivation of oligotrophiles/slow-growing bacteria (Konopka, 2000)
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; High-pressure gas or a compressor can be combined to	Characterization of processes under high-hydrostatic-pressure (Parkes et al., 2009;

	induce high-gas-pressure	Pradillon et al., 2004; Wright et al., 2003; Zeng et al., 2009) Increase gas-form substrate concentration (Deusner et al., 2009; 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).

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 (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)

Box 1. Bio Electrochemical Systems

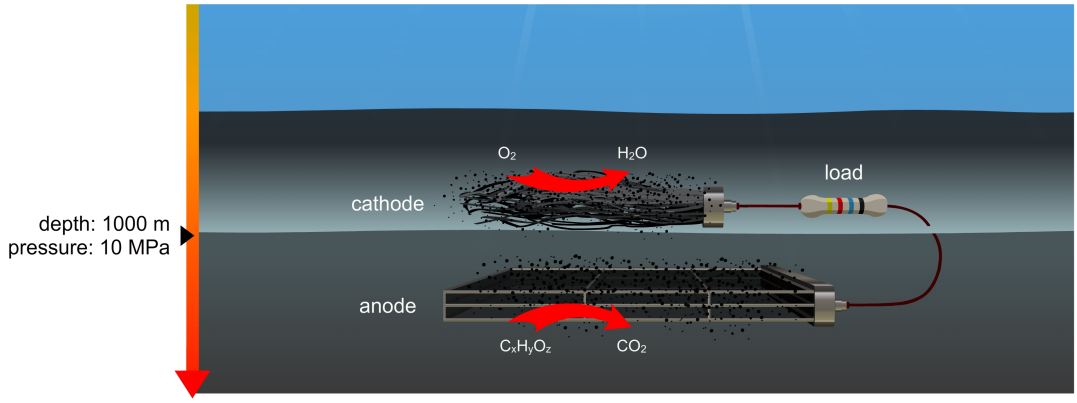
Here, a brief description of a BES with examples applicable to a marine environment will be provided. Extensive reviews on general BES functioning can be found elsewhere (Logan et al., 2006; Pham et al., 2009; Rabaey and Verstraete, 2005). A BES is a system that is capable of harvesting electrons from microbial respiration. When current is harvested it is called a Microbial Fuel Cell (MFC) 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 microorganisms 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 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 work. The most common reduction reaction in the cathode is O_2 reduction to H_2O. Important is that the anode and cathode reactions are separated in space to prevent a short circuit. 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 protons compared to electrons. This movement of protons is needed for charge balancing of the overall reaction. No research has been presented up till now that investigated the buffering capacity of 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. (Logan et al., 2006). The true energy produced is also heavily dependent on mass transfer and electrode kinetics.

Reference:

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. Microbial Fuel Cells: Methodology and Technology. Environmental Science & Technology, 2006; 40, 5181-5192.

Pham, H., Boon, N., Marzorati, M., Verstraete, W. Enhanced removal of 1,2-dichloroethane by anodophilic microbial consortia. Water Research, 2009; 43, 2936-2946.

Rabaey, K., Verstraete, W. Microbial fuel cells: novel biotechnology for energy generation. Trends in Biotechnology, 2005; 23, 291-298.



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 saturation concentrations of methane are 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 ($\Delta G^{\circ\prime}$) is in the first order of the standard redox potential ($\Delta E^{\circ\prime}$): $\Delta G^{\circ\prime}=-nF\Delta E^{\circ\prime}$, (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 sulphate became the dominant electron acceptors to support growth of 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. Geochimica Et Cosmochimica Acta, 2006; 70, 3369-3386.