Online Monitoring of Crude Oil Biodegradation at Elevated Pressures

Ana Gabriela Valladares Juárez, Hari Spandana Kadimesetty, Daniela E. Achatz, Martina Schedler, and Rudolf Müller

***Abstract*—In order to study the biodegradation of crude oil spilled in the deep sea, incubations of deep-sea-bed sediments and crude oil were carried out in a high-pressure reactor, but monitoring the biodegradation of oil at high pressure is limited by sampling because the volatile crude oil components are partly lost during depressurization. Moreover, the seawater-oil-sediments multiphase system cannot be sampled representatively. The aerobic oil biodegradation can also be monitored indirectly by measuring the oxygen consumed and the carbon dioxide produced. In this paper, the O_2 and CO_2 concentrations were monitored in a reactor with transparent windows using chemical-optical sensors. To compare the effect of pressure on the biodegradation of oil, two pressure regimes were compared: atmospheric pressure (1 bar) and 150 bar, corresponding to 1500 m depth of the Deepwater Horizon's well at the Gulf of Mexico. Only in the experiments where deep-sea sediments were added, the oxygen concentration decreased while the carbon dioxide and the bacterial concentration increased. In experiments where no sediment was added, the values for the oxygen and carbon dioxide remained constant. This proved that deep-sea sediments contained microorganisms, which could degrade crude oil at both 1 and 150 bar. To our knowledge, this is the first time where O_2 and CO_2 were monitored online during crude oil biodegradation at high pressure in the laboratory.**

***Index Terms*—Biodegradation, cells (biology), chemical sensors, image analysis, monitoring, oil pollution, sea floor.**

I. Introduction

A. Oil Extraction in the Deep-Sea

THE DEEPWATER HORIZON (DWH) accident is to date, the largest man-caused deep-sea oil spill. About 800 million liters of crude oil were released at 1500 m depth in the Gulf of Mexico over a period of 84 days [1]. The search and extraction of oil in deeper waters are a growing global trend, as the shallow water or land reservoirs dry out and the oil price remains high [2]. The average depth of the ocean is around 4000 m with a pressure of 408 bar (for every 10 m of water column, the pressure increases in approximately 1 bar) [3]. The extreme conditions, such as high pressure, low temperatures, hard rock or salt layers above the reservoirs, make the deep-sea oil exploration challenging. According to the International Energy Agency since the year 2000, more than half of all the oil reserves have been discovered in deep-water [2]. From 2007 onward, new oil reserves are being discovered offshore Brazil, including a deep-water oil reservoir located at 1830 m depth under a fragile salt layer. Along the West Africa coast, there are also offshore (less than 400 m deep) to deep-water (400–1500 m deep) oil fields being exploited in Angola and Nigeria [2], [4]. New medium to deep oil fields in Ghana, Liberia, and Sierra Leone have been recently discovered and put into production [2].

The U.S. Department of Energy reported that the offshore oil production (mostly in the Gulf of Mexico) will grow 18% from 2011 to 2020, as technology for deeper extraction of oil (1500 m depth) advances [5]. The Mexican oil company Pemex, announced in 2014 the discovery of an ultradeep-water (at 2500 m depth) light-oil field in the Gulf of Mexico [6]. The deep-water production of oil is expected to be 13% of the global output of crude oil and gas by 2020 [5].

Energy companies are investing large sums of money in new deep-sea drilling technologies and claim that those will allow safer drilling for both the environment and the rig personnel [5]. Nevertheless, any man-caused or technology mistake could cause a new oil spill accident. The spill of the DWH taught us that even closing the oil rig was a very complicated operation. The acute and chronic environmental sequels of hydrocarbons spilled into the deep sea are unknown.

B. Biodegradation of Oil and Influence of Elevated Pressure

One of the principal mechanisms that remove oil from the environment is biodegradation, a process in which indigenous bacteria metabolize the pollutants. Hydrocarbon-degrading microorganisms are vastly present in the environment. They generally represent less than 1% of the total microbial community, but in the presence of oil pollutants, they can be enriched up to 10% of the total community. The rates of oil biodegradation are lower in pristine marine waters than in polluted waters [7]. In case of the DWH, the indigenous microorganisms of Gulf of Mexico at the spill site readily degraded part of the oil [8]. According to Deming and Baross, the microbial diversity in the deep-sea sediments is higher than in the upper ocean layers, and pulses of nutrients can enhance the microbial diversity of the otherwise oligotrophic and static habitat [9]. The Gulf

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of Mexico is a natural hydrocarbon seepage zone and therefore enriched in hydrocarbon degrading bacteria.

The composition of the crude oil and environmental factors (e.g., temperature) affects the ability of microorganisms to degrade the oil [10]. Some authors have demonstrated that elevated pressure can have an effect on the metabolism of microorganisms. The alcoholic fermentation by *Saccharomyces cerevisiae* was three times faster at 100 bar of hydrostatic pressure compared to ambient pressure; above 200 bar, the fermentation slowed down until it was completely inhibited at 870 bar [11]. Other authors collected core samples off the east coast of Florida at 4940 m depth. They enriched an indigenous mixed deep-sea bacterial culture from the cores and tested the biodegradation of hexadecane with it. The culture grew slower on hexadecane at the simulated deep-sea pressure than at the atmospheric pressure and also lost viability in comparison to the one incubated at 1 bar [12]. It was recently reported that even mild variations of pressure (7 bar) elevated oxygen tension and elevated carbon dioxide tension caused widespread changes in the transcriptome of *Pseudomonas putida* but not in the cell physiology [13].

Middleburg *et al.* reviewed previous studies done about the rates of mineralization of organic matter in marine systems. The rates measured in the laboratory were difficult to relate to *in situ* rates because of incorrect sediment dilution or homogenization; also changes in temperature and pressure (water depth) affect microbial activity and thermodynamic equilibrium and were not considered. Therefore, the determination of *in situ* organic matter mineralization rates was restricted to shallow environments [14]. The study of deep-sea microbia at *in situ* pressure and temperature would complement the knowledge of the deep ocean.

We study the biodegradation of crude oil by benthic sediments. Measuring the biodegradation rates of pollutants in the deep-sea is challenging because of the extreme elevated pressures and low temperatures. That is why we simulated the deep-sea pressure in laboratory-scale reactors. The experiments were done at room temperature and solely the effect of high pressure on the biodegradation of oil by indigenous marine bacteria present in seabed sediments was studied.

C. Monitoring the Biodegradation of Oil at Elevated Pressure

Crude oil is a mixture of thousands of paraffinic, aromatic, and naphthenic compounds [15]. Crude oils can vary greatly in composition and in physical and chemical characteristics, even within the same geological formation. They span from highly flammable liquids to viscous and heavy tar-like material [16]. The oil spilled in the DWH accident was Louisiana light sweet crude oil.

Monitoring a bioreaction at high pressure is limited by sampling because the volatile crude oil components are partly lost during depressurization. The abiotic mass losses are higher for the lighter oils than for the heavy oils, because the low-molecular-weight components volatilize [10]. Cycles of decompression and recompression for subsampling could also affect the bacteria and bias the experiment. Moreover, the seawater-oil-sediments multiphase system cannot be sampled representatively. Until now, we had to sacrifice one reactor per time point to determine the increase in biomass and the disappearance of the hydrocarbon by gas chromatography, which is a resource and time-consuming approach to measure biodegradation at elevated pressures. Another drawback of using various parallel reactors to determine the biodegradation of oil by the deep-sea sediments is the variability within the microbial community. Lowit *et al.* [17] state that the experimental reproducibility is dependent on the one hand on the variance of the analytics and on the other hand on the heterogeneous distribution of the bacteria in the environment.

The use of online chemical, optical, and biological sensors for monitoring bioprocesses is challenging. Several techniques, such as culture turbidity, light scattering, and fluorescence, have been developed but are not sufficiently tested to be used reliably in industrial processes. Interferences such as air bubbles or background particles affect the readings, whereas the fluorescence is affected by pH, dissolved oxygen tension, and substrate levels; further difficulties are the high risk of microbial contamination and blockage of the sensors [18]. Fluorescence spectroscopy was used to monitor online the biodegradation of phenanthrene, a component of crude oil, by the bacteria *Sphingomonas yanoikuyae* [19], but the crude oil contains a large amount of nonfluorescent aliphatic compounds that cannot be quantified with fluorescence. On top of that, the measurement of any parameter at elevated pressure is even more complicated.

The aerobic oil biodegradation can also be monitored indirectly by measuring the oxygen consumed and the carbon dioxide produced, as in (1). The stoichiometry was adapted from Doran [18]

$$\text{oil} + a\text{O}_2 + b\text{NH}_3 \rightarrow c\text{CH}_2\text{O}_{0.52}\text{N}_{0.23} + d\text{CO}_2 + e\text{H}_2\text{O}. \tag{1}$$

It was assumed that the biomass has the formula of the *Pseudomonas* strain, $\text{CH}_2\text{O}_{0.52}\text{N}_{0.23}$ [18]. The analysis of carbon dioxide and oxygen concentrations is an established technology for monitoring the growth of microorganisms in an aerobic bioprocess at atmospheric pressure. Generally, the exhaust flow of the fermenter is analyzed. However, the commercially available sensors cannot be operated at deep-sea pressures.

The approach of using only one reactor per experiment and measuring online the parameters of interest decreases the experimental error, but for monitoring the biodegradation of oil at elevated pressure online, it is necessary to have analytical tools that can withstand or circumvent the enormous pressure. In this work, the oxygen and carbon dioxide concentrations were monitored in real time in a pressurized reactor with two transparent windows using chemical-optical sensors. To our knowledge, this is the first time that both parameters, oxygen and carbon dioxide, were simultaneously measured in a high pressure system. This technological advancement will be fundamental for the study of the effect of high pressure on the biodegradation of pollutants and organic matter in general in the deep ocean.

We hypothesized that elevated pressure has an influence on the biodegradation of the polluting hydrocarbons that were released into the deep-sea. In order to prove this thesis, it is necessary to monitor the degradation progress at elevated pressure. As a proof of principle, we monitored the biodegradation

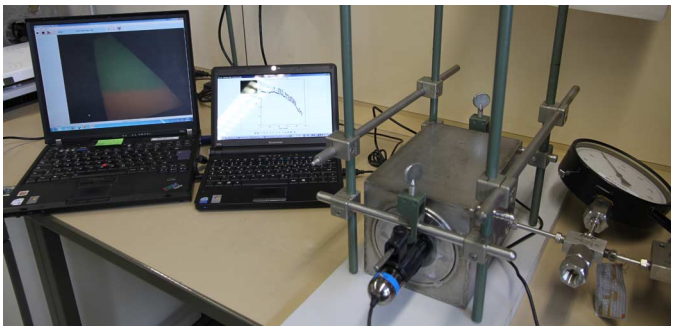

Fig. 1. High-pressure reactor with VisiSens camera installed in the front window. The images in the computers correspond to the sensing-patch glued inside the reactor window (shown in Fig. 2).

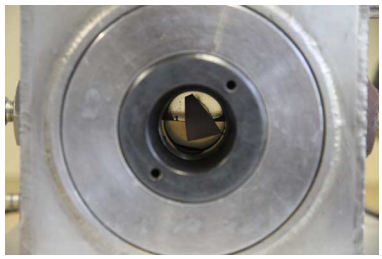

Fig. 2. Window of the high pressure reactor with an oxygen sensing-patch glued to the inner side.

(via oxygen and carbon dioxide curves) of crude oil by bacteria contained in deep-sea sediments at two different pressures: 1 and 150 bar.

II. Materials and Methods

A. Materials

The experiments were done in a stainless-steel, 100 mL high-pressure reactor, which can withstand up to 400 bar. The reactor has two glass windows; one at each end (see Figs. 1 and 2).

In order to monitor the O_2 concentration, the chemical-optical sensor system VisiSens1 consisting of the portable camera DU01 and the sensor foil SF-RPSu4 was used. The sensor foil was glued to the inner side of the window (see Fig. 2). For measuring the CO_2, the VisiSens3 system, consisting of the camera DU03 combined with the foil SF-CD1R, was placed in the other window [Precision Sensing GmbH (PreSens), Regensburg, Germany]. The sensing foils were glued to the inner side of the reactor's windows and were in direct contact with the reaction mixture. The images were collected, processed, and evaluated with the software VisiSens AnalytiCal 1 and AnalytiCal 3 for O_2 and CO_2, respectively (PreSens GmbH). A scheme of the high-pressure reactor setup with the VisiSens camera and the recording computer is shown in Fig. 3.

Unconsolidated material from the top of a deep-sea sediment core collected in August 2010 at 1520 m depth near the DWH site was used as the source of microorganisms. The surface scratch of the sediment core, corresponding to the sea bottom, was used as inoculum, as this is the aerobic zone that most likely contains aerobic bacteria. The sediment slurry was aliquoted in 1 mL portions and frozen; it was thawed as needed for the experiments. The biodegradation experiments

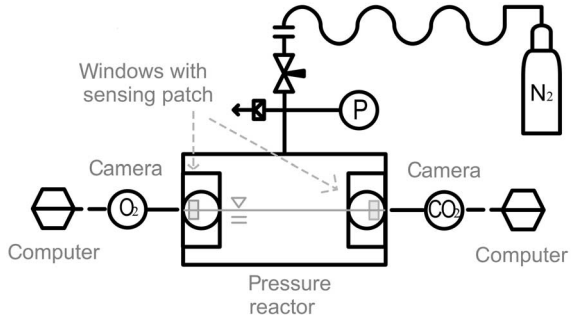

Fig. 3. Scheme of the high-pressure reactor with transparent windows where the VisiSens cameras connected to computers were used for monitoring the O_2 and CO_2 concentrations online. The nitrogen bottle was used for pressurizing the reactor.

were performed in batch mode. The reactor was half-filled with liquid, leaving in the other half an air cushion to provide the oxygen required by the microorganisms. The reactor was supplied with 50 mL of minimum-mineral medium, 50 μL of Louisiana sweet crude oil as the only carbon source, and finally, 1 mL of deep-sea sediments as bacterial inoculum. In order to overcome oxygen limitation, 2.8 bar of compressed air was filled into the reactor. For the high-pressure experiments, additional 150 bar of nitrogen were added. The blank-controls were made as described before but without the addition of sediments.

The partial pressure of oxygen and carbon dioxide is equivalent to the concentration of those gases in the liquid phase at ambient pressure since pressure was incremented with inert nitrogen gas; therefore, we will use the term "concentration" within this work.

B. Sensors

The measurement of oxygen and carbon dioxide is based on fluorescence ratiometric imaging (FRIM), which is a method for reading out the signal of a fluorescent chemical optical sensor. Ratiometric measurements (2-wavelenght) are more robust than intensity measurements at a single wavelength against interferences of ambient or scattered light; they use simple instrumentation for measuring emission intensities and are suitable for a wide range of applications [20]–[22]. The sensors based on fluorescence are more convenient for detecting oxygen than conventional sensors (i.e., Clark electrodes) because they do not consume oxygen and the sensing element (foils) can be placed separately from the emitting light source and electronics (noninvasive measurement). This spatial separation is an advantage for using the sensors in extreme environments. The sensor foils used in this study were composed of an inert polymer support, the fluorescent sensor layer, and a white optical isolation layer. The sensor layer contained an indicator dye and a reference dye. The fluorescence intensity of the indicator dye changed due to different oxygen or carbon dioxide partial pressures, whereas the fluorescence intensity of the reference dye remained constant. The camera collected the fluorescence signal of the sensor foils. A light-emitting diode (LED) array integrated in the camera was used as a light source. The proprietary software (AnalytiCal 1 and AnalytiCal 3, PreSens GmbH)

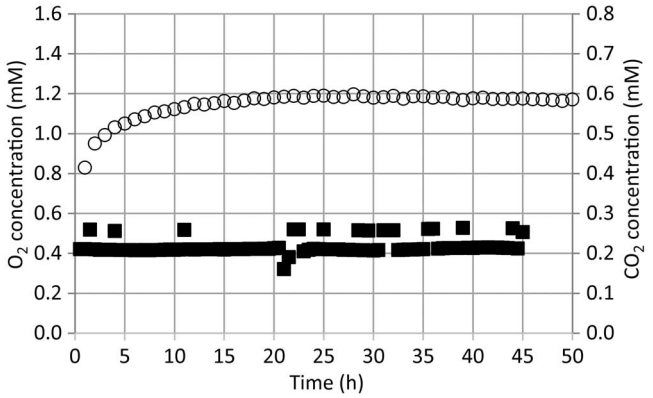

Fig. 4. O_2 (◯) and CO_2 (■) concentration in the control experiment where crude oil was incubated in mineral medium at 1 bar.

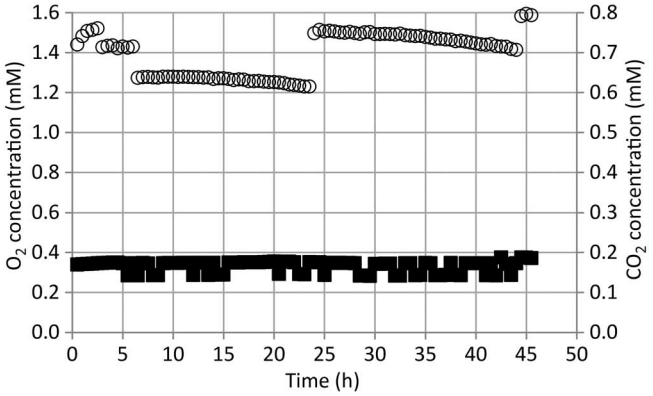

Fig. 5. O_2 (◯) and CO_2 (■) concentration in the control experiment where crude oil was incubated in mineral medium at 150 bar.

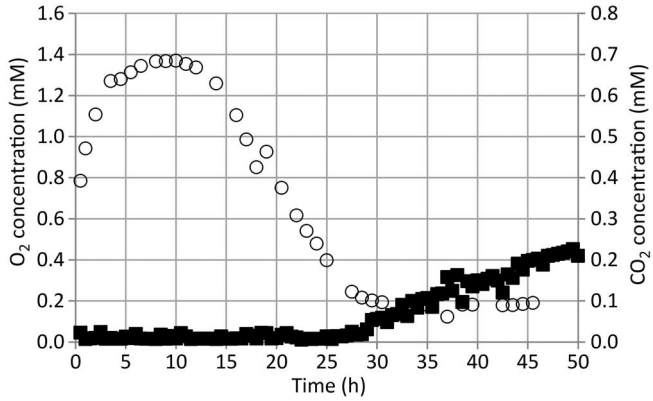

Fig. 6. O_2 (◯) and CO_2 (■) concentration in the experiment where marine sediments were incubated with crude oil in mineral medium at 1 bar.

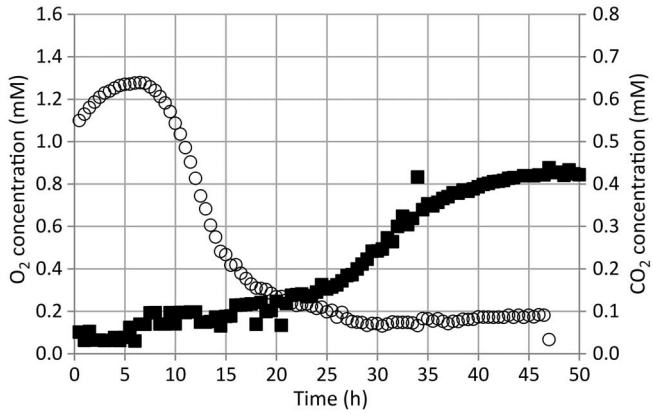

Fig. 7. O_2 (◯) and CO_2 (■) concentration in the experiment where marine sediments were incubated with crude oil in mineral medium at 150 bar.

calculated the oxygen concentration in percent air saturation and the carbon dioxide concentration in volume per volume percent. The conversion to milimolar was done using a calibration curve with solutions of known concentration of the analyte.

C. Methods

The reaction was monitored in the liquid phase until the values were constant. For the oxygen calibration, the 0% point was obtained using a sodium dithionite solution and the 100% point was obtained using water saturated with air. For the carbon dioxide calibration, a solution from 0 to 60 mg/L of CO_2 was used. Samples from the supernatant were collected at the start and at the end of the incubation to determine the viable bacteria reported as colony-forming units (CFU).

III. RESULTS

In the blank control experiments where oil and media were incubated at 1 and 150 bar, the O_2 and CO_2 values were fairly constant (Figs. 4 and 5). In contrast, in experiments where deep-sea sediment was added (Figs. 6 and 7), a sharp decrease in the O_2 concentration and an increase in CO_2 concentration were observed. For both pressure regimes, the carbon dioxide evolved mainly after the oxygen had been depleted. For the 1 bar incubation, the ΔCO_2 was about 0.19 mM and for the 150-bar, incubation was 0.35 mM (Figs. 6 and 7).

The oxygen consumption rate during the 1-bar incubation, from 16 to 27 h (Fig. 6), was 0.07 mM/h and afterward, the oxygen value stagnated. In this experiment, the CO_2 was detected with a time delay in comparison with the 150 bar incubation (Fig. 7). The oxygen consumption rate from 10 to 17 h at 150 bar was 0.10 mM/h. The time at which half of the initial oxygen was consumed was 21 h for the 1-bar incubation and 13 h for the 150-bar incubation.

The depletion of oxygen corresponds to an increase in bacterial concentration (Fig. 8), only in the incubations where the deep-sea sediments were added, the number of bacteria increased. The final bacterial concentration was higher for the 1 bar (2×10^5 cells/mL) incubation than for the 150 bar (1×10^5 cells/mL) incubation. Preliminary 16S rDNA analysis with Denaturing Gradient Gel Electrophoresis (DGGE) suggests that the bacterial community evolved differently for the two pressure regimes (unpublished results).

In Fig. 9, an oxygen sensing patch placed in the oil-biodegradation incubation is shown at start, middle, and end time of the experiment. The reactor was half-filled with air; the top part of the patch was in the gas phase; and the bottom part was in the liquid phase, as shown in Fig. 2. The upper part of the panels shows the color image captured by the camera. The

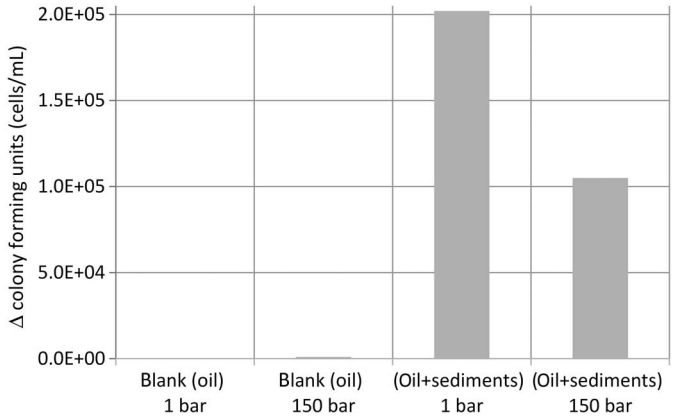

Fig. 8. Bacterial density difference (end time minus start time) of blanks and of incubations with marine sediments, at both 1 and 150 bar (of experiments shown in Figs. 4–7).

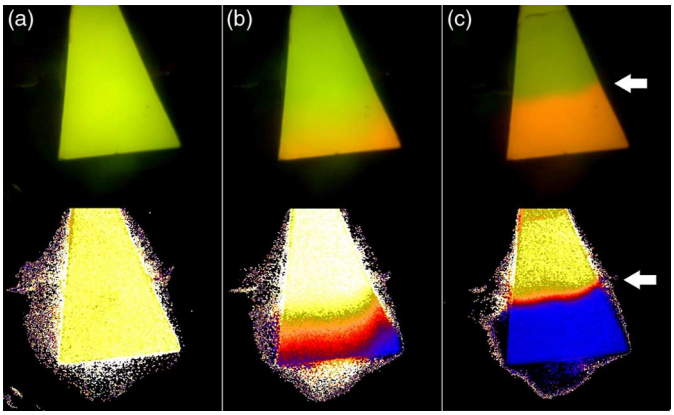

Fig. 9. Images of the (a) start, (b) middle, and (c) end times of an oil biodegradation incubation, where an oxygen-sensing patch was glued to the window of the high-pressure reactor (as shown in Fig. 2). The reactor was half-filled with media, the top part of the patch was in the gas phase; and the bottom part was in the liquid phase. The upper part of the panels shows the color image captured by the camera; the lower part shows the processed, ratiometric images (false color representation). A decrease in the oxygen concentration changed the color of the patch from light to dark; from green to red in case of the camera fluorescence images and from yellow to blue for the processed ratiometric images. In panel (c), the gas–liquid interphase can be seen as a defined line (arrows).

lower part shows the processed images. In both cases, it can be seen that a decreasing concentration of oxygen changes the color from light to dark. The oxygen gradients are clearly visible and for the results shown in Figs. 4–7, only the part of the patch submerged in the liquid was used for calculating the oxygen or carbon dioxide concentrations.

IV. Discussion

There was no biodegradation of oil in the blank control experiments because the O_2 and CO_2 values were fairly constant and the bacterial density did not increase (Figs. 4, 5, and 8). In contrast, in the deep-sea-sediment amended incubation, a sharp decrease in the O_2 concentration and an increase in CO_2 concentration were observed because the crude oil was biodegraded; this was corroborated with the increase in cell density (Fig. 8). For both pressure regimes, the carbon dioxide evolved mainly after the oxygen had been depleted. For the 150-bar incubation, the ΔCO_2 was larger than in the 1 bar incubation (Figs. 6 and 7). This could be explained by the $\sim$10 times greater solubility of carbon dioxide in water at 150 bar in comparison to 1 bar at 18°C [23]. Probably in the 150 bar pressure reactor, the produced CO_2 gas was pushed into the liquid phase by the headspace overpressure and, therefore, detected earlier and in larger amount than in the low pressure incubation. However, since the carbon dioxide can only be measured in the liquid phase, it is unknown if the total concentration of it in both liquid and gas phases is equivalent for both tested pressures. An important issue to consider is that the oxygen and carbon dioxide sensors have been designed and calibrated for working at ambient pressure and never tested before at 150 bar. The calibration could not be corrected for effects of pressure and ionic strength. Although the sensors gave us a trend of the biodegradation of oil, more deep research into the effect of pressure on the sensor's chemistry is needed to obtain accurate absolute values for the oxygen and carbon dioxide concentration.

The measured initial oxygen and carbon dioxide concentrations of Figs. 6 and 7 fit to the theoretically expected values, which are 1.0 mM for oxygen and 0.05 mM for carbon dioxide, considering that each reaction was supplemented with 2.8 bar pressurized air. For the blank reactions, the carbon dioxide baseline was 0.2 mM and remained stable in this value. The higher baseline value was probably because a different calibration was required for the sensing patches produced in different lots or to the low sensitivity of the CO_2 patches at low concentration.

Equation (1) was used to perform a mass balance. It was assumed that the crude oil was a paraffinic oil with the formula $C_{25}H_{52}$ and that the oil was completely oxidized to carbon dioxide and water without biomass production, a respiratory quotient (RQ) of 0.66 was calculated (RQ: moles of CO_2 produced divided by the moles of O_2 consumed) [14]. Other researchers found that for the biodegradation of hexadecane by marine alkane-degrading bacterial communities, the RQ was in the range of 0.4–1.3, depending on the oxygen supply rate to the culture [24]. The calculated RQ for the 150 bar incubation with oil was 0.32 (0.35 mmol of $CO_2/1.10$ mmolO_2). This value is similar to the reported values for oil biodegradation and is lower than the maximal calculated RQ of 0.66. Making a mass balance from the measured data, oxygen consumption, carbon dioxide production, and biomass production, it turns out that the amount of biomass produced was two orders of magnitude smaller than the calculated. This might be because some of the bacteria from the deep-sea might not be viable and culturable in the agar plates. It is well known that some aquatic bacteria cannot be cultured or form clumps, which leads to an underestimation of the aquatic bacterial population [25]. Another bias source is that the bacterial density was measured from the liquid phase only, and the reaction system is a multiphase system where bacteria could adhere to the sediments or to the oil, since the amount of oxygen consumed in the reactions was higher than what was predicted by the mass balance. Another possibility is that intermediate metabolites were produced and because they cannot be detected, the mass balance is not complete.

The initial increase of oxygen "hump" detected from 3 to 10 h in Figs. 4–7 can be due to diffusion of the oxygen from the gas phase into the liquid phase, since this oxygen hump was not observed in the gas phase (data not shown). Chadwick *et al.* described the diffusion of oxygen in a batch reactor filled with anoxic water and left in contact to the open air, where an oxygen increase from nondetectable to a stable plateau after 10 h was reached [26]. The initial oxygen increment was smaller in the 150 bar experiment, which can be explained by mixing of the system with the pressurized nitrogen, but on the other hand, the solubility of oxygen in water increases with depth of water body as the pressure of the water column increases, so an increase in pressure shifts the oxygen from the gas to the liquid phase. There are physical and chemical phenomena that the pressure parameter causes in the system; to understand the biodegradation of oil at elevated pressure, it is necessary to recreate the conditions at the sea floor and be able to measure some parameters online, that is why applying online monitoring is of great use.

Since the reactor was not mixed, there were depth strata containing different concentrations of oxygen and carbon dioxide in the reactor at different times, as shown in Fig. 9. The oxygen in the bottom of the reactor was depleted first and the depletion zone rose up to the air–water interface until the end of the incubation. The image analysis allows temporal and spatial determination of the carbon dioxide and oxygen profiles in the incubation.

Some of the disturbances in the baselines might be due to the sensitivity of VisiSens sensors to light, and albeit the reactor was covered with a dark plastic foil. Some of the jumps in the O_2 and CO_2 values, as in Figs. 4 and 5, can be attributed to this, whereas others can be attributed to an accidental movement of the camera position in the reactors. Another encountered challenge was that the sensing patches detached from the glass with time and high pressure. When air or liquid entered the space between the glass and the patch, the measurements were disturbed.

The different evolution of the O_2 and CO_2 curves for the different incubations, low (1 bar) and high pressure (150 bar), can be partially attributed to the effect of the pressure and the mixing caused when pressurizing the reactor. It is also highly likely that the heterogeneity of the sediments used as inocula also plays a role in the difference in behavior in the incubations. The bacteria present in the sediments are not distributed uniformly in the sediment slurry and can grow at different rates. The biodegradation of oil was faster in the high pressure incubation than that at ambient pressure (Figs. 6 and 7). Turley and Lochte found that deep-sea bacteria mineralized organic carbon (from detritus) at a faster rate at 456 bar than at 1 bar; but there was no significant difference in the biomass produced. They suggested that the water-sediment interface in the deep-sea is a zone of high remineralization of organic carbon because the benthos is exposed to seasonal carbon enrichments [27].

Until now, we had monitored the biodegradation of oil by sacrificing one reactor per time point and analyzing the bacterial density and the concentration of oil offline. This approach was time- and resource-intensive. Monitoring the evolution of oxygen and carbon dioxide online makes the study of the biodegradation of oil under simulated deep-sea conditions faster and more reliable, since the inherent variability of the environmental sediments used as inocula for the different sacrificed reactors was avoided.

V. Conclusion

Oxygen is a key substrate for aerobic microbial growth and biodegradation of crude oil. Carbon dioxide is a major product of the biodegradation and both parameters can be used to estimate the amount of oil that has been degraded. The metabolism of the bacterial communities present in deep-sea sediments can be analyzed in real time by the method and with the reactor described in this work. In this paper, we observed that the biodegradation of oil was faster in the high pressure incubation than that at ambient pressure (Figs. 6 and 7). To our knowledge, this is the first time that O_2 and CO_2 concentrations were monitored online during the crude oil biodegradation at high pressure in the laboratory. The technology presented here could be used in other fields of oceanography, e.g., to study the degradation of detritus and organic matter in general in the ocean bottom or to study the effect of acidification in the deep-sea caused by climate change. The online, noninvasive detection of oxygen and carbon dioxide are convenient for high-pressure experiments. Further experiments of the biodegradation of oil, changing various parameters like temperature, pressure, and using other types of oils and sediments will be pursued to obtain a valuable insight into biodegradation of crude oil under artificial deep-sea conditions.

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