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An insight into the growth of *Alcanivorax borkumensis* under different inoculation conditions

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ABSTRACT

Alcanivorax borkumensis is a hydrocarbon degrading bacterium found to dominate bacterial communities in marine regions containing high levels of hydrocarbons. It has been linked to oil degradation around oil spill sites; thus, it has potential to be used actively in oil spill remediation. Here, we investigate the effect of solution and interfacial conditions on the growth of *A. borkumensis*. We show that providing *A. borkumensis* with dissolved organic nitrogen as an additional nutrient in solution leads to shorter lag times prior to hydrocarbon utilization at the oil–water interface. Hence, *A. borkumensis* can be encouraged to utilize *n*-alkanes present at the surface of the system quicker by supplementing the system with dissolved organic nitrogen. For fixed oil–water interfacial areas, the growth rates of bacteria show weak dependence on the initial bacteria concentrations; however, increasing bulk interfacial area leads to higher bacterial growth rates due to an increased amount of available surface area for degradation. To our knowledge, this is the first study to offer quantitative insight into how *A. borkumensis* can be actively supported in their utilization and degradation of oil for the bioremediation of marine oil spills.

1. Introduction

The BP oil spill in 2010 has led to a great increase in discussions about the effect of oil pollution on the environment, especially the marine environment. This new interest inspired a closer look at the need for sustainability plans for marine areas (Levy and Gopalakrishnan, 2010). The 2010 Deepwater Horizon Spill claimed 11 lives (Joye and MacDonald, 2010) and released 4 million barrels of oil into the Gulf of Mexico (Water, 2011), the effects of which, both ecological (DeLaune and Wright, 2011; Williams et al., 2011; Lin and Mendelssohn, 2012) and economical (Force et al., 2010; McCrea-Strub et al., 2011; Upton, 2011), are still being dealt with today and are why it is necessary to understand the ways in which oil spills can be remediated.

Deep-water oil spills, like the Deepwater Horizon disaster, result in insoluble hydrocarbons being released into, and ultimately residing on the surface of, the water. Additionally, short-saturated hydrocarbons and aromatics remain in the deep water column (Reddy et al., 2012). It is often left to sunlight and naturally occurring marine bacteria to degrade the insoluble hydrocarbons that reside on the water surface. Several species of bacteria have been reported to be able to degrade these hydrocarbons (Golyshin et al., 2002; Hara et al., 2003; Liu and Shao, 2005). Many of these have also been found to grow in abundance in oil spill regions (Cappello et al., 2007; Hazen et al., 2010). Some of these oil-degrading bacteria synthesize their own biosurfactant that emulsify the oil into distinct droplets, which then serve to improve the bacteria's ability to degrade the oil (Yakimov et al., 1998; Ron and Rosenberg, 2002; Calvo et al., 2004). It is thought that this process increases the available surface area of the oil/water (o/w) interface for the bacteria to attach to, improving the degradation ability (Lessard and DeMarco, 2000). Although it has been shown that an increase in interfacial area on the droplet level generally improves the ability of the bacteria to access the oil, the bulk interface between the oil and water phases has yet to be studied.

Alcanivorax borkumensis was first reported as a biosurfactant-producing, hydrocarbon degrading bacterium in 1998 (Yakimov et al., 1998). It is found in high concentrations as the predominant bacterial species around oil spill regions (Cappello et al., 2007; Rojo, 2009) and produces a glycolipid biosurfactant to aid in accessing hydrocarbons within an emulsified droplet (Kasai et al., 2002; Qiao and Shao, 2010). *A. borkumensis* growth is extremely dependent on nutrient concentrations in the region of hydrocarbons, namely the amounts of phosphorous and nitrogen (Rojo, 2009), and it has been found to grow on both *n*-alkanes–water as well as branched alkanes–water interfaces (Hara et al., 2003). Previous studies have looked at how droplet size, supplemented nitrogen and phosphorous and carbon chain length can affect

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bioremediation by *A. borkumensis*. It is known that the growth curve of *A. borkumensis* follows the same trends seen for most bacteria including a lag phase followed by a growth phase and then a saturation phase (Cappello et al., 2007). To date, no studies have looked at how the bulk oil–water interface impacts bioremediation. Furthermore, the effect of different starting concentrations has never been examined. Finally, although fertilizers, or supplemented nutrients, have been studied extensively, dissolved organic nitrogen as a fertilizer has never been reported on. Here we use *A. borkumensis* to probe its growth in contact with the bulk octane–broth interface when there is and is not dissolved organic nitrogen in solution. We also investigate the changes in bulk oil/water interfacial area. Lastly, we explore how bacteria concentration in the bulk aqueous solution affects the growth of bacteria with an insoluble hydrocarbon source.

2. Materials and methods

2.1. Bacteria inoculation

Marine broth solution (BD Difco 2216) was mixed into de-ionized water at a concentration of 37.4 g/L, along with 2 g/L-supplemented KNO_3. Solution was supplemented with KNO_3 based on previous reports of nutrients being a limiting factor (Leahy and Colwell, 1990; Head et al., 2003). The solution was mixed and boiled for 1 min and then autoclaved at 121 °C for 40 min. A solution of marine broth without the organic nitrogen was also prepared by excluding the 5 g/L peptone, 1 g/L yeast extract available in Difco marine broth. This custom broth contained 19.45 g/L NaCl, 5.9 g/L $MgCl_2$, 3.24 g/L $MgSO_4$, 1.8 g/L $CaCl_2$, 0.55 g/L KCl, 2 g/L KNO_3 and 0.126 g/L HK_2O_4P. *A. borkumensis* (ATCC® 700651™) was suspended in 3.5–7 mL of the prepared broth, depending on the test, and cultured on a shaker plate at 30 °C. Oil phase was introduced into the system as a 1–3 mL layer of octane (C_8H_{18} density=0.7 g/mL, M_w=114.23 g/mole) resting above the aqueous phase.

2.2. Bacteria cell viability measurements

After suspending bacteria in broth, an initial concentration reading was conducted. 500 μL of bacterial solution was removed and stained for reading. Live/dead stain (LIVE/DEAD® BacLight™ Bacterial Viability Kit, Life Sciences) was mixed into the solution at 0.75 μL of each dye per 1 mL of bacteria solution. At individual time points, 500 μL of solution was removed, stained and read for live/dead signals. Live/dead signals were read using a PHERAstar Plus microplate reader (BMG labtech).

Absorbance $A_{600} = 0.95\exp(0.002I)$

The equation above, where I is the live cell intensity, was used to convert the live cell intensity readings to absorbencies. Finally, the absorbance values were converted to live cell concentrations using the conversion of measured value A_{600} of 1 at 8×10^8 cells/mL.

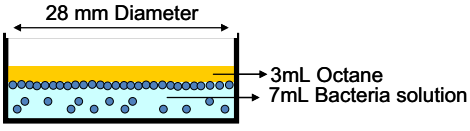

Fig. 1. Schematic of apparatus for insoluble hydrocarbon tests.

2.3. Qualitative assessment of bacteria's emulsifying capabilities

After suspending bacteria in broth, cultures were vortexed for 30 s at 1800 rpm. After 5 min, the oil–water interface of each culture was imaged to show any evidence of emulsion. At certain time points, the experiment was repeated.

3. Results

Fig. 1 shows the schematic of our experimental setup to understand the growth of bacteria at the interface. At various time points, a LIVE/DEAD stain was performed to quantify the amount of bacteria growth and then converted to live cell concentration using the calibration curve above.

This simple setup was chosen to look at the bulk oil–water interface as compared to the droplet interface that has been studied in the past. Additionally, it mimics an actual oil slick on the surface of the ocean on a laboratory scale.

3.1. Effect of dissolved organic nitrogen on bacteria growth

Using the apparatus shown in Fig. 1, we investigated the growth of *A. borkumensis* under different amounts of nitrogen, as seen in Fig. 2.

The effect of dissolved organic nitrogen and limited inorganic nitrogen was probed. Here, initial bacteria concentrations were kept around $c_o \approx 7.7 \times 10^7$ cells/mL. The growth curves show typically four phases: an initial lag phase, an exponential growth phase, the stationary phase (maximum growth), and a death phase, which is the result of the toxic effects of the octanol product of degradation (Naether et al., 2013). The lag time λ is defined as the time it takes the bacteria to enter its exponential phase of growth. For our experiments, we determined that a threshold normalized live cell concentration of 2 would determine the switch from the lag phase to the growth phase. The cells are inoculated in a new, fresh medium under laboratory conditions. Since the environment of bacteria changes suddenly at time $t=0$, the initial period of bacteria growth is heavily inhibited as they need to spend their energy for adaptation. In the growth phase, the bacteria concentration, c, can be described by a well-known autonomous model (Vance, 1990).

$$\frac{dc}{dt} = \beta(c)c, \quad c(0) = c_o, \quad c_o \le c \le c_{max} \tag{1}$$

In this equation, β(c), the specific growth rate of the population, is dependent on the bacteria type, the concentration of bacteria,

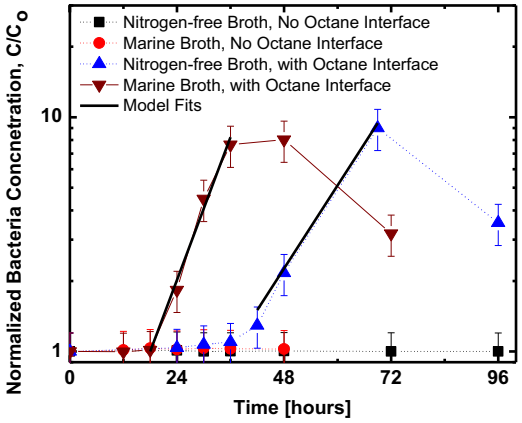

Fig. 2. The concentration of live *Alcanivorax borkumensis* versus time with and without dissolved organic nitrogen. The black lines on the growth curves with octane represent the fitted model in Eq. (3).

and the environment. The specific growth rate is the number of bacteria divisions per unit time. Our specific growth rate values were determined using the average slope of the growth phase between the maximum concentration and the threshold concentration used to signify leaving the lag phase and entering the growth phase. Each experiment was run twice with 4 replicates each time and error was calculated based on the standard deviation of all 8 replicates. An error of 20% can be assumed for all calculated growth rates. The bacteria reached a peak concentration c_{max} after which the bacteria growth rate stabilized due to depleting nutrients. We first tested the growth of bacteria in both broths with no added hydrocarbon layer. As expected, there was no growth because hydrocarbons are an essential nutrient for *A. borkumensis*. Subsequently, we performed experiments to quantify the growth of bacteria in a solution containing only a nominal amount of inorganic nitrogen and another containing dissolved organic nitrogen both with an added insoluble hydrocarbon layer on top. Table 1 shows measured values of average lag times, average specific growth rate and the maximum concentration of bacteria observed in these experiments.

When bacteria were grown in the organic nitrogen free solution with an octane layer above the aqueous phase, the lag time was ~42 h. In these experiments, bacteria grew at a rate of $\beta = 2.20 \times 10^7$ cells/h and reached a peak concentration of $c_{max} = 6.93 \times 10^8$ cells/mL in 69 h. Note that the aqueous solubility of octane in water is extremely low (~7 μM), which amounts to about 1.14 nL/mL of water. Since decreases in volumes of octane observed were much more than soluble amounts, the bacteria consumed almost all oil directly interacting with the interface.

Finally, experiments were performed in order to look at the growth of *A. borkumensis* when there was both dissolved organic nitrogen in solution and an octane layer at the surface. These experiments showed that the bacteria experienced much shorter lag times of $\lambda \sim 18$ h and then grew at a rate of $\beta = 2.84 \times 10^7$ cells/h before peaking at 6.18×10^8 cells/mL in just 36 h.

3.2. Effect of initial concentration

We examined the growth of *A. borkumensis* in relation to the starting concentration of bacteria is the system, as seen in Fig. 3a.

Microscope images showing the three starting concentrations can be seen in Fig. 3b. In the images, the green represents the live cells, while the red is the dead cells. *A. borkumensis* has been reported to be roughly 0.7 μm wide and 1.5 μm long (Yakimov et al., 1998). Some of the larger green spots, that are roughly 20 μm in size, indicate that the bacteria aggregate in solution. The middle image shows what was chosen to be the mid-level starting concentration of bacteria, and is the same starting concentration used in the organic nitrogen tests, $c_o \approx 7.7 \times 10^7$ cells/mL. The lowest starting concentration was chosen to be 1/10 of the middle value and was calculated based on the number above to be $c_o \approx 7.7 \times 10^6$ cells/mL. Finally, the highest concentration was chosen to be 10× higher than the mid level and was calculated to be around $c_o \approx 7.7 \times 10^8$ cells/mL based on the starting concentration from the organic nitrogen tests.

At the mid-level starting concentration of bacteria, a lag time of roughly $\lambda \sim 42$ h and a growth rate of $\beta = 2.2 \times 10^7$ cells/h were exhibited. At the highest concentration, the bacteria showed a lag time of about $\lambda \sim 24$ h and an average growth rate of roughly $\beta = 6.93 \times 10^6$ cells/h. Finally, the lowest starting concentration displayed a lag time of more than $\lambda \sim 60$ h and a growth rate of only $\beta = 2.12 \times 10^6$ cells/h. The lowest starting concentration was not able to reach its maximum concentration in the 96 h experiment. This was determined based on the fact that it never surpassed the threshold concentration for leaving lag phase. Therefore, these numbers do not represent the true growth curve since it may have continued to grow after the 96 hours at which we ended the experiment.

3.3. Effect of interfacial area

The effect of the bulk oil/water interface was probed using different containers for the inoculation, as seen in Fig. 4a.

Tests were conducted using two containers of varying sizes, a schematic for which can be seen in Fig. 4b. The smaller tube has half the diameter as the normal tube. This amounted to a surface area about 70% less than the surface area of the larger tube (176.63 mm^2 and 615.44 mm^2). Because of differences in both the diameter and the conical bottoms of the tubes, ½ the volume of solution was used in the smaller tube so that both sets of experiments would begin at the same depth. Each experiment was started with 7.7×10^7 cells/mL. The smaller tube exhibited a growth rate of 1.04×10^7 cells/mL and a lag time of just over 54 h.

4. Discussion

4.1. Effect of organic nitrogen on bacteria growth

We assume that certain critical intra-bacterial substances are the bottleneck in the process of growth (such as certain enzymes or other metabolites to metabolize substrates in the different environments). Following a previous study, which showed that *A. borkumensis* produces a low molecular weight biosurfactant that acts by decreasing the surface tension of water insoluble hydrocarbons in order to improve the hydrocarbon's bioavailability (Ron and Rosenberg, 2002), we assume that biosurfactant is necessary for *A. borkumensis* to degrade the hydrocarbon. Hence, this increase in lag time is indicative of the time it takes the bacteria to produce enough biosurfactant to decrease the surface tension of the oil enough in order to utilize it. Note that any predictions of mechanisms of biosurfactant induced digestion of oil by *A. borkumensis* are outside the scope of this study.

The results show that by including dissolved organic nitrogen in the bacteria's growth inoculation, the lag time decreased by more than half (~57%). The growth rates were much closer on the other hand (dissolved organic nitrogen increased the growth rate by just ~25%) as were the maximum concentrations, which were within the margin of error.

The rate of growth of bacteria, following its lag time, becomes proportional to its concentration. The growth can be described by Eq. (2), where λ is the lag time and b is the growth parameter.

$$\frac{dc}{dt} = bc, \quad \lambda \le t \le t_{max}, \quad c(\lambda) = c_o \tag{2}$$

Table 1
Summary of results.

Bacteria environment	Average lag time, λ (h)	Average specific growth rate, β (cells/h)	Max bacteria concentration, c_{max} (cells/mL)
Organic nitrogen free broth only	∞	0	7.75E+07
Marine broth (dissolved organic nitrogen) only	∞	0	7.96E+07
Organic nitrogen free broth with octane layer	42	2.20E+07	6.93E+08
Marine broth with octane layer	18	2.84E+07	6.18E+08

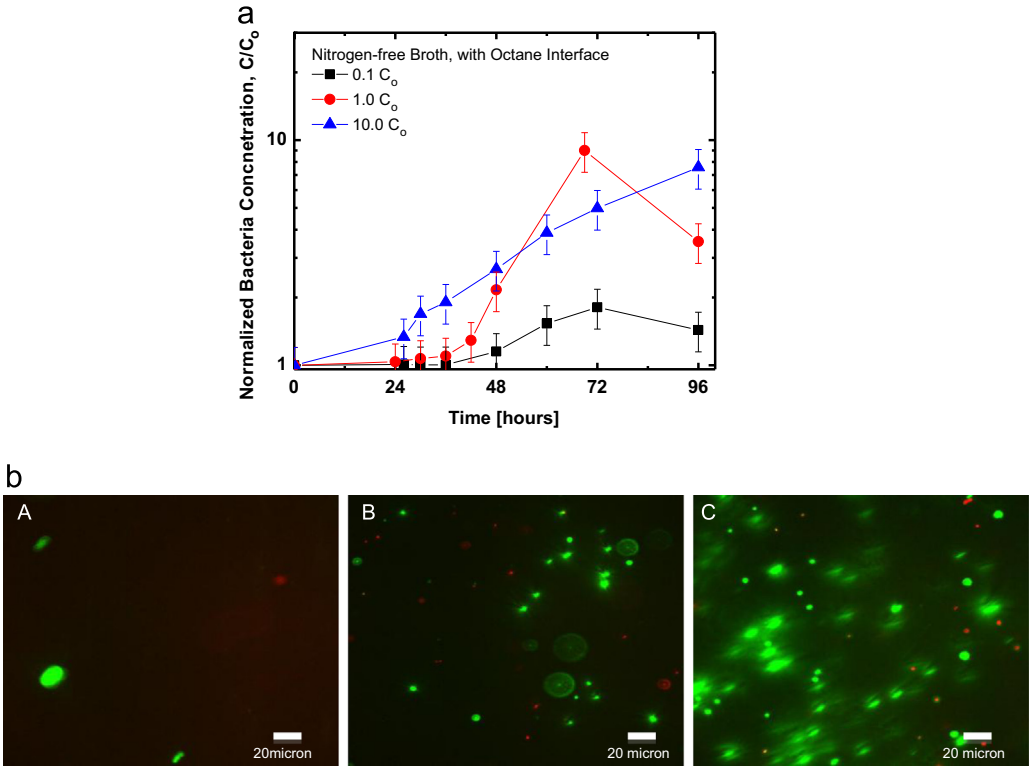

Fig. 3. (a) *A. borkumensis* concentration versus time in hours for different starting concentration of bacteria. (b) Images of the (A) lowest ($0.1c_o$), (B) mid level (c_o), and (C) highest ($10c_o$) starting concentration of bacteria tested. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

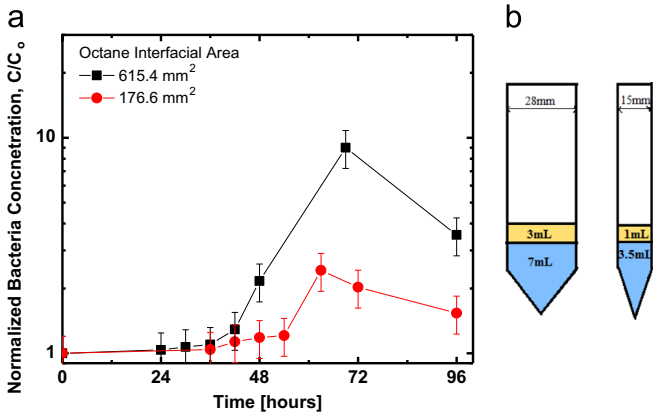

Fig. 4. (a) Live cell concentration of *Alcanivorax borkumensis* versus time in hours for different bulk interfacial areas. (b) Schematic of the apparatus for the surface area test.

Eq. (2) can be integrated to Eq. (3), which describes an exponential growth rate.

$$c = c_o e^{b(t-\lambda)} \tag{3}$$

The growth curves for both conditions with the oil layer were fit to Eq. (3), where c is the concentration, c_o is the initial concentration, and t is the time. In this equation, an increase in b corresponds to an increase in the specific growth rate. It can be assumed that the variable b is independent of t and c. The variable b was fitted to the growth curves and exhibited no correlation with either t or c. It is possible that a different model would give a relationship between b and t and c but that was outside the scope of this model. After fitting the growth curves to the model, it was found that providing the bacteria with organic nitrogen increased the value of b. The system with organic nitrogen yielded a b value of 0.117, while that without organic nitrogen yielded a b value of just 0.068. This model fit well with the experimental results, as seen in Fig. 2, and proves that providing the bacteria with organic nitrogen can improve its growth. This suggests that *A. borkumensis* can be encouraged to utilize hydrocarbons faster by fertilizing the system with dissolved organic nitrogen.

To further probe this decrease in lag time, the effect of dissolved organic nitrogen on the bacteria's ability to form an emulsion was investigated (Fig. 5).

The results indicated that an emulsion is formed 24 h earlier when there is organic nitrogen in solution. Upon further examination though, it was clear that the degree of emulsion was a function of the bacteria concentration in solution. At time 24 in the broth containing organic nitrogen, there were 1.41×10^8 cells/mL in solution. A similar degree of emulsion was observed at time 48 in the broth without organic nitrogen, and there were 1.67×10^8 cells/mL in solution. Time 48 in the broth with organic nitrogen showed a similar emulsion to the broth without organic nitrogen at time 72. At these times, there were 6.18×10^8 cells/mL and 6.93×10^8 cells/mL, correspondingly. With this in mind, it can be inferred that adding dissolved organic nitrogen to the system allows the bacteria to better utilize the oil layer initially, but does not affect the bacteria's ability to lower the surface tension of the oil interface or create an emulsion. The lack of a difference in maximum concentration supports this inference as well. Because it is known that emulsions increase the growth of bacteria, the fact that both experiments created equivalent emulsions and yielded similar maximum concentrations is expected. In addition, the results indicated that an increase in bacteria concentration, and therefore biosurfactant, which is part of the bacteria's cell membrane (Abraham et al., 1998), increases the emulsifying ability of the bacteria.

4.2. Effect of initial concentration

The data shows that the decrease in lag time is nonlinear with respect to initial *A. borkumensis* concentrations. It can be represented by the model in Eq. (4), where λ is the lag time and c_o is the

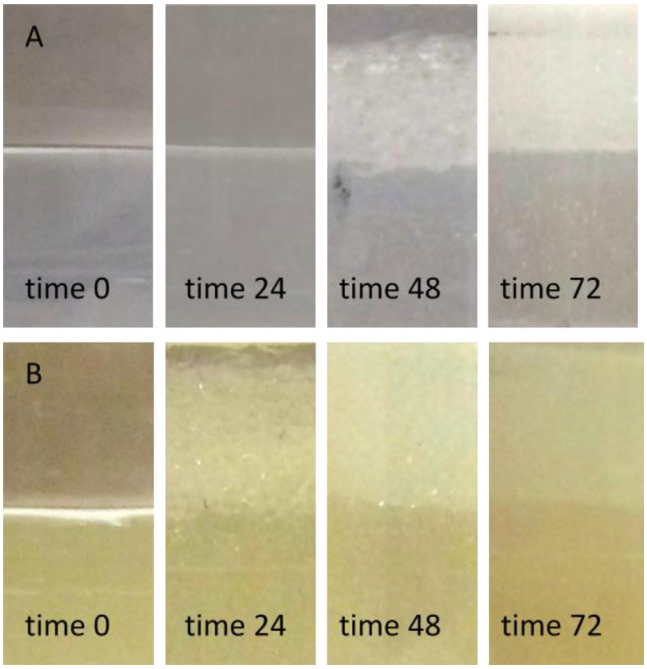

Fig. 5. Images of the oil/water interface at various time points for (A) a system without organic nitrogen and (B) a system with organic nitrogen. The bottom layer for both is the broth, and the upper layer is the octane.

initial concentration.

$$\lambda \sim 1521 c_o^{-0.2} \tag{4}$$

The model shows that as the initial concentration is increased, the lag time is decreased. It is likely that there is some limit to this model, but the limit was not determined in this paper. Due to the exponential nature of the model though, it is clear that the lag time decreases less rapidly after an initial concentration on the order of 10^8. *A. borkumensis* is a biosurfactant producing bacteria as mentioned above. Although its biosurfactant is not entirely necessary to begin degradation of the oil layer, its production is likely the bottleneck process in entering the exponential phase of growth. Assuming a critical biosurfactant concentration is needed before reaching end of the lag phase, the data suggests that rate of production of biosurfactant by *A. borkumensis* depends on the size of its surrounding population (which is called quorum-sensing).

The growth curves for the middle and high initial concentrations were fit to the growth model in Eq. (3) just like with the nitrogen tests. The starting concentration 7×10^7 cells/mL yielded a b value of 0.068 compared to a b value of 0.031 for the tests that began with 7×10^8 cells/mL. It is clear from the obtained b values that the larger initial concentration grows at a much slower rate. As the initial concentration increases, there is a decline in the growth rate but no correlation exists between the growth rate and the starting concentration. Although this seems counterintuitive, it is actually logical. Despite the increased amount of bacteria, the actual number of bacteria that can fit at the interface is finite so the growth rate of the bacteria would become almost linear once the interface is saturated. This implies that *A. borkumensis* growth rates depend more on the exposed area of oil interfaces. The above conclusion is also supported by the fact that the maximum *A. borkumensis* concentrations c_{max} were observed to be the same for 2 orders of concentrations, excluding the lowest since it never peaked.

By using the typical size of *A. borkumensis*, we can estimate about $\sim 5.86 \times 10^8$ bacteria can fit at the interface in monolayer conformation. Since the middle starting concentration was $c_o = 7.7 \times 10^7$ cells/mL and there were 7 mL, there was $\sim 4.9 \times 10^8$ bacterium in the initial inoculation. These *A. borkumensis* would have almost entirely covered the interface making the surface area a limiting factor after a very short period of time. The highest concentration started with 10 times more bacteria than could fit at the interface. This is why, at initial times (after lag phase), no exponential growth phase was seen and the growth followed a more linear trend at the early time points. Hence, the growth phase is entirely a function of the surface area for the highest starting concentration. Previous studies have also shown that when interfacial area becomes limiting, growth becomes linear (Ron and Rosenberg, 2002).

4.3. Effect of interfacial area

The slight difference in lag time observed for the two different diameter tubes can be explained by the smaller tube starting with a smaller mass of bacteria. Since half the volume of broth was being used, half the mass of bacteria was added in order to achieve the same starting concentration. The longer lag time is consistent with the starting concentration experiments since in order to achieve different starting concentrations in that experiment, different masses of bacteria were added to each inoculation. The growth rate observed for the larger interface was $\sim$2.12 times faster than that observed for the smaller interface (2.2×10^7 cells/h compared to 1.04×10^7). The larger interface is roughly 3.4 times the smaller interface so the difference in growth rate is indirectly proportional to the bulk oil/water surface area. The same growth model shown in Eq. (3) was fit to the surface area experiments just as with the Nitrogen tests. For the smaller diameter tube, a b value of 0.053 was obtained versus 0.068 for the larger diameter tube. As noted above, an increase in b value corresponds to an increase in the growth rate. Increasing the diameter of the tube, experimentally and theoretically increased the growth rate.

This relationship suggests that *A. borkumensis* can be encouraged to utilize surface hydrocarbons quicker by increasing the bulk oil/water surface area in the system. The smaller interface can only fit 1.68×10^8 bacteria at the surface, but the experiment began with 2.7×10^8 cells. Therefore, at the beginning of the experiment not every cell would be able to access the interface. We showed before that increasing the starting bacteria concentration would decrease the lag time. For a smaller interface though, since the interface is already saturated, increasing the amount of starting bacteria would have no effect on the growth rate. The lag time would be decreased, but the growth rate would be so much slower than a larger bulk interface that it would still take more time to degrade the oil layer. Additionally, the smaller diameter tube yields a drastically cut maximum concentration. Since it is ultimately the maximum concentration of bacteria that determines the rate of degradation, it is not suggested using a smaller interface. It has been shown that by using surfactants to emulsify the oil into discrete droplets bacteria is more readily able to degrade the oil due to an increase in surface area on the droplet level (Yakimov et al., 1998; Ron and Rosenberg, 2002; Calvo et al., 2004). Our experiments show that allowing the oil to spread over a larger surface area can also increase the bacteria's ability to degrade the oil.

4.4. Conclusion

We have investigated the growth of *A. borkumensis* in a variety of different situations that could be encountered in the marine environment during an oil spill situation. We have seen that *A. borkumensis* grows faster when the system is supplemented with dissolved organic nitrogen. We have observed that by supplying *A. borkumensis* with dissolved organic nitrogen when first introduced to a system containing hydrocarbons, the lag time prior to degrading the hydrocarbon molecules can be shortened. We

have studied the effect of different starting concentrations of bacteria and found that higher starting concentration can lead to less of a lag time before utilization of the hydrocarbon layer. We have also shown that by increasing the surface area between the oil and water phases, we can increase the growth rates and decrease the lag time.

These insights could have great implications on the future of bioinspired oil spill remediation. Studies have shown that nitrogen, in the form or KNO_3 and uric acid, and phosphorous access is a rate limiting step in the growth of hydrocarbonoclastic bacteria (Ron and Rosenberg, 2014). Our investigation has revealed that providing dissolved organic nitrogen to an oil spill can also increase the rate of bioremediation. This is significant because dissolved organic nitrogen would not be toxic to other species and would not add to the toxic waste of an oil spill. It is also known that contact with the oil layer is necessary for bacteria degradation, which is why emulsifying surfactants and particles have been studied extensively (Ron and Rosenberg, 2014). During the BP Deepwater Horizon oil spill, COREXIT was used as a dispersant to emulsify the oil layer because it was thought that it would aid the bacteria in degradation (Hamden and Fumer, 2011). Unfortunately, it was later discovered that COREXIT made the oil 52 times more toxic (Rico-Martínez et al., 2013). Our findings show how simply increasing the bulk surface area of an oil spill can increase the rate of bioremediation. Therefore, surfactants, which may prove toxic to marine organisms, are not necessary for enhanced bioremediation. Future studies will involve using this same technique to quantify the growth of other types of bacteria under certain conditions. Furthermore, the same technique can be modified for larger scale experiments. *A. borkumensis* has been shown to yield higher growth rates when using alkanes with a chain length of C_{12}–C_{19} in the past (Naether et al., 2013). We are currently testing the effect of certain surfactant and dispersant-stabilized crude oil emulsions on the growth of *A. borkumensis* for application in future oil spills.

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References

Abraham, W.-R., Meyer, H., Yakimov, M., 1998. Novel glycine containing glucolipids from the alkane using bacterium *Alcanivorax borkumensis*. Biochim. Biophys. Acta (BBA) – Lipids Lipid Metab. 1393 (1), 57–62.

Calvo, C., Toledo, F.L., González-López, J., 2004. Surfactant activity of a naphthalene degrading *Bacillus pumilus* strain isolated from oil sludge. J. Biotechnol. 109 (3), 255–262.

Cappello, S., Denaro, R., Genovese, M., Giuliano, L., Yakimov, M.M., 2007. Predominant growth of Alcanivorax during experiments on "oil spill bioremediation" in mesocosms. Microbiol. Res. 162 (2), 185–190.

DeLaune, R.D., Wright, A.L., 2011. Projected impact of Deepwater Horizon Oil Spill on U.S. Gulf Coast Wetlands. Soil Sci. Soc. Am. J. 75 (5), 1602–1612.

Force, R., Davies, M., Force, J.S., 2010. Deepwater Horizon: removal costs, civil damages, crimes, civil penalties, and state remedies in oil spill cases. Tulane Law Rev. 85, 889.

Golyshin, P.N., Chernikova, T.N., Abraham, W.-R., Lünsdorf, H., Timmis, K.N., Yakimov, M.M., 2002. Oleiphilaceae fam. nov., to include *Oleiphilus messinensis* gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons. Int. J. Syst. Evol. Microbiol. 52 (3), 901–911.

Hamden, L.J., Fumer, P.A., 2011. Effects of COREXIT EC9500A on bacteria from a beach oiled by the Deepwater Horizon spill. Aquat. Microb. Ecol. 63 (2), 101–109.

Hara, A., Syutsubo, K., Harayama, S., 2003. Alcanivorax which prevails in oil-contaminated seawater exhibits broad substrate specificity for alkane degradation. Environ. Microbiol. 5 (9), 746–753.

Hazen, T.C., Dubinsky, E.A., DeSantis, T.Z., Andersen, G.L., Piceno, Y.M., Singh, N., Jansson, J.K., Probst, A., Borglin, S.E., Fortney, J.L., Stringfellow, W.T., Bill, M., Conrad, M.E., Tom, L.M., Chavarria, K.L., Alusi, T.R., Lamendella, R., Joyner, D.C., Spier, C., Baelum, J., Auer, M., Zemla, M.L., Chakraborty, R., Sonnenthal, E.L., D'haeseleer, P., Holman, H.-Y.N., Osman, S., Lu, Z., Van Nostrand, J.D., Deng, Y., Zhou, J., Mason, O.U., 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330 (6001), 204–208.

Head, I.M., Jones, D.M., Larter, S.R., 2003. Biological activity in the deep subsurface and the origin of heavy oil. Nature 426 (6964), 344–352.

Joye, S., MacDonald, I., 2010. Offshore oceanic impacts from the BP oil spill. Nat. Geosci. 3 (7), 446.

Kasai, Y., Kishira, H., Sasaki, T., Syutsubo, K., Watanabe, K., Harayama, S., 2002. Predominant growth of Alcanivorax strains in oil-contaminated and nutrient-supplemented sea water. Environ. Microbiol. 4 (3), 141–147.

Leahy, J.G., Colwell, R.R., 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54 (3), 305–315.

Lessard, R.R., DeMarco, G., 2000. The significance of oil spill dispersants. Spill Sci. Technol. Bull. 6 (1), 59–68.

Levy, J.K., Gopalakrishnan, C., 2010. Promoting ecological sustainability and community resilience in the US Gulf Coast after the 2010 Deepwater Horizon Oil Spill. J. Nat. Resour. Pol. Res. 2 (3), 297–315.

Lin, Q., Mendelssohn, I.A., 2012. Impacts and recovery of the Deepwater Horizon Oil Spill on vegetation structure and function of coastal salt marshes in the Northern Gulf of Mexico. Environ. Sci. Technol. 46 (7), 3737–3743.

Liu, C., Shao, Z., 2005. Alcanivorax dieselolei sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment. Int. J. Syst. Evolut. Microbiol. 55 (3), 1181–1186.

McCrea-Strub, A., Kleisner, K., Sumaila, U., Swartz, W., Watson, R., Zeller, D., Pauly, D., 2011. Potential impact of the Deepwater Horizon oil spill on commercial fisheries in the Gulf of Mexico. Fisheries 36 (7), 332–336.

Naether, D.J., Slawtschew, S., Stasik, S., Engel, M., Olzog, M., Wick, L.Y., Timmis, K.N., Heipieper, H.J., 2013. Adaptation of the hydrocarbonoclastic bacterium *Alcanivorax borkumensis* SK2 to alkanes and toxic organic compounds: a physiological and transcriptomic approach. Appl. Environ. Microbiol. 79 (14), 4282–4293.

Qiao, N., Shao, Z., 2010. Isolation and characterization of a novel biosurfactant produced by hydrocarbon-degrading bacterium *Alcanivorax dieselolei* B-5. J. Appl. Microbiol. 108 (4), 1207–1216.

Reddy, C.M., Arey, J.S., Seewald, J.S., Sylva, S.P., Lemkau, K.L., Nelson, R.K., Carmichael, C.A., McIntyre, C.P., Fenwick, J., Ventura, G.T., Van Mooy, B.A.S., Camilli, R., 2012. Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. Proc. Natl. Acad. Sci. USA 109 (50), 20229–20234.

Rico-Martínez, R., Snell, T.W., Shearer, T.L., 2013. Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). Environ. Pollut. 173 (0), 5–10.

Rojo, F., 2009. Degradation of alkanes by bacteria. Environ. Microbiol. 11 (10), 2477–2490.

Ron, E.Z., Rosenberg, E., 2002. Biosurfactants and oil bioremediation. Curr. Opin. Biotechnol. 13 (3), 249–252.

Ron, E.Z., Rosenberg, E., 2014. Enhanced bioremediation of oil spills in the sea. Curr. Opin. Biotechnol. 27 (0), 191–194.

Upton, H.F., 2011. The Deepwater Horizon Oil Spill and the Gulf of Mexico Fishing Industry. Report No. R41640. Congressional Research Service, Washington, DC.

Vance, R.R., 1990. Population-growth in a time-varying environment. Theor. Popul. Biol. 37 (3), 438–454.

Water, D., 2011. The Gulf Oil Disaster and the Future of Offshore Drilling. National Commission on the BP Deepwater Horizon Spill and Offshore Drilling.

Williams, R., Gero, S., Bejder, L., Calambokidis, J., Kraus, S.D., Lusseau, D., Read, A.J., Robbins, J., 2011. Underestimating the damage: interpreting cetacean carcass recoveries in the context of the Deepwater Horizon/BP incident. Conserv. Lett. 4 (3), 228–233.

Yakimov, M.M., Golyshin, P.N., Lang, S., Moore, E.R.B., Abraham, W.-R., Lünsdorf, H., Timmis, K.N., 1998. *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. Int. J. Syst. Bacteriol. 48 (2), 339–348.