Journal of Industrial Microbiology, 13 (1994) 279–286
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Published by The Macmillan Press Ltd

Effects of nitrogen source on crude oil biodegradation

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(Received 19 October 1992; accepted 6 June 1993)

Key words: Crude oil; Biodegradation; Nitrogen source; Respirometry

SUMMARY

The effects of NH_4Cl and KNO_3 on biodegradation of light Arabian crude oil by an oil-degrading enrichment culture were studied in respirometers. In poorly buffered sea salts medium, the pH decreased dramatically in cultures that contained NH_4Cl, but not in those supplied with KNO_3. The ammonia-associated pH decline was severe enough to completely stop oil biodegradation as measured by oxygen uptake. Regular adjustment of the culture pH allowed oil biodegradation to proceed normally. A small amount of nitrate accumulated in all cultures that contained ammonia, but nitrification accounted for less than 5% of the acid that was observed. The nitrification inhibitor, nitrapyrin, had no effect on the production of nitrate or acid in ammonia-containing cultures. When the culture pH was controlled, either by regular adjustment of the culture pH or by supplying adequate buffering capacity in the growth medium, the rate and extent of oil biodegradation were similar in NH_4Cl- and KNO_3-containing cultures. The lag time was shorter in pH-controlled cultures supplied with ammonia than in nitrate-containing cultures.

INTRODUCTION

The use of fertilizers to stimulate biodegradation of oil in natural environments is not new. Many researchers have examined the fate of oil in various environments including soils, water, and seawater [3,5–7,10–12,14,15,17,21], and concluded that nutrient addition, especially nitrogen and phosphorus, can stimulate oil biodegradation. Mineral nutrients (e.g. KNO_3, NH_4NO_3, K_2HPO_4, $MgNH_4PO_4$) and organic nutrients, such as urea, paraffin-supported mineral nutrients, and octyl phosphate, are the most common compounds used for bioremediation. In most of these studies, only one type of nutrient compound was used. Few researchers have studied different nutrient types concurrently. In one case the addition of mineral nutrients stimulated biodegradation more than the organic nutrients [6]. In another study a proprietary organic nutrient was equivalent to nitrate in closed systems [3].

Observations made in our laboratory suggest that the source of nitrogen added to oil-degrading enrichment cultures may have a powerful effect on degradative ability. We have routinely used NH_4NO_3 as the nitrogen source, but recently we observed that KNO_3 permitted greater degradation of oil as measured by oxygen consumption. Preliminary results showed that, after oxygen consumption had ceased, the pH of flasks containing NH_4NO_3 was two to three units lower than flasks supplied with KNO_3. This series of experiments was undertaken to establish the effect of nitrogen source on biodegradation of crude oil and to determine the likely cause of the pH shift.

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MATERIALS AND METHODS

Respirometry

The effects of different nitrogen sources and pH adjustments on oil degradation were measured in analytical respirometers. The respirometers, Model WB512 from N-CON Systems, Larchmont, NY, USA, supply pure O_2 to 500-ml reaction flasks in response to pressure drops created by O_2 consumption. Calibrated microsolenoid valves meter oxygen into the flasks to balance pressure against temperature-controlled reference cells. A computer monitors the solenoid valve pulses, records the data, and calculates the O_2 uptake rates. The respirometers are equipped with a temperature-controlled water bath and magnetic stirrers. Each flask contains a CO_2 trap consisting of a polymethylpentene centrifuge tube that holds a KOH solution of known concentration. The KOH solution can be changed through a syringe valve on top of the flask. The KOH is changed when the pH indicator, Alizarin Red S (10 mg L^{-1}), in the CO_2 trap changes color. The amount of CO_2 trapped is determined by measuring the pH of the KOH solution.

Measurement of nitrogen species

Ammonia concentrations in experimental flasks were determined using an ion-specific electrode with an Orion 720A pH/mV meter (Orion Research Inc., Boston, MA,

USA). The electrode was calibrated daily using freshly prepared standards. Particles and residual oil were removed by filtration through 0.45-μm pore size Millipore filters (Millipore Corp., Bedford, MA, USA) with glass fiber prefilters. The ionic strength and pH of each sample were adjusted by addition of 0.25 ml of 10 M NaOH prior to measurement.

Nitrite and nitrate were measured by adapting the standard US EPA method 353.1 [20]. Samples were filtered as described above. For nitrite analysis, 3.0 ml of sample was mixed with 1.0 ml of color reagent (200 ml concentrated H_3PO_4, 7.5 g sulfanilamide, and 0.75 g *N*-1-naphthylethylenediamine dihydrochloride in a total volume of one liter of water). For nitrate analysis, 3.0 ml of sample, 0.1 ml 10 N NaOH, 1.0 ml $CuSO_4 \cdot 5H_2O$ (0.025 mg ml^{-1}), 1.0 ml hydrazine sulfate (1.4 mg ml^{-1}) were mixed and allowed to react for 15 min at room temperature. Finally, 1.0 ml of the color reagent was added and the absorbance at 520 nm was measured. Standard curves were prepared daily for nitrite and nitrate, and samples were compared with the standards. The nitrate method was modified for samples containing sea salts medium, because divalent cations present in this medium interfere with the hydrazine reduction step. The interference was eliminated by precipitating these ions at pH 11, then removing the precipitate by filtration through 0.5-μm pore size glass fiber filters (MSI, Westboro, MA, USA). The filtrate was analyzed as described above.

Measurement of heterotrophic bacterial numbers

Heterotrophic populations in the flasks were measured by standard spread plate methods. Samples collected for the chemical analysis were also used for microbiological analysis. Serial tenfold dilutions of each sample were prepared in 20 g L^{-1} saline solution. Triplicate plates of appropriate dilutions were prepared and spread with flame-sterilized glass rods. The agar was Difco marine agar 2216 (Difco Laboratories, Ann Arbor, MI, USA). The plates were incubated 6 days at 20 °C and colonies were then counted. Average heterotrophic populations per ml were calculated from the results.

Experimental procedures

Three separate experiments were conducted to determine the effects of nitrogen source on oil degradation. In the first experiment, we investigated the relationship between ammonia concentration and depression of the culture pH. The cultures were grown in a sea salts medium [13] that contained (in g L^{-1}) NaCl (23.476), $MgCl_2 \cdot 6H_2O$ (10.634), Na_2SO_4 (3.917), KCl (0.664), KBr (0.096), $Na_2HPO_4 \cdot 7H_2O$ (0.043), $FeNH_4(SO_4)_2$ (0.0005), $CaCl_2 \cdot 2H_2O$ (1.460), and H_3BO_3 (0.026). The cultures were supplied with one of the following nitrogen sources (in g L^{-1}): NH_4NO_3 (0.284), KNO_3 (0.722), or NH_4Cl (0.190, 0.095, 0.048, or 0.024). A single respirometer flask was used for each treatment of nitrogen source with or without pH adjustment. All media were adjusted to pH 8.0 before autoclaving them for 15 min at 121 °C. Each flask contained 250 ml of the salts medium. Light Arabian (LA) crude oil was added to each flask at a concentration of 5 g L^{-1}. The flasks were inoculated with 1.0 ml of a concentrated suspension of oil-degrading microorganisms that had been previously grown on Prudhoe Bay (PB) crude oil for 3 weeks. The pH of each flask was measured daily, and half of the flasks were adjusted as necessary by adding sterile 1N KOH. The experiment was run for 2 weeks.

The inoculum was a mixed culture of oil-degrading bacteria that was originally isolated from an oil-contaminated beach in Prince William Sound, Alaska. This mixed culture was maintained by transfer from batch cultures after growth in Bushnell–Haas medium [4] containing PB and NH_4NO_3 (1 g N L^{-1}). The cultures were transferred at 3-week intervals.

The second experiment tested the effects of nitrogen source, nitrification inhibitor, and pH adjustment on O_2 uptake and CO_2 production by cultures growing on LA. The sea salts medium [13] for this experiment contained (in g L^{-1}): NaCl (23.476), $MgCl_2 \cdot 2H_2O$ (10.634), Na_2SO_4 (3.917), $CaCl_2 \cdot 2H_2O$ (1.460), KCl (0.664), KBr (0.096), $SrCl_2 \cdot 6H_2O$ (0.040), H_3BO_3 (0.026), and K_2HPO_4 (0.225). Either NH_4Cl (0.190 g L^{-1}) or KNO_3 (0.361 g L^{-1}) was added as the source of nitrogen. A nitrification inhibitor (nitrapyrin, Hach Chemical 2533, Hach Chemical, Loveland, CO, USA) was added to half of the cultures containing NH_4Cl at a concentration of 0.8 g L^{-1}. Each flask received 350 ml of the appropriate medium, LA oil (5000 mg L^{-1}), and 1.0 ml of a mixed culture that had been growing on PB oil. One group of flasks with either KNO_3, NH_4Cl, or NH_4Cl plus nitrification inhibitor was pH adjusted as necessary over the course of the experiment. The pH of a second, otherwise identical, group was not adjusted. The six treatments were prepared in duplicate. The experiment was run at 20 °C, and O_2 consumption and CO_2 production were measured for 20 days. Samples were withdrawn from the flasks periodically for measurement of pH, NH_3, NO_2^-/NO_3^-, total dissolved organic carbon, and heterotrophic bacterial numbers. Oxygen uptake and CO_2 production data were adjusted for the volume changes that accompanied sampling.

The effects of ammonia-associated acid production were examined in a well buffered system in a third experiment. Biodegradation of LA oil (5000 mg L^{-1}) was studied in a modified Bushnell–Haas medium [4] that contained (in g L^{-1}): NaCl (20), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.02), KH_2PO_4 (1.0), K_2HPO_4 (1.0), $FeCl_3 \cdot 6H_2O$ (0.05), $MnSO_4 \cdot 7H_2O$ (0.06 mg L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.085 mg L^{-1}), $(NH_4)_6Mo_7O_{24}$ (0.079 mg L^{-1}), H_3BO_3 (0.114 mg L^{-1}), and either KNO_3 (0.361) or NH_4Cl (0.190). The pH was adjusted to 7.4 before autoclaving the medium. Half of the cultures containing NH_4Cl received nitrification inhibitor (0.8 g L^{-1}). Each flask contained 350 ml of the appropriate medium and was inoculated with 1.0 ml of an actively growing culture of oil degrading microorganisms. As before, one set of flasks was pH adjusted as necessary, and another set was not adjusted. Each treatment was prepared in duplicate. The experiment was conducted at 20 °C, and O_2 consumption and CO_2 production were measured for 13 days. Samples

were withdrawn from the flasks periodically to measure pH, NH_3, NO_2^-/NO_3^-, total dissolved organic carbon, and heterotrophic bacterial numbers.

RESULTS

Effects of nitrogen source on culture pH, oxygen consumption, and growth

The effects of several common nitrogen sources on crude oil biodegradation in a poorly buffered sea salts medium, as measured by oxygen consumption, are summarized in Table 1. Since oil was the only electron donor added to the cultures, the amount of oxygen that was consumed at the end of the experiment (O_u) is assumed to be proportional to the mass of oil that was degraded. Based on oxygen uptake, approximately 35% of the total oil had been degraded at the end of the experiment. It is likely that this represents the bulk of the readily degradable components, because other experiments with LA degradation by this mixed culture show that 96% of the normal alkanes (between 10 and 35 carbon atoms in length) and 29% of the resolvable aromatic compounds were degraded at comparable levels of O_2 uptake [9].

The final pH of cultures that contained ammonia was substantially lower than that of cultures that were supplied with nitrate as the sole source of nitrogen, unless they were regularly neutralized by addition of KOH. Since neither nitrate nor ammonia have buffering capacity in this pH range, the difference cannot be attributed to additional buffering capacity in the nitrate-containing medium. In most cases, the pH of ammonia-containing cultures became low enough to inhibit oil biodegradation, but pH-adjusted, ammonia-containing cultures performed as well as cultures that were supplied with KNO_3. Thus, the inhibitory effect of ammonia on oil biodegradation appears to be a result of its effect on the culture pH.

The relationships among the nitrogen source, culture pH, and the kinetics of oil-dependent oxygen consumption and growth were studied in greater detail in the second experiment. The pH of oil-degrading cultures grown in sea salts medium was different in the presence of ammonia and nitrate, and the rate and extent of oxygen consumption was strongly affected by pH (Fig. 1). The cultures that were supplied with KNO_3 exhibited only minor fluctuations in pH, and oxygen consumption proceeded equally well in pH-adjusted and non-adjusted cultures. In fact, pH adjustment was not required in any of the KNO_3-containing cultures. The pH of cultures that contained NH_4Cl, however, decreased sharply after 2–3 days. Oxygen uptake by cultures that contained ammonia was strongly inhibited if the pH was not regularly adjusted by addition of base. Oxygen consumption in the pH-adjusted ammonia-containing cultures was similar to that in the cultures containing nitrate, but the lag time was shorter. Nitrification inhibitor had no effect: the NH_4Cl and NH_4Cl plus nitrapyrin cultures behaved in an almost identical manner (data not shown).

Bacterial growth, as measured by total heterotrophic plate counts, was also affected by culture pH (Fig. 2). The growth rates and maximum bacterial density were approximately equal in all of the cultures containing KNO_3, but pH-adjusted, NH_4Cl-containing cultures achieved maximum cell densities that were nearly ten-fold higher than those of the non-adjusted cultures. Furthermore, the heterotrophic bacterial density declined after 10 days in the non-adjusted ammonia-containing cultures, but the maximum population density was stable in the pH-adjusted cultures with ammonia and the cultures with KNO_3 until the experiment was terminated. Biomass yields were approxi-

TABLE 1

Effect of nitrogen source on oil biodegradation (O_u) and culture pH after 14 days of growth in sea salts medium

Nitrogen source	pH adjusted?	$[NH_4^+]$ (mg N L^{-1})	$[NO_3^-]$ (mg N L^{-1})	O_u (mg O_2 L^{-1})	Final pH
NH_4NO_3	no	50	50	1458	4.46
	yes			4805	7.93
KNO_3	no	0	100	4514	7.40
	yes			4223	8.00
NH_4Cl	no	50	0	1390	4.52
	yes			4672	7.38
NH_4Cl	no	25	0	1372	4.39
	yes			4401	7.37
NH_4Cl	no	12.5	0	1441	5.62
	yes			5092	7.46
NH_4Cl	no	6.25	0	1067	6.34
	yes			1169	7.55

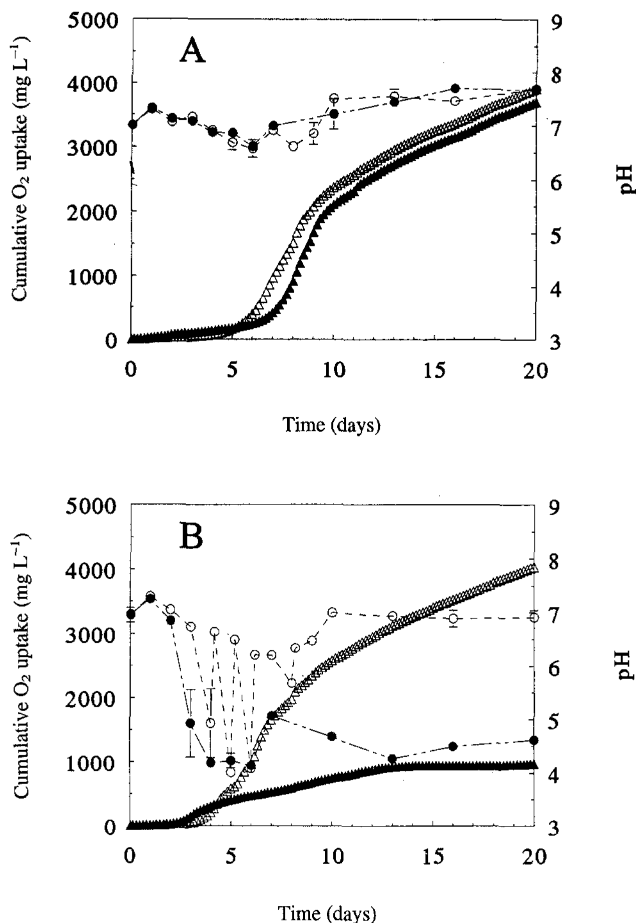

Fig. 1. Oxygen consumption (△, pH-adjusted; ▲, not adjusted) and pH (○, pH-adjusted; ●, not adjusted) in respirometer flasks containing light Arabian crude oil and (A) KNO_3 or (B) NH_4Cl.

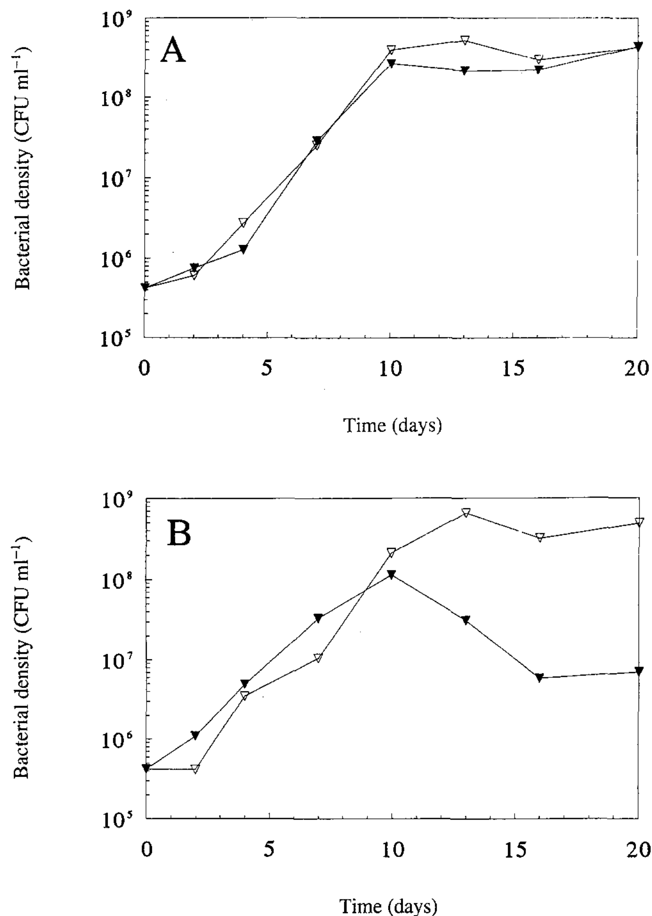

Fig. 2. Heterotrophic bacterial density in pH-adjusted (▽) and non-adjusted (▼) cultures in respirometer flasks containing light Arabian crude oil and (A) KNO_3 or (B) NH_4Cl.

mately the same in pH-adjusted cultures with ammonia and in the cultures that contained KNO_3.

Comparison of the behaviors of the cultures containing NH_4Cl with those containing KNO_3 implicates ammonia as the source of the acid that caused the pH reductions. Additional support for this conclusion is provided by the relationship between the observed pH changes in the pH-adjusted ammonia cultures and the time course of ammonia consumption. This relationship is illustrated for the pH-adjusted NH_4Cl culture in Fig. 3. The culture pH stabilized after ammonia was completely consumed. The behavior of the pH-adjusted NH_4Cl plus nitrapyrin cultures was identical to the pH-adjusted NH_4Cl cultures. Very little ammonia was consumed in any of the ammonia-containing cultures that were not pH-adjusted. Thus, acid production in these oil-degrading cultures was directly related to the presence (and presumably to the metabolism) of ammonia. Acid production was not related to oxygen consumption (i.e. oil degradation), which continued for at least 2 weeks after the ammonia was exhausted, or to growth, which occurred equally well in pH-adjusted cultures with ammonia and in cultures with KNO_3.

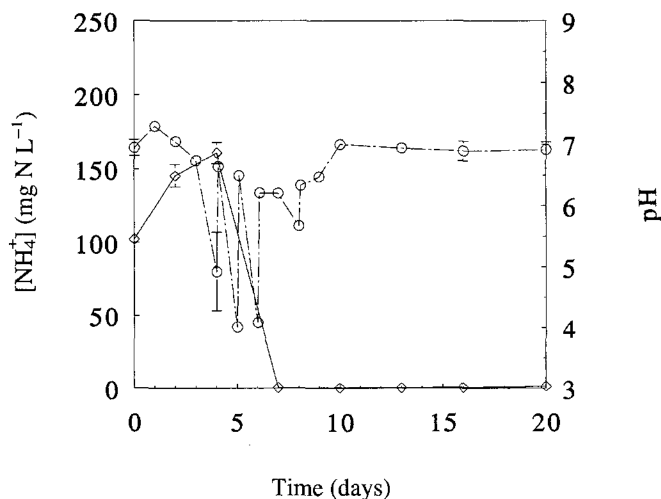

Fig. 3. Ammonia concentration (◇) and pH (○) in the pH-adjusted, NH_4Cl-containing cultures.

The role of nitrification in ammonia-associated acid production

The inability of nitrapyrin to affect acid production in ammonia-containing cultures argues against the hypothesis that nitrification is the cause of the observed pH changes. Nevertheless, nitrate production was observed in all of the treatments that contained NH_4Cl. The relationship between nitrate concentration and pH is shown in Fig. 4 for the pH-adjusted and -nonadjusted NH_4Cl treatments. The behavior of the cultures containing NH_4Cl plus nitrapyrin was similar to the cultures containing NH_4Cl. In each case, the first appearance of nitrate above its background level coincided with the first observed reductions in pH. In pH-adjusted cultures, nitrate concentrations rapidly stabilized at approximately twice their initial concentrations. In the cultures that were not pH-adjusted, nitrate accumulated to six to seven times its initial concentration.

The difference in the extent to which nitrate accumulated in the pH-adjusted and -nonadjusted cultures is probably related to the ability of oil-degrading bacteria in each culture to use nitrate as a nitrogen source. In the pH-adjusted cultures, ammonia was rapidly consumed and the culture conditions favored continued oil-degradation and growth. Thus, heterotrophic bacteria in these cultures probably were able to use most of the nitrate that was formed by nitrification. Nitrate that accumulated in pH-adjusted cultures reflects a balance between the rate at which nitrate was produced by nitrifiers and the rate at which it was consumed by heterotrophs. The low pHs of the cultures that were not regularly neutralized, however, inhibited metabolism, including growth and consumption of ammonia. It is unlikely that utilization of nitrate by heterotrophs occurred in these cultures, because the ammonia concentration remained very high throughout the experiment (ammonia represses expression of the genes involved in assimilatory nitrate reduction [2]). Therefore, the nitrate that accumulated in the cultures that were not pH-adjusted probably reflects the total amount that was produced.

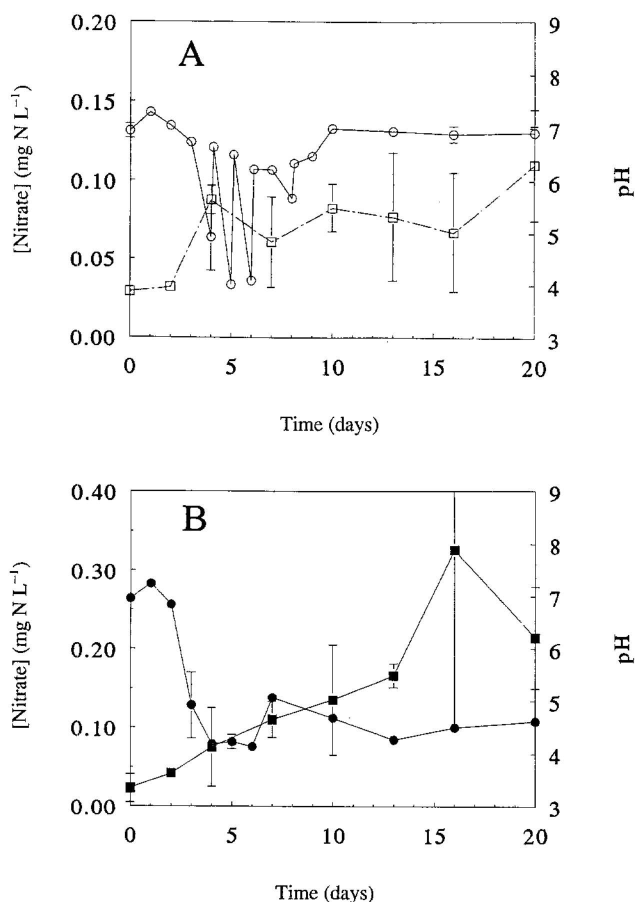

Fig. 4. Nitrate concentration (□, ■) and pH (○, ●) in (A) pH-adjusted and (B) non-adjusted cultures containing NH_4Cl.

The data presented in Fig. 4 demonstrate that nitrification occurred in the cultures that were supplied with ammonia and that the timing of nitrate and acid production coincided. However, insufficient nitrate was produced to account for the observed acid production. The reaction stoichiometry, neglecting growth, that relates production of acid and nitrate by nitrifiers is [8]:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

Thus, two moles of acid equivalents are produced per mole of nitrate that is formed by oxidation of ammonia. Based on this ratio and the amount of nitrate that accumulated, less than 1% of the acid that was observed in the pH-adjusted cultures can be attributed to nitrification (Table 2). More nitrate accumulated and less acid was produced in the cultures that were not pH-adjusted, but less than 5% of the acid observed in these cultures can be attributed to nitrification (Table 2). Since the nitrate that accumulated in the cultures that were not pH-adjusted is probably equal to the amount that was produced, nitrification cannot be the source of the ammonia-associated acid.

Effect of nitrogen source on lag time

In poorly-buffered media, acid production associated with ammonia metabolism inhibited oil biodegradation. Therefore, nitrate is a superior nitrogen source when the buffering capacity of the oil-contaminated environment is low. However, because nitrate must be reduced to ammonia before it can be assimilated into biomass, ammonia is a preferred nitrogen source for many bacteria [2]. The implications of this preference for oil biodegradation kinetics are shown in Fig. 5, in which the oxygen consumption curves for pH-adjusted ammonia cultures and for nitrate cultures are compared. Oil biodegradation started more quickly in the pH-adjusted ammonia cultures than it did in the cultures containing nitrate (Fig. 5(A)). Oil biodegradation in a well-buffered medium, where ammonia-associated acid production did not cause a substantial reduction in pH, followed the same pattern (Fig. 5(B)). Rapid oxygen consumption usually started after 3–4 days in cultures that were supplied with ammonia, but 5–6 days usually passed before biodegradation began in the cultures containing nitrate. In both poorly-buffered sea salts and well-buffered Bushnell–Haas media,

TABLE 2

Comparison of the amount of acid observed in NH_4Cl-containing cultures to the amount of acid that can be attributed to nitrification

Treatment	pH_o	pH_f	A_{obs}[d] (moles H^+)	$M_{nitrate}$[e] (moles NO_3^-)	$A_{nitrate}$[f] (moles H^+)	$\frac{A_{nitrate}}{A_{obs}}$
NH_4Cl[a]	6.94	6.91	9.9×10^{-4}	1.3×10^{-6}	2.5×10^{-6}	0.003
NH_4Cl + nitrapyrin[a]	6.97	7.02	1.0×10^{-3}	1.0×10^{-6}	2.1×10^{-6}	0.002
NH_4Cl[b]	6.96	4.43[c]	2.1×10^{-4}	4.6×10^{-6}	9.1×10^{-6}	0.043
NH_4Cl + nitrapyrin[b]	6.98	4.46[c]	2.1×10^{-4}	3.9×10^{-6}	7.8×10^{-6}	0.037

[a]Culture pH regularly adjusted by addition of 0.1 M NaOH.
[b]Culture pH not adjusted.
[c]Average of culture pH days 5 to 20.
[d]Total amount of acid produced during the experiment: for pH adjusted cultures, A_{obs} was calculated from the total volume of 0.1 M NaOH added to adjust the pH; for the non-adjusted cultures, A_{obs} was calculated from the average final pH and a titration curve for sea salts medium.
[e]Moles of nitrate produced.
[f]The acid that can be attributed to nitrification: $A_{nitrate} = 2*M_{nitrate}$.
pH_o = initial pH; pH_f = final pH.

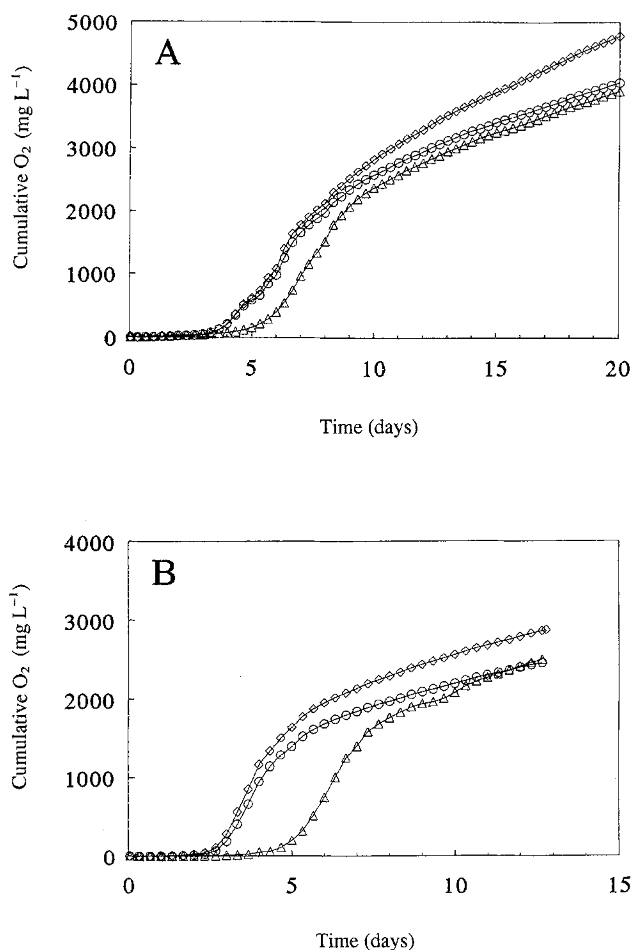

Fig. 5. Oxygen consumption in (A) pH-adjusted sea salts and (B) Bushnell–Haas media for the following treatments: KNO_3 (△), NH_4Cl (○), NH_4Cl plus nitrapyrin (◇).

once oil biodegradation began, the rates of oxygen consumption were similar in nitrate and pH-controlled ammonia cultures. Thus, the nitrogen source had a strong effect on the duration of the lag phase that preceded active oil degradation, but it did not affect the growth rate.

DISCUSSION

The rate of biodegradation of crude oil and refined petroleum products in natural environments is frequently limited by nutrient availability. Bioremediation of contaminated environments usually involves addition of nitrogen and phosphorus fertilizers, but comparisons of the relative efficacy of different types of fertilizers have usually considered only mineral versus organic (i.e. lipophilic) fertilizers. We have demonstrated that important differences exist between two common mineral fertilizers, ammonia and nitrate. Both of these compounds have important advantages and disadvantages, and these should be considered along with the characteristics of the contaminated environment, when choosing a fertilizer to effect bioremediation.

We have identified two major effects of the nitrogen source on crude oil biodegradation in respirometer flasks. In poorly-buffered media, acid production associated with ammonia metabolism can reduce the culture pH to a level that inhibits oil biodegradation. When the culture pH is controlled, either by regular addition of base or by providing sufficient buffering capacity in the medium, both nitrogen sources support extensive biodegradation of crude oil, but biodegradation starts more quickly in the presence of ammonia than in the presence of nitrate.

The time required for initiation of active oil biodegradation can be an important consideration under some circumstances. In open water systems, for example, where nutrients can be rapidly removed from the contaminated zones, maximum

efficiency of nutrient utilization will be achieved when biodegradation starts quickly. Also, because oil slicks can move, they must be degraded and dispersed quickly to prevent contamination of coastal areas.

Although the source of the ammonia-associated acid was not identified, it appears to be directly linked to ammonia metabolism by the oil-degrading enrichment culture that was used in these experiments. Our experience suggests that this phenomenon is widespread, because all of the oil-degrading enrichment cultures that are maintained in our laboratory exhibit some degree of acid production in the presence of ammonia. This phenomenon is not simply a result of the biochemistry of ammonia assimilation, however. Two pathways are available for incorporation of ammonia into amino acids: glutamate dehydrogenase and glutamine synthetase/glutamate synthase [22]. The net reaction for both pathways involves production of glutamate from ammonia and α-ketoglutarate. Neither pathway produces acid. The glutamate dehydrogenase reaction consumes one proton, and the glutamine synthetase/glutamate synthase pathway is acid neutral—a proton is produced in the first step and consumed in the second.

Ammonia-associated acid production could limit the rate and extent of oil biodegradation in environments that have limited buffering capacity and limited dilution effects. Bioremediation of poorly-buffered environments that are contaminated with oil or refined petroleum products will probably be more effective if a nitrate-based fertilizer is used or if sufficient buffering capacity is supplied with the fertilizer mixture.

Our original hypothesis—that nitrification was the cause of the pH changes that we observed—is not supported by our data. A small amount of nitrate was produced in the ammonia-containing cultures, but this could account for only a small fraction of the acid that was produced. Nitrapyrin, an inhibitor of autotrophic nitrification, did not affect the production of nitrate or acid in ammonia-supplemented cultures. Nitrapyrin, which is poorly soluble in water [16], might have partitioned into the oil phase, reducing its effectiveness as an inhibitor of autotrophic nitrifiers that may be present in the oil-degrading enrichment culture. Due to the extremely long generation times that are characteristic of these organisms [18,19], however, it seems unlikely that autotrophic nitrifiers could be present in these oil-degrading enrichment cultures. Formation of nitrate by heterotrophic oxidation of ammonia has been reported [1], and such a process might be responsible for the traces of nitrate that were observed in these cultures.

The results of this research clearly demonstrate that oil biodegradation can be strongly affected by the source of nitrogen provided to support bacterial growth. Ammonia and nitrate each have advantages and disadvantages, and these should be considered when choosing a fertilizer mixture to enhance bioremediation at specific sites. Biodegradation of crude oil begins more quickly when nitrogen is supplied as ammonia than when it is supplied as nitrate. Ammonia utilization, however, can be accompanied by acid production, and under some conditions this acidity can inhibit the rate of oil biodegradation or cause it to cease entirely. Thus, successful bioremediation of oil spills might depend as much on supplying an appropriate nitrogen source as it does on supplying enough nitrogen.

ACKNOWLEDGEMENTS

We are grateful to Mr Charles Jones and Ms Edith Holder for excellent technical assistance.

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