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Hydrocarbon Biodegradation in Utah's Great Salt Lake

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Abstract: The Great Salt Lake comprises two high salinity arms, the North at 34% salinity, and the larger South at 16%. The biodegradation of gasoline range alkanes, cycloalkanes, aromatics, alkenes and cycloalkenes was extensive in samples from both arms, although slower than in fresh- and sea-water. Less volatile hydrocarbons in weathered crude oil were degraded less extensively, and again more slowly than in sea or fresh-water. The substrates subject to degradation are substantially more diverse than has previously been reported, and indicate that biodegradation will likely be the eventual fate of any petroleum hydrocarbons that enter the lake and do not evaporate. The biodegradation is, however, much slower than in other environments, and we discuss whether it might be increased to meet anthropogenic pollution, perhaps by nutrient supplementation with organic nitrogen.

Keywords: salinity; biodegradation; gasoline; crude oil; primary biodegradation

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1. Introduction

Utah's Great Salt Lake is the largest saltwater lake in the western hemisphere, typically varying in area from 3000 to 6000 km^2 [1]. It is quite shallow (average 4–6 m), and since the completion of a railway causeway in 1959, has been effectively two lakes with very different salinities. This distinction is readily seen from the air, since the North Arm is routinely faintly purple due to archaeal *Halobacteriaceae* populations, while the South Arm is greenish due to algal, principally *Dunaliella*, blooms [2]. A red form of *Dunaliella* may also contribute to the color of the North Arm [3]. We measured the salinity of the North Arm to be 34% solids in July 2016, while the Southern part was 16% solids. A new 50 m opening in the causeway was installed in December 2016, and the enormous difference in salinity is likely to gradually disappear; salinity before the causeway was built was about 22% [4]. Seawater, of course, is about 3% salinity.

Despite the lake's proximity to Salt Lake City, with its plethora of oil driven transport, the frequent train traffic across the causeway, a small recreational harbor with sail and motor boats at the southern end of the lake, and limited oil seeps in the North Arm [5,6], little work has been done on the potential fate of any hydrocarbons that might get into the lake [7]. Ward and Brock [8] concluded that salinity severely inhibited the biodegradation of hexadecane, even in samples from the highly saline North Arm where there are small heavy oil seeps [5,6]. They could not enrich hydrocarbon-degrading consortia at salinities above about 20%. Nevertheless, Sei and Fathepure [9] isolated an enrichment culture from the North Arm that was able to grow on benzene and toluene, although not on xylenes, at salinities approximating the North Arm.

We have investigated the ability of the indigenous microbiota in water from both the northern and southern parts of the lake to degrade trace levels of a broad range of hydrocarbons under close to natural aerobic conditions. We used lake water, with its indigenous microbes and natural levels of nutrients [10], which are at least two orders of magnitude lower than those used by Ward and Brock [8] and Sei and Fathepure [9]. We carried out two parallel sets of experiments, one with ~75 ppm unleaded gasoline in small sealed vials that were analyzed for volatile hydrocarbons (C_4 to ~C_{12}), and the

other with 2.5 ppm weathered crude oil in large open bottles that were analyzed for larger hydrocarbons (C_{15} to ~C_{35}). These mixtures span the potential pollutants that might enter the lake, and allow potential co-metabolism such as that identified by Sei and Fathepure [9], who reported that ethylbenzene biodegradation required the presence of benzene. We emphasize that these experiments were designed to understand the background biodegradation capabilities of the indigenous microbiota in the lake. Future experiments might attempt to stimulate this process, perhaps by adding nutrients to oiled shorelines as has proven effective at sea [11].

2. Materials and Methods

Water was collected from the Southern Arm of the Great Salt Lake at the marina at Magna, UT (40.73 N, −112.21 W), and from the Northern Arm near the Spiral Jetty (41.44 N, −112.67 W) in July 2016. Approximately 50 L were collected at each site into polyethylene containers, and shipped overnight to ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA, where the experiments were conducted. The water was aerated gently for 1–2 days before being decanted into the experimental vessels, and was subject to 16 h light: 8 h dark days throughout at 21 °C, appropriate for summer conditions in the lake.

Replicate samples for volatile analytes were prepared with 10 mL of lake water in a 40 mL VOA vial, and ~0.75 µL of gasoline was added [12]. The sealed vials were incubated with gentle horizontal rotation (5 rpm) until analyzed, in duplicate, by purge-and-trap GC/MS at 7, 11, 28 and 90 days [12]. Individual hydrocarbons (Table 1) were identified from known standards [13].

Samples for weathered crude oil experiments were carried out in 5 L Kimax bottles with 4 L of water and ~10 µL of a European crude oil (initial API Gravity 32.7°) that had been artificially weathered to have lost hydrocarbons $<C_{13}$ by evaporation (20% weight loss [14]). After addition of the oil, the bottles were transiently capped with a Teflon sheet and shaken vigorously to disperse the oil. A stir bar was then added, the bottles loosely capped, and stirring maintained at about 100 rpm, which generated a ~1 cm vortex at the surface. Evaporation was replaced by the occasional addition of deionized water (~10%) to maintain constant volumes. Immediately on assembly, and at 9, 16, 28, 39 and 60 days, duplicate replicates were sacrificed by the addition of methylene chloride, and the oil was extracted. After drying and concentrating to ~10 µL/mL, the oils were analyzed by GC/MS [14]. The hydrocarbon concentrations were quite different in the two types of experiments because of analytical detection limits; the gasoline was nominally present at 75 ppm by volume, although much was in the gas phase until the material dissolved in the aqueous phase had been degraded. The weathered crude oil was initially present at 2.5 ppm, and was open to the air so that some material may have been lost by evaporation, which is somewhat enhanced at high salinities [15].

In both cases, primary biodegradation was identified and quantified as the preferential loss of individual hydrocarbons with respect to a potentially conserved molecule within the hydrocarbon mixtures. We used 2,2,4-trimethylpentane (*iso*-octane) in the gasoline [12], and hopane [14] in the crude oil. If either of these molecules disappeared in the incubations, potentially by biodegradation, our estimates of biodegradation would be underestimates. 2,2,4-trimethylpentane (*iso*-octane) is one of the last molecules in gasoline to be degraded in fresh and seawater, but it is eventually consumed (median half-life in freshwater and seawater 8.4 days, [12]); we did not detect biodegradation in the experiments reported here, even after 90 days.

Triplicate 10 mL water samples from both parts of the lake were dried at the beginning of the experiment to ascertain the solids content. After initial drying at 60 °C, final drying was achieved overnight at 110 °C.

Table 1. The biodegradation of gasoline hydrocarbons in 90 days.

Aromatics	% Consumed in 90 Days South Arm	% Consumed in 90 Days North Arm	Linear and *iso*-Alkanes	% Consumed in 90 Days South Arm	% Consumed in 90 Days North Arm
benzene	100	60	butane	35	25
toluene	100	81	pentane	100	22
ethylbenzene	100	83	hexane	100	31
p-xylene	100	97	heptane	100	43
m-xylene	100	90	octane	100	63
o-xylene	100	74	nonane	100	91
butyl benzene	100	97	decane	100	100
propylbenzene	100	87	undecane	100	100
isopropylbenzene	100	51	dodecane	100	100
1-ethyl-2-methylbenzene	68	63	2-methylbutane	9	5
1-ethyl-3-methylbenzene	100	89	2-methylpentane	40	14
1-ethyl-4-methylbenzene	100	97	3-methylpentane	37	11
1,2,3-trimethylbenzene	100	75	2-methylhexane	95	15
1,2,4-trimethylbenzene	100	91	3-methylhexane	79	13
1,3,5-trimethylbenzene	73	70	2-methylheptane	100	21
(1-methylpropyl) benzene	87	47	3-methylheptane	94	15
(2-methylpropyl) benzene	100	56	3-methyloctane	100	37
1,2-diethylbenzene	74	69	2-methylnonane	100	84
1,4-diethylbenzene	97	80	3-methylnonane	100	74
1-methyl-2-propylbenzene	70	69	4-methylnonane	100	62
1-methyl-3-propylbenzene	99	91	5-methylnonane	99	34
1-methyl-4-propylbenzene	100	97	2,2-dimethylpentane	6	14
1-methyl-2(1-methylethyl) benzene	48	44	2,3-dimethylpentane	19	12
1-methyl-3(1-methylethyl) benzene	77	66	2,3-dimethylpentane	14	9
1-methyl-4(1-methylethyl) benzene	100	77	3,3-dimethylpentane	23	18
1-ethyl-3,5-dimethylbenzene	68	67	2,4-dimethylpentane	37	14
2-ethyl-1,3-dimethylbenzene	62	61	2,4-dimethylhexane	44	6
1,2-dimethyl-4-ethylbenzene	89	85	2,5 dimethylhexane	81	9
1,4-dimethyl-2-ethylbenzene	98	79	2,5-dimethylheptane	100	18
2,4-dimethyl-1-ethylbenzene	97	86	2,6-dimethylheptane	85	21
1,2,3,4-tetramethylbenzene	74	79	3,5-dimethylheptane	100	18
1,2,3,5-tetramethylbenzene	75	77	2,5-dimethyloctane	99	38
1,2,4,5-tetramethylbenzene	81	83	2,6-dimethyloctane	99	42
naphthalene	100	94	2,2,3-trimethylpentane	11	2
1-methylnaphthalene	97	77	2,3,3-trimethylpentane	9	1
2-methylnaphthalene	100	97	2,3,4-trimethylpentane	10	3
tetralin	77	76	**Cyclic Alkanes**		
indan	97	96	methylcyclopentane	20	4
1-methylindan	93	92	*cis*1,3-dimethylcyclopentane	29	11
4-methylindan	99	99	*trans*1,3-dimethylcyclopentane	9	4
5-methylindan	91	96	cyclohexane	9	2
1,2,3,4-tetramethylbenzene	74	79	methylcyclohexane	17	0
1,2,3,5-tetramethylbenzene	75	77	ethylcyclohexane	76	9
1,2,4,5-tetramethylbenzene	81	83	1,1-dimethylcyclohexane	0	0
naphthalene	100	94	*cis*1,2-dimethylcyclohexane	11	2
1-methylnaphthalene	97	77	*trans*1,2-dimethylcyclohexane	6	0
2-methylnaphthalene	100	97	*cis*1,3-dimethylcyclohexane	7	0
tetralin	77	76	*trans*1,3-dimethylcyclohexane	11	0
indan	97	96	propylcyclohexane	100	34
1-methylindan	93	92	butylcyclohexane	100	49
4-methylindan	99	99	**Linear and *iso*-Alkenes**		
5-methylindan	91	96	2-methyl-1-butene	10	12
			2-methyl-2-butene	40	26
Cyclic Alkenes			*cis*2-pentene	95	44
cyclopentene	52	22	*trans*2-pentene	84	33
1-methylcyclopentene	71	34	3-methyl-2-pentene	15	13
3-methylcyclopentene	16	9	*cis*2-hexene	69	25
4-methylcyclopentene	14	7	*trans*2-hexene	100	37
1-ethylcyclopentene	81	12	*cis*3-hexene	90	41

3. Results

3.1. Salinity of the North and South Arm of the Great Salt Lake

As expected, water from the southern end of the lake was much more saline than seawater—16% solids. Water from the North Arm was close to saturated, since large balls of crystals could be seen in the shallow water where samples were collected—we measured 34% solids. Some crystals formed in the North Arm incubations during the oil incubations, despite periodic replacement of evaporation with deionized water.

3.2. Gasoline Degradation

Figure 1A shows representative total ion chromatograms of the gasoline before and after 90 days of incubation in water from the North and South Arms, while Figure 1B shows that the apparent half-life of the gasoline was about 60 days in the South Arm (16% salinity), and more than 100 days in the saturated conditions (34% solids) of the North Arm.

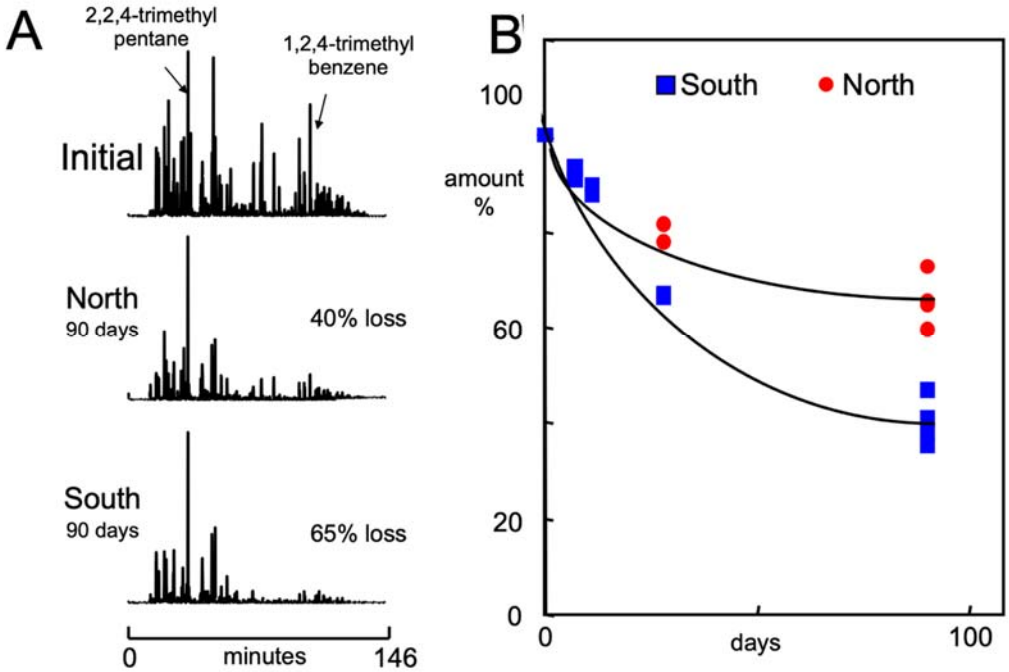

Figure 1. Purge-and-trap total ion chromatograms of the initial gasoline, and of gasoline exposed to water from the South and North Arms of the Great Salt Lake for 90 days of incubation (**A**). The chromatograms are normalized to 2,2,4-trimethylpentane (*iso*-octane), and the lines in panel (**B**) are to guide the eye.

Although biodegradation was by no means as rapid or as complete as that seen in fresh and seawater, where the apparent half-life was 5 days under otherwise identical experimental conditions [12], it was extensive. The losses of individual compounds after 90 days are listed in Table 1, and here we graphically present some of the data. Figure 2A shows that the BTEX (benzene, toluene, ethylbenzene and the xylenes) were fully consumed in the sample from the South Arm, and more than 50% consumed in the sample from the North Arm, in 90 days. We note that Sei and Fathepure [9] saw no degradation of the xylenes in their experiments with enrichment cultures that had been enriched for several transfers with benzene as the only carbon source, but they are clearly lost in Figure 2A. Figure 2B shows chromatograms of the C_3-benzenes in the same samples. Again, biodegradation was extensive in both samples. Note that the most volatile compound in this group (*iso*-propylbenzene) has been conserved in the North Arm incubation, indicating that the loss of the other compounds cannot be ascribed to evaporation from a poorly sealed vial. Similarly, the obvious isomer specificity of the losses indicates this must be biodegradation. Isomer

specificity is further explored in Table 1, which we emphasize is a snapshot in time; we confidently expect that biodegradation of compounds identified as having some loss in our experiment would likely be complete in a longer period.

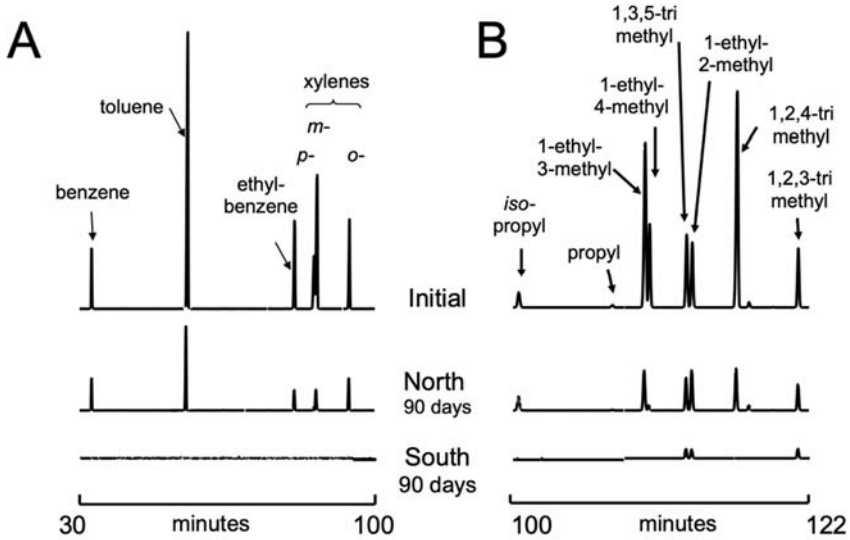

Figure 2. Biodegradation of BTEX (**A**) and the C3-benzenes (**B**) in gasoline in water from the South and North Arms of the Great Salt Lake after 90 days of incubation. Panel (**A**) shows the chromatograms of ions (m/z) 78 (benzene) and 91 (toluene and the others), while Panel (**B**) shows the chromatograms for m/z = 105.

Gasoline alkanes were also extensively degraded in water from both arms of the lake, and again rather more extensively in the South Arm; Figure 3 shows an example of the data, normalized to 2,2,4-trimethylpentane. Figure 4A shows the loss of *n*-alkanes, while 4B shows that branched alkanes are degraded less effectively as branching increases. The loss of trimethylpentanes in this figure is close to the resolution of the analysis, which is based on the conservation of 2,2,4-trimethylpentane, but the data are consistent with earlier work that the other trimethylpentanes were degraded slightly faster than the 2,2,4-isomer in fresh- and sea-water [12]. Cyclic alkanes were degraded in both Arms of the Lake (Table 1), although we saw no evidence for the degradation of 1,1-dimethylcyclohexane, which was amongst the more recalcitrant hydrocarbons in our work with fresh- and sea-water [12]. It is notable that this compound contains a tertiary carbon.

Alkenes are not significantly present in crude oils [16], but they are generated in the refining of gasoline, and they are degraded in fresh- and sea-water [12]. Even the most recalcitrant cyclic compounds, such as the methylcyclopentenes (Figure 5), were degraded here, again showing pronounced isomer specificity (Table 1). Surprising complexities are apparent; for example, *cis*2-pentene seems to be degraded more rapidly than the *trans* isomer, while *cis*2-hexene is degraded subtly more slowly than its *trans* isomer.

3.3. Crude Oil Degradation

The biodegradation of crude oil in the sea has been extensively studied for many years, and it is now clear that the surface area of the oil is a major determinant of the rate of biodegradation. Providing the oil is dispersed as small droplets (<100 μm), the biodegradation has an apparent half-life of 10–20 days in the sea [14,17–19]. Biodegradation was somewhat slower in water from the South Arm of the Great Salt Lake (apparent half-life about 30 days) but much slower in the almost saturated North Arm, where only some 20% had been lost in 66 days (Figure 6). Alkane degradation was significantly retarded in the North Arm, and there was very little loss of the three and four ring polycyclic aromatic hydrocarbons detectable by GC/MS in either part of the lake (Table 2). The loss of the naphthalenes and fluorenes may include significant evaporation, but nevertheless there

was greater loss from the lower salinity South Arm, giving some confidence that at least part of the loss was biodegradation. Naphthalene biodegradation was clearly seen in the sealed experiments with gasoline (Table 1).

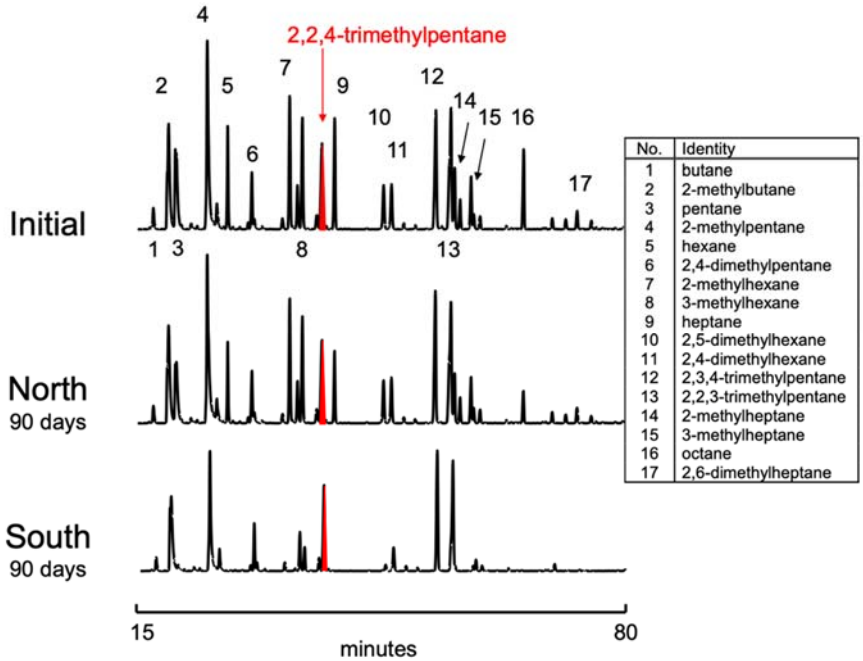

Figure 3. Purge-and-trap selected ion monitoring (m/z = 43) chromatograms of C_4 to C_8 alkanes in the initial gasoline, and of gasoline exposed to water from the South and North Arms of the Great Salt Lake for 90 days of incubation. The chromatograms are normalized to 2,2,4-trimethylpentane (*iso*-octane), shown in red, and the principal components are identified.

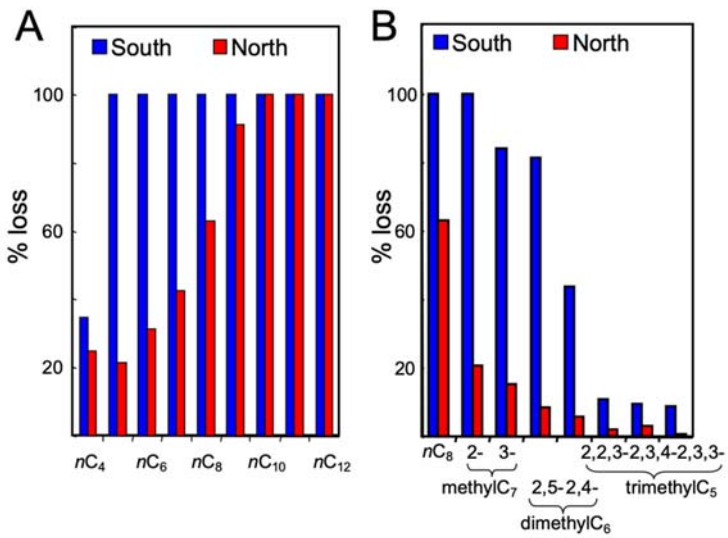

Figure 4. Biodegradation of the *n*-alkanes (**A**) and *iso*-octanes (**B**) in gasoline in water from the South and North Arms of the Great Salt Lake after 90 days of incubation. Panel (**B**) shows the detected isomers of octane.

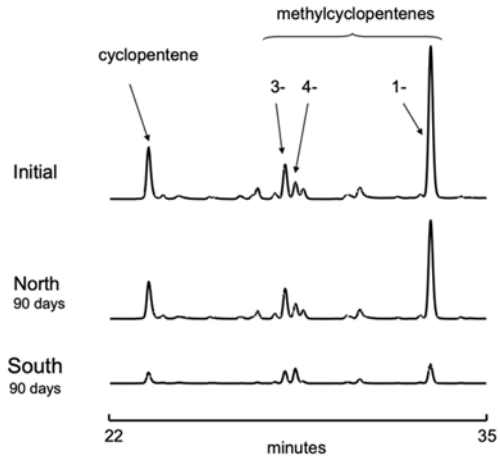

Figure 5. Purge-and-trap selected ion monitoring (m/z = 67) chromatograms of cyclopentenes in the initial gasoline, and of gasoline exposed to water from the South and North Arms of the Great Salt Lake for 90 days of incubation. The chromatograms are normalized to 2,2,4-trimethylpentane (*iso*-octane).

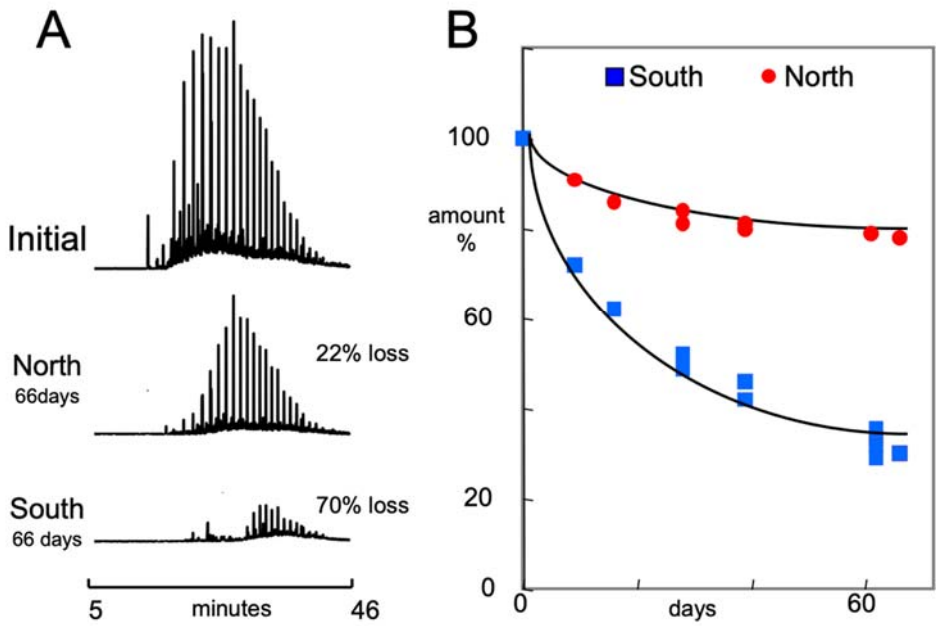

Figure 6. Total ion chromatograms of the initial crude oil, and of the oil exposed to water from the South and North Arms of the Great Salt Lake for 66 days of incubation (**A**). The chromatograms are normalized to hopane [14], and the lines in panel (**B**) are to guide the eye.

The Cn nomenclature indicates the number of pendant carbons on the aromatic nucleus. For example, C2-fluorenes includes all the dimethyl- and ethyl-fluorenes.

Table 2. The biodegradation of crude oil hydrocarbons in 66 days.

Aromatics	% Consumed in 66 Days South Arm	% Consumed in 66 Days North Arm	Linear Alkanes	% Consumed in 66 Days South Arm	% Consumed in 66 Days North Arm
naphthalene	100	100	tetradecane	100	100
C1-naphthalenes	100	100	pentadecane	100	86
C2-naphthalenes	100	90	hexadecane	100	86
C3-naphthalenes	100	70	heptadecane	100	85
C4-naphthalenes	100	50	octadecane	100	75
fluorene	100	25	nonadecane	100	66
1-methylfluorene	60	0	icosane	100	57
2/3-methylfluorene	80	0	henicosane	100	5
4-methylfluorene	80	0	docosane	99	5
9-methylfluorene	80	0	tricosane	95	2
C2-fluorenes	40	0	tetracosane	90	1
C3-fluorenes	25	0	pentacosane	86	2
phenanthrene	30	10	hexacosane	83	0
1-methylphenanthrene	15	7	heptacosane	81	0
2-methylphenanthrene	15	7	octacosane	80	0
3-methylphenanthrene	20	10	nonacosane	79	2
9-methylphenanthrene	20	12	triacontane	79	1
C2-phenanthrenes	0	0	hentriacontane	78	0
C3-phenanthrenes	0	0	dotriacontane	76	3
C4-phenanthrenes	0	0	tritriacontane	66	0
benz[a]anthracene	20	10	tetratriacontane	34	1
chrysene	10	0			
1-methylchrysene	5	0	***iso*-Alkanes**		
2-methylchrysene	5	0	pristane	99	67
3-methylchrysene	0	0	phytane	95	63
4/6-methylchrysene	5	0			
C2-chrysenes	0	0			

4. Discussion

Oil spill responders have given considerable thought on to how to respond to significant oil spills on inland lakes, and rely on microbes to remove residual hydrocarbons that are not collected or burned [20]. Uncollected oils are potentially subject to biodegradation and photooxidation, and these processes show very different preferences for hydrocarbon removal [21]. Biodegradation prefers small and less alkylated hydrocarbons, with a preference for *n*-alkanes, while photo-oxidation shows almost the opposite preferences, removing larger and more alkylated aromatic hydrocarbons before smaller aromatic species, and showing only minimal activity towards *n*-alkanes [22,23]. The losses seen here show no evidence for photo-oxidation, but many of the specificities expected for biodegradation. This is particularly evident for the larger aromatics, such as chrysene and phenanthrene, which show minimal loss in samples that have lost most of their alkanes (Figure 6 and Table 2). We thus attribute all the losses here to biodegradation, and discuss them in that light. We note, however, that our laboratory studies were aimed at studying biodegradation, and run at far lower light intensities than spilled oil might encounter on the surface of the Great Salt Lake. It is very likely that photo-oxidation will contribute to the weathering of spilled oils and fuels on the Lake.

Our results show that hydrocarbon biodegradation in Utah's Great Salt Lake extends to far more classes than previously reported [8,9] in both the South Arm at 16% salinity and the North Arm at 34% salinity. Aromatics, alkanes, cycloalkanes, alkenes and cycloalkenes all showed evidence of at least significant, if not complete, primary biodegradation in 90 days (Table 1). Larger aromatics, such as phenanthrene and chrysene were more recalcitrant (Table 2), but degradation is likely the eventual fate of most of the hydrocarbons emanating from the seeps reported by Bortz [5] and Sinninghe Damsté et al. [6].

We have recently reviewed hydrocarbon biodegradation in salinities significantly above seawater [7]. Several genera of bacteria contain species that have been shown to degrade hydrocarbons at greater than 10% salinity (*Achromobacter*, *Acinetobacter*, *Actinopolyspora*, *Alcanivorax*, *Bacillus*, *Cellulomonas*, *Delftia*, *Dietzia*, *Exiguobacterium*, *Geobacillus*, *Gordonia*, *Halomonas*, *Marinobacter*, *Mesorhizobium*, *Salinisphaera*, *Ochrobactrum*, *Pseudomonas*, *Rhodococcus*, *Stenotrophomonas*, *Streptomyces* and *Thalassospira*, see [7]) but few of these have been formally identified in the Great Salt Lake. Haws [24] found *Bacillus* and *Pseudomonas* in the North Arm, and noted that earlier studies had found *Achromobacter.* Almeida-Dalmet and Baxter [25] found *Halomonas* in the North Arm, and Meuser et al. [2] found a *Marinobacter*, a *Rhodococcus* and a *Streptomyces* in the South Arm. Similarly, Tazi et al. [26] found 16S rRNA sequences that clustered with environmental taxa known to degrade hydrocarbons: *Shewanella*, *Halomonas*, *Idiomarina*, *Alcanivorax*, *Pseudomonas* and *Marinobacter*. To our knowledge, no single organism from the Great Salt Lake has yet been shown to grow on hydrocarbons as sole carbon source.

The most abundant bacterial genus in the North Arm seems to be *Salinibacter* [25], which has been associated with oil contamination of hypersaline waters from a pond in France [27], but it is not yet clear whether the organism can degrade hydrocarbons [27].

Several genera of archaea contain species shown to degrade hydrocarbons at greater than 20% salinity; *Haloarcula*, *Halobacterium*, *Haloferax*, *Halorubrum*, *Halovivax* and *Natrialba* [7,28], and members of all have been found in the North Arm [2,25,26,29,30].

Some fungi are also able to grow on hydrocarbons as sole carbon source, and many more can degrade aromatic hydrocarbons [31]. Baxter and Zalar [30] have isolated members of 11 Ascomycete genera from the North and South Arm of the Great Salt Lake, and several of these (*Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*) contain known hydrocarbon degraders [31]. We note, however, that the importance of fungal degradation of hydrocarbons in aquatic environments is not well understood.

As expected from the pioneering work of Ward and Brock [8], the biodegradation reported here was much slower than in fresh- and salt-water [12,14], and even slower than biodegradation in experiments with concentrated seawater at 16% salt [15]. Nevertheless, the biodegradation in the Great Salt Lake follows a similar pattern to that seen in fresh- and sea- water: increased branching of alkanes slows biodegradation, tertiary carbons slow biodegradation, smaller aromatics are degraded preferentially to larger ones, and biodegradation shows significant isomer specificity—even in the alkanes (compare the four isomers of methylnonane in Table 1), but especially in the aromatics (Figure 2A,B and Table 1). We have previously shown that different freshwater bacterial species show specific preferences for the different isomers of the C3-benzenes [32], but that together they completely consume them all, albeit with subtly different kinetics. The isomer specificity means that a partially degraded product taken from the environment has a chemical composition quite different from the initial spill, posing potential complications for forensic identification [33]. The biomarkers in crude oils, such as the hopanes, are more recalcitrant than any of the hydrocarbons typically analyzed in gasoline, and provide a robust fingerprint for identifying the source oil of a spill [34].

It is not clear why biodegradation in the Great Salt Lake is so slow. The concept of bioremediation is that hydrocarbon-degrading microbes are ubiquitous although likely at low concentrations in the absence of hydrocarbon foodstuffs [35]. The arrival of hydrocarbons from a seep or an anthropogenic source gives these organisms a preferred substrate, and their number increases as they consume the substrate, and the substrate is converted to biomass and CO_2 [35]. Quite likely the extreme salinity of the Lake exerts many challenges to microbes that grow there, especially in the North Arm. The salinity we measured in the North Arm (340 g/L) corresponds to 4.6 M NaCl, 0.2 M KCl and 0.5 M $MgSO_4$ [36], with an ionic strength of 6.4 M. The sample from the marina in the South Arm was approximately half this concentration (Seawater is 0.7 M). Such high salinities significantly limit oxygen dissolution, such that oxygen saturation in the North Arm is only around one third the level it is in seawater, while that in the South Arm is about

one half [37]. The high salinities also affect hydrocarbon solubility. Most hydrocarbons are only very poorly soluble in water and this is further decreased with increasing ionic strength [38]. Perhaps counterintuitively, the decreased solubility at high salinity causes increased volatility due to Henry's Law, and volatile loss of hydrocarbons from our open system was likely a small contributor to total hydrocarbon loss in the open experiments [15]. Microbes degrading gasoline hydrocarbons in our sealed vials (which contained more than enough oxygen for the complete mineralization of the added gasoline [12]) may thus have been hindered by the low solubility of both oxygen and hydrocarbons. Nevertheless, this seems an unlikely sole explanation of the slow biodegradation—for example the *Halovivax* and *Haloarcula* isolated by Kebbouche-Gana et al. [28] grew on diesel as a sole carbon source with a doubling time of much less than 1 day in a medium of similar ionic strength (4.2 M), although with casamino acids in abundance as a potential nitrogen source (1 g/L).

Hydrocarbons are energy-rich substrates, but they lack the other elements required for microbial growth, which must be provided by the environment. The Great Salt Lake is severely limited in both nitrate and ammonium. Post [39] reported from his studies of the North Arm from 1975–1977 that "ammonia varied considerably being undetectable about half the time. Nitrates and nitrites were not detected. Organic nitrogen was plentiful averaging about 8 mg L^{-1} N (0.6 mM) over several years and was fairly constant"; "phosphate was plentiful". Nitrate (summer 0.7 μM) and ammonium (summer 9 μM) were detectable in the Southern Arm [10], but Marcarelli et al. [40] found that algal growth in the area was nitrogen limited, and that cyanobacterial nitrogen fixation was inhibited at salinities above 7%. Thus inorganic nitrogen limitation may be one reason for the slow biodegradation seen here, when compared to freshwater and marine environments [12,14,17–19]. Nevertheless, most halophilic Archaea, the principal organisms in the North Arm [25,26,29,30], are said to grow best on organic nitrogen, and all the recommended ATCC media use casamino acids, yeast extract, tryptone, casein or glutamate as the recommended nitrogen source [41]. It is noteworthy that Corsellis et al. [27] found that organic nitrogen, in their case casamino acids, substantially increased the growth of the bacterium *Salinibacter* at 30.8% salinity.

Perhaps some other element is limiting growth, although this seems unlikely given the diverse elements in the brine [36]. Perhaps there are inhibitory compounds, but again this seems unlikely given the reasonable primary productivity associated with the lake; 2.13 g C m^{-2} d^{-1} [10] which is very similar to that measured in Lake Erie [42].

Understanding whether there are additives, perhaps organic nitrogen, that could stimulate hydrocarbon biodegradation under high salinity conditions would be a valuable contribution to oil spill response should the need ever arise.

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References

1. Mohammed, I.N.; Tarboton, D.G. An examination of the sensitivity of the Great Salt Lake to changes in inputs. *Water Res. Res.* **2012**, *48*, W11511. [CrossRef]
2. Meuser, J.E.; Baxter, B.K.; Spear, J.R.; Peters, J.W.; Posewitz, M.C.; Boyd, E.S. Contrasting patterns of community assembly in the stratified water column of Great Salt Lake, Utah. *Microb. Ecol.* **2013**, *66*, 268–280. [CrossRef] [PubMed]
3. Brock, T.D. Salinity and the ecology of *Dunaliella* from Great Salt Lake. *J. Gen. Microbiol.* **1975**, *89*, 285–292. [CrossRef]
4. Post, F.J. The microbial ecology of the Great Salt Lake. *Microb. Ecol.* **1977**, *3*, 143–165. [CrossRef] [PubMed]
5. Bortz, L.C. Heavy-Oil Deposit, Great Salt Lake, Utah: Section V. Exploration Histories. In *SG 25: Exploration for Heavy Crude Oil and Natural Bitumen*; AAPG Special issue; AAPG: Tulsa, OK, USA, 1987; pp. 555–563.

6. Sinninghe Damsté, J.S.; De Leeuw, J.W.; Kock-van Dalen, A.C.; De Zeeuw, M.A.; De Lange, F.; Irene, W.; Rijpstra, C.; Schenck, P.A. The occurrence and identification of series of organic sulphur compounds in oils and sediment extracts. I. A study of Rozel Point Oil (USA). *Geochim. Cosmochim. Acta* **1987**, *51*, 2369–2391. [CrossRef]
7. Abou Khalil, C.; Prince, V.L.; Prince, R.C.; Greer, C.W.; Lee, K.; Zhang, B.; Boufadel, M.C. Occurrence and biodegradation of hydrocarbons at high salinities. *Sci. Total Environ.* **2021**, *762*, 143165. [CrossRef] [PubMed]
8. Ward, D.M.; Brock, T.D. Hydrocarbon biodegradation in hypersaline environments. *Appl. Environ. Microbiol.* **1978**, *35*, 353–359. [CrossRef]
9. Sei, A.; Fathepure, B.Z. Biodegradation of BTEX at high salinity by an enrichment culture from hypersaline sediments of Rozel Point at Great Salt Lake. *J. Appl. Microbiol.* **2009**, *107*, 2001–2008. [CrossRef]
10. Stephens, D.W.; Gillespie, D.M. Phytoplankton production in the Great Salt Lake, Utah, and a laboratory study of algal response to enrichment 1. *Limnol. Oceanogr.* **1978**, *21*, 74–87. [CrossRef]
11. Bragg, J.R.; Prince, R.C.; Harner, E.J.; Atlas, R.M. Effectiveness of bioremediation for the *Exxon Valdez* oil spill. *Nature* **1994**, *368*, 413–418. [CrossRef]
12. Prince, R.C.; Parkerton, T.F.; Lee, C. The primary aerobic biodegradation of gasoline hydrocarbons. *Environ. Sci. Technol.* **2007**, *41*, 3316–3321. [CrossRef] [PubMed]
13. Uhler, R.M.; Healey, E.M.; McCarthy, K.J.; Uhler, A.D.; Stout, S.A. Molecular fingerprinting of gasoline by a modified EPA 8260 gas chromatography-mass spectrometry method. *Int. J. Environ. Anal. Chem.* **2003**, *83*, 1–20. [CrossRef]
14. Prince, R.C.; Butler, J.D.; Redman, A.D. The rate of crude oil biodegradation in the sea. *Environ. Sci. Technol.* **2017**, *51*, 1278–1284. [CrossRef] [PubMed]
15. Abou Khalil, C.; Fortin, N.; Prince, R.C.; Greer, C.W.; Lee, K.; Boufadel, M.C. Crude oil biodegradation in upper and supratidal seashores. *J. Hazard. Mater.* **2021**, *416*, 125919. [CrossRef] [PubMed]
16. Tissot, B.P.; Welte, D.H. *Petroleum Formation and Occurrence*; Springer: Berlin/Heidelberg, Germany, 1984. [CrossRef]
17. Prince, R.C.; McFarlin, K.M.; Butler, J.D.; Febbo, E.J.; Wang, F.C.; Nedwed, T.J. The primary biodegradation of dispersed crude oil in the sea. *Chemosphere* **2013**, *90*, 521–526. [CrossRef]
18. Brakstad, O.G.; Nordtug, T.; Throne-Holst, M. Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Mar. Pollut. Bull.* **2015**, *93*, 144–152. [CrossRef]
19. Wang, J.; Sandoval, K.; Ding, Y.; Stoeckel, D.; Minard-Smith, A.; Andersen, G.; Dubinsky, E.A.; Atlas, R.; Gardinali, P. Biodegradation of dispersed Macondo crude oil by indigenous Gulf of Mexico microbial communities. *Sci. Total Environ.* **2016**, *557*, 453–468. [CrossRef]
20. Owens, E.H.; Taylor, E.; Marty, R.; Little, D.I. An inland oil spill response manual to minimize adverse environmental impacts. *Proc. Int. Oil Spill Conf.* **1993**, 105–109. [CrossRef]
21. Prince, R.C.; Garrett, R.M.; Bare, R.E.; Grossman, M.J.; Townsend, T.; Suflita, J.M.; Lee, K.; Owens, E.H.; Sergy, G.A.; Braddock, J.F.; et al. The roles of photooxidation and biodegradation in long-term weathering of crude and heavy fuel oils. *Spill Sci. Technol. Bull.* **2003**, *8*, 145–156. [CrossRef]
22. Garrett, R.M.; Pickering, I.J.; Haith, C.E.; Prince, R.C. Photooxidation of crude oils. *Environ. Sci. Technol.* **1998**, *32*, 3719–3723. [CrossRef]
23. Aeppli, C.; Mitchell, D.A.; Keyes, P.; Beirne, E.C.; McFarlin, K.M.; Roman-Hubers, A.T.; Rusyn, I.; Prince, R.C.; Zhao, L.; Parkerton, T.F.; et al. Oil irradiation experiments document changes in oil properties, molecular composition, and dispersant effectiveness associated with oil photo-oxidation. *Environ. Sci. Technol.* **2022**, *56*, 7789–7799. [CrossRef] [PubMed]
24. Haws, E.S. Ecology of culturable organisms at Rozel Point, Great Salt Lake, Utah. Master's Thesis, Brigham Young University, Provo, UT, USA, 2007. Available online: https://scholarsarchive.byu.edu/etd/857 (accessed on 21 July 2022).
25. Almeida-Dalmet, S.; Baxter, B.K. Unexpected complexity at salinity saturation: Microbial diversity of the North Arm of Great Salt Lake. In *Great Salt Lake Biology*; Baxter, B., Butler, J., Eds.; Springer: Berlin/Heidelberg, Germany, 2020; pp. 119–144. [CrossRef]
26. Tazi, L.; Breakwell, D.P.; Harker, A.R.; Crandall, K.A. Life in extreme environments: Microbial diversity in Great Salt Lake, Utah. *Extremophiles* **2014**, *18*, 525–535. [CrossRef] [PubMed]
27. Corsellis, Y.Y.; Krasovec, M.M.; Sylvi, L.L.; Cuny, P.P.; Militon, C.C. Oil removal and effects of spilled oil on active microbial communities in close to salt-saturation brines. *Extremophiles* **2016**, *20*, 235–250. [CrossRef]
28. Kebbouche-Gana, S.; Gana, M.L.; Khemili, S.; Fazouane-Naimi, F.; Bouanane, N.A.; Penninckx, M.; Hacene, H. Isolation and characterization of halophilic Archaea able to produce biosurfactants. *J. Ind. Microbiol. Biotechnol.* **2009**, *36*, 727–738. [CrossRef] [PubMed]
29. Baxter, B.K.; Zalar, P. The extremophiles of Great Salt Lake: Complex microbiology in a dynamic hypersaline ecosystem. In *Model Ecosystems in Extreme Environments*; Elsevier Inc.: Amsterdam, The Netherlands, 2019; pp. 57–99. [CrossRef]
30. Bayles, M.; Belasco, B.C.; Breda, A.J.; Cahill, C.B.; Da Silva, A.Z.; Regan, M.J., Jr.; Schlamp, N.K.; Slagle, M.P.; Baxter, B.K. The Haloarchaea of Great Salt Lake as models for potential extant life on Mars. In *Extremophiles as Astrobiological Models*; Seckbach, J., Stan-Lotter, H., Eds.; Wiley Online Library: Hoboken, NJ, USA, 2020; pp. 83–124. [CrossRef]
31. Prince, R.C. *Eukaryotic Hydrocarbon Degraders. InTaxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes. Handbook of Hydrocarbon and Lipid Microbiology*; McGenity, T., Ed.; Springer: Cham, Switzerland, 2019; pp. 53–72. [CrossRef]
32. Prince, R.C.; Walters, C.C. Biodegradation of oil hydrocarbons and its implications for source identification. In *Standard Handbook Oil Spill Environmental Forensics*; Stout, S.A., Wang, Z., Eds.; Academic Press: Cambridge, MA, USA, 2016; pp. 869–916. [CrossRef]

33. Stout, S.A.; Douglas, G.S.; Uhler, A.D. Assessing temporal and spatial variations of gasoline-impacted groundwater using relative mole fractions and PIANO fingerprinting. *Environ. Forensics* **2010**, *11*, 328–341. [CrossRef]
34. Wang, Z.; Stout, S.A.; Fingas, M. Forensic fingerprinting of biomarkers for oil spill characterization and source identification. *Environ. Forensics* **2006**, *7*, 105–146. [CrossRef]
35. National Research Council. *In Situ Bioremediation: When Does It Work?* National Academies Press: Washington, DC, USA, 1993. Available online: https://nap.nationalacademies.org/catalog/2131/in-situ-bioremediation-when-does-it-work (accessed on 21 July 2022).
36. Domagalski, J.L.; Orem, W.H.; Eugster, H.P. Organic geochemistry and brine composition in Great Salt, Mono, and Walker Lakes. *Geochim. Cosmochim. Acta* **1989**, *53*, 2857–2872. [CrossRef]
37. Geng, M.; Duan, Z. Prediction of oxygen solubility in pure water and brines up to high temperatures and pressures. *Geochim. Cosmochim. Acta* **2010**, *74*, 5631–5640. [CrossRef]
38. Burant, A.; Lowry, G.V.; Karamalidis, A.K. Measurement of Setschenow constants for six hydrophobic compounds in simulated brines and use in predictive modeling for oil and gas systems. *Chemosphere* **2016**, *144*, 2247–2256. [CrossRef]
39. Post, F.J. Microbiology of the Great Salt Lake North Arm. *Hydrobiologia* **1981**, *81*, 59–69. [CrossRef]
40. Marcarelli, A.M.; Wurtsbaugh, W.A.; Griset, O. Salinity controls phytoplankton response to nutrient enrichment in the Great Salt Lake, Utah, USA. *Can. J. Fish. Aquat. Sci.* **2006**, *63*, 2236–2248. [CrossRef]
41. ATTC. Microbial Media Formulations. 2019. Available online: https://www.atcc.org/en/Documents/Learning_Center/Microbial_Media_Formulations.aspx (accessed on 21 July 2022).
42. Fitzpatrick, M.A.; Munawar, M.; Leach, J.H.; Haffner, G.D. Factors regulating primary production and phytoplankton dynamics in western Lake Erie. *Fundam. Appl. Limnol.* **2007**, *11*, 137–152. [CrossRef]