Some Chemical and Microbiological Studies of Surtsey

by

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The eruption of the volcano Surtsey provided us with an unusual opportunity to conduct certain investigations of importance to exobiology. The interest was two-fold: chemical and biological. From the chemist's point of view the volcano simulates some of the conditions that may have occurred on the primitive earth during the genesis of organic compounds. While it is to be expected that the composition of the atmosphere has changed since primordial times, the possibility of detecting any abiogenic synthesis from the outgassing of a volcano today would still substantiate the hypothesis of the primordial synthesis of biological molecules. For the biologist, the volcano provided an unusual locale to test techniques which may be eventually used for the detection of life on other planets. A sterile piece of land which begins to be invaded by living organisms provides a rare opportunity to study the phenomenon of biological succession, beginning with very primitive microorganisms and later, higher forms of life. At the same time, methods of detecting extremely small numbers of microorganisms and the presence of unusual types which may survive under very rigorous conditions, can be tested.

With this end in view, Drs. R.S. Young, C. Ponnamperuma, and I. Breger (of the U.S. Geological Survey, Washington, D.C.) participated in a study of the volcano under the auspices of the National Research Council of Iceland, the Surtsey Research Society and the National Aeronautics and Space Administration. On the 4th of October, a party of several investigators landed on Surtsey by helicopter and obtained samples from various locations starting with the crater down to sea level. During the time this

sampling was being done, Syrtlingur was in eruption and fresh uncontaminated ash was collected from the atmosphere fallout.

The following samples were collected:

Sample Number	Description of Sample
1	Dry surface dust collected from a crater fumarole which was protected from the fallout of Syrtlingur; temperatures in the fumarole ranged from 120°C to at least 150°C.
2	Moist dust and a piece of hard granular rock collected around a fumarole in the crater; temperature was about 130°C.
3	Moist sand collected from the crater, where the temperature was slightly over 100°C.
4	Ash collected on the slope on the northeast side of the island, where the surface temperature was 10°C; it is unlikely that this ash was from Syrtlingur since this locale was not open to Syrtlingur and the prevailing wind was in a different direction.
5	Surface dust from the ocean-side of the lagoon on Surtsey, where the temperature was 12°C; sample was probably contaminated with fallout from Syrtlingur and sea spray.
6	(a) Freshly-fallen surface ash collected within 300 yards of Syrtlingur at a depth of about 1 centimeter; sample probably not more than 10 minutes old; sample may have absorbed some atmospheric moisture. (b) Sample collected by Dr. Breger near Syrtlingur in the path of the fallout; falling ash was caught on aluminium foil before it touched the surface of Surtsey; ambient temperature was about 12°C during the hour spent collecting the sample; a strong wind was blowing while the sample was collected.

All samples except 6 (b) were collected aseptically in sterilized metal containers using sterilized implements. Sample 6 (b) was collected on aluminum foil as described above.

The samples were brought back to the Ames Research Center and several studies were conducted: (1) analysis for amino acids; (2) analysis for hydrocarbons; (3) determination of total organic carbon; (4) biological studies.

Section I - Amino Acid Analysis: C. Ponnamperuma, J. Williams and L. Caren

In order to determine the amino acid content of Surtsey samples 1 and 6 (b), extractions were made with water and 6N HCL and analyzed for acidic and neutral amino acids on a amino acid analyzer. A sample of sea water collected near Surtsey was also analyzed for its acidic and neutral amino acid content.

Experimental

Twenty-five g. of ash was refluxed with 150 ml. water in a soxhlet for 40 hours. Another aliquot of 25 g. ash was similarly extracted with 100 ml. 6N HCL for 48 hours. The extract was evaporated to dryness, dissolved in 25 ml. water, evaporated to dryness again, and then added to 4 ml. 0.2N sodium citrate buffer, pH 2.2. Particulate matter was removed by centrifugation.

One ml. of this concentrated extract was applied directly to the amino acid analyzer (Phoenix, Model K8000 VG-B). Another ml. of the concentrated extract was mixed with 200 ul. of a mixture of C^{14}- labelled amino acids which served as internal standards. The effluent from the column of the amino acid analyzer was split into two streams; in one stream, the ninhydrin-positive material was monitered, whereas in the other stream, radioactivity was recorded. Coincidence of the radioactive standards with ninhydrin-positive peaks from the Surtsey sample were used to identify the amino acids present.

Another ml. of the concentrated extract was vacuum-sealed, hydrolyzed at 105^{o}C for 48 hours, evaporated to dryness, neutral-

ized with NH_4OH, filtered, evaporated to dryness, and then dissolved in 0.5 ml. of 0.2N sodium citrate buffer, pH 2.2. The sample was then applied to the amino acid analyzer.

Results

The water extract of the Surtsey ash contained 0.003 uM each of aspartic acid and alanine as well as 0.004 uM each of glycine and serine. The HCl extract contained traces of glycine, serine and alanine. Traces of cystine, valine, and methionine were detected in the sea water collected near Surtsey. The presence or absence of basic amino acids were not assayed.

Discussion

The presence of amino acids in these samples is extremely interesting. However, it must be borne in mind that we cannot directly conclude an abiogenic origin for them. There are a number of possible sources of contamination, such as the sea water which could have rushed into the crater, the possible contamination with sea spray in the atmosphere, and the possible breakdown of organic matter from the earth's crust through which the gasses were being ejected during eruption. However, the presence of only four amino acids seems to suggest some type of abiogenic origin. If the amino acids were a result of contamination, one would expect to see more of those commonly found in natural protein. The suggestion has also been made that on account of the charge separation generated when sea water splashes on molten lava, the tephra particles would remain relatively uncontaminated by the sea water.

The analysis of a sample of sea water equal to the volume of sea water which could have saturated the samples we analyzed did not reveal the presence of the same amino acids found in the Surtsey ash samples. The evidence therefore seems to suggest an abiogenic origin for the amino acids identified in the Surtsey samples. Further investigation and more rigorous controls will be necessary to clarify this point completely.

Section II - Hydrocarbons: C. Ponnamperuma and K. Pering.

Approximately 25 g. of a Surtsey sample were assayed for aliphatic hydrocarbons. The sample was extracted in an all-glass soxhlet apparatus for 6 hours with benzene-methanol. The extract was evaporated to about 0.5 ml. and analyzed by gas chromatography. No aliphatic hydrocarbons were detected.

Section III - Determination of Total Organic Carbon: Richard D. Johnson and Catherine C. Davis

Abstract

Ash samples from Surtsey were analyzed by a new and highly sensitive technique for total organic carbon content. The method is described in detail, and the results are compared with those from other exotic soils.

Introduction

Classical analysis for total organic carbon is based upon the oxidation of the organic material to carbon dioxide followed by gas chromatographic, infrared, gravimetric, acidimetric, turbidimetric, or nephlometric determination of the carbon dioxide. To achieve sensitivities below 1 part per thousand, these techniques generally use large (1 g.) samples with wet "combustion". There are also several completely automated modes of analysis, most often using gas chromatography, which use smaller samples, but which suffer from problems of incomplete oxidation and thus require an intimate mixture of sample and catalyst. On a highly sensitive basis, such analysis can give large blank determinations. The primary drawback to the classical method is in the analysis of soil samples which may contain large amounts of inorganic carbon, primarily carbonates. These samples must first be treated with acid to drive off the inorganic contribution before the oxidation. With solid samples, unless the solid inorganic matrix is completely dissolved by hydrofluoric acid, there will remain small portions of the carbonate which will interfere with subsequent organic determination.

The method herein described is highly sensitive, uses small sample sizes, and is totally immune to inorganic carbonates.

Experiment 1

The apparatus used in the organic carbon determination is shown schematically in Figure 1. The sample of 25 mg. is weighed into small vycor boats, and a series of these boats are then placed into a closed "Y" tube under a helium atmosphere. With a group of magnetically coupled implements, the boats are pushed into a tube furnace at 860°C. The pyrolysis products are then swept in the helium through a small quartz wool scrubber into the jet of a hydrogen flame ionization detector, where the sample in helium is mixed with hydrogen and burned in air. The ion current above the flame, resulting from the burning of organic matter and the resulting chemi-ionization of the organic fragments, is amplified by an electrometer amplifier, and the resulting single peak is integrated with the area being proportional to the organic content of the sample. This apparatus was built from commercially available gas chromatography components (Perkin Elmer pyrolysis unit, Beckman GC-4 flame detector, electrometer, and gas controls). More recent efforts have been to improve instrumental performance while building a small portable field model, which will be described elsewhere.

The detector was operated with 430 cc/min of air, 76 cc/min of hydrogen, and 200 cc/min of helium carrier.

Results and Discussion

In Figure 2 are shown both the calibration data and the results from various soil samples. The pyrolysis under helium occurs with varying results, giving products which are detected with varying efficiencies in the flame detector, depending upon the number and kind of functional groups attached to each carbon atom. Two calibration compounds were used as known amounts mixed into incinerated (1000°C in air) soil. The dextrose represents the case where high oxygen to carbon ratio leads to low detection efficiency, while the benzoic acid represents the case where the efficiency approaches

that for hydrocarbons. The results from unknown samples are then read from the center of the band formed from the calibration curves for these two compounds. At higher carbon contents, the detector response begins to level off, providing an upper analyzable limit of 1 percent organic matter, based upon 25 mg. samples. At the lower end, blank determinations resulting from traces of contamination limit detection at approximately 5 parts per million. In the case where the soils were also analyzed by classical procedures, it was found that any differences between the techniques were in the direction of the classical analysis being too high, or the flame ionization technique being too low. The second alternative is considered to be highly unlikely when compared with the first and the previously discussed problems of trapped inorganic carbonates.

Two Surtsey samples were run with this technique. Sample no. 1, collected directly from a fumarole, gave 50 parts per million of organic carbon. Sample no. 6a, a freshly collected falling ash sample, contained 100 parts per million. Because of the uncertainties associated with detection efficiency of pyrolysis fragments, there is an estimated uncertaintly of approximately $\pm$ 30 percent in all results (the width of the calibration band).

Conclusions

A new technique for the analysis of soil samples for total organic content has been described. The method, based upon the gas chromatographic flame ionization detector, has been used to analyze two Surtsey samples, both of which are considered relatively clean. The samples were found to contain between 50 and 100 parts per million of organic matter.

Section IV - Studies of Surtsey Island Ecology: Edward L. Merek and Richard S. Young

Nine samples of Surtsey material were collected in sterile containers by Dr. R.S. Young. These samples were used (1) to determine whether or not a general heterotrophic microflora has developed on the island, and (2) to define some of the parameters

encountered by any invading organisms.

The heterotrophic population was assayed by plating serial dilutions on Trypticase Soy Broth agar plates. This media was chosen because at that time, highest colony counts from soils had been obtained on this medium. The plates were incubated aerobically at 25°C for three weeks. Results are indicated in Table I.

TABLE I. Colony counts from Surtsey ash

SAMPLE	Counts, number/g. ash BACTERIA	FUNGI
1-1	< 10	< 10
1-2	< 10	< 10
1-3	< 10	< 10
4-1	< 10	700
4-2	< 10	250
4-3	300	3000
5-1[2]	1500	500
5-2	< 10	< 10
5-3[3]	< 10	3700
6	< 10	< 10

1. No colonies developed on plates sprinkled with .1 g of ash.
2. Cans accidently dented enroute - seal broken

Chemical analyses, such as normally used for agricultural soils, include organic carbon (wet combustion), organic nitrogen (Kjeldahl), pH and Eh in paste, and cation and anion analyses by electrodialysis of a 1:5 suspension in 0.05 N boric acid solution.

TABLE 2. pH, Eh, and organic carbon and organic nitrogen

SAMPLE	Organic Carbon ppm	Organic Nitrogen ppm	pH	Eh. Volts Uncorrected
1-1	0	0	6.5	.13
1-2	0	0	4.8	.25
1-3	0	0	5.8	.21
4-1	20	0	4.6	.26
4-2	0	0	4.5	.20
4-3	800	0	4.2	.23
5-1	120	0	4.5	.25
5-2	42	0	5.4	.25
5-3	80	0	4.8	.20

TABLE 3. Cation analysis, ppm

SAMPLE	N as NH_4	Ca	Mg	Mn	K	Na
1-1	5	220	87	1	60	52
1-2	0	2170	360	8	165	1320
1-3	0	7	0	0.2	30	105
4-1	0	900	180	3	215	3090
4-2	5	205	135	8	96	9750
4-3	5	410	110	0.2	160	3100
5-1	0	525	205	2	105	1350
5-2	2	470	150	1.5	72	720
5-3	0	345	495	0.5	306	6810

TABLE 4. Anion analysis, ppm

SAMPLE	N as NO_3	P as PO_4	B as BO_4	S as SO_4	Cl	C as CO_3	C as HCO_3
1-1	0	13	0.3	70	26	0	0
1-2	2	2	1.5	160	2600	0	0
1-3	0	0.3	0.9	72	51	0	0
4-1	10	0.6	0.7	70	2390	0	0
4-2	0	1	0.7	50	310	0	0
4-3	0	2	0.6	120	227	0	0
5-1	18	0	0.1	65	840	0	3
5-2	13	0	3.1	80	340	0	2
5-3	30	2	0.6	190	11,500	0	12

The low pH and low Ca/Na ratio suggests that actinomycetes will be excluded from developing heterotrophic population and that fungi may be the best heterotrophic competitors in this environment. Samples collected adjacent fo fumeroles in northern California have yielded mostly fungus colonies on heterotrophic media. If we assume that an invasion of marine microorganisms will initially colonize Surtsey, these microorganisms will encounter a new environment with respect to pH, except perhaps at the ocean-beach interface. The variability of the solution extracted from different samples further suggests that marine organisms may become established at certain sites while different organisms may become established at other sites. Since a large percentage of the microorganisms in soil can grow well in sea water media, the ability to grow in such media would not necessarily indicate a marine-derived population. However, the presence of a large population of microorganisms which are unable to grow in a sea water medium would provide some evidence for the establishment of a land-derived microflora. The types and abundance of these microorganisms will be the subject of any future investigations.

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ORGANIC CARBON DETERMINATION APPARATUS

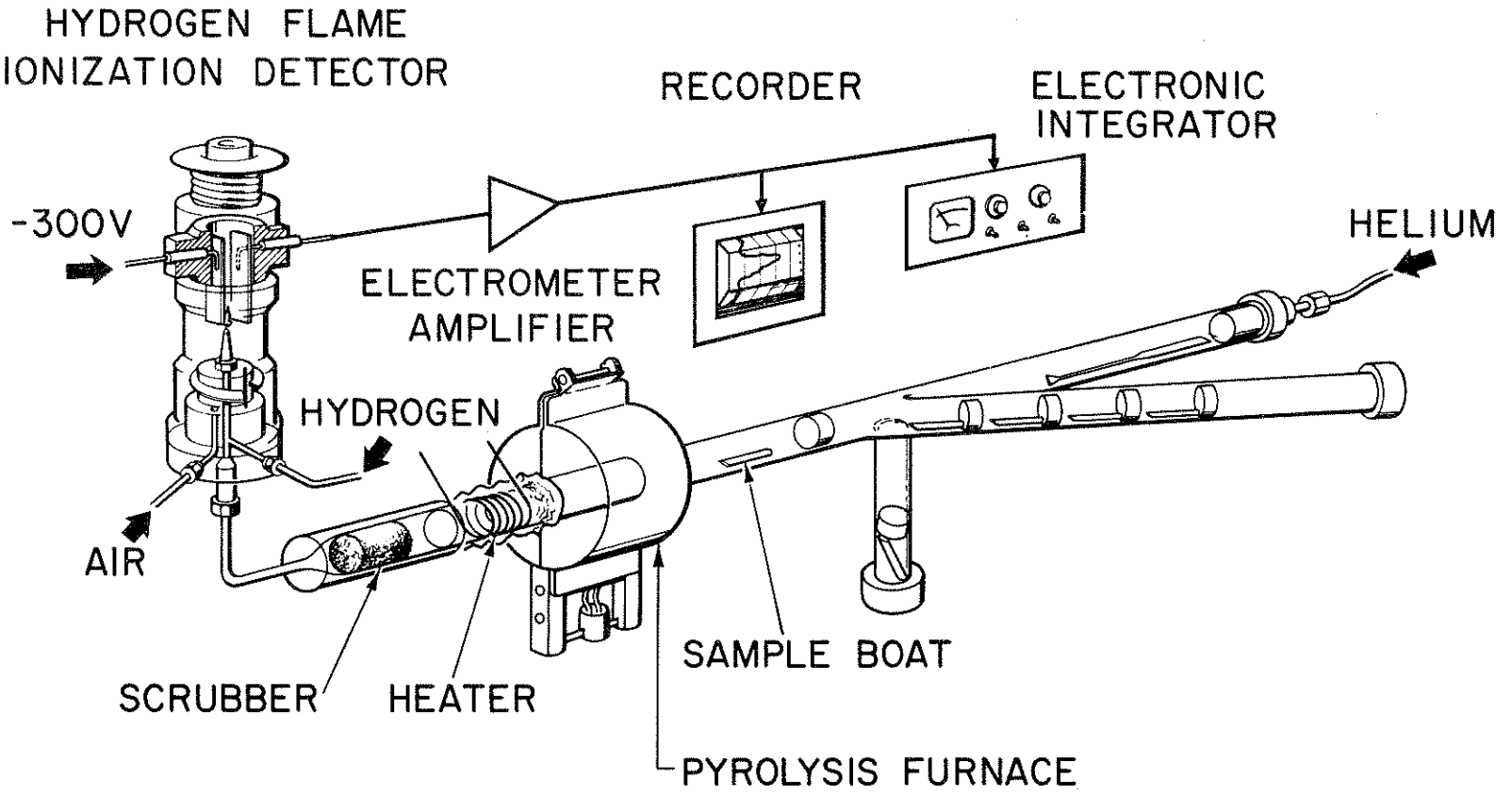

Figure 1

DETECTION OF ORGANIC CARBON BY HYDROGEN FLAME DETECTOR

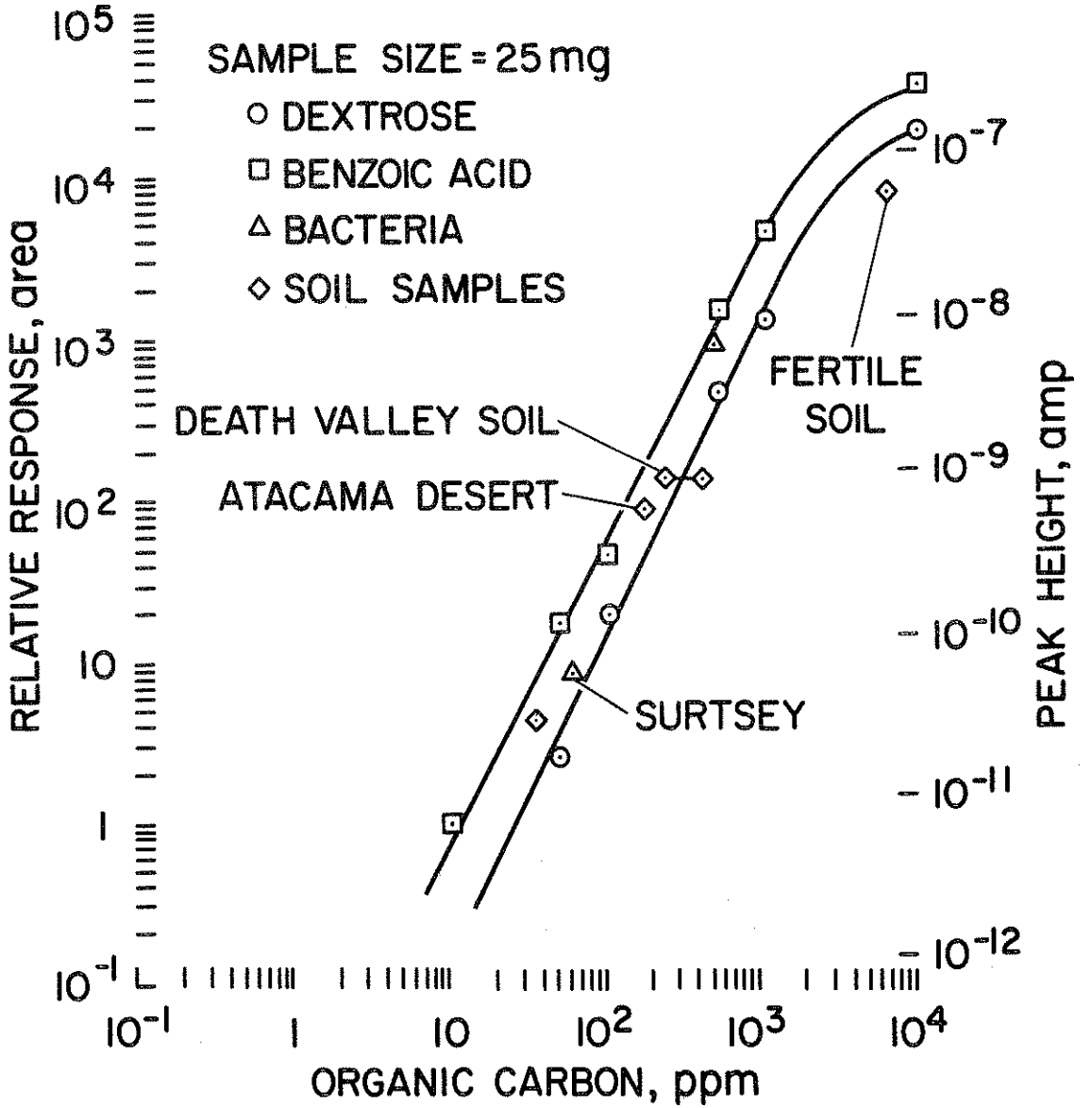

Figure 2

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION