

• • • • • **GAS PRODUCTION BY STORED HUMAN WASTES IN A
SIMULATED MANNED SPACECRAFT SYSTEM**

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INTRODUCTION

Treatment of human waste generated during manned space flight is an unavoidable necessity that is as much a function of such flights as providing fuel for the booster rockets. With our present outlook, human waste may be treated in accordance with two general concepts. The first of these employs waste as all or part of the nutrient source in biological systems capable of furnishing food for the astronauts and maintaining a balanced atmosphere within the capsule. This concept applies to future long trips in which all of the necessary food and oxygen cannot be supplied at the time of launch. The second general concept involves treatment and storage of the waste in an innocuous condition. This concept applies to the immediate problem of short duration trips with high weight and volume penalties. Common methods envisioned for the latter concept are heat and chemical sterilization, desiccation, refrigeration, freezing, and sealing the waste in impervious containers. All of these methods are intended to prevent formation of gas or to prevent gas and volatile materials from escaping into the atmosphere. A waterproof bag would be sufficient to retain the waste and attendant microorganisms if no gases were formed. It is obvious that gas produced from waste and not the waste itself is the major facet of this problem. Therefore, we feel that a sound knowledge of the gases produced in stored waste is essential to the design of any waste storage system. This information must determine the design, material, and operation of such systems.

In addition to its application to the short term waste storage concept, a knowledge of the gases produced by human waste could have considerable bearing on the operation of future bioregeneration systems. Fecal waste will have to be collected one stool at a time and these may be stored for periods ranging from minutes to days depending upon the system and the flight. During these storage periods gases will be produced, especially if putrefaction is allowed to occur either intentionally or accidentally.

SAMPLE COLLECTION, VESSEL CONSTRUCTION, AND SEALING

Collection of Fecal Matter

Members of the laboratory staff volunteered to furnish fecal matter on a regular basis, and scheduled requirements were easily met, despite occasional absences of the donors and individual irregularities. Each donor was given a folding camp stool type commode, a supply of plastic bags with drawstrings, and a number of wide-mouth glass jars with screw-on lids. With this equipment at his home, as well as a duplicate set-up at the laboratory, the donor was able to conveniently collect samples and transport them to the laboratory. Only feces were collected, urine and toilet tissue being disposed of in the water closet.

Construction of Storage Vessel

Fecal matter was stored in commercial No. 2½ tin cans, fitted with a pressure-vacuum gage and sampling head, as illustrated in Figure 1. Before lids were attached to the can body, a centrally located hole was drilled and a brass fitting with 1/8" internal pipe threads was soldered at the underside of the lid, leaving no material projecting above the upper surface. This was necessary to avoid interference with the canner chucks. A brass 1/8" pipe tee and three 1/8" close pipe nipples permitted connection of the lid fitting, pressure-vacuum gage and sampling head, as shown. The sampling head consisted of a 1/8" brass pipe cap, with a 1/8" hole drilled through the end, and the surface below the threads machined flat. A rubber disc diaphragm, 1/4" thick and a close fit with the pipe threads, was placed in the cap, covered with a brass washer, 1/8" inside diameter, and the assembly was then screwed to the nipple. All pipe threads were liberally coated with glyptol before assembly.

Sealing Procedure

Fecal matter was sealed in the vessels as soon as possible after collection. After the sample, in the bag and collection jar, had been weighed, and net weight of feces determined, it was removed from the jar and placed in the can. The plastic bag was slit to avoid trapping of gases, and the lid was attached to the can with an automatic can sealer. To eliminate the possibility of gas leakage, both top and bottom seams of the can were soldered carefully. The gage and sampling head assembly was attached, and the vessel was stored in an incubator held at 30°C. Time of sealing, donor's cod letter, type of atmosphere, and barometric pressure were written on the can body with a felt pen to insure proper identification of each sample. The above procedure, which applies to samples canned in air, was followed for those canned in argon with the following additional steps. A hypodermic needle, attached by a hose to a vacuum pump, was inserted through the rubber disc in the sampling head. When the pressure-vacuum gage indicated that essentially all air had been removed from the vessel, the needle was withdrawn. A similar needle, attached to a cylinder of argon, was then inserted through the rubber disc, and argon was forced into the vessel until the gage reached its zero reading.

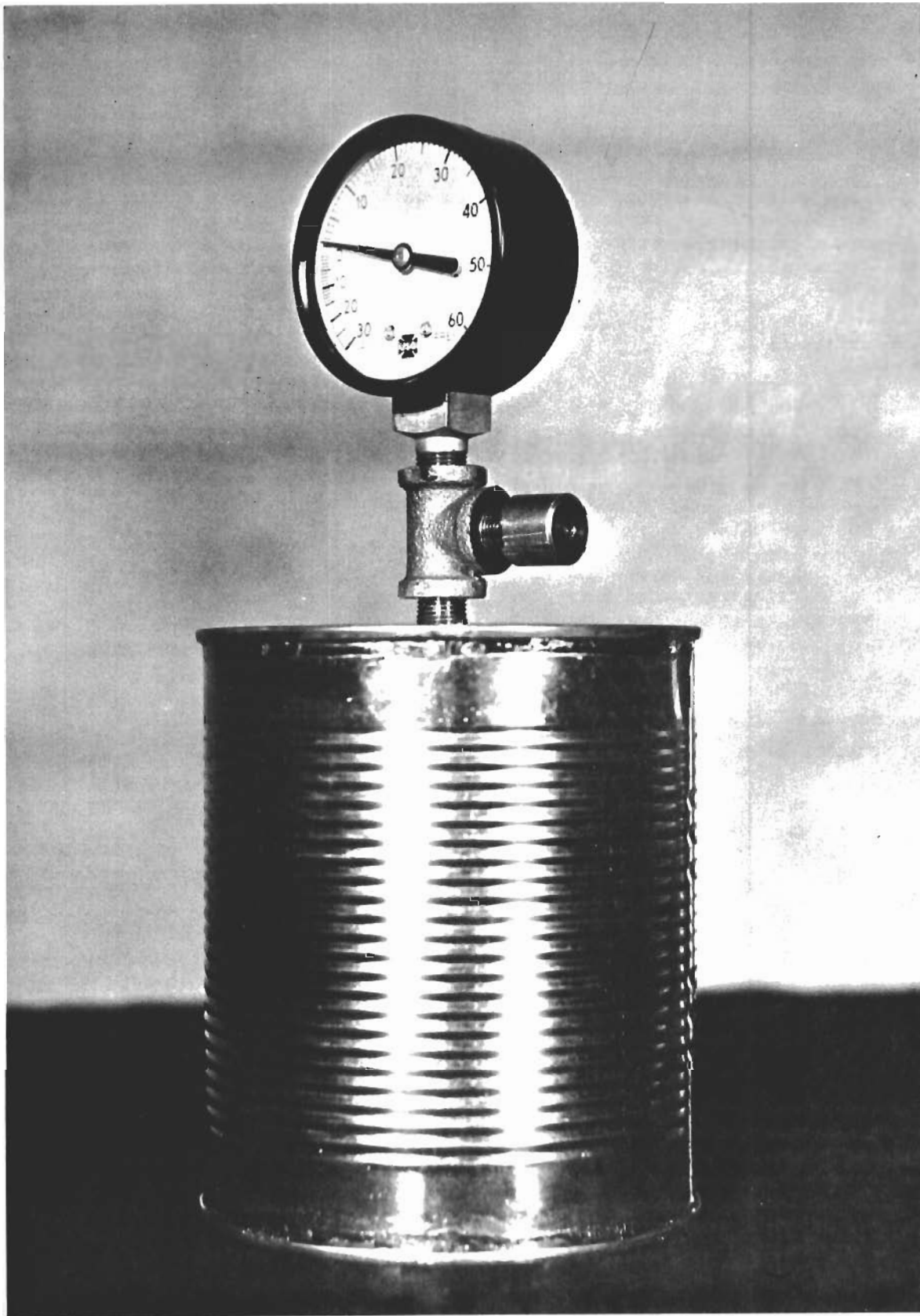


Fig. 1

PRESSURE MEASUREMENT

Gage Calibration

The small pressure-vacuum gages used on the vessels were not of exceptional accuracy, so it was necessary to calibrate each gage and prepare a calibration curve. It was found that cycling the gage several times through its entire range, by means of compressed air and a vacuum pump, was sufficient to stabilize the readings.

Data Collection

Following the initial reading at the time of sealing, the gages were read at 8:00 a.m. and 4:00 p.m. each day, and the barometric pressure was recorded at each reading. A data sheet was maintained for each sample, covering date, time of day, gage reading, corrected gage reading, barometric pressure, total hours in storage, and absolute pressure in the vessel. The time of gas sampling was also recorded on the data sheet. Each sample was stored for a period of 14 days, or until gas leakage forced termination of the test. It might be added that many of the first attempts failed because of gas leakage, and exceptional care was required in soldering seams and sealing fittings to avoid this problem. Leakage was checked by immersing the vessel, up to the gage stem, in deaerated water and observing the formation of bubbles. Serious leaks, of course, were apparent from the odor produced.

Volume Determination

To obtain gas volumes from pressure data, it was necessary to determine the head space in each vessel. The volume of the vessel, complete with gage and fittings, was 875 cc, and the volume of the plastic bag was 18 cc. Assuming that fecal matter has a mean specific gravity of 1.0, head space was determined by subtracting the net weight of the sample, in grams, from the 857 cc net volume of the vessel containing the plastic bag. It is interesting to note that the weight of fecal samples processed varied from 51.5 grams to 305.5 grams, all samples consisting of a single bowel movement. Head space, therefore, ranged from 805.5 cc to 551.5 cc.

Assuming that the gases produced in this experiment behave as perfect gases, it is possible to apply the general gas law to determine the volume of gas at standard conditions (14.7 psi and 0°C) produced if the increase in pressure is known. This relationship can be expressed as follows, as pressures were measured at 30°C:

$$\text{ml. gas produced (STP)} = \frac{(\text{Headspace in ml.})(273^{\circ}\text{K.})(\text{Pressure Increase, psi})}{(303^{\circ}\text{K})(14.7 \text{ psi})}$$

The volume of gas (STP) in the vessel at a given time, therefore, would be the sum of ml. gas produced (STP) and the headspace expressed in ml. (STP).

GAS PRESSURE DATA

Pressure Increase During Storage

Typical observed values of absolute pressure as a function of time, in a sealed vessel containing untreated fecal matter, are shown in Figure 2. In this particular case, the initial atmosphere in the vessel was air, but the characteristic shape of the curve was similar in the case of initial argon atmosphere. It was generally true that the rate of increase of pressure decreased with time, and eventually reached zero. Between samples, however, there was considerable variation in the maximum pressure attained, and in the actual rate of increase at any given time. Much of this variation might be expected to arise from differences in sample weights and head spaces in the vessels.

Volume of Gases Produced

In order to compare gas production by different samples on a more uniform basis, the volume of gas produced (reduced to standard temperature and pressure) per gram of fecal matter was calculated and plotted as a function of time. These values for several samples are shown in Figure 3. There was some indication that the initial weight of the fecal sample had some influence on gas production, with the smaller samples producing a greater volume of gas per gram. This relationship, however, was not consistent in all cases. Another observation may be made regarding the difference in rates of gas production measured for Type A and Type B samples. (These types will be discussed in more detail later in the paper.) Type A samples exhibited a higher early gas production rate, but tended to level off at an earlier time, than did type B samples.

Logarithmic Characteristics of Gas Production

Another observation is of interest. In most cases, the relationship of volume of gas produced per unit weight, as a function of time, closely followed a logarithmic curve over a certain portion of the 14 day period. Figure 4 illustrates this relationship for one sample, and it can be seen that from the first to the fourteenth day there was little deviation from theoretical values. The slope of this line, however, was different for each sample, and in some cases the relationship held for only four or five days. The equation shown holds true only for this sample. Small variations in the two numerical values shown would be required to fit this plot to data obtained from other samples.

Effect of Vessel Head Space on Pressure

Figure 5 illustrates the theoretical variation in pressure that could be attained in 14 days by a typical 100 gram fecal sample sealed in vessels of varying head space. As head space approaches and becomes less than the volume of the sample, gas pressures rise rapidly. An increase of head space to several times greater than the volume of sample results in a gradually decreasing pressure.

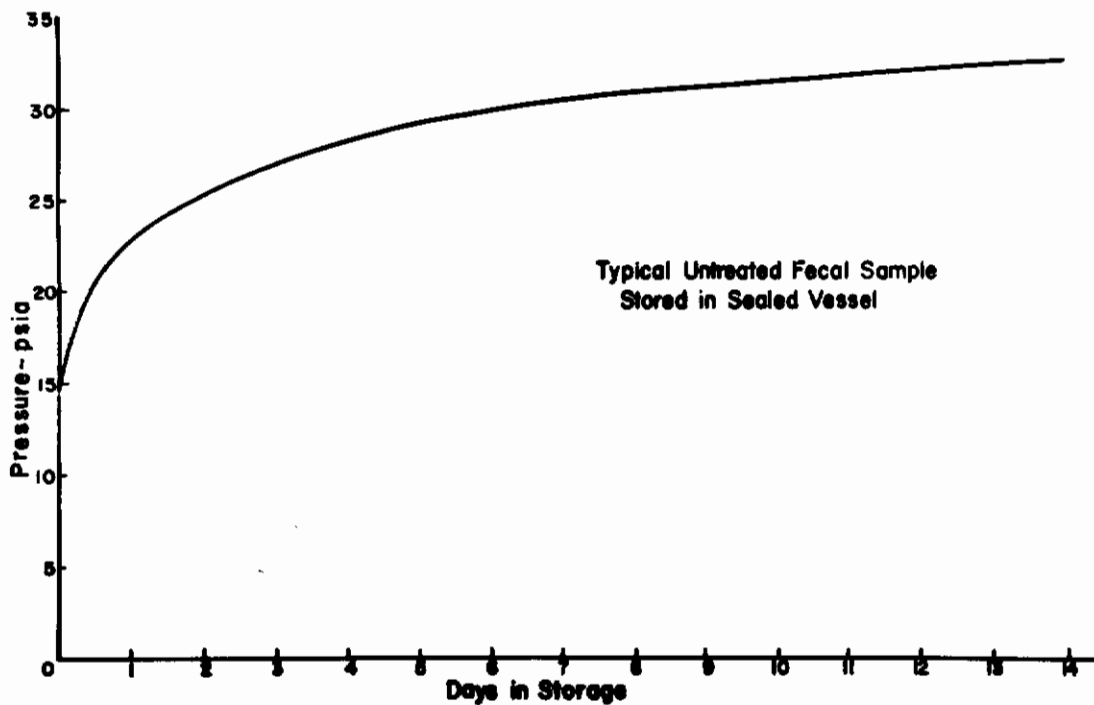


Fig. 2. Absolute Pressure Increase

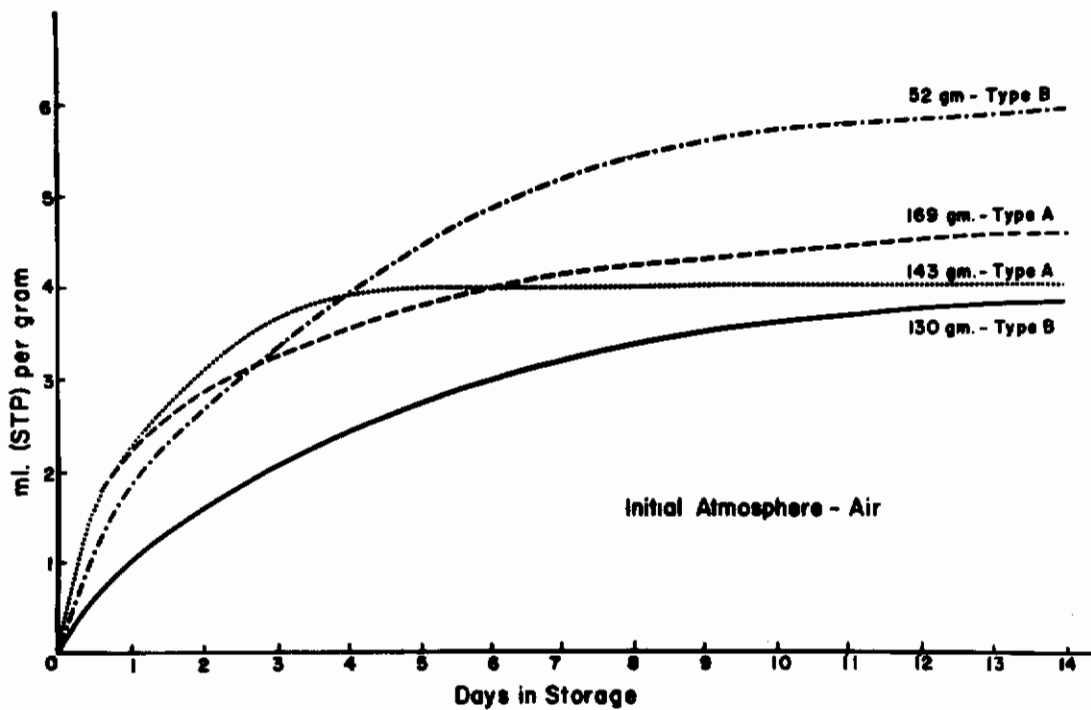


Fig. 3. Volume of Gas Produced per Gram of Feces

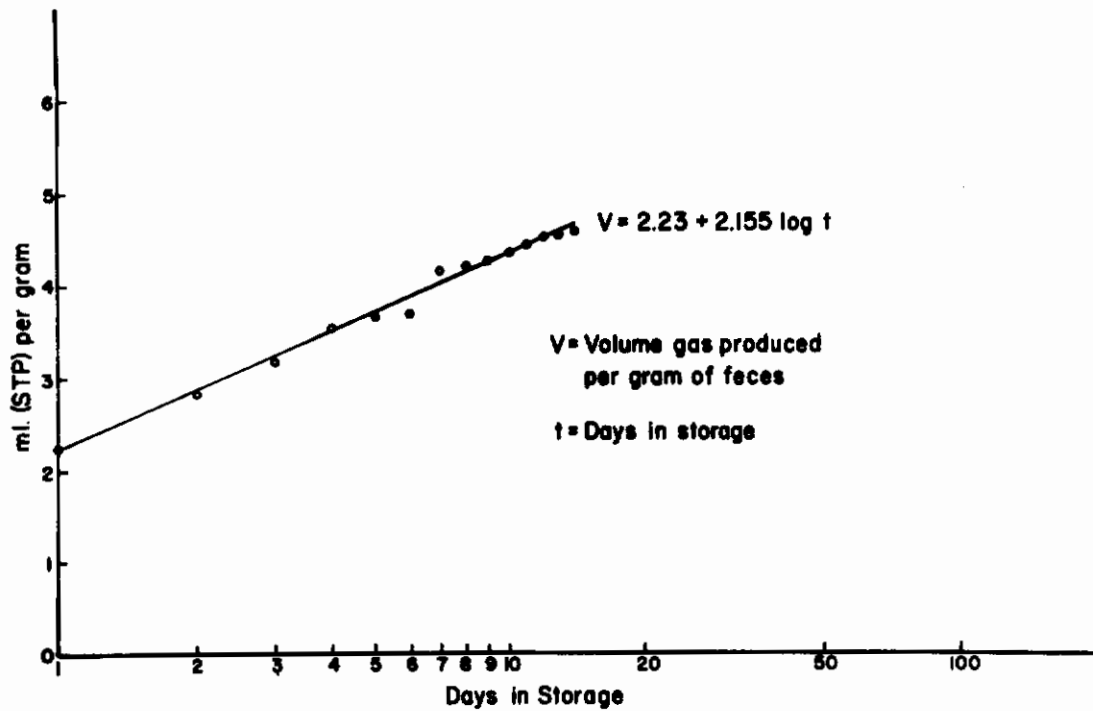


Fig. 4. Logarithmic Plot of Gas Volume Produced

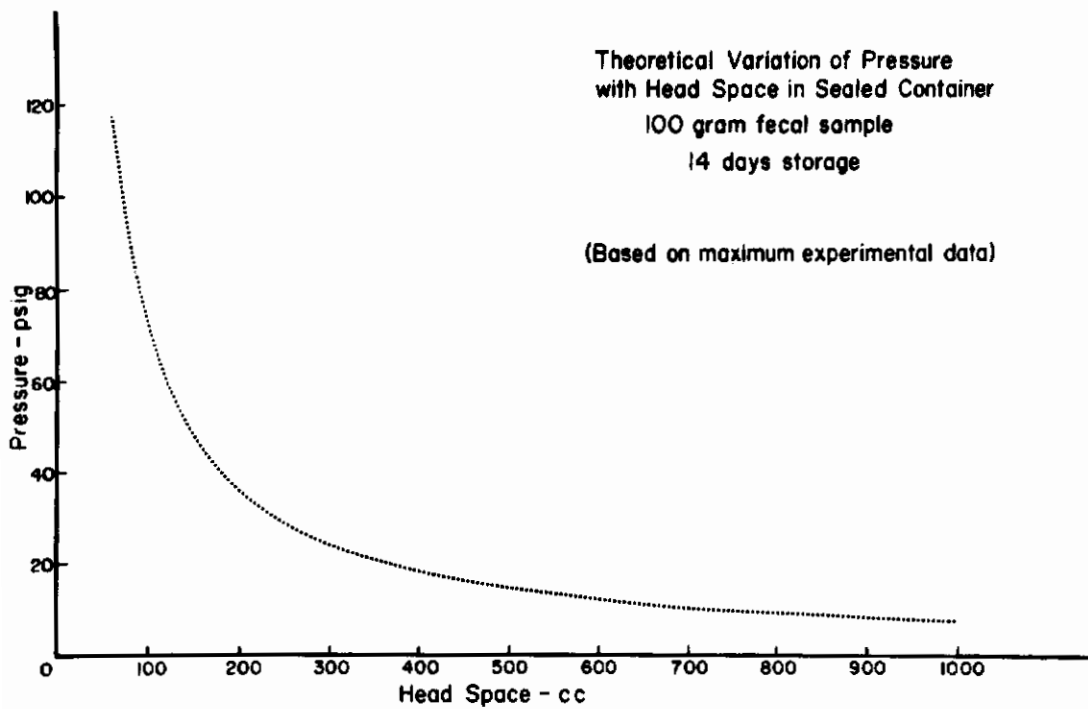


Fig. 5. Effect of Head Space on Pressure

GAS CHEMISTRY PROCEDURE

Gas produced by the samples was analyzed daily by gas chromatography. This provided continuous data on the nature of gases produced during the entire storage period. Because of the multiplicity of gas samples taken for analysis, they had to be relatively small so that their accumulative effect would not appreciably distort the final gas composition. To accomplish this, one ml. samples were taken through the rubber sampling diaphragm already described using a modified hypodermic syringe. This syringe consisted of a standard glass barrel with a teflon plunger. The plunger was fitted with an "O" ring seal. A standard 5/8 inch 25 gauge needle completed the device. This modified syringe was gas tight and, with it, we could consistently handle the one ml. gas samples without fear of contamination.

These samples were introduced directly into a Perkin-Elmer, Vapor Fractometer for analysis by gas chromatography. Argon was used as the carrier gas for these analyses. Three different chromatographic columns were used. A 2 meter silica gel column was used at 50°C with a carrier gas flow of 5 ml/min. to detect hydrogen, methane, carbon dioxide and combined nitrogen and oxygen. A one meter Molecular Sieve 5-A column was used at 25°C with a carrier gas flow of 3.5 ml/min. to separate the nitrogen and oxygen. This also provided a check of the methane and hydrogen. A one meter Fluro Pak 80 column was used at 25°C with a carrier gas flow of 1 ml/min. to detect the presence of ammonia. The mol. percent concentrations of the various gases was determined from corrected peak areas on the chromatograph recorder charts. The mls. of each gas present were calculated from the mls. of total gas present and their mol. percent concentration.

After termination of the tests, gas samples were analyzed by standard Orsat and Mass spectrometry techniques to verify the gas chromatography results. These samples were necessarily large and therefore were destructive to the stored fecal samples.

GAS CHEMISTRY DATA

During the 14 day storage period, the oxygen in the initial atmosphere was consumed and the nitrogen remained unchanged. The major gases produced by our samples were carbon dioxide, methane, hydrogen and hydrogen sulfide. These were the only gases observed to occur in sufficient abundance to effect the gas pressure of the sealed samples. It is interesting to note that ammonia was not detected in any of the samples either by gas chromatography or by mass spectrometry.

In addition to these major gases, minor constituents such as indole, skatole, and mercaptans were undoubtedly present, but in concentrations well below 0.1%. These were not detected by our procedure. This is not surprising when one realizes that concentrations in the part-per-billion range are

sufficient to cause an appreciable odor.

All of these gases were not produced by each fecal sample. In fact, two distinct types of fecal samples were distinguishable by their gas production. The first of these we shall call Type A or non-methane producers. A typical example of this type of sample is shown in Figure 6. This figure shows the mls. of each gas produced or absorbed versus storage time. Carbon dioxide was by far the most abundant gas produced. Most of it was produced early in the storage period i.e., during the first 100 hours. The only other gas produced was a rather small amount of hydrogen. Oxygen was consumed slowly over the 14 day period. Its rate of consumption did not appear to correlate with the rapidly changing rate of carbon dioxide production. The quantity of nitrogen remained constant throughout the storage period.

Figure 7 shows the same type A sample. In this case the mol. percent concentration of the various gases are plotted with respect to time. The slow rate of oxygen consumption and small change in percent composition of the gases after 100 hours of storage is quite obvious.

The second category of fecal samples we shall call type B or methane producers. A typical example of this type is shown in figure 8. This again shows the mls. of each component plotted versus time. This sample, like the first, was sealed in an air atmosphere. Carbon dioxide was still the principal gas produced and again most of it was produced within the first 100 hours of storage. Contrary to the type A sample, however, methane was apparent in less than a day and this became the second most abundant gas produced during the first 100 hours. Also contrary to the type A samples, oxygen was absorbed rapidly. None remained after 100 hours of storage. Nitrogen was again unchanged throughout the storage period.

Figure 9 shows the mol. percent concentration of various gases present in the same type B sample shown in the previous figure. The formation of methane is quite obvious from this graph. About 3.8% of methane ultimately appeared in the gas produced by the sample. This is an average sample. Methane produced by type B samples varied from 1.5 to 10% of the total gas. The rapid utilization of oxygen is well illustrated in this graph. In addition to the gases shown, 0.1% of hydrogen sulfide was also produced. This was detected by mass spectrometry at the end of the storage period, since our chromatographic procedure did not detect this gas. Again, the fact that little or no change in gas composition occurred after 100 hours storage is well illustrated.

A procedure was designed to determine the effect of oxygen on the gas production of stored feces. To accomplish this, the initial atmosphere was replaced with the inert gas, argon. This accomplished two things. An anerobic initial atmosphere was provided and, since argon was also used as the carrier gas, the chromatograph was insensitive to its presence. Consequently, the gases produced by the sample could be detected with greater accuracy at lower concentrations. Also, it provided a more sensitive test for production or utilization of nitrogen.

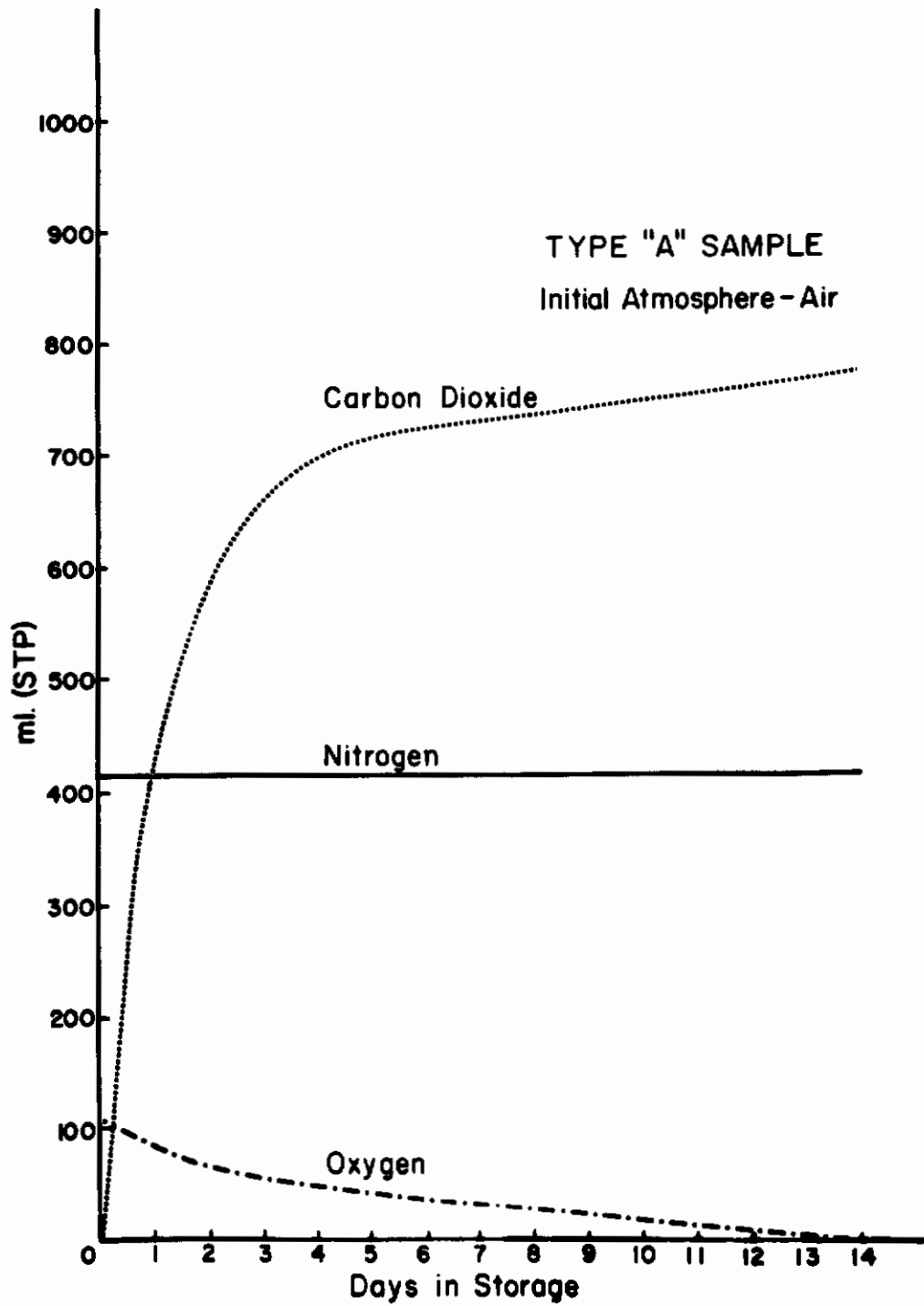


Fig. 6. Volume of Gases in Container

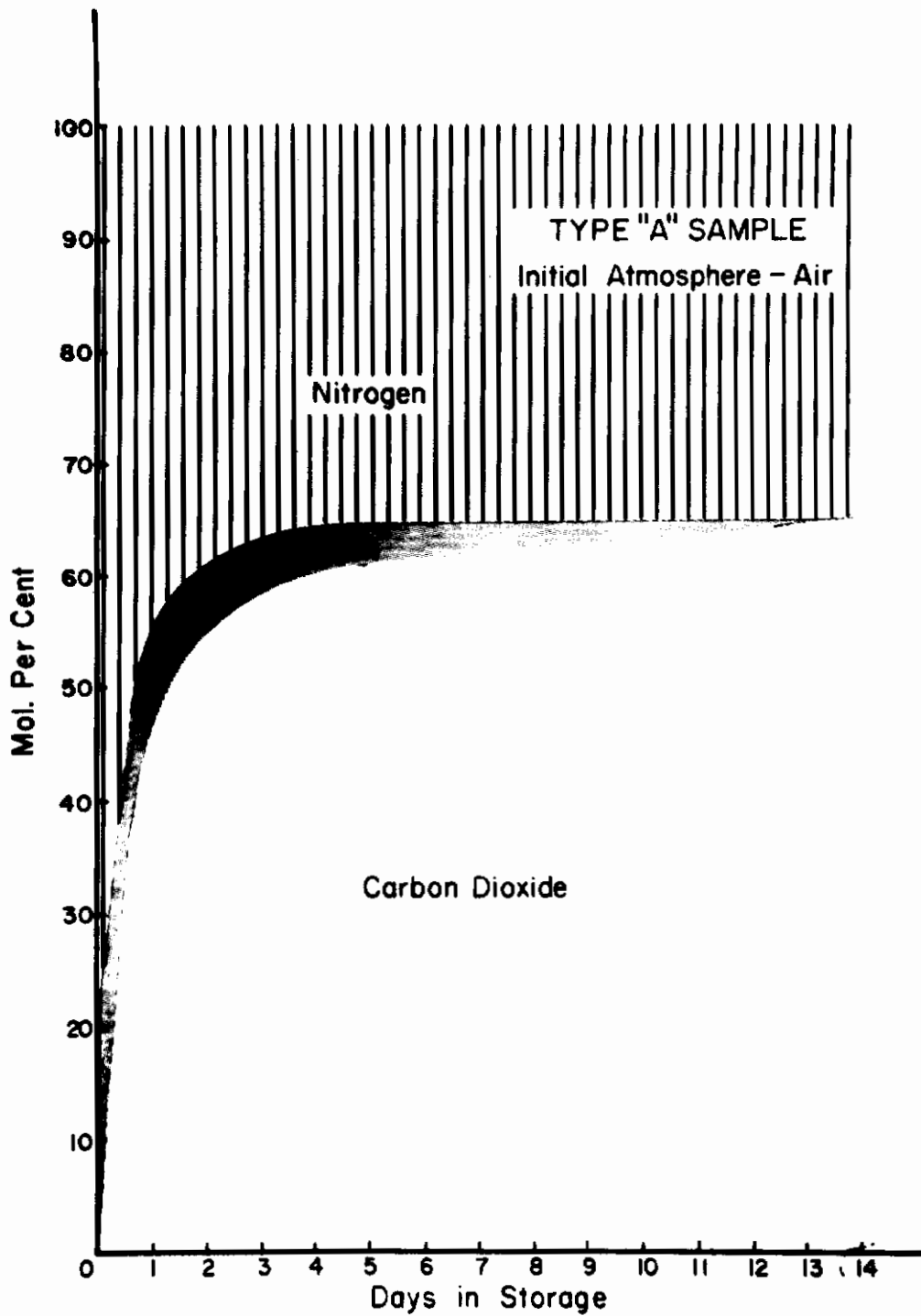


Fig. 7. Per Cent Composition of Gases in Container

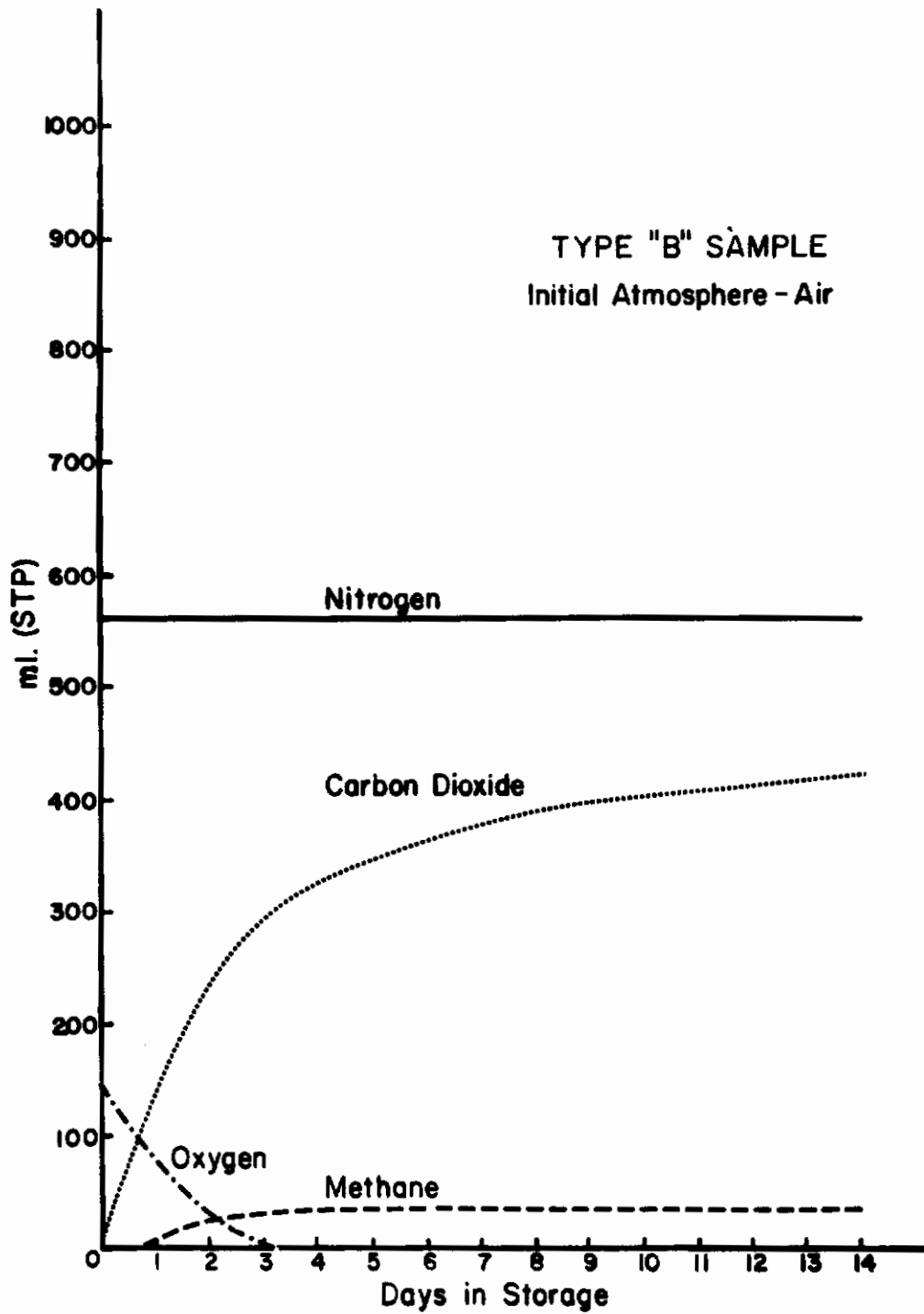


Fig. 8. Volume of Gases in Container

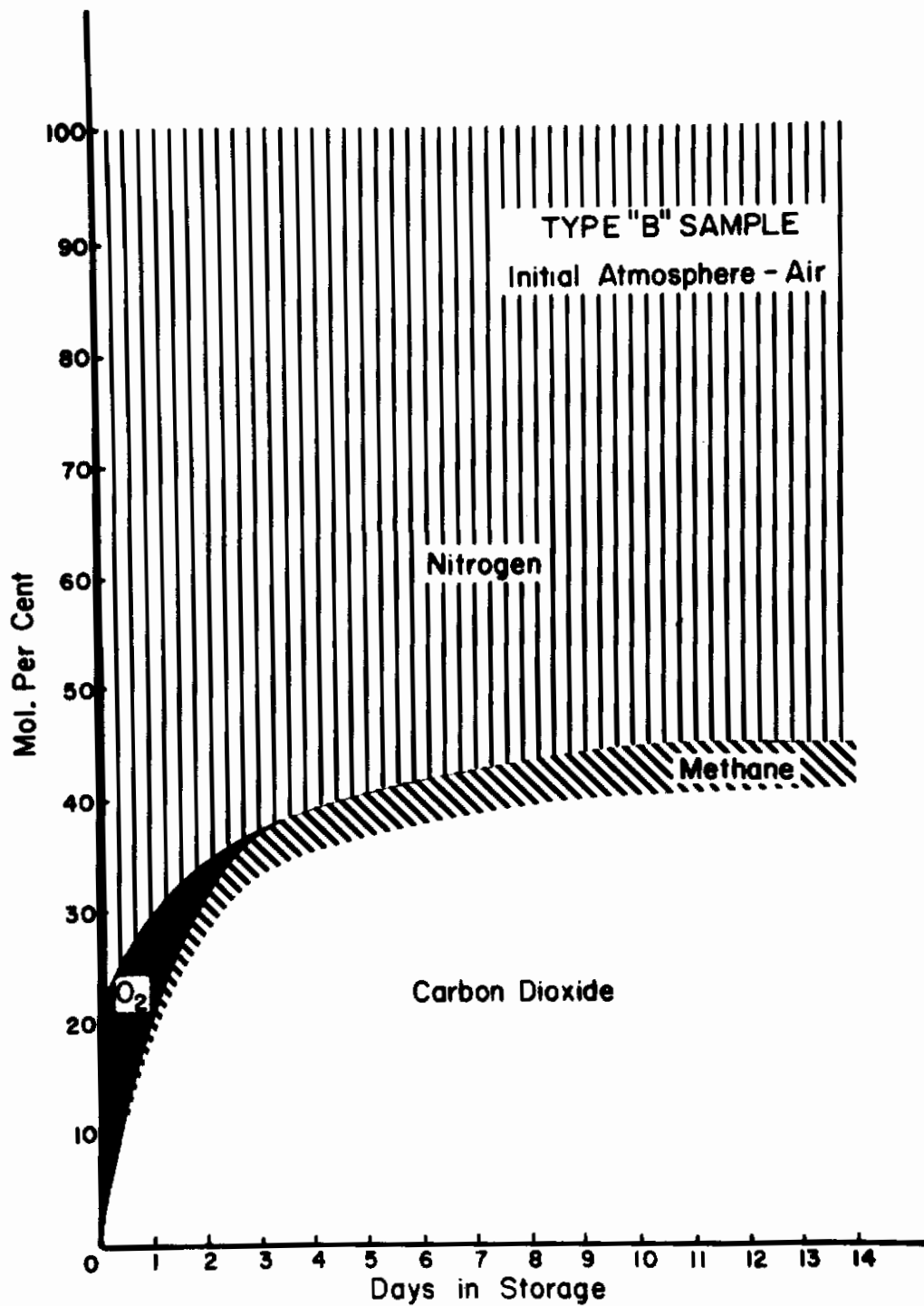


Fig. 9. Per Cent Composition of Gases in Container

Contrails

Figure 10 shows the volume of gas produced by a typical type A sample sealed in an initial argon atmosphere. Carbon dioxide was again the only gas produced in measurable quantity. This agrees exactly with data obtained from type A samples sealed in air as already shown. The slight amount of nitrogen represents part of the original air that was not removed before the argon was introduced into the sealed container. It is noteworthy that even this rather small amount (4.4 ml) of nitrogen remained unchanged throughout the storage period.

Figure 11 shows the volume of gas produced by a typical type B sample sealed in an initial argon atmosphere. Carbon dioxide and methane were again the major gases produced by this type of sample. In this test about 1/10 of an atmosphere of air was allowed to remain in the initial atmosphere so that any changes in nitrogen quantity could be easily followed. Obviously, no change occurred.

A comparison of the data obtained from these typical type A and B samples provides some interesting observations. Type A fecal samples are essentially carbon dioxide producers and slow oxygen consumers. Type B fecal samples are essentially carbon dioxide and methane producers and rapid oxygen consumers. Perhaps the most interesting observation was the fact that certain individuals consistently produced type A or B fecal samples. No individual to date cooperating with this study has been observed to produce both type A and B samples. Further, the presence or absence of oxygen in the initial atmosphere did not appear to affect the kinds of gases produced by the two types of fecal samples. There was no evidence of nitrogen production or utilization by any of the fecal samples during the 14 day storage period. The type of fecal sample appears to be determined by the individual producing it. There are many possible reasons for this. Diet is the first consideration; however, it is unlikely that diet alone could be responsible for this phenomenon. The diets of the various individuals involved although uncontrolled, were roughly similar, and certainly the diet of any individual varied as much or more from day to day as the diets of all individuals on any given day. Nature of the intestinal flora could account for the phenomenon. Type B samples could be those containing methane producing organisms; whereas, type A samples contained no organisms capable of producing methane. The ubiquitous occurrence of methane producers and the fact that most individuals produced type A samples tend to rule against this conclusion. Relative oxidation of the samples could also account for this phenomenon. Methane production is known to take place only under anaerobic or reducing conditions (Wood, ref. 7). The presence of sufficient oxygen or hydrogen acceptors could prevent or greatly delay the formation of methane, even when methane producing organisms are present. The amount of available oxygen present in the fecal sample could be effected by the amount of air eaten with the food and the retention time of the food within the digestive system. The rapid utilization of oxygen from the initial atmosphere could indicate a reduced or "oxygen starved" condition; whereas, the slow utilization of oxygen from the initial atmosphere by type A samples could indicate a more highly oxygenated or "less starved" condition.

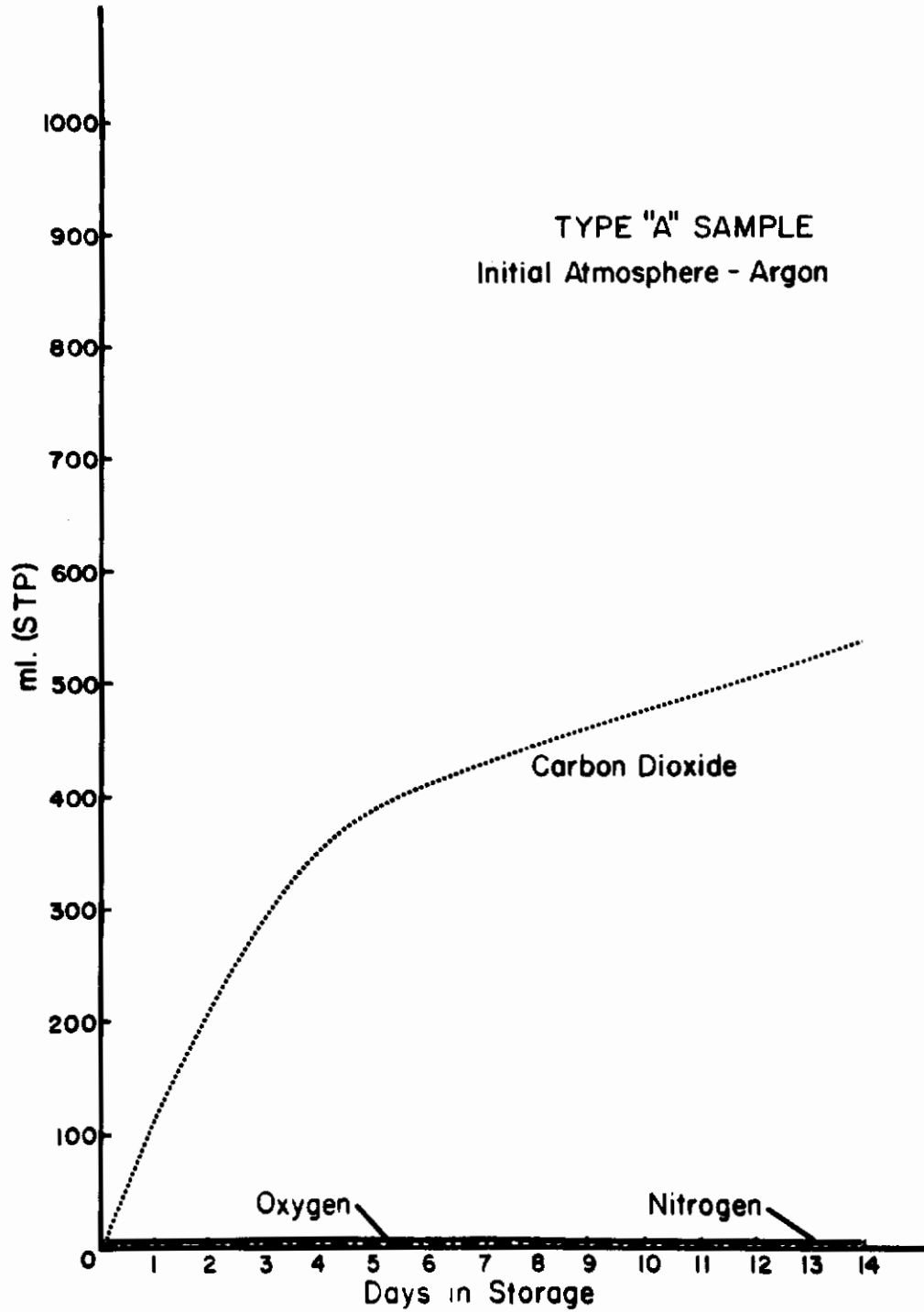


Fig. 10. Volume of Gases in Container

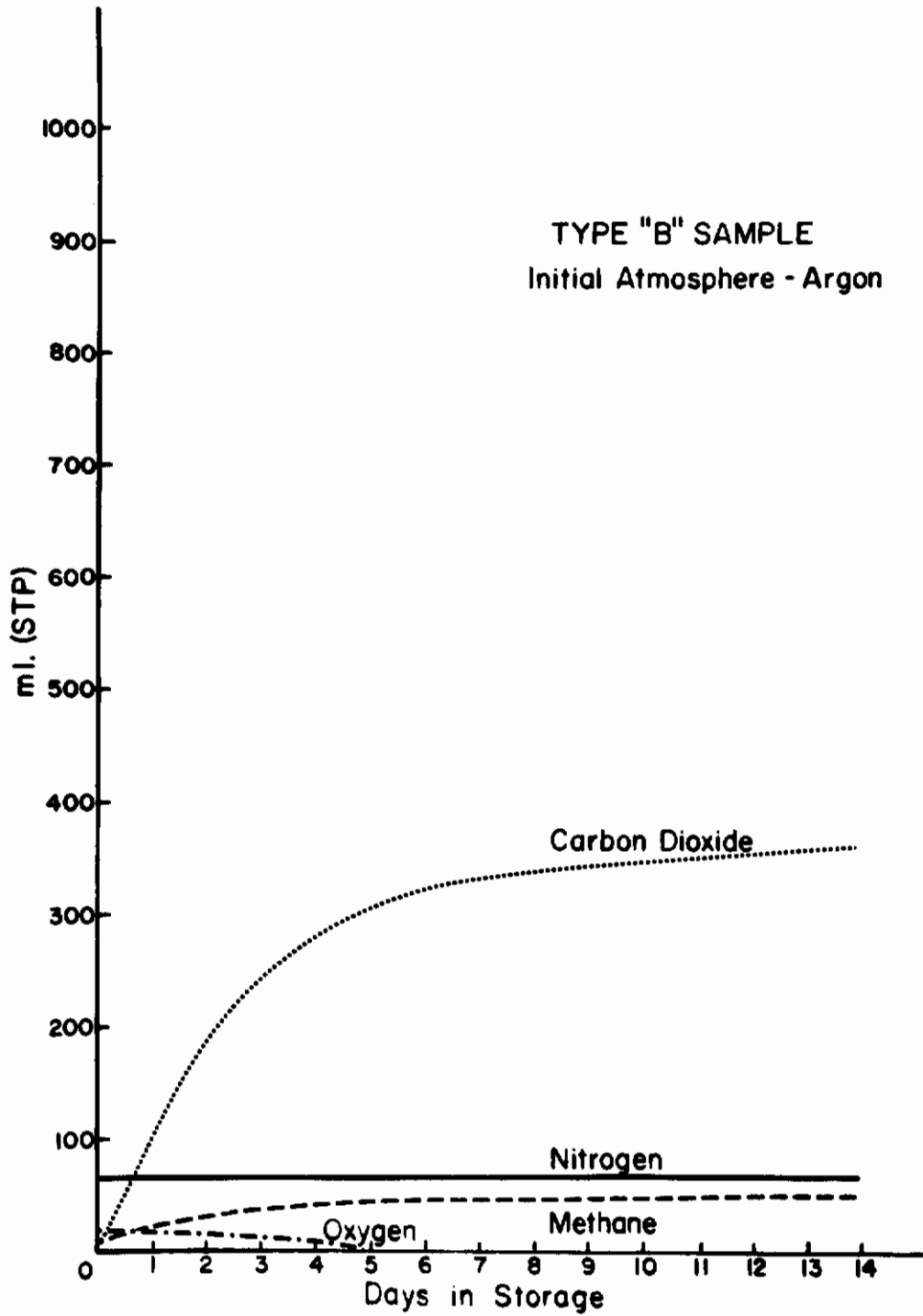


Fig. II. Volume of Gases in Container

DISCUSSION OF MICROBIAL GAS PRODUCTION

The production of gas in stored fecal waste is the result of microbial metabolism during the storage period. Such waste, treated with an effective germicide, will not produce gas.

Production of carbon dioxide by microorganisms is a very common phenomenon. This gas is a byproduct of many aerobic and anaerobic metabolic processes. It is probably the most abundantly produced gas of microbial origin. It would be very difficult, if not impossible, to attempt to pinpoint the specific metabolic reactions responsible for carbon dioxide production in stored fecal samples. The variation in samples and extremely diverse microbial flora they contain would insure that carbon dioxide emanates from a variety of metabolic reactions. Further, it is likely that the changing conditions during storage affect these reactions.

It is obvious, from data presented, that the volume of oxygen consumed falls well below that required for the amount of carbon dioxide produced, if the process were entirely dependent on aerobic metabolism. Obviously, gas produced by stored feces is largely the result of anaerobic reactions and the employment of hydrogen acceptors other than oxygen. A variety of hydrogen acceptors are used by some organisms. Wood (ref. 7) lists aldehydes and carboxylic acids as the most common. Nitrate could also serve as a hydrogen acceptor in denitrification reactions. However, if this occurred, the reaction must have stopped before the nitrate was reduced completely to molecular nitrogen. Otherwise nitrogen would have accumulated in the containers. Certain strains of Escherichia coli reduce nitrate to nitrite but no further (Lamanna and Mallette, ref. 3). This might have occurred.

The possibility of dissolved oxygen occurring in the fecal material has already been suggested.

The production of methane in stored fecal waste should be expected. The anaerobic digestion process common to sewage disposal plants results in the formation of large quantities of methane. Gotaas (ref. 2), states that anaerobic composting of barnyard manure results in the formation of gas composed of about 2/3 methane and 1/3 carbon dioxide. Methane bacteria are notoriously strict anaerobes and exhibit rather slow rates of reproduction. As a consequence they are difficult to isolate in pure culture. They have not been as thoroughly studied as many other groups of bacteria (Clifton, ref. 1). They do not attack carbohydrates or amino acids. Some alcohols and fatty acids are, with a few exceptions, the only compounds fermented (Wood, ref. 7). In some of these fermentations, carbon dioxide can serve as the hydrogen acceptor, and it is reduced to methane in the process. Stadtman and Barker (ref. 6) have shown that methane is formed from carbon dioxide in the fermentation of buterate, valerate, and caproate by Methanobacterium suboxydans. A similar reduction of carbon dioxide was reported for the fermentation of propionate by Methanobacterium propionicum. This is not the only route of methane formation. Pine and Vishniac (ref. 5) reported that crude enrichment cultures of methane bacteria ferment methanol to methane intact. A coincident oxidation of the substrate to

carbon dioxide through formate as an intermediate is a part of this metabolic route. The actual method of methane production by these fecal samples is not known, and probably the end product is tempered by many variables. What determines the presence or absence of methane production; i.e., the difference between type A and B samples remains unsolved. The presence of various substrates, differences in flora, and degree of anaerobiosis have already been advanced. We have observed, however, that whatever the reason it is consistent as to individual.

The formation of hydrogen sulfide by the type B samples suggests a more anaerobic environment.

The effect of storage temperature on gas production is essentially a rate effect. Lowering the storage temperature from 30°C to 20°C did not appear to reduce the total amount of gas produced; however, gas was produced at a lower rate. Gotaas (ref. 2) reported that the amount of gas produced by anaerobic waste composters was approximately the same for temperatures between 15°C and 35°C. Elevated temperatures should be expected to increase the rate of gas production.

Storage of fecal waste does not appear to result in a profound growth of microorganisms. Growth takes place to be sure, but it usually only amounts to a few generations at most. This is not too surprising. Miner, et al. (ref. 4) reported that bacterial cells constitute about 1/3 of the dry weight of feces. Any profound multiplication of this situation would produce a significant change in appearance of the stored feces. This has not been observed. Further, it is doubtful whether the remaining dry weight of material would contain sufficient nutrient to support appreciable prolonged growth. Gas production in stored feces may be the result of essentially a "resting cell" metabolism. If this is the case, a large part of the substrate for gas production early in the storage period could result from endogenous catabolism. This would place considerable emphasis on the fecal flora as the determining factor of the type and quantity of gases produced.

DISCUSSION OF DESIGN CRITERIA

Major Factors in Design of Vessel

If storage of untreated fecal matter is considered for aerospace application, a number of factors must be evaluated. a.) Vessels cannot be completely filled with fecal matter without drastically increasing the internal pressure that will develop during the storage period. Figure 5 illustrates this point graphically. If pressures are to be held to values not requiring excessively heavywall sections, head space must approach the volume of the waste in a single container. The weight and volume penalty for manned space missions of unfilled pressure vessels will be severe.

b.) Leakage of gases into the cabin cannot be tolerated, so elaborate means for sealing the vessels will be required. This factor will again complicate design and add a weight penalty. c.) The capacity of the vessel will be limited to the amount of waste that can be placed in an empty vessel at one time. It will not be possible to open the vessel later to add more waste without escape of noxious gases, unless a bulky air-lock system is provided. The weight penalty of using a large number of small vessels is obvious.

SUMMARY

1. Untreated fecal material stored in sealed containers at 30°C produced an appreciable gas pressure within the containers. The rate of pressure increase, varied inversely with time. The pressure increased most rapidly during the first 4 days of storage.

2. Most fecal samples produced from 3.5 to 6 mls. of gas (at STP) per gram of feces. There was a tendency for smaller (lighter weight) stools to produce more gas per gram of feces.

3. The volume of gas produced per weight unit plotted as a function of time followed a logarithmic curve during at least part of the 14 day storage period. The slopes of these plots varied from sample to sample.

4. The pressure produced by any sample was very dependent on the head space of the container. When head space became less than the volume of feces, excessively high pressures were reached.

5. The major gases produced under these conditions by sealed individual stools were carbon dioxide, methane, hydrogen and hydrogen sulfide. Most of this gas was produced in the first 4 days of storage.

6. Two types of fecal samples were observed in this investigation. Type A samples were carbon dioxide producers and slow oxygen utilizers. Type B samples were carbon dioxide and methane producers and rapid oxygen utilizers. Certain individuals always produced either type A or B stool samples, but so far no one has been observed to produce both.

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