ANALYTICAL TECHNIQUES FOR RESEARCH ON THE ABATEMENT OF BACTERIAL ACID GENERATION IN PYRITIC TAILINGS

M. Silver
Extractive Metallurgy Laboratory

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M. Silver*

Abstract

Over the past 20 years, CANMET has conducted investigations on the production and movement of acidic mine drainage within uranium tailings. These studies have gradually evolved into investigations on the prevention of acidic drainage production by water-table manipulation and tailings surface coverings of wetland vegetation. The following compendium of analytical procedures has been prepared to provide analytical uniformity in order that the data from various investigations on mine tailings can be compared.

* Research Scientist, Biotechnology Section, Mineral Sciences Laboratories, CANMET, Energy, Mines and Resources Canada, Ottawa, K1A 0G1.

MÉTHODES D'ANALYSE POUR LA RECHERCHE SUR LA RÉDUCTION DE LA FORMATION D'ACIDES BACTÉRIENS DANS LES RÉSIDUS PYRITEUX

M. Silver*

Résumé

Au cours des vingt dernières années, le CANMET a effectué des études sur la formation et le mouvement du drainage minier acide dans les résidus d'uranium. Peu à peu, ces études ont été orientées vers l'étude de la prévention de la formation du drainage acide par le changement du niveau de la nappe phréatique et l'étude des plantes de marais recouvrant les résidus. Le présent résumé de méthodes d'analyse a été préparé dans le but d'uniformiser ces méthodes afin de pouvoir comparer les données obtenues des différentes études sur les résidus miniers.

*Chercheur scientifique, Section de la biotechnologie, Laboratoires des sciences minérales, CANMET, Énergie, Mines et Ressources Canada, Ottawa, K1A 0G1.

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1.0 INTRODUCTION

Pyrite-containing mine/mill wastes deposited in surface impoundment areas result in the generation of acidic seepage containing high concentrations of heavy metals that are environmentally unacceptable. The iron-oxidizing bacteria oxidize pyrite (1) according to the equation:

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4 \qquad \text{Eq 1}$$

with the formation of sulphuric acid and soluble ferric sulphate. The ferric iron thus formed oxidizes the residual metal sulphide minerals in the tailings:

$$Fe_2(SO_4)_3 + MS \rightarrow MSO_4 + 2FeSO_4 + S^\circ \qquad \text{Eq 2}$$

This produces soluble metal sulphates, ferrous sulphate, and elemental sulphur, which can then be further oxidized by these bacteria to ferric sulphate and sulphuric acid:

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \qquad \text{Eq 3}$$

$$2S^\circ + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \qquad \text{Eq 4}$$

Hydrolysis of ferric sulphate results in the precipitation of secondary iron precipitates, such as hydronium jarosite, and the generation of sulphuric acid:

$$3Fe_2(SO_4)_3 + 12H_2O \rightarrow 2O\{H[Fe(SO_4)_2 \cdot 2Fe(OH)_3]\} + 5H_2SO_4 \qquad \text{Eq 5}$$

Increasing awareness of the deleterious environmental impact of mine wastes has resulted in increased research into processes for the abatement of this pollution. Current research at the Canada Centre for Mineral and Energy Technology consists of compost production (2), as well as laboratory (3) and field studies (4) on tailings leaching. The following remedial procedures have been suggested for the abatement of acidic tailings seepage containing heavy metals:

- intercept the oxygen supply;
- chemically inhibit the iron-oxidizing bacteria;
- confine the seepage to the tailings deposit;
- neutralize the seepage from the tailings (5).

Of these procedures, the inhibition of bacterial pyrite-oxidation by eliminating oxygen from the tailings appears to be the most effective and economical in a climate in which precipitation exceeds evaporation. Sulphuric acid generation was shown to be completely arrested by either a layer of compost (5) or a layer of water (6,7). Most recent studies (8) have shown that either compost or wetland soil eliminated both acid generation and radon emanation from uranium tailings.

Research on the vegetation of tailings deposits in Canada has been conducted by government (9), private consultants (10), and public mining companies (11,12) since the 1970's. However, examination of the available literature on this subject reveals that investigative and analytical procedures used in these studies are diverse, thus making correlation of data between them difficult. This report attempts to present standardized procedures for the chemical, physical, and microbiological analysis of soils and tailings to facilitate comparison of research data.

2.0 LYSIMETER DESIGN AND CONSTRUCTION

Accelerated laboratory leaching studies can be used to predict the leaching characteristics of tailings in a shorter period of time than can be determined by hydrogeological research (7). Investigations using laboratory lysimeters have been correlated with field-test data; these investigations have shown to be reproducible and to allow for comparison between different but similar tailings (6,7,5). Lysimeters are leaching vessels, located either in laboratories or in the field exposed to the atmosphere, that contain soil or other test materials such as tailings, through which a known volume of leachant can be passed,

and from which effluents can be collected for chemical and physical analysis. Data obtained from lysimeter leaching tests can be used to determine the leaching characteristics of the materials contained in the lysimeter under controlled conditions.

Experimental details of the design and operation of lysimeter leaching tests are important in the generation of data for the prediction of chemical, physical, and biological occurrences of the test material. The size of the lysimeter may range from a few grams to a few tonnes, and it is dependent on the data to be obtained or the manipulations required. Small lysimeters, for example, can be placed in refrigerators to determine the effects of temperature changes (13), but they are limited with respect to investigation of vertical and horizontal changes within the test mass. Small lysimeters are also more sensitive to wall effects, such as the influx of atmospheric gasses and leachate channelling. Lysimeters must be sufficiently robust to withstand any required manipulations to prevent leaking. Thus, they must be made either from existing plastic containers (Fig. 1), or from plexiglass that is held together with screws; has mortice and tennon joints; or is externally braced. Construction materials should be inert, preferably glass or plastics, and all fittings should be free from leaks.

The simplest leaching tests, which use burette-type columns, can be used for gram quantities of test materials in Pasteur pipettes, to masses of tens of kilograms in glass and plastic columns. Plastic containers, such as those illustrated in Figure 1, each contain 20 kg of tailings with or without a layer of soil placed on the tailings surface; are inexpensive; are easy to construct, maintain, and manipulate; but they tend to deteriorate with prolonged use. This type of lysimeter is suitable for leaching tests that do not exceed two years' duration and involve a minimum of movement. A more robust type of lysimeter is made from 1- or 2-cm-thick plexiglass sheets, of screwed or channelled construction, externally braced with metal strapping (Fig. 2). This type of lysimeter has been used successfully to investigate the leaching characteristics of 25- to 50-kg masses of tailings in various deposition configurations, and is sufficiently robust to endure repeated freezing and thawing cycles. Externally braced cylindrical plastic tubs can be used as lysimeters for tailings masses of 100 to 250 kg or more (8,5). Larger lysimeters, such as the one shown in Figure 3 that contains 1 t of tailings, can be constructed from externally braced plexiglass. Even larger lysimeters have been constructed of externally braced plywood (14) containing 11 t of tailings; outdoor pits containing 200 t of tailings have also been described (15).

Regardless of the size and nature of the material to be investigated in lysimeter leaching tests, several factors are important in using this technique. The test materials must be as similar as possible in their physical, chemical, and biological properties; they must also be placed in the leaching vessels in a configuration as similar as possible to that of the material being compared. Any variation from the field conditions must also be controlled so that correct correlations may be made. Finally, the effluent from the lysimeters must be collected to yield samples for analysis that are truly representative of the test conditions.

3.0 SAMPLING OF SOILS AND TAILINGS

Because of the wide variability inherent in any given soil mass, satisfactory sampling methods must be chosen for a given situation. Sampling in duplicate, or using the entire soil mass, may be suitable for small-scale leaching tests, whereas investigations of larger tests and outdoor areas may require composite sampling procedures. The requirements of a valid composite sample are that enough individual samples of the same size are collected randomly using the same technique, that there is no chemical or physical interaction between the individual samples, and that the composite sample is homogenous for the objectives of the analysis (16). Soil-sampling methods are described in detail by Jackson (16,17), Petersen and Calvin (18), Allen et al. (19), Black (20), and the Association of Official Analytical Chemists (21).

A variety of equipment is commercially available for obtaining soil samples, the choice of which will depend on the physical properties of the soil and the objectives of the investigation. For well-developed soils and soft coherent tailings, trenches may be dug with a spade to study the development of different horizons, a trowel being used to collect samples. For samples whose volume must be known, a graduated metal cylinder can be

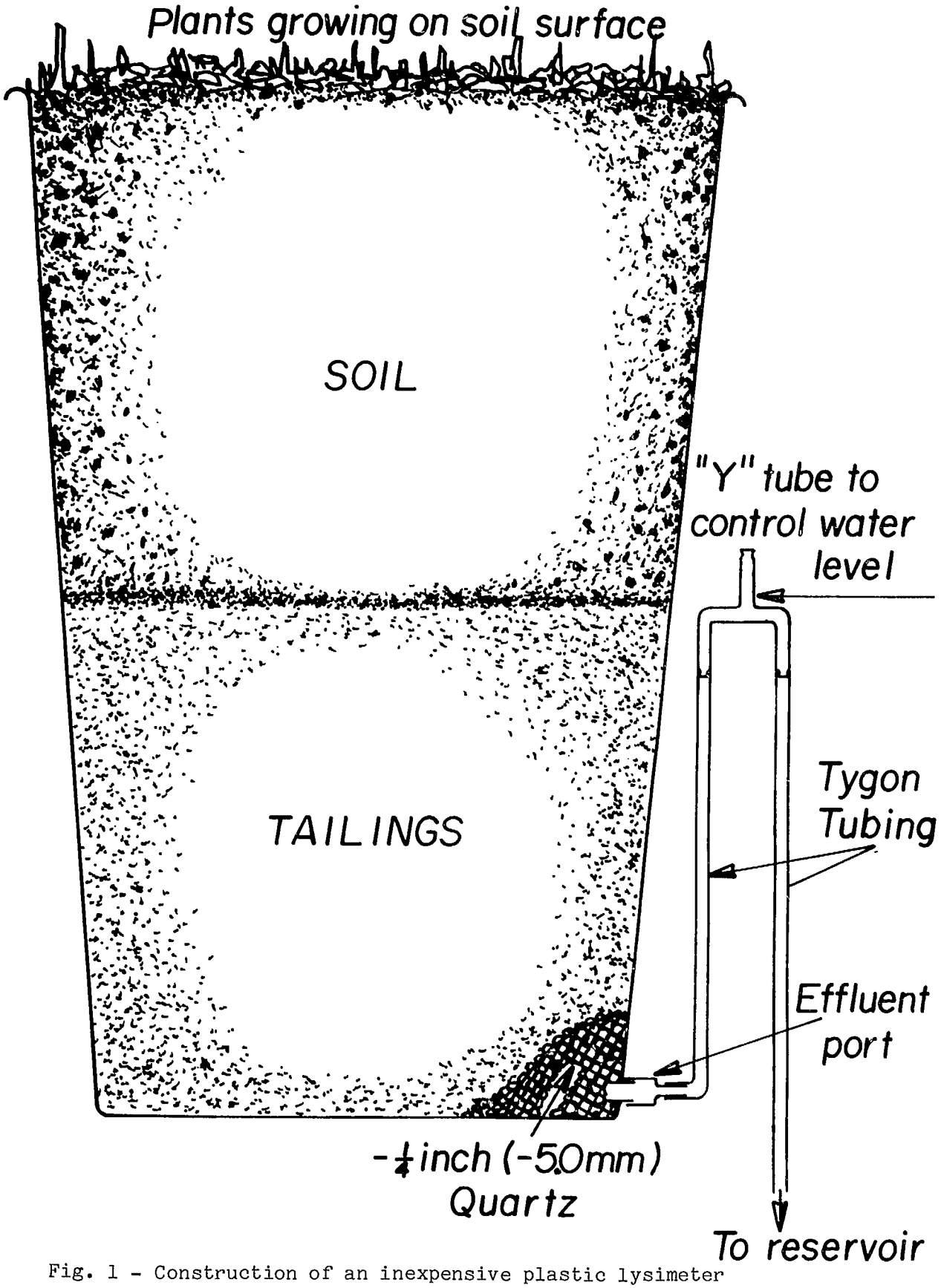

Fig. 1 - Construction of an inexpensive plastic lysimeter

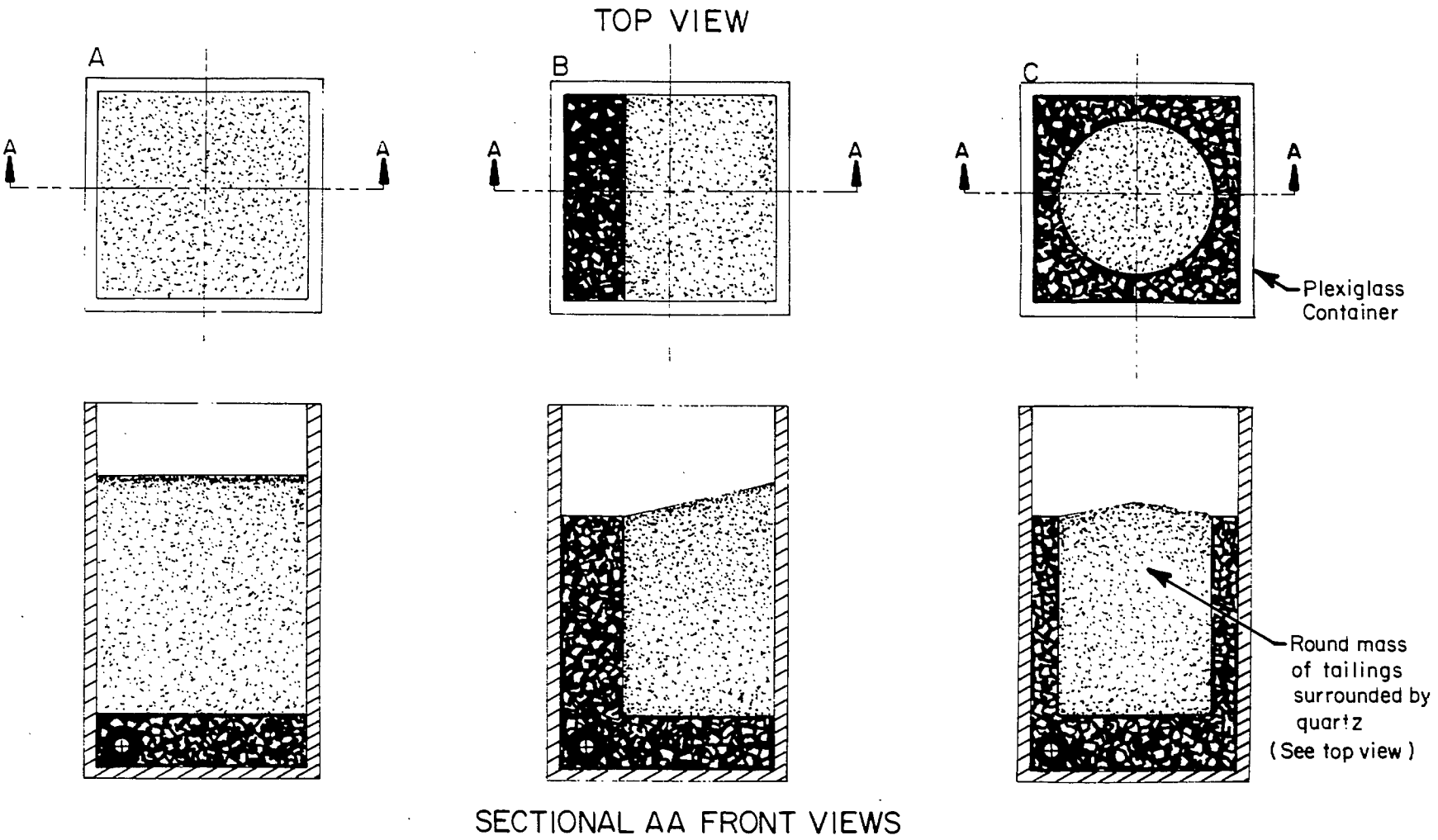

Fig. 2 - Construction details of a square plexiglass lysimeter used for determining the leaching characteristics of tailings deposited by different methods. Each lysimeter is 30 cm square and 63 cm in height, and contains a 10 cm base of -1/4 in. (-5.0 cm) quartz. The tailings mass contained in each lysimeter is: A-53 kg; B-39 kg; C-29 kg

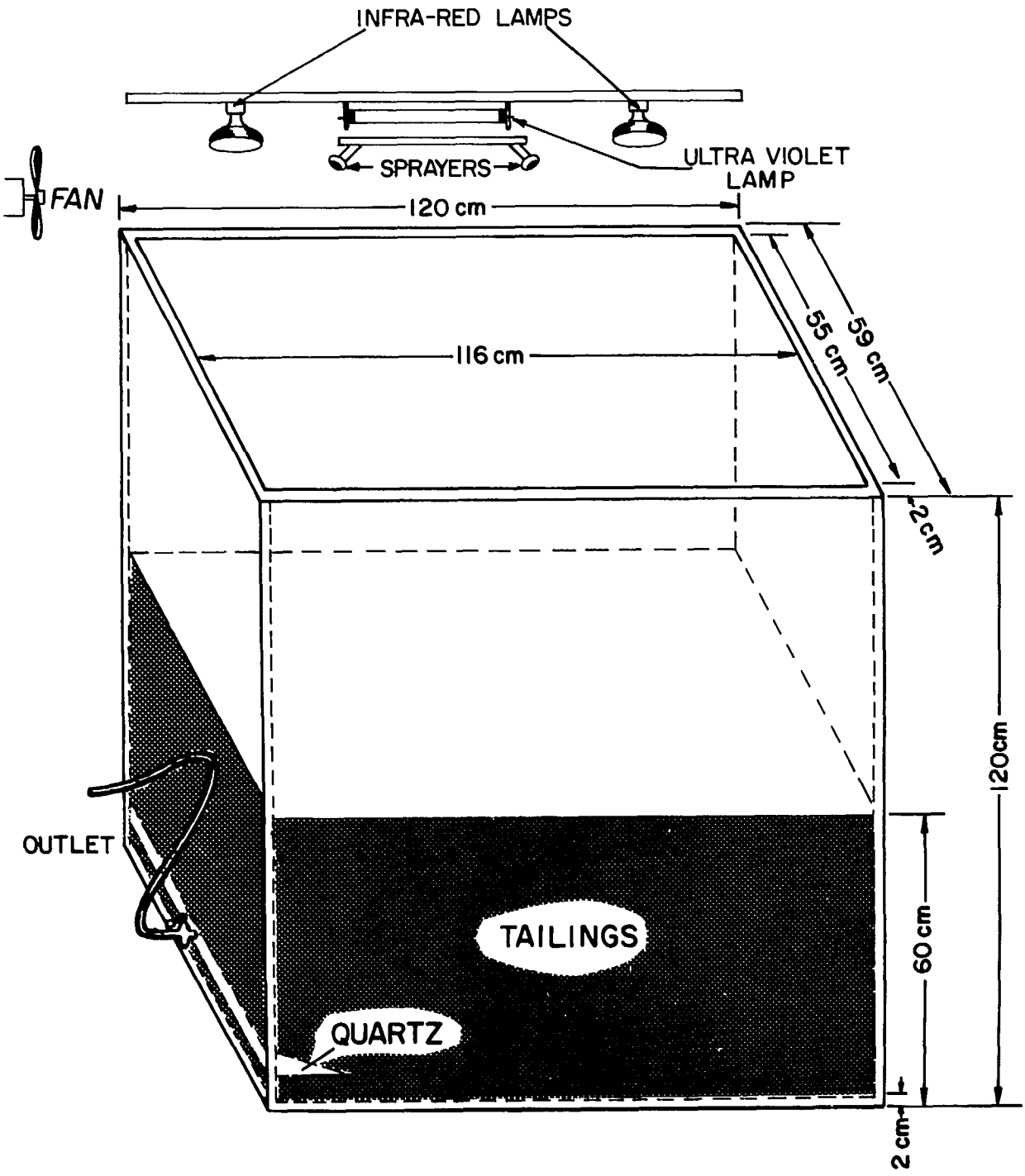

Fig. 3 - Construction details of a large plexiglass lysimeter capable of containing one tonne of tailings

used to obtain soil cores. Compression of the soil core samples can be minimized by using core samplers of large diameter fitted with detachable cutting heads. Continuous sampling of soil and tailings profiles to depths not exceeding one metre can be accomplished using split-spoon core samplers, or a metal pipe inserted manually. Deeper continuous or semi-continuous samples may be obtained to depths of more than 10 m using metal pipe, inserted into the soil or tailings through a hollow stem auger using a drop hammer. As tailings are often inhomogeneous, core length is limited to the depth that the metal pipe can be advanced without simply pushing the sample through the tailings. Multiple samples must then be obtained by collecting a limited depth of sample, withdrawing the samples, boring a hole into the tailings to the depth of the previous sample, and collecting another sample. Aluminum tubing is useful for collecting core samples, because it can be easily cut with a pipe cutter to yield samples of uniform volume.

Augers and fence-post-type borers are often used to obtain soil samples; although these are easy to use, they do not preserve the original soil structure. Poorly decomposed peat is sampled using commercially available, auger-type samplers with sharp cutting edges; well-humified peat may be sampled using a piston-type instrument described by Tibbets (22).

4.0 PHYSICAL ANALYSIS OF SOILS AND TAILINGS

4.1 PARTICLE-SIZE DISTRIBUTION OF ORGANIC SOILS

The particle-size distribution of a soil is a description of the proportion of the various sizes of particles present. It is one of the most stable of soil characteristics, being minimally modified by cultivation, and is used as the basis of soil textural classification. Because soil particles are not perfectly spherical but tend to be anisometric, different methods of analysis yield different analytical results. Thus, the results of particle-size measurements must always be accompanied by notation of the method used. The method of Dinel and Lévesque (23) is suitable for organic soils, yields reproducible results, and uses equipment (Fig. 4) that is easily constructed from readily available supplies.

Place 25 g of moist soil samples broken into small pieces in 300 mL of distilled water and agitate for 16 h. Determine the water content by drying at 105°C and reweighing. Resuspend the sample in 300 mL of distilled water and pour into a 200-mesh-screen sieve. Wash with water to remove material finer than 200 mesh. Dry at 105°C and weigh. Place the retained material on the top sieve of a series of sieves of mesh size 10, 20, 40, 100, and 200 assembled in a cylinder with a hole near the base for the entry of air and the outlet of water. Fill the cylinder with water to just above the level of the screen in the top (10 mesh) sieve. Bubble air through the cylinder for 1 h at a rate sufficient to agitate the nest of sieves. Remove the sieves, collect the material, dry each fraction at 105°C, and weigh. Calculate the proportion of the sample in each size range as a percentage of the dry weight of the total sample.

4.2 DEGREE OF DECOMPOSITION OF THE ORGANIC MATERIAL OF SOILS

Soils are composed of inorganic materials such as minerals originating in surrounding rocks, and organic materials. The organic matter consists of mixtures of plant, animal, and microbial residues in various stages of decomposition, as well as substances synthesized chemically and biologically from breakdown products (24). The extent to which this organic matter is decomposed is important in the classification of soil type; it also affects the availability of organic compounds for interaction with the inorganic constituents of soils and the nutrient status for soil microorganisms. The degree of decomposition of soils may be defined according to the rubbed and unrubbed fiber content (25), the extent of humification (by determining the methoxyl content with sodium pyrophosphate) (26), or by visual examination according to the method of von Post (27) as modified by Nygard (28).

4.2.1 Fiber Content of Unrubbed Soil

The fiber content provides a measure of the degree of decomposition of organic soil material. Place ≈25 mL of a moist soil sample on a strip of paper towelling and roll into a cigar shape. Squeeze lightly to remove surplus water. Remove sample from towelling and cut into cylinders ≈6-mm long.

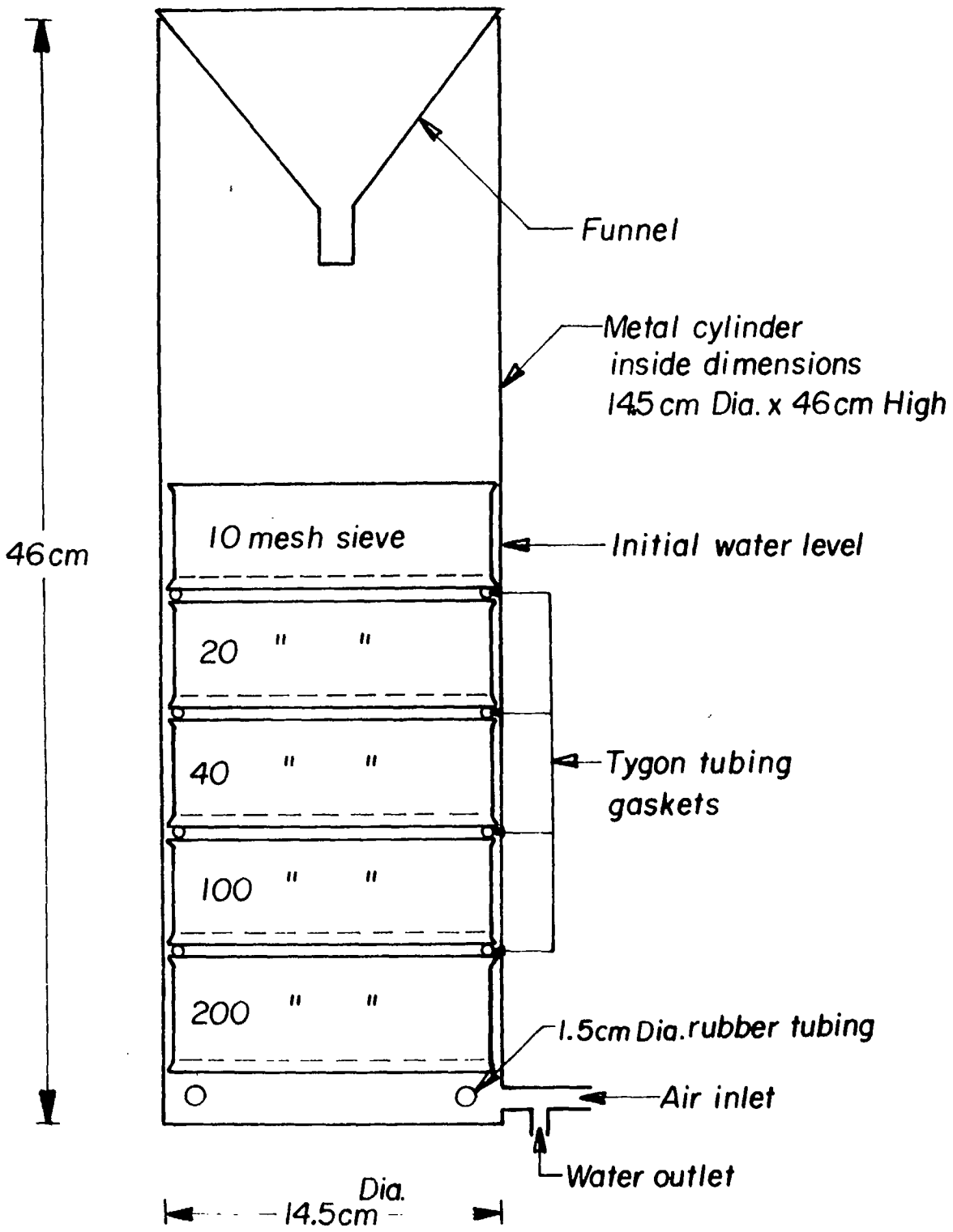

Fig. 4 - Construction details of an apparatus for particle-size distribution analysis

Mix thoroughly in a beaker, and pack a 5- or 10-mL plastic hypodermic syringe, modified by cutting away half of the cylinder wall in a longitudinal direction, between the 0- and 5-mL marks (Fig. 5). Press the sample into the syringe to release excess air, but not water, to exactly the 5-mL capacity limit with the syringe piston. Transfer the soil material onto a 100-mesh sieve and wash with flowing water, until the water passing through the sieve appears clear against a white background. Collect the sample, remove excess moisture with paper towelling, and transfer into the modified syringe. Pack the sample into the smallest volume by simultaneously pushing on the syringe piston and levelling the surface with a spatula, ensuring that the water content is the same as the initial sample. Record the volume of the sample in the syringe and determine the proportion of the unrubbed fiber as a percentage of the initial volume, 5.0 mL.

4.2.2 Fiber Content of Rubbed Soil

Transfer the sample of unrubbed fiber into a 100-mesh sieve, then rub lightly between the thumb and fingers under a stream of water until the water passing through the sieve appears clear against a white background. Continue until the fibers roll between the thumb and fingers like fragments of string, without gliding or smearing. Collect and dry the sample in paper towelling. Transfer the sample into the modified syringe and pack into the smallest volume by simultaneously pushing on the syringe piston and levelling the surface with a spatula, ensuring that the water content is the same as the initial sample. Record the volume of the sample in the syringe and determine the proportion of the rubbed fiber as a percentage of the initial volume, 5.0 mL.

4.2.3 Pyrophosphate Index

The sodium pyrophosphate solubility index is an estimation of the degree of humification of organic soil. To 0.5 g of air-dried soil in a 125-mL Erlenmeyer flask, add 50 mL of 0.025 M sodium pyrophosphate (prepared in distilled water) and agitate for 18 h. Filter through Whatman 2V prepleated filter paper, dilute 1:4 with distilled water, mix, and determine the optical density at 550 nanometres. Calculate the pyrophosphate index by multiplying the optical density by 100.

4.2.4 Degree of Decomposition of Peat

Nygard's modification (27) of the method of von Post (28) designates the degree of decomposition of peat. The classification of Nygard is divided into 5 categories as opposed to 10 of von Post; it is determined by squeezing a handful of wet organic material, then observing the material and expressed liquid. The five stages of decomposition recognized by this method are:

a) very poor - undecomposed to very slightly decomposed peat. Liquid removed is clear, colourless to slightly turbid; no peat substance oozes between the fingers; plant structure is intact.

b) poor - slightly decomposed peat. Liquid is very turbid; some peat oozes between fingers; plant structure easily identifiable.

c) medium - moderate to moderately well decomposed peat. Liquid is very turbid; about one-third of peat substance oozes between fingers; residue is mushy and friable; plant structure easily recognized.

d) well - well-decomposed peat. One-half to two-thirds of peat substance passes between fingers like mush (porridge); residue friable; and plant structure of more resistant remnants easily recognized.

e) very well - muck derived from almost fully decomposed peat with no plant structure recognizable. Nearly all mass passes between the fingers like a mush.

4.3 BULK DENSITY

The bulk density (D_b) of a soil is the ratio of the mass-to-volume of an oven-dried sample. It is useful in converting the water content as a function of weight-to-content by volume, for calculating porosity, and for estimating the weight of very large volumes of soil. The most commonly used methods of measuring soil bulk density are the "clod" and "core" methods. Although the clod

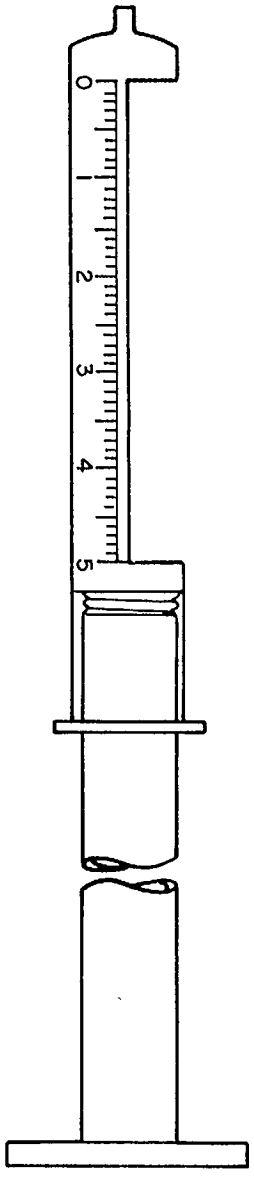

Fig. 5 - Modified plastic hypodermic syringe used to determine the fiber content of soil

methods usually yield greater bulk density values in poorly compacted soils, they are applicable to a wider variety of soils, including those that are cemented or stony. One of the most convenient methods is that of Brasher et al. (29), described by Sheldrick (25), because it does not require specialized apparatus. It is based on Archimides' principle: the density of a soil mass is calculated from the weight difference of this mass when weighed in air as compared to its weight in water. The bulk density is expressed in grams per cubic centimetre. Multiplication by 62.4 converts this expression to pounds per cubic foot.

To clods of soil of 50- to 200-mL volume each, attach a weighed nylon string and identification tag (W1). Dip the sample (in a fume hood) in Saran (Dow Saran Resin 310): methyl ethyl ketone (1:8), withdraw rapidly, and allow to dry for 30 min. Weigh (W2). Repeat the coating procedure until a thin continuous coat of resin forms on the clod surface, then record the number of coatings (N). Weigh the sample in air (W3) and in water (W4). Oven dry the clod for three days, gradually increasing the temperature from 50°C to 105°C. Cool to room temperature and weigh (W5). Recoat the clod by dripping several times in the Saran:methy lethyl ketone as before, allow to dry for 30 min, then weigh in air (W6) and in water (W7). Break the clod apart and separate all material larger than particle size of 2 mm by selving with a 10-mesh screen. Wash the coarse material on the screen with water, dry and weigh (W8). Determine the volume of the coarse material (W9) by measuring the displacement of water in a graduated cylinder.

Calculation 1. Weights and volumes of Saran coatings applied before oven drying

$$\text{Air-dried weight} = A1 = \frac{W3-W2}{N-1} + W3-W2 \qquad \text{Eq 6}$$

$$\text{Oven-dried weight} = A2 = 0.9\ A1 \qquad \text{Eq 7}$$

$$\text{Air-dried volume} = V1 = \frac{A2}{1.3} \qquad \text{Eq 8}$$

$$\text{Oven-dried volume} = V2 = \frac{A2}{1.3} \qquad \text{Eq 9}$$

Calculation 2. Weights and volumes of Saran coatings applied after oven drying

$$\text{Air-dried weight} = A3 = W6-W5 \qquad \text{Eq 10}$$

$$\text{Air-dried volume} = W3 = \frac{A3}{1.3} \qquad \text{Eq 11}$$

Calculation 3. Net weight of clods corrected for Saran and nylon string weights

$$\text{With field moisture} = A4 = W3-A1-W1 \qquad \text{Eq 12}$$

$$\text{Oven-dried} = A5 = W5-A2-W1 \qquad \text{Eq 13}$$

Calculation 4. Net volume of clods corrected for Saran volume

$$\text{With field moisture} = V4 = W3-W4-V1 \qquad \text{Eq 14}$$

$$\text{Oven-dried} = V5 = W6-W7-V2-V3 \qquad \text{Eq 15}$$

Calculation 5. Bulk density

$$\text{With field moisture} = Dbm = \frac{A5}{V4} \qquad \text{Eq 16}$$

$$\text{Oven-dried} = Dbod = \frac{A5}{V5} \qquad \text{Eq 17}$$

Calculation 6. Field moisture (water) content

$$\text{Per cent by weight} = Mc = \frac{A4-A5}{A5} \times 100 \qquad \text{Eq 18}$$

$$\text{Per cent by volume} = Mc \times Dbm \qquad \text{Eq 19}$$

Calculation 7. Clod bulk density corrected for coarse fragment content

$$\text{With field moisture} = Dbm(c) = \frac{A5-W8}{V4-W9} \qquad \text{Eq 20}$$

$$\text{Oven-dried} = Dbod(c) = \frac{A5-W8}{V5-W9} \qquad \text{Eq 21}$$

4.4 WATER AVAILABILITY

The availability of water to support the growth of plants is directly related to the ability of a soil to absorb and retain water. The method for the determination of the water content is a simple gravimetric comparison of fresh and oven-dried soil, as described by Sheldrick (25). The available moisture content is determined by the difference between the field-water retention capacity and the water content at the wilting point, these being the measurements of the upper and lower limits of available water. Field-water retention capacity is determined in lighter sandy soils at 1/10 bar (1.5 psi) and in heavier soils, such as clay loams, at 1/3 bar (5 psi). Field-water retention capacity can be measured by a simple proce-

dure to determine the weight of water retained by an oven-dried sample of soil, according to a method suggested by K.C. Ivanson (30). The water-retention capacity is determined by the method of Richards (31), as described by Sheldrick (25), using a commercially available, pressure-plate apparatus (Fig. 6) such as that manufactured by Soilmoisture Equipment Co., Santa Barbara, California.

4.4.1 Water Content

The water content can be determined per unit mass without determining the bulk density of the soil; however, the water content per unit volume requires that the bulk density be determined (Section 4.3, Calculation 5).

In a tared (W1) weighing vessel (i.e., beaker), add a moist soil sample and weigh (W2). Heat at 105°C for three days, cool in a desiccator, and reweigh (W3). Calculate the percentage of water by weight (θ_w) using the formula:

$$\theta_w = \frac{W2-W3}{W3-W1} \times 100 \qquad \text{Eq 22}$$

or calculate the presence of water by volume (θ_v) using the formula:

$$\theta_v = \theta_w \times Dbm \qquad \text{Eq 23}$$

4.4.2 Water-Retention Capacity

The method described is a simplification of the much more complex procedure of determining soil water desorption curves by tension. Place ≈5 mL of moist soil sample into a metal, rubber, or plastic retainer ring (1-cm high and 4 cm in diametre); set the ring on a porous ceramic plate fitted with a plastic diaphragm. Place the plate in a container of water such that the sample absorbs water by capillary action. Cover with a plastic sheet and let stand for ≈18 h. Remove excess water, place the plate in a pressure filter, and apply pressure at 1, 4, or 15 atmospheres (15, 60, or 225 psi) for 24 h. Apply a 4-psi pressure differential above the samples until water expression ceases (6-10 days), remove the samples, transfer to tared beakers (W1), and weigh (W2). Oven dry at 105°C for three days and reweigh (W3). Calculate the water content as a percentage of oven dry weight by the formula:

$$\text{Water retention capacity at X atmospheres} = \frac{W2-W3}{W2-W1} \times 100 \qquad \text{Eq 24}$$

The water retention capacity at the wilting point is determined at 15 atmospheres of pressure.

4.4.3 Field Water Retention Capacity

The field water retention capacity of a soil is the water content of a saturated soil at atmospheric pressure.

Place filter paper into a conical funnel and moisten the filter paper with water until saturated. Add 100 g (accurately weighed) (W1) of oven-dried soil (soil dried at 105°C for three days) into the funnel. Measure or weigh precisely 1 L (1000 g) of water and pour through the soil, recovering the eluate into a tared (W2) flask. Pass the eluate through the soil three more times, or until no more water is absorbed. Measure the volume of the eluate accurately by weighing (W3) and calculate the field-water retention capacity by the equation:

$$\text{\% Field water retention capacity} = \frac{1000 - (W3-W2)}{W1} \times 100 \qquad \text{Eq 25}$$

4.5 CARBON-TO-NITROGEN RATIO

The ratio of the carbon and nitrogen contents of soils is of value in the classification of fertility. C:N ratios range from less than 10 in very rich soils to greater than 50 in nitrogen-poor, upland peats. Nitrogen is not limiting at C:N ratios of less than 10, the average C:N ratio of microbial cells. At higher C:N ratio values, the rate of decomposition of carbon compounds will be related to the exogenous nitrogen supply.

The carbon:nitrogen ratio of a soil is determined by measuring the total carbon and the total nitrogen contents of the soil. The optimum method of carbon analysis is made with an induction furnace (LECO). Alternatively, the organic carbon is

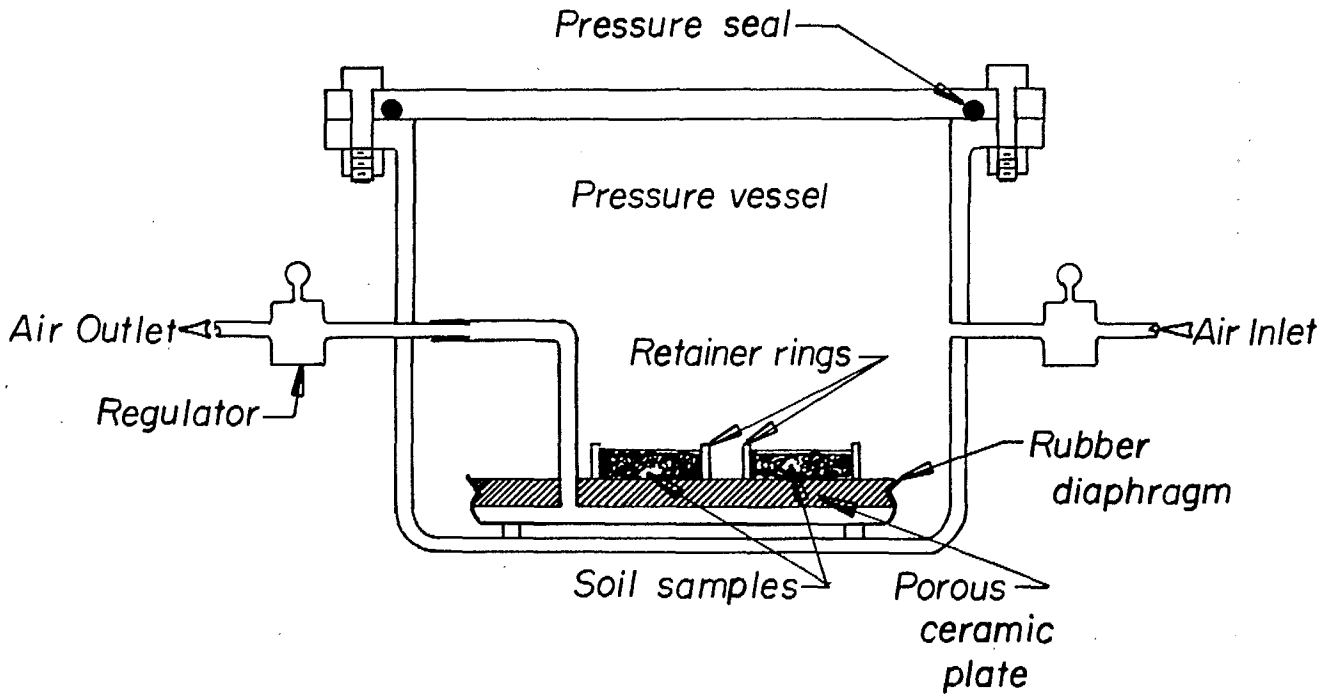

Fig. 6 - Pressure plate apparatus for the determination of water retention capacity of soils

determined by potassium dichromate reduction, either by titration with ferrous sulphate (25) or by colorimetry (32).

4.5.1 Total Organic Carbon Determination by Wet Oxidation and Ferrous Sulphate Titration

To 0.1-2.0 g of dried, crumbled soil samples in a 1-L flask, add 10 mL of 0.1 N potassium dichromate solution that has been prepared by dissolving 49.04 g of $K_2Cr_2O_7$, previously dried for 1 h at 105°C in 1 L of distilled water. In a fume hood, add 20 mL of concentrated (>96%) sulphuric acid, stirring vigorously with a magnetic stirrer. Mix by swirling by hand for 1 min and let stand on a sheet of asbestos for 30 min. If the solution is green in colour, add 1 mL of the potassium dichromate solution and 2-mL aliquots of concentrated H_2SO_4, until the green colour disappears. Record the volume of potassium dichromate added (A). Add 500 mL of distilled water, 10 mL of 85% phosphoric acid (H_3PO_4), and 1 mL of 0.16% barium diphenylamine sulphonate indicator dissolved in distilled water. Stir with a magnetic stirrer, then titrate rapidly from a burette with standardized 0.5 N ferrous sulphate dissolved in 4% sulphuric acid, until the liquid in the beaker is purple or blue. Continue titration slowly until the colour flashes to green, and record the volume of titrant used (B).

The true normality of the titrant (N) is determined by titrating 10 mL of 1 N $K_2Cr_2O_7$ standard in 500 mL of distilled water, 10 mL of 85% phosphoric acid and 1 mL of 0.16% barium diphenylamine sulphonate indicator with the ferrous sulphate solution. The percentage of organic carbon of the sample is calculated by dividing the number of mL of dichromate reduced, by the weight of the sample (W) in grams, and multiplying by the empirical factor 0.4, according to the equation:

$$\% \text{ Organic carbon in sample} = \frac{A-(BN)}{W} \times 0.4 \qquad \text{Eq 26}$$

$$\% \text{ Total organic matter} = \% \text{ organic carbon} \times 1.732 \qquad \text{Eq 27}$$

If more than 16 mL of titrant is required to determine the amount of dichromate not reduced, decrease the size of the original sample and repeat.

4.5.2 Colorimetric Determination of Total Carbon

To a 2- to 10-mg dried soil sample (accurately weighed) in 2 mL of distilled water, add 5.0 mL of dichromate solution (prepared by dissolving 5 g of sodium dichromate in 20 mL of distilled water and diluting to 1 L with concentrated sulphuric acid). To compensate for colour imparted by the soil sample, prepare a blank by adding 5 mL of concentrated sulphuric acid to an identical mass of dried soil sample in 2 mL of distilled water. The blanks and samples are heated in a boiling water bath for 20 min, cooled to room temperature, and the optical density (O.D.) at 660 nanometres is determined with a spectrophotometer or a colourimeter fitted with a red filter. The concentration of carbon in the sample is determined by subtracting the optical density of the sample from the optical density of the blank; the resultant O.D. is then compared to a standard curve, which was prepared using solutions of an anhydrous sugar (i.e., dextrose) that contained 0.5 to 1 mg of carbon per 2 mL.

4.5.3 Organic Nitrogen Determination

Organic nitrogen may be determined either by direct nesslerization or by distillation followed by titration. The latter method is more accurate due to the larger volumes used, thus decreasing the volume ratio factor (33). Accurately weigh 1 to 5 g of dried soil sample (A) and place in a 500-mL Kjeldahl flask. Add 20 mL of concentrated H_2SO_4, 13.4 g of K_2SO_4, and 3 mL of mercuric sulphate solution [8 g of mercuric oxide (HgO) dissolved in 50 mL of 16% sulphuric acid and diluted to 100 mL with distilled water]. Mix thoroughly and digest by boiling until frothing ceases, and continue boiling for 30 min after the liquid becomes clear. Cool and dilute to 350 mL with ammonia-free (distilled) water. Add disodium phenolphthalein indicator [0.5% in distilled water, or 0.5% in 50% ethyl, or isopropyl alcohol]; also add a sodium hydroxide-sodium thiosulphate solution [500 g of NaOH and 25 g of $Na_2S_2O_3.5H_2O$ in 1 L (final volume) of distilled water] until just alkaline. Distill into 50 mL of indicating boric acid solution [20 g of H_3BO_3 dissolved in 100 mL of water and 10 mL of mixed indicator (2 volumes of 0.2% methyl red in 95% ethyl alcohol, plus 1 volume of 0.2% methylene blue in 95% ethyl alcohol) diluted to 1 L with distilled water], with

the condenser tip below the surface of the boric acid solution until ≈200 mL of distillate has been collected. Titrate with 0.02 N H_2SO_4 until the indicator turns a pale lavender colour (B). Repeat the titration on a blank comprising an identical volume of the indicating boric acid solution diluted to 250 mL with distilled water (C). Calculate the percentage nitrogen in the sample using the equation:

$$\% \text{ Nitrogen in sample} = \frac{(B-C) \times 0.028}{A} \qquad \text{Eq 28}$$

The carbon:nitrogen ratio is determined by calculating the ratio of the percentage of carbon in the sample to the percentage of nitrogen in the sample.

4.5.4 Loss-on-Ignition

Weight loss of a sample heated to 550°C is approximately equal to the total amount of organic matter present in the sample, and does not include structural water or carbonates (25). To a tared porcelain or Vycor crucible, add 1-10 g of sample that has been passed through a 2-mm (10 mesh) screen. Dry for ≈18 h at 105°C and weigh to determine the oven-dried weight of the sample (A). Place the crucible containing the sample into a muffle furnace, increase the heat slowly (≈2°C/min) to 550°C, and maintain at that temperature for ≈18 h. Remove crucible, cool in a desiccator, remove, and weigh (B). Calculate the loss on ignition at 550°C by the equation:

$$\% \text{ Loss on ignition (550°C)} = \frac{A-B}{A} \times 100 \qquad \text{Eq 29}$$

The loss on ignition at 550°C will include virtually all of the organic material in the sample, but may also include some of the more volatile complex sulphide minerals. The residue will contain most of the structural water and virtually the total mineral content.

4.6 CATION EXCHANGE CAPACITY

The ability of a soil to store cations, which can be liberated for use by plants and microorganisms, can be determined by measuring the cation-exchange capacity. Cation exchange is a reversible reaction in which the cations associated with soil minerals and organic materials are reversibly replaced with cations of salt and acid solutions. Salts of ammonium, barium, calcium, potassium, or sodium can be used for determining the cation-exchange capacity of soils. Barium is recommended by the Association of Official Analytical Chemists (21) because it reacts more completely with the exchangeable cations, including hydrogen and aluminum. Soils containing high concentrations of organic matter, clay minerals and calcite are also more accurately determined with barium acetate than with ammonium acetate, since ammonium may be trapped by clay minerals such as vermiculite, may be absorbed by organic matter in soil, or may be metabolized by soil microorganisms. The method described by Sheldrick (25) is presented below.

This method of determining the cation-exchange capacity of soils, by titration of barium acetate with sodium hydroxide, does not require the use of atomic absorption spectrophotometry and can be used on soils containing appreciable concentrations of sodium, calcium, and magnesium.

Add 0.5 to 2.0 g of accurately weighed (A) dried soil to a 250-mL Erlenmeyer flask, add 50 mL of 0.5 N HCl, seal the flasks with parafilm, and shake for 30 min. Filter through Whatman #41 filter paper in a large funnel, then wash with water until all the chloride is removed from the soil. This is determined when 1-mL samples do not become cloudy when added to 0.1 mL of a 1% silver nitrate solution. Discard the filtrate. Puncture the filter paper and wash the sample into a flask, using exactly 100 mL of 0.5 N barium acetate (63.86 mL of barium acetate in 1 L of distilled water). Shake the flask for 15 min and filter through Whatman #41 filter paper into an Erlenmeyer flask. Wash the sample with three successive 100-mL portions of distilled water and add to the filtrate. Add 0.3 mL of phenolphthalein (1% phenolphthalein in 95% ethyl alcohol) and titrate to a pink colour with 0.1 N NaOH, the exact normality (N) of which has been previously determined by titration against 10 mL of 0.1 N potassium phthalate standard. Record the volume of titrant used (B). Calculate the cation-exchange capacity in milliequivalents per 100 g by the equation:

$$\text{CEC (meq/100 g)} = \frac{BN}{A} \times 100 \qquad \text{Eq 30}$$

4.7 pH

The hydrogen ion activity or pH is one of the most important chemical properties of a soil for plant growth (16). Because of its accuracy and convenience, pH is measured with universally available pH metres fitted with glass electrodes. Soil pH values vary with the methods of preparation of a sample; the water, salt, and CO_2 content; and normal variations from sample to sample. Thus, these factors must be stated when pH values are reported. Dilution of most soils from a thick paste to a 1:10 (soil:water ratio) suspension causes increases in pH values from 0.2 to 0.5 pH units, but increases of more than one unit have been observed in certain alkaline soils. Determination of pH values at 1:10 dilution has the advantage that the interference due to the cation exchange capacity, especially for sodium-saturated soils, is eliminated; pH values determined at 1:10 (soil:water) are termed the hydrolytic pH values. Most commonly, however, soil pH is determined in 1:2 (soil:water) suspensions. Interference due to the variability of salt contents of soils can be eliminated by determining pH values in soil suspended in salt solutions. However, the pH values of some soils measured in 1 N KCl may be as much as 2 pH units lower; the pH values measured in 0.01 M $CaCl_2$ are always approximately 0.5 pH units lower than when measured in water.

To measure the pH of a soil, add sufficient water to a soil sample or a composite soil sample to yield the desired soil:water ratio. Stir for at least 1 h and determine the pH value with a glass electrode pH metre.

4.8 POROSITY

The porosity of a soil influences the movement of liquids and gasses, the development of root systems, soil strength, and heat transfer. For materials that do not absorb water, such as ion exchange resins or tailings, the pore volume can be determined by weighing the quantity of water that occupies the void volume of the test material contained in a column (34). For materials such as humified soils that do absorb water, the porosity (the percentage of the bulk volume not occupied by solids) is determined using the difference between the bulk and particle densities as a percentage of the particle density. The procedures for determining porosity of soil samples are described by Vomocil (35).

4.8.1 Porosity of a Tailings Sample

The pore volume of a given volume (V) of a tailings sample, or a sample of material that does not absorb water, can be determined by packing a glass column with a known volume of material, then adding water until the level coincides with the upper surface of the material. The water is drained into a tared beaker, the remaining water being recovered by flowing air through the column. The amount of water recovered is determined by weighing (A). The porosity is then determined using the equation:

$$\% \text{ Pore volume} = \frac{A}{V \times 0.95} \times 100 \qquad \text{Eq 31}$$

with a correction made for the absorption of water on the particle surfaces.

4.8.2 Porosity of a Soil Sample

The determination of the porosity of a sample which absorbs water requires that the values of both the bulk density and the average particle density be known. Bulk density is determined as described in Section 4.3. Particle density is determined by the method of Blake (20).

To a tared 100-mL-volumetric flask (W1), add approximately 50 g of soil sample and reweigh (W2). Subtract the water content of the sample determined by drying a duplicate sample at 105°C for three days (W3). Add distilled water, ensuring that all entrapped air is expelled by heating gently, cooling, and adjusting the water level to the mark. Weigh (W4) and record the temperature of the contents. Discard the contents of the flask, wash and refill with distilled water, and weigh (W5). Calculate the particle density (D_p) in g/cm^3 using the formula:

$$D_p = \frac{d_w (W2-W3-W1)}{(W2-W3-W1) - (W4-W5)} \qquad \text{Eq 32}$$

where d_w is the density of water at the observed temperature.

The porosity (S_+), a percentage of the bulk volume not occupied by solids, is calculated by the formula (35):

$$S_+ = \frac{D_p - D_b}{D_p} \times 100 \qquad \text{Eq 33}$$

5.0 MICROBIOLOGY OF SOILS AND TAILINGS

Bacteria are present in soils and tailings deposits in larger numbers than any other form of life, although they frequently comprise only one-quarter of the living biomass (13), due to their small size. Because of their small size and high metabolic rates, they have a large influence on the chemistry of their environment, decomposing organic materials as well as transforming the organic and inorganic chemical constituents. In tailings, bacteria are the principal catalytic agents in the transformation of minerals. Pyrite and other sulphide minerals are oxidized by the iron-oxidizing bacteria to soluble and insoluble metal sulphates, sulphuric acid, and ferric iron (1). The ferric iron can oxidize sulphide minerals with the generation of elemental sulphur, which can be further oxidized by the sulphur-oxidizing bacteria to sulphuric acid. Under anaerobic conditions and in the presence of suitable organic carbon compounds, sulphate can be reduced to sulphide by the sulphate-reducing bacteria.

The size of bacterial populations is dependent on the availability of nutrients, temperature, moisture and gas content, and the pH of the environment. Varying conditions result in wide temporal and spatial variations in the soil populations. Because of the diverse nutritional characteristics of bacteria in soils and tailings, bacterial populations cannot be assessed using one medium, but different media and cultural conditions must be used for each group of bacteria. The pour-plate method, which uses various media solidified with agar, is the procedure most widely used for the enumeration of bacteria in soil. Agar plates prepared in this way may be incubated in the presence of air to determine the concentration of aerobic bacteria; or, they may be incubated in the absence of air to determine the concentrations of anaerobic bacteria. For bacteria that grow poorly on solid media, enumeration must be accomplished using a dilution method, such as the most probable number (MPN) estimation using a suitable liquid medium.

5.1 ESTIMATION OF IRON-OXIDIZING BACTERIA AND SULPHUR-OXIDIZING CONCENTRATIONS

The locations of the iron-oxidizing bacteria in the tailings indicate areas of potential pyrite oxidation, and the concentrations of these bacteria indicate the influence of the environment on the population of these bacteria. The concentrations of the iron-oxidizing bacteria have been estimated using a 9-tube, most-probable-number (MPN) method (37) in which 0.1, 1.0, and 10 mL aliquots of soil or tailings slurries, or serial dilutions of these suspensions, are placed in sterile test tubes containing 4 mL of the 9K ferrous iron medium of Silverman and Lundgren (38) that contains (g/L): $(NH_4)_2SO_4$ - 3; KCl - 0.1; NaH_2PO_4 - 0.35; $MgSO_4$ - 0.5; $Ca(NO_3)_2$ - 0.01; $FeSO_4$ - 3.3; pH 2.5 adjusted with H_2SO_4. An incubation period of three weeks is used, with verification after five weeks to check for late positives. The medium can be modified to contain lower concentrations of $(NH_4)_2SO_4$, NaH_2PO_4, and $MgSO_4$. For the enumeration of sulphur-oxidizing bacteria, the same medium with 1 g/L sodium thiosulphate replacing the ferrous sulphate and an initial pH of 6.0 may be used.

Estimation of bacterial concentrations using the 9-tube MPN method may be made from Table 1.

5.2 ASSESSMENT OF AEROBIC AND ANAEROBIC HETEROTROPHIC MICROORGANISM POPULATIONS IN TAILINGS AND SOIL

The presence and concentrations of heterotrophic bacteria indicate areas containing metabolizable organic compounds. These organic compounds may arise as excretions from autotrophic bacteria (19); excretions from plants and algae (33); and products of organic (soil) detritus breakdown (39). The term "aerobic" is used to denote organisms capable of growing in the presence of oxygen, and the term "anaerobic" is used to denote organisms capable of growing in the absence of air; both terms include microorganisms that are both obligate and facultative.

Table 1 - Determination of the most probable number by using nine tubes with three samples of 10 mL, three samples of 1.0 mL, and three samples of 0.1 mL

10-mL tubes positive	1-mL tubes positive	Most probable number (cells/mL) 0.1-mL tubes positive			
		0	1	2	3
0	0	<0.03	0.03	0.06	0.09
0	1	0.03	0.06	0.09	0.12
0	2	0.06	0.09	0.12	0.16
0	3	0.09	0.13	0.16	0.19
1	0	0.04	0.07	0.11	0.15
1	1	0.07	0.11	0.15	0.19
1	2	0.11	0.15	0.20	0.24
1	3	0.16	0.20	0.24	0.29
2	0	0.09	0.14	0.20	0.26
2	1	0.15	0.20	0.27	0.34
2	2	0.21	0.28	0.35	0.42
2	3	0.29	0.36	0.44	0.53
3	0	0.23	0.39	0.64	0.95
3	1	0.43	0.75	1.2	1.6
3	2	0.93	1.5	2.1	2.9
3	3	2.4	4.6	11	>11

The total population of heterotrophic microorganisms is determined by the pour-plate method. This method uses suitable solid media such as nutrient agar or soil extract agar, in which 1.0 mL aliquots of soil or tailings suspensions (or serial dilutions thereof) are placed in sterile petri dishes. Melted sterile media at 40-45°C are then added to each petri dish, swirled to distribute the bacteria uniformly, and allowed to set. The plates used for the determination of aerobic bacteria are incubated aerobically, whereas the plates used for the determination of anaerobic bacteria are incubated in anaerobic jars. Moulds and yeasts are enumerated using a solid medium containing (in g/L): glucose - 10; peptone - 5; KH_2PO_4 - 1; $MgSO_4$ - 0.5; Rose Bengal - 0.033; agar - 20 incubated aerobically. Nitrogen-fixing aerobes are enumerated using a solid medium containing (in g/L): KH_2PO_4 - 5; $MgSO_4$ - 2; $CaSO_4$ - 1; $FeSO_4$ - 0.2; $MnSO_4$ - 0.2; MoO_3 - 0.1; KI - 0.1; mannitol - 10; agar - 20 incubated aerobically. The above media are sterilized prior to use by autoclaving at 121°C for 20 min.

Sulphate-reducing bacteria can be detected using a modification of the medium of Butlin et al. (40) (R.G.L. McCready, personal communication) containing (in g/L): K_2HPO_4 - 0.5; NH_4Cl - 1; Na_2SO_3 - 0.5; Na_2SO_4 - 0.5; $CaCl_2$ - 0.1; $MgSO_4$ - 2; yeast extract - 1; sodium lactate (70%) - 5 (mL); $FeSO_4$ - 0.002. The pH is adjusted to 7 with H_2SO_4 and the medium is sterilized by filtration instead of by autoclaving. The inoculated medium is incubated in completely filled, screw-cap test tubes. These bacteria can also be enumerated using a 9-tube MPN method, the roll-tube method of Hungate (41), or by the pour-plate method with incubation in anaerobic chambers.

All manipulations used in the assessment of microbial populations of soils and tailings must be conducted using correct microbiological techniques. All materials, media, and reagents must be sterile. Disposable petri dishes, pipettes and mechanical pipette tips, as well as test tubes may be purchased presterilized ready for use; all other glassware must be sterilized by autoclaving at 121°C for 30 min. Media are sterilized either by autoclaving or by filtration into sterile flasks. Sterile techniques must also be used when transferring samples and media.

5.3 RESPIRATION RATE

The respiration rate, a measurement of the metabolic activity of the heterotrophic bacteria in a soil sample, is based on the formation and evolution of carbon dioxide. The carbon dioxide is trapped in and neutralizes an alkaline solution, the residual alkali being determined by oxalic acid titration. The use of barium hydroxide is favoured over other bases because a dense white precipitate of barium carbonate forms, permitting rapid visual confirmation of the efficiency of carbon dioxide entrapment (42).

Determine the water content (Section 4.4.1) and the field water retention capacity (Section 4.4.3) of a soil sample. Accurately weigh a fresh soil sample so that it contains the equivalent of 25.0 g of oven-dried soil and place in the bottom of a 1- or 2-L Erlenmeyer flask. Add sufficient water to bring the moisture content to 70% of the field-retention capacity, then swirl to form a uniform layer of soil on the bottom of the flask. The depth of the layer of soil in the bottom of the flask should be ≈0.5 cm. Humidified air that is free of carbon dioxide is passed through the flask into a CO_2 trap containing solid soda lime or 20% NaOH solution (Fig. 7). After 24 h, pass the gas effluent from the reaction flask through three containers in series, each containing 100 mL of 0.1 M barium hydroxide. When the barium hydroxide solution is efficiently trapping the carbon dioxide that has evolved, the liquid in the first two containers will become cloudy, while the liquid in the third will remain clear.

After 24 h (T), transfer and combine the trapping solution into a 1-L Erlenmeyer flask, add 1 mL of phenolphthalein indicator (as prepared for the method described in Section 4.6), and titrate with 0.1 M oxalic acid standardized against 0.1 M phthalic acid standard. The exact molarity (M) and the volume of the titrant (V) are recorded. Replace the trapping solution with fresh 0.1 M barium hydroxide and repeat each day for 3-10 days. The respiration rate is calculated by the equation:

$$\text{mg } CO_3\text{/C evolved per gram per hour} = \frac{30\text{-}V\left(\frac{N}{0.1}\right)}{TW} \qquad \text{Eq 34}$$

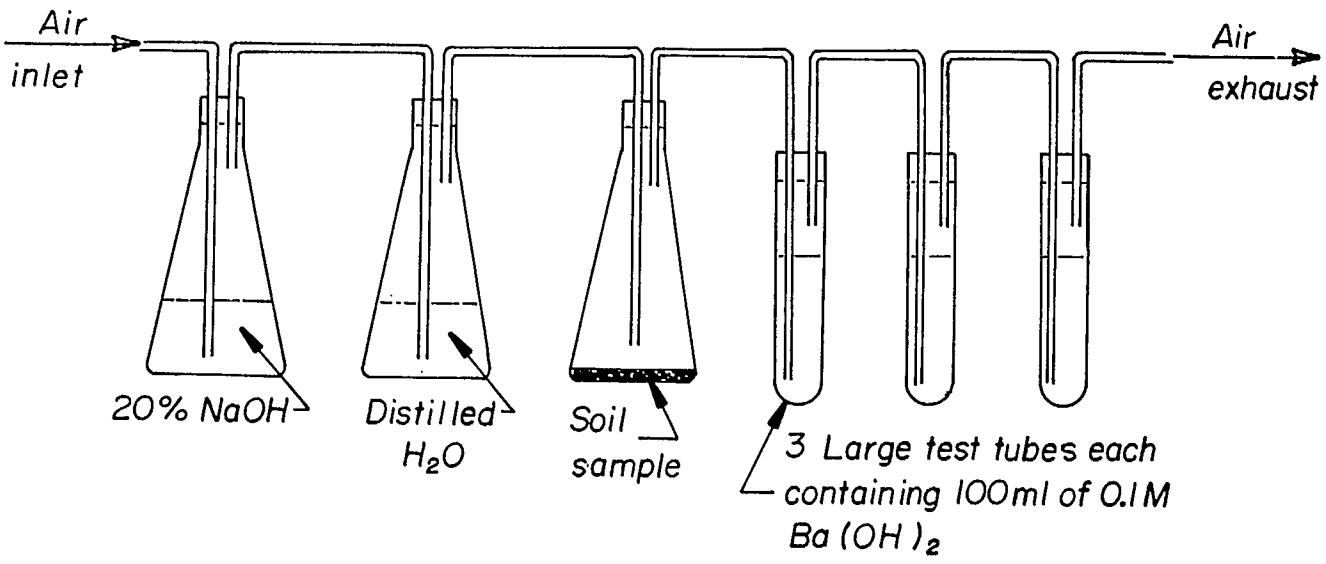

Fig. 7 - Apparatus for the determination of the respiration rate of micro-organisms in a soil sample

6.0 ANALYSIS OF ORGANIC CHEMICAL COMPOUNDS IN SOILS AND TAILINGS

Heterotrophic microorganisms use sugars, polymers of sugars, and organic acids as their substrates for growth, and all bacteria, both heterotrophic and autotrophic, excrete organic compounds. The bacteria exist in nature in symbiotic association, with the excretion products of one species often serving as the growth substrate for other species. For example, the autotrophic sulphur- and iron-oxidizing bacteria use CO_2 as a carbon source; they use inorganic sulphur and iron compounds, including minerals, as their energy sources, generating sulphuric acid and excreting hydroxy as well as keto acids such as lactate and pyruvate. Should these compounds migrate to anaerobic locations, they could be metabolized by the sulphate-reducing bacteria as their carbon and energy source, and simultaneously they will use sulphate as their terminal electron acceptor. Thus, detection and quantification of organic compounds are useful in elucidating the role of microorganisms in soils and tailings.

6.1 CARBOHYDRATES

Total carbohydrate concentration is determined in supernatents of tailings or soil slurries by the anthrone (9-oxyanthracene) method described by Umbreit, Burris, and Stauffer (32). A 3-mL aliquot is placed in a test tube, and 6 mL of anthrone reagent (0.2% anthrone in 95% H_2SO_4) is added and mixed immediately. The test tubes are placed in a boiling water bath for 3 min, cooled, and the optical density determined at 620 nanometres. The sugar content of the sample is determined by comparison of the O.D. value to that of a set of standards containing 10 to 120 µg of glucose.

Individual carbohydrates may be identified by paper, thin layer, or high-performance liquid chromatography. A dried soil or tailings sample is extracted with water-free pyridine, which dissolves the carbohydrates but not the salts, and an aliquot of the extract containing 20 to 50 µg in 2 to 10 µL is applied to Whatman #1 chromatography paper, or to microcrystalline cellulose or silica gel, thin-layer chromatography plates. After drying, the chromatograms are developed using appropriate solvent systems. Smith (43) describes several solvent systems that may be used for paper chromatography of carbohydrates. Two of the solvent systems recommended are propanol:ethyl acetate:water (7:1:2) and isopropanol:n-butanol:water (7:1:2). Similar procedures have been described for thin-layer chromatography (44) using solvent systems containing ethyl acetate:pyridine:water (12:5:4), isopropanol:n-butanol:water (5:3:2), and chloroform:acetic acid:water (6:7:1). For better resolution, chromatography in two dimensions using two different solvent systems may be employed. To locate individual carbohydrates, the dried chromatograms are sprayed with 0.1 M $AgNO_3$ in 5 N NH_4OH (44).

A high-performance liquid chromatography system that employs multiple ion-exchange columns connected in series (45), in which carbohydrates can be identified and quantified in a single operation, has been described.

6.2 ORGANIC ACIDS

Pyruvate is excreted by the iron-oxidizing bacteria (46) and, if not removed by other microorganisms, inhibits the oxidation of sulphide minerals (47,48). Pyruvate and other organic acids such as lactate, propionate, and acetate may arise from the catabolism of lignin, a polymer of phenylpropane units, and may serve as substrates for both aerobic and anaerobic bacteria. Their location, identification, and quantification will aid in the elucidation of the interactions of biological entities in soils and tailings. The colourimetric methods for total keto acids and total hydroxy acids are described by Umbreit, Burris, and Stauffer (32). These components are extracted from soils and tailings by distilled water.

Total keto acid concentration is determined using the method of Friedmann and Haugen, in which 1.0 mL of sample is incubated for 30 min with 1 mL of 0.1% 2-4 dinitrophenylhydrazine in 2 N HCl, then 1 mL of 20% NaOH and 7 mL of water are added. The optical density at 440 nanometres is determined and compared to O.D. of a set of standards containing 5 to 100 µg of sodium pyruvate.

Total hydroxy acid concentrations are determined by the method of LePage, by adding 0.5 mL of sample to 6 mL of sulphuric acid, mixing immediately, heating in a boiling water bath for 5 min, cooling to room temperature, adding 0.05 mL (1 drop) of

20% $CuSO_4.5H_2O$, and 0.05 mL (1 drop) of 1.5% p-hydroxydiphenyl in 0.5% NaOH, incubating for 30 min at 30°C, heating at 100°C for 2 min, and measuring the optical density at 565 nanometres. The O.D. of each sample is then compared to the O.D. values of a set of standards containing 3 to 30 mg of the zinc salt of lactic acid.

Organic acids can be identified by paper or thin-layer chromatography using a variety of solvent systems. Suitable solvents for paper chromatography on Whatman 54 paper include butanol:formate:water (7:3:12) or ethanol:ammonia:water (20:1:4) (42); solvents for thin-layer chromatography on silica gel plates include ethanol:chloroform:ammonium hydroxide:water (53:30.3:15.2:1.5) or ethanol:ammonium hydroxide:water (20:1:4) (49). For increased separation, chromatography in two dimensions can be used.

Aliphatic, keto, and hydroxy acids can be detected on dried paper and thin-layer chromatograms by spraying with one of several acid/base indicators, such as a 0.04% solution of bromphenol blue in ethanol adjusted to pH 5 with citric acid, or 0.03% methyl red in 0.05 N sodium borate buffer (pH 8.0), or with 0.05 M silver nitrate in 0.05 N sodium hydroxide.

Organic acids can also be separated and quantified by high-performance liquid chromatography (50) or by ion chromatography (51).

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