Use of Anaerobic Respirometers for Measuring Gas Production in Toxicity and Treatability Tests

by

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INTRODUCTION

Anaerobic processes are used widely for stabilizing domestic sludges, treating industrial wastes, and converting biomass to methane. These processes frequently receive complex organic chemicals that are classified as being hazardous or toxic. Despite advances in our understanding of anaerobic reactions, there is limited information regarding the impact of toxic chemicals on the kinetics of anaerobic reactions. One means of determining these impacts is to measure the volume and rate of gas produced in anaerobic reactors. Laboratory tests designed for this purpose usually are based on the use of small bench-scale reactors, typically 50 to 500 mL, containing anaerobic cultures that are dosed with various amounts of test waste or chemical. The decrease in the rate or volume of gas production indicates the adverse effect of the test substance. The challenge of such tests is to measure the gas production accurately and precisely. Previous means have included use of volumetric displacement devices, wet test meters, calibrated pressure manometers, or manual removal with syringes. Either method is time-consuming or subject to numerous sources of error or both.

A new device -- named the anaerobic respirometer -- has been developed to help correct these deficiencies. This device measures gas production in incremental volumes as small as 0.10 mL. Tests with the device show precisions better than 3% coefficient of variability including natural biological variations. Calibration error is less than 1%. The anaerobic respirometer saves considerable test time and provides output in the form of a computer file that can be transported directly to spreadsheets or other data processing software.

The objective of this paper is to review the operating characteristics of gas flow measuring methods and to show how automatic respirometers that provide essentially instantaneous response to changes in gas flow rate allow much greater insight into the reaction of an anaerobic culture to a change in environmental or input conditions.

GAS MEASUREMENT METHODS

Methods available for measuring the rate and volume of gas produced by laboratory-scale anaerobic reactors include volume displacement devices, wet-test or wet-tip meters, lubricated syringes, manometer-assisted syringes, calibrated pressure manometers or transducers, and automatic anaerobic respirometers. Each of these methods has specific advantages and disadvantages that must be considered when they are used.

Volume Displacement Devices

Volume displacement devices typically include cylinders that are inverted in baths of acidified water with gas piped to the

cylinder from the anaerobic reactor using flexible tubing (Figure 1A). Alternately, the fluid can be displaced from a storage vessel to a receiving container (Figure 1B). The displaced volume can be read from markings on the cylinder or by changes in surface level in the barrier liquid. Volume displacement devices are low cost and convenient to use but are subject to considerable error. These devices require careful control of the pH of the barrier liquid, and to produce accurate measures of gas production, adjustments must be made to account for the weight of the cylinder or differences in liquid levels between vessels.

Some volume displacement devices have been automated by using enclosed chambers and three-way valves to evacuate a fixed volume of gas when a preset displacement is reached[1] (Figure 2A). This approach requires careful maintenance and frequent attention to prevent plugging the valve by water condensate or by corrosion. Viega, et al[2] presented the design of a device in which gas is evacuated in fixed increments through an inverted siphon placed between two legs of a U-tube filled with acidified water (Figure 2B). This device can be automated with the use of electrodes to detect changes in liquid surface level and thereby count the number of discharge events. The volume displacement is a function of the size of the device, but increments of about 50 mL per cycle have been reported.

The relatively large volumes released during each discharge cycle for both the Moletta and the Viega designs makes these devices more applicable to laboratory-scale reactors producing about 10 to 50 mL per hour. Calibration errors of less than 1% are reported but data on test precision is limited.

Wet-Test or Wet-Tip Gas Meters

Wet-test gas meters use the buoyancy of gas to impart movement to a rotary device suspended in a fluid[3]. The number of revolutions of the device produces a measure of gas production. These devices are quite expensive, and since they require a minimum flow rate above about 10 L/hr, they are most suitable for use with pilot-scale anaerobic reactors. Lower rates can result in erratic and erroneous readings. Wet-tip meters measure gas volume by fluid-induced movement of a counting device in increments of about 100 mL and thus are suitable for use with relatively large laboratory-scale reactors but are not sensitive enough for use with small bench-scale units. Most of these meters also have metal parts that are subject to corrosion by acidic gases.

Lubricated Syringes

Syringes having large-diameter glass plungers may be used to measure gas production rates less than about 100 mL/day (Figure 3A)[4]. In this method, the syringes are placed in a horizontal

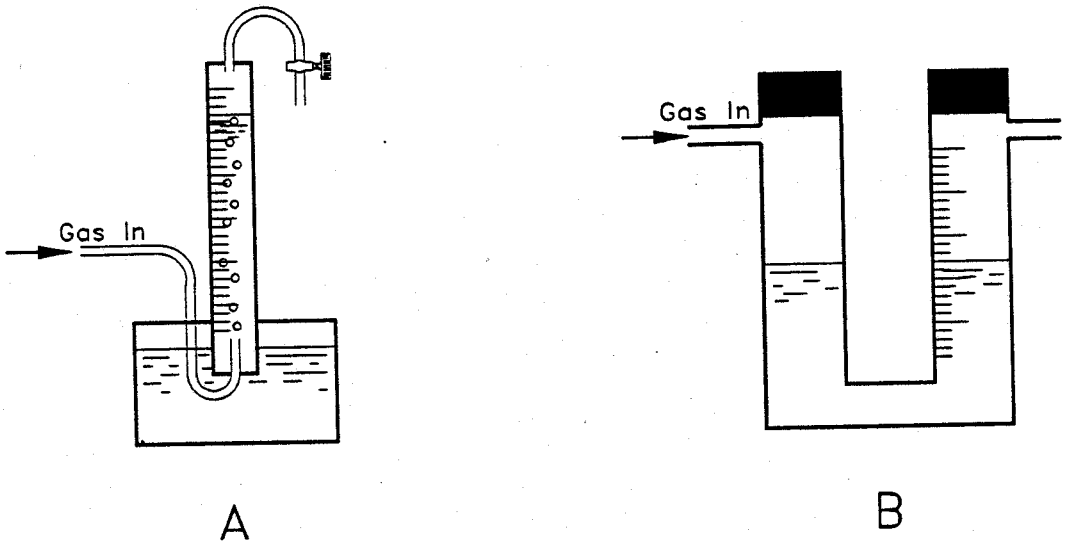

Figure 1. Schematic diagrams of typical volume displacement gas flow-measuring devices.

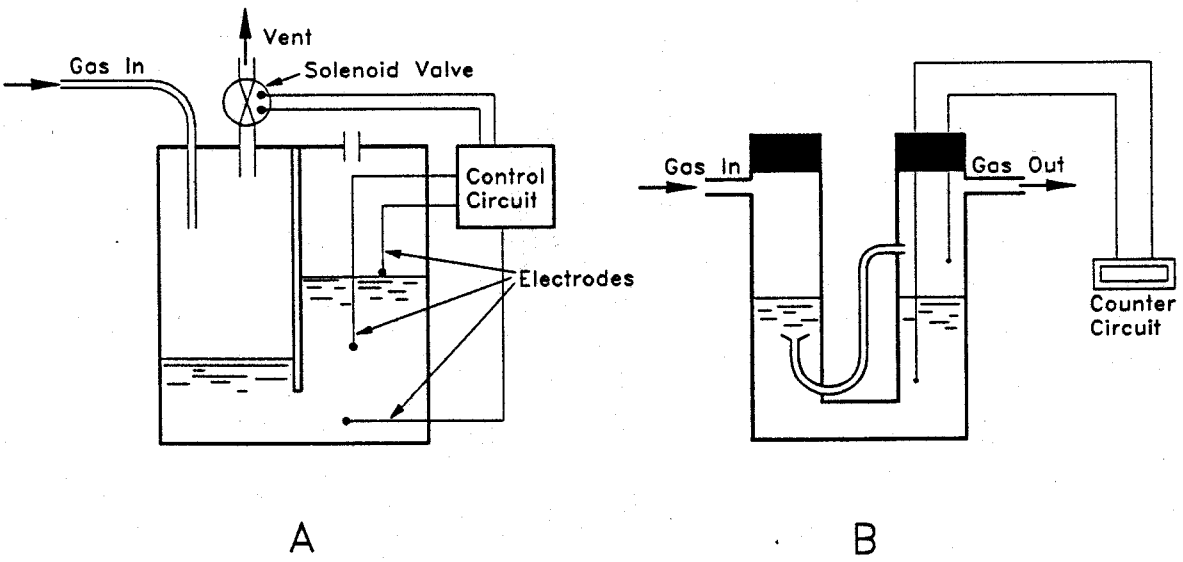

Figure 2. Schematic diagrams of automated volume-displacement gas flow measuring devices as reported by Moletta and Albagnac[1] (A) and Viega, et al.[2] (B).

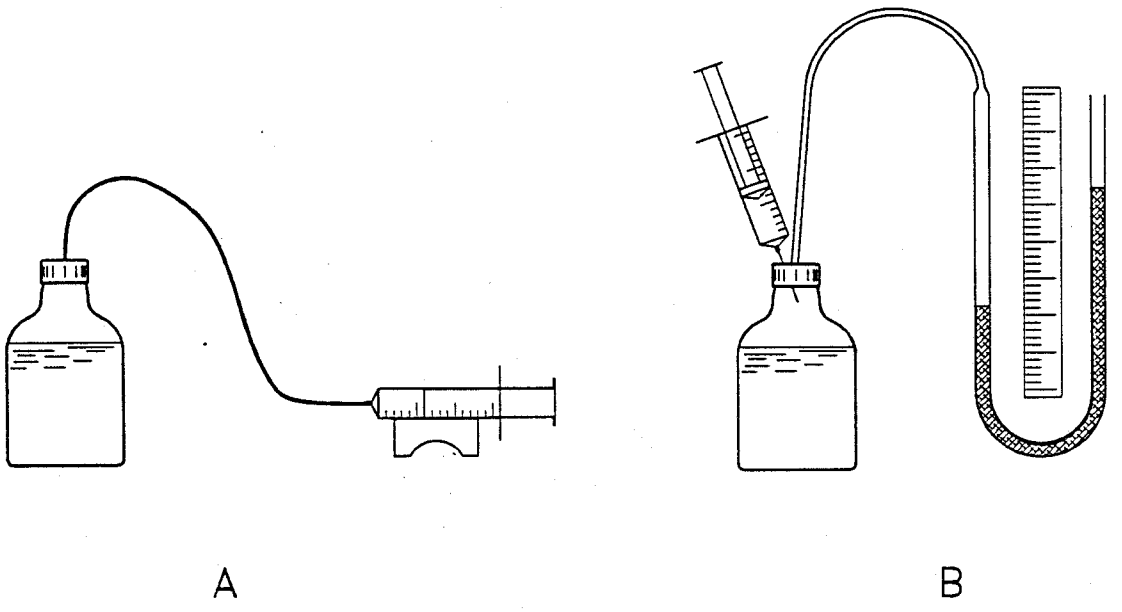

Figure 3. Schematic diagrams of gas flow measuring apparatus using lubricated syringes (A) and manometer-assisted syringes (B).

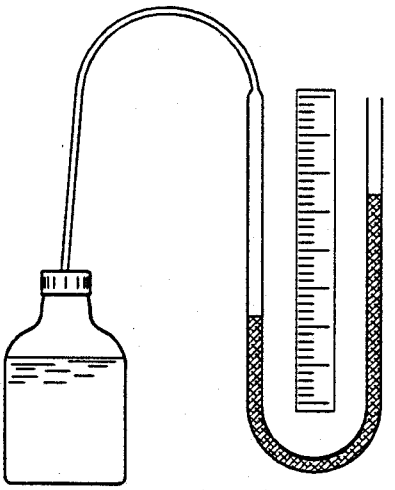

Figure 4. Schematic diagram of a pressure manometer used as a gas flow measuring apparatus.

position and the plungers are lubricated using mineral oil, glycerine, or other fluid so that when the syringe is connected to an anaerobic reactor, the gas causes a displacement of the plunger. This method requires close attention including manually evacuating and re-setting the syringe when its capacity is reached. Sources of error include resistance of the syringe to movement and loss of gas through the fluid seal. This method is reasonably accurate for measuring cumulative gas production rates greater than about 100 mL/d but is quite erratic for hourly measurements and is not amenable to automation.

Manometer Assisted Syringes

Gas-tight plastic syringes having rubber plungers may be used to withdraw gas from small test reactors at frequent intervals during a test program[5]. Gas is withdrawn until the pressure in the vessel, as read on a manometer, is equal to that at the start of the test (see Figure 3B). A thermal-barometric pressure vessel and manometer may be used to provide a reference pressure for maximum precision and accuracy. This method is quite accurate if gas is withdrawn at frequent intervals so that the pressure remains low but is quite inconvenient for measuring gas production rates greater than about 100 mL/d. Permitting the units to reach excessive pressures before evacuation can allow soluble carbon dioxide to change the pH and gas composition significantly. Frequent piercing of septa with needles on syringes and manometer attachments can contribute to the occurrence of leaks.

Calibrated Pressure Manometers

Since pressure is related to the volume of gas produced, the height of a fluid column in a manometer or the electrical response from a pressure transducer may be used to measure gas production[6,7] (Figure 4). The pressure manometer method requires careful calibration of the gas volume versus pressure, and the calibration is related to gas composition and solubility, temperature, and the ratio of liquid to gas volume. James, et al.[8] described the use of a Warburg respirometer for measuring gas production volumes up to about 50 mL over a 24 to 36-hour test period. While sensitive to small changes in gas production, the investigators reported that this method is labor intensive and time consuming.

Accurate measurement of the production of specific gases is difficult when using calibrated pressure manometers because the solubility of carbon dioxide changes significantly under pressure. Analysis of gas samples removed under pressure may show inaccurate percentages of the amount of methane and carbon dioxide produced. The calibration also is a function of the headspace volume and can change during a test if the composition of the gas changes appreciably. Also, the headspace gas volume must be sufficiently large to store the gas without causing

pressures so high that leaks occur. Typically, headspace volumes with this method should be about two to four times the volume of gas produced daily. Infrequent recording of gas pressure and its associated volume can lead to smoothing of gas production rates, thereby masking significant reaction events.

Automatic Anaerobic Respirometers

Anaerobic respirometers are specially designed instruments that measure gas production in increments as small as 0.1 mL (Figure 5)[9]. These increments are measured automatically, essentially on a continuous basis, and the data is recorded by counters or computers. The incremental volumes may be measured as frequent evacuations of gas in response to small pressure changes or as the volume of bubbles passed through specially-designed flow-measuring cells. The precision of anaerobic respirometers typically is less than 3% coefficient of variability including biological variations, and calibration errors are less than 1%.

One advantage of anaerobic respirometers is that they respond instantaneously to changes in gas production and do not allow significant pressure buildup. Consequently, the headspace volume can be reduced to as little as 10 mL, thereby minimizing changes in gas composition due to dilution of a large volume of headspace gas. This feature allows the attachment of carbon dioxide adsorption devices in the gas line so that direct measurement of methane can be accomplished (Figure 6).

COMPARISON OF GAS MEASURING METHODS

Manual methods of measuring gas production -- including the use of syringes and pressure manometers -- typically are labor-intensive and time-consuming, and unless data are recorded at frequent intervals the sensitivity of measurement is reduced and critical events may not be observed. Examples of gas production from small bench-scale reactors (50 to 500 mL) as measured using the syringe withdrawal method described by Johnson and Young[5] are shown in Figure 7. The tests were designed to determine the effect of phosphorus on anaerobic reactions[10]. While the gas production curves are generally well defined, the long times between samples mask small changes in gas production.

This situation is illustrated clearly in Figure 8 which shows a gas production curve as measured using an automatic anaerobic respirometer that recorded gas flow in incremental volumes of 0.14 mL (CHALLENGE ANR-100 system; Figure 5)[9]. A plot of the data at 30-min intervals clearly shows two major reaction events. The first steep-sloped portion of the curve represents the gas production associated with the conversion of ethanol to acetate and hydrogen which in turn are converted rapidly to methane and carbon dioxide. The section of the curve having the lower slope represents the conversion of acetate and

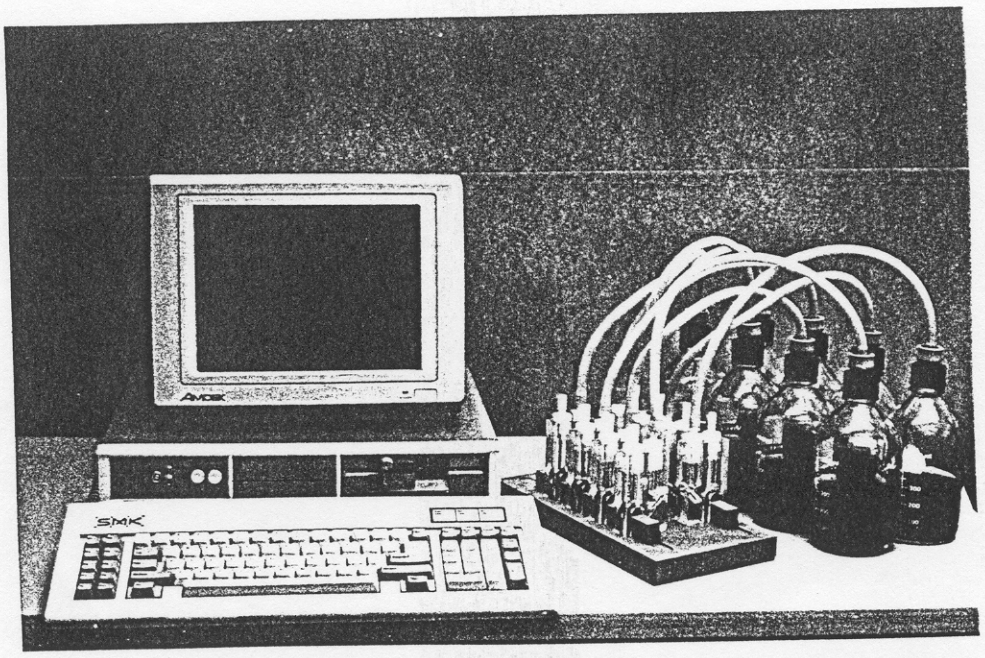

Figure 5. Photograph of an automatic anaerobic respirometer (Courtesy Challenge Environmental Systems, Inc.)[9].

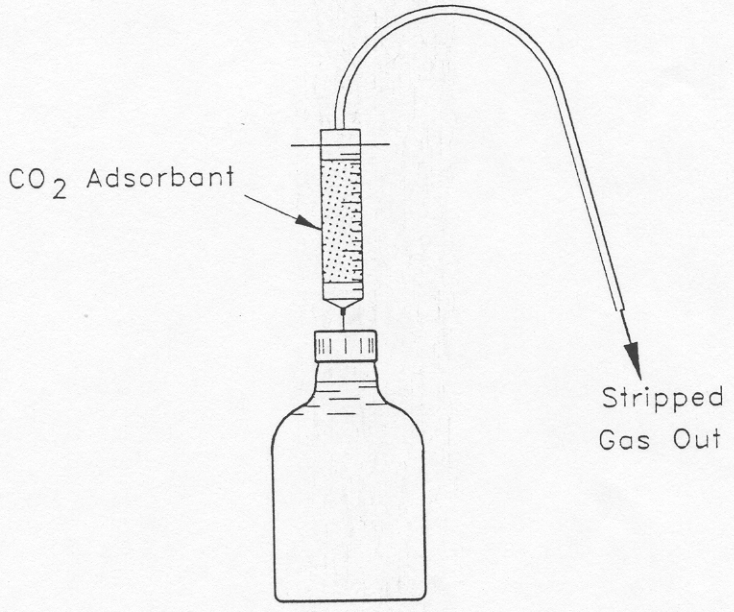

Figure 6. Test apparatus for direct measurement of the methane produced by a laboratory-scale anaerobic reactor.

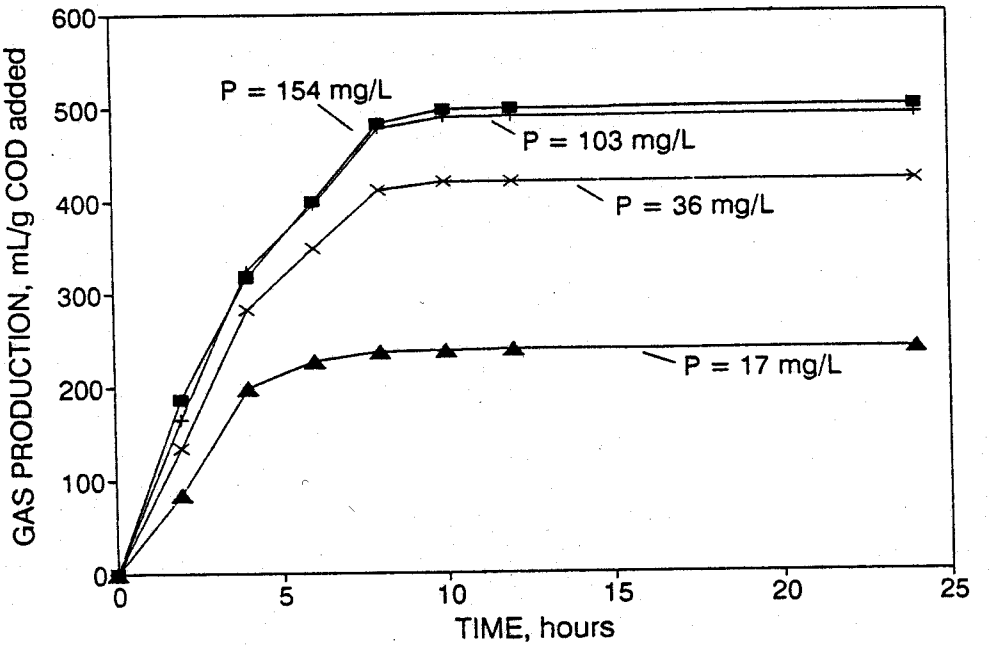

Figure 7. Examples of total gas production measurements using the manometer-assisted syringe method with gas withdrawal at relatively infrequent intervals.

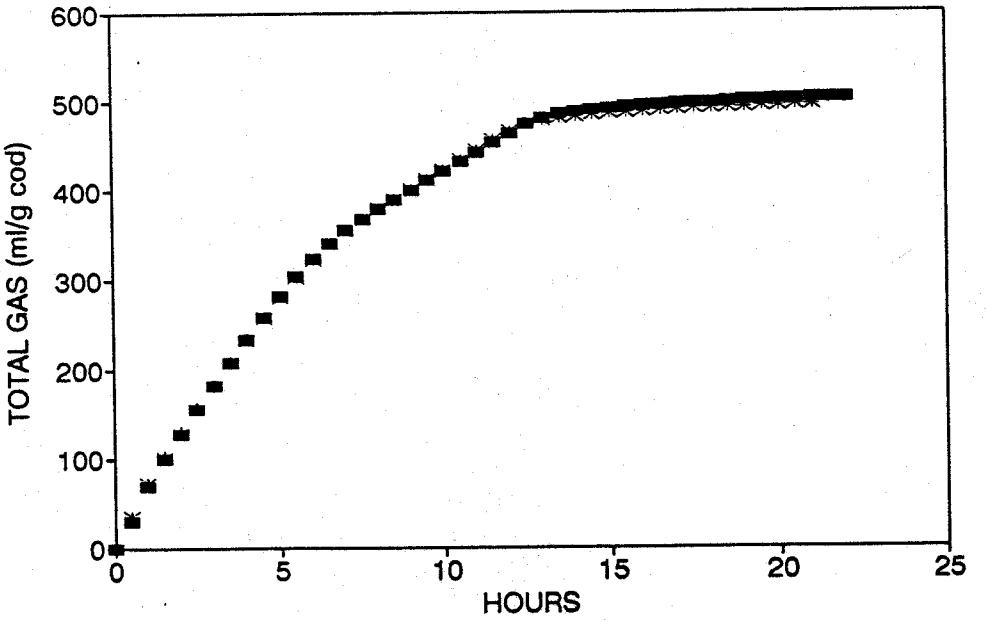

Figure 8. Example of total gas flow measurement using an anaerobic respirometer (CHALLENGE ANR-100 system)[9].

hydrogen to methane and carbon dioxide. These events, while obviously present in the manually-collected data shown in Figure 7, are not as well defined and the break-points in the curves cannot be located precisely.

Figure 9 shows the results of directly measuring methane production from an ethanol-enriched test reactor using a device similar to that illustrated in Figure 6 for adsorbing carbon dioxide and an anaerobic respirometer for measuring gas flow. The lower curves in Figure 9A represent the production of methane as calculated from conversion of hydrogen and acetate. Figure 9B shows the respective conversions of ethanol, acetate, and other intermediates.

The substrate and gas production curves shown in Figures 8 and 9 are representative of a steady-state culture that is not affected by the stress of environmental changes or the presence of toxic chemicals. When such stresses do occur, significant changes in methane production rate and organic substrates take place. For example, addition of toxic organic chemicals or industrial wastes can cause the methane production curves to deviate significantly from those for control cultures. As illustrated in Figure 10, 100 mg/L of 3-chlorophenol caused significant changes to occur both in methane production and substrate conversion.

Modeling the methane production from acetate and hydrogen shows significant reduction in the rate of substrate conversion. In this case, the toxicant caused a significant increase in the concentration of other intermediates consisting primarily of propionic acid, which accounted for the lower rate of methane production from hours 3 through 20. Further increases in the toxicant dose caused major changes in methane production as illustrated in Figure 11. These changes can be used to verify and model the impact of toxic chemicals on biological growth and substrate conversion reactions.

CONCLUSIONS

Gas flow measurement is an important test parameter when assessing the effect of toxic chemicals or industrial wastes on the performance of anaerobic treatment processes. The selection of gas measuring devices must be based on the size of anaerobic reactor used and the sensitivity and capacity of the gas measuring apparatus. Anaerobic respirometers provide a significant improvement in the ability to measure gas production accurately and precisely for small bench-scale reactors, and the resulting data provide greater insight into the reaction mechanisms than normally is possible using manual gas flow measuring methods.

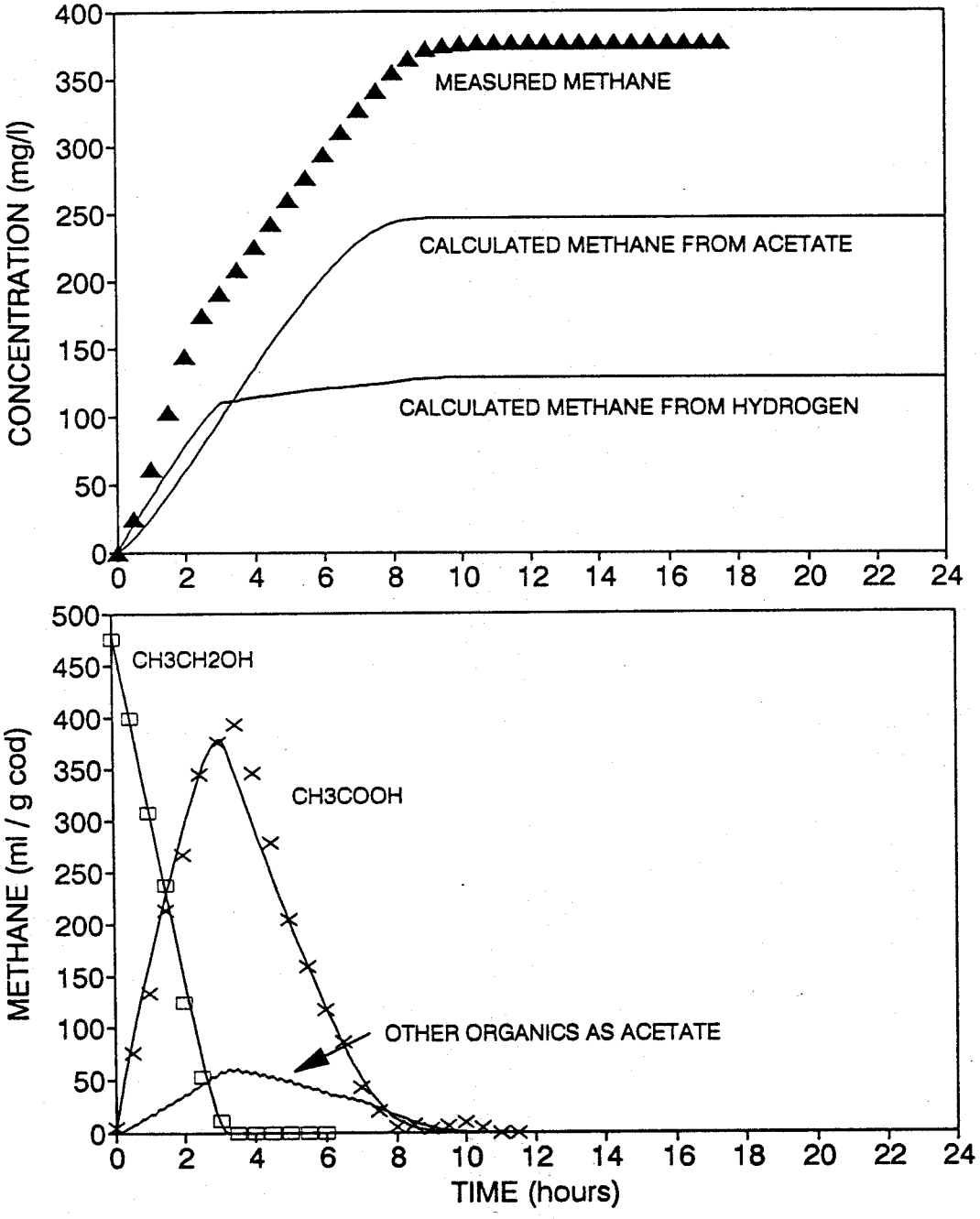

Figure 9. Direct measurement of methane production from an ethanol-enriched culture (A) using an automatic respirometer and an apparatus similar to that shown in Figure 6 plus associated concentrations or organic substrates (B). Symbols represent measured data, lines represent calculated quantities.

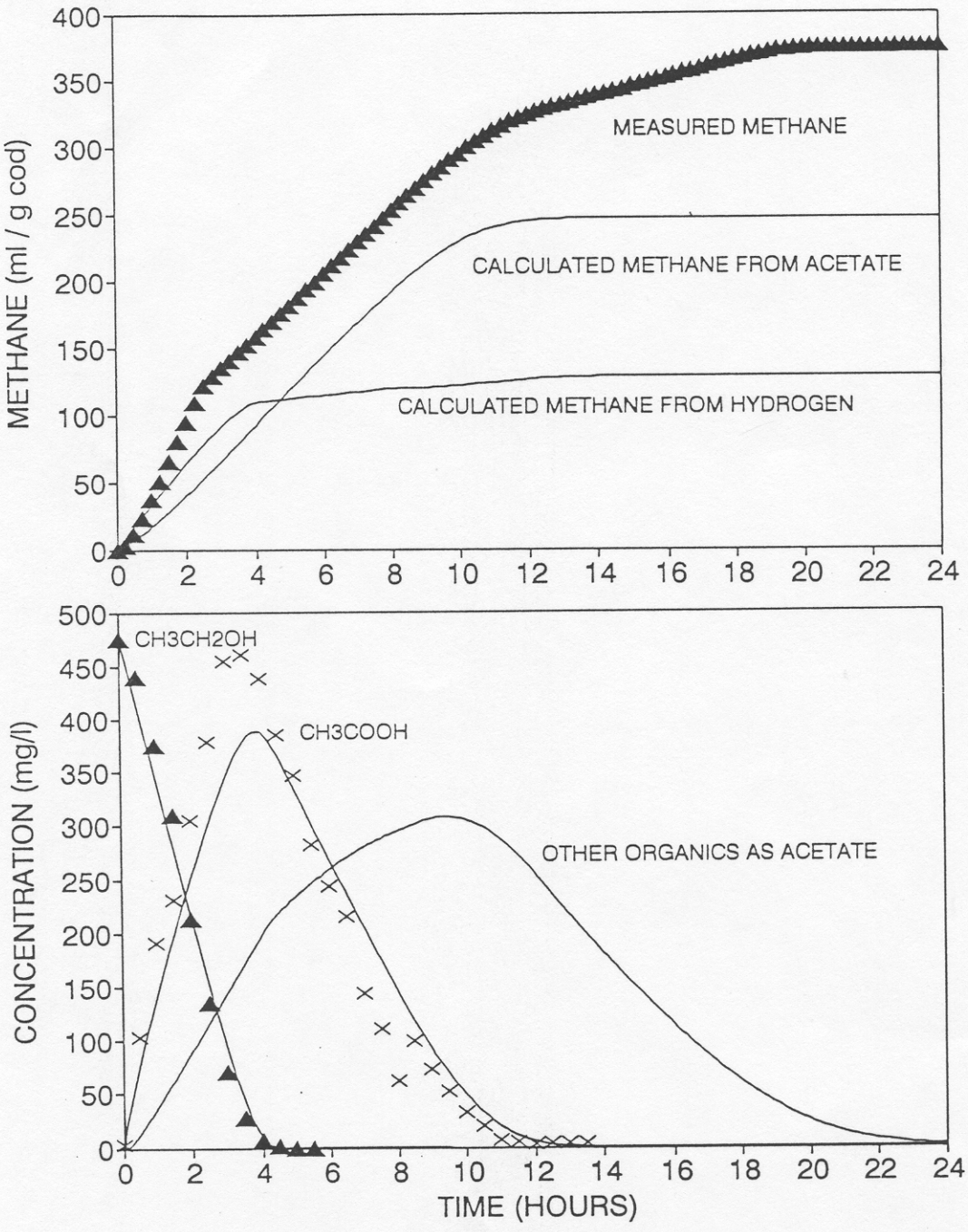

Figure 10. Methane production as measured and as calculated for the conversion of hydrogen and acetate (A) plus associated organic substrates (B) in an ethanol-enriched culture receiving 100 mg/L of 3-chlorophenol.

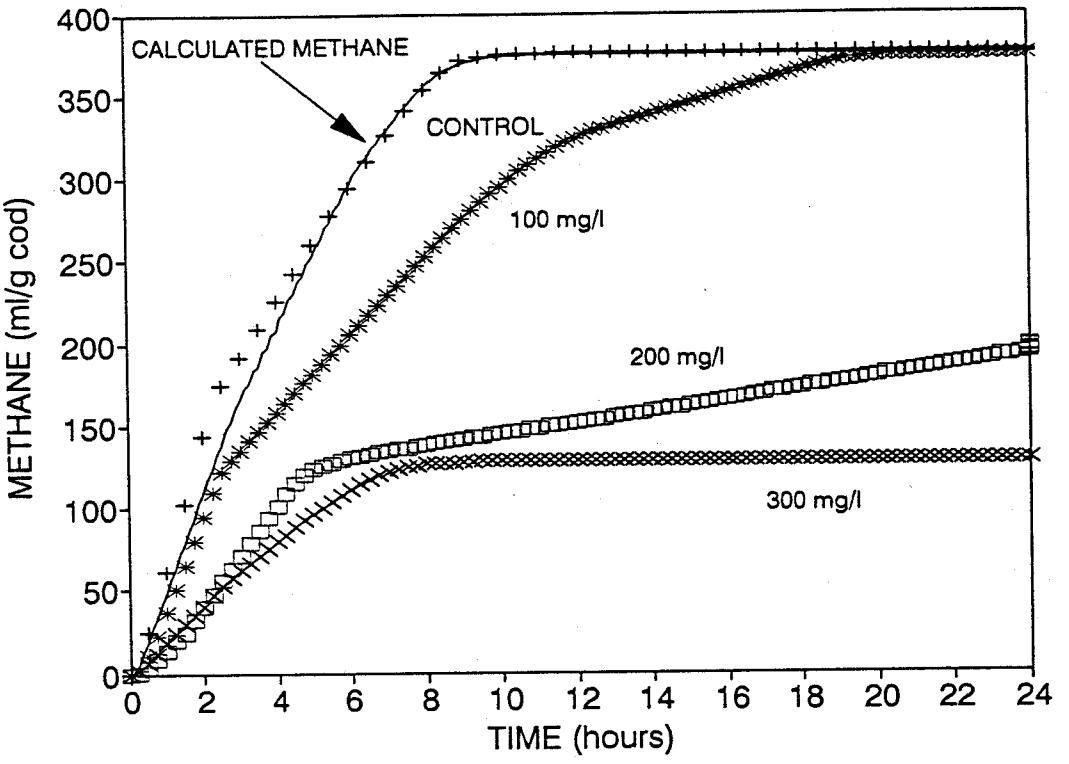

Figure 11. Methane production from reactors receiving different amounts of 3-chlorophenol.

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