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Automatic pH Control and Soluble and Insoluble Substrate Input for Continuous Culture of Rumen Microorganisms

LEONARD L. SLYTER[1]

U.S. Department of Agriculture, Agricultural Research Service, Nutrition Institute, Beltsville, Maryland 20705

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An artifical rumen continuous culture with pH control, automated input of water-soluble and water-insoluble substrates, controlled mixing of contents, and a collection system for gas is described.

Several devices for continuous culture of rumen microorganisms in vitro have been described. One of these (2) has been modified several times during the past 10 years (1, 3). The system as modified and described herein is improved over the previous systems in that it allows automatic pH control, less air leakage and greater ease in gas collection, more controlled mixing rates, and automated dispensing of insoluble substrate. Also, depending upon the diameter of the pulleys used to rotate the Alnico magnets, the same or different mixing rates can be provided to the six fermenters.

The continuous culture apparatus is shown in Fig. 1. The device consists of a water bath with six 3.175-cm (diameter) by 15.24-cm bronze (length) tubes, inside of which a glass tube connects the overflow tube of a 500-ml fermenter (3013 SPL, Bellco Glass, Inc., Vineland, N.J.) to a 1-liter separatory funnel to collect effluent. The water bath (3010-12, Labline Inc., Chicago, Ill.) is mounted on a mobile metal cart (width, 61 cm; length, 91 cm; height, 91 cm) to which is attached a one-eighth horsepower (1,725 rpm) motor (type NS1-54R, Bodine Electric, Chicago, Ill.) with reducing gears used to drive a 0.95-cm texthane belt (Potomac Rubber Co., Washington, D.C.) and a series of six pulleys (diameter, 5 cm; width, 0.9 cm) at 88 rpm. On top of each pulley is positioned an Alnico C-shaped magnet (12-012, Fisher Scientific Co., Silver Spring, Md.). Holes cut into the stainless steel plate covering the heating coils allow the fermenters to be permanently positioned directly above the magnets. For pH control and monitoring, a combination autoclavable pH electrode (F1155-S, Scientific Products, Columbia, Md.) is mounted in a rubber stopper and placed in a fermenter port. The electrode is connected to a pH control module (F1150, Scientific Products) in which a Speedomax H recorder (Leeds and Northrup, Columbia, Md.) is substituted for the module chart recorder. The pH control module, recorder and dual pumps (F1166-2, Scientific Products) are used to monitor and/or control pH's within each of six fermenters at 6-min intervals. A rubber stopper with three tubes is placed into a second fermenter port. One 16-cm long 4-mm inside diameter glass tube is used for sample removal. A second tube allows an artificial saliva and tap water solution to be infused, and a third tube allows alkali solution for pH control to be infused. An acid solution for pH control may be infused through a third port.

The fermenter dome has two outlets. One is a ground-glass joint for gas collection. The other is a 20-mm port through which insoluble substrate is added. The gas is collected in a 7.08-liter butyl-rubber sampling balloon (Seamless Rubber Co., New Haven, Conn.) whose stem is cemented over a 0.635-cm stainless-steel tube with contact cement (No. 169, B. F. Goodrich, Columbia Rubber Corp., Beltsville, Md.) which is attached to a male stainless-steel quick connect (400-1/4 QC-200-316-DESO; Potomac Value and Fitting, Inc., Rockville, Md.). The male quick connects and the stainless-steel female quick connects facilitate the collection and removal of the gas for analysis. The female quick connects are attached to a rubber tubing which allows gas to be collected from a 10/30 ground joint in the fermenter dome. A pellet dispenser (7746-5001, Bellco Glass Inc., Vineland, N.J.) mounted directly above the fermenter dome is held in place by a flexiframe assembly. The cam-operated aluminum fingers of the dispenser operate in a

[1] Present address: U.S. Department of Agriculture, Agricultural Research Service, Animal Physiology and Genetics Institute, Feed Energy Conservation Laboratory, Beltsville, Md. 20705.

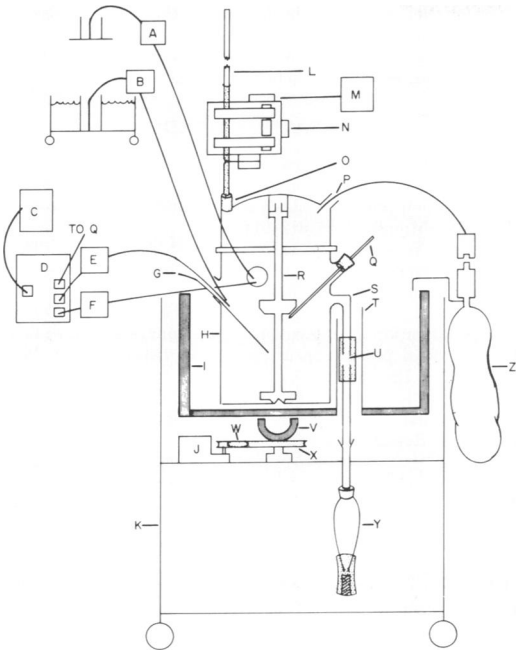

FIG. 1. *Fermenter and accessories: (A) pump used to infuse the nitrogen solution; (B) pump used to infuse the artificial saliva, tap water, and soluble energy solution from refrigerated cart; (C) recorder; (D) pH control module; (E) pump used to infuse acid solution; (F) pump used to infuse base solution; (G) sampling glass tube; (H) fermenter; (I) water bath; (J) one-eighth horsepower motor with reducing gears and drive pulley; (K) mobile cart; (L) glass tube for pelleted substrate; (M) timer; (N) pellet dispenser; (O) pellet delivery port; (P) gas collection ground-glass joint with rubber tubing and female quick connect; (Q) line leading to pH electrode and port; (R) fermenter stirring device; (S) effluent outlets; (T) bronze tube; (U) effluent rubber tubing; (V) magnet; (W) texthane belt; (X) pulley; (Y) effluent collection funnel; (Z) gas collection balloon connected to male quick connect. The fermenter base has three ports; one for infusing solutions A and E, another for infusing solutions B and F and sampling from G, and a third for providing a pH electrode, Q.*

sequence to allow the substrate contained between the fingers to be dropped by gravity from the sealed glass tube into the fermenters at the preselected time intervals (2 min to 60 h). Moisture condensation into the area housing the pellets is prevented by a cam-driven metal frame which pinches the rubber inlet tube against a block which closes the tube near the bottom of each pellet dispenser in the time interval between substrate additions. The amount of substrate released can be varied by adjusting the space interval between the fingers or by varying the size of the pellet, or by a combination of both methods.

In experiments in which both water-soluble energy and nitrogen solutions are infused, the energy and artificial saliva solutions are maintained in a refrigerated cart (model CR49, New Brunswick Scientific Co., Inc., New Brunswick, N.J.) and pumped into each fermenter by a peristaltic pump (model 600–1200, Harvard Apparatus Co.). A second peristaltic pump is used to infuse the nitrogen solution. For control of insoluble substrate input, the pellet dispenser and timer unit (7746-5002, Bellco Glass, Inc.) are used.

Anaerobic conditions are maintained while initiating an experiment, by flushing CO_2 through a needle at the base of the effluent collector during and for a minimum of 10 min after inoculum is placed into the fermenters. The CO_2 is passed from a cylinder to the fermenters through a series of rubber tubing and 6-mm glass Y-tube connectors. Once the experiment has started, air is kept from entering the system by keeping the fermenter closed, by passing CO_2 into the artificial saliva solution continuously, and by leaving a few milliliters of the effluent undrained when the effluent is removed. Air entry into the system is also prevented by allowing the fermentation gases to pass back into effluent collector during effluent drainage.

Once a study is started with the system described herein a predetermined pH can be maintained in six fermenters on a continuous basis. The unit allows water-soluble and/or water-insoluble substrates to be dispensed automatically. The six dispensers (when set to deliver 0.47-cm diameter pellets with maximal finger spacing, and a 37-min feeding interval) delivered an average of 28.8 ± 0.6 g daily during a 3-week period. The length of the pellets used varied from 5 to 12 mm. Pellet sides must be smooth and the ends must be fairly blunt if good reproducibility from gravity feeding is to be accomplished. The unit allows the complete recovery of gases, liquids, and solids for subsequent analysis with a minimum of air contamination. Atmospheric air injected into a chromatograph gave a N_2 peak of about 100. At the same sensivity 55 of 90 samples tested from six fermentors during 6 weeks of continuous culture of a mixed rumen bacterial population gave no indication of an N_2 peak at all while a total of 82 samples had N_2 peak heights of less than 3. The pellet-dispensing system has not been tested with pure cultures. However, continuous cultivation of pure cultures of anaerobes should be possible with pelleted substrates provided the

substrate is properly irradiated or otherwise made sterile. Sterile environments should be possible because all the components of the system that come in contact with the substrate delivery system or the culture growth chamber are autoclavable. The pellet dispenser system has been used successfully with mixed cultures (A. K. Mosi et al., J. Anim. Sci. **41**:411, 1975; L. L. Slyter et al., J. Anim. Sci. **41**:432, 1975). Also the system without the pellet dispenser and pH module system has been used successfully to cultivate a rumen anaerobe, *Butyrivibrio fibrisolvens* as a pure culture during a 7-day trial (4).

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