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Short Communication

An economical bioreactor for evaluating biogas potential of particulate biomass

Ann C. Wilkie *, P.H. Smith, F.M. Bordeaux

Soil and Water Science Department, University of Florida, P.O. Box 110960, Gainesville, FL 32611-0960, USA

Abstract

An economical bioreactor designed for evaluating the biogas potential of particulate biomass is described. The bioreactor uses a simple stirring apparatus, called the *Bordeaux stirrer*, to enable gas-tight mixing of fermentation cultures. The apparatus consists of a low-rpm motor connected to a bent steel stir rod, which is placed in a length of flexible plastic tubing inserted through a rubber stopper in a gas-tight manner. This stirrer is suitable for providing intermittent or continuous mixing in bench-scale anaerobic cultures containing particulate biomass. The reactor system may be operated as a batch-fed or semi-continuously fed digester. This communication documents the advantages of the stirring apparatus, describes the details of reactor fabrication and operation, and outlines the type of experimental work for which the bioreactor is suitable.

Keywords: Anaerobic digestion; Treatability studies; Intermittent mixing; Gas-tight bioreactor; Enrichment cultures; Particulate biomass; Biogas; Biochemical methane potential; Bordeaux stirrer

1. Introduction

In fermentation processes, gas production is often proportional to substrate utilization, product formation and microbial growth. When measured, fermentation gas production can provide experimental verification of biochemical transformations through a mass or carbon balance. Yet, gas production is most often measured only in anaerobic digestion experiments where the product biogas has value as a fuel. A barrier to the measurement of gas production is the challenge of fabricating an inexpensive gas-tight fermentation reactor that allows mixing, substrate addition and culture removal. While other gas-producing fermentation research may also have use for a simple gas-tight reactor, anaerobic digestion research has a continual need for such an apparatus.

Prior to designing and constructing full-scale anaerobic digesters for treatment of a particular waste, a treatability study is often recommended (Tchobanoglous and Burton, 1991). The operation of a heated and mixed bench-scale anaerobic reactor, at a conservative hydraulic retention time (15–30 d) and a moderate organic loading rate (1–4 kg COD m^3 d^{-1}), fed a representative waste sample for a period equivalent to several retention times, can yield optimal estimates for degradability, methane yield, and process stability (WPCF, 1987). The recommended set-up for a continuously stirred anaerobic treatability study often suggests using a magnetic stirrer to keep the reactor contents mixed (Zickefoose and Hayes, 1976; WPCF, 1987; Tchobanoglous and Burton, 1991).

The use of a magnetic stirrer in anaerobic digestion laboratory studies, however, has several disadvantages. First, the stir bar can be thrown off-center (decoupling) and become lodged at the edge of the reactor vessel, which terminates effective mixing (Kleppinger, 1994). Also, operating an anaerobic reactor using a magnetic stirrer with particulate feeds and an opaque culture presents challenges to stable operation of the stirring mechanism. When particulate solids settle on the bottom, they may impede stir bar rotation and also prevent reactivation of the magnetic stirrer. Since sulfides produce an opaque solution in many anaerobic cultures, it is often difficult to detect whether mixing is occurring or is effective.

With particulate biomass feedstocks, there is an intimate spatial relationship between cellulolytic organisms

* Corresponding author. Tel.: +1-352-392-8699; fax: +1-352-392-7008.
E-mail address: acwilkie@ufl.edu (A.C. Wilkie).

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and their substrates, which enhances hydrolysis (Aldrich, 1993). Also, syntrophic relationships between anaerobic bacteria are enhanced by close spatial proximity (Thiele, 1991). Due to these ecological constraints, intermittent mixing was found to enhance digestion rates over continuous mixing since vigorous mixing disrupts the spatial arrangement between bacteria and particulates as well as with syntrophic organisms (Fannin, 1987). Empirical observation of culture settling rates allows the effective use of intermittent stirring, which in our work was chosen to be a stirring interval of 15 min/h for sewage sludge and particulate biomass feeds. Intermittent stirring would be difficult or impossible using a magnetic stirrer. Finally, the need to verify stir bar rotation practically eliminates the use of reactors that are not transparent. The simple stirrer and bioreactor described in this communication overcome these limitations.

Other than by using a magnetic stirrer, a magnetically coupled bearing, biogas recirculation using an explosion-proof compressor, an extra pump for recirculating culture media, or a shaker table, stirring of an anaerobic culture requires a penetration through the vessel exterior that accepts a rotating shaft. Sealing this penetration in a gas-tight manner can require expensive sealed bearings (Willimon and Andrews, 1969) that may fail prematurely when exposed to the hydrogen sulfide in the gas or organic acids in the culture broth. The *Bordeaux stirrer*, described herein, seals the rotating shaft inside a flexible tube, effectively eliminating the gas-tight bearing in a cost-effective manner by replacing the bearing with a steel rod, a stopper, and a length of tubing sealed at one end.

The ability to derive meaningful information on variations in operating conditions or perturbations to the anaerobic digestion process in bench-scale experiments requires numerous small reactors (0.5–10 L) that can be maintained in semi-continuous or batch operation for extended runs without upset. The slow growth rates of many strictly anaerobic microorganisms in the digestion consortium results in long lag times (2–6 months) before steady-state populations are reached. Therefore, a reliable and inexpensive reactor design is required to enable simultaneous operation of numerous reactors. Such a bioreactor has been in operation at the University of Florida for many years with satisfactory results including reproducible operating conditions and minimal stirrer maintenance requirements. While a cursory description of the bioreactor system can be found in the referenced literature, the authors have received enough inquiries from researchers, particularly from developing countries, to acknowledge that previous descriptions have been inadequate to allow other researchers to build this simple device. The construction and operation of this inexpensive apparatus are described in this communication.

2. Methods

2.1. Bioreactor

Bench-scale bioreactors are constructed from 4 L glass aspirator bottles, as shown schematically in Fig. 1. The bioreactor components are itemized in Table 1. A 40 cm × 3.175 mm diameter stainless steel rod is bent in a gentle J-shape with a bend of 30° from the vertical over the lower 15 cm of its length and any sharp edges are removed from the lower end by grinding. The bent length of the stir rod is lightly coated with silicone grease and inserted into a 32 cm length of 6.4 mm ID × 12.7 mm OD flexible plastic tubing, which is sealed at the end with a 2.54 cm length of 7 mm OD glass rod (Table 1). The open end of the tubing is inserted through an 11.2 mm hole in the center of the reactor stopper (no. 10, solid rubber) such that it protrudes 1 cm above the top of the stopper, with the steel rod extending 9 cm above the tubing. The protruding rod is coupled to a 60-rpm motor (W.W. Grainger, Inc., Lake Forest, IL), with a 15.9 mm × 50.8 mm metal cylinder, bored to accept the 7.9 mm OD motor shaft and the stir rod, which are secured with set screws. The aspirator bottle (bioreactor) is mounted in a temperature-controlled water bath with

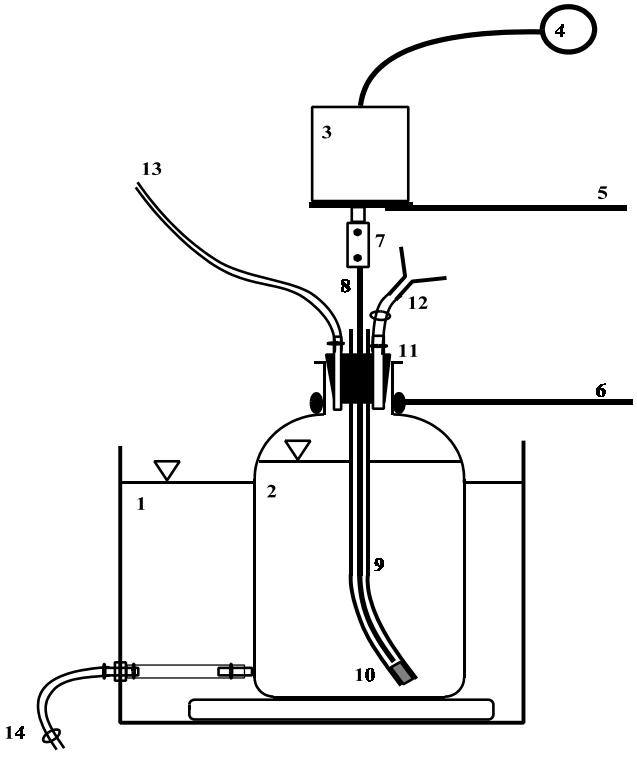

Fig. 1. Schematic of bioreactor with Bordeaux stirrer. Components are: (1) water bath with temperature control; (2) reactor vessel (4 L); (3) stirrer motor; (4) timer; (5) motor support; (6) reactor support; (7) coupler with set screws; (8) bent stir rod; (9) stirrer tube; (10) stirrer tube stopper; (11) reactor stopper; (12) feed inlet with funnel and clamp; (13) gas outlet to gas collector; (14) effluent outlet with clamp.

Table 1
Bioreactor components

Item	Source code[a]	Catalogue no.	Estimated retail cost[b]	Cost per bioreactor[b]
Reactor vessel, glass aspirator bottle (4 L)	F	02-972F	4 @ $414.54	$103.64
Reactor support, chain clamp	F	05-745	$35.42 each	$35.42
Reactor stopper, solid rubber no. 10 (holes drilled in-house)	F	14-130P	8 @ $22.30	$2.79
Stopper retainer, stainless steel wire (0.76 mm OD)×1 m	G	3TY76	274.3 m @ $17.02	$0.06
Stirrer motor, 60 rpm motor (w/0.53 N·m torque output)	G	2Z810	$49.25 each	$49.25
Stirrer motor support	I	na[c]		$10.00
Coupler w/set screws	I	na		$10.00
Stirrer, stainless steel rod (3.175 mm diameter)×40 cm	G	3PA79	80×91.4 cm @ $66.90	$0.37
Stirrer tube, Tygon clear tubing (6.4 mm ID×12.7 mm OD)×32 cm	F	14-169-1L	15.2 m @ $114.73	$2.42
Stirrer tube stopper, glass rod (7 mm OD)×2.54 cm	F	11-377E	108×1.2 m @ $303.11	$0.06
Reactor feed inlet, glass tubing (13 mm OD)×8 cm	F	11-362H	93×1.2 m @ $149.80	$0.11
Reactor gas outlet, glass tubing (7 mm OD)×8 cm	F	11-362E	223×1.2 m @ $149.80	$0.05
Feed and effluent lines, natural rubber tubing (12.7 mm ID×19.1 mm OD)×15 cm×2	F	14-178-5G	3.7 m @ $36.95	$3.00
Feed funnel, short stem	F	10-371C	6 @ $38.67	$6.45
Feed and effluent clamps, steel swing jaw clamp (20 mm)×2	F	05-834-5	3 @ $45.68	$30.46
Hose clamps, stainless steel clamp (7.9–22.2 mm)×5	F	14-198-5B	10 @ $18.59	$9.30
24-h electronic timer	G	1XC66	$67.40 each	$67.40
Total				$330.78

Sources are: F—Fisher Scientific, Pittsburgh, PA (www.fishersci.com); G—W.W. Grainger, Inc., Lake Forest, IL (www.grainger.com); I—fabricated in-house.

[a] Listing of sources is for example purposes only and does not constitute endorsement.

[b] Costs are in US $ as of August 2003.

[c] na—not applicable.

a chain clamp. The motor is bolted to a stainless steel plate and support such that the shaft is centered over the stopper (Fig. 1). Ten or more motors can be controlled by a single 24-h timer if suitable wiring is employed, allowing simultaneous intermittent stirring of multiple bioreactors.

The bioreactor stopper is also bored with an 11.2 mm hole for a 13 mm OD glass tube for substrate addition and bored with a 6.4 mm hole for a 7 mm OD glass tube for gas collection. The stopper is fixed onto the aspirator bottle by wiring it in place using 0.76 mm OD stainless steel wire. A short-stem funnel is inserted into 12.7 mm ID natural rubber tubing that is fixed to the feed inlet by a hose clamp. A steel swing jaw clamp is used to seal the feed tube between feeding events. The effluent is expelled through the glass nipple at the base of the aspirator bottle through a length of 12.7 mm ID natural rubber tubing that passes through the wall of the water bath and is sealed with a swing jaw clamp.

2.2. Gas collector

Gas is collected and measured by water displacement in a calibrated, inverted 9.5 L glass bottle, as illustrated in Fig. 2. Components of the gas collection system are itemized in Table 2. Natural rubber tubing (6.4 mm ID) carrying biogas from the reactor passes through a serrated tee (for gas sampling and venting) to a 7 mm OD glass tube, which passes through a 2-hole solid rubber stopper (no. 12) and extends to within 1 cm of the top of the inverted bottle. The stopper is fixed onto the glass bottle by wiring it in place using 0.76 mm OD stainless steel wire. Accumulated gas displaces an aqueous liquid, which is forced from the bottle through a second 7 mm OD glass tube and continues through a 6.4 mm ID flexible plastic tube into a storage reservoir consisting of a 10 L plastic carboy, open to the atmosphere. The displaced liquid passes through a spigot on the plastic storage reservoir, which is positioned just above the gas collection bottle (Fig. 2). Gas production for a chosen time interval is measured by the change in the level of the meniscus in the glass bottle and the gas pressure is determined from the difference between the levels of the menisci in the glass bottle and the storage reservoir.

2.3. Operation

Operation of the system for daily feeding entails turning on the stirrer for 30 min prior to feeding. All ports into the bioreactor are sealed except for the gas

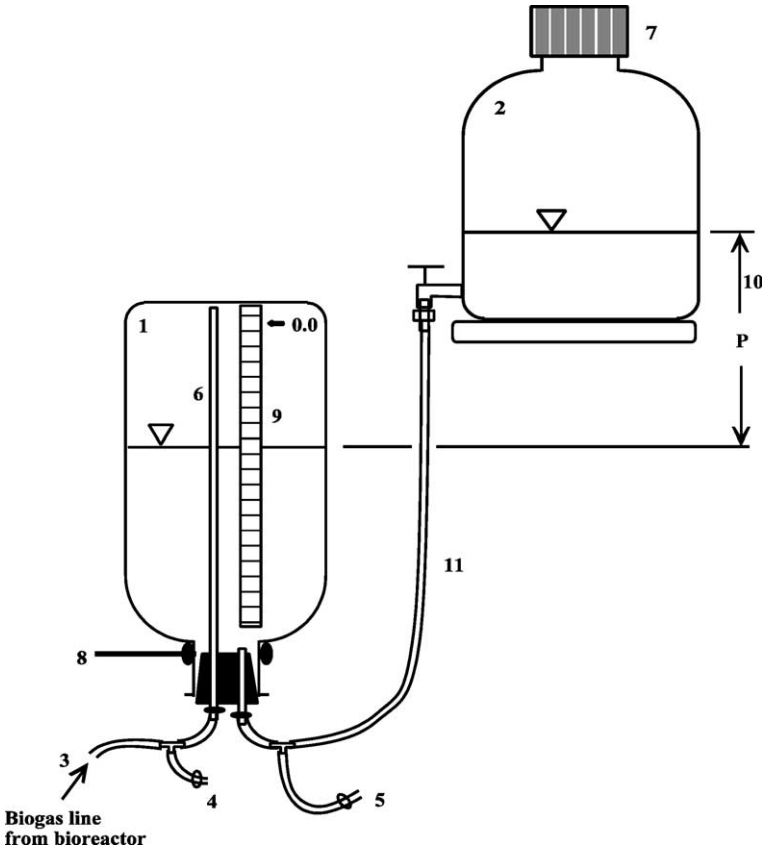

Fig. 2. Schematic of liquid–displacement gas collection apparatus. Components are: (1) gas collector (9.5 L); (2) water reservoir (10 L); (3) gas line from bioreactor; (4) gas vent with clamp; (5) drain line with clamp; (6) gas inlet; (7) vent cap; (8) gas collector support; (9) graduated scale; (10) dynamic pressure head; (11) displacement line.

Table 2
Gas collector components

Item	Source code[a]	Catalogue no.	Estimated retail cost[b]	Cost per bioreactor[b]
Gas collector, glass bottle (9.5 L, graduated)	F	02-887-1	4 @ $558.24	$139.56
Collector support, chain clamp	F	05-745	$35.42 each	$35.42
Collector stopper, solid rubber no. 12 (holes drilled in-house)	F	14-130T	5 @ $22.70	$4.54
Stopper retainer, stainless steel wire (0.76 mm OD)×1 m	G	3TY76	274.3 m @ $17.02	$0.06
Water reservoir, polypropylene carboy w/spigot (10 L)	F	02-963-2A	6 @ $544.76	$90.80
Collector gas inlet (51 cm) and water outlet (8 cm), glass tubing (7 mm OD)×59 cm	F	11-362E	223×1.2 m @ $149.80	$0.33
Gas line, natural rubber tubing (6.4 mm ID×9.5 mm OD)×40 cm	F	14-178-2C	15.2 m @ $53.14	$1.40
Gas and water line tees, T-shaped connector (7.9 mm OD)×2	F	15-319D	12 @ $16.51	$2.75
Water line, Tygon clear tubing (6.4 mm ID×12.7 mm OD)×1 m	F	14-169-1L	15.2 m @ $114.73	$7.55
Gas vent and drain line clamps, steel day pinchcock×2	F	05-867	12 @ $27.77	$4.63
Hose clamps, stainless steel clamp (7.9–22.2 mm)×2	F	14-198-5B	10 @ $18.59	$3.72
Total				$290.76

Sources are: F—Fisher Scientific, Pittsburgh, PA (www.fishersci.com); G—W.W. Grainger, Inc., Lake Forest, IL (www.grainger.com).
[a] Listing of sources is for example purposes only and does not constitute endorsement.
[b] Costs are in US $ as of August 2003.

outlet, which is connected to the gas collector. This enables the reactor to operate under anaerobic conditions since positive pressure is maintained to minimize air leakage into the system. The gas collection system allows for mixed-liquor sampling and feeding without oxygen intrusion. The biogas volume and pressure,

water bath temperature, ambient temperature, ambient pressure and time are manually recorded. The biogas is analyzed for methane content and the volume corrected to dry gas at standard temperature and pressure (0 °C and 760 mmHg) using the ambient temperature and gas pressure measurements and table values of saturated vapor pressure. A mixed-liquor sample is removed from the bioreactor through the effluent line. The biogas line is opened to a vent and the displacement line to the water reservoir is closed at the spigot. The feed tube is opened and the blended feed is poured into the feed funnel. The feed tube is clamped and the line to the water reservoir is reopened, allowing the liquid level in the gas collector to return to the "zero" level. The gas vent line is clamped and the level of the meniscus in the gas collector is recorded, along with the time. A dye may be added to the water to facilitate easier reading of the liquid level. In addition, the liquid may be acidified to reduce the migration of CO_2 from the biogas into the liquid phase, or a strong base (NaOH or KOH) may be added to the liquid to remove CO_2 from the biogas allowing direct measurement of daily methane production.

3. Discussion

3.1. Experimental work using the Bordeaux stirrer

This gas-tight bioreactor design is suitable for various types of anaerobic culture work and has been operated continuously for more than three years without encountering a materials failure. A series of four mesophilic (35 °C) reactors fed Napier grass, amended with N and P, were used to investigate the potential benefit of micronutrient additions. This study resulted in enhanced catabolism of organic acids and a 40% increase in methane production with the appropriate additions of Ni, Co, Mo, Se and S (Wilkie et al., 1986; Smith et al., 1988a; Smith et al., 1992). A series of five reactors fed Bermuda grass were operated simultaneously at thermophilic temperatures (55 °C) and the culture bacterial lipids were examined to characterize the effect of propionate, butyrate and nitrate amendments on microbial biomass and community structure compared to control cultures (Henson et al., 1985). Using a substrate of Bermuda grass, the reactors were also used to provide a source of inoculum for enrichment studies leading to the isolation of a thermophilic butyrate-utilizing bacterium in co-culture with *Methanobacterium thermoautotrophicum* (Henson and Smith, 1985; Smith et al., 1988b). Another study using this bioreactor design examined the influence of propionate and butyrate infusions on thermophilic cultures also maintained on Bermuda grass (Henson et al., 1986; Smith et al., 1988a).

Further research using this reactor design investigated the effects of soluble oxidants on thermophilic digestion of Bermuda grass, which confirmed that nitrate was preferentially reduced in the presence of sulfate (Rivard et al., 1988; Smith et al., 1988a). Additional studies successfully isolated six new xylan-degrading strains of *Butyrivibrio fibrisolvens* from a mesophilic reactor fed Napier grass (Sewell et al., 1988). The reactor system was also used to develop thermophilic enrichment cultures on Bermuda and Napier grass, which allowed direct isolation of functional genes encoding cellulases from the microbial consortia (Healy et al., 1995). The Bordeaux stirrer was also employed in studies of artificially imposed extreme environments of high hydrogen and propionic acid inputs, high organic loading rates, and low hydraulic retention times in both primary sewage sludge-fed and Napier grass-fed cultures (Wilkie and Smith, 1989). More recently, the bioreactor was used in comparing the effects of retention time on performance and effluent odor concentrations in anaerobic digestion of flushed dairy manure (Powers et al., 1997, 1999).

3.2. Additional applications and modifications

The biochemical methane potential (BMP) assay (Owen et al., 1979; Owens and Chynoweth, 1993) is cited as a means to estimate the ultimate methane yield and first-order methane production rate for biomass and waste substrates. This simple batch assay relies on low substrate levels to assure digester imbalance does not occur. In the standard 100 mL assay, only 0.2 g of degradable sample should be added to the serum bottles (Owens and Chynoweth, 1993). For most biomass and waste substrates, no more than 0.4 g of dry material should be placed in the assay bottles. When substrates are reasonably homogeneous and finely ground, this limited sample size is sufficient for reliable estimates of ultimate methane yield. However, some biomass and solid waste samples are more heterogeneous and obtaining 0.4 g of a representative subsample is not reproducible. This increases the measurement errors on replicate samples. The simple bioreactor as described is ideal for treatability studies to estimate methane yield and methane production rate parameters from either semi-continuous or batch operation on samples of heterogeneous biomass and waste samples. Since much larger samples of substrate may be employed (8–16 g of dry sample per day), the precision of yield measurements can be high.

The bioreactor can be used for "pure culture" work, since the reactor (glass) and components can be autoclaved. Other materials (plastic) may be substituted for the reactor vessel. Also, the stirring mechanism is suitable for larger volumes (up to 20 L). While the cited studies used an intermittently stirred, daily feeding regime

for particulate substrates, with minor modification the bioreactor can be configured for continuous feeding experiments using soluble substrates. If a constant-head gas metering device is used to monitor gas production, the outlet of the effluent line from the bioreactor can be placed above the culture level to allow effluent to spill continuously into a receiving flask. Continuous feeding also requires a low-volume, positive displacement metering pump.

3.3. Particulate mixing

At 60 rpm, the tip of the stir rod (assuming a radius of 7.6 cm) travels at a velocity of 48 $cm\,s^{-1}$ during stirring. This is sufficient for suspending particulate matter including grit, which is often present in primary sewage sludge and livestock waste. Agitation with a magnetic stirrer in glass reactors fed primary sludge fails to adequately suspend these heavier particles and tends to scour the glass bottom until failure (unpublished observation). In addition, the depolymerization of particulate organic matter often requires surface attachment of microorganisms, which can be disrupted by excessive mechanical agitation (Aldrich, 1993; Fannin, 1987). Observations of daily biogas production, in continuously stirred Napier grass-fed reactors, indicated lower gas production than in similar intermittently stirred reactors (unpublished data). Also, biofilm thickness was shown to decrease by more than half when tip velocity increased from 40 to 120 $cm\,s^{-1}$ (Liu and Tay, 2001). The ability of the Bordeaux stirrer to suspend particulates at moderate tip velocities and to operate intermittently is a distinct advantage.

Routinely, the bioreactors were operated with mixed-liquor total solids concentrations of 3.4% (Rivard et al., 1988) and were fed biomass slurry of 8% total solids without problems. In some cases, feed-slurry concentrations of up to 11% TS were fed without suffering mixing problems (Smith et al., 1988a). The 60-rpm stirrer motor used produces a torque of 0.53 $N \cdot m$, and is sufficient to mix the slurry. The rheology of plant-matter slurries can impede agitation in digester systems (Fannin, 1987), but the Bordeaux stirrer is capable of handling slurries with solids concentrations greater than 10%, based on observed mixing and settling of particulates during operation.

3.4. Economic assessment

The total cost per bioreactor is approximately US $622 (based on 2003 prices). This includes the stirring mechanism and the biogas collection system. The components of the fabricated water bath are not included, but a Styrofoam cooler, thermometer, and aquarium heater could be substituted to satisfy these requirements at minimal cost.

4. Conclusions

This low-cost bioreactor system has been used effectively in a variety of fermentation research projects over a 15-year period. The Bordeaux stirrer is a simple apparatus for gas-tight stirring of fermentation cultures and has been found to be inexpensive and very reliable, based on reproducibility of reactor conditions and minimal maintenance requirements. The stirrer seals the rotating shaft in a flexible tube and is suitable for providing intermittent or continuous mixing in bench-scale bioreactors. This economical bioreactor design offers a useful laboratory tool and represents a valuable contribution to basic research in anaerobic digestion and gas-tight microbial cultures fed particulate biomass.

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