

CHAPTER 3

A novel anaerobic bioreactor for laboratory scale experiments

(Provisional patent applied for)

3.1. Introduction

The study of anaerobic bacteria has intensified tremendously over the past years. Such microorganisms, in addition to being of clinical interest, have considerable ecological importance as well as potential use in industrial processes. Experimentation has however been hampered by the lack of inexpensive, readily available equipment.

Currently a variety of techniques and equipment are used for anaerobic studies. These include custom made bioreactors, anaerobic cabinets, Gaspack systems (Figure 1) and a variety of other devices. Professionally manufactured anaerobic bioreactors are prohibitively expensive and also expensive to operate.

Sulphate reducing bacteria (SRB) are strictly anaerobic microorganisms. During anaerobic culturing, all oxygen must be excluded from the system (Prescott *et al.*, 1990).

This can be accomplished by:

- Using special anaerobic media containing reducing agents such as thioglycollate or cysteine. The reducing agents will eliminate any dissolved oxygen present within the medium so that anaerobes can grow beneath the surface.

- Oxygen may also be eliminated by removing air with a vacuum pump and flushing out residual oxygen with nitrogen gas. Often CO₂ as well as nitrogen is added to the chamber since many anaerobes require a small amount of CO₂ for best growth (Figure 2) (Prescott *et al.*, 1990).

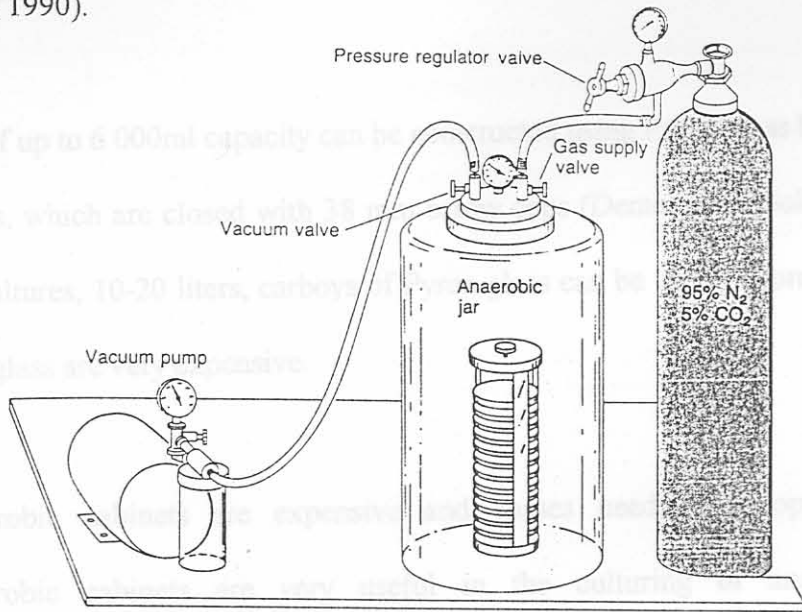


Figure 1. Vacuum and gas displacement method of anaerobic culture. The anaerobic jar is evacuated at least three times and refilled each time with nitrogen or a nitrogen-carbon-dioxide mixture (Prescott *et al.*, 1990).

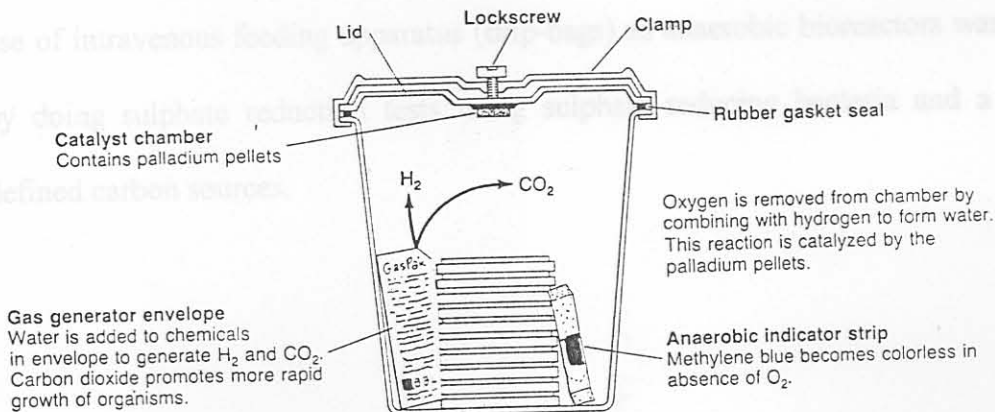


Figure 2. The GasPak anaerobic system (Prescott *et al.*, 1990).

One of the most popular ways of culturing small numbers of anaerobes is through the use of a GasPak jar (Figure 2) (Prescott *et al.*, 1990). In this procedure, the environment is made anaerobic by using hydrogen and a palladium catalyst to remove oxygen through the formation of water.

Cultivation vessels of up to 6 000ml capacity can be constructed using Pyrex glass bottles or Erlenmeyer flasks, which are closed with 38 mm screw caps (Demain and Solomon, 1986). For larger cultures, 10-20 liters, carboys of Pyrex glass can be used. Bioreactors manufactured from glass are very expensive.

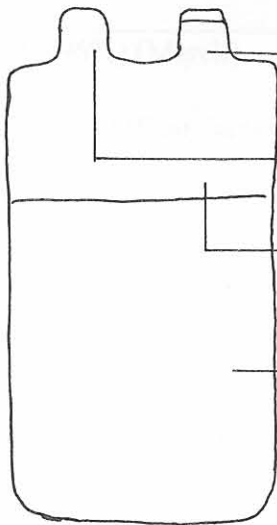
Conventional anaerobic cabinets are expensive and gasses needed for operation expensive. Anaerobic cabinets are very useful in the culturing of anaerobic microorganisms but not practical for the use as anaerobic bioreactors.

From the above it is clear that there is a lack of inexpensive, anaerobic bioreactors, especially suited for training purposes and laboratory experimentation. In this study, the use of intravenous feeding apparatus (drip-bags) as anaerobic bioreactors was evaluated by doing sulphate reduction tests using sulphate reducing bacteria and a variety of defined carbon sources.

Figure 4. The anaerobic bioreactor (the expansion of the bag indicates gas production)

3.2 Materials and methods

Fermentor design



Total volume: 1000ml

Rubber seal through which excess gasses were removed and samples were taken.

Port through which mixture was poured and sealed with heat

Head space for gasses produced (H_2S , CO_2 or methane)

900ml of medium: mixture of artificial AMD, inoculum and carbon source

Figure 3. A schematic representation of the anaerobic bioreactor



Figure 4. The anaerobic bioreactor (the expansion of the bag indicates gas production).

Media composition

Table 1. Artificial acid mine drainage (AMD) composition.

Component	Quantity
MgSO ₄ .7H ₂ O (Merck)	1.31g
H ₂ SO ₄ (96%) (Merck)	0.30ml
FeSO ₄ .7H ₂ O (Saarchem)	4.56g
NH ₄ Cl (Labchem)	0.19g
H ₂ PO ₄ (85%)(Merck)	0.02ml

The above chemicals (Table 1) were dissolved in one litre of distilled water. The pH was adjusted to 7.2 using 10M Sodium hydroxide (Merck). By using this formula, theoretically, an average sulphate concentration of 2 500mg/L should be achieved. The SQ118 spectroquant (Merck) (kit no. 1.14791.00010) was used to determine the sulphate concentration of the AMD.

Standardization of the inoculum

In order to find the most suitable concentration of inoculum (digester sludge), the following experiment was done. Mixtures of 1 litre AMD (Table 1) and 5.56ml lactic acid (92%) were prepared. The pH was adjusted to 7.2-7.5 before the digester sludge obtained from the Daspoort Water Purification Plant in Pretoria, South Africa was added. Volumes of 100ml, 200ml and 300ml of inoculum were added to the AMD and lactic acid mixtures, respectively.

Chemical analysis

A SQ118 spectroquant (Merck) was used to determine the amount of sulphate reduction using kit no. 1.14791.0001.

Preliminary sulphate reduction tests on AMD

A volume of 300ml inoculum was added to 1000ml of artificial AMD (Table 1). The respective carbon source was also added to this mixture (Table 2). Of this, 900ml were added to the drip-bags, respectively, which were used as bioreactors. The pH was adjusted to 7.1-7.5.

Table 2. Defined carbon sources used during this experiment

Carbon sources	Quantity per 100ml AMD
Lactic acid (92%) (Merck)	5.56ml
Acetic acid (Merck)	5.95ml
Butyric acid (BDH)	4.80ml

All experiments were done in triplicate. The sulphate concentration of the different bioreactors were monitored every 2-3d. As a control experiment, only AMD and inoculum was used with no added carbon sources.

3.3 Results and Discussion

Media composition

The average sulphate concentration of the artificial AMD was 2356mg/l. This resembles the sulphate concentration of AMD.

Standardization of the inoculum

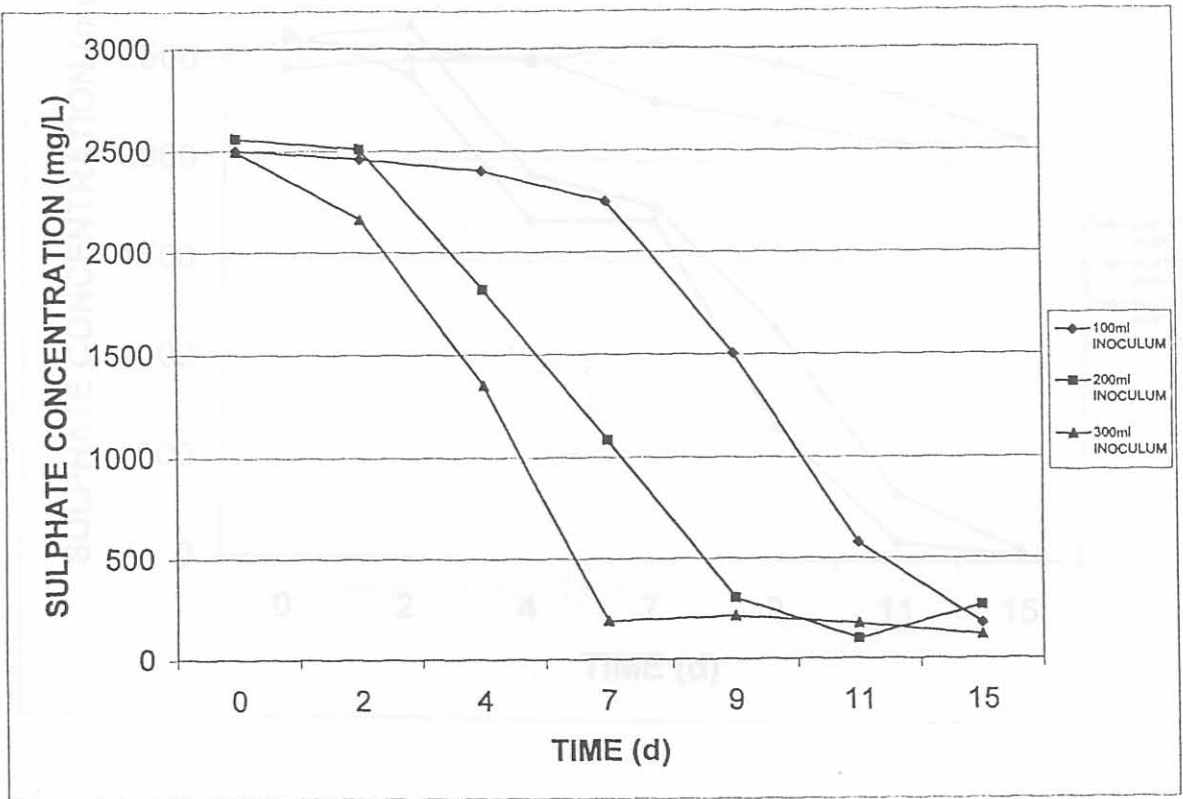


Figure 6. Preliminary sulphate reduction tests using lactic acid, acetic acid and butyric acid.

Figure 5. Sulphate reduction using 100ml, 200ml and 300ml of inoculum respectively.

Digester sludge consists mostly of fermentative bacteria and have low numbers of SRB (Zhender, 1988). The 300ml inoculum proved to be the most suitable concentration of

inoculum (Figure 5). A larger volume of inoculum would be impractical, due to the 1000ml limit of the bioreactor. However, this did not pose a problem, since effective sulphate reduction was obtained using 300ml inoculum (Figure 5).

Preliminary sulphate reduction tests with AMD

It was shown that with regard to sulphate reduction, acetate alone was not a good carbon source for SRB (Figure 6). In the presence of acetate, acetotrophic methanogens

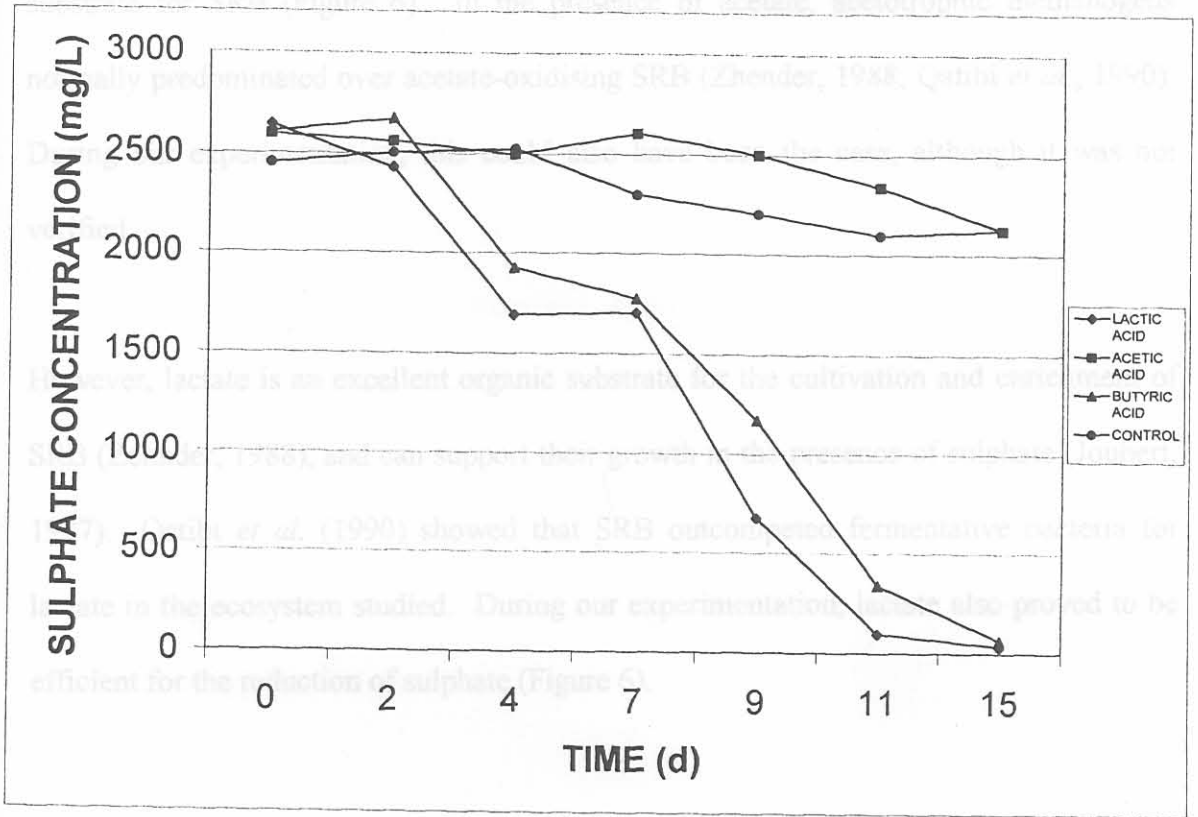


Figure 6. Preliminary sulphate reduction tests using lactic acid, acetic acid and butyric acid as carbon sources.

Preliminary sulphate reduction tests with AMD were done to see if the bioreactor was suited to be used as an anaerobic bioreactor. It was found that during the two week

period, lactic acid and butyric acid proved to be good carbon sources for the removal of sulphate while acetic acid was not efficient (Figure 6). These results confirmed studies done by other researchers (Isa *et al.*, 1986; Ahring and Westerman, 1987; Joubert, 1987; Zhender, 1988; Qatibi *et al.*, 1990; Visser *et al.*, 1993).

It was shown that with regard to sulphate reduction, acetate alone was not a good substrate for SRB (Figure 6). In the presence of acetate, acetotrophic methanogens normally predominated over acetate-oxidising SRB (Zhender, 1988; Qatibi *et al.*, 1990). During our experimentation, this could also have been the case, although it was not verified.

However, lactate is an excellent organic substrate for the cultivation and enrichment of SRB (Zehnder, 1988), and can support their growth in the presence of sulphate (Joubert, 1987). Qatibi *et al.* (1990) showed that SRB outcompeted fermentative bacteria for lactate in the ecosystem studied. During our experimentation, lactate also proved to be efficient for the reduction of sulphate (Figure 6).

Bioreactor design

In view of the above results, the drip-bag proved to be an effective, inexpensive small-scale anaerobic bioreactor. During all of the sulphate reduction tests, gasses were produced (Figure 4). The drip-bags were air-tight and no gas leakage occurred. This was illustrated by the fact that the drip-bags expanded to their full capacity. The rubber seal provided a convenient sampling port through which gasses could be removed by syringes.

Only minimal contamination of oxygen, if any, occurred during the taking of samples. This inexpensive, easy to use anaerobic bioreactor did not require the addition of catalysts to achieve anaerobic conditions and no contamination of oxygen occurred. Therefore we concluded that the intravenous feeding apparatus could be used as an anaerobic bioreactor for various laboratory experiments.

3.4 References

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