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A fermentation system for rapid and accurate modelling of rumen function

Report for MAF

August 2008

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1. Introduction

Currently, New Zealand rumen microbial/nutritional scientists use a simple batch culture system to evaluate the relative quantities of fermentation gases produced from fresh forage substrates incubated with rumen contents. This technique is a static system that can only be used for short periods and is non-representative of dynamic rumen processes, since it does not allow an adaptation of the microbial community. In batch cultures, gas composition is measured at the end of the fermentation period, which does not account for the release rates. At present, batch cultures are carried out manually which severely limits the number of samples that can be handled and introduces additional analytical variation.

To address the limitations of the existing fermentation technology, we constructed a 'state of the art' continuous dual flow fermentation system for use by rumen microbiologists/rumen nutritionists comparable to the system described by Teather and Sauer (1988) (see Appendix 1). The continuous fermentation system enables solid and liquid substrates to be fed by computer control into the fermentor, where solid particles are retained longer in the raft mat than liquid components, leading to different liquid and solid turnover rates as observed in the rumen. The pH in the fermentors can be monitored and controlled by the system and the system is interfaced with gas analysers to facilitate continuous and automated monitoring of fermentation gases. This facility will speed up work on developing mitigation solutions for enteric methane emissions. In particular, this system will facilitate:

- screening of potential mitigation agents or forages;
- the assessment of dose response rates, prior to expensive animal trials;
- real-time and long term assessment and characterisation of microbial and biochemical responses;
- the testing of new tools for monitoring changes in rumen microbial populations.

In addition to speeding up development of methane mitigation strategies, the continuous flow systems will facilitate a broader range of assays allowing us to model aspects of plant cell wall degradation and microbial nitrogen utilisation. The facilities will provide the NZ animal feed industry and researchers a means to evaluate; strategies for improving feed digestibility, forages or formulations for reducing methane emissions or improved nitrogen utilisation using a dynamic rumen-like system prior to animal based evaluations or cultivation of forages for future animal studies.

Besides being a useful tool for screening of anti-methanogenic materials, the system will also allow us to study the effect of basic parameters like rumen turnover rates and pH on methane production, which cannot be controlled independently in living animals. The unit is designed as an open system to which components can be added in order to improve it, based on needs arising when the system is in use in various projects.

2. Description of the system of the system

2.1 General

The fermentation system consists of a linear array of 6 vessels with a working volume of 1000 ± 10 ml mounted on a mobile stand (Figure 1). The vessels are situated in a temperature controlled box (working temperature, 39 °C). The vessels are fitted with an overflow that leads into a 2 litre bottle situated in a cooled (4 °C) water bath below the fermentor unit to stop fermentation of the out-flowing material. Temperatures of both the heated box and the water bath are computer controlled. Each vessel is fitted with a solid feeder, a pH and temperature probe, a gas flow meter and is connected to three pumps. One pump is for buffer that controls the liquid turnover, another is to pump liquid substrates and a base pump controls pH. Stirring is done magnetically via an interfaced stepper motor mounted below the fermentor. The fermentation gases are distributed via a solenoid valve to pass a cell for the measurement of methane and hydrogen concentration.

2.2 Interface

The program that controls the unit is LabView. Gas volume and composition are recorded for each fermentor continuously. In addition, pH and temperature are recorded at intervals that can be chosen for each fermentor individually. The system can be set to just monitor the pH, or control it. These measurements are logged in a data file along with the vessel identification, date and time of the measurements.

The unit also controls the feeder, the stirrer and the pumps for buffer, liquid feed and base. These controls are programmed for a 24 hour period but can be changed at any time during the experiment. The system can be programmed to dose a certain amount of substrate over various length of time from 1 dose to continuous feeding. The application of liquid feed can be also set independently for each fermentor over time. Stirring speed can be set faster to mix in feed better during feeding time and slower during the time when no substrate is applied. When sampling of total fermentor contents is required, the stirring program can be set to a mixing function in order to a receive a representative sample of the whole fermentor contents. Buffer flow can be

varied independently to modify liquid turnover in the system and can be synchronised with the application of substrates. The application of base is dependent on pH, but stirring will be slightly faster by default for a short period of time after the application of base to ensure the mixing of the base into the fermentor contents before the next pH reading. The front panel of the interface is shown in Figure 2 showing the readings and control settings for each fermentor and a graphic display of the measurement data of the last few hours.

2.3 Fermentation vessel

The fermentation vessel is a 2L borosilicate glass container with several outlets fitted (Figure 3). The overflow is fitted at a 45° angle to give an effective volume of 1000 ± 10 ml. On top of the overflow, a small geared motor with a rubber flap will be mounted, which turns whenever buffer is dispensed to the system to prevent a clogging of the overflow and an increase in liquid volume in the system. Into a smaller, second, 45° inlet, the pH and temperature probe is fitted beside the overflow. One of the small ports in the front will be the sampling port and the second is a provision for filtering liquid contents in order to manipulate the liquid turnover independently from the solid turnover of the system. One of the two back ports is for the application of buffer, the second for liquid feeds and base.

The lid of the vessel is airtight. Besides sealing the fermentor, the main purpose of the lid is to support the feeding unit. For refilling the feeder, a shutter located within the lid will keep the fermentor airtight while the new substrate is applied to the feeder (Figure 4). In addition to the feeder inlet, there are two gas outlets fitted in the lid. One outlet leads to the gas meter and the other is the application of tracer gases and for calibration purposes.

2.4 Stirring unit

In order to maintain a feed mat in the system, the stirring is generally very slow (~10 - 20 rpm). This is achieved by a stepper motor that drives a magnet which moves the stirring unit (Figure 5). The stirring unit consist of a paddle at the bottom of the fermentor and a coil that slices through the feed mat gently pressing the material down. Stirring regimes can be controlled via the interface and a manual override allows the collection of a whole content fermentor sample by setting mixing the contents for a short time.

2.5 Solid feed dispenser

The solid feed dispenser is a new development based on a design of Peter Lawrence, University of Hohenheim. The unit is basically a stainless steel tube with 35 mm inner

diameter into which the substrates are applied. After fitting on the fermentor, the substrates are forced into the fermentor by a piston driven by a stepper motor. (Figure 6A) The feed is prevented from freely falling into the fermentor by a silicone diaphragm (Figure 6B). The unit fits airtight into the fermentor lid which can be sealed off when the feeder is removed to be refilled with substrate. The feeder holds dried substrate for at least 48 hours and in principle, can also handle fresh forages. At present the use of fresh substrates is a feature that is not fully tested and a cooling unit might be necessary to prevent the fresh feed from decaying within the feeder. However, this feature was not part of the original application, but could be an added value of the design.

2.6 pH measurement and control

The pH probe is inserted from the side into the fermentor and also includes a PT100 sensor for monitoring the temperature in the vessel. Temperature within the vessels is used to control the heater located at the back of the box in which the fermentors stand. The system is capable of monitoring the pH of the fermentation, but also to control pH by the infusion of a base to maintain a preset value. pH is measured at intervals that can be preset. Data are stored in a file along with temperature data from the temperature probe. In the case of controlled pH, a reading below the threshold value will trigger the base pump to deliver a preset volume of base into the fermentor, stirring is increased for 2 minutes slightly and the next reading will be taken after 5 minutes in order to have the contents mixed properly before the next infusion of base.

2.7 Gas flow meter

The gas measurement is done volumetrically in a cell that fills and then flips to release the gases (Figure 7). One of these units is attached to each fermentor and the gas volume is measured continuously by a little magnetic switch. The volumetric measurement was chosen, because in contrast to electronic devices, the measurement is not influenced by gas composition, water vapour and fermentation acids present in headspace gases. The data from the cells are recorded continuously and logged in a data file.

2.8 Gas composition detector

There are two detector cells, one for hydrogen and one for methane. The cells have a range of 0-10% and 0-100% with a resolution of 0.01 and 0.5%, respectively. After the gas volume measurements, the gases from each fermentor are forced through the two cells where gas composition is determined. In between measurements, the system is flushed with nitrogen to avoid cross-contamination. Gases are distributed via a computer-controlled solenoid valve to the methane and hydrogen cell. Gas

composition cells are mounted in the centre of the main panel above the fermentors (Figure 8).

2.9 Pumps

Each fermentor has three pumps for the delivery of liquid substrates, buffer, and if chosen, base. The pumps are mounted on the main panel above the fermentors (Figure 9). These pumps are interfaced with the central unit and the outputs can be preset for every fermentor individually. The buffer pump is the principal means of liquid turnover within the system, while base and liquid feed will be dispensed only at times of feeding or when the pH drops below the preset value.

3. Design and manufacturing

The system was designed by Stefan Muetzel and Mike Tavendale based on a principle design of Teather and Sauer (1988), a system that was set up at the University of Hohenheim by S. Muetzel. The system developed will be the first continuous culture system where gas production and gas composition can be monitored online.

Sourcing of the components and manufacturing was done at Lincoln Research centre under the lead of Steve Gebbie with Scot Sevier and Hong Zhang responsible for modelling and interfacing respectively. Assembly of the system is being completed at Lincoln and Stefan Muetzel will commission the system at Lincoln before it is brought to the Palmerston North site of AgResearch.

Appendix 1

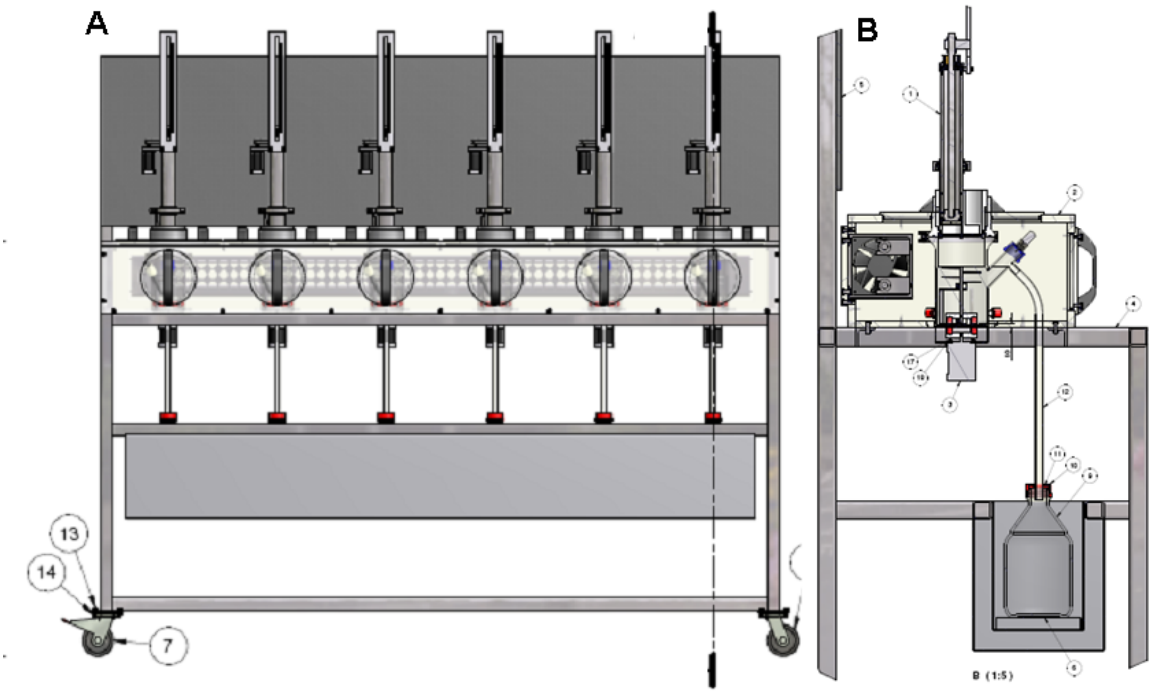

Figure 1 Overview of the unit of 6 vessels in front view (A) and side view (B)

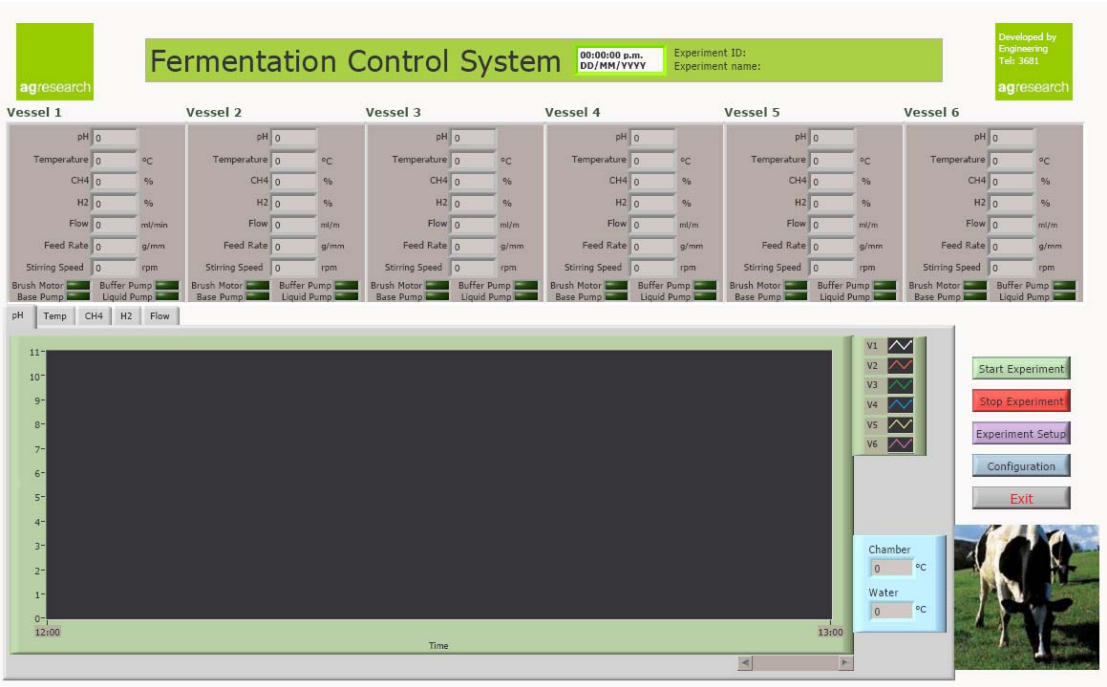

Figure 2 Interface front view showing the system settings from each fermentor and a graphic display of measurements

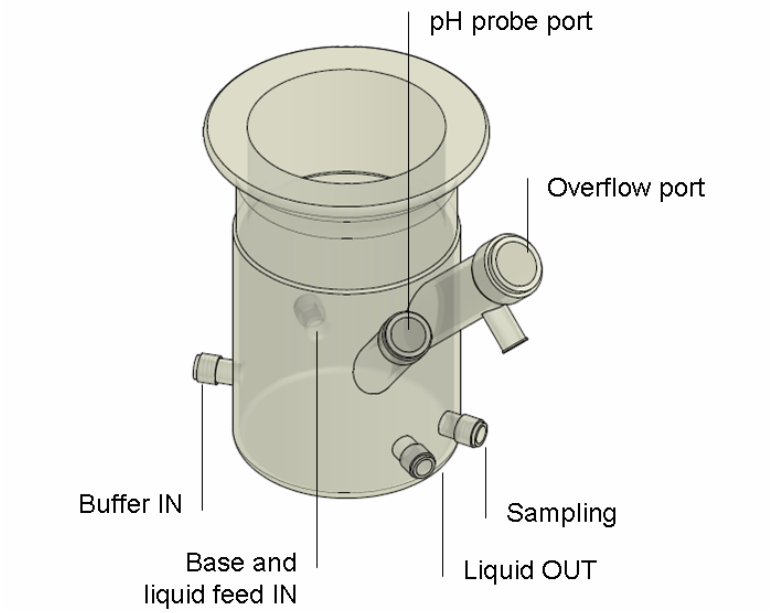

Figure 3 Glass vessel with overflow and ports for sampling and introducing liquid components and pH measurement

Figure 4 Vessel lid with shutter and an two gas outlets

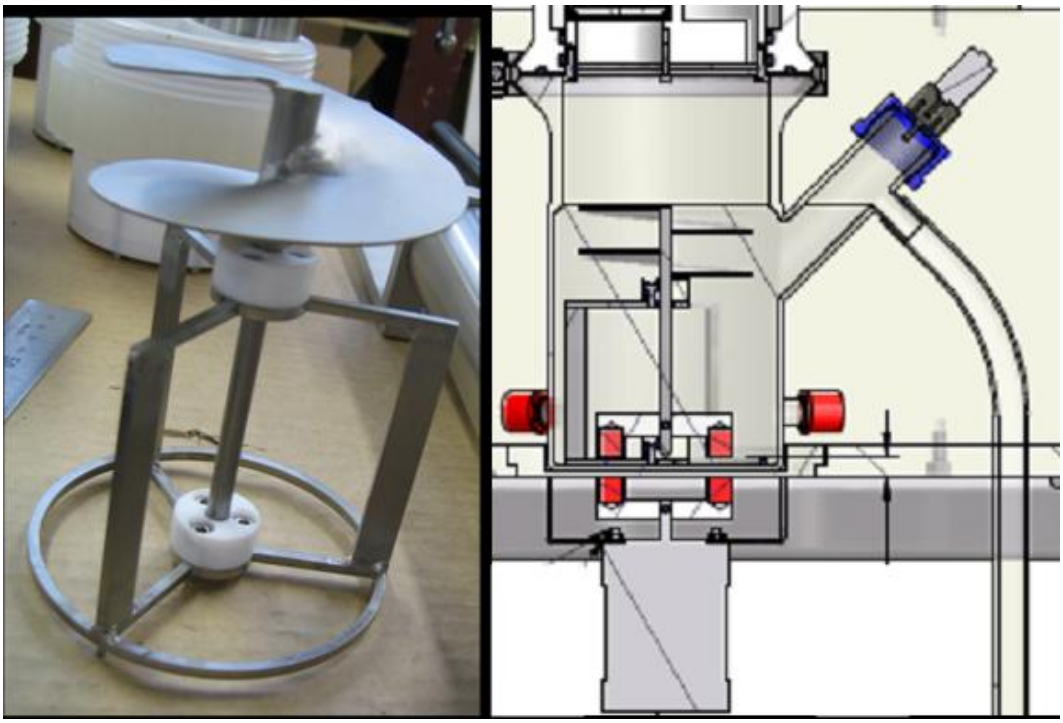

Figure 5 Stirring unit with coil and schematic view of the unit within in the fermentor and the motor located beneath the fermentor.

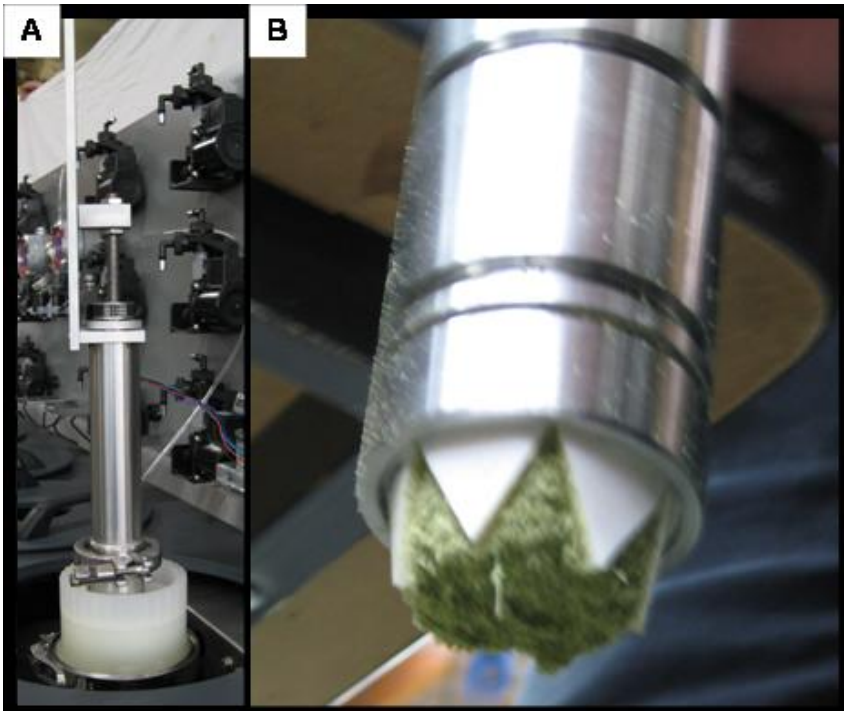

Figure 6 Solid feed dispenser mounted on a fermentor (A) and end piece showing substrate held by the silicon diaphragm (B)

Figure 7 Gas flow meter unit

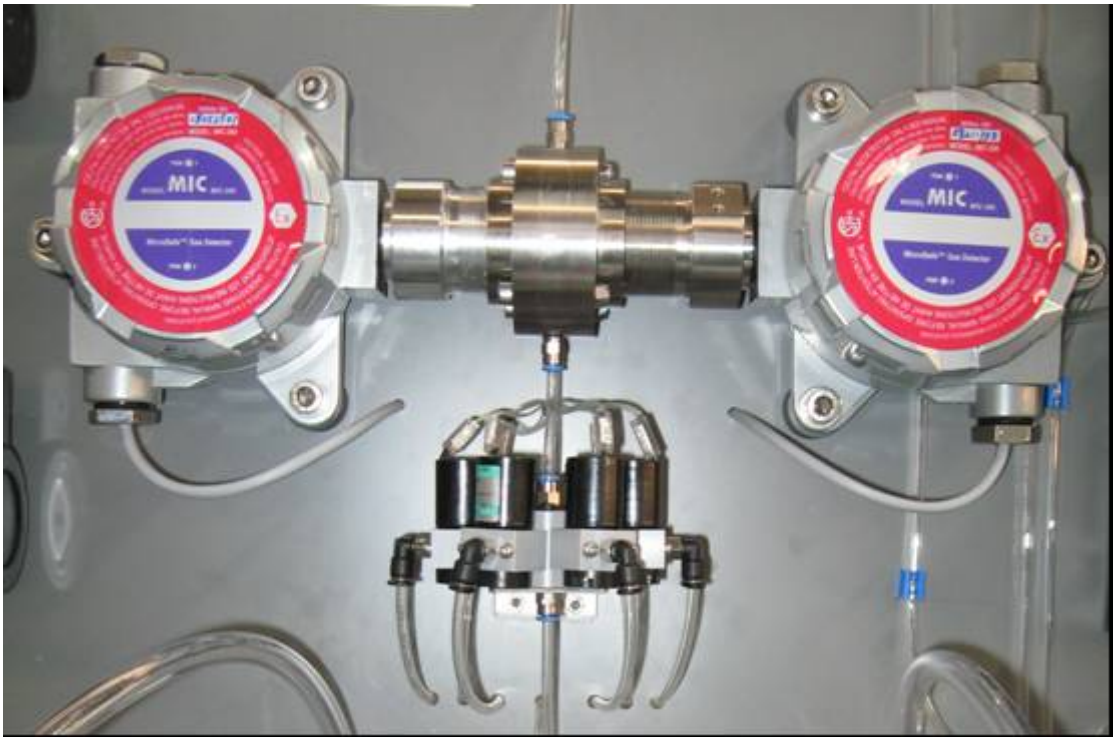

Figure 8 Gas composition cells and solenoid valve (below) mounted at the central panel

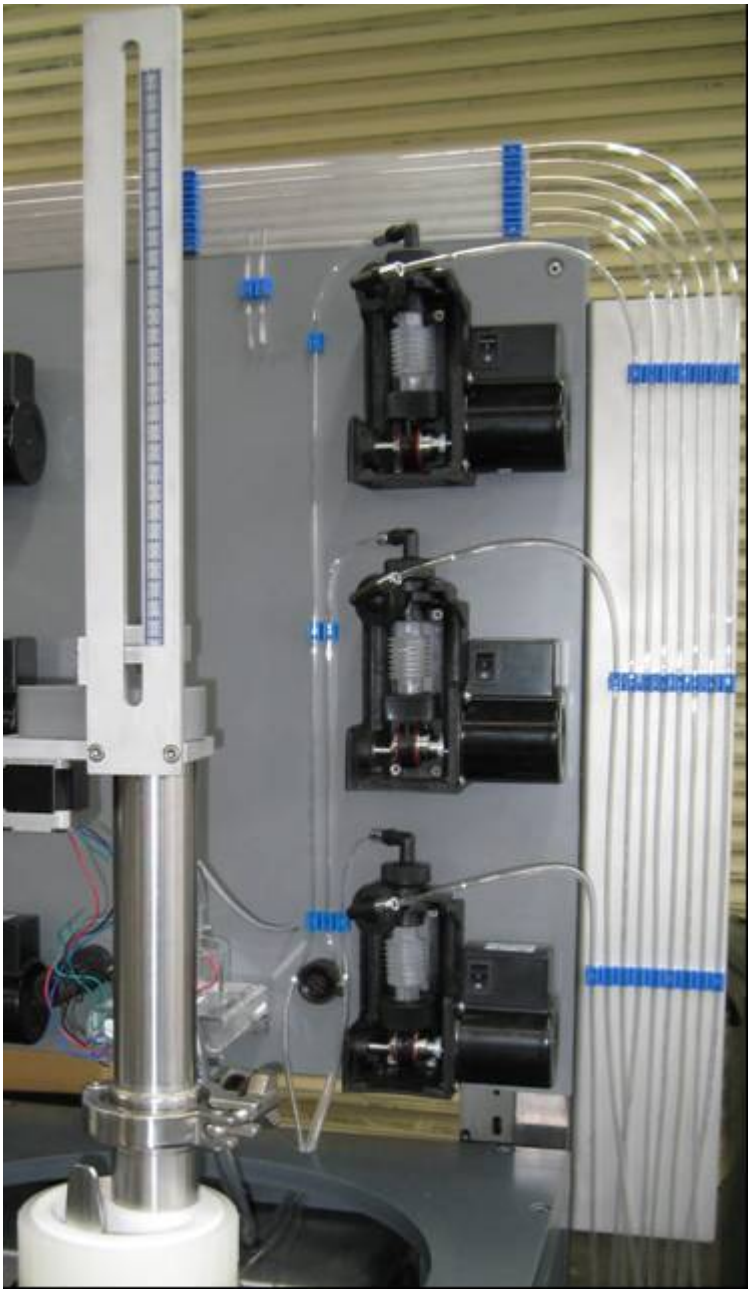

Figure 9 Array of pumps to deliver buffer, liquid feed and base into the fermentor.