# AUTOMATIC ASEPTIC SAMPLING APPARATUS

This invention is concerned with an automatic aseptic sampling apparatus, especially one suitable for drawing uniform sterile culture samples from a bioreactor at preset intervals.

## BACKGROUND OF THE INVENTION

Few sampling systems supplied with laboratory fermenters are easily modified for unattended aseptic sampling. Most commercial sampling systems are manually operated. They are either hooded samplers or bottom-harvesting valves. The former operate on a vaccum principle and are widely accepted as being safe and simple for culture sampling. They can be sterilized either separately or with the culture vessel. During operation, aseptic conditions are maintained by a break in the liquid stream, along with the hood on the exit line. The latter — bottom harvesting valves — are usually found on larger (2–25 L) fermentation vessels. They use in situ steam sterilizable diaphragms or piston valves. These valves are easily sterilized between sampling times and are very reliable.

An automated, computer controlled HPLC system is described by R.C. Dinwoodie et al in Biotechnology and Bioengineering, Vol. XXVII, pp. 1060–1062 (1985). The system is equipped with a continuous flow-through sample vial for the on-line analysis of fermentation broths. A peristaltic pump withdraws a stream from the fermenter, passes it through a filtration unit and then through the vial. The stream and the filtered cells are returned to the fermenter.

Another sampling device is described in Biotechnology and Engineering, Vol. XXVIII, pp. 119–121 (1986) by M. Ghoul et al. This device has a recirculation loop with a proportioning peristaltic pump and four three-way sterilizable valves. A continuous band of ultrafiltration membranes is provided to filter the sample drawn from a fermenter. The filtration surface is renewed after each sample. A cuvette is provided to receive the samples after filtration. The entire sampling system is controlled by a microcomputer. Steam sterilization of the circuit is available to prevent its contamination with microorganisms.

While these prior art devices are useful, there is still a need for a simple and reliable sampling system that is useful, for instance, for batch yeast fermentation lasting 12 to 24 hours. In particular, it is an object of the present invention to develop a sampling system wherein the often large dead volume would be reduced to a minimum.

## STATEMENT OF THE INVENTION

According to the present invention, there is provided an apparatus for taking liquid samples from a container such as a bioreactor, or fermenter, which comprises

(a) a three-way valve having a first port communicating with the container, a second port and an outlet port,

(b) a waste liquid vessel,

(c) a reversible pump communicating on its one side with the second port and, on the other side, with the waste liquid vessel,

(d) a first flow control means associated with the first port for only permitting the flow of liquid therethrough from the container to the three-way valve, and

(e) a second flow control means associated with the outlet port for only permitting the discharge of liquid therethrough from the three-way valve.

Preferably, the reversible pump is a peristaltic pump. A control means is provided to alternate the operation of the pump in both directions. The control means may include, for instance, a timer and a controller which are adapted to operate the pump for a selected period of time at preset intervals in either direction.

## DESCRIPTION OF PREFERRED EMBODIMENT

The invention will be explained in more detail in and by the following description to be taken in conjunction with the drawing, in which

FIG. **1** is a schematic representation of the invention as associated with a fermenter and

FIG. **2** is a cross-sectional view of an embodiment of the three-way valve.

Referring to FIG. **1**, the apparatus comprises a three-way valve **10**, a reversible peristaltic pump **12**, a waste container **14** and a sample collector **40**. In the embodiment illustrated, the apparatus is adapted to take uniform liquid samples from a fermenter **18**, e.g. to determine the kinetics of fermentation of certain cultures.

The waste container **14** is a simple sterilizable vessel having an inlet port **15**. The vessel is provided with a porous plug **20** to prevent the ingress of contaminants, e.g. bacteria to the system while permitting an easy flow of air to and from the container **14**.

The peristaltic pump **12** (Cole-Parmer Masterflex Pump with pump head #7015-20) is installed on a length on non-collapsible silicon tubing **22** which connects the port **15** of the waste container **14** to the three-way valve **10**. As shown in FIG. **2**, the three-way valve **10** has a first port **26**, a second port **28** and an effluent port **30**. The first port **26** is provided with a check valve **32** which is a sterilizable machined teflon valve but may be replaced with a ball check valve since the branch, or leg, of the valve **10** ending with the first port **26** is normally disposed vertically. The branch of the valve **10** having the outlet port **30** is normally positioned horizontally and is provided with a machined teflon check valve **34** which is held against its seat by means of a spring **36**.

The pump **12** is connected to the second port **28** of the three-way valve **10**. The first port **26** of the valve **10** is in communication with a fermenter **18** through a conduit **38** which ends with a probe, or a transfer needle, not illustrated in the drawing.

The effluent port **30** is connected to a sample collector **40** via a tubing **41**. A Gilson Model 201 programmable fraction collector with a 27-position multipurpose rack has been employed in this embodiment of the invention. The samples are distributed to separate sample tubes situated in a cooling bath **42**.

A timer **44** and a controller **46** are coupled electrically with the peristaltic pump **12** and with the sample collector **40**. They serve to automate the sampling proce-dure by reversing the operation of the peristaltic pump **12** at selected intervals and by controlling the duration of pumping. Also, the sample collector is controlled correspondingly for the successive samples to be passed to separate sample tubes.

It will be noted that the timer **44** and the controller **46** are not mandatory for the operation of the apparatus. The pump may be operated and reversed manually where only a few samples are to be taken. Alternatively, for long processes to be monitored, it may be expedient