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Automatic whole broth multi-fermentor sampling

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SUMMARY

An efficient, aseptic method of obtaining whole broth fermentation samples was developed based on a piston-valve, a local sample loop, and an ability to drive the entire sample volume with sterile air through a sample line and into a remote tube. The configuration delivers 10-ml samples 10 m away with about 4 ml of broth wasted in the sampling process. An autosampler was enhanced and programmed to control acquisition into chilled tubes. The autosampler-based system represents a convenient way to provide frequent samples to profile intracellular and extracellular components for yeast and bacterial fermentations. A configuration to provide sampling from six fermentors with a multi-rack autosampler will be presented.

INTRODUCTION

The ability to characterize a fermentation process is often constrained by the analysis work required. Analytical support for fermentation research can be enhanced using automatic sampling. Automatic sample acquisition is a highly regarded convenience for fermentation process characterization. Automatic sampling and on-line fermentation analysis of extracellular components typically utilize a recycle loop with tangential flow filtration [7,9,11,12] or filtration probes [2,8] for the combined functions of sample acquisition and sample preparation. For cell-associated analyses, automatic whole broth sample acquisition is the necessary first step for subsequent analysis. Systems for automatic whole broth fermentor sampling have also been documented [1,3–6]. We developed an automatic system to provide highly efficient sampling with a number of desirable features. The resulting method for obtaining fermentation samples into chilled tubes has proven to be a most useful research tool and will be described. The sampling configuration will first be presented. An efficient method of controlling delivery from multiple units will then be covered. Finally, features of the autosampler-based program will be discussed and a typical application presented.

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MATERIALS AND METHODS

Sampling configuration

A piston-type sampling valve (Biolafitte, Soissons, France) was implemented for the automatic sequence developed. This valve, which was already in place for manual sampling, provided a number of advantages for automatic sampling.

The piston-valve assembly is comprised of an inner piston, an outer cylinder, and a lever which drives the piston in and out. The outer cylinder fits into a standard 25-mm port. A steam line is tapped into the port for valve sterilization. The piston is hollow along its length from the side inlets to the valve outlet. In the non-sampling position the piston inlets are lined up with the steam tap (Fig. 1a). In the actuated position, the piston moves in and the piston inlets are exposed to the fermentor contents (Fig. 1b). To sample a fermentor manually, the valve is steamed, a sample tube is held at the valve outlet and the lever is pulled to actuate the piston and collect the sample.

The piston-valve provides an ideal mechanism for steaming the sampling line from as close to the fermentor as possible. Furthermore, by augmenting the steam inlet with a sterile air inlet, almost all of the sample which exits the fermentor can be blown into the remote sample tube. This provides a zero-dead-volume design. No sample is left to stagnate in the line between samples, the entire sample flow patch can be steamed, dried and cooled between uses, and no sampling pump is required in the configuration.

Use of the piston-valve for automatic sampling required customization. Automatic actuation of the valve

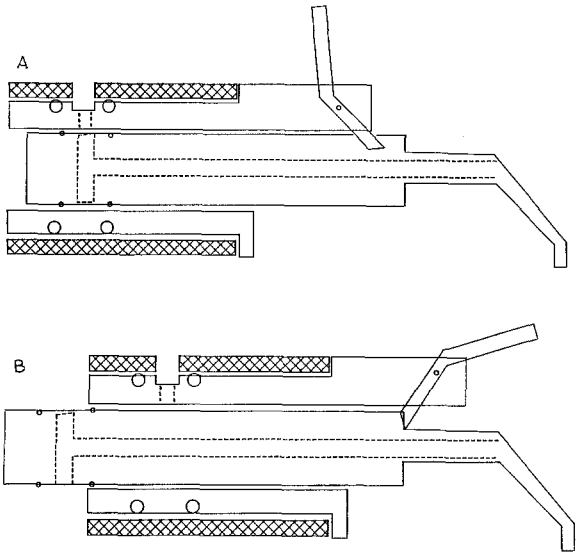

Fig. 1. The piston-type sampling valve. **A.** Non-actuated. **B.** Actuated.

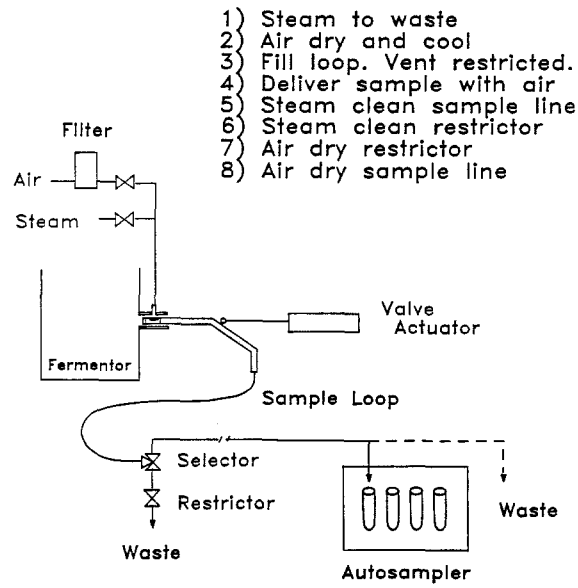

Fig. 3. The sampling sequence configuration.

was enabled for our application by mounting a double-acting air cylinder onto the valve (Fig. 2). The cylinder-body was mounted to the valve-piston and the cylinder rod was attached to the valve lever. Pneumatic operation of the sampling valve was thus enabled.

Sample acquisition and delivery requires additional valving (Fig. 3). Automatic diaphragm valves already in place for nutrient feed port sterilization were reconfigured to provide sterile air or steam to the sample valve steam tap. A flexible line from the sample valve to the common port of a three-way ball-valve provides a local sample loop. Fermentor pressure drives sample through the loop to waste upon sample valve actuation. The sample aliquot is driven with sterile air to the remote location. Steam is used to sanitize the loop before and after sampling. The valve and loop are cooled and dried with air before sampling. (Note: the port is not open to contamination during sampling, so sterilization between samples is not necessary. The sample loop is steam-sterilized along with the batch prior to inoculation.) Sterility tests were performed with the system. Media with 10 g/l of both yeast extract and glucose was sampled hourly from two fermentors. No contaminant growth was detected after 10 days of automatic sampling.

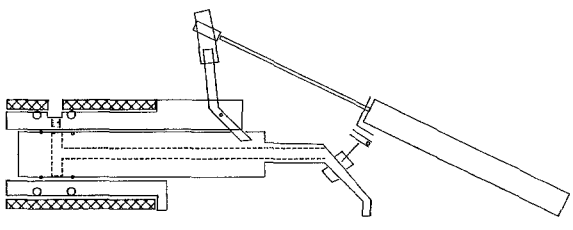

Fig. 2. Automatic actuation of the sampling valve.

Waste minimization

The question of how to actuate the sampling valve for a long enough time period to completely fill the loop without purging excess broth to waste was a key design issue. Our solution was to use a valve to restrict the flow to waste from the sample loop. Since flow through a restriction decreases with viscosity, it was possible to size the valve closure so that displaced air would vent rapidly (being relatively non-viscous) while the flow of liquid through the restriction would be greatly inhibited. The loop purge-time could thus be adjusted for the 'worst case' situation where a relatively low fermentor pressure is driving a high-viscosity sample through the loop. Low-viscosity broths sampled within the operating pressure range of the fermentor do not cause additional waste since the sample flow practically stops when the loop is filled and liquid reaches the restriction.

The restriction is comprised of a needle-bonnet valve which is adjusted to remain slightly open in the 'closed' position. The two-position valve is thus either fully open or almost closed. The restriction is most valuable for minimizing waste, but is also used to provide back-pressure during sample port sterilization. A fixed orifice would not suffice mainly because the restriction must be cleared after each use. A blast with steam and then air in the fully open position effectively clears the restriction for re-use.

The sample size is set by the volume of the sample loop. Programmatic sample volumes can conceivably be obtained using a variable number of loop volume deliveries. The loop volume includes the volume in the piston (about 5 ml) plus the volume in the line to the three-way valve (about 3 ml minimum). The maximum working volume for the standard sample tube is about 35 ml. Higher volumes caused excessive splashing with the air flow rates used for delivery. The sample volume can thus be adjusted from 8 to 35 ml. Combined volume loss between the loop and the restrictor, and loss due to wall adhesion totals about 4 ml per sample. The impact of sampling on process volume can be put into perspective: 24 10-ml samples will provide 240 ml of sample, wasting an additional 96 ml in the process. (Waste can be directed to a kill tank if desired.)

The reproducibility of sampling depends on the broth. Water can be delivered with a standard deviation of 0.1 ml using a 10-ml loop. A viscous broth with particulate media components showed a standard deviation of 1.1 ml using the same loop. The absolute precision is not, however, an important consideration for the sampling application described.

Sampling sequence

The entire sampling sequence can be summarized as follows:

(1) Steam the valve and loop to waste, unrestricted, to drain condensate (60 s).
(2) Steam the valve and loop to waste, restricted, to sanitize the port and maintain sterility (120 s).
(3) Steam the sample line (20 s).
(4) Air dry and cool the sample line (180 s).
(5) Air dry and cool the sample loop and the restriction (120 s).
(6) Position the remote autosampler cannula to the sample tube.
(7) Actuate the piston valve and fill the sample loop (6 s).
(8) De-actuate the piston valve and deliver the sample (30 s).
(9) Position the autosampler cannula back to waste.
(10) Steam-clean the sample line (20 s).
(11) Steam clean the restrictor-valve (10 s).
(12) Air dry the sample line (10 s).

Additional details of sampling

Sample delivery using various sample line IDs (inner diameters), air pressures, and sample viscosities was investigated. Sample losses on tubing walls were found to increase with increases in the tubing ID and sample viscosity, and with decreases in the air pressure. The air pressure required for reliable delivery increased with viscosity, and lower ID. Higher air pressures (above 30 psi) produced excessive entrainment of sample into the requisite air stream exiting the sample tube upon delivery. Tubing with 1/8″ ID and air pressures between 15 and 20 psi produced good characteristics with respect to reliability (no occlusion), minimal losses and acceptable splash upon delivery. Polypropylene tubing with 1/4″ OD was used. Its temperature characteristics have been acceptable, though some deformation at unions is apparent after numerous sterilizations with steam. (Teflon is equally or better suited for the application, though it too will tend to flow at the sterilization temperatures). The use of clear tubing is advantageous: the ability to see the sample through the tubing is valuable for troubleshooting and provides visual verification that sample is not getting past the restriction after the loop is filled. A poorly adjusted restriction could lead to excessive volume losses if not corrected.

The sample line is terminated at the autosampler cannula. The cannula tip is normally inserted tightly into a waste line. After the sample line is sterilized and dried, the cannula travels to the next sample tube and lowers into the tube for delivery. Afterwards, the cannula is directed back to the waste location so the sample line can be steamed clean.

It is interesting to note that during delivery the air should be pulsed off and on if there are any dead-ended flow path junctions in the sample line. During development of our multi-fermentor manifold, we found that a steady stream of air would deliver 6 ml, while a stream of air pulsed off and on would deliver 10 ml. Sample apparently was being trapped in the tees and crosses of our check-valve manifold during sample delivery. Turning the air off accessed the lost sample as it backed out the dead-ends as pressures equalized. A single pulse off during delivery proved adequate to minimize the sample volume loss.

The standard cannula for the autosampler was too small for some of our target whole broth sampling applications. A larger bore cannula was fabricated using 1/8″ OD, thin-walled stainless steel (surgical) tubing. This bore proved too large for piercing septa and a practice of using tubes 'sealed' with aluminum foil was adapted for autosampler-based acquisition. The cannula tip is angled to pierce the foil during sample delivery.

Sterility can be maintained right up to the cannula tip from the sample port using steam and sterile air as described. If necessary, aseptic samples could be provided using a suitable sterile environment at the autosampler. With the chilled rack, however, it is unlikely that the perforated tube seal will cause a problem with either contamination or evaporation.

The sampling sequence was developed using a micro-

processor-based multi-rack autosampler (Gilson Medical Electronic, Middleton, WI). The device has ample programmability and eight digital outputs. Five outputs were required to drive the sequence. Open-collector transistor outputs from the autosampler were used to drive a relay for each valve. The contact closures activated air and steam pneumatic control valves in the fermentor control cabinet. Pneumatic signals for the three remaining valves were generated with additional four-way solenoid valves. A program was written to sequence the valves and deposit samples into sequential tubes into a chilled rack on a periodic basis.

Multi-fermentor sampling

A strategy to control sample acquisition from up to six fermentors was also developed. The six designated fermentors were situated in close proximity (i.e. two rows of three, back-to-back, with an aisle in between). A common sample destination for all of the units was thus feasible. The question of whether to have separate sampling controllers for each fermentor, or centralized control for all the fermentors can be a key design issue. The advantages of distributed control with autosampler/fermentor pairs are increased capacity, simplicity of design and independent sampling. The advantages of centralized control with a single autosampler are reduced capital and lab space conservation. Given the eventuality of implementing robotic sample processing for the six fermentors, the centralized approach was clearly indicated for our application. The high capital investment and large size of a robotics system precludes the distributed approach for a group of proximate fermentors. The decision is more a matter of preference for autosampler-based multi-fermentor sampling control. For the current application, a pneumatic control cabinet was designed (in-house), fabricated (Panel Oven Eng., Edison, NJ), and installed to control sampling from the six designated fermentors (Fig. 4).

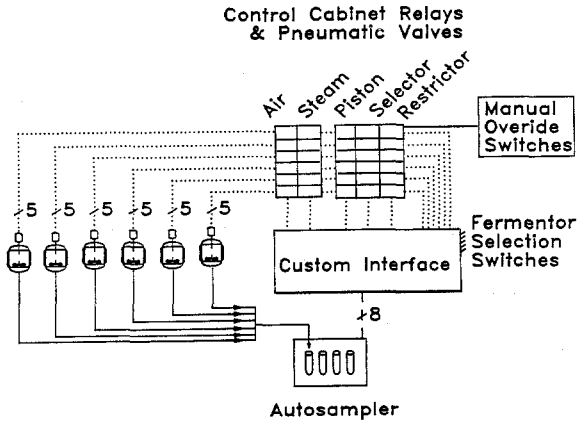

Fig. 4. Multi-fermentor sampling.

Sampling system actuator matrix

Using centralized control, the number of primary control signals can be much less than the number of actuators controlled in the system. To accomplish this, a matrix is wired so that a positive voltage is supplied to select the desired function, and a ground is supplied to select the fermentor set. In our system for example, six sets of five actuators are controlled with 11 control signals (Fig. 4). The control programming is simplified with the matrix configuration because only the enabling signal is fermentor-dependent. The same five valve signals are sequenced regardless of the fermentor being sampled.

The control signals are supplied through a multi-conductor cable from the control relays to the cabinet. For convenience, the control signals in our system can also be generated using a portable switch-box. This manual-override input/output (I/O) box was specified primarily for trouble-shooting, but is also useful for taking manual samples. The I/O box is connected to the cabinet through a multi-conductor cable and can be taken to the fermentor of interest for interactive control of the automatic valves. A rotary switch is used to select the desired fermentor, and ten toggle switches are available to operate the relays and solenoid valves (SOVs) as desired (i.e. steam, air, piston, selector and restrictor, plus five spares for future applications). To enable the I/O box for input, it is necessary to select the 'manual' mode on the cabinet. A key is required to change the mode of operation. When not in use for manual control, the I/O box is centrally located where its schematic indicator lights show the valve positions and the identity of the enabled fermentor.

Multi-fermentor on-line sampling and analysis

In addition to automatic sample acquisition, a system of automation for on-line sample analysis was desired. A laboratory robotics system (Zymark, Hoptington, MA) was designed, specified, delivered and developed for multi-fermentor on-line analysis [10]. Whereas the autosampler was originally configured for single-fermentor sampling, the robotics system was configured for multi-fermentor sampling and analysis. Sample acquisition alone was still, however, the most generically useful application of the sampling capabilities. Multi-fermentor capabilities were thus developed for the autosampler.

Multi-fermentor sampling with an autosampler

To upgrade the autosampler for multi-fermentor capability, several enhancements in the hardware and software were required. With only three outputs left (i.e. 8 total minus 5 used in the sequence) a mechanism to provide the enabling signal to activate one of six fermentors was required. A binary encoder was thus installed to activate

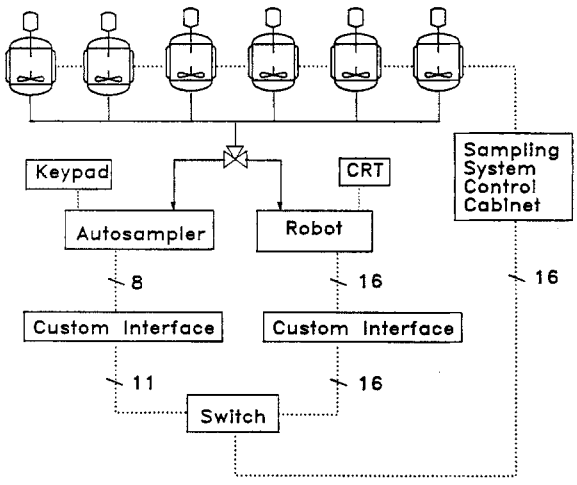

Fig. 5. Shared control of the sampling system for on-line analysis development.

the proper set of actuators with (only) the three remaining autosampler outputs.

In addition to the hardware modifications, the software had to be substantially enhanced to enable multi-fermentor sample scheduling with all the desired features. A logic loop for five fermentors and five racks was programmed (i.e. only five of the six fermentors could be sampled at a time). Switches added to the interface were scanned to denote on-line fermentors. User-friendly variable modification was enabled, and the option to stop after the last (14th) sample, or to loop back to the first tube was included.

Shared control of the sampling system by the autosampler and the robot is depicted in Fig. 5. The control-relay signal source is selected using a multi-pole (25-pin) switch. The sample destination is selected using a three-way valve. Selection of the controller source is thus quite straight-forward.

RESULTS AND DISCUSSION

Operation

The ability to schedule multi-fermentor sample acquisition with the autosampler constitutes the primary result of the development work described. A summary of the operating sequence serves to demonstrate the user-friendly sampling capabilities developed.

To use the system, sample tubes are first prepared with foil seals and are loaded into the appropriate autosampler racks. Autosampler-based control is selected with a switch and valve as described above. Switches are set to denote the fermentors to be sampled, and the autosampler program is run. Four variables are prompted for input as follows:

(1) Analysis time – This display prompts for the sampling rate period in minutes.

(2) Rack position – This is the prompt to enter the next tube into which to sample (i.e. 1–14).

(3) Process sample – This prompt is for the identity of the fermentor associated with switch No. 5 (i.e. fermentor No. 5 or 6. There are only 5 racks available, and 6 on-line fermentors.)

(4) Replay 0/1 - This flag directs the autosampler to reset the tube number from No. 14 back to No. 1 automatically. If zero, sampling will halt after tube No. 14.

The sterilization sequence for the first on-line fermentor is implemented with sample directed into the first tube of the corresponding rack. The step number and wait period are displayed during timed wait states. The valving status and fermentor ID are also indicated on the I/O switch box. The tube last filled is displayed between sampling sets. The sampling rate period is precise to within seconds per day, regardless of how many fermentors are on-line because the wait period is updated for each sampling sequence implemented.

If it is desired to modify the sampling rate period, the program is halted and rerun. The ability to specify any tube as the 'first' tube eliminates the inconvenience of starting at tube No. 1 when modifying any parameters. The sampling sequence times can readily be viewed or modified by running a separate program designed for this purpose.

Typical implementation

The system is typically used to acquire samples to measure cell growth and product formation for multiple yeast and bacterial fermentations. Fig. 6 shows the optical density and product profiles for a bacterial fermentation currently under development. Hourly samples were scheduled automatically and processed manually on an intermittent basis. Relief from manual sampling was an enormous convenience, especially since multiple tanks were run and overnight samples were required.

Industrial applications

Recycle loops typically implemented for sampling laboratory-scale fermentors are not practical for most industrial fermentations. The associated risk of contamination, along with labor intensive operation normally preclude their use for process monitoring. The implementation of a sampling sequence like the one described could be of great value for production level process monitoring, control, and quality assurance.

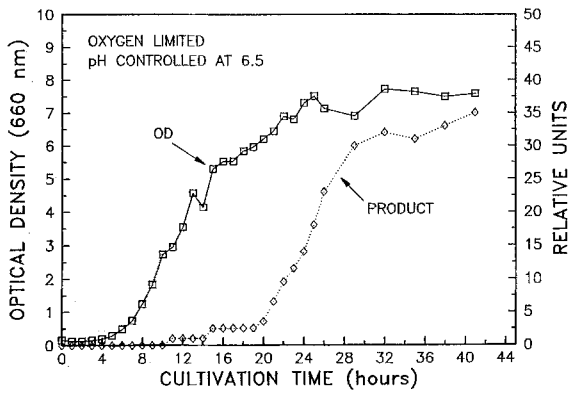

Fig. 6. A typical application: optical density and product titer profiles for a bacterial fermentation process.

For sample acquisition alone, autosampler-based sampling can be configured as in the application described. If some level of processing is desired, a robust processing sequence of pneumatic operations can be developed, configured and controlled with a customized programmable logic controller (PLC). Devices already available could be assembled to minimize development. For example, an autosampler can be programmed to trigger periodic sampling while the PLC is programmatically synchronized to control the customized processing sequence, with the autosampler storing the final processed sample. An on-line analysis feature could be included with the system. The stored sample would provide a mechanism for subsequent manual or semi-automatic analysis in case the on-line analysis feature is not available or if the analysis feature fails.

Feasibility studies and development work can conceivably go a long way to improve industrial process monitoring and control. An aggressive approach to implementing novel sampling and analysis systems in the process area can provide more information with less effort than otherwise possible. This could conceivably provide a competitive edge which may be critical for some industrial applications.

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REFERENCES

1 Appelquist, R. and G. Johansson. 1989. A chemically sterilizable barrier for the protection of a fermentor during automatic sampling into a flow-injection system. Anal. Chim. Acta 216: 299–306.

2 Dincer, A.K., M. Kalyampur, W. Skea, M. Ryan, T. Kierstead. 1982. Continuous on-line monitoring of fermentation processes, Dev. Industr. Microbiol. 25: 603.

3 Geppert, G. and L. Asperger. 1987. Automatic on-line measurement of substrates in fermentation liquids with enzyme electrodes. Bioelectrochem. Biotechnol. 17: 399–407.

4 Gunilla, G.K.E. and P. Matteau. 1988. An automatic aseptic bioreactor sampling system. Biotechnol. Bioeng. 32: 923–926.

5 Hill, F.F. and J. Thommel. 1982. Continuous measurement of the ammonium concentration during the probogation of Baker's yeast. Process Biochem. 17: 16.

6 Holst, O., H. Hakanson, A. Miyabayashi and B. Mattiasson. 1988. Monitoring of glucose in fermentation processes using a commercial glucose analyzer. Appl. Microbiol. Biotechnol. 28: 32–36.

7 Kroner, K.H. and N. Papamichael. 1988. Continuous sampling techniques for on-line analysis. Process Biochem. 23: iii–vi.

8 Niehoff, L., J. Moller, R. Hiddessen and K. Shugerl. 1986. The use of an automatic on-line system for monitoring penicillin cultivation in a bubble-column loop reactor. Anal. Chim. Acta 190: 205–212.

9 Reda, K.D. and D.R. Omstead. 1990. Automatic fermentor sampling and stream analysis. In: Computer Control of Fermentation Processes. pp. 73–106. CRC Press, Boca Raton, FL.

10 Reda, K.D., R.L. Greasham and M.P. Thien. 1990. On-line robotics for multi-fermentor process monitoring and control. ISLAR90 Symposium Proceedings (submitted for publication).

11 Schugerl, K. 1988. On-line analysis and control of production of antibiotics. Anal. Chim. Acta 213: 1–9.

12 Wolfgang, W.S., G. Pommerening, C. Wandrey, M. Kula. 1989. On-line measurement of extracellular proteins in the continuous cellulase production by flow injection analysis. Enzyme Microb. Technol. 11: 96–105.