PROC. N.S. INST. SCI (1987)
Volume 37, pp. 53-62

FERMENTATION VOLUME CONTROL IN STIRRED VESSELS OF WORKING CAPACITY TEN LITERS[1]

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The volume of fermentation liquid in stirred vessels of working capacity ten liters can be controlled during 14 days fermentation within the range 9.95-10.05 L by supporting the vessels on a spring the expansion of which is used to magnetically close a reed switch thus activating a pump to add sterile water (or medium) to the fermentor. As the weight of the fermentor increases by this addition, the spring is compressed and the reed switch opened. The success of the system depends on the use of suitable flexible couplings to the fermentor head-plate including a sliding spline coupling between the motor-drive and the impeller shaft. Drawings of this coupling and its associated electronics are given. The system has been used for more than 25,000 hours without break-down and can be easily adapted to computer control and hence chemostat operation.

La volume de liquide de fermentation dans des vaisseaux agités de capacité fonctionnelle de 10 litres peut être contrôlé pendant 14 jours de fermentation entre 9.95 - 10.05 L en soutenant les vaisseaux sur un ressort dont l'expansion est employé pour fermé magnétiquement un interrupteur à roseau ainsi activant une pompe qui ajoute de l'eau stérile (ou du médium) à la cuve de fermentation. Quand le poids de la cuve de fermentation est augmenté par cette addition, le ressort est comprimé et l'interrupteur s'ouvre. Le succès de ce système dépend de l'emploie des manchons élastiques à la tête du fermenteur qui incluent un accouplement glissant à rainures entre le moteur et l'arbre de transmission. Les dessins de cet accouplement et des circuits électroniques sont donnés. Le système est en service depuis plus de 25000 heures sans panne et peut être facilement adapté a contrôle d'ordinateur et donc a fonctionnement chémostatique.

Introduction

In many applications the accurate maintenance of the volume of a small scale fermentation is not critical. Fermentations, where bacteria or yeasts are being cultivated on rich media, are usually of 24-72 h duration, with low aeration rates. In these circumstances, and particularly where the cells or metabolites are the product(s) desired, concentration of the medium can usually be disregarded. However, many fermentations of fungi require 7-14 days growth and high aeration rates which result in the evaporation of 25-30 mL h^{-1}. Such losses are intolerable and several devices have been reported (Kroll *et al.*, 1956; Quinn, 1962) that claim to control evaporation rates and/or fermentation volumes.

In our laboratory 6 fermentors of working capacity about 10 L are exhausted via a manifold and incinerators to atmosphere, and it has been found that condensation and return equipment is inefficient and difficult to maintain in a sterile condition. We have, therefore, examined methods of control of fermentation volume by the addition of sterile water or medium and our experiments along these lines are reported, together with an assessment of the equipment that finally evolved.

Methods

The fermentors used in this work are glass jars of capacity 15 L and a normal working capacity of 10 L. The jars support stainless steel head-plates, that carry baffles

[1] NRCC No. 28474.

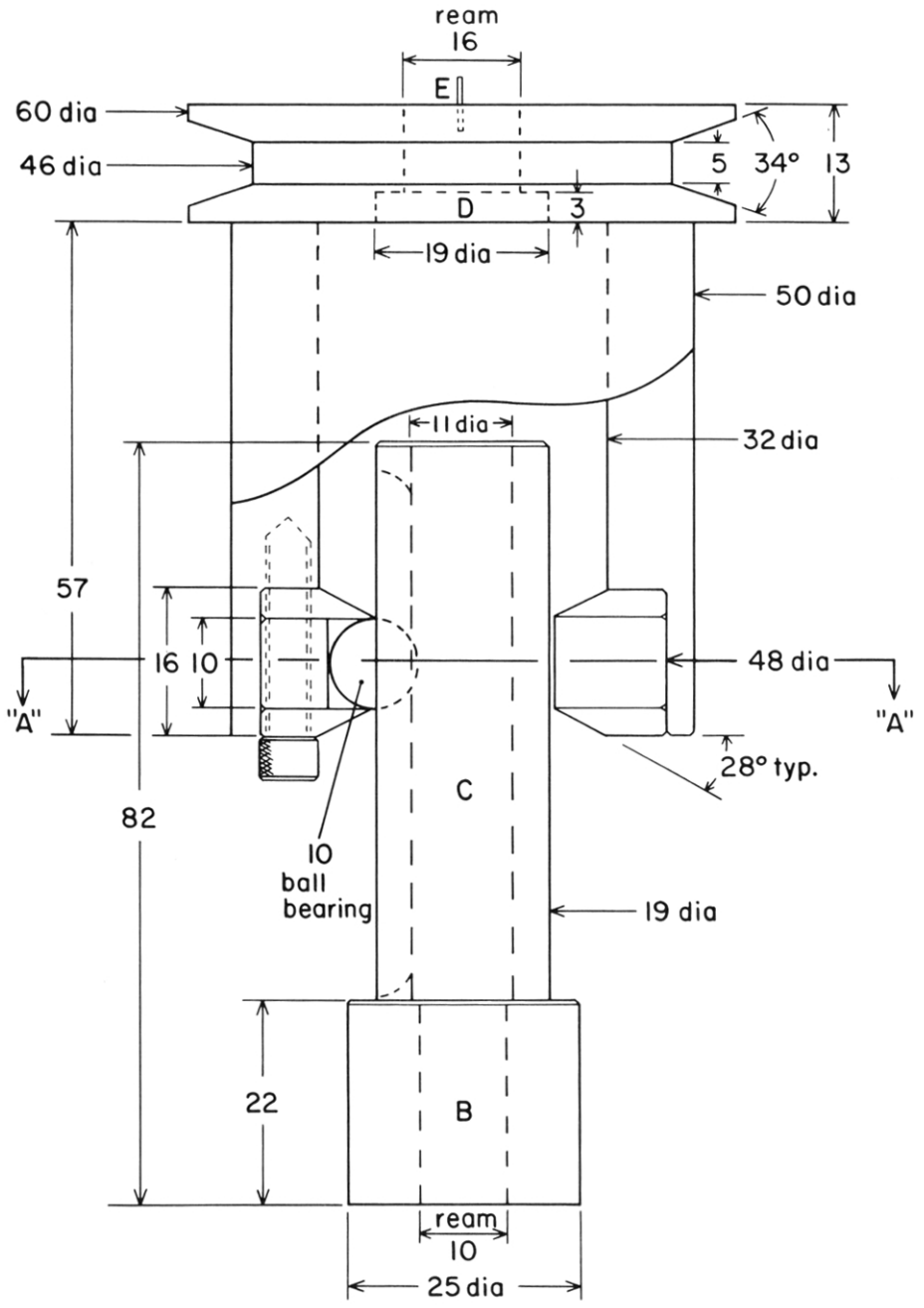

Fig 1. Construction drawing of drive-unit; all dimensions in mm; B = coupling to impeller shaft of fermentor, set screws not shown; C = spline bearing to permit vertical motion; D = plate limiting upward motion in a vertical direction: E = adaptor shrunk fitted into pulley axle, used when connected to a low voltage DC motor e.g. Radio Shack part number 273-223, to generate a voltage proportional to r. min^{-1}.

through which water circulates to control the temperature of the culture, roller-bearings that support the stirrer shaft, a variety of instruments, air intake and exhaust etc. The weight of the assembly is about 23.2 Kg when it contains about 10 L of medium. The equipment was originally purchased from New Brunswick Scientific, Edison, New Jersey, U.S.A.

The modifications of these fermentors to achieve constant fermentation volume can be divided into 3 parts: the construction of the spring support, the mechanism of the modified drive and thirdly the electrical circuitry to drive peristaltic pumps on closure of a reed switch. Each will be described in the following sections.

Spring support

The springs were constructed of 6.5 mm steel rod to give about 11 turns of outside diameter 15.2 cm and a length when uncompressed of 70 cm. Spring guides were constructed of steel pipe of internal diameter 15.25 cm and were welded in a perfectly vertical position at the top and the bottom of the frame-work provided by New Brunswick Scientific. Molybdenum sulfide was used to lubricate the spring in its channel and as a retaining adhesive for the plate at the top of the spring that was in contact with the base of the fermentor jar.

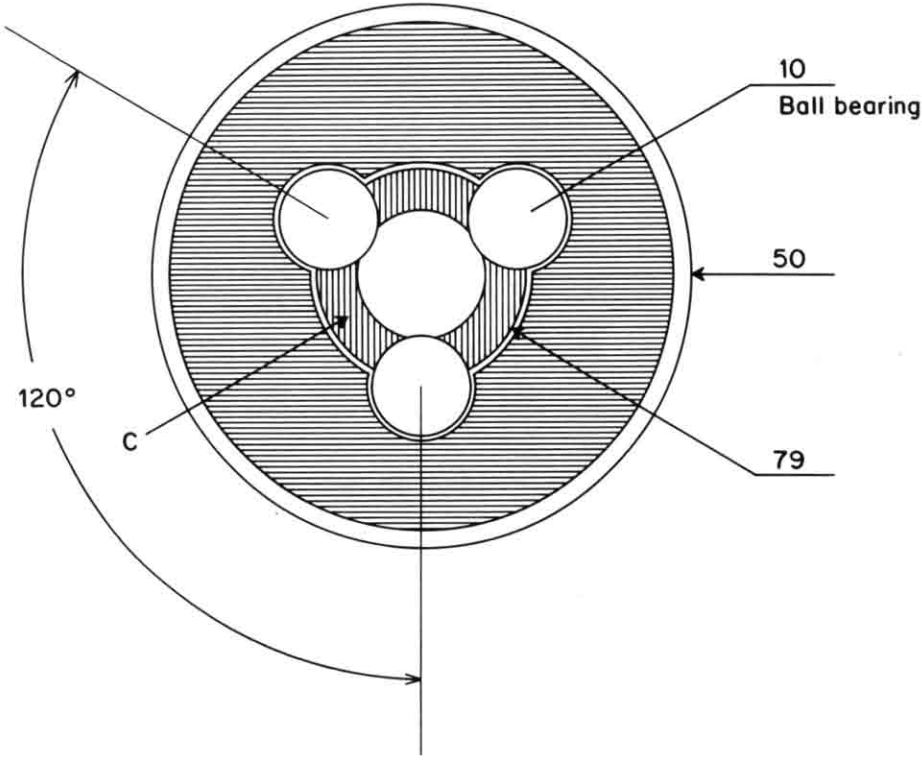

Fig 2. Construction drawing of section across A-A′ in Fig 1; all dimensions are diameters in mm; C = spline bearing to permit vertical motion.

Modification of the drive-unit to allow vertical motion of the fermentor

A drawing of the drive mechanism is shown in Fig 1. The pulley at the top was driven by a D.C. motor of 0.25 h.p. and the pulley and belt connection shown in Fig 1 were hinged to the cabinet. The hinge was spring-loaded to ensure that the resting position of the assembly was about 30° above the horizontal plane. This allowed installation of the fermentors (after they had been sterilized) and precise adjustment, by means of set-screws, of the mechanism in a perfectly horizontal position. The lower segment B (Fig 1) served as a clamp to the end of the impeller shaft of the fermentor and was secured with 2 set-screws. The limits of the sliding bearing C were controlled by the ball-race C' (lower) and by the contact plate D (upper). The bearing surfaces shown in Fig 2 were accurately machined to provide smooth vertical motion and were used to transmit the drive via the unit B-C (Fig 1). Since the bearing was the sole support of the latter unit the assembly was sufficiently flexible to absorb small imbalances of the fermentor assembly which sometimes developed during long fermentations.

Electrical circuitry for controlling addition of liquids to the fermentors

The vertical position of the fermentor was determined by the use of magnetically closed reed switches (obtainable from any electronics outlet e.g. Radio Shack). These were installed on the frame-work supporting the fermentors as shown in Fig 3. The

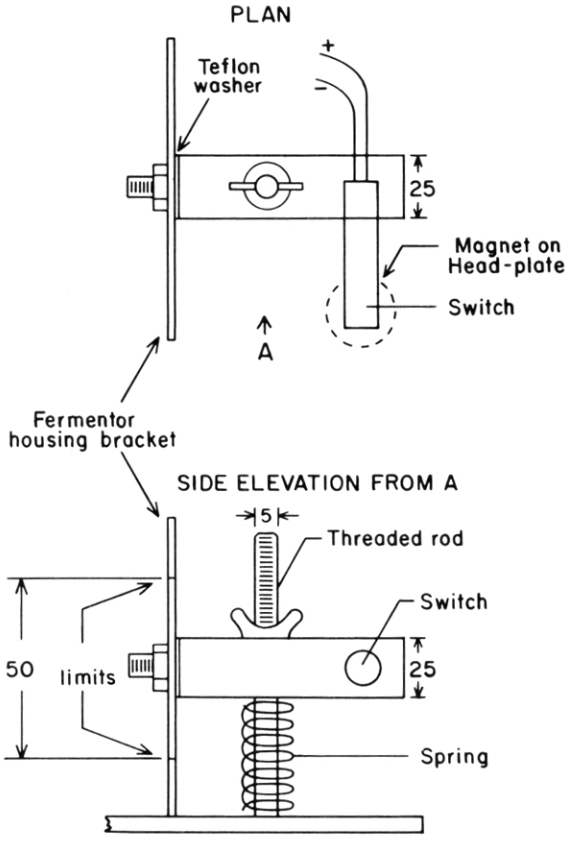

Fig 3. Construction drawing of reed switch mounting; all dimensions are in mm. The block holding the switch was made of high density polyvinyl chloride.

position of the switch above the fermentor was used to determine the fermentation working volume i.e. its height was inversely proportional to the volume. The switches were closed by the field of a small teflon coated laboratory magnet which was carried in a stainless steel fitting attached to the fermentor head-plate by one of the bolts used to secure the head-plate to the vessel. A tight press-fit of the magnet in the carrier enabled the assembly without the magnet to be sterilized, the magnet being pressed into position during assembly of the fermentors on the frame. For several years the relay switching circuits shown in Fig 4 were used to activate one peristaltic pump dedicated to each fermentor. The series combination of 0.1 μF and 510 ohms was used to reduce sparking at the reed switch contacts. Alternatively the data aquisition and control system, recently described (Brewer et al., 1987) can be used in place of the circuitry shown in Fig 4. This has the advantage that only one peristaltic pump is required for 3 fermentors. The system is shown diagrammatically in Fig 5 and it has been used for many of the measurements reported in the Results section of this paper. Each fermentor was assigned one multiplexer card (option 020) in a Hewlett-Packard data aquisition and control unit (catalogue no. HP 3421A). The power supply of about 5 v was delivered to the data aquisition unit when the reed switch was closed and hence the fermentor required water (medium) to replace that lost by evaporation. The locations 0 to 1 on each multiplexer card were used as switch controllers. One was assigned to each fermentor (3 in all) and the remaining 3 opened or closed solenoid valves that determined whether acid, or base or water (medium) were added to the fermentors. The peristaltic pump was then activated by means of the circuitry shown in Fig. 6, the logic of which assures that the pump is only switched on if two switches on the data aquisition units are closed, one corresponding to one of

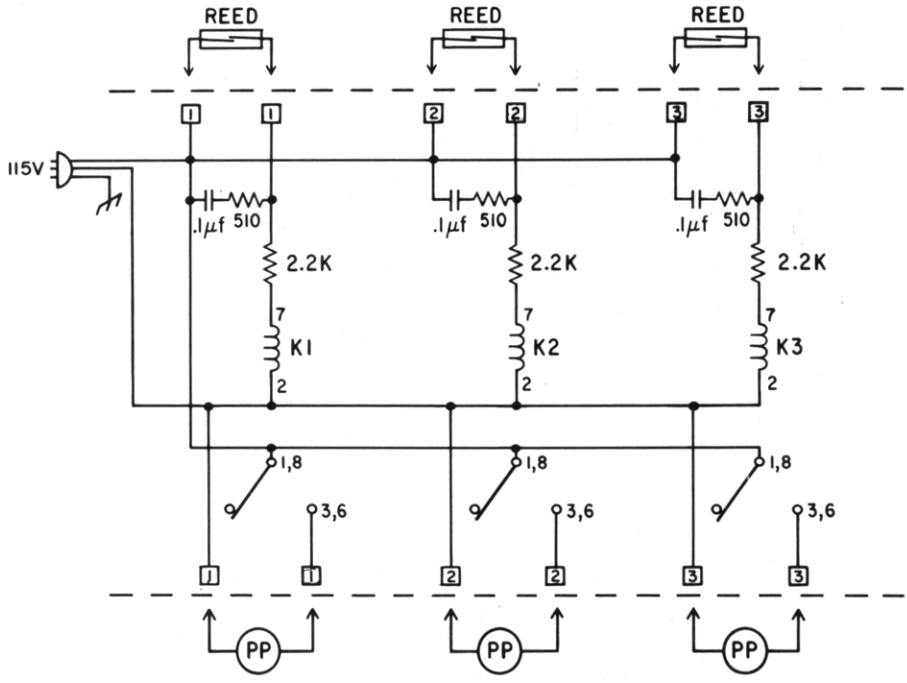

Fig 4. Circuit diagrams of relays used to activate peristaltic pumps (PP). The relays K_1, K_2, and K_3 were Potter and Brumfield type KRP 11A.

the fermentors and the other attached to the acid, or base, or water reservoir. These boundary conditions were set by the algorithms in the controller of the HP-IL loop. These programmes are available to other workers on request.

Results

The mechanical parts of the constant level system were installed on our fermentors in 1977 and the fermentors so equipped have been used for about 25,000 h since that time. The only modifications that have been made are those relating to the security and position of the magnet and reed-switch. The locations of the magnet below the reed switch was not critical for control of the volume of broth in the fermentor, but of course, was important with respect to the absolute fermentation volume. As most of the work done in these fermentators were comparisons of the effect of changing fermentation parameters on product yield it was important to be able to set the fermentation volumes of all fermentators at the same value. The working volume of the fermentors was determined by their exact height and this depended on the tension in the supporting spring. This tension of course varied from one spring to another and was offset by calibration of the position of the reed switch after inoculation. This calibration was achieved by supporting the switch on the locked, threaded adjustment shown in Fig 3.

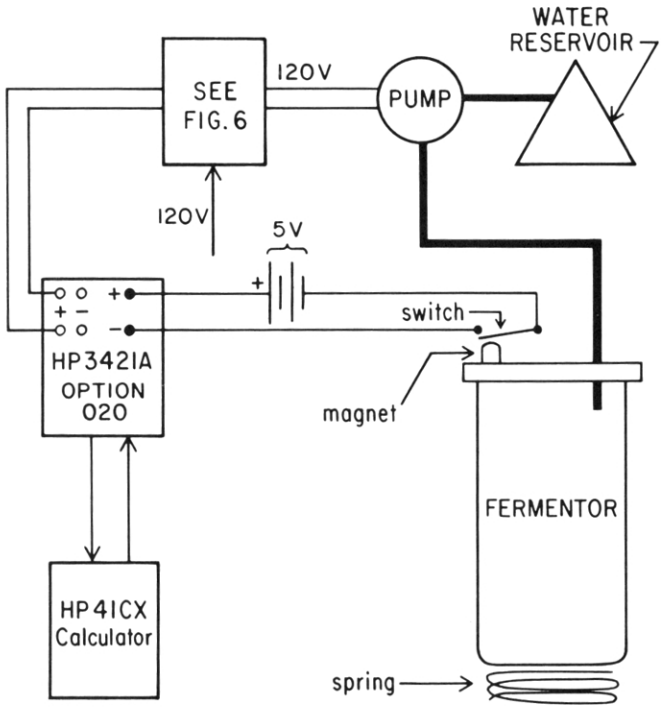

Fig 5. Diagram of hydraulic and electrical circuitry for one fermentor using data logging and control equipment. Only 3 switches (whose state is determined by the HP-IL controller) on the HP-3421A card are shown. The junction on the right of the card senses the status of the switch. Both pairs of terminals on the left of the card must be closed to switch on the pump. Thick lines show liquid transfer.

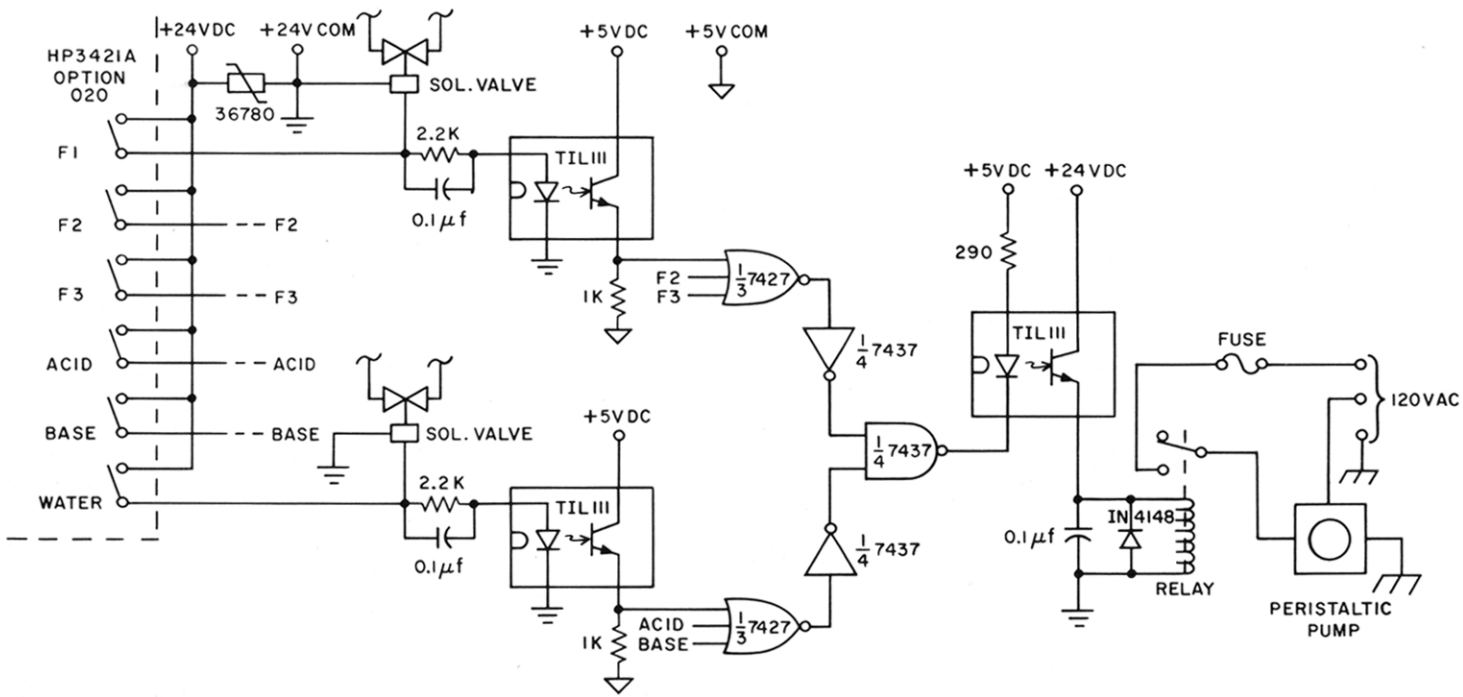

Fig 6. Circuitry for interface between HP3421A control unit and the peristaltic pump. Only the circuitry for water (medium) addition to one fermentor is shown; the other 2 fermentors and the acid/base addition connections are indicated. The solenoid valves were Radiometer type MNV 1E and required a 24 v supply. The 5 v supply was used in logic circuitry as is customary. The following symbols are used: ⏚ is the circuit common in the 24 v section; ⏚ is the circuit common in the logic circuits and ⏚ is safety ground in the 120 v a.c. section. The symbol ⊐ indicates that the integrated circuit is being used in its logical sense. The following components indicated on the Fig were: type V36ZA80 metal oxide varistor; 36Z80, surge suppressor; TIL 111 optically coupled circuit isolator, 7427 TTL triple 3-input NOR gate; 7437 TTL quad two imput NAND gate buffer; IN4148 signal diode.

Table I Variability of volume of water added to a stirred tank losing fluid at a constant rate of 14.2 mL min^{-1}.

Measurement Number	Time Elapsed (m,s)	Time of addition(s)	Volume
1	—	—	
2	2:47	11	58.6
3	7:23	11	58.6
4	10:50	14	74.6
5	18:54	11	58.6
6	23:36	11	58.6
7	25:25	21	111.9
8	33:27	12	64.6
9	38.35	11	58.6
10	42:28	11	58.6
11	46:16	11	58.6
12	49:39	14	74.6
13	55:00	11	58.6
14	59:30	11	58.6
15	62:57	11	58.6
16	66:32	17	90.6
17	73:11	11	58.6
18	78:03	11	58.6
19	82:21	11	58.6
20	87:28	16	85.3
21	97:13	11	58.6
22	101:31	12	64.0
23	102:57	17	90.6
24	113:35	11	58.6
25	117:05	11	58.6
26	122:38	11	58.6
27	125:14	12	64.0

The electrical circuitry associated with the system has also proved to be very robust and the relay system shown in Fig 4 was used for the first 7 or 8 years. The reliability of this circuitry is perhaps surprising since slight arcing at the contacts of the reed switch due to the inductance of the relay coil might be expected to shorten the life of the switch.

The efficiency of the level control system was assessed at first by the simple device of a volumetric scale engraved on the sides of the fermentors, and by recording the observed level at regular intervals. Over a considerable period of time we believed that the volume was controlled to $\pm$ 100 mL or $\pm$ 1%. Lately, with the development of the data logging and control system a more accurate estimation of the sensitivity of the level control became possible. The data in Table I was obtained by removing water from the fermentor at a constant rate of 14.2 mL min^{-1}. Over a period of about 125 min, 27 additions of water were made by the system and the mean volume added was 66 $\pm$ 13.6 mL. The data in Table II are a closer simulation of fermentation conditions, the water in the fermentor being removed by evaporation and as an aerosol in the usual way. The mean volume of water added on each of 30 occasions over a period of about 4 days was 100 $\pm$ 10 mL and the average time of addition was 22 s. Hence the system is capable of controlling the fermentation volume to $\pm$ 50 mL or $\pm$ 0.5%. Over the range 4-10 mL s^{-1} the rate of addition of water or medium did not change the accuracy.

Table II Variability of volume of water added to a stirred tank losing fluid by evaporation and aerosol formation.

Measurement Number	Fermentor Conditions			Time		Volume Added (mL)
	Temp (°C)	Air Flow (L min^{-1})	r min^{-1}	Elapsed (h:min)	of addition (s)	
1	43.9	10	270	0	20	85
2	43.6	10	254	3:09	24	105
3	43.5	9	240	7:12	23	100
4	43.3	9	272	10:50	20	85
5	44.2	9	255	14:05	25	113
6	43.2	10	278	17:51	24	99
7	44.3	10	290	21:15	24	109
8	43.6	10	266	24:46	18	82
9	43.0	10	266	27.40	20	91
10	42.9	10	254	30:34	21	96
11	43.1	10	245	33:41	27	122
12	43.0	10	272	37:23	23	105
13	43.1	10	238	40:55	21	95
14	43.0	10	263	43:45	22	98
15	43.6	10	258	47:03	24	110
16	42.9	10	246	50:33	24	108
17	43.1	10	264	53:53	22	100
18	42.9	9.5	266	57:07	23	104
19	43.2	9.5	229	60:12	20	92
20	42.9	9.5	279	63:31	22	99
21	43.2	9	273	66:43	19	86
22	43.5	9	238	69:58	20	87
23	43.1	9	245	73:08	20	90
24	43.0	9	266	76:22	24	110
25	43.5	10	282	79:48	21	96
26	43.8	10	280	82:47	25	114
27	43.5	10	240	86:07	25	114
28	43.9	10	246	89:35	22	101
29	43.1	10	252	92:35	24	109
30	43.4	10	256	95:50	21	96

Discussion

Many different ways of controlling the fermentation volume in our fermentors were investigated. These included condensers in the exhaust line, level sensing electrically conductive probes such as those used for foam detectors, ultrasonic radiation absorption detection devices and strain-gauge load cells. The first method was inefficient because of the small temperature difference between the condenser and the fermentation liquid, and it had no effect on the mechanical transfer of liquids as aerosols. The second and third methods are inefficient in the presence of the stable foams often encountered in cultures of fungi. The results of using load-cells were disappointing probably because of friction in the mounting assembly, but also because of the tendency of the signal to drift under the harsh environmental conditions over a long period of time.

By contrast, the system described here performed satisfactorily from the onset. The data in Tables 1 and 2 consolidate impressions of the sensitivity of this volume control device during several years experience. During this time almost all the other mechan-

ical features of these fermentors have failed or required repair but no malfunction of the level control equipment has occurred.

The ability to control within defined limits the fermentation volume in a number of fermentors throughout the fermentation with an overall discrepancy of about 0.5% has enabled workers in this laboratory to develop optimum conditions for the production of a number of fungal metabolites. These have varied from the straightforward production of 3-acetoxy-7,15-dihydroxy-12,13-epoxytrichothec-9-en-8-one (Greenhalgh et al., 1984) to the production of the highly unstable 3-(3'-isocyano*cyclo* pent-2-enylidene)propionic acid (Brewer et al., 1982) and to some control of the very complex mixture of peptide antibiotics produced by *Trichoderma* spp. (Brewer et al., 1987).

With the development of the HP-IL control and data-logging system this constant level device can obviously be used for other applications, notably chemostat operations. Thus cultures can be harvested continuously, on the model illustrated by the data in Table 1, or periodically in fermentations designed to induce synchrony (Dawson and Glättli, 1972).

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Received 26 August 1987.