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On-line Automated Monitoring and Control Systems for CO_2 and O_2 in Aerobic and Anaerobic Solid-State Fermentations

G. Saucedo-Castañeda,‡ M. R. Trejo-Hernández‡

Departamento de Biotecnología, Universidad Autónoma Metropolitana, Iztapalapa, A.P. 55-535, C.P. 09340, Mexico D.F., Mexico

B. K. Lonsane

Fermentation Technology and Bioengineering Discipline, Central Food Technological Research Institute, Mysore 570 013, India

J. M. Navarro

Laboratoire de Génie Microbiologique, Université Montpellier II, Sciences et Techniques du Languedoc, Place E. Bataillon, 34095 Montpellier Cédex, France

S. Roussos, D. Dufour & M. Raimbault

Laboratoire de Biotechnologie, Centre ORSTROM, 911 Av. Agropolis, B.P. 5045, 34032 Montpellier Cédex 1, France

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Two systems for monitoring and control of gases from solid-state cultures have been developed. The first involves on-line automated monitoring of CO_2 and O_2 concentration in exhaust gases from eight fermenters or eight gas sampling ports in a large fermenter. It proved to be efficient in obtaining information on the physiological state and respiration rate of the culture in a real-time process. Furthermore, the specific growth rate (μ) can be estimated reliably by gas measurements in aerobic cultures. The second system is for automated control of exit gases from aerobic solid-state fermentations. It permitted elimination of biomass and temperature gradients in a large fermenter due to the maintenance of culture under non-limiting conditions on oxygen. These two systems have applicability in aerobic and anaerobic solid-state processes and were found to be reliable in a number of fermentation experiments as well as optimization of solid-state fermentation. To our knowledge no earlier report of such versatile and reliable on-line automated monitoring/control systems has appeared.

INTRODUCTION

The monitoring and control of various fermentation parameters are of critical importance for achieving higher productivity in solid-state fermentation (SSF).[1,2] The lack of such facilities has been, in part, responsible for the neglect of SSF processes up to 1980 in Western and European countries.[3] Some, recent reports on SSF processes has involved the development of strategies for overcoming the problems of monitoring and control of fermenters.

‡Present address: Lab. Biotechnologie, ORSTOM, 911 Av. Agropolis, B.P. 5045, 34032 Montpellier Cédex 1, France.

Corresponding author: Dr G. Saucedo-Castañeda. Telephone: 67.61.75.75; Fax: 67.54.78.00.

The aeration of moist solid medium is one of the critical factors which governs productivity[2] since it not only supplies O_2 but also removes metabolic heat, gaseous and volatile products from the fermenting mass.[2] Recently, the rate of aeration has been integrated with the control of temperature and moisture content by evaporative cooling of water.[5,6]

Measurements of the consumption of O_2 or the evolution of CO_2 are often used for the indirect estimation of biomass[7] and these data are important in the scale-up of SSF processes.[8] Nevertheless, the systems available so far[9–14] for the monitoring of CO_2 and O_2 concentrations in SSF processes are not on-line and automated and therefore have limited utility and analytical results could be influenced by sampling errors. Furthermore, no simple and reliable control system for maintaining constant O_2 and CO_2 in the fermenting mass or fermenter exhaust gases is yet available except for a laboratory-scale closed aeration system.[15] This shaker-mounted fermenter is a research tool and not practicable at larger scales.

The monitoring/control of O_2 and CO_2 levels in SSF systems can be achieved through the measurement of exit gases coming from fermenters. A need for a simple and efficient system for the monitoring and/or control of CO_2 and O_2 in SSF processes is a prerequisite for the optimization and scale-up of SFF processes.

Two versatile systems for on-line automated monitoring/control of these gases in SSF processes have been developed. Their design, functioning comparison, evaluation and utility are reported in this communication.

PRINCIPLES AND DETAILS OF INSTRUMENTATION AND ASPECTS OF AUTOMATION

Monitoring system for aerobic and anaerobic processes

This system, which allows automated on-line monitoring of aerobic and anaerobic SSF processes without disturbing the culture, is based on the sampling of the exhaust air from the fermenter, the separation and estimation of CO_2 and O_2 by a gas chromatograph, all functions being connected to a personal computer and integrated into an on-line automated system. A prototype (Fig. 1) consists of a gas chromatograph equipped with a thermal conductivity detector and automatic injector (Model IGC 11, Delsi, France); a gas separating concentric column (CTRI, Alltech, USA); a gas sampler (B.C.V., 8, Delsi, France); a personal computer (PC 3865XB/16, Copam, UK); and an integrator program (Chroma, Biosystèmes, France).

A program has been developed in BASIC (Fig. 2) which controls the whole monitoring system. Gases from fermenters are dried and then ranged in a sampler. The latter is under the control of the computer and works by ON/OFF action commands on the selection valves. The computer selects the desired fermenter and directs the injection of the sample of gas into the chromatograph. The system can simultaneously monitor eight different fermenters or an equal number of samples from different gas ports in a large solid-state fermenter. The analysis cycle involves a total number of eight measurements and takes around 30 min to complete. In a sequential estimation of the gases, the first valve in the gas sampler is closed after gas is injected in the chromatograph. Subsequently, the second valve is opened and the pipe-line, from sampler to injector, is purged during the course of analysis of the first fermenter. Once the first analysis is over, the estimation of gases coming from the second fermenter is carried out. The cycle continues in this fashion until all eight valves are operated. Unless otherwise commanded, the cycle is repeated continuously. The exit air from the fermenters which are not selected for analysis are vented in the atmosphere.

Integration of chromatograms is carried out immediately by the computer. Data are permanently saved indicating date and time of injection and one file is created for each fermenter. Each chromatogram analysis takes nearly 10 Kb. Our experience in this research indicates that 50–150 analyses are required to monitoring one fermentation process.

Some specific problems were faced while developing and designing the system. For instance, humidified air is generally employed in SSF processes to compensate for the loss of water due to forced air flow passing through the fermenter. The presence of water in the exhaust air was, however, found to lead to erroneous results. This problem was efficiently overcome by passing the exhaust air through a condenser which removed the major part of the water. The left-over traces of water in the gases were subsequently removed by passing through a silica gel column. The dry gases thus

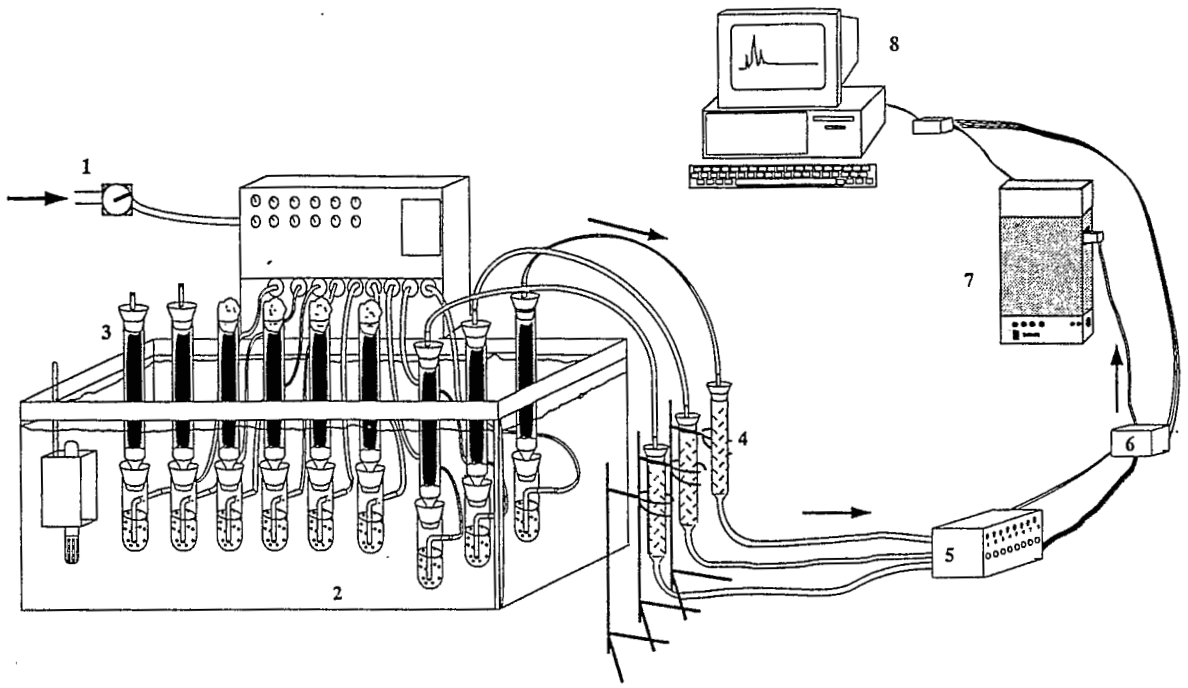

Fig. 1. A diagrammatic representation of the prototype system developed for on-line automated monitoring of gases in solid-state fermentation. 1: Regulated pressure air inlet, 2: temperature-controlled water bath, 3: column fermenters, 4: silica gel tube, 5: gas sampler, 6: gas injector, 7: gas chromatograph, 8: computer.

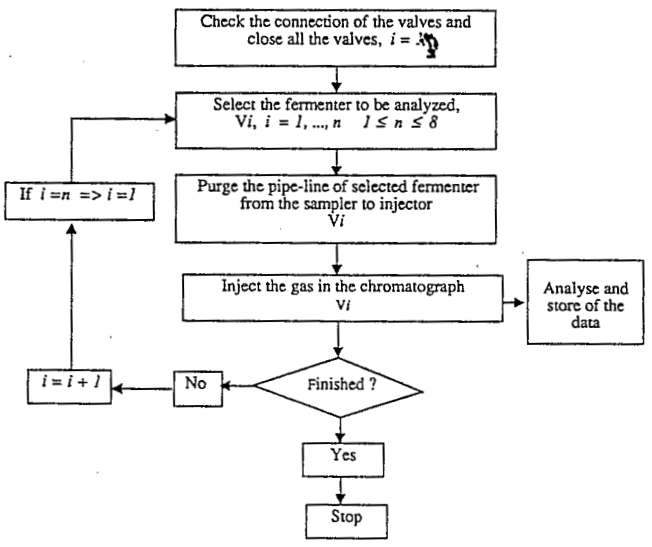

Fig. 2. Structure of the BASIC programme developed for on-line automated monitoring of gases in the system based on the use of gas chromatography.

obtained were injected into the gas chromatograph.[16]

Another problem faced was the variation in the air flow rates to the fermenter and consequently from the bioreactor to the gas chromatograph. An accurate determination of the concentration of gases in the exhaust air is directly related to the air flow rate. Therefore, two air pressure regulators were installed in the air-line to obtain an air supply at constant pressure. In addition, a fine needle valve was used to control accurately the air flow to the fermenter.

System for monitoring and control of gases in aerobic processes

The aim of developing this system for the control of gases in SSF was to maintain the culture under non-limiting conditions of O_2 and to supply the air according to the requirements of the culture, which varies depending upon the phase of growth. This can be carried out by varying the air supply to the fermenter.[17]

The system developed has the ability to monitor the concentrations of CO_2 and O_2 in exit air continuously and to modify the air flow rate to the fermenter automatically to maintain the levels of these gases in the exit air at constant value. All these functions are performed by a computerized on-line automated system. The sample of the exit air goes directly to the chilled-water condenser for moisture separation and is then continuously analyzed. The control system can be used only for one fermenter and is suitable for all kinds of aerobic SSF processes.

The prototype developed (Fig. 3) consists of a refrigerated glass condenser for moisture separation; cryostat (Polystat, Prolabo, France); a silica

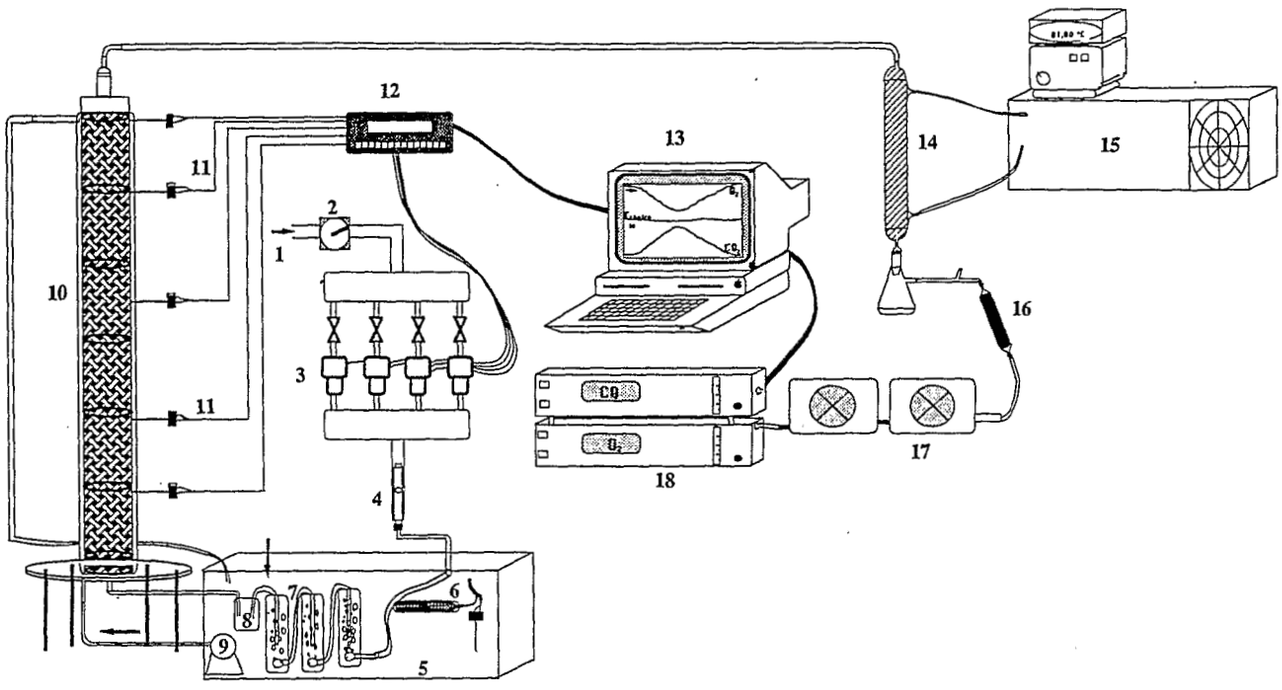

Fig. 3. Diagrammatic sketch of the system developed for monitoring and control of exhaust gases in aerobic solid-state cultures. 1: Compressed-air pipeline, 2: air-pressure regulator, 3: electro-valves, 4: rotameter, 5: temperature-controlled water bath, 6: heater, 7: air humidification column, 8: water trap, 9: pump for circulating water through jacket of the fermenter, 10: six-segmented column fermenter, 11: temperature probes, 12: analogic–numerical interface, 13: computer, 14: condenser, 15: chilled water bath, 16: silica gel tube, 17: CO_2 and O_2 analyzers, 18: pumps.

gel column, individual analyzers for O_2 and CO_2 (polarographic and infrared, models OM-11 and LB-2, respectively, Sensor Medics, California, USA); membrane pumps (Sensor Medics, California, USA); an analogic-numerical interface card (Digimetrix, GS-ADC12B16V4G, USA); a personal computer (Apple IIe, USA); and four electrovalves (Siral Protello, Italy) in the inlet air pipeline to the fermenter. The air was supplied at 0·2 bar while the electrovalves were calibrated for the supply of 100, 200, 400 and 800 ml air/min. Thus, the system can automatically vary the air supply at a step of 100 ml/min. The maximum air supply is 1500 ml/min when all the valves are open. Furthermore, the system was provided with five thermister probes (YSI, Ohio, USA) for the measurement of the temperature of the fermenting solids at different points. The computer program allows the selection of the time interval between two analyses of CO_2, the minimum interval being 1 min. The concentration of gases, temperature and flow rate values are permanently saved.

As far as CO_2 and O_2 analysis is concerned, the functioning is similar to that of the system described above. However, individual analyzers for CO_2 and O_2 were selected because of their higher sensitivity and quicker response when compared to those of the gas chromatograph. For example, the time taken by individual analyzers to estimate O_2 and CO_2 concentrations is 1 and 0·13 s, respectively, as against that of 3·1 min in the case of the gas chromatograph.

The system simultaneously analyzes both CO_2 and O_2 concentrations. It is emphasized that either of these gases can be used as a control parameter for aerobic SSF processes. The shape of the curve of O_2 evolution is the inverse of that for CO_2 production. However, only one of these parameters was taken into account while designing the control program. The choice of CO_2 concentration, instead of O_2, was made due to the higher sensitivity and quicker response of the former for the analysis when compared to the O_2 analyzer. As indicated above, the control of CO_2 at the set value in the exhaust air can be achieved by increasing or decreasing the air flow rate to the fermenter.[17]

The functioning of the CO_2 control program is presented in Fig. 4. It involves two sections, the first one, in BASIC, commands start-up of the program, on-line display of results under graphic

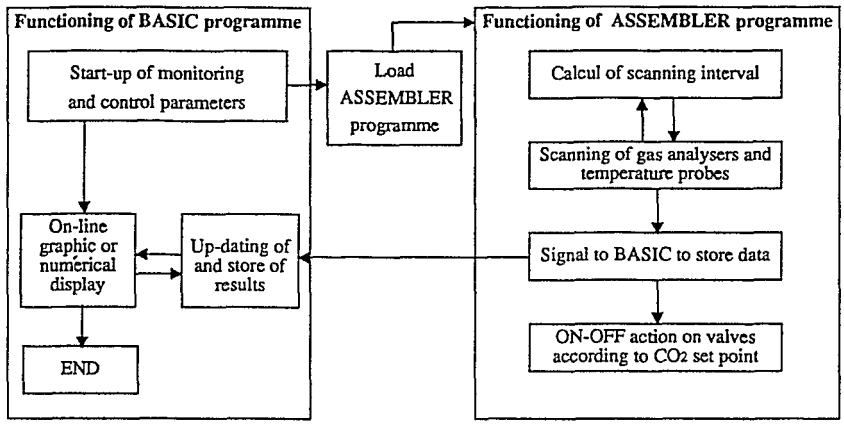

Fig. 4. Functioning of the programme developed for automatic control of CO_2 and O_2 concentrations in aerobic solid-state culture using the system on the use of individual gas analyzers.

or numerical mode, data saving and loads the ASSEMBLER section. The latter constitutes the second section and commands scanning of gas analyzers and temperature probes at desired time intervals. It also automatically directs ON–OFF action on the valves to change the air flow rate.

MATERIALS AND METHODS

Evaluation of analytical performances of the systems

The analytical performances of the systems, involving either the gas chromatograph or individual gas analyzers, were compared using a large column fermenter (4 cm diameter × 90 cm height)[16] in the aerobic SSF process for biomass formation by *Schwanniomyces castelli* CBS 2863, an amylolytic fermenting yeast. The exhaust gases from the fermenter were analyzed simultaneously by both systems. Experimental details are given below, the exception being the fixed aeration flow rate of 0·4 ml air/g moist material.

Evaluation of the utility of the gas chromatograph system

The gas chromatograph system was evaluated for its utility in aerobic and aerobic–anaerobic SSF systems involving *Schw. castelli* CBS 2863. Aerobic cultures of yeasts were carried out in small column fermenters[18] (20 cm height × 4 cm diameter) as described below. The levels of gases were continuously monitored. In contrast, the monitoring of aerobic–anaerobic cultures was carried out by analysis of gases during the production of alcohol from starch by *Schw. castellii* in a large column fermenter (4 cm diameter × 90 cm height) as mentioned below.[16]

Evaluation of the efficacy of the CO_2 control system

The efficacy of the CO_2 control system was also evaluated in a large column fermenter (4 cm diameter × 90 cm height) during the aerobic growth of *Schw. castellii*.[17] Culture conditions were as indicated below, except for the automatic control of the aeration rate.

Technique for aerobic cultures of *Schw. castellii*

Sugar cane pith bagasse was used as a support for absorbing the nutrient medium containing (g/litre): Soluble starch (Prolabo) (100), peptone (Merck, pancreatically digested casein) (1), yeast extract (1), KH_2PO_4 (5), NaCl (1), $MgSO_4.7H_2O$ (2) and urea (10). The pH of the medium was adjusted to 3·5 prior to sterilization by autoclaving at 121°C for 15 min and was raised to 6·5 just before inoculation at a 10% (v/v) level. Vitamins and trace elements were added and the bagasse was prepared as indicated elsewhere.[16] Bagasse was blended with liquid medium at a ratio of 14:86 (v:w). The resulting moist solid medium was charged in fermenters at an apparent density of 0·35 g/ml. Under these conditions, the initial moisture content was about 77%. An inoculum containing 10^8 cells/ml was prepared as indicated earlier.[16] Fermentation was carried out at 30°C with an aeration rate of 1·4 ml air/min/g of moist material. Cultures were analyzed for starch, biomass and pH by following the methodology described elsewhere.[16,19]

Technique for aerobic–anaerobic cultures of *Schw. castellii*

A solid-state ethanol fermentation system was selected as the experimental model for monitoring the gases from aerobic–anaerobic cultures. Details of culture conditions and experimental methodology have been described previously.[16]

RESULTS AND DISCUSSION

Comparative analytical performance of the systems

The data of the aerobic growth of *Schw. castelli* in the large column fermenter (Fig. 5) showed that the O_2 estimation by these two systems were equal during the entire course of 30-h fermentation. Thus, any of these two systems can be employed for accurate monitoring of O_2 in the exit air from aerobic SSF processes. In contrast, CO_2 estimations in the exit air were identical only up to the first 11 h and during the last 10 h of the fermentation. The values for the estimation of CO_2 between 11–50 h were much lower in the case of the monitoring system involving the gas analyzer. Such wide differences were due to the use of the infrared CO_2 analyzer limited to measurements below 10%. CO_2 concentrations above 10% are rarely encountered in fermentation processes except those involving anaerobic cultures. The data indicate that both these systems are suitable for real-time monitoring of aerobic SSF.

Utility of the gas chromatograph monitoring system for the optimization of culture conditions and the calculation of specific growth rates in aerobic SSF processes

As in the submerged culture, a massive inoculum and favourable conditions of culture should enhance better development and metabolism of the microorganism under study. An assessment of the gas chromatograph system for such purposes was carried out by studying the effect of the inoculum level in aerobic solid-state cultures of *Schw. castelli*. The inoculum levels employed were $5{\cdot}7 \times 10^7$, $3{\cdot}9 \times 10^6$, $5{\cdot}8 \times 10^5$, $3{\cdot}8 \times 10^4$ and $3{\cdot}6 \times 10^3$ cells/ml of the liquid culture medium used for absorbing on treated pith bagasse. The data on the effects of inoculation levels on volumetric rate and total production of carbon dioxide are shown in Fig. 6(a) and 6(b).

The data were found to be useful in demonstrating that the duration of the lag phase is directly related to the inoculum level. A progressive increase in the duration of the lag phase is observed with the increase in the inoculum level. In the same way, a gradual reduction of the maximum volumetric rate of CO_2 production was observed as the inoculum decreased (Fig. 6(A)). On the other hand, the total gas production (Fig. 6(B)) seemed to be less sensitive to inoculum variations from 10^4 to 10^7 cells/ml.

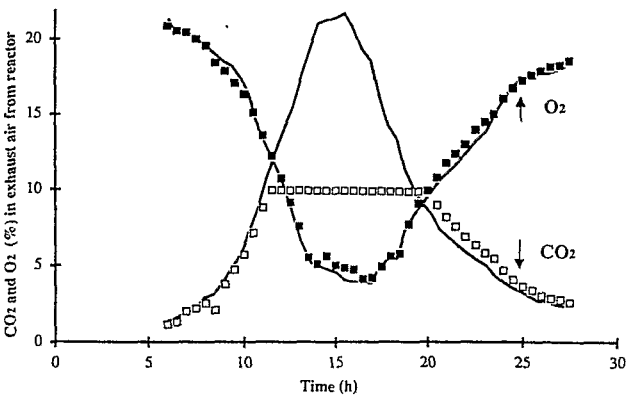

Fig. 5. Comparison of analytical performances of gas chromatography and individual analyzer systems for monitoring CO_2 and O_2 in exhaust air during SSF aerobic cultures of *Schw. castellii*. Solid lines: gas chromatograph; symbols: individual analyzers. □: CO_2, ■: O_2.

Another use of the data is for deriving useful information from the respiratory activity (μ_r) which can be calculated from the natural logarithm of total production of CO_2 during the fermentations with different levels of inoculum (Fig. 6(C)). This activity was almost constant for inoculation levels varying from 10^5 to 10^7 cells/ml (Table 1). A slight decrease was observed with the 10^4 cells/ml inoculum. Microscopic observations of samples showed the presence of contaminating bacteria and moulds with an inoculation level lower than 10^5 cells/ml. From 10^5 to 10^7 inoculum cells/ml, the axenic population of *Schw. castellii* rises to 10^9 cell/ml at the end of the culture (Table 1). The data proved useful in deciding that a minimum level of 10^5 cells/ml is required to impose *Schw. castellii* as the predominant flora in our culturing conditions. A higher level seemed suitable to reduce the lag phase. These results are closest to those used in SSF for yeasts as well as moulded spores.[20–24]

The results of O_2 and CO_2 analysis during cultivation of *Schw. castellii* in the small column fermenter under the best conditions (10^7 cells/ml) are shown in Fig. 7(A). The data showed an O_2 consumption slightly lower than that of CO_2 pro-

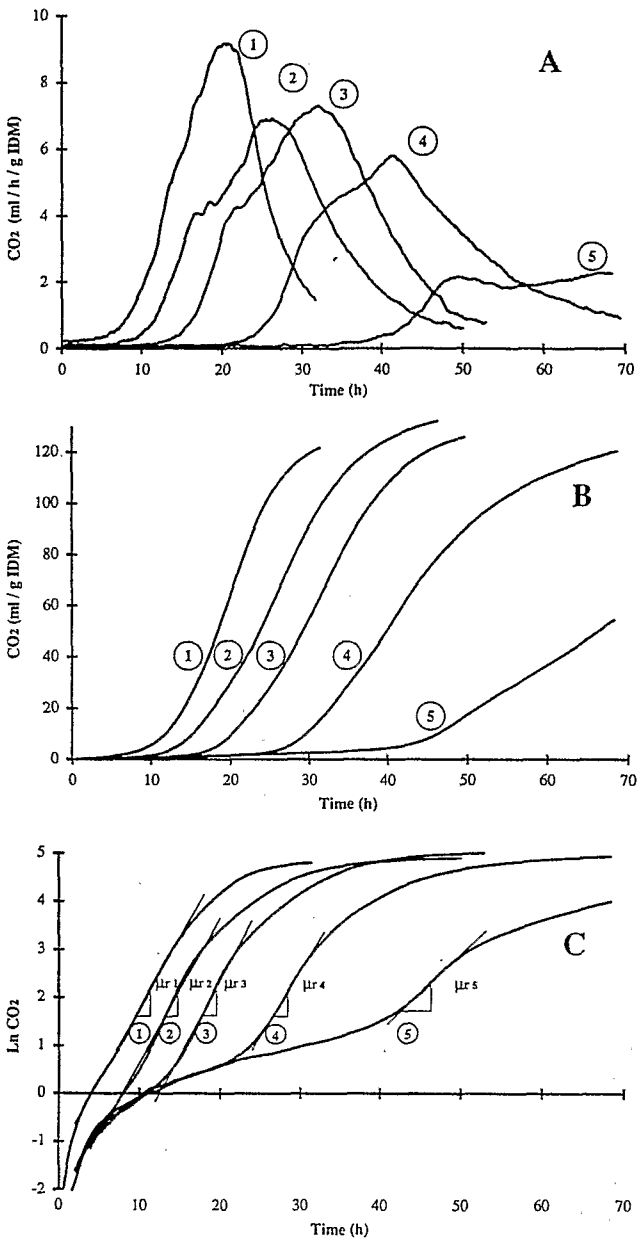

Fig. 6. Effect of inoculum level on CO_2 evolution during the course of aerobic SSF cultures of *Schw. castellii*. Numbers 1, 2, 3, 4, 5 indicate the inoculum levels: $5{\cdot}7 \times 10^7$, $3{\cdot}9 \times 10^6$; $5{\cdot}8 \times 10^5$; $3{\cdot}8 \times 10^4$ and $3{\cdot}6 \times 10^3$ cells/ml, respectively. A: Carbon dioxide evolution rate (ml/h/g IDM); B: total amount of CO_2 produced (ml/g IDM); C: estimation of respiratory activity (μ_r) through CO_2 measurements.

duction (Fig. 7(B)). The values of μ_r calculated from these O_2 and CO_2 determinations were 0·25 and 0·30 h^{-1}, respectively, i.e. generation times of 2·3 and 2·7 h, data which are acceptable for yeasts. Thus, the variation in μ_r values was around 20%, depending upon the gas considered. The mean value of respiratory quotient ($RQ = \Delta CO_2 / -\Delta O_2$), between 12 and 27 h of fermentation, was $1{\cdot}12 + 0{\cdot}01$ as obtained from 180 pairs of automatically registered data (Fig. 7(A)). However, the quantity of O_2 consumed and CO_2 produced were equimolar during respiration, thereby giving an RQ equal to 1·0.[25] The observed difference may be explained by the presence of urea in the culture medium, a obligatory component for growth, which is acting as a nitrogen source and also as a neutralizing agent for pH control of the medium.[19]

Another use of the monitoring system developed is for the calculation of specific growth rate (μ). On-line measurement of SSF process parameters is a useful tool for real-time assessment of cultures and reactor conditions. In this way, several authors have used gas analysis as a practical feature for fermentation monitoring.[9,13,14,23,26-28]

The relationships between O_2 consumption, CO_2 production and biomass have been established for yeasts on SSF systems.[12,13,14] These results permit the calculation of biomass production, cell yields against O_2 and CO_2 (Yx/O_2 and Yx/CO_2) and μ. This methodology is suitable when culture conditions are well known and yields are kept constants throughout the fermentation process. Nevertheless, in practice, these conditions cannot be ensured and some problems appear, especially concerning the values of these yields. It is felt that gas analysis can help to overcome this problem.

Data on the comparison of μ calculated from the cell number and μ_r calculated from CO_2 measurements are given in Fig. 8. These results

Table 1. Effect of inoculum level on major fermentation parameters of aerobic solid state cultures of *Schw. castellii*

	Inoculum level (cells/ml)	μ_r (h^{-1})	*Lag phase (h)*	*Final biomass (cells/ml)*	*Contamination*
1	$5{\cdot}7 \times 10^7$	0·30	3	$4{\cdot}0 \times 10^9$	−
2	$3{\cdot}9 \times 10^6$	0·30	4	$4{\cdot}4 \times 10^9$	−
3	$5{\cdot}8 \times 10^5$	0·31	13	$5{\cdot}4 \times 10^9$	−
4	$3{\cdot}8 \times 10^4$	0·28	25	$3{\cdot}1 \times 10^9$	+
5	$3{\cdot}6 \times 10^3$	0·16	42	—	+ + +

Symbols − and + correspond to absence and 25% of contaminations, respectively.

have emanated from the experiments involving two inoculum ages, 12 and 24 h, during aerobic cultivation of *Schw. castellii* on impregnated bagasse in a small-column fermenter. It was found

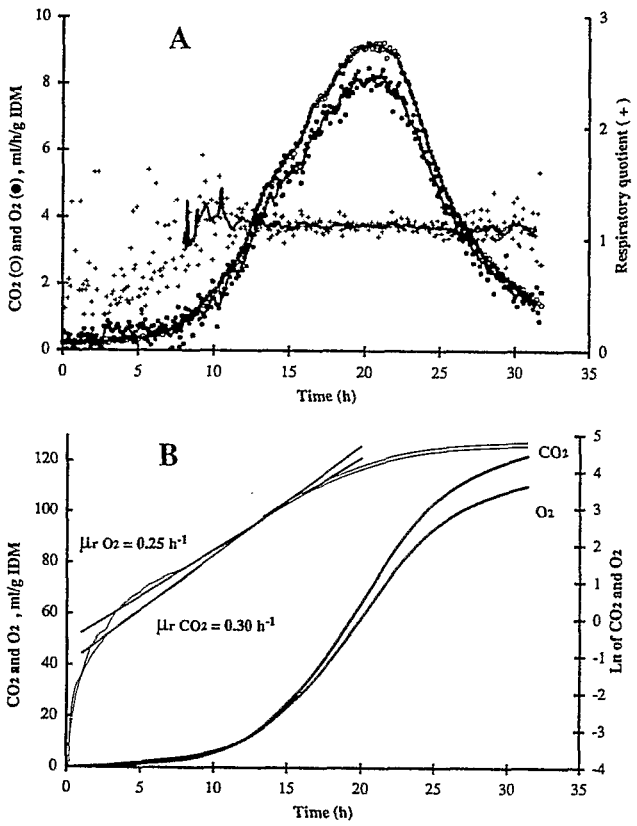

Fig. 7. CO_2 and O_2 profiles during aerobic cultivation of *Schw. castellii* on impregnated bagasse. A: Carbon dioxide production and oxygen consumption rates and respiratory quotient; B: total production of CO_2 and consumption of O_2 and estimation of respiratory activity (μ_r) by Ln of these gases.

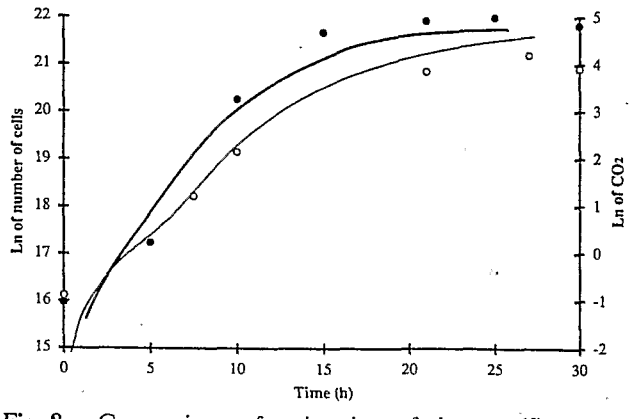

Fig. 8. Comparison of estimation of the specific growth rate (μ) by ln of cell numbers (symbols) and estimation of respiratory activity (μ_r) from Ln of CO_2 measurements (solid lines) during aerobic solid-state cultures of *Schw. castellii* on impregnated bagasse. Inoculum age: ——, ● = 12 h; ——, ○ = 24 h.

that the gradients of the graphs of Ln of cell number and Ln of CO_2 against time resembled each other very closely in the case of the same inoculum age (Table 2), thus giving similar values for μ and μ_r. These values for 12-h inoculum were higher than that of the 24-h inoculum. Such a trend was expected, since the inoculum was in exponential phase in the case of the 12-h inoculum. The estimation of μ from the Ln of the cell numbers is not very accurate in the present case because of the very low number of data point used, while the estimation of μ_r from Ln of CO_2 measurements is assumed to give a smaller error in calculation because of the large number of data points (Table 2). Thus, the experimental evidence showed that the specific growth rate could be estimated precisely through respiratory activity by continuous measurements of CO_2. The difficulties in and non-availability of biomass estimation methods for SSF processes[2,3] have been experienced as major constraints by many workers in the optimization and scale-up of SSF. The reliability of the present methodology could be of immense help for the on-line evaluation of solid-state cultures. It is also important to note the significance of μ_r as a specific parameter.

It is thus apparent that important information can be obtained by monitoring the exhaust gases from SSF. O_2 consumption, CO_2 production, the physiological stage of the cells and the respiration rate of the culture can be known on a real-time basis without disturbing the culture. It is worth pointing out that this prototype has been tested successfully in the ORSTOM laboratory (Montpellier, France), over the last 3 years, for monitoring diverse microbiological systems including solid-state cultures of yeasts[16,19] and moulds.[26]

Utility of the gas chromatograph system for monitoring aerobic–anaerobic SSF systems

SSF by *Schw. castellii* for alcohol production was selected as the experimental model for the automated monitoring of aerobic–anaerobic cultures. This fermentation consists of two phases: an aerobic stage required for biomass build-up and amylase synthesis and an anaerobic phase for the transformation of starch into alcohol. The experimental set-up and methodology were as described earlier.[16] A close gas-recirculation system was used to shift the metabolism from an aerobic to an anaerobic pattern. The concentration of all the components of culture medium, used for absorbing on bagasse, was increased by a factor of 2 and

Table 2. Comparison of specific growth rate (μ) calculated on the basis of cell number and the respiratory activity (μ_r) calculated on the basis of CO_2 measurements from the data on two inoculum ages during aerobic SSF by *Schw. castellii*

Inoculum age (h)	μ (h^{-1})	μ_r (h^{-1})	*Number of points used in regression*		*Correlation coefficient*	
	Ln X	*Ln CO_2*	*Ln X*	*Ln CO_2*	*Ln X*	*Ln CO_2*
12	0·40 ± 0·2	0·43 ± 0·02	4	22	0·970	0·993
24	0·30 ± 0·2	0·30 ± 0·01	3	97	0·998	0·996

3. Thus $1X$, $2X$ and $3X$ correspond to the three different concentrations used, where X expresses the concentration of the basic culture, where the starch represented nearly 85% of total nutrients.

The gas profiles of the exhaust air for these three starch concentrations are presented in Fig. 9(A) for O_2 and 9(B) for CO_2 levels. After a lag phase of nearly 6–7 h, the O_2 concentration decreased rapidly and was maintained at a very low level (< 1%) between 15–60 h, thereby shifting the metabolism from an oxidative to a fermentative type. The oxygen consumption expressed on a dry-matter basis decreased with the increase in the starch content (Fig. 9(A)). Nevertheless, the total oxygen consumption was the same, since the air supply was identical for all three cases. On the other hand, the carbon dioxide evolution increased progressively during the aerobic and anaerobic phases of the fermentation, for all three starch concentrations examined (Fig. 9(B)).

The data in Table 3 summarize the results concerning these experiments. The starch concentration in this aerobic–anaerobic SSF culture was inversely related to the quantity of water present in the medium culture. Therefore a change in the quantity of substrate gave rise to a specific decrease in the mositure content of the medium. An increment of a factor of 3 in the concentration of all the components of the medium decreased the moisture content from 77·1 to 62·8%. Amylases were produced during the aerobic phase of the fermentation.[29] A substrate utilization higher than 90% suggested that amylolytic activity from yeast was not limited by starch hydrolysis for the $1X$ and $2X$ cultures (Table 3). The apparent overall yield of alcohol production was slightly affected when the starch content was increased from 100 to 200 g/litre (−3%). Nevertheless, a substantial reduction was observed with with a concentration of 300 g/litre (−48·8%). This result could be explained mainly by a limitation of water availability and an increase of ionic strength in the culture medium. Sato and Yoshizawa[14] have already pointed out the importance of water content in alcoholic SSF systems. Our previous paper[30] has indicated the potential of using an amylolytic fermenting yeast for the production of alcohol from starch. The relatively low yield obtained was explained because part of starch was consumed for biomass build-up and

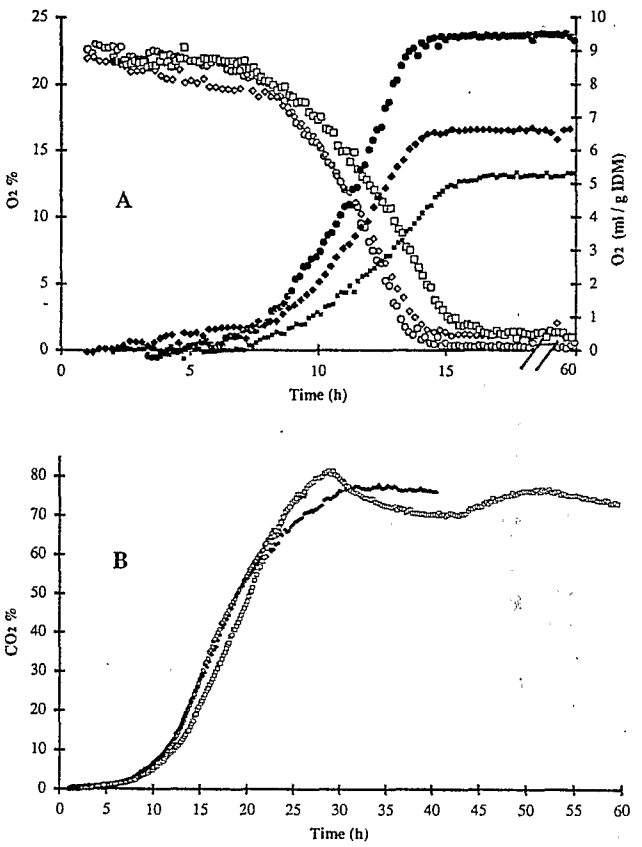

Fig. 9. Influence of starch concentration on profile of gases during aerobic–anaerobic solid-state cultures of *Schw. castellii* for production of alcohol from starch. A: O_2 concentration (hollow symbols) and total oxygen consumption (solid symbols); B: profiles of CO_2 concentration. Starch concentration codes: ○, ● = 100 g/litre; ◇, ◆ = 200 g/litre; □, ■ = 300 g/litre.

Table 3. Effect of starch concentration on performance of alcoholic fermentation by *Schw. castellii* in solid-state culture

Concentration factor	*Starch (g/litre)*	*Yx/s*	*Substrate consumption (%)*	*Alcohol yield (% theoretical)*	*Initial moisture (%)*
1*X*	100	0·05	95·7	62·5	77·1
2*X*	200	0·03	93·7	60·5	69·6
3*X*	300	0·04	93·9	32·0	62·8

C/N ratio of 65 and total air volume of 3700 ml.

amylase synthesis during the aerobic phase of the culture.

It appears to be difficult to apply a thermal mass flow meter for the continuous measurement of CO_2 evolution since the output signal fluctuated too much whilst recirculating gases from the fermenter.[27] Our system, reported in the present studies, overcomes this problem as it permits on-line continuous monitoring of SSF under aerobic and anaerobic conditions. Different phases of fermentation, thus, can be evaluated through the physiological state of the culture which is known in real time and without perturbation of the fermenting mass. This monitoring system could be an important tool for large-scale solid-state ethanol fermenters.[31]

Utility of the system involving individual gas analyzers for automatic gas control in aerobic solid-state culture

The results of the controlled aerobic solid-state cultures of *Schw. castellii* are presented in Figs 10(a), 10(b) and 11. The automatic control of CO_2, at a set value of 2%, resulted in a CO_2 concentration of $1{\cdot}8 \pm 0{\cdot}2\%$ (Fig. 10(a)). It has, in turn, maintained the O_2 concentration at $19{\cdot}3 \pm 0{\cdot}2\%$ (Fig. 10(a)). The control system worked/functioned intensively from 8 to 25 h, the most active phase of growth. In this period, the air flow rate was extensively altered automatically by the system and a total of 700 measurements and corrections were effected to maintain the CO_2 around the set value. The CO_2 levels in the exhaust air at other fermentation times were slightly below the set value. The use of the control system resulted in a nearly equal biomass yield ($Ys/s = 0{\cdot}40 \pm 0{\cdot}02$) at all bed heights of the controlled fermenter, in contrast to large variations in the fermenter without such control.[17] The plot of the air flow rate (ml/min) and the total air supply (litres/g IDM) against time showed interesting results (Fig. 10(b)). The shape of these curves

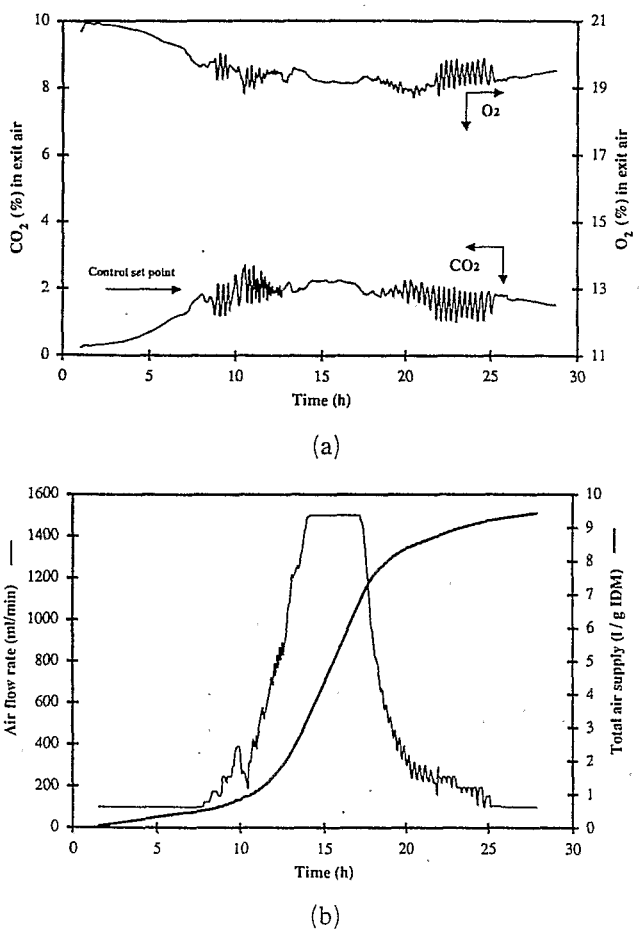

Fig. 10. Profile of gases during controlled solid-state cultures of *Schw. castellii* on impregnated bagasse. (a) CO_2 and O_2 concentrations (%) in exhaust gas from fermenter; (b) rate of air supply to fermenter and total air supply.

indicated the dependence of aeration requirements on the growth phases of the fermentation. The air-transfer capability of the system met the O_2 demand of the culture in an economic fashion as it supplied more air only when it was required. Thus the air flow rate will be low during lag or germination phases but will then increase gradually during the exponential phase and finally decrease progressively in the stationary phase.

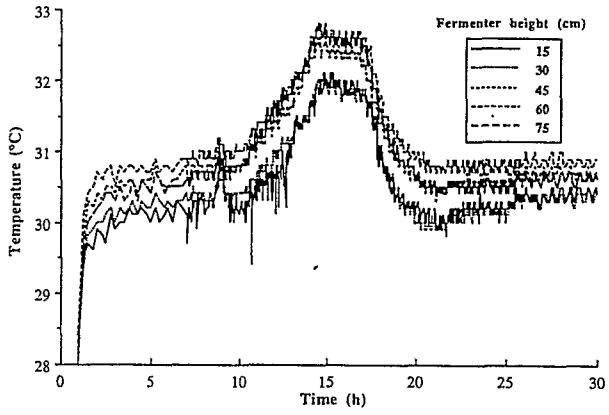

Fig. 11. Variations in the temperature in gas-controlled fermentations of *Schw. castellii* cultured on sugar-cane bagasses impregnated with nutrient medium.

An additional bonus of the control of CO_2 in exhaust air was the maintenance of the temperature of the culture in a reasonable range. During the exponential growth period an increase in temperature of 2°C was observed with respect to the set point (Fig. 11).

The development of an efficient automatic system for control of CO_2 in exhaust air constitutes an outstanding development for aerobic SSF. It will be of great significance in eliminating biomass and temperature gradients in the fermenting solids. Coupled with other control systems and reactors developed recently.[5,6,27,31,32] The control of CO_2 at a specific value can be used as a criterion for the scale-up of SSF processes. This new concept of control of exit gases in SSF could be an analog to that of dissolved oxygen in SmF. In our case, the O_2 in the headspace of the fermenter was maintained at 92% of the maximum value.

CONCLUSIONS

An automated system for on-line monitoring of gases from aerobic and anaerobic solid-state cultures, as developed in the present studies, is suitable for analysis of gases from eight fermenters or the same number of sampling ports in a large fermenter. It permits real-time assessment of different phases of SSF without disturbing the culture. For aerobic cultures, the specific growth rate (μ) can be estimated from carbon dioxide measurements. A system for the automatic control of CO_2 and O_2 in exhaust gases from aerobic solid-state cultures can maintain the cultures under non-limiting conditions with respect to O_2. Additionally, it considerably reduces the biomass and temperature gradients in a large fermenter.

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